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Dear participants,

Welcome to the 24th Meeting of the European Society for Animal Cell Technology to be held in Barcelona, Spain, from May 31st to June 3rd, 2015. I am very honoured to have received the responsibility from ESACT to become the meeting Chairman. I had the privilege to work together with an outstanding group of persons planning and supporting the preparation of this meeting, to whom I want to express my profound gratitude. These are the members of our Scientific Committee, Organizing Committee, ESACT Executive Committee, the emerging board of the ESACT Frontiers, the session chairs, the organizers of the pre-meeting Workshops and indeed the team of Grupo Pacifico providing support to all organization aspects.

ESACT has become a leading conference in the field of Animal Cell Technology, attracting around 1000 persons from both academia and industry. The three main components of this meeting: scientific programme, trade exhibition and social programme have been planned to provide in a short time an up-date on the advances in the field, both at the basic and applied levels, and an opportunity of interaction between the participants. I do hope that the complete programme will meet your expectations and you will find the meeting stimulating and inspirational for your own activities.

The Palau de Congressos de Barcelona, in the area of Fira de Barcelona – Plaça Espanya, is the venue for the 24th ESACT Meeting. It is conveniently located in the City, with good connections to the underground system and many hotels at a walking distance. The facilities in the venue will enable to run the meeting efficiently, providing good proximity among the different areas where the activities will run: auditorium, trade exhibition, poster area and catering.

The scientific program has been built in six sessions, each one of them with invited oral speakers, selected oral speakers from the abstracts presented by authors, and poster presentations. Many thanks to all of you, the scientific inputs are the pillars of our meeting.

All abstracts received have been reviewed in an anonymous process (author names and affiliations were not known through the evaluation) by the Scientific Committee members, and have been ranked strictly according their quality. This ranking has been then used to select the abstracts to be presented as oral contributions, and as well to select the best 100 posters that will be eligible for the poster prize. During the first poster session, anonymous poster prize jury members will evaluate these posters, and a short list of 20 posters will be selected by June 2nd. The authors of these posters will be invited for a short oral presentation on June 3rd afternoon session, and the attendants to this session will be voting for the three best posters, receiving the Poster Award at the Gala Dinner. I want to express here my gratitude to the persons serving as poster jury and to the organizers of the poster prize.

As a pre-meeting activity, six workshops are offered on Sunday morning, before the official opening of the Meeting. They include three topical workshops and three company workshops. We are very grateful to all individuals and companies putting efforts to prepare these workshops and give an additional value to our participants.

An intrinsic part of the ESACT meeting series is the exhibition area, where leading companies are presenting their products, services and capabilities, and meeting with the users of their technologies. The exhibition area has been designed to offer an atmosphere encouraging the interactions around the many stands of companies.

The social program has been conceived with the target to keep the outstanding quality of previous events, contributing to the unique atmosphere of ESACT meetings. Barcelona is a well-known city with many attractions for the visitors, and provides an exceptional scenario for the ESACT meeting. The selection of the sites for our social program has been done to expose the participants, in a short time, to some of the highlights of Barcelona and the Catalan culture. It includes two jewels of the Modernistic period, Sagrada Familia and Sant Pau Hospital, a visit to the splendid Codorniu winery with some cultural demonstrations, including food, and the Gala Dinner in the Catalan National Art Museum, with a visit to the Romanesque painting exhibition.

The program offered to our participants would not be possible without the generous support from our sponsors, to whom we are very grateful for their contributions, helping us in many aspects. Among them I would like to highlight the large number of grants that have been provided to young researchers to participate in the meeting, fact that is an intrinsic part of ESACT commitment to work for the younger generations in Animal Cell Technology.
Finally, a very important remark: ESACT has grown thanks to the contribution of a group of individuals who have generously provided their best for the advancement of Animal Cell Technology in Europe and the ESACT meeting is the occasion to acknowledge this by presenting the ESACT Medal to them. In our meeting, the persons receiving such recognition, for outstanding contributions to ESACT, are Martin Fussenegger, Stefanos Grammatikos and Sabine Geisse. Congratulations!!

I wish you a very successful meeting.

Warm welcome to Barcelona!!

---

Welcome from the Executive Committee

With great proudness we are looking to set-up of the forthcoming ESACT meeting. It is the 24th time that this meeting is organized under the umbrella of the European Society for Animal Cell Technology. Within the 39 years since its foundation ESACT has developed the meetings to become the most important European conference in the field of Animal Cell Technology. These are always based on a scientific programme, a trade exhibition and a rich social programme. While this is not the only activity of ESACT, it is the most important one and is regarded as the hallmark of the society. Looking to the set-up, the programme and the location I am can imagine that your expectations will be fulfilled in attending this meeting.

It is important to note that ESACT is initiating the meeting, but that its execution is in the hand of the meeting chairman. The decision to hand this responsibility to Francesc Gòdia was easily made. He had already chaired the ESACT meeting in Granada, an unforgettable event for those who had the privilege to participate. Apart from this, many other features qualified him to overtake this task. For him a driving force was the fact that after having organized a meeting in Spain he wanted to get another one to his home in Catalonia. In fact, Barcelona is a perfect place and its only disadvantage is that participants could be detracted from the meeting to enjoy the city. In this context it should be mentioned that the social programme will satisfy many touristic demands and will give you an insight into many aspects of the location.

Bringing such a meeting to fly needs many efforts from different organizations and individuals. We thank Grupo Pacifico for the organization, the scientific committee for setting up an interesting programme, the organizing committee for assembling all different activities, the speakers and poster presenters for their preparations, the exhibitors for setting up their booths and the sponsors for their generous support. Our main thanks go to Francesc Gòdia for his wholehearted engagement in this event. We wish all of us a meeting that meets our expectations, success for the presentations, new impulses and interactions amongst the participants.

On behalf of the ESACT Executive Committee

---

Hansjörg Hauser
Chairman

Paula Alves
Vice Chairwoman

Francesc Gòdia
Chairman 24th ESACT Meeting
Barcelona 2015
# COMMITTEES

## CHAIRMAN

**Francesc Gòdia**  
Universitat Autònoma de Barcelona (Spain)

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<td>UCB Pharma (Belgium)</td>
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<td>Elisabeth Fraune</td>
<td>Sartorius Stedim Biotech (Germany)</td>
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<td>Paula Alves</td>
<td>IBET (Portugal)</td>
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<td>Mercedes Segura</td>
<td>bluebird bio (USA)</td>
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<td>Matthieu Stettler</td>
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<td>Martin Fussenegger</td>
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<td>Elephterios Papoutsakis</td>
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<td>Amine Kamen</td>
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<td>Alain Bernard</td>
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<td>Helmholtz Centre for Infection Research (Germany)</td>
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<td>IBET (Portugal)</td>
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<td>Nicole Borth</td>
<td>BOKU, University of Vienna (Austria)</td>
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<td>Alan Dickson</td>
<td>University of Manchester (UK)</td>
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<td>Dethardt Müller</td>
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<td>Yvonne Genzel</td>
<td>Max Planck Institute for Complex Technical Systems (Germany)</td>
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VENUE MAP

LEVEL 3
- AUDITORIUM
- ROOM 5
- ROOM 6
- ROOM 3
- ROOM 1 and ROOM 2

LEVEL 2
- Restaurant
- Clock Room
- Poster

LEVEL 1
- Secretariat
- Poster
- Street level: Main Entrance

LEVEL 0
- Exhibition
## EXHIBITION MAP

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Innovation in
Cell Culture Technology

Pre-Conference Workshop
31st May, 14:00 - 15:00

During the workshop we will discuss how innovative single use solutions in cell culture technology help to speed up media and process development and improve large scale production.

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September 27th - October 1st, 2015
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Confirmed lecturers include Terry Papoutsakis (University Delaware, USA), Manuel Carrondo and Paula Aves (IBET, Portugal), Hansjörg Hauser (HZI, Germany), Francisco Gómez (UAB, Spain), Stefanos Grammatikos (UCB Pharma SA, Belgium) and Anne Tostrup (Biogen-Idec, Denmark). Other information can be found in www.esact.org

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Learn about high quality cell source and production, cellular assay optimization and cell assay applications.

The main topics (1) Target Identification and validation, (2) Preclinical testing, (3) Assay Development and validation, (4) Cell line selection, (5) Cell material production and validation, (6) Frequently used cellular HTS assays, (7) Complex cellular assays, and (8) Equipment and logistic.

Confirmed lecturers include Rosalía Arebola (Sanofi, France), Catarina Brito (IBET, Portugal), Christa Burger (Merck, Germany), Hansjörg Hauser (HZI, Germany), Stephanie Hennig (Uni Bonn, Germany), Eberhard Krauß (VIB Headquaters, Belgium), Heinz Ruffner (Novartis, Switzerland), and Carsten Pieck (Merck, Germany). Other information can be found in www.esact.org.
SOCIAL EVENTS

MONDAY | 18:00-22:00

Sagrada Familia + Sant Pau Hospital + cocktail

At 17:30 sessions will finish.

After the session closure, buses will start departing from the venue main entrance. The last bus will depart at 18:00. In each bus, there will be volunteers of the Congress guiding the group, first to Sagrada Familia and then to Sant Pau Hospital. At the arrival to Sagrada Familia, each group will meet a guide and have a 30 minutes visit, followed by free time, until 20:00.

Sagrada Familia is one of the greatest works of Antoni Gaudí, currently under construction. The origins of La Sagrada Familia go back to 1866, when Josep Maria Bocabella i Verdaguer founded the Spiritual Association of the Devotees of St Joseph, which from 1874 promoted the construction of an expiatory church dedicated to the Holy Family. When the church will be completed, it will have 18 towers: 12 dedicated to the apostles, 4 to the evangelists, one to Jesus and another to Mary. La Sagrada Familia is located in the heart of Barcelona and is frequented by millions of tourists every year.

The visit will finish at 20:00. Participants should gather at the same place they entered in Sagrada Familia, and will then take a short walk to Sant Pau Hospital. Volunteers will help with indications, although the street to walk, Passeig Gaudi, is straight and Hospital Sant Pau can be seen from Sagrada Familia very easy.

The architectural complex of Hospital de la Santa Creu i Sant Pau is a landmark in the culture and heritage of Barcelona city in particular and in Catalonia in general. It was founded by the partnership between the City, the Church and very generous donors, among them Pau Gili. Its architectural and patrimonial value extends beyond its urban location and the site has received international acclaim. In 1997, the Hospital was declared World Heritage by UNESCO for its singular architectural and artistic beauty.

A cocktail will be served for all participants, who will also be able to visit the Sant Pau Hospital complex and enjoy the richness of its decoration. Cocktail will finish at 22.00 pm. Buses will take all the members back from Sant Pau Hospital to Plaza Catalunya and Plaça Espanya at the end of the activity.
TUESDAY | 18:00-22:30

Outing to Codorniu winery + dinner

At 17:30 sessions will finish.

After the session closure, buses will start departing from the venue main entrance. The last bus will depart at 18:00. Travel journey is 30 minutes.

Codorniu winery It is a modernist complex, a jewel of the winery architecture designed by Josep Puig i Cadafalch and declared National Historical-Artistic heritage in 1976.

At their arrival, the participants will have the opportunity to join a short guided visit to the winery or enjoy a glass of cava wine in the gardens of the winery. Also, there will be a demonstration some of the Catalan oldest cultural traditions, including the famous human tours, Castellers.

After the visit, participants will enjoy dinner in one of the spectacular old cellars of Codorniu winery.

Dinner will finish around 22:30. Buses will take all the participants back to Plaça Catalunya and Plaça Espanya at the end of the activity.

With support of
WEDNESDAY | 21:00-24:00

Gala Dinner at MNAC

Palau Nacional, Parc de Montjuïc, s/n, Barcelona

From 20:45 buses will be departing from the venue to MNAC, Museu Nacional d’Art de Catalunya, in Palau Nacional de Montjuïc. Alternatively, participants can also use the escalators to reach the museum (5 minutes in bus, 10 minutes walking).

From 21.00, at the arrival to MNAC, participants will have the opportunity to have a drink in the cocktail terrace and also to visit the collection of art. Particularly, the participants will discover the secrets of Romanesque art, visiting the paintings from several churches from the Pyrenees mountains that were transferred to MNAC. These are paintings from XII and XIII Centuries. The Palau Nacional is one of the most emblematic buildings in Barcelona.

Over the years, since its opening in 1929, the building has been the scene of various events that have impacted the social life of Barcelona. MNAC is the balcony of Barcelona, from which you can get spectacular panoramic views of the colorful light show of the Magic Fountain and the city. The Magic Fountain will be operating for the ESACT meeting from 21:00 to 22:00, approximately.

The Gala dinner will begin around 22:00. The dinner will be held in the Oval Hall. The Oval Hall is one of the largest event rooms in Europe, where majestic classical architecture and modern productions meet.

Once the dinner will finish, the prizes for the posters and video contest will be presented and life music will close the meeting up to 02:00. From the end of the Gala dinner, up to 02:00, there will be a continuous service of buses connecting MNAC and Plaza Espanya.
A cell line for life

Part of our gene to GMP cell culture capability, Apollo™ is a mammalian expression platform developed by FUJIFILM Diosynth Biotechnologies’ scientists. Created with manufacture in mind, it will deliver a high quality recombinant cell line to take your biopharmaceutical from pre-clinical through to commercial production - a cell line for life.

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IMPORTANT INFORMATION

VENUE

Palau de Congressos de Barcelona (Fira de Barcelona)
Avda. Reina Maria Cristina s/n
08004 Barcelona

TECHNICAL SECRETARIAT

GRUPO PACIFICO
Marià Cubí 4 | 08006 Barcelona (SPAIN)
T. +34 932388777 F. +34 932387488
esact2015@pacifico-meetings.com

OFFICIAL LANGUAGE

The official language of the Congress is English. No simultaneous translation will be provided.

BADGES AND SECURITY

It is essential that you wear your personal badge at all times while in the Congress venue and events, as it is the official entrance pass to scientific sessions, and other Congress activities.

For the Social Events, it will also be necessary to present the corresponding Ticket that will be provided with the registration package.

MEDICAL ASSISTANCE AND INSURANCE

Participants are advised to make their own arrangements regarding travel insurance and medical assistance during the Congress. Neither the Organization nor the Congress Secretariat are able to accept any responsibility whatsoever for damage or injury to persons or their belongings during the Congress.

OPENING HOURS

Sunday, May 31st: From 9:00 am to 05:30 pm
Monday, June 1st: From 8:00 am to 05:30 pm
Tuesday, June 2nd: From 8:00 am to 05:30 pm
Wednesday, June 3rd: From 8:00 am to 05:00 pm
SPEAKERS READY ROOM

Speakers have to provide their presentations in Room 1. This has to be done no later than two hours before the scheduled time of the session.

Speaker's room opening hours:

- Sunday, May 31st: From 9:00 am to 05:30 pm
- Monday, June 1st: From 8:00 am to 05:30 pm
- Tuesday, June 2nd: From 8:00 am to 05:30 pm
- Wednesday, June 3rd: From 8:00 am to 03:00 pm

WIFI ACCESS

Wifi access: ESACT
Password: 2015

ACCESS TO THE CONGRESS

The delegate fee gives access to the following:

- Access to all the conference of the ESACT Congress
- ESACT Congress Lunches
- Traders Cocktail on Sunday, May 31st
- Social Events

Accompanying person fee give access to:

- Congress Lunches
- Social Events
ESACT Frontiers Program (EFp) is an initiative within the European Society for Animal Cell Technology focused on promoting career progression and active engagement of young generations to ESACT and to the field of Animal Cell Technology (ACT).

EFp mission is to facilitate the cooperation and knowledge exchange among young scientists of the ACT community and to provide an easy-to-access platform to connect young researchers with ESACT senior members.

EFp will also launch initiatives to challenge early-career researchers to promote academia-industry interaction, and to support innovative and/or unconventional ideas helping to open ESACT to future trends of science and technology. The ultimate goal is to provide the next generation of ESACT scientists a platform to influence the development of the society and to give the young generation of scientists a voice in our community.

EFp was founded in 2014 and the first group was selected out of 15 candidates by the ESACT Executive committee. The group represents scientists and engineers from academic, governmental and private institutions:

- Mercedes Segura, bluebird bio (Cambridge, MA, USA)
- Simon Ausländer, ETH Zurich (Basel Area, Switzerland)
- Emma Petiot, University Claude Bernard Lyon 1 (Lyon, France)
- Ana Filipa Rodrigues, iBET (Oeiras, Portugal)
- Markus Heine, Fraunhofer Institute for Toxicology and Experimental Medicine, ITEM (Braunschweig, Germany)
- Christopher Sellick, MedImmune (Cambridge, UK)
- Verena Lohr, Sanofi (Frankfurt, Germany)

This group will be partially renewed every two years (next renewal: ESACT meeting 2017). Application procedures for the next Frontiers group will be available on EFp webpage (through www.esact.org) from Jan-May 2017. Until then, EFp welcomes and encourages suggestions and ideas from all members of the ACT community, particularly our youngest members. Contact us at ESACT_Frontiers@esact.org.

During ESACT meeting 2015, EFp is chairing the Poster Prize Contest and, in the spirit of supporting innovative ideas, is also chairing the “Synthetic Biology” workshop and co-chairing the “Emerging Technologies” session. Additionally, EFp organized a new video contest: C2P2 in 90 sec. All authors from submitted abstracts and registered attendees were invited to upload a video, up to 90 seconds, connecting their research to the ESACT 2015 theme: C2P2 – Cells, Culture, Patients, Products. The best videos will be presented during the meeting and three winners will be selected.

EFp intends to organize an ESACT Frontiers meeting that will take place in-between ESACT meetings for the youngest ACT community members which will provide a platform for interaction, exchange of ideas, an opportunity to present their work and also sessions and workshops on career management and development. Additional actions under consideration include visiting grants, support on industry-academia interaction, science hack days, new teaching formats and grant awards for best MSc and PhD thesis.
ESACT MEDALS 2015
The ESACT medal is awarded to Dr. Fussenegger for his activities to promote ESACT towards a society that merges academic achievements with industrial development for the creation and production of new therapeutics and prophylactics through animal cell engineering.

Martin, a Swiss citizen started his career with basic work in molecular biology and infection in several famous laboratories in Basel and at Max-Planck-Institutes in Tübingen and Berlin. When he joined Jay Bailey in Zürich (1996) he moved towards bioengineering and within a very short period developed his own profile in this field by successful engineering of cell cycle and apoptosis with the emphasis of improving bioproduction. He continued as an independent investigator at ETH Zürich, first as a group leader then as Full Professor before becoming the founder and Head of the Department of D-BSSE of ETH in Basel. Unlike many academic scientists who confine themselves to a narrow field of inquiry, Martin has been pushing the boundary and has been continuously identifying new exciting approaches to target specific disease problems.

Martin was member of the ESACT Executive Committee (2001-2013), serving as Treasurer (2003-2005), Vice Chairman (2005-2009) and Chairman (2009-2013). He was member of numerous Scientific Committees for ESACT meetings and in this function he acted as a guarantor for scientific quality and originality of the programmes. During his chairmanship ESACT was reoriented and brought to the current status as a legal society. His leadership was instrumental for the success of ESACT.
Stefanos Grammatikos, Ph. D.
Vice President, Head of Biotech Sciences, UCB Pharma SA

The ESACT medal is awarded to Dr. Grammatikos for his leadership role in ESACT, and the development of the cell-culture biotechnologies to promote the growth of the protein therapeutics industry in Europe.

He is a native of Greece with broad educational and international experience: 12 years in the USA, 13 years in Germany, and 7+ years Belgium/UK. He was raised on Corfu, Greece and educated in the US in Chemical and Biochemical Engineering (U.C. Berkeley, Northwestern).

He was elected member of the ESACT Executive Committee (2001-2013), serving as Secretary (2005-09) and Treasurer (2009-13), and was instrumental to the growth and success of ESACT. He was the initiator of JIN, Job Information Network and other activities focused on providing support to the professional development of young people. He was very active in the organization of the ESACT Meetings in Dresden, Lille and Barcelona, in 2007, 2013 and 2015.

His industrial career of more than 20 years in bioprocess engineering and the management of biopharmaceutical R&D is profound in scope and diverse in activity. He worked for 12 years at Boehringer Ingelheim (Biberach, Germany) and since 2008 at UCB, Belgium. He had key roles in: the development of enabling technologies leading to platform expression systems, cell line and process development platforms for mAbs; and the organizational, operational and licensure success of new multimillion biopharmaceutical manufacturing facilities. He has extensive leadership experience in organizing, inspiring, motivating, mentoring, developing and managing large groups of scientists and staff.
The ESACT medal is awarded to Dr. Sabine Geisse for her activities to promote ESACT and her contributions to the field of cell based technology for the production of bio-therapeutics.

Sabine Geisse’s major focus of expertise is in eukaryotic cell biology. She holds PhDs in Nutrition and Human Biology awarded by the Justus-Liebig-University in Giessen and Philipps-University in Marburg, respectively. Following a postdoc period at the University of Essen, Germany, she joined Sandoz/Novartis in Basel/Switzerland in 1988 within the newly formed Discovery Technologies department. In 2004, she received the Novartis Leading Scientist award.

As a group leader, her team was responsible for the early stage production of recombinant biomolecules such as antigens, antibodies and therapeutic proteins within Research.

She built up and implemented a platform for transient and stable expression technologies in eukaryotic cell lines and developed expression systems for transient expression in several cell lines on small and large scale for the rapid generation of recombinant proteins.

Sabine Geisse organized for several years the Swiss/European BioTechnology Workshop (in Ittingen). Her good humor and very open heart created an unique atmosphere of joyful creativity in an international workshop that became a reference in this field and where scientific concepts and technologies for heterologous gene expression in animal cell systems catalyzed the exchange of know-how among engineers and scientists in academia and industry contributing to the success and progress of novel biopharmaceuticals.
Call for Applications for ACTIP Fellowships

ACTIP (the Animal Cell Technology Industrial Platform) is a scientific and informal European forum of companies employing animal cell technology (www.actip.org). Twice a year the representatives of the member companies meet to discuss research, development, technology and regulatory issues of mutual interest. Besides the representatives of the member companies, invited speakers and observer companies (SMEs working on the themes covered by the meeting) meet together and participate to these meetings. The meetings are characterized by a friendly atmosphere and very lively discussions and take place in the home towns (and sometimes on the premises) of ACTIP members.

ACTIP wants to open up these informal industrial meetings to you, young scientists working on a project in biomanufacturing or related activity of industrial interest, i.e. development of new expression systems, new mammalian cell lines, test development... ACTIP wants to offer you the floor to present your project and personal expertise to our community; not only an excellent opportunity for you to present your project to experts but also a great way to expand your personal network on a European scale.

The Fellowship consists of all costs paid to attend a two-day meeting of ACTIP. For the next two years a total of 4 Fellowships will be awarded to young professionals following an evaluation by experts from the member companies. The awarded Fellows will be invited to attend one of the ACTIP meetings taking place over the course of the next two years following the application. In order to be selected for such an invitation, ACTIP invites you to send in an application for the ACTIP Fellowship.

How to apply?
Applicants should be younger than 35 years of age and educated to at least degree level in one of the disciplines underlying biomanufacturing. The applicant should describe his/her involvement in a recent project on biomanufacturing or related activity of industrial relevance. Project descriptions should not exceed one A4 page. A recent curriculum vitae with full contact details should accompany the application.
Applications should be sent to:
Dr. Chandra Eskes, Executive Secretary ACTIP, email: actip@secam-ce.eu

Timeline
Applications can be sent from 15 June until 23 August 2015. Evaluations by ACTIP experts will take place from 24 August to 16 October 2015. Experts will be assigned by the ACTIP Steering Committee. Selected Fellows will be notified by October 30 at the latest. The shortlist of selected fellows will be published on the ACTIP website. The meetings to which the selected ACTIP Fellows shall be invited will take place during the period from December 2015 to June 2017.
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<td>Registration opens</td>
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<td>16.00-17.30</td>
<td>Opening + Keynote</td>
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<td>17.30-19.00</td>
<td>Session 1</td>
<td>18.00-22.00</td>
<td>Sagrada Familia + Hospital Sant Pau + Cocktail</td>
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<td>19.00-21.00</td>
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All sessions will be in auditorium and workshops in rooms 3, 5 and 6 as indicated in the programme.
# PRE-MEETING WORKSHOPS

## SUNDAY 31ST MAY

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<td>09.00</td>
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<tr>
<td>11.30-13.00</td>
<td><strong>Topical Workshops (in parallel)</strong></td>
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<td></td>
<td>Single-cell analysis – A new tool for bioprocess engineering</td>
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<td>From Glycoscience to Glycobiotechnology</td>
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<td>Synthetic biology: New tools for engineering mammalian cells</td>
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<td>13.00-14.00</td>
<td><strong>Lunch</strong> (level 3 – Hall)</td>
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<td>14.00-15.30</td>
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<td>Sartorius Stedim Biotech: Innovation in Cell Culture Technology</td>
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<td>SAFC: Applying an integrated design approach to create a novel high performance fed-batch media system for mammalian cells</td>
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**Topical Workshop** (Level 2 - Room 3)

**SINGLE-CELL ANALYSIS – A NEW TOOL FOR BIOPROCESS ENGINEERING**

Timo Frensing\(^1\), Hansjörg Hauser\(^3\)

\(^1\)Max Planck Institute for Dynamics of Complex Technical Systems, Sandtorstr.1, 39106 Magdeburg, Germany

\(^2\)Otto von Guericke University Magdeburg, Cellular Systems for Bioprocess Engineering, Universitätsplatz 2, 39106 Magdeburg, Germany

\(^3\)Helmholtz Centre for Infection Research, Scientific Strategy, Inhoffenstr. 7, 38124 Braunschweig, Germany

The smallest unit of cell culture-based production processes is the individual cell. So far, however, most analytical methods and tools used for bioprocess optimization have been focusing on bulk measurements of cell populations. These measurements typically provide only average results and cell-to-cell variability cannot be determined. Yet, it is well known that heterogeneities exist even in isogenic cell populations due to epigenetic alterations and stochasticity in signaling, gene expression and protein formation. To better understand the behavior and productivity of cells in biotechnological production processes and to support selection of high-yield cell clones it is important to study the performance of cells on a single-cell level. Novel technologies such as microfluidics, improvements in live cell imaging and flow cytometry, and increased sensitivity of omics platforms provide deeper insights into cell-to-cell variability and will help to define the characteristics of high-yield cells. The combination of single-cell analysis, bioinformatics tools and stochastic mathematical modeling will lead to a comprehensive understanding of the cell biology and will provide new strategies for the optimization of cell culture-based production processes.

The workshop aims to present an overview on the state-of-the-art methods for single-cell analysis and to discuss benefits and limitations. Additionally, new insights into cellular processes derived by single cell analysis shall be presented. The following questions will be addressed:

- What is the molecular basis of heterogeneity and how can it be overcome?
- What technologies and assays have been developed over the recent years?
- What are the current limits in analyzing individual cells?
- What options are available to design improved cell lines?
- What is the status of bioinformatics tools and mathematical modeling approaches?
- What would be required for a comprehensive description of cell growth and product formation in manufacturing of biologicals?

**Presentations:**

**1. Origin of cellular heterogeneity and its visualization.**

Hansjörg Hauser

*Helmholtz Centre for Infection Research, Scientific Strategy, Inhoffenstr. 7, 38124 Braunschweig, Germany.*
2. Temporal heterogeneity of expression onset from natural and synthetic regulatory circuits in isogenic cell populations.
Dagmar Wirth
Helmholtz Centre for Infection Research, Model Systems for Infection and Immunity, Inhoffenstr. 7, 38124 Braunschweig, Germany.

3. Engineering a stem cell niche-on-a-chip.
Yoji Tabata, Matthias Lutolf
Ecole Polytechnique Federale de Lausanne, Laboratory of Stem Cell Bioengineering, CH-1015 Lausanne, Switzerland.

Timo Frensing
Max Planck Institute for Dynamics of Complex Technical Systems, Sandtorstr.1, 39106 Magdeburg, Germany
Otto von Guericke University Magdeburg, Cellular Systems for Bioprocess Engineering, Universitätsplatz 2, 39106 Magdeburg, Germany.

Topical Workshop (Level 3 - Room 5)

FROM GLYCOSCIENCE TO GLYCOBIOTECHNOLOGY

With nucleic acids, proteins and lipids complex carbohydrates are one of the four fundamental classes of macromolecules that comprise living systems. Their complexity is extremely high due to fact that they are made up of a relatively large variety of individual sugar units linked to one another in a multitude of ways. Understanding their structures and functions is crucial to further explore biology and biotechnology.

Glycoscience, the fundamental study of complex carbohydrates and how they affect all our lives, is becoming more and more important in medical applications and biopharmaceutical industry. Research in this challenging field covers questions regarding the pathogenicity of viruses and bacteria, the way in which organs develop and are repaired and new ways of disease treatment. Thus, over the last two decades, glyco-biotechnology has been established as part of the big biotechnological market.

Covering state-of-the-art analytics, process technologies and medical applications in glyco-biotechnology this workshop aims to provide an overview on from basic research to industrial application with the following lectures:

- From Glycobiology to Glycoimmunology (Bernd Lepenies, MPI Golm/Dahlem).
- The Glycoanalytical Toolbox (Erdmann Rapp, MPI Magdeburg).
- Antibody glycans - functional impact and options for modification (Volker Sandig, ProBioGen).
- Mab production in CHO cells and the impact of process parameters and conditions on their glycosylation (Michael Butler, University of Manitoba).
- GlycoDelete technology for removing undesired glycan heterogeneity in mammalian cells, yeast and plants (Nico Callewaert, VIB).
Topical Workshop (Level 3 - Room 6)

SYNTHETIC BIOLOGY: NEW TOOLS FOR ENGINEERING MAMMALIAN CELLS

Chair: Simon Ausländer (ETH Zürich) and Markus Heine (Fraunhofer ITEM)

Abstract

Current producer cell lines in biopharmaceutical manufacturing exhibit remarkable production capacities, however, lack of any direct control option to adjust essential biological processes, such as the biopharmaceutical production itself or cell proliferation and viability. Synthetic biology now offers a wide range of ligand-responsive gene switches that provide precise gene control systems to fine-tune gene dosage of endogenous or synthetic genes. Next-generation cell lines could be engineered in the future to have the ability to integrate and process key information inside or outside of the cell (such as nutrient concentrations, pH level changes, miRNA expression patterns or light illumination) and link them to the activation of specific output proteins to adjust cellular processes.

In addition, engineered biosensors that monitor available metabolite levels inside the cell could complement these gene switches to timely and precisely control the production process. However, these tools are encoded in genetic elements and only reach their full potential when stably integrated into engineered cells. The CRISPR/Cas9 technology promises to revolutionize genome engineering and enables multiplexed modifications of DNA sequences within the cellular genome at any site of interest. In this workshop, we introduce you to these technologies and together discuss how they could improve the biopharmaceutical manufacturing process.

Moderator:

Simon Ausländer (ESACT Frontiers; ETH Zürich, Switzerland)

Speakers:

Markus Heine (ESACT Frontiers; Fraunhofer ITEM, Germany):
“State-of-the-art cell line engineering”

Karen Polizzi (Imperial College London, UK):
“Engineered biosensors in living mammalian cells provide on-line monitoring of metabolites”

David Ausländer (ETH Zürich, Switzerland):
“Identifying suitable ligand-responsive gene switches for bioprocessing”

Benjamin Häfliger (ETH Zürich, Switzerland):
“Engineering miRNA sensor-based gene circuits”

Helene Fastrup Kildegaard (Technical University of Denmark, Denmark):
“CRISPR/Cas9-mediated genome engineering in mammalian cells”
1) CELL CULTURE: “HIGH OCTANE FED-BATCH: PROCESS INTENSIFICATION REIMAGINED”

Abstract:

Multiple approaches are available for fed-batch media and process development. The differences in the approaches reflect challenges that process development scientists have taken on and addressed, either through process change or media/feed formulation change.

During this session, we will explore case studies that demonstrate how changes in media development are intended to assist process development scientists in addressing today’s media/process development challenges.

Specific topic discussion areas will include:

- Platform process considerations
- Feeding supplement process development constraints
- Management of product quality parameters

Presenter: Rebecca Moore, PhD. - Research & Development Senior Scientist - Thermo Fisher Scientific
- BioProduction

2) SINGLE-USE TECHNOLOGY: “INNOVATIVE SINGLE USE TECHNOLOGIES FOR BIOPHARMA PROCESSES”

Define your solutions with Innovative technologies like Single Use fermentor, Harvestainer for adherent cell culture based vaccines, Onsite Integrity testing, single use heat exchanger, Freeze/Thaw and shipping solutions.

Presenter: Jacob McNeil - Senior Product Manager - Thermo Fisher Scientific – BioProduction
Company Workshop Sartorius Stedim Biotech GmbH (Level 3 - Room 5)

INNOVATION IN CELL CULTURE TECHNOLOGY

Driven by a growing pipeline of biopharmaceutical drugs and the cost pressure that the Pharma industry is experiencing, more and more companies are adopting highly automated mini-bioreactors in process development and single-use production approaches up to the 2000 L scale. Especially continuous processing has gained tremendous interest as it enables to reduce production scale and facility footprint. The remaining step in upstream processing that has been challenging to address via single use techniques is clarification of crude harvests. As single use solutions are getting mature, the debate is moving towards definition of specific standards in support of users and suppliers.

During the workshop we will discuss how innovative single use solutions in cell culture technology help to speed up media and process development and improve large scale production.

Agenda

Novel, efficient approach of CHO media and feed optimization using ambr15 and DoE.
Dr. Wolfgang Kusser and Brian Lee, Media Applications Specialists, Sartorius Stedim Biotech.

Rapid, single use high throughput cell culture process development using ambr250.
Christopher Kistler, Associate Principal Scientist Merck and Co.

Case Study: Continuous Processing at 2000L Single-Use Bioreactor Scale.
Jessica Martin, Field Marketing Manager Single Use North America, Sartorius Stedim Biotech.

Katharina Blaschczok; Research Assistant, ZHAW.

New Single-Use Solutions for Harvesting High Density Mammalian Cell Cultures at Cubic Meter Scale.
Anja Regner, Process Engineer, USP Production, Rentschler Biotechnologie.

Company Workshop SAFC (Level 3 - Room 6)

APPLYING AN INTEGRATED DESIGN APPROACH TO CREATE A NOVEL HIGH PERFORMANCE FED-BATCH MEDIA SYSTEM FOR MAMMALIAN CELLS

Kevin Kayser, Ph.D., Senior Director R&D, SAFC, Cell Sciences and Development, SAFC -Sigma Aldrich, 2909 Laclede Avenue, Saint Louis, MO 63103, USA

Over the past 20 years, significant advances have been made in media and feed design. New design approaches have been established at SAFC to optimize industrial cell culture medium and feeds. Dr. Kayser will reflect on the various methodologies that have been employed over the years both in SAFC and the industry as a whole. He will discuss SAFC’s current approach which is an integrated design strategy. SAFC approaches optimization of both medium and feed simultaneously using fundamental nutritional biochemistry analysis, high-throughput testing and multivariate statistics. We applied this new integrated design strategy to create a new chemically defined fed batch media for Chinese hamster ovary cells. Over one-hundred biopharmaceutical manufacturing characteristics were considered in the media and feed design specifications. Dr. Kayser will focus on the design and testing of this new media and feed system.
## SCIENTIFIC PROGRAMME

### SUNDAY 31ST MAY

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<td>Chairperson: Martin Fussenegger</td>
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<td></td>
<td>• Biological Functions and Therapeutic Potential of Vascular</td>
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<td>Endothelial Growth Factors</td>
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<td>Kari Alitalo (Translational Cancer Biology Program and Wihuri Research</td>
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<td><strong>17.30-19.00</strong></td>
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<td>Chairpersons: Hitto Kaufmann and Hansjörg Hauser</td>
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<td>17.30-18.00</td>
<td>• Front-line of genome editing technology for animal cell engineering</td>
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<td>Tetsushi Sakuma (Hiroshima University, Japan)</td>
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<td>18.00-18.20</td>
<td>• Efficient genome engineering of CHO cell factories using CRISPR</td>
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<td>Helene Fastrup Kildegaard (Technical University of Denmark, Denmark)</td>
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<tr>
<td>18.20-18.40</td>
<td>• Optimization of protein production via genetic engineering of CHO</td>
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<td>Pierre-Alain Girod (Selexis, Switzerland)</td>
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<tr>
<td>18.40-19.00</td>
<td>• Enabling tools for virus producer cell line development and</td>
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<td>Ana Sofia Coroadinha (IBET, Portugal)</td>
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<td><strong>19.00-21.00</strong></td>
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### MONDAY 1ST JUNE

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<td>09.00-09.30</td>
<td>• C/ EBP α creates an elite cell state in B cells. Thomas Graf (Center of Genomic Regulation, Spain)</td>
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<td>09.30-09.50</td>
<td>• Adventitious agent contamination risk mitigation: engineering mmv virus resistance into CHO Host Cells. Joaquina Mascarenhas (Sigma Aldrich, USA)</td>
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<td>09.50-10.10</td>
<td>• Finding the needle(s) in the haystack: identification of mirna master regulators improving productivity independent of cell type and product. Simon Fischer (University of Applied Sciences Biberach, Germany)</td>
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<td>10.10-10.30</td>
<td>• Production of IgGs with a human-like sialylation in CHO cells. Céline Raymond (National Research Council of Canada, Canada)</td>
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<td>11.00-13.00</td>
<td>Session 2: Cell based assays to drive drug development (Auditorium)</td>
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<td>11.00-11.30</td>
<td>Chairpersons: Manuel Reina and Christa Burger</td>
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<td>11.00-11.30</td>
<td>• Cell-based methods for early detection of hepatotoxicity in drug development: high content multiparametric screening and toxico-metabolomics. José Vicente Castell (Hospital La Fe, Spain)</td>
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<td>11.30-12.00</td>
<td>• Novel iPSC-derived cellular systems for in vitro disease modeling. Giorgia Salvagiotto (Cellular Dynamics, USA)</td>
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<td>12.00-12.15</td>
<td>• Next-generation 96-well tissue culture plates re-engineered via 3D printing: A screening platform for measuring contractile force in human pluripotent stem cell-derived cardiac microtissues. Nimalan Thavandiran (University of Toronto, Canada)</td>
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<td>12.15-12.30</td>
<td>• A “Human-on-a-Chip” platform-aiming for a paradigm shift in substance testing. Uwe Marx (TissUse Berlin, Germany)</td>
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<td>12.45-13.00</td>
<td>• Immortalized lung epithelial cells with in vivo like characteristics. Tobias May (InSCREENeX GmbH, Germany)</td>
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<td>16.00-16.30</td>
<td>The beauty and the beast: a perspective on Biopharmaceuticals.</td>
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<td>16.30-16.50</td>
<td>Improved production and quality of enveloped viral particles in BEVS:</td>
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<td>16.50-17.10</td>
<td>Identification of process parameters influencing product quality in</td>
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<td>17.10-17.30</td>
<td>Non-fucose level as a function of glycoenzyme transcription in a</td>
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<tr>
<td>18.00-22.00</td>
<td>Sagrada Familia + Hospital Sant Pau + Cocktail</td>
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### Session 3: Linking process engineering to product quality (Auditorium)

**Chairpersons:** Ashraf Amanullah and Alain Bernard

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<tr>
<td>09.00-09.30</td>
<td>When bad things happen to good proteins: effects of cell culture process on protein characteristics.</td>
<td>Robert Kiss (Genentech, USA)</td>
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<td>09.30-09.50</td>
<td>Understanding the impact of mild hypothermia on monoclonal antibody glycosylation in cho cell cultures through experimental and computational analyses.</td>
<td>Si Nga Sou (Imperial College London, UK)</td>
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<td>09.50-10.10</td>
<td>Dynamic flux analysis of GS-CHO cells: Identifying the burden of protein glycosylation on cellular metabolism.</td>
<td>Ioscani Jiménez Del Val (University College Dublin, Ireland)</td>
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<td>10.10-10.30</td>
<td>Process parameters impacting product quality</td>
<td>Jan Bechmann (Boehringer Ingelheim Pharma GmbH &amp; Co. KG, Germany)</td>
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#### 10.30-11.00 Coffee break (level 0 – Exhibition area)

### Session 4: Emerging technologies (Auditorium)

**Chairpersons:** Martin Fussenegger and Ana Filipa Rodrigues

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<td>Targeting cancer and chronic inflammation with armed antibodies and small molecule drug conjugates</td>
<td>Dario Neri (ETH Zurich, Switzerland)</td>
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<td>Complex primary cell-based models for drug discovery</td>
<td>Heinz Ruffner (Novartis Institute of Biomedical Research, Switzerland)</td>
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<td>12.10-12.25</td>
<td>DNA methylation in CHO dp-12 cells: landscape and effects on gene expression</td>
<td>Ana Wippermann (Bielefeld University, Germany)</td>
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<td>12.25-12.40</td>
<td>High-Density 3D Cell Culture and Analysis in an Integrated Microfluidic Platform</td>
<td>Sebastien Sart (Ecole Polytechnique LADHYX, France)</td>
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<td>12.40-12.55</td>
<td>Mammalian designer cells for biomedical applications</td>
<td>David Ausländer (ETH Zürich Basel, Switzerland)</td>
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<td>12.55-13.10</td>
<td>Multi-omics approach for comparative studies of monoclonal antibody producing cho cells</td>
<td>Camila A. Orellana (AIBN, The University of Queensland, Australia)</td>
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#### 13.10 -14.30 ESACT General Assembly (lunch provided) (Room 5)

#### 13.10-14.30 Lunch (Level 2 - Restaurant)
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| 14.30-16.00  | **Poster Session and Coffee** (Level 1 and 0)  
Presenting authors must be at their poster for discussion.  
From 14.30 to 15.15 for even poster numbers and from 15.15 to 16.00 for uneven numbers. |
| 16.00-17.30  | **Session 5: New Therapeutic Approaches** (Auditorium)  
Chairpersons: Terry Papoutsakis and Margarida Serra |
| 16.00-16.30  | • *Antibody-drug conjugates: implications on the design of mAbs*  
* Aad van de Leur (Synthon Biopharmaceuticals, The Netherlands) |
| 16.30-16.50  | • *Expression and manufacture of a new bispecific drug for her2+breast cancer: the beat-gbr 1302 antibody*  
* Pierre Moretti (Glenmark Pharmaceuticals, Switzerland) |
| 16.50-17.10  | • *Development of an advanced cell therapy product indicated for the treatment of gonarthrosis*  
* Joaquim Vives (Banc de Sang I Teixits, Spain) |
| 17.10-17.30  | • *Optimization of hpsc production from distinct pluripotent and metabolic states*  
* Yonatan Lipsitz (University of Toronto, Canada) |
| 18.00-22.30  | **Outing to Codorniu winery + dinner** |
**WEDNESDAY 3RD JUNE**

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<td>Auditorium</td>
<td>Terry Papoutsakis and Margarida Serra</td>
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<td>09.00-09.40</td>
<td>• AAV at 50: A golden anniversary of discovery research, and gene therapy success in the Samulski lab.</td>
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<td>R. Jude Samulski (U. North Carolina, USA)</td>
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<td>• Rapid Response Ebola Antibody Development Enabled by High Throughput Technologies.</td>
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<td>Kevin Bailey (Regeneron, USA)</td>
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<td>10.10-10.30</td>
<td>• Protein aerosol for intranasal nose to brain (N2B) delivery.</td>
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<td>11.00-13.00</td>
<td><strong>Session 6: Cell based Vaccines</strong></td>
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<td>Amine Kamen and Yvonne Genzle</td>
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<td>11.00-11.30</td>
<td>• Recombinant vaccine made in Insect cells.</td>
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<td>Mannon Cox (Protein Sciences, USA)</td>
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<td>11.30-12.00</td>
<td>• Fighting Ebola Virus infection: the Development and Use of Ebola Monoclonal Antibodies and Vaccines.</td>
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<td>Xiangguo Qiu (Public Health Agency, Canada)</td>
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<td>12.00-12.20</td>
<td>• Development of an anti-idiotypic Mab as cancer vaccine: from discovery to approval.</td>
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<td>Adolfo Castillo (Center of Molecular Immunology, Cuba)</td>
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<td>12.20-12.40</td>
<td>• Development of a mammalian cell culture process for the rapid clinical-scale production of novel influenza nanoparticle vaccines.</td>
<td>Auditorium</td>
<td>Payal Biswas (VPP-VRC-NIAID-NIH, USA)</td>
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<td>12.40-13.00</td>
<td>• Purification of Modified Vaccinia Virus Ankara from suspension cell culture.</td>
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<td>Ingo Jordan (ProBioGen AG, Germany)</td>
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<td>13.00-14.30</td>
<td><strong>Lunch</strong></td>
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<tr>
<td>14.30-16.00</td>
<td><strong>Poster session: presentation of posters selected for poster prize</strong></td>
<td>Auditorium</td>
<td>Mercedes Segura and Verena Lohr</td>
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16.00-17.30  **Keynote + Closing**

Chairperson: Hansjörg Hauser

- *Gene Therapy of Inherited Muscle Diseases*
  *Fulvio Mavilio* (Genethon, France)

21.00-24.00  **Gala Dinner at MNAC**
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   IN CELL BIOLOGY AND MEDICINE .......................................................................................76
6. CELL CULTURE BASED VACCINES ..........................................................................................81
7. CLOSING KEYNOTE ...................................................................................................................86
BIOLOGICAL FUNCTIONS AND THERAPEUTIC POTENTIAL OF VASCULAR ENDOTHELIAL GROWTH FACTORS

Kari Alitalo.
Translational Cancer Biology Program and Wihuri Research Institute, Biomedicum Helsinki, University of Helsinki, Helsinki, Finland.
kari.alitalo@helsinki.fi

Because of the importance of the growth of new blood vessels, or angiogenesis, in tumor progression, the first anti-angiogenic agents have been approved for clinical use. Although these treatments have been successful in the treatment of many types of solid tumors, most patients are either refractory or eventually acquire resistance to anti-angiogenic therapeutics. A combination of angiogenesis inhibitors based on based on solid knowledge of the major interacting angiogenesis signaling pathways could be used to significantly advance the efficacy of tumor therapy. - The idea of proangiogenic therapy is to grow new functional blood vessels and thus restore blood flow to ischemic tissue. In addition to angiogenesis of blood capillaries, growth of larger arterioles/arteries (arteriogenesis, or collateral formation) is especially beneficial for this goal. Several attempts have been made to stimulate angiogenesis and arteriogenesis in tissue ischemia, with limited success. One of the obstacles has been the property of angiogenic growth factors to promote vascular leakage, leading to tissue edema and fibrin deposition. Despite intensive efforts, growth factors suitable for angiogenic therapy have not yet provided significant help in the treatment of cardiovascular disease. – A better understanding of the biology of the vascular growth factors may facilitate therapeutics development for cardiovascular diseases. - The growth of lymphatic vessels, lymphangiogenesis, is actively involved in a number of pathological processes including tissue inflammation and tumor dissemination but is insufficient in patients suffering from lymphedema, a debilitating condition characterized by chronic tissue edema and impaired immunity. Lymphangiogenic growth factors provide possibilities to treat these diseases.
FRONT-LINE OF GENOME EDITING TECHNOLOGY
FOR ANIMAL CELL ENGINEERING

Tetsushi Sakuma.
Department of Mathematical and Life Sciences, Graduate School of Science,
Hiroshima University, Hiroshima 739-8526, Japan.
tetsushi-sakuma@hiroshima-u.ac.jp

Genome editing technology heralds a new era for animal cell engineering. Programmable site-specific nucleases, such as transcription activator-like effector nucleases (TALENs) and clustered regularly-interspaced short palindromic repeats (CRISPR)/Cas9, enable to induce DNA double-strand breaks (DSBs) at any desired genomic locus, resulting in efficient gene knockout and knock-in in broad range of cultured cells1. In this presentation, I introduce a brief overview and provide latest information regarding genome editing technologies. Our group has so far developed various systems in genome editing field, such as the Platinum Gate TALEN system for constructing highly-active Platinum TALENs2 and the Multiplex CRISPR/Cas9 Assembly System for creating all-in-one CRISPR/Cas9 vector enabling highly-efficient multiplex genome editing3. Recently, our group newly established the PITCH (Precise Integration into Target Chromosome) system, which facilitates convenient gene knock-in in cultured cells and organisms4. Homologous recombination (HR)-assisted gene knock-in has generally been used for spontaneous or programmable nuclease-mediated donor DNA integration. It enables precise gene knock-in, but the labor for constructing targeting vector with long homology arms and limited applicability due to the lower HR activity have been technical hurdles to utilize this method. On the other hand, our PITCH system utilizes alternative DSB repair pathway, microhomology-mediated end-joining (MMEJ), which enables easy, efficient and precise gene knock-in without relying on HR.
Currently, we have been improving our systems and broadening their applicability. This presentation includes such state-of-the-art information of genome editing technology, hopefully contributing the advancement and expansion of this technology in animal cell engineering.

References:
EFFICIENT GENOME ENGINEERING OF CHO CELL FACTORIES
USING CRISPR CAS9 TECHNOLOGY

Helene Fastrup Kildegaard, Jae Seong Lee, Lise Marie Grav, Thomas Kallelhauge, Signe Gerling Thomsen,
Carlotta Ronda, Henning Gram Hansen, Anders Holmegaard Hansen, Lasse Ebdrup Pedersen.
Novo Nordisk Foundation Center for Biosustainability,
Technical University of Denmark, Denmark.
hef@biosustain.dtu.dk

Background and Novelty:
Chinese hamster ovary (CHO) cells are widely used in the biopharmaceutical industry as a host for the
production of complex pharmaceutical proteins. Thus, genome engineering of CHO cells for improved product
quality and yield is of great interest. Here, we demonstrate the applicability and efficacy of the CRISPR Cas9
genome editing technology for generating knock-in and knock-out CHO cell lines with desirable properties.

Experimental Approach:
Transient transfection and expression of a CHO codon-optimized Cas9 protein together with sgRNAs against
genes involved in glycosylation (COSMC and FUT8) and in apoptosis (BAK and BAX) was performed to
generate knock-out CHO cell lines. To generate knock-in CHO cell lines, donor DNAs were designed and co-
transfected with Cas9 and sgRNA to facilitate site-specific integration of transgenes into the CHO genome. The
efficacy of the CRISPR Cas9 system and the indels generated were analyzed by Sanger sequencing and deep
sequencing. Off-target analysis was also performed.
Knock-in and knock-out cell lines were generated using limited dilution or single cell sorting using FACS. The
engineered cell lines were further analyzed in respect to growth, transgene expression, glycoprofiles, protein
level, gene copy number and apoptosis.

Results and Discussion:
Application of the CRISPR Cas9 technology for CHO cell engineering facilitated the disruption of the four
genes with an indel frequency of up to 47% in COSMC. Enrichment of cells transfected with Cas9 and sgRNAs
against FUT8, BAK and BAX simultaneously increased the average frequency of indels generated at the three
genomic sites from 11% to 68%. Single cell sorting of the enriched multiplexed cells facilitated the generation
of single, double and triple knock-out cell lines. Further characterization of selected triple knock-out cell lines
confirmed improved resistance to apoptosis and removal of fucosylation as expected.
In addition, site-specific integration of transgenes mediated by CRISPR Cas9 and homology directed repair
resulted in the generation of targeted integrants with improved clonal homogeneity compared to random
integrants.
The proven efficacy of genome engineering mediated by CRISPR Cas9 technology has a large potential to
accelerate current CHO engineering efforts and to pave the way for accelerated generation of desirable CHO
cell factories with predicted culture performance.
OPTIMIZATION OF PROTEIN PRODUCTION VIA GENETIC ENGINEERING OF CHO CELL LINE

Girod Pierre-Alain.
Selexis, Plan-les-Ouates, Switzerland.
pierre-alain.girod@selexis.com

Background:
Optimizing protein expression is a multidimensional problem. Mammalian expression systems are influenced by mRNA sequence, host and production conditions. These factors are closely connected and impact each other. Decoding the complex nature of factors influencing expression has identified adjustable parameters (mRNA and protein structure, vector system, culture conditions). In some cases, it still does not impact on abundance of the end-product. The reason is optimal productivity of some recombinant proteins (ie Fc-fusion, blood clotting factors) requires more than elevated transcription (1). CHO cells have some limitations in their intrinsic capacity to manage high level of protein synthesis as well as folding of some recombinant proteins.

Experimental Approach:
We exploited the transcriptome data of our CHO-K1M host cell line to monitor transcript levels of genes involved in protein synthesis pathways. Our engineering design was based on the observation that variability of gene expression level among the different pathway steps may contribute to the recombinant product differences. We constructed libraries of engineered CHO-K1M cells using unique proprietary transposon vectors harboring SGE DNA elements to compensate for rate-limiting factors (2). Each CHO-K1Mplus cell library displays a diversity of auxiliary proteins involved in secretory pathway machineries and cellular metabolism. Collectively, the libraries address a broad range of expression issues.

Results and Discussion:
Application of CHO-K1Mplus cell libraries enabled the selection of cell lines expressing the correct product (Fig.1B) by comparison to the unmodified host cell (Fig.1A). Our results demonstrate that ‘omic’ profiling by co-expressing components of the cellular metabolism can improve the secretion efficiency of some difficult-to-express therapeutic proteins from CHO cells (3).

References:
ENABLING TOOLS FOR VIRUS PRODUCER CELL LINE DEVELOPMENT AND ENGINEERING


(1) iBET, Oeiras, Portugal; (2) iBET and ITQB-UNL, Oeiras, Portugal.

avalente@itqb.unl.pt

Background and Novelty:
Stable cell line development or cell engineering substrates for virus production is an intensive and time consuming work. Several genetic constructs are often needed thus, requiring the transfection, clonal selection and screening for each. Virus titration methods are also laborious and in some cases lengthy. Herein, we describe a novel method for fast screening of high-titer virus producing clones that allows to merge cloning and titration in a single step.

Experimental Approach:
The method makes use of split-GFP, a green fluorescent protein separated into 2 fragments – S10 and S11 – which fluoresce only upon transcomplementation. A cell population producing infectious virus with a S11 transgene is cloned and co-cultured with a target cell line harboring the S10 fragment. S11 viruses produced by the clone infect the target cells and reconstitute the GFP signal. Only the clones yielding high fluorescence signal are isolated, avoiding growth/titration studies of the low titer clones. Additionally, this method is being combined with targeted integration which confers flexibility to modify the producer cell by the use of recombinase mediated cassette exchange (RMCE) methodology.

Results and Discussion:
The method was first validated by establishing a retrovirus producer from a nude cell line allowing the screening of 200 clones in two weeks; clones producing up to 1x10⁶ infectious particles per mL were isolated. Since this method combines both the power of single-cell resolution with the dynamics of cell population analysis it has a wide potential of application such as the evaluation of different cell substrates, expression vectors or metabolic cell engineering strategies. We used it to compare the production performance of different cell substrates – HEK293 vs. HEK293T – showing that the later sustain increased titers; this performance was shown to result from physiological changes induced to the cells rather than at the level of viral vector transcriptional activity, substantiating the need of large T antigen to support high viral titers. Presently the method is being used in cell metabolic engineering. Thirty gene targets are being evaluated. For example, the over-expression of glutathione metabolism genes originated clones producing up to 12 fold more.

The method herein developed, demonstrated its potential in important aspects of viral vector production, from high-titer clone selection to cell metabolic engineering and screening for high-producing cell substrates. Current undergoing work is its adaptation to other virus production systems, either in stable or transient and its conversion to a label free methodology making it possible to be used with wild type viruses.

This enabling tool will contribute for the progress of virus based biopharmaceuticals by accelerating cell line development and host engineering.
C/EBPα CREATES AN ELITE CELL STATE IN B CELLS

Thomas Graf.
Center of Genomic Regulation, Barcelona, Spain.
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Our earlier work has shown that the myeloid transcription factor C/EBPα induces B cells to transdifferentiate into macrophages at high efficiencies. More recently we reported that the transient expression of this factor in B cells, followed by expression of Oct4, Sox2, Klf4 and c-Myc (OSKM), poises them for nearly deterministic reprogramming into induced pluripotent stem cells. We have now found that reprogramming of the poised B cells by OSKM can be further enhanced under culture conditions of naïve pluripotent cells, resulting in the activation of endogenous Oct4 within 24 hours. The ‘elite’ cell state created in B cells by the transient activation of C/EBPα appears to be akin to that of granulocyte/macrophage progenitors, which are exquisitely sensitive to OSKM induced reprogramming and whose generation strictly depends on C/EBPα. Our findings have removed a major obstacle in studying the early reprogramming events and permitted us to investigate how the transient expression of C/EBPα in B cells leads to the almost immediate accessibility of pluripotency genes to Oct4 binding. Our new data provide unprecedented insights into the earliest events leading to activation of the pluripotency gene regulatory network, resulting in somatic cell reprogramming.
ADVENTITIOUS AGENT CONTAMINATION RISK MITIGATION: ENGINEERING MMV VIRUS RESISTANCE INTO THE CHO HOST CELLS

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Background:
The introduction of animal origin free (AOF) media has significantly reduced the incidence of adventitious virus contamination in biological production systems. Nevertheless, contamination by the parvovirus Mouse Minute Virus (MMV) remains a continuing challenge. Although infrequent, infection of a fermenter can be catastrophic for a manufacturer, and can also have a potential impact on drug supply, patient safety and have regulatory implications. In this work, we evaluated engineering the CHOZN+ GS+ parental cell line to create a new host cell line that would be resistant to MMV infection by modifying the major receptors used by the virus to enter cells. The goal was to engineer a host cell line resistant to MMV infection, while maintaining productivity and product quality profiles. Our strategy is outlined below.

Experimental Approach:
Attachment to a cell surface receptor is a key first step in the infection cycle for viruses. MMV has been shown to preferentially bind to α-2,3 sialylated glycans with a type-2 Galβ1-4GlcNAc motif.

Our approach was to systematically knock out genes affecting sialylation and then challenge each cell line for their ability to resist viral entry. To test the importance of sialylation the following genes were knocked out:
1. The CMP-sialic acid transporter, solute carrier family 35A1 (Slc35A1) which results in a complete absence of sialylated glycans.
2. The core 1-beta-1,3-galactosyltransferase-1 specific chaperone (COSMC) which leads to truncated O-glycosylation and an absence of sialic acid on these structures, while N-linked glycosylation remains unaffected.
3. The core 1-beta-1,3-galactosyltransferase-1 (C1GalT1) enzyme required for synthesis of the core 1 structure of mucin-type O-glycans.

MMV infectivity studies were conducted with different strains of virus at multiplicities of infection of 0.8, 3 and 0.3 viruses per cell, far higher than would be encountered in a fermenter infection. Infection of the cells was analyzed at several time points by Western blot for expression of replication associated viral proteins and by Southern blot and PCR for viral genomic sequences.

Results:
While the complete absence of sialic acid on the SLC35A1 knock-out cell line led to almost complete resistance to MMV infection, the COSMC knock out clones were about 10x more resistant to MMV infection. The knock out of the COSMC gene exclusively truncates O-glycosylation, leaving the N-glycosylation pathway and the terminal sialic acids intact. Given the importance of N-glycosylation on most monoclonal antibody therapeutics and the absence of O-glycosylation on human IgG, we went ahead with this target for further characterization and validation. These analyses included evaluating multiple COSMC knock out clones for genotypic and phenotypic stability. To asses recombinant protein production and quality, a model recombinant IgG was transfected into the new host cell lines and high titer producing single cell clones were generated.

Growth, IgG productivity and product quality verification studies on the recombinant clones were performed. Our data demonstrate that viral resistance against MMV virus can be incorporated into CHO production cell lines, adding another level of “defense”, against the devastating financial consequences of this virus infection, without compromising monoclonal antibody yield or quality.
FINDING THE NEEDLE(S) IN THE HAYSTACK: IDENTIFICATION OF MIRNA MASTER REGULATORS IMPROVING PRODUCTIVITY INDEPENDENT OF CELL TYPE AND PRODUCT

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Background and Novelty:
MicroRNAs (miRNAs) constitute an important class of non-coding RNAs and are imperative for the regulation of gene expression in mammalian cells. By post-transcriptionally modulating the expression of hundreds of different genes, miRNAs are capable of regulating entire pathways to control cellular phenotypes. Therefore, miRNAs represent promising tools for cell engineering of biopharmaceutical production cells. In contrast to classical cell engineering approaches where individual genes are overexpressed or knocked-down, miRNAs represent a versatile and novel engineering tool which does not add any translational burden to the cell. However, mammalian cells express hundreds of different miRNAs and finding the perfect candidate seems like looking for the needle in the haystack. Here, we show the identification of universal ‘master regulators’, that positively influence productivity independent of cell type or produced protein.

Experimental Approach:
An unbiased, functional miRNA screen was conducted in recombinant Chinese hamster ovary (CHO) cells to identify miRNAs improving bioprocess relevant cellular characteristics. 1,139 murine miRNAs were individually transfected in a high-throughput RNA delivery approach. A multiparametric cell analysis procedure enabled the parallel assessment of changes in protein expression, cell growth, apoptosis and necrosis. Impactful miRNAs were validated in secondary screenings using monoclonal antibody (mAb) and secreted alkaline phosphatase (SEAP) producing CHO cell lines as well as by stable miRNA overexpression. Finally, mAb-producing CEVEC’s Amniocyte Production (CAP) cells were used for a cross-species transfection approach using the 48 top pro-productive miRNA hits to identify species-independent miRNA master regulators.

Results and Discussion:
By taking advantage of a functional high-content miRNA screening we were able to identify >750 miRNAs significantly affecting protein expression, cell growth, apoptosis and necrosis in CHO cells. Functionality of impactful miRNAs could be successfully validated in secondary screenings as well as by stable miRNA overexpression of selected miRNAs in SEAP and mAb-producing CHO cells. Furthermore, we demonstrate that identified pro-productive miRNAs were able to substantially increase mAb yields of two industrial high-producing CHO cell lines expressing different antibodies. This suggests that miRNAs can act cell line and product independent and could be used as universal cellular enhancers. Moreover, we provide detailed results of multifunctional miRNAs influencing two or three obviously non-interrelated cellular functions. We show that the majority of these impactful miRNAs are actually expressed in CHO cells or at least could be found as pre-miRNA hairpin on the genome. Our findings suggest that miRNA regulation is highly redundant in CHO cells and crucial cellular processes are controlled by a plethora of different miRNAs in parallel. Strikingly, a cross-species miRNA transfection approach revealed impactful miRNAs improving antibody productivity and cell growth in human CAP production cells. In summary, many miRNAs were found to influence specific phenotypes presumably due to the regulation of one or multiple genes involved the same pathway. Intriguingly, a much greater number of miRNAs was identified to modulate more than one phenotype most likely by controlling multiple cellular pathways. Our results highlight the presence of multifunctional miRNAs in mammalian cells underscoring the unique potential of miRNAs for next-generation cell engineering.
PRODUCTION OF IGGS WITH A HUMAN-LIKE SIALYLATION IN CHO CELLS

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Background and Novelty:
IgGs that possess α2,6-sialylated Fc-glycans are involved in the anti-inflammatory properties of intravenous immunoglobulins (IVIGs), through a mechanism which has not been elucidated yet. The impact of this sialylation on the classic IgG’s effector functions also remains unclear. The understanding of these mechanisms has been impeded by the complexity of the sialylated glycan species together with the relative rarity of α2,6-sialylated IgGs. In this study, we show that the α2,6-sialylation of IgG1’s Fc domain can be efficiently achieved by the transient co-expression of the human β1,4-galactosyltransferase 1 (GT) and α2,6-sialyltransferase 1 (ST6) in CHO cells, whereas the expression of one or the other glycosyltransferase alone yields very limited sialylation. The process allows for the production of milligrams of human-like sialylated mabs within two weeks. We present a panel of four orthogonal assays for the fine characterization of the mabs’ glycoprofile that are in very good agreement with each other.

Experimental Approach:
CHO cells in suspension were transfected with polypelexes composed of polyethylenimine and plasmids encoding the mab and the enzymes. The proteins were expressed transiently, or stable pools were obtained in the GS system with MSX selection in glutamine free medium. The mabs were purified on protein A resin from the supernatants after four days to avoid sialylation degradation (potentially caused by sialidases, pH and ammonia levels increase in late culture). The glycoprofiles were characterized through a set of four assays: lectin-blotting, capillary isoelectric focusing (cIEF), liquid chromatography coupled to electrospray ionisation mass spectrometry (LC-ESI-MS) and hydrophilic interaction liquid chromatography (HILIC). The relative glycan abundances obtained by LC-ESI-MS and HILIC correlated very well (Pearson coefficient 0.96). The evaluation of SA linkage type was accomplished by HILIC analyses after α2,3-specific or broad range sialidases.

Results and Discussion:
The transient expression of both GT and ST6 resulted in IgG1s where G2FS(6)1 was predominant, and 88% of the SA were of α2,6 type. 75% of the glycan branches were galactosylated and 25% sialylated, in comparison with 23% and 1% respectively in IgG1s expressed alone. In contrast, the co-expression of GT or ST6 led to less than 5% of sialylated glycan antennae. While the low galactosylation level in IgG1+ST6 can explain this result, it is surprising in the case of IgG1+GT where 70% of the branches were galactosylated.

With this approach, the IgG1s were produced at yields around 15 mg/L. In order to reach yields closer to 200 mg/L, stable pools of CHO cells expressing GT, ST6 and a mab were selected. However, the high mab productivity was achieved to the detriment of the sialylation level. Parameters of transfection and selection were thus modified to reach high sialylation levels.

Our method allows the fast production of milligrams of IgGs with a human-like Fc-sialylation, providing material for further functional studies, and also initiating the development of a recombinant substitute for anti-inflammatory IVIGs. The association of Fc/2 analysis by LC-ESI-MS with glycan analysis by HILIC constitutes a highly reliable platform for the fine characterization of antibodies’ glycoprofiles.
CELL-BASED METHODS FOR EARLY DETECTION OF HEPATOTOXICITY IN DRUG DEVELOPMENT: HIGH CONTENT MULTIPARAMETRIC SCREENING AND TOXICO-METABOLICs

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Hepatotoxicity is a major reason for drug non-approvals and withdrawals. When this occurs at a later stage in drug development, the economic loses may be considerable. In order to minimize failures, predictive experimental approaches that can be used at an early stage of drug development are desirable. Our laboratory has developed several approaches to pursue this goal. The multiparametric analysis of xenobiotic toxicity by cellular imaging-based approaches (high-content screening, HCS) and metabolomics could play a key role in the early detection of toxicity and the classification of compounds based on patterns of cellular injury.

Our strategy aims at developing and validating robust and reproducible, in vitro multiparametric cell-based analysis to identify drugs potentially hepatotoxic to humans and their mechanisms of action. 78 reference compounds have been investigated in microcultures of the human hepatic cell line HepG2 which was genetically manipulated to confer xenobiotic metabolic capabilities. This was achieved by simultaneous transfection with recombinant adenoviruses encoding CYP1A2, CYP2C9 and CYP3A4 (ADV-HepG2). Five fluorescent probes to assess cellular parameters were simultaneously loaded in the cultures and analysed with the high-content screening station “Scan′R” to investigate different cell parameters (BODIPY493/503, lipid content; 2′,7′-dihydrodichlorofluorescein diacetate, ROS; tetramethyl rhodamine methyl ester, mitochondrial membrane potential; propidium iodide, cell viability; and Hoechst 33342, nuclei staining). ADV-HepG2 and HepG2 cells were exposed to bioactivable and non-bioactivable compounds, and this approach could identify early and late events in the course of hepatotoxic, giving clues about the toxicity mechanism(s) implicated.

A mass spectrometry-based approach has been established to identify toxicity mechanisms using biomarker pattern recognition of cells incubated with drugs. The metabolomic profiles displayed after exposure of human hepatocytes (HepG2) to model drugs with well-described mechanisms of hepatotoxicity were analyzed. Multivariate data analysis allowed deciphering biomarker fingerprints related to each toxicity mechanism and severity of damage. A predictive model allowing discrimination among non-hepatotoxic compounds and those eliciting toxicity through one or more mechanisms was setup. The model integrates biomarker relative weights and estimates the likelihood of a drug to cause hepatocyte damage through one or more toxicity mechanisms. The strategy so far developed is robust allowing precise and reproducible results indicating metabolomics-based assays may be a suitable in vitro approach for hepatotoxicity screening of new drug candidates.

The combination of these approaches allows early identification of potential hepatotoxicity of new drugs, as well a better estimation of the relative contribution of adverse outcome pathways in the toxic event.
NOVEL IPSC DERIVED CELLULAR SYSTEMS FOR IN VITRO DISEASE MODELING

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A major challenge in disease research and drug discovery is modeling human biology in physiologically relevant and predictive in vitro systems. Human induced pluripotent stem cell (iPSC) technology allows for the generation of virtually any cell type of the human body in unlimited quantities from a donor. This technology also enables access to human disease models which have been shown to recapitulate the native human phenotypes in vitro. The functional relevance of human iPSC-derived cells in research and drug discovery programs is being demonstrated by a rapidly growing body of literature. Here, we present case study examples of induced, engineered, and innate disease models generated by the production of iPSC-derived cell types environmentally stimulated to elicit a disease phenotype, genetically modified to introduce a disease mutation, and from patient-derived material, respectively.

We describe examples of induced disease models in three different cell types: 1) the application of iPSC-derived hepatocytes in hepatitis C virus (HCV) infectivity and in \textit{Plasmodium} parasites-mediated malaria model; 2) an amyloid-beta induced neuron toxicity assay using iPSC-derived cortical neurons used in a pilot screen to identify compounds protective against Alzheimer’s Disease; 3) an assay for cardiac hypertrophy using iPSC-derived cardiomyocytes, designed with a workflow suitable for high throughput screening. Additionally, we provide data from iPSC lines genetically modified to carry point mutations: 1) in the myosin heavy chain generating cardiomyocytes as a model of hypertrophic cardiomyopathy; 2) in the amyloid precursor protein yielding a neuron based model of Alzheimer’s Disease. Finally, we present an iPSC-derived diabetic cardiomyopathy model, in which culture conditions were optimized to induce the disease in apparently normal iPSC-derived cardiomyocytes. This model was used in a phenotypic screen for rescue from the pathological phenotype during diabetic stress and identified candidate protective molecules for iPSC-derived cardiomyocytes generated from diabetic patient samples.

The data presented show how the iPSC technology offers reliable and predictive model systems not otherwise attainable using currently available primary and immortalized cells, thus creating new tools and opportunities in drug discovery.
NEXT-GENERATION 96-WELL TISSUE CULTURE PLATES RE-ENGINEERED VIA 3D PRINTING: A SCREENING PLATFORM FOR MEASURING CONTRACTILE FORCE IN HUMAN PLURIPOTENT STEM CELL-DERIVED CARDIAC MICROTISSUES

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Background and Novelty:
Advancing translational research in the field of cardiac regeneration to treat myocardial infarction requires critical initiatives, one of which is the establishment of a reproducible in vitro heart tissue model. The use of human pluripotent stem cell-derived cardiomyocytes (hPSC-CM) for routine screening is garnering increasing interest within the research community and the pharmaceutical industry. While a number of groups have shown that hPSC-CM can be used to evaluate drug response in vitro, the assay formats employed generally focus on immunohistochemistry and/or electrophysiology at the scale of single cells or monolayers. Along with these readouts, contractile force, at the tissue scale, is an additional functional parameter that is an important assessor of cardiac function and health. High content functional screens using cardiac microtissue-based in vitro models, which closely mimic native heart tissue, can be used as a highly accurate and informative tool for validating hits determined by preliminary screening techniques. We have created a screening platform with a focus on contractile force measurements of functional 3D hPSC-derived cardiac microtissues that we term Cardiac MicroRings (CMRs).

Experimental Approach:
We have employed high-resolution 3D printing to construct custom next-generation 96-well tissue culture plates which can be used to formulate, culture, and assay CMRs. We are able to formulate these rings of cardiac microtissues using the passive remodeling ability of cardiomyocytes and non-myocytes in a collagen 1-based extracellular matrix. The CMR forms around two elastic micro-cantilevers located at the base of each well. As the CMR contracts around the micro-cantilevers, the contractile force of the cardiomyocytes deflects the cantilevers. The displacement of the deflection can be imaged in each well and used to calculate a total magnitude contractile force, using both the structural and material properties of the micro-cantilevers. These tissues can also be layered to create more complex tissue structures, and can be used as a model to study tissue-tissue interactions.

Results and Discussion:
We show the utility of the platform by assessing the range of contractile function presented by CMRs formulated with cardiac cells derived from various cardiac differentiation methods. Next we demonstrate construction of higher-complexity co-culture cardiac tissues and the emergence of intratissue spatial re-organization by combining cardiomyocytes with epicardial cells. We then conduct a cardiotoxicity screen of a selection of compounds which have adverse effects on the contractile machinery of cardiomyocytes. Finally, we plan to generate CMR models of disease using hPSC-CMs derived from patients with heart disease to assess effects in contractile function. We aim to have this platform set a new gold-standard in the functional screening and validation of cardiac-associated drugs.
A “HUMAN-ON-A-CHIP” PLATFORM - AIMING FOR A PARADIGM SHIFT IN SUBSTANCE TESTING

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Background and Novelty:
Strategies to develop “human-on-a-chip” platforms are applying micro-physiological systems towards the in vitro combination of miniaturized human organ equivalents into functional human mini-organisms, which aim to replace systemic toxicity testing and efficacy assessment of new drug candidates in animals. Data derived from such platforms are expected to substantially reduce attrition rate in clinical trials. Therefore, such platforms need to emulate human biology at the smallest possible scale at reproducible operation under physiological or pathological conditions over long exposure periods. Oral, dermal and intravenous administration routes have to be integrated into such platforms and a homeostasis of organ equivalents have to be ensured. We have developed a universal multi-organ-chip platform for long-term culture of human 3D organ equivalents interconnected within a common capillary microfluidic network, mimicking physiological blood flow.

Experimental Approach:
Standard microscopic slide format has been selected for our microfluidic multi-organ-chip platform. A glass layer at the bottom of the chips allow for life tissue imaging. An on-chip micro-pump has been integrated into the platform to support physiological pulsatile fluid flow through the organ culture compartments. The platform is universally applicable to co-culture of human barrier organ models, such as skin or intestine, and 3D parenchymal organs, e.g. miniaturized liver or brain equivalents. The human organs are scaled down by a factor of 1:100000.

Results and Discussion:
Proof of concept combining miniaturized human liver and skin equivalents at steady culture conditions over 28 days was achieved in 2012. A repeated dose (7 day) exposure assay has been established based on that two-organ-chip and transferred into routine commercial use. Dose dependent liver response to troglitazone served as a qualification parameter. Subsequently, repeated dose substance test assays with co-cultures of human skin interconnected with dendritic cells and human liver in combination with neuronal spheroids have been qualified. Finally, in addition to the two-organ-chip assays, a four-organ-chip combining human intestine, liver, skin and kidney equivalents into a functional ADMET test assay has been established. Tissue engineering data and assay performance data for repeated dose substance exposures through topical and systemic administration routes will be presented. Finally, worldwide development status, challenges and next development steps in the field of “organ-on-a-chip” and “human-on-a-chip” developments will be highlighted.
METABOLIC PROFILING OF HUMAN NEURAL CELLS: COMBINING 3D 
IN VITRO MODELS AND ^{13}C-NMR SPECTROSCOPY

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Background and Novelty:
The high attrition rates registered in the pharma industry demand a paradigm shift towards human cell models 
of higher relevance, which can closely recapitulate the main features of target tissue, as well as more predictive 
and accurate assays. In this context, the generation of human neural cells by directed differentiation of stem 
cells holds great promise for disease modeling, drug discovery and toxicology. Here, 3D culture systems are 
useful complementary tools towards more accurate preclinical evaluation, enabling to mimic the in vivo cell-
cell and cell-extracellular matrix interactions while presenting an intermediate degree of complexity between 
traditional 2D cultures and the organ. In this work, we combined the use of a human 3D in vitro neural model 
with 13C-NMR spectroscopy to study the metabolic features of stem cell-derived human neural cells and their 
response to known toxic compounds.

Experimental Approach:
Dynamic culture system was adopted for aggregation and neural differentiation of NT2 pluripotent stem cell 
line. Differentiated aggregates were characterized phenotypically and functionally, using confocal microscopy, 
quRT-PCR and electrophysiological recordings. For the identification of neural metabolic signatures, extracts 
generated from differentiated aggregates incubated with ^{13}C labeled substrates were analyzed by ^{13}C-NMR 
spectroscopy, allowing tracking of the fate of the substrates through different metabolic pathways.

Results and Discussion:
Neural aggregates composed by neurons and astrocytes were characterized. Incubation with [1-^{13}C]glucose 
resulted in similar labeling in [4-^{13}C]glutamine and [4-^{13}C]glutamate indicating that more astrocytes, expressing 
glutamine synthetase, than neurons, compared to brain tissue, were present in the 3D cultures. Higher labeling of 
glutamine than glutamate derived from pyruvate carboxylase activity was observed. This is in accordance with 
the presence of this enzyme in astrocytes and not in neurons, as described for primary murine cells. Incubation 
with [2-^{13}C]acetate resulted in labeling patterns reflecting cell-type specific metabolism, in agreement with 
previous reports describing that acetate is mostly metabolized by astrocytes. Moreover, [2-^{13}C]GABA was 
detected after incubation with labeled glucose or acetate, showing efficient neuronal differentiation. Importantly, 
the establishment of functional metabolic networks namely the glutamine-glutamate-GABA shuttles between 
neurons and astrocytes were observed. Further characterization was performed by challenging the cells with 
acrylamide, a prototypic neurotoxic compound to specifically target the neuronal population. This led to an 
increase in the astrocytic metabolic signature and decrease in neuronal metabolism, such as increased content 
of intracellular glutamine and no synthesis of GABA.
The metabolic profiling presented here showed that the human 3D neural cell model displayed similar 
metabolic signatures previously associated with primary murine neurons and astrocytes, revealing its relevance 
for preclinical research and suggesting that these pathways are also present in human neural cells in culture. 
Moreover, these results show that this methodology can be explored to increase the mechanistic knowledge 
on human neural metabolism, as well as for the development of more relevant human disease models, drug 
screening and toxicological studies.

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and SFRH/BD/52473/2014, FCT).
IMMORTALIZED LUNG EPITHELIAL CELLS WITH IN VIVO LIKE CHARACTERISTICS

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Background and Novelty:
The lungs are the central organ of the respiratory tract. Their major function is the gas exchange that is achieved in the alveoli. The alveolus is composed of two different types of epithelial cells (Type I and Type II cells) which form a tight barrier. The physiological role of this barrier is to prevent foreign organisms/substances to penetrate the body. As drug delivery through the lung epithelium becomes more and more important, in vitro test systems are highly desirable that mimic the in vivo situation. Such a cell system such reflect the barrier properties with regard to passive and active transport processes and in addition, should produce surfactant (a lipoprotein complex) which is e.g. involved immune reactions as well as in regulating physical function of the lung.

Experimental Approach:
For the generation of novel lung epithelial cell systems we established with our functional immortalization technology (CI-SCREEN) >40 different novel cell lines. Next, a process was established that assessed different parameters like morphology, gene expression, protein expression, protein localization and functional properties to identify a cell line that best reflects the in vivo situation.

Results and Discussion:
By this experimental approach we were able to generate a cell line that expresses lung epithelial specific markers on a RNA level as well as on the protein level. Importantly, this cell line is polarized and forms specific epithelial structures like microvilli, desmosomes and caveolae. Furthermore, it is characterized by an extremely tight barrier (TEER values >2000\(\Omega\)/cm\(^2\)) with low passive diffusion. These phenotypic characteristics were stable in culture for more than 30 population doublings, which renders this cell line an ideal tool for infection or drug absorption studies.
THE BEAUTY AND THE BEAST: A PERSPECTIVE ON BIOPHARMACEUTICALS

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Biopharmaceuticals are among the most sophisticated and elegant achievements of modern sciences. That the biopharma industry exists and rapidly grows at all is testament to the persistence and effectiveness of the scientific community. Now, the industry has moved into the era of recombinant proteins, pioneered by agonistic replacement therapies like the recombinant insulins and another class of proteins - monoclonal antibodies (mAbs) - has become a mainstream technology today tackling previously untreatable diseases. The emerging picture of the longer term is even more exciting, with innovations like immunotherapies and radically new concepts like antibody-drug conjugates and gene and cell therapies making it to the market.

As Biopharma moves from scientific frontier into business mainstream, the industry will increasingly be forced to confront the same challenges faced by other businesses: maintaining competitiveness by ensuring affordability, quality and delivery performance with speed while new markets, players and top talents are emerging globally. Advanced diagnostic and rapid genetic profiling combined with big data analytics will accelerate research but increase complexity. Ultimately, the biopharma community will have to heal through very personalized medicines and at the same time treat a growing population by far cheaper medication.

To keep pace, biopharma players have to revisit and fundamentally reassess many of the strategies, technologies and operational approaches they currently use. Only through a combination of strong science, deep operational excellence and longer-term strategic view will the biopharma industry be able to fulfill its real potential - and today's actions will shape Biopharma communities' readiness to grasp these vast opportunities as they come to fruition.
IMPROVED PRODUCTION AND QUALITY OF ENVELOPED VIRAL PARTICLES IN BEVS: A HOST CELL-DRIVEN AND TARGET-ORIENTED APPROACH

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Background and Novelty:
The Insect Cell-Baculovirus Expression Vector System (IC-BEVs) is widely used for the production of recombinant proteins for pre-clinical research and industrial vaccine manufacture. The combination of a tunable viral vector with the high productivities achieved in insect cells is major driver for the success of this platform. Nonetheless, the knowledge on insect cells’ physiology and their dynamics after infection is still scarce, hampering the design of robust optimization plans. In this regard the present work aims at providing a toolbox for IC-BEVs bioprocess engineering in a target-oriented perspective. Previously, we addressed the impact of the baculovirus infection on the host cell physiology, stressing out key cellular features that support highly productive phenotypes(3). This information was applied in the design of tailored supplementation schemes to boost IC-BEVs productivity. Ultimately, the developed schemes were evaluated on the basis of their effect on the production of three targets with increasing complexity: recombinant influenza neuraminidase; multimeric influenza VLPs expressing hemagglutinin and matrix protein 1 and the baculovirus.

Experimental Approach:
An in-depth metabolomic profile of the IC-BEVs was traced, comparing Sf9 and Hi5 cell lines. The respective exo- and endo-metabolome were assessed before and after infection. The results were subjected to multivariate data analysis and, in parallel, contextualized in a pathway analysis framework. With the information gathered several supplementation schemes were designed. An orthogonal screening was undertaken for the evaluation of the supplementation schemes, varying the MOI, addition timing, cell line and target product. This was firstly performed at the micro-scale level, exploring the AMBR robot system, and afterwards transposed to shook flask cultivation. The best culture scheme was implemented in a small-scale bioreactor setting. Cellular growth, viability, specific productivity and final product quality were evaluated as readouts of systems performance.

Results and Discussion:
The metabolic profiling revealed that Hi5 cells are better suited to cope with proteotoxic stress, which can further justify their higher protein production yields. Overall, pathway analysis highlighted that better producer phenotypes seem to be correlated with the capacity of cells to shift their metabolism in favor of energy-generating pathways to fuel biosynthesis, a scenario observed in Hi5 cells(1). Media manipulation strategies were designed aiming at reinforcing cellular pathways associated with higher productivity. Namely, nucleotides, lipids, cofactors and redox balancers were selected for culture supplementation schemes. The results show that Sf9 and Hi5 cells respond differently to the supplements addition, indicating that they have different biosynthetic requirements. In addition, the supplements effect is MOI-dependent, which suggests that the virus load can be mandatory in dictating the cellular responsiveness to environmental manipulations. Ultimately, the fine tuning of the MOI along with the addition of a defined supplement in a minimal fed-batch mode yielded an improvement of the systems’ productivity up to 6 fold.

IDENTIFICATION OF PROCESS PARAMETERS INFLUENCING PRODUCT QUALITY IN MAMMALIAN CELL CULTURE

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Background and Novelty:
Product quality of monoclonal antibodies (mAbs) during manufacturing of the biopharmaceuticals is a major concern. Higher molecular weight aggregates can be formed during upstream (USP) and downstream (DSP) processing, which negatively influence product yields, reduce the therapeutic efficacy of the mAbs and trigger immunogenic responses upon administration. Reducing the level of aggregates during USP, could improve the production of the biopharmaceuticals and reduce the burden on expensive DSP removal of the HMW species. However, the lack of analytical tools to detect mAb aggregates in USP restricts understanding the origin of the aggregates and finding cell culture conditions influencing product quality to reduce the level of mAb aggregates. We present a high-throughput compatible method which allows quantification of mAb aggregate formation directly in cell culture samples of Chinese hamster ovary (CHO) cells replacing falsifying, laborious and time-consuming chromatographic methods. Furthermore, we have screened for different culture conditions effecting mAb aggregate formation in mammalian cell culture and identified important process parameters to maintain product quality. In conclusion, our work demonstrates that the formation of mAb aggregates can be assessed directly in mammalian cell culture and product quality can be controlled by the selection of certain cell culture process parameters.

Experimental Approach:
Aggregation of two mAb-producing CHO cell lines was investigated using different analytical methods. Formation of mAb aggregates was assessed using size exclusion chromatography (SEC), multi-angle light scattering (MALS), dynamic light scattering (DLS), fluorescence spectroscopy and microscopy. In order to distinguish between the aggregates formed in cell culture, mAb aggregates differing in size and morphology were induced using different stress methods. The induced aggregates controls were used to identify extrinsic fluorescence dyes and instrument settings for high throughput analysis of bioprocess samples. Aggregate formation of the cell lines was studied under different cell culture conditions by varying culture parameters such as temperature, pH-value, osmolality, agitation and culture additives.

Results and Discussion:
We show that product quality can be assessed directly in cell culture samples without falsifying pre-purification procedures. To identify the aggregates formed in cell culture different mAb aggregate controls were induced resulting in mAb dimers, tetramers, oligomers and large particles with hydrodynamic diameters greater than 100 nm. Different extrinsic fluorescence dyes were identified regarding their ability to detect mAb aggregates using high-throughput fluorescence spectroscopy and microscopy. Using our developed methods we screened for cell culture conditions influencing mAb aggregate formation of two different CHO cell lines. Finally, we identified critical parameters influencing product quality in mammalian cell culture. Our results indicate how much protein can be lost due to upstream protein aggregation and emphasize quality assessment directly in bioprocess operation samples, which helps to improve production of biopharmaceuticals and reduce the burden on expensive DSP.
NON-FUCOSE LEVEL AS A FUNCTION OF GLYCOENZYME TRANSCRIPTION IN A GLYCOENGINEERED CHO CELL LINE

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Background and Novelty:
Antibody-dependent cellular cytotoxicity (ADCC) is one important mode of action for therapeutic mAbs in the field of oncology. It is strongly dependent on the glycan pattern of the Fc N-glycan: low core fucose levels typically result in an increase in ADCC.
Within Roche Pharma we are working with CHO cell lines designed to produce therapeutic antibodies based on the GlycArt system. These cell lines contain in addition to the recombinant gene for the therapeutic antibody (mAb), also recombinant genes for two glycosyltransferases, N-acetylgalactosaminyltransferase-III (GntIII) and mannosidase-II (ManII). As a result the CHO cells produce antibodies with a modified glycosyl structure characterized by a low-fucose Fc fragment part.

Experimental Approach:
In this study we analyzed recombinant ManII and GntIII cell lines regarding their ability to produce low fucose mAb dependent on the in vitro cell age. The mRNA level of the recombinant glycosylation enzymes ManII and GntIII was determined over the course of seed- and inoculum-train (shake flask) using RT-qPCR and related to corresponding glycosylation data of the mAb at the end of the production run simulated in our cell culture robotics facility.

Results and Discussion:
An efficient high throughput RT-qPCR has been developed in collaboration with Roche Diagnostics which can now be used also for transcription analysis of other CHO genes. Data from the robotics experiment closely mirror the production scale. The results of this study provide insight into the mechanisms of glycosylation of recombinant ManII and GntIII cell lines and enable recommendations regarding in vitro cell age and selection pressure for the seed- and inoculum-train of the production process.
WHEN BAD THINGS HAPPEN TO GOOD PROTEINS: EFFECTS OF CELL CULTURE PROCESS ON PROTEIN CHARACTERISTICS

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Keywords:
CHO cell culture, protein quality, protein product variants, protein product impurities.

Background and Novelty:
In producing therapeutic proteins from cell culture processes, it is necessary to develop processes capable of delivering consistent protein characteristics, particularly those that could affect the structure or function of the biomolecule and potentially impact the product safety or efficacy. Over the past thirty years, cell culture scientists and engineers have navigated the waters of process understanding in tackling protein product characterization challenges. In most cases, the root cause of protein product attribute variation is related to cell culture process conditions.

Experimental Approach:
Laboratory-scale (high throughput and up to 2-L) designed studies and analysis of pilot-scale and manufacturing-scale CHO cell cultures are used to evaluate the effects of processing conditions on product attributes.

Results and Discussion:
The impacts of process conditions on protein attributes may be due to biosynthetic (biological origin) or degradative (chemical origin) effects, and these effects may be driven by intracellular or extracellular factors. This presentation will review industrially significant experiences, and discuss approaches toward better understanding and control of recombinant protein product characteristics through cell culture manipulations, including medium composition and process design. Specific attribute variation addressed will include amino acid sequence, glycosylation (micro and macroheterogeneity), and size and charge variant distributions.
UNDERSTANDING THE IMPACT OF MILD HYPOTHERMIA ON MONOCLONAL ANTIBODY GLYCOSYLATION IN CHO CELL CULTURES THROUGH EXPERIMENTAL AND COMPUTATIONAL ANALYSES

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Background and Novelty:
With positive outcomes from medical treatments, monoclonal antibodies (mAbs) are to date the best-selling biologics in the pharmaceutical market. Their medical values and safety have been reported to rely on the carbohydrate structures that are attached to the mAb N-linked glycosylation site on each constant region. It was also reported that different bioprocess conditions during recombinant mAb production directly impact glycan compositions and their distribution on the molecules, although the mechanism behind this change is not fully understood. This lack of understanding limits process design and optimisation. To address this issue we examined the effect of mild hypothermia (32°C) on mAb N-linked glycosylation, using experiments, flux balance analysis (FBA) and mechanistic modelling to identify resulting differences in cell metabolism. A defined mathematical model that mechanistically and quantitatively describes CHO cell behaviour and metabolism, mAb synthesis and its N-linked glycosylation profiles before and after the induction of mild hypothermia was also constructed, which we believe is the first quantitative model that relates mild hypothermia to the four elements mentioned above. Not only does the model aid understanding of the way bioprocess conditions affect product quality, it also provides a platform for bioprocess design, control and optimisation in industry and helps the implementation of the Quality by Design principles.

Experimental Approach:
In this study we experimentally examined CHO culture performance at physiological and mild hypothermic (introduced during late-exponential cell growth) conditions, with respect to cell growth, nutrients and metabolite concentrations, mAb production at transcriptional, translational and secretion levels through quantitative real-time PCR, western blotting and affinity chromatography, N-linked glycan profiles of the product via a LabChip method, as well as mRNA and protein expressions of N-linked glycosyltransferases. To better understand CHO cell metabolism, intracellular carbon fluxes were estimated using FBA that was constrained with our experimental exometabolite data. Next we relate the effect of mild hypothermia with mAb glycosylation with the help of a modular mechanistic model previously developed, that describes CHO cell growth, nutrient and nucleotide sugar (NSD) metabolisms, mAb synthesis and Golgi N-linked glycosylation.

Results and Discussion:
CHO cultures at 32°C possess prolonged cell viability, lower rates of nutrient metabolism and reduced carbon fluxes towards nucleotide, NSD and lipid syntheses. The specific mAb productivity at 32°C is 20% higher, accompanied by increased transcription, translation and transport rates of mAb molecules. Glycan studies show limitation in terminal galactosylation on the mAb Fc region at 32°C. Our mechanistic model suggests reduced rates of NSD production and lower galactosyltransferase expression at 32°C, which is later experimentally validated. The model describes the time-course data well and reproduces the N-linked glycosylation profiles of mAb produced in cold-shocked CHO cells. Results from both experimental and computational studies show lower expression of N-linked glycosyltransferases to be the main cause of glycan variation in mild hypothermia and manipulating the expression of these enzymes is suggested as a way to improve mAb glycosylation. In this way it allows biopharmaceutical industry to implement quality into products during bioprocessing to satisfy the Quality by Design regime.
DYNAMIC FLUX ANALYSIS OF GS-CHO CELLS: IDENTIFYING THE BURDEN OF PROTEIN GLYCOSYLATION ON CELLULAR METABOLISM

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Background and Novelty:
All commercially-available therapeutic monoclonal antibodies (mAbs) contain a consensus N-linked glycosylation site on the constant fragment (Fc) of their heavy chains. The distribution and monosaccharide composition of the carbohydrates (glycans) present on this site determine the therapeutic mechanism, serum half-life and immunogenicity of these products. In addition, the glycosylation process is heavily influenced by the conditions under which the producing cell line is cultured. Because of this, Fc glycosylation is widely regarded as a critical quality attribute (CQA) of mAbs within the Quality by Design (QbD) framework. When considering mAb Fc glycosylation as a CQA, a fundamental goal is to develop robust strategies for understanding and controlling bioprocesses to ensure mAb glycosylation-associated quality. In this context, we have developed an integrated experimental and modelling platform that dynamically and mechanistically links the extracellular environment with cellular metabolism and protein glycosylation.

Experimental and Modelling Approach:
Experimentally, IgG4-producing GS-CHO cells were cultured using three different amino acid feeding strategies. In addition to typical culture data (viable cell density, glucose, lactate, ammonia and mAb titre), the extracellular concentration of amino acids was determined by RP-HPLC. The intracellular availability of nucleotide sugars (NSs) – the direct metabolic substrates for glycosylation – was determined daily using anion exchange chromatography. Finally, mAb glycan analysis was performed for days 5, 8, 10, 12 and 13 of culture using ultra performance hydrophilic interaction chromatography (UPLC-HILIC) coupled with QTOF mass spectrometry.

The modelling approach used for this work consisted in generating a dynamic metabolic flux model (dMFA), where the specific production and consumption rates (q) calculated with a cell culture dynamics (CCdyn) model were used as inputs for a fully determined metabolic flux network (MFA) consisting of 55 material balances and 55 metabolite fluxes. Glycosylation was represented by including eight NSs and outlets for host cell N and O-linked glycans as well as mAb glycosylation. The end result is a model that describes how intracellular metabolite fluxes (including those destined for HCP and mAb glycosylation) change with respect to time as a function of changes in the extracellular environment. The unknown parameters of the dMFA model were determined from the experimental data by performing non-linear parameter estimation using the gPROMS modelling software.

Results and Discussion:
Galactosylation of the mAb glycans was observed to decrease during culture across all amino acid feeding strategies. This drop in glycan complexity did not correlate with the different amino acid feeding strategies or with the intracellular availability of UDP-Gal. The dMFA results indicate that, for this particular cell line, decreases in mAb galactosylation over time are due to insufficient availability of cellular glycosylation machinery relative to mAb specific productivity (q). Using these results, we define “glycosylation capacity” as the maximum amount of total glycoprotein (HCP and mAb) the cells can process without generating truncated glycoforms. The framework presented herein contributes to further understanding the process of mAb glycosylation and indicates potential glycosylation control strategies where mAb q can be modulated throughout culture to minimise changes in mAb glycan distribution.
PROCESS PARAMETERS IMPACTING PRODUCT QUALITY

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Background and Novelty:
Product quality is a result of the entire production process including protein sequence, cell substrate and process parameters. Many of the desired product properties are defined by posttranslational modifications with impact on biological activity, immunogenicity, half-life or stability. In-depth process understanding enables the targeted modulation of product quality attributes by rationally designed bioprocesses. This is valuable for new biological molecules in order to improve efficacy, reduce side effects, access new patient populations. For biosimilars this allows developing into defined quality attribute profiles. The identification of suitable process parameters and media compositions to modulate quality attributes is challenging due to the complexity of cell culture processes. Here, this challenge was approached by comprehensive data analysis, in-depth characterization of charge variant formation and high-throughput screening of process parameters and media compounds.

Experimental Approach:
The impact of process parameters on product quality attributes was analyzed with special focus on acidic charge variants and glycosylation pattern. Initially a database was created including process and analytical data from twelve projects. Data sets of more than 2500 fed-batch processes with 6300 analytical data sets enabled a cross-project analysis and correlation of process parameters with product quality attributes. The formation of charge variants was explored by uni- and multivariate techniques within the database to identify potentially impacting process parameters. These were then further investigated in experimental work. Cell culture parameters impacting growth and product formation rates like media osmolality and pH profiles were tested in bioreactor cultivations. In addition, post-harvest experiments exploring different pH, temperature, light and buffer conditions were studied in storage stress studies. Data from both studies were integrated to establish predictive modeling of charge variant formation in upstream process supernatants. In addition, the impact of cell culture conditions and media compounds on the glycosylation pattern was assessed by an integrated screening approach. Multi parallel small scale bioreactors, robotics based product capture and high throughput analytics were combined to minimize hands-on-time to gain data for correlation analysis.

Results and Discussion:
Said setups supported the identification of numerous media supplements and upstream process conditions that were applied for rational modulation of glycosylation patterns and charge variants. For the latter, the analysis of formation kinetics enabled modeling of charge variant formation in process supernatants. A mechanistic model that predicts a time and productivity dependent increase of acidic isoforms by up to 2.8 % per day will be presented. Moreover, case studies focusing on the optimization of glycan patterns and antibody dependent cellular cytotoxicity by using metal ions as media supplements will be shown. The overall derived database and toolbox is applied for ongoing projects for fine tuning of product quality attributes to meet the desired characteristics. After gap analysis, process parameters can be chosen for application in process development to finally achieve quality products.
TARGETING CANCER AND CHRONIC INFLAMMATION WITH ARMED ANTIBODIES AND SMALL MOLECULE DRUG CONJUGATES

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Antibodies can be used to deliver bioactive molecules (drugs, cytokines, photosensitizers, radionuclides, etc.) to the tumor environment, thus sparing normal tissues. The antibody-based targeting of certain modified extracellular matrix (e.g. splice isoforms of fibronectin and of tenascin-C) is particularly attractive, as these antigens represent accessible, abundant and selective tumor-associated antigens [Refs. 1-5].

We have recently explored the development of linkerless strategies for the coupling of potent cytotoxic drugs to tumor-targeting antibodies [Refs. 6,7]. Furthermore, we have compared the performance of antibodies and of small organic ligands (e.g., those isolated from large DNA-encoded chemical libraries) [Refs. 8-11], as vehicles for pharmacodelivery applications.

References:

COMPLEX PRIMARY CELL-BASED MODELS FOR DRUG DISCOVERY

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The ultimate goal of drug discovery is the identification of efficacious and safe treatments that will address a medical need. Thus, model systems employed towards that aim should closely reflect the patho-physiology of the disease being investigated. Organoid and organotypic cultures comprise cellular systems grown under close-to-physiological conditions, providing a framework for proper cell function by mimicking the natural environment. Primary mouse intestinal epithelial organoids, referred to as “mini-guts”, contain tissue-specific stem cells which self-renew and maintain a multi-lineage differentiation program reflective of the normal tissue. Incorporation of disease-relevant insults provide powerful and tractable models for the identification of a) molecular pathways of stem cell renewal, differentiation and apoptosis, which are central to regenerative medicine applications, and b) drug candidate leads. It is therefore of high importance to characterize and profile such organoid cultures at the molecular level in order to develop predictive models with relevance to normal human physiology and disease. We will present data describing an ex vivo model of radiation-induced, intestinal mucositis and its potential applications for drug discovery.
DNA METHYLATION IN CHO DP-12 CELLS: LANDSCAPE AND EFFECTS ON GENE EXPRESSION

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Background and Novelty:
Chinese hamster ovary (CHO) cells represent the major host for recombinant protein production. Although genomic and transcriptomic CHO data became recently available and enables rational process design and metabolic engineering, information is missing about DNA cytosine methylation in CHO cells as a crucial epigenetic modification and an important element in the regulation of mammalian gene expression. As a comprehensive understanding of epigenetic effects in biopharmaceutics production requires a global characterization of the CHO cell epigenome, we applied whole genome bisulfite sequencing and microarray experiments to display the CHO DP-12 cell DNA methylation landscape and analyze its impact on gene expression.

Experimental Approach:
Genomic DNA from IgG-producing CHO DP-12 cells (clone#1934, ATCC CRL-12445) was subjected to bisulfite conversion and next generation Illumina sequencing in order to construct a single-base DNA methylation map of the CHO cell genome. A second library from native DNA was sequenced to account for single nucleotide polymorphisms (SNPs) present between the reference K1 and the CHO DP-12 genome. In parallel, gene expression was measured using novel one-color CHO-specific microarrays and allowed for the identification of 3,120 highly expressed genes in our samples.

Results and Discussion:
CHO DP-12 cells exhibited 61 % DNA methylation, suggesting global hypomethylation in comparison to the majority of published mammalian DNA methylomes (e.g. 82 % DNA methylation in HUES64 cells or 78 % DNA methylation in fetal brain cells [1]). Analysis of the distribution of DNA methylation levels across the CHO genome showed a heterogeneous pattern of partially and highly methylated domains covering all analyzed scaffolds. Interestingly, microarray analysis revealed that partially methylated domains contained functional clusters of genes that were either highly or weakly expressed. Highly expressed genes contributed to translation, RNA processing, energy metabolism and cell cycle. Therefore, the observed correlation between epigenetic features of the CHO DP-12 cell genome and the expression of functional gene clusters showed evidence of being related to production cell properties such as high proliferative capacities and deregulated apoptosis. Weakly or not expressed genes within CHO DP-12 partially methylated domains had functions in neurological processes, calcium homeostasis and cellular adhesion. Taken together, our results prove that DNA methylation represents an important link between CHO cell genotype and phenotype and allow for a deeper understanding of CHO cell characteristics.

HIGH-DENSITY 3D CELL CULTURE AND ANALYSIS IN AN INTEGRATED MICROFLUIDIC PLATFORM

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Background and Novelty:
The development of robust high-throughput screening devices combining cell culture and detection systems is required for drug screening or for the applications of stem cells in therapy. In these fields, droplet-based microfluidics has recently shown avenues for controlled biological compartmentalization and high throughput cellular analysis, together with a significant reduction of the volume of reagents.

3D cultures have demonstrated improved biological functions of various mammalian cell types. In particular, the spheroids, which are tissue-like cellular aggregates, were reported to represent a physiologically relevant environment to mimic in vivo behavior. Various methods enable the formation of spheroids including hanging drops, low attachment wells etc. However, the application of these tools for high content screening is hindered by the limits on the number or the reproducibility of the spheroids. Moreover, these tools show limited compatibility with detection systems for in situ spheroid monitoring and analysis.

Here we demonstrate a microfluidic platform that integrates high-density spheroid formation, long-term culture and analysis. This platform is based on droplet techniques, with modifications that allow long term 3D culture at time scales relevant with drug or cellular microenvironment screening.

Experimental Approach:
Nanoliter-sized aqueous droplets containing liquid agarose and hepatocytes were generated in fluorinated oil by flow focusing, in a microfabricated PDMS chip. The confined droplets were trapped in an array of 500 capillary anchors, through the reduction of their surface energy. After spheroid formation and hydrogel gelation, the oil phase was replaced with culture medium. The on-chip immobilized spheroids were cultivated and analyzed in situ during a 7 days culture period.

Results and Discussion:
The individual spheroid formation kinetics was obtained by live imaging. Representative size and shape evolutions were determined and deviations from the mean behavior were identified. In situ live/dead, immuno-cytchemistry and BrdU staining were performed on-chip at various time points of the culture period. The analysis of over 10,000 spheroids indicated sustained viability and proliferation as well as improved expression of functional markers (i.e. intracellular albumin) compared to conventional 2D cultures. Moreover, the interspheroid analysis shed light on a significant correlation between albumin expression and morphometric parameters (i.e. spheroid index and size). The results were further validated by RT-PCR analysis on extracted spheroids from the chip. Furthermore, on chip intra-spheroids analysis was performed on over 100,000 cells. It was found a highly significant correlation between cellular organization (distance between cells) and the cell function.

Altogether, this study contributes to understanding the unique properties of 3D cultures by correlating micro-structural cues with the cell function. More importantly, this study demonstrates for the first time, the application of the droplet-based microfluidics into a robust and integrated long-term 3D culture and analysis platform, with potential applications in drug screening, stem cell and cancer research.
MAMMALIAN DESIGNER CELLS FOR BIOMEDICAL APPLICATIONS

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Background and Novelty:
Mammalian designer cells have been engineered with synthetic gene circuits to operate as information-processing systems that dynamically integrate and respond to input signals such as small-molecules or proteins. These gene circuits fine-tune the expression of specific output proteins for the tailored execution of specialized biological tasks. Gene circuits sensitive to disease-related trigger molecules offer great opportunities in biomedical applications. Here, we report the engineering of two unique designer cell lines and their successful implementation of three biomedical applications: i) a histamine-sensitive designer cell line was established for use in next-generation diagnostics producing personalized allergy profiles employing human blood, ii) a \( \text{H}^+/\text{CO}_2 \)-responsive designer cell line was implemented in biotechnology to exactly program and remote-control biopharmaceutical manufacturing processes using the traceless gas molecule \( \text{CO}_2 \), and iii) similar pH-sensing cells were pioneered in cell-based therapy for the development of a closed-loop circuit that enables autonomous treatment of diabetic ketoacidosis in mice utilizing therapeutic cell implants.

Experimental Approach:
Synthetic trigger-inducible gene switches represent basic building blocks for the design of gene circuits in mammalian cells. Here, we take advantage of cell-surface receptors that induce an endogenous signaling cascade, which can be rewired to synthetic promoters leading to the production of reporter or functional proteins, e.g. biopharmaceuticals. Cells engineered with these gene switches are able to score specific metabolites derived from human blood cells in vitro as well as in vivo when encapsulated into alginate beads and convert input signals into an appropriate production of desired output molecules.

Results and Discussion:
We present a cell-surface receptor-based design strategy of mammalian sensor devices that are responsive to the two disease-related triggers histamine (Ausländer, D. et al., Nature Communications 5, 4408 (2014)) and protons (Ausländer, D. et al., Molecular Cell 55, 397-408 (2014)) and are functional in the relevant physiological range, which is crucial for their successful application in biomedicine. The first designer cell line is engineered with a histamine sensor device enabling the detection of immune cell-derived histamine in human blood. Depending on the level of allergen-induced histamine release a correlating amount of reporter protein is produced and thereby provides a personalized allergy profile of human subjects mapping (non-)allergic reactions. The second designer cell line is engineered with the pH-Sensor, a gene switch responding to protons that enables traceless induction of the production process of biopharmaceuticals in bioreactor setups utilizing gaseous \( \text{CO}_2 \). This first-of-its-kind system depends on an intrinsic component of cell culture medium (\( \text{H}^+ \)), can be remote-controlled by gaseous \( \text{CO}_2 \), and relies on common pH/\( \text{CO}_2 \) control modules of state-of-the-art bioreactors. Also, the closed-loop gene circuit pH-Guard was developed that detects acidosis states in diabetic ketoacidosis (DKA) and induces a therapeutic response by secreting insulin. When pH-Guard-engineered cells are microencapsulated into alginate beads and implanted into mice that develop DKA the designer cells operate autonomously inside the organism and efficiently restore glucose homeostasis. In summary, we present an advanced animal cell technology to equip living cells with useful sensor devices for next-generation cell-based biomedical and biotechnological applications.
MULTI-OMICS APPROACH FOR COMPARATIVE STUDIES OF MONOCLONAL ANTIBODY PRODUCING CHO CELLS

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Background and Novelty:
Monoclonal antibody (mAb) therapy has revolutionized the treatment of a vast range of diseases, mostly in the areas of oncology and autoimmune/inflammatory disorders. With a world market exceeding 60 billion USD and six mAb related products in the top 10 selling drugs, the industry continues to grow at a fast rate. Chinese hamster ovary (CHO) cells are the preferred production host for therapeutic mAbs. Despite the high homology between mAbs, each production cell line is still developed through exhaustive screening, which is time-consuming and expensive because the factors controlling expression are largely unknown. Moreover, production lines generated from the same screen vary not only in productivity but in fermentation performance. Systems biology can be a powerful tool to identify key markers of good production lines, with the aim of engineering superior host lines that more reliably produce good production clones. To date it has been hampered by the need to use the mouse, rat and/or human genome as a reference and has suffered from the inherent limitation in coverage of 2-dimensional gel electrophoresis or mouse or CHO cDNA microarrays. The development of new techniques such as RNA sequencing for transcriptome analysis and LC-MS/MS for proteome analysis combined with the recent release of the CHO genome has reigned interest in using quantitative proteomics and transcriptomics to study high productivity.

Experimental Approach:
Here we applied the latest generation of tools to a standard contrast of a relatively good and an average CHO producer line generated from one transfection. For each cell line, three independent vials were thawed and passaged for two weeks prior to bioreactor inoculation. Cells were harvested in mid exponential phase and samples were analysed using RNA sequencing and two different proteomic techniques, iTRAQ and SWATH, for the identification of expression differences between the two cell lines.

Results and Discussion:
More than 11,000 transcripts and 2,000 proteins were quantified. Despite the fact that both clones come from the same transfection pool, approximately 60% of the quantified transcripts and proteins varied significantly. Proteomics identified three key biological processes as up-regulated in the high producer cell line: glutathione biosynthesis, actin filament processes and intracellular transport, and the down regulation of several growth-related processes. Metabolomic analysis confirmed that the high producing cell line displayed higher intracellular levels of glutathione. These processes may be important for conferring high mAb production, and as such two gene candidates have been nominated for targeted engineering of high-expression cell lines.
ANTIBODY-DRUG CONJUGATES: IMPLICATIONS ON THE DESIGN OF MABS

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Antibody-drug conjugates (ADCs) are monoclonal antibodies (mAbs) that are covalently linked to cytotoxic agents. Although the concept has existed for decades, only recent advances in linker, drug, and antibody technologies have turned ADCs into valuable therapeutic agents as illustrated by the recent approvals of Adcetris® and Kadcyla® (T-DM1). In addition to these approved ADCs, over 30 other ADCs are currently in clinical trials and many more are in preclinical development.

Whereas the function of a naked mAb in a clinical setting can be very diverse, within an ADC the mAb has the function to target the highly potent drug to its destination and secure internalization while the function of the drug is to kill the target tumor cells, resulting in different quality attributes for the parent mAb. With the development of new linker technologies and site-directed conjugation, mAbs are being (re)designed to include engineered cysteine or non-natural amino acids impacting on the design of cell lines used for manufacturing ADCs (1).

Synthon Biopharmaceuticals has built a linker-drug (LD) platform based on a cleavable linker-duocarmycin payload for the development of novel generation ADCs (2). SYD985, the lead ADC originating from that platform, is an interchain cysteine-coupled HER2-targeting ADC based on trastuzumab. We have demonstrated that SYD985 has superior in vitro and in vivo potency compared to T-DM1 in low HER2-expressing models, and shows complete remission in breast cancer HER2 2+ and 1+ animal models where T-DM1 doesn’t show any efficacy at all (3). SYD985 is presently being tested in a Phase I clinical study.

The manufacturing of ADCs is significantly more complex than for a naked mAb, as not only the mAb, but also a highly potent LD as well as the conjugate of these two have to be produced. Synthon possesses the unique capabilities to produce the LD, the mAb as well as the ADC in-house, which provides optimal flexibility in the development and control of quality aspects.

During this presentation, I will focus on the implications of the design of the mAb to be used for ADCs, the manufacturing of an ADC, and the progress of our SYD985 lead ADC compound.

2. Elgersma R et al; Molecular Pharmaceutics 2015 FEB, ahead of print.
3. Dokter W et al; Mol Cancer Ther 2014; 13(11); 1–12.
EXPRESSION AND MANUFACTURE OF A NEW BISPECIFIC DRUG FOR HER2 BREAST CANCER: THE BEAT-GBR 1302 ANTIBODY

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Keywords:
Bispecific antibody; BEAT; HER2; CD3; breast cancer.

Background and Novelty:
While the idea of bispecific drugs was brought up over 30 years ago, the development of formats mature enough for the clinic remained for a long time a challenge. The whole field was hampered by major problems of manufacturability and immunogenicity [1]. With the recent arrival of new bispecific formats, either as antibody-like molecules (containing an Fc) or scFv fragments without an Fc, at least 18 bispecific antibodies have entered clinical trials showing very promising results. The BEAT® format has been developed as bispecific antibodies maintaining the pharmacokinetics and the low immunogenicity of human IgG with excellent manufacturability properties [2]. This work describes the production of a new bispecific drug targeting HER2 on tumor cells and CD3 on cytotoxic T-cells soon entering the clinic for the treatment of breast cancer: the GBR1302-BEAT molecule. It highlights the generation of stable cell lines, the large scale production, the purification and formulation and demonstrates the in vivo efficacy of the molecule in a mouse xenograft model.

Experimental Approach:
The BEAT GBR1302 molecule consists of a Hc, a Lc and a Fc-scFv. The FAB arm binds to CD3 and the scFv arm binds to the HER2 cell receptor. A proprietary engineered CH3 interface mimics the natural association of the heterodimeric T-cell surface receptors α and β driving heterodimerization of the molecule. The protein A binding site of one of the Fc fragments is abrogated to facilitate the isolation of the BEAT-antibody by affinity chromatography. Stable cell lines are generated by co-transfection of proprietary expression vectors in CHO-S cells. A dedicated CLD platform was developed for the selection of high BEAT® secretors. Large scale expression was performed in fed-batch (250 L). The GBR1302-BEAT was purified using a standard DSP process (ProtA, VI, CEX, UF/DF, AEX, VF).

Results and Discussion:
The asymmetry of the GBR1302-BEAT allows the characterization of the secretion profiles of clones using high throughput analytics based on the molecular weight. Using this approach, GBR1302 expressing cell lines were generated with a volumetric productivity of several g/L and a high heterodimerization level (>90%) in a time efficient manner. Based on the built-in purification approach the GBR1302-BEAT was purified using a standard DSP process with yield and purity comparable to standard mAbs. It was eventually formulated with a low level of aggregation (< 2 %). GBR1302-BEAT effectively recruited cytotoxic T cells against HER2 positive breast cancer cells including the trastuzumab-resistant breast cancer cell line JIMT-1 and showed strong tumor cell lysis activity. The excellent manufacturing attributes and pre-clinical efficacy of our T cell redirecting BEAT antibody justify further clinical development as a treatment for HER2 positive cancers. The GBR1302 BEAT aims entering the clinic mid-2015.

DEVELOPMENT OF AN ADVANCED CELL THERAPY PRODUCT INDICATED FOR THE TREATMENT OF GONARThROSIs

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Background and Novelty:
Gonarthritis is the most common cause of pain and disability in middle-aged and older people because the successful repair of degenerated articular cartilage has not been attained yet and it is a major clinical challenge. Our group has developed an advanced cell therapy product up to phase I/IIa clinical trial (NCT01227694) with the aim to improve the outcome of the patient’s articular cartilage after intra-articular injection of autologous mesenchymal stromal cells (MSC).

Experimental Approach:
This endeavor required the design and execution of 1) non-clinical studies, 2) GMP-compliant bioprocess for cell production and 3) a Phase I/IIa clinical trial.

Results and Discussion:
1) Three preclinical studies were performed in sheep and murine animal models to assess pharmacodynamics/pharmacokinetics, subchronic toxicology and dose response demonstrating the safety and efficacy of the cell-based product application.
2) A GMP-compliant closed-system process was developed for high titer cell expansion of MSC in a relative short period of time (21 days). The drug product consisted in 40x10⁶ ± 10x10⁶ MSC in a saline solution and more than 50 cell batches have been produced up to date demonstrating production consistency.
3) Fifteen patients were included in a single-arm clinical trial for the treatment of gonarthritis grade II-III (Kellgren & Lawrence). Pain amelioration was observed in the first 3 months post-treatment and it was maintained over the next 9 months.

Our future work will focus on studying the long-term effect of MSC on cartilage regeneration and pain reduction.
OPTIMIZATION OF HPSC PRODUCTION FROM DISTINCT PLURIPOTENT AND METABOLIC STATES

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Background and Novelty:
As human pluripotent stem cell (hPSC) based cell therapies begin to enter clinical trials, an important contributor to their success or failure is the development of robust, efficient processes for cell production. Quality-by-Design (QbD) is a methodology that is readily applicable to the cell therapy field wherein product knowledge is integrated into manufacturing process development to plan for product safety and efficacy. We have applied QbD-based experimental design strategies to investigate key bioprocess parameters in hPSC expansion, identify critical metabolites in hPSC production, and evaluate the significance of cell state in hPSC manufacturing.

Experimental Approach:
The effects of three key bioprocess parameters (dissolved oxygen, seeding density, and feeding strategy) on the production of two hPSC lines (HES2, an embryonic stem cell line, and 110, a reprogrammed hPSC line) were first investigated in a controlled parallel microbioreactor system. The effect of metabolic substrates on iPSC production was then analysed by combining proteomics and flux balance analysis modelling. Finally, the importance of hPSC states to attributes critical to their manufacture is being investigated by changing cell state with small molecule pathway inhibitors.

Results and Discussion:
A screening bioreactor system (Micro-24 Micoreactor System, Pall Corporation) with 24 parallel, small scale (2-7mL) bioreactor units was selected to conduct suspension screening experiments. When operating at the best conditions tested this system produces 3.0±0.6 fold hPSC (~90% Oct4+Nanog+ cells) expansion over six days. Potentially critical metabolites were identified through collaboration in the Project Grandiose Consortium, an international effort to describe the cellular and molecular changes that occur during PSC reprogramming. A transient upregulation of glycolytic enzymes early in the reprogramming process was identified, as was a sustained upregulation of cysteine and methionine metabolic enzymes late in the reprogramming process, consistent with recent reports describing the importance of bioenergetic and epigenetic changes in the acquisition of pluripotency. Using a tissue-specific flux balance analysis technique, flux distributions for 2950 metabolic fluxes were generated, and 45 exchange fluxes identified. These metabolites may be critical for pluripotency maintenance during expansion and will be validated in our screening bioreactor apparatus.

In the bioreactor, the impact of hPSC cell state on attributes related to cell quality (pluripotency marker expression, expansion potential, and differentiation capacity) was identified. Combinations of inhibitors of pathways associated with a state of reduced manufacturing robustness in mouse PSCs (ERK, GSK3b, and others) resulted in increased expansion potential of the hPSCs, though negative effects on pluripotency marker expression and differentiation capacity were observed. This integrated strategy, combining metabolic analysis with alterations to cell state in a screening bioreactor system will describe an important design space relevant to PSC generation and expansion bioprocesses for clinical translation.
AAV AT 50: A GOLDEN ANNIVERSARY OF DISCOVERY, RESEARCH, AND GENE THERAPY SUCCESS IN THE SAMULSKI LAB

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Fifty years after the discovery of adeno-associated virus (AAV) and more than 30 years after the first gene transfer experiment was conducted, dozens of gene therapy clinical trials are in progress, one vector is approved for use in Europe, and breakthroughs in virus modification and disease modeling are paving the way for a revolution in the treatment of rare diseases, cancer, and possibly HIV. Critical to advancing this preliminary phase I data is the ability to scale up AAV vector manufacturing. We will walk through these early efforts to generate AAV productions in human vs. insect cells for preclinical vector through to the current need for large scale bio-reactor capability. This review will provide a historical perspective on the progression of AAV for gene therapy from the discovery to the clinic, focusing on contributions from the Samulski lab regarding the basic science and cloning of AAV, optimized large-scale production of vectors, preclinical large animal studies and safety data, vector modifications for improved efficacy, and successful clinical applications.

RAPID RESPONSE EBOLA ANTIBODY DEVELOPMENT ENABLED BY VELOCIMAB® TECHNOLOGIES

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Rapid transmission of infectious diseases leading to the global spread is a growing concern in the modern world. The recent Ebola crisis in the West Africa is an example of highly lethal infection, killing up to 70% of infected individuals. Rapid response to these medical emergencies is critical for controlling their spread. Regeneron’s VelociMab® technologies enable the development and rapid production of fully human antibodies. This approach integrates the sorting of VelociImmune® Mouse B cells with stable CHO production cell line generation to quickly move candidate antibodies into preclinical and cGMP clinical manufacturing. This case study of a “rapid response” drug development program will illustrate the strategies and critical technologies used to achieve unprecedented speed to patients.
PROTEIN AEROSOL FOR INTRANASAL NOSE TO BRAIN (N2B) DELIVERY

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Background and Novelty:
For the treatment of neurodegenerative diseases, drug delivery to the central nervous system (CNS) has gained considerable recent interest. But many concepts for the delivery of therapeutic agents have failed by the natural blood-brain-barrier (BBB). The N2B route for drugs could provide an adequate alternative of CNS drug delivery - bypassing the BBB. N2B drug transport takes mainly place at the olfactory area, unfortunately this area is well hidden at the skull basis. In this study, we simulated numerically the characteristics of aerosols for optimal deposition for N2B delivery and confirmed those data with experimental deposition in 3D human nose models. With these aerosol characteristics we generated protein formulation to maintain stability and function and to avoid shear stress during dispersion. Currently, we are testing the formulated proteins in an in vitro exposition system and in vivo. Thereby, we are presenting a comprehensive approach for N2B drug delivery of proteins.

Experimental Approach:
Two human nose models – a standardized and an individual one – were constructed from CT scans and display the complex 3D geometry of the human nose and its olfactory cleft. The deposition of liquid airborne aerosols were simulated numerically by computational fluid dynamic (CFD) and confirmed experimentally in positive rapid prototyped model. For aerosol generation, protein formulations were dispersed and evaluated for stability applying SE-HPLC and photometry. Identified suitable formulations were exposed to a nasal cell culture growing on trans-well inserts to investigate transport, permeability and activity. Moreover, nasal epithelial cells (RPMI) were co-cultured with macrophages (THP-1) to study immunogenicity of dispersed proteins.

Results and Discussion:
In the present study, we found that aerosol parameters had to be defined very precisely to enable deposition at anatomically hidden areas. Cross-validation of CFD simulation and deposition studies in a realistic nose model helped to get an idea of particle size and flow rate for N2B delivery. Protein formulation needs to be adapted for dispersion. Compared to the parenteral formulation, the aerosol formulation revealed improved protein stability. Nevertheless shear stress and dispersion effects had an increasing impact on protein aggregation when generating nanometer-sized particles. Here, we present the establishment and optimization on an in vitro exposition system. Currently, protein formulations are tested with different aerosol generators for bioactivity, immunogenicity and transport behavior in the Vitrocell® Cloud exposition system. These results will be verified in vivo.

In conclusion, a portfolio for protein aerosol could be established that shows different hurdles – particle size deposition in experimental and in silico nose model, protein stability, in vitro and in vivo studies – that have to be overcome for a successful N2B delivered protein aerosol.
RECOMBINANT VACCINES MADE IN INSECT CELLS

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The baculovirus-insect cell expression system is well known as tool for the production of complex proteins. The technology is also used for commercial manufacture of various veterinary and human vaccines. The speaker will review how this technology can be applied to produce a multitude of vaccine candidates.

The key advantage of this recombinant protein manufacturing platform is that a universal “plug and play” process may be used for producing a broad range of protein-based prophylactic and therapeutic vaccines for both human and veterinary use while offering the potential for low manufacturing costs. Large scale mammalian cell culture facilities previously established for the manufacturing of monoclonal antibodies that have now become obsolete due to yield improvement could be deployed for the manufacturing of these vaccines. Alternatively, manufacturing capacity could be established in geographic regions that do not have any vaccine production capability. Dependent on health care priorities, different vaccines could be manufactured while maintaining the ability to rapidly convert to producing pandemic influenza vaccine when the need arise.

Flublok is a recombinant hemagglutinin influenza vaccine produced in this production platform and provides an attractive alternative to the current egg-based influenza vaccine (TIV) manufacturing process. Protein Sciences Corporation was awarded a contract in June 2009 from the U.S. Department of Health and Human Services to further develop this technology for the production of recombinant influenza vaccines for pandemic preparedness. Flublok is approved by the FDA for the prevention of influenza in adults 18 and older. In addition to Flublok other product opportunities to support the versatility of the platform will be presented.

An existing U.S. based bacterial production facility was modified to support the manufacturing of Flublok by adapting the bioreactor train to support insect cell culture. Within 100 days the first batch of Flublok was produced at the 2000L scale. The feasibility of insect cell based production is further demonstrated by the successful start-up of the Flublok production plant in Japan where the product is manufactured at the 21,000L scale.
FIGHTING EBOLA VIRUS INFECTION: THE DEVELOPMENT AND USE OF EBOLA MONOCLONAL ANTIBODIES AND VACCINES

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*Ebola virus* causes a severe haemorrhagic fever in humans with a case fatality rate from 50-90%. Sporadic small to medium outbreaks in Africa over 38 years have resulted in approximately 2300 infections. Currently an outbreak in West Africa of the lethal *Zaire ebolavirus*, named Ebola virus (Makona), has seen >25,000 infections with >10,000 deaths. There are currently no approved vaccines or post-exposure therapeutics available so rapid deployment of a vaccine or therapeutic is urgently needed. A humoral immune response raised against the EBOV glycoprotein (GP) has shown to be sufficient for protection from an EBOV infection. We have developed an effective post-exposure therapy and a vaccine, both targeting the EBOV GP. The ZMapp (MappBio) post-exposure therapy consists of a combination of three monoclonal antibodies (c2G4 + c4G7 (PHAC/Defyrus) + c13C6 (USAMRIID)) that have been chimaerized with the human constant region. The VSVG-EBOVGP vaccine is a live attenuated vaccine using Vesicular Stomatitis Virus (VSV) as the viral vector, in which the VSV glycoprotein gene has been replaced with the EBOV GP gene. When initiated on day 5 after an EBOV infection, when fever, leukocytosis and thrombocytopenia were already present, the 3 dose ZMapp treatment given 3 days apart saw 100% of rhesus macaques survive, in comparison to the mock treated controls which were euthanized on day 8. The VSVG-EBOVGP vaccine protects 100% of cynomolgus macaques from a lethal high dose EBOV challenge. At 28 days post-immunization, the endpoint EBOVGP-specific IgG titre ranged from 32,000-128,000. As a post-exposure therapeutic VSVG-EBOVGP protected 33-50% of NHPs when given within 1 hour after being challenged with EBOV. The strategy of developing vaccines and post-exposure therapeutics against the EBOV GP has been very successful. As a result of the outbreak in West Africa, both the ZMapp/ ZMab (20 patients) and VSVG-EBOVGP (2 patients) has been used as a post-exposure therapy under emergency use protocols. The VSVG-EBOVGP vaccine is currently in Phase II/III clinical trials and ZMapp will be in phase I clinical trial by end of 2014/Jan 2015.
DEVELOPMENT OF AN ANTI-IDIOPTYPIC MAB AS CANCER VACCINE: FROM DISCOVERY TO APPROVAL

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Background and Novelty:
Racotumomab (1E10) is a murine anti-idiotypic antibody that mimics N-glycolyl-GM3 gangliosides. This antibody has been tested in some countries as an anti-idiotypic vaccine adjuvanted in Al(OH)₃, in several clinical trials Phase II and III for NSCLC, melanoma and breast cancer. Recently this product was approved for treatment for NSLC by regulatory authorities in Cuba and Argentina. This study describe the novel and complete development strategy allowed to take the product from the original idea in the laboratory to test the concept in advanced clinical trials and approval, including its scale-up and comparability studies.

Experimental Approach:
Initially the product was obtained from mice ascites fluid (AF), however a new pilot scale cGMP process based in stirred tank continuous mode cell culture using protein free medium was developed (ST). Further the production was scaled up to 1000 L bioreactor and a culture medium was optimized in order to increase productivity and cell growth. Bioequivalence between vaccine products obtained from ascites, different stirred tank scales and different cell culture media through a comparability tests was studied. Also, the influence of pH, ionic strength and phosphate concentration of buffer on the adsorption of the Mab to the aluminum gel was investigated. Formulations with different adsorption percentages and antibody/adjuvant ratios were characterized by DLS.

Different characteristics as primary, secondary and tertiary structure, microheterogeneity, identity, purity and biological activity of obtained Mabs, as well as vaccine formulation characteristics as stability, absorption to AlOH₃, immunogenicity and antitumor activity “in vivo” were analyzed. Techniques like mass spectrometry, liquid chromatography, circular dicroism, fluorescence, SDS-PAGE and immunodetection. In case of in vivo analysis, two animal models were used (Leghorn chickens for immunogenicity and mouse for antitumoral activity of vaccine).

Results and Discussion:
Some differences were observed like charge heterogeneity and glycans attached to Fc-γ, mainly due to varying amount of sialylated species, asparagine deamination and oxidation in each condition. These characteristics did not affect the immune response elicited in chicken and antitumor effect on F3II carcinoma model.
Moreover optimization of product formulation significantly increased the percentage of adsorbed protein to alumina and stability. In vivo experiments using Leghorn chickens were performed to evaluate effects on immunogenicity of the different vaccine formulations. However no influence of the adsorption and the racotumomab/Alumina ratio on the immunogenicity of the vaccine was observed.
In summary, the introduction of a well-established process platform for the production of monoclonal antibodies, which led to increased levels of vaccine safety and reducing production costs, yielded a product very similar in each case, enabling the scaling and clinical development of the product until its health record.
DEVELOPMENT OF A MAMMALIAN CELL CULTURE PROCESS FOR THE RAPID CLINICAL-SCALE PRODUCTION OF NOVEL INFLUENZA NANOPARTICLE VACCINES

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Background and Novelty:
The influenza viruses cause seasonal epidemics and affect millions of people worldwide. Each year new influenza vaccines are produced to match circulating viruses. The limitations of currently available vaccines along with the complex time consuming manufacturing processes, underscore the need for more effective vaccines and rapid production technologies. Here we demonstrate a mammalian cell culture based production of novel influenza vaccines using self-assembling nanoparticles (NPs).

Experimental Approach:
Ferritin, a protein that naturally forms NPs was genetically fused to the viral hemagglutinin (HA) and was expressed in mammalian cells to produce HA-Ferritin nanoparticles (HA-F NPs). A transient gene expression process based on Polyethylenimine (PEI) mediated transfection was developed and scaled up for 50L scale GMP production of different HA-F NPs. The different HA-F constructs used were- H1 A/California/04/09, H2 A/Singapore/1/57, H1 A/New Caledonia/20/99 and H5 A/Indonesia/05/05.

Results and Discussion:
Transfection conditions, media, feed, process parameters and scale up conditions were optimized to achieve a high titer process. 50L-scale production of an influenza nanoparticle vaccine has been successfully completed for Phase I clinical trials.
PURIFICATION OF MODIFIED VACCinia VIRUS ANKARA FROM SUSPENSION CELL CULTURE

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Background and Novelty:
Modified Vaccinia Virus Ankara (MVA) is a host-restricted poxvirus and a promising candidate for vectored vaccine applications. The high level of attenuation of MVA prevents productive infection in most mammalian (including human) cells. However, MVA replicates efficiently in chicken embryo fibroblasts (CEF) and cultures of the continuous muscovy duck-derived CR.pIX suspension cell line. The advantage of vaccine production in CEF cultures is extensive regulatory experience whereas production in CR.pIX cultures in chemically defined media would be associated with lower logistical costs, vastly reduced opportunity for contamination with adventitious agents, and process options in modern bioreactors. Upstream yields beyond \(10^9\) infectious units/mL (pfu/mL) have been demonstrated in CR.pIX cultures, greater than those usually reported with CEF cultures. We now describe developments towards downstream processes for purification of injectable vaccine preparations.

Experimental Approach:
Regulatory and process requirements need to be reconciled if a virus vaccine such as MVA is to be produced on a continuous cell line. For example, the poxvirus particles are 200-400 nm in diameter and thus too large for most filtration steps to remove contaminants. The complete process therefore depends on continuous sterility. Furthermore, infectivity of MVA must be maintained, while a substantial reduction of host-cell derived proteins and DNA must be achieved. The currently admissible level for host-cell derived DNA is \(<10\) ng per parenteral vaccine dose \((10^8\) pfu). Our approach to purify MVA obtained from CR.pIX bioreactor cultures is to combine a treatment with chaotropic agents prior to cell lysis with tangential flow filtration (TFF) and chromatography. Monolithic chromatography columns with average pore sizes of 6 \(\mu\)m were selected to ensure binding and interaction of the large virus particles.

Results and Discussion:
We screened different monolith chemistries that ranged from cation and anion exchange to hydrophobic interaction. The binding and elution properties of a weak cation exchanger material (CM) were most suitable for purification of MVA particles in these studies. Purification was robust and largely independent of a cell-free supernatant or a complete, specially treated, lysate of infected cells as source. Influence of pH and conductivity of load material, as well as the dynamic binding capacity was determined. Main product peaks were obtained reproducibly by elution with NaCl at conductivity of 90 mS/cm. The product peaks contained approximately \(10^8\) pfu, corresponding to a recovery of 40-60\%. Singly, or in combination with a strong cation exchanger monolith, DNA content could be reduced to 60-100 ng per dose. Such levels already correspond to only 6 to 10-fold above the admissible threshold. Implementation of an enzymatic digestion step in combination with TFF allowed a further reduction of the DNA content. Maintenance of infectivity of MVA was confirmed by titration assays.

In summary, although further optimization is required, we can already demonstrate that monolith chromatography in combination with TFF steps is suitable for the purification of large poxviral particles. Infectious units were eluted in discrete product peaks and host-cell derived DNA was depleted significantly even without nuclease treatment.
GENE THERAPY OF INHERITED MUSCLE DISEASES

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Mutations in a large number of genes affect development and function of skeletal and heart muscles, and lead to severe, often fatal genetic diseases. These include Duchenne muscular dystrophy (DMD), limb-girdle muscular dystrophy and many other severe, though less frequent disorders for which no therapy is currently available. Recent progress in the design and manufacturing of adeno-associated viral vectors (AAVs) allow for the first time the development of gene therapies for monogenic muscle disease, based on systemic administration of high doses of AAV vectors carrying therapeutic gene expression cassettes. We will discuss the example of DMD and X-linked myotubular myopathy.
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CELL LINE ENGINEERING
STABILITY DIFFERENCE OF EACH CHROMOSOME IN CHINESE HAMSTER OVARY CELL LINE

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Background and Novelty:
Biopharmaceutical products represented by therapeutic antibodies are increasing in pharmaceutical industries. Chinese hamster ovary (CHO) cell lines are widely used in the field of biotechnology to produce therapeutic antibodies. CHO-DG44 cell line is dihydrofolate reductase (DHFR)-deficient CHO cell line, which is frequently used as a host cell for gene amplification method. We have previously constructed CHO genomic bacterial artificial chromosome (BAC) library that is expected to cover entire CHO-DG44 genome [1]. More than 300 BAC clones were corresponded to the chromosomal location of CHO-DG44 and CHO-K1 cell lines [2]. Thirteen BAC clones were identified to distinguish all the 20 individual chromosomes in CHO cells [1]. Since chromosome of CHO cell is unstable, variation of chromosome number occurs in the CHO cells. In this study, we focused on the dynamics of each chromosome during changes in the number of chromosomes. In addition, sequences of 304 BAC clone DNA that identified the chromosomal location in CHO cell lines were determined. In recent years, control of the expression vector integration site by gene targeting is becoming a new approach to construct stable cell line. Our study leads to an understanding of chromosomal location of the genome and the stability of each chromosome to establish high-producing CHO cell line.

Experimental Approach:
Ratio of chromosome number in CHO-DG44 cell line was examined. Six cell lines were isolated: three with 20 chromosomes (normal) and three with over 30 from the CHO-DG44 cell line. BAC-fluorescence in situ hybridization (FISH) technique was used to confirm each chromosome and to analyze the combination of chromosomes in these cell lines. HiSeq sequencing system was used to analyze 304 BAC clone DNA sequences.

Results and Discussion:
While most CHO-DG44 cells show the chromosome number around 20, four percent of cells were found to have more than 30 chromosomes. Comparison between CHO-DG44 cells with 30 or more chromosomes and those with the normal 20 reveals that specific chromosomes increased or decreased with the changes in the number of chromosome. In detail, chromosome D (chromosomes are named A to T in order of length, long to short) was easily decreased in number despite total chromosomes being increased in a cell. On the other hand, chromosomes E,F,G,H,I,K,M,O,Q,S were doubled in individual three cell lines with high chromosome number. Furthermore, either chromosome A or B was doubled in high chromosome number cells. Chromosomes of CHO cell lines are changed significantly from the original Chinese hamster except for chromosome A and B. Chromosome A and B are the only chromosome pair that exists as homologous chromosomes in CHO cell lines. This result suggests that the chromosome A and B are very stable. By reading the sequence of BAC clone DNA, genome sequence of each chromosome in CHO-DG44 and CHO-K1 cell lines were revealed. Overlapping sequences on stable chromosomes appear to be attractive candidate locations to establish high-producing CHO cell line.

CONSTRUCTION OF GENE KNOCKOUT CHO CELL LINE BY SIMPLE GENE TARGETING METHOD

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Background and Novelty:
Demand for therapeutic antibodies is increasing. Although Chinese hamster ovary (CHO) cell lines are the major hosts for producing therapeutic antibodies, it is difficult to establish stable and high-producing cell lines. Random integration of transgenes increases variation in antibody productivity because genes are integrated into unspecified locations on chromosomes. Gene targeting is a method that uses homologous recombination by adding homologous sequences to the genomic region on both sides of the exogenous gene. CRISPR-Cas9 system induces double-strand break by guide RNA and Cas9 which increases efficiency of homologous recombination. Stable production of antibodies is important in industrial production. However, cellular productivity of antibody decreases during long-term cultivation. DNA methylation is considered as one of the reasons for its reduction. In this study, we constructed simple gene targeting method in CHO cells by using CRISPR-Cas9 system and tried to knockout de novo DNA methyltransferase gene in CHO cells to attain stable production.

Experimental Approach:
Previously, we classified CHO-DG44 chromosomes by fluorescence in situ hybridization (FISH). Chromosomes were aligned by length from A to T [1]. We constructed a CRISPR-Cas9 vector that expresses guide RNA sequence targeting a region on chromosomeO. Antibody expression vectors with/without target site homology arms were also constructed. The percentage of gene integration into chromosomeO was compared among the cells transfected using random integration and cells transfected with CRISPR-Cas9 vector and antibody expression vector with/without homologous regions. FISH analysis was used to identify the chromosomal integration sites. The mRNA expression of de novo methyltransferases in CHO-K1 cells was investigated by RT-PCR.

Results and Discussion:
In order to investigate the efficiency of CRISPR-Cas9 system gene-targeting in CHO cells, the percentage of gene integration into chromosomeO was analyzed by FISH. In random integration, 29% of transfected cells showed specific integration into the chromosomeO and 71% of cells showed integration into other chromosomes. In case of the integration using the antibody vectors with homology arms, 33% of transfected cells showed specific integration into the chromosomeO, 23% of the cells showed integration into other chromosomes, and 44% of the cells were not observed. On the other hand, 74% of cells showed the specific integration into the chromosomeO and 26% of cells showed integration into other chromosomes in the experiment using the antibody vectors without homology arms. These results indicate that we could efficiently insert exogenous genes into the specific region of the genome using CRISPR-Cas9 vectors and exogenous genes without adding homologous region on either side of the genes. Using this method, we tried to knockout de novo DNA methyltransferase gene in CHO cells. The expression levels of de novo DNA methyltransferases Dnmt3a, 3b, and 3L in CHO cells were not reported previously. We investigated the expression levels of these three methyltransferases using RT-PCR analysis. Only Dnmt3a is expressed in CHO-K1 cells, while Dnmt3b and 3L were not detected. Therefore, we focused on Dnmt3a expression and are constructing the Dnmt3a-knockout antibody-producing CHO cell line for stable production of antibodies.

ESTABLISHMENT AND CHARACTERIZATION OF NEW HUMAN CELL LINES FOR RECOMBINANT THERAPEUTIC PROTEIN PRODUCTION

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Background and Novelty:
Recombinant therapeutic proteins are increasingly requested with advances in tissue engineering using stem cells. Human cell line is an attractive host for the production of such glycoprotein, but there are few reports on human cells for a commercial production. In this study, we established new human lymphoid cell lines from peripheral blood mononuclear cells (PBMCs) by treatment with phorbol 12-myristate 13-acetate (PMA) under a non-GMP condition, and characterized them by gene and protein expression analyses.

Experimental Approach:
The human PBMCs (2 x 10⁶ cells/ml) were cultured in 24 well plates in 12.5% FBS-ERDF medium supplemented with 10 ng/ml of IL-4 and/or IL-6 and 1 μg/ml of PMA for three months. The medium was changed every two or three days.
The gene expression of telomerase reverse transcriptase (TERT), a marker of immortalization, was examined by RT-PCR. The immunoglobulin (Ig) isotype was confirmed by ELISA. The CD markers on cell surface were detected by flow cytometry.

Results and Discussion:
Although normal human cells are difficult to be transformed by chemical reagents such as PMA, we succeeded in obtaining three human cell clones under above condition. The key point in our transformation may be continuous stationary culture without passage. All obtained clones were able to be subcultured over one year and were found to express TERT. In addition, they produced any isotype of immunoglobulin in the medium, indicating B lymphocytes. The results of flow cytometry also supported it. Therefore, these clones may be suitable for the production of secreted human glycoprotein because mature B lymphocytes have the extensive endoplasmic reticulum. In the next step, we will establish GMP compliant new human lymphoid cell lines.
CLEAVAGE EFFICIENT 2A PEPTIDES FOR HIGH MONOCLONAL ANTIBODY EXPRESSION IN CHO CELLS

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Background and Novelty:
Commonly used vectors for monoclonal antibody (mAb) cell line development express the heavy chain (HC), light chain (LC) and the selection marker gene(s) either on two separate vectors or a single vector with multiple promoters. Each gene is under the control of its own promoter and transcribed separately. Poor coupling of the mAb and selection marker genes for such designs results in a significant proportion of non-expressing clones surviving drug selection. Another disadvantage of having separate expression units is the lack of accurate control of the relative LC and HC expression levels. The ratio of LC:HC expression affects both mAb expression level and quality, such as aggregation and glycosylation. Expressing HC, LC and selection marker genes in one transcript using 2A peptides should minimize non-expressing clones and provide accurate control LC over HC expression at balanced ratios. Four 2A peptides derived from the foot-and-mouth disease virus (F2A), equine rhinitis A virus (E2A), porcine teschovirus-1 (P2A) and Thosea asigna virus (T2A) have been used commonly in biomedical research but have not been evaluated for mAb expression in Chinese hamster ovary (CHO) cells.

Experimental Approach:
Different tricistronic vectors containing F2A, E2A, P2A and T2A were designed and compared for expression of three biosimilar IgG1 mAbs in CHO DG44 and K1 cell lines in both transient and stable transfections. HC and LC were linked by different 2A peptides both in the absence and presence of GSG linkers. A furin recognition site was inserted upstream of 2A to remove 2A residues that would otherwise be attached to the HC. DHFR was under the control of a mutated IRES with attenuated translation efficiency for enhancing the stringency of selection for high producing cell lines. The stably transfected and amplified CHO DG44 pools were characterized for growth and productivity in shake flask cultures. The cleavage efficiency of 2A peptides was determined using western blotting and mass spectrometry. Product aggregate level was measured using size exclusion chromatography (SEC).

Results and Discussion:
Different 2A peptides exhibited different cleavage efficiencies that correlated to the mAb expression level. The relative cleavage efficiency of each 2A peptide remains similar for expression of different IgG1 mAbs in different CHO cells. While complete cleavage was not observed for any of the 2A peptides, GSG linkers did enhance the cleavage efficiency and thus the mAb expression level. T2A with the GSG linker (GT2A) exhibited the highest cleavage efficiency and mAb expression level. Stably amplified CHO DG44 pools generated using GT2A had titers of 357, 416 and 600 mg/L for the three mAbs in shake flask batch cultures. Incomplete cleavage likely resulted in incorrectly processed mAb species and aggregates, which were removed with a chromatin-directed clarification method and protein A purification. The vector and methods presented provide an easy process beneficial for both mAb development and manufacturing.
AMPLIFIED FRAGMENT LENGTH POLYMORPHISM (AFLP): A NEW METHOD TO IDENTIFY LARGE SCALE GENOMIC rearrangements IN CHINESE HAMSTER OVARY (CHO) CELLS

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Background and Novelty:
CHO cells are the most popular production system for the manufacturing of therapeutic protein products. Although ease of handling, fast growth in suspension culture and the capability to perform human-like post-translational modifications are strong advantages of this cell line, a clear draw-back is the frequent occurrence of chromosomal rearrangements which affect cell line and process development as well as reproducibility and the required screening effort. Here we describe a method that enables rapid assessment of the rate of genomic rearrangements in a given cell line with time in culture and the identification of subclones or cell lines characterized by genomic differences using Amplified Fragment Length Polymorphism (AFLP).

Experimental Approach:
The principle of AFLP is a restriction enzyme digest of genomic DNA, followed by ligation of the fragments to adapters with a predefined sequence. DNA amplification of restriction fragments is performed using selective AFLP primers complementary to the annealed adapter sequence, but containing extra nucleotides. An initial pattern of bands of digested genomic DNA is defined and allows quantification of chromosomal changes over time or between subclones and parental cell lines using sophisticated statistical techniques.

Results and Discussion:
A variety of host cell lines as well as a stable and an unstable producer clone were analyzed over a period of six-months in culture. Genomic rearrangements were identified for each cell line over time by a change revealing different rates of genomic changes in the analyzed cell lines as well as degrees of relationship between the cell lines and clones at the starting point. Different approaches to optimize and standardize the method were tested leading to a reliable and reproducible protocol.
The method could now be used to assess genomic changes over time within a given cell line, thus providing an additional databased decision parameter for choice of stable cell lines during producer cell development. In addition, it may be used for cell line/clone identification and for analysis of end-of-production material.
USE OF A NEW GENETIC ELEMENT TO IMPROVE EXPRESSION LEVEL OF CELL LINES FOR RECOMBINANT PROTEIN PRODUCTION

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Background and Novelty:
The establishment of a high expressing cell line for therapeutic protein production remains challenging. It is well known that the expression level of production cell lines can be improved at the genetic level by the implementation of regulatory elements (MAR, UCOE) in the expression vector to recruit the transcription machinery more effectively or to prevent epigenetic silencing. The goal of this work was the implementation of a new genetic element in our in-house vector system for improved transgene expression. The focus was put on the flanking sequences of the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) housekeeping gene, which is an ubiquitously expressed enzyme. It was hypothesized that its surrounding genetic environment would favor sustained expression. In this study, we present the effect of the upstream (5′) and downstream (3′) regions flanking the GAPDH gene on stable and/or transient expression in CHO-S and HEK293 EBNA cells.

Experimental Approach:
For the development of the “GAPDH” expression vectors, 3.2 kilobases of genomic DNA flanking the human GAPDH gene were amplified by PCR. The upstream GAPDH sequence was cloned in 5′ and the downstream GAPDH sequence was cloned in 3′ of the expression cassette. The expression cassette contained from 5′ to 3′: a promoter, a sequence coding for a kappa light chain, an internal ribosome entry site (IRES), a sequence coding for an IgG1 heavy chain, an IRES, a sequence coding for the green fluorescent protein and a sequence coding for a poly(A) tail. The GAPDH plasmids were transfected in transient and stable in suspension-adapted CHO-S and HEK293 EBNA cells. Four different expression plasmids (A, GAPDH_A, B and GAPDH_B) were tested and compared to the original backbone (pGLEX41). Plasmid A is a modification of pGLEX41 with codon optimization of the bla gene and the use of the R6K origin of replication instead of pUC origin of replication. Plasmid B corresponds to plasmid A with an additional reduction in the number of CpGs in certain region of the backbone. The expression level was studied by quantification of the IgG titer in the supernatant.

Results:
In CHO cells, a 2-fold higher expression level was obtained with the GAPDH plasmids compared to vectors without the GAPDH flanking sequences. This was true for both A and B constructs. The data demonstrate that the beneficial effect of the vector is solely due to the GAPDH flanking sequences and not the A and B modification. Compared to pGLEX41, a 3-fold higher expression could be observed. In HEK293 EBNA cells, the GAPDH_B vector is showing a 3-fold increase in expression, whereas the GAPDH_A vector shows even a 5-fold increase in expression compared to the pGLEX41 vector. In transient, the GAPDH flanking regions seem to be favorable for the production of IgG in both cell lines. CHO-S stable pools transfected with GAPDH_A and GAPDH_B showed a higher expression of IgG than pGLEX41 transfection (2.7 and 3.5 fold more respectively). Therefore, the GAPDH flanking regions are beneficial not only for transient expression but also for stable cell lines generation.
ENGINEERING OF STABLE *DROSOPHILA MELANOGASTER* CELL LINES FOR RECOMBINANT PROTEIN PRODUCTION BY RECOMBINASE-MEDIATED CASSETTE EXCHANGE

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**Background and Novelty:**

Insect cells are a cost-efficient and robust system for the production of complex eukaryotic proteins. Their capability of growing in suspension to high cell densities even at room temperature makes them an attractive alternative to CHO cells. The conventional way of generating stable insect cell lines is based on random insertion of the expression cassette into the host cell genome. An approach to overcome problems associated with the random insertion – like unintended activation or silencing of host genes – is the recombinase-mediated cassette exchange (RMCE) which has been successfully used in numerous cell lines, but not *Drosophila melanogaster* Schneider 2 (S2) cells up to now. Our aim is therefore the development of RMCE as a tool for targeted gene integration in S2 cells.

**Experimental Approach:**

In this study, we use flipase for the exchange of DNA sequences between a wild type flipase recognition target (FRT-WT) site and the mutant site FRT-F5. Our RMCE approach consists of two steps: 1.) Transfection of S2 cells with a tagging vector that contains a *gfp* gene between the two FRT sites and subsequent isolation of a master cell clone; 2.) Co-Transfection of the master cell line with a) a targeting vector that contains the final expression cassette and b) a flipase vector.

The tagging vector contains *hygro*\(^{6}\) for antibiotic selection, the targeting vector *puro*\(^{8}\). To distinguish between random integration of the targeting cassette and successful cassette exchange, a promoter trap technique is used.

**Results and Discussion:**

The transfection method was optimized to maximize transfection efficiency. A maximum transfection efficiency of 30% was reached using lipofection. Generation of a stable cell line by hygromycin selection took about four weeks. The resulting stable cell pool was very heterogeneous regarding GFP expression. Moreover, the distribution shifted over the time, because low expressing subpopulations grew faster than high expressing ones. These instabilities in the polyclonal cell pool underlined the need for single cell cloning.

Single cell cloning was carried out by limiting dilution. To promote viability and proliferation of single cells in a microtiter plate, culture media with varying amounts of conditioned medium and FBS were tested. Viability of S2 cells during single cell cloning in SF900 II medium was low under different conditions: 100% conditioned medium or 50% conditioned medium combined with 0% or 10% FBS.

After single cell cloning, a high producer that has incorporated the expression cassette in a transcriptionally active locus was identified by GFP quantification. This master clone is used for all further experiments. The master cell line is co-transfected with the targeting vector and a flipase-encoding vector and the resulting production cell lines are selected by puromycin.

As all production cell lines carry the expression cassette at the same genomic locus, comparative studies, e.g. to characterize promoter strengths, can be easily conducted. RMCE for S2 cells is a versatile tool box for engineering protein production cell lines. Compared to traditional generation of monoclonal cell lines, the generation process for the production cell lines is significantly shortened.
SITE-SPECIFIC INTEGRATION IN CHO CELLS MEDIATED BY CRISPR/CAS9 AND HOMOLOGY-DIRECTED DNA REPAIR PATHWAY

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Background and Novelty:
Chinese hamster ovary (CHO) cells are the most widely used mammalian hosts for production of therapeutic proteins. However, development of recombinant CHO cell lines has been hampered by unstable and variable transgene expression caused by random integration. With draft genome of several CHO cell lines and targeted genome editing technologies recently being made available, rCHO cell line development based on site-specific integration has the potential to overcome the limitations of clonal heterogeneity. Notably, CHO cells have previously been described as a cell type recalcitrant to homology-based integration of large DNA constructs, thus targeted integration in CHO cells has been confined to error-prone NHEJ based approaches. Here, we demonstrate for the first time efficient and precise targeted gene integration into site-specific loci in CHO cells using CRISPR/Cas9 genome editing system and compatible donor plasmid harboring short-homology arms, a gene of interests (GOI), and a fluorescent marker gene as an indicator for random integration.

Experimental Approach:
We aimed at developing a CRISPR and HDR mediated targeted integration system to obtain controlled and precise integration of transgenes. The system is based on donor plasmids harboring short homology arms, which flank the Cas9 cleavable sgRNA target site, used as the integration sites. The donor plasmids were constructed as follows: the homology arms towards each of the active sgRNAs were designed to facilitate CRISPR/Cas9-mediated targeted integration into three loci via HDR pathway. An mCherry expression cassette and a neomycin resistance gene expression cassette were placed within the homology arms. A ZsGreen1-DR expression cassette was placed outside the homology arms to detect random integration events. Upon correct targeting of the donor plasmids to the desired locus via HDR, cells will lose ZsGreen1-DR expression and only express mCherry together with neomycin resistance. We targeted a total of four sites in three different genomic loci of CHO cells; one site in C1GALT1C1 (COSMC) encoding the C1GALT1-specific chaperone 1, two sites in Mgtat1 encoding the mannosyl (alpha-1,3-)glycoprotein beta-1,2-N-acetylglucosaminyltransferase, and one site in LdhA encoding the lactate dehydrogenase A.

Results and Discussion:
Simultaneous introduction of active single guide RNAs, Cas9 nucleases, and donor plasmid have enabled precise HDR-mediated integration of large gene expression cassettes into desired sites with a frequency between 7.4–27.8%, following a simple drug-selection. The low level of off-target mutations in most potential off-target sites and more stable and highly reproducible expression levels of GOI were observed in targeted integrants, which supports the robustness and efficiency of current strategy. Our results can help pave the way for the targeting of GOI to specific loci in CHO cells, increasing the likelihood of generating isogenic cell lines with consistent protein production.
USE OF GENETICALLY ENCODED FLUORESCENT BIOSENSOR TO MONITOR INTRACELLULAR REDOX CHANGES IN CHO-K1 RECOMBINANT PROTEIN-PRODUCING CELLS

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Background and Novelty:
Mammalian cell lines have become the dominant system for the production of recombinant proteins for clinical applications. Despite the fact that the process yield of recombinant protein production has been increased, further improvements in cell productivity are still needed. Some parameters such as cellular growth, proliferation, metabolic activity and cellular stress are monitored for process optimization. One of the most important stresses the cells face involves the generation of radicals and oxidants during mitochondrial respiration. Recently, redox-sensitive variants of the green fluorescent protein (roGFP2 and rxYFP) have been developed [Antioxid Redox Signal.2010;13(5):621-50]. The biosensors were engineered to equilibrate with the intracellular pool of oxidized and reduced glutathione, the major redox buffer of most eukaryote, allowing the in situ and time-resolved analysis of the cellular redox state.
In our lab, we have engineered a recombinant protein producer CHO-k1 cell line to express a redox biosensor. This sensor displayed a sensitive and reversible response to different redox stimuli. In this work, we deepen the characterization of the sensor response during a batch culture.

Experimental Approach:
CHO-K1-hGM-CSF (CHO-k1 producing human granulocyte macrophage colony-stimulating factor) expressing rxYFP biosensor was grown in batch cultures. Glucose, lactate, ammonia and amino acid concentrations were quantified in culture supernatant and hGM-CSF activity was estimated by a bioassay. The functional analysis of the biosensor was performed by flow cytometry, using the median fluorescence intensity (MFI) of rxYFP+ cells for analysis.

Results and Discussion:
During batch culture the biosensor revealed a significant and sustained shift to a more oxidative intracellular milieu prior to entrance to the late log-phase (growth retardation). On day 3, complete renewal of the culture medium restored, only marginally (10%), the intracellular reducing milieu. On the other hand, addition of a 5 days-conditioned medium (which stem from cells that underwent a 20% oxidation of the biosensor) to a fresh cell culture (day 1) caused only a minor change (oxidation) in the intracellular redox state. Furthermore, the addition of hGM-CSF to the culture did not affect the redox balance. These results suggest that cells entering the late-log phase undergo a metabolic reprogramming.
Quantification of amino acids level in culture supernatants revealed that Ser, Glu and Pro were significantly consumed throughout a 6-days batch culture, whereas Gly and Ala were produced. The decrease in the cell specific growth rate and biosensor oxidation detected on day 3 correlate with the complete depletion of Glu occurring on this day. Interestingly, Glu is required for glutathione synthesis.
Taken together, our data suggest that the biosensor is capable to anticipate metabolic deficiencies that alter the cellular redox balance in batch culture systems. Future work will attempt to apply this non-invasive monitoring technology to increase cell line productivity in a controlled process.

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PRODUCTION OF RECOMBINANT COAGULATION FACTORS IN HUMAN CELL LINES

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Background and Novelty:
The currently available recombinant coagulation factor products have been produced in non-human murine cell lines. Experience with cross species-produced recombinant human proteins suggests that non-human cellular processing can affect protein functionality, impacting on efficacy and raising the immunogenic potential of the product. Considering that inhibitor antibodies are one of the major concerns in the Hemophilia treatment, it is necessary to evaluate the production of such proteins in human cell lines, ideally suitable to serum-free suspension cultures.

Experimental Approach:
Here we describe the susceptibility of the promising human cell lines HKB-11 (ATCC CRL-12568), SK-HEP-1 (ATCC HTB-52), HEP-G2 (ATCC HB-8065) and 293T (ATCC CRL-11268) to growth under serum-free suspension and to the production of recombinant factors VIII and IX (lentiviral vector-mediated gene transfer).

Results and Discussion:
To achieve an efficient recombinant protein production process, two properties of the host cell are essential: ability to grow to a high cell density in a serum free suspension culture and the production of the protein of interest at high levels with proper biological activity. On that basis, human cells were adapted and cultured under serum free suspension cultures. HKB-11, SK-HEP-1 and 293T cells were able to grow and be cryopreserved in serum free conditions. Kinetic characterization showed that the HKB-11 adapted to FreeSyle (Life Technologies); SK-Hep-1 adapted to SFMII (Life Technologies) and 293T cells adapted to FreeSyle (Life Technologies) medium reached cell densities as high as 8.6x10⁶; 1.9x10⁶ and 2.3x10⁶ cells/mL, respectively. The maximum specific growth rates (μ_max) were similar for SK-Hep-1 (0.0159 h⁻¹) and HKB-11 (0.0186 h⁻¹) and higher for 293T cells, 0.032 h⁻¹. We further evaluated the potential for recombinant coagulation factors production in these cells. The potential for rFVIII production was carried out in Sk-Hep-1 cells because hepatic cell lines have already been described as a good host cell for the production of such protein. rFVIII producer cells presented μ_max values as high as 0.064 h⁻¹ and production levels around 1 IU/mL (1.5 IU/10⁶ cells) in adherent serum-containing conditions. The serum-free suspension adaptation for this cell line and the transduction of the serum-free adapted cells is ongoing with promising results. The rFIX production was first assessed in 293T cells, because of the superior growth properties. Before serum-free adaptation, the cells presented rFIX production level of 0.74 μg/10⁶ cells (48 hours). After the adaptation of the recombinant cell, the production increased to 1.64 μg/10⁶ cells (24 hours). Alternatively, with the genetic modification of previously serum-free suspension adapted wild-type 293T cells, it was possible the production of 2.14 μg/10⁶ cells (24 hours), values higher than the ones described in the literature. Taking together, these results suggest that the human cells tested, here successfully cultured under serum-free suspension conditions, could be suitable for the manufacturing of recombinant proteins, including coagulation factors, reducing the potential to induce immunogenic reactions.
HYPERGLYOSYLATED INTERFERON-ALPHA PRODUCED IN CHO-K1 AND HEK 293 CELLS: DIFFERENCES IN GLYCAN STRUCTURE, BIOLOGICAL ACTIVITY AND PHARMACOKINETIC

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Background and Novelty:
Glycosylation is one of the most important post-translational modifications that proteins can undergo because it defines their biological and physicochemical characteristics. Even though oligosaccharide structures attached to proteins are conserved among eukaryotes, many differences have been found between therapeutic glycoproteins expressed in different hosts.

Chinese Hamster Ovary (CHO) cells are the most commonly used mammalian hosts for the production of commercially approved biopharmaceuticals. Human Embryonic Kidney (HEK) cells have gained importance due to their ability to produce glycoproteins that are very similar to the human naturally secreted proteins in terms of post-translational modifications, specifically glycosylation. Another advantage is that this system avoids the expression of antigenic glycoforms like N-glycosylation (Neu5Gc) present in CHO derived products. For these reasons, there is an increasing interest in using human cell lines for the production of recombinant therapeutic proteins.

IFN4N is a hyperglycosylated variant of human IFN-α2b that contains 4 N-glycosylation sites and was developed in our laboratory through glycoengineering. This molecule showed an increment in its molecular mass and charge, improved pharmacokinetic properties and higher in vivo activity when expressed in CHO cells. These findings allowed us to propose IFN4N as a new therapeutic candidate for viral and tumor diseases.

Experimental Approach:
The benefits demonstrated by IFN4N prompted us to develop CHO-K1 and HEK 293 producer cell lines in order to investigate the differences in the carbohydrate composition of both products as well as its influence on their biological activity and pharmacokinetic properties.

Results and Discussion:
IFN4N$_{CHO}$ and IFN4N$_{HEK}$ were successfully produced and purified. SDS-PAGE analysis showed that IFN4N$_{CHO}$ exhibited a higher average molecular mass compared to IFN4N$_{HEK}$. IEF assays revealed that IFN4N$_{CHO}$ glycoforms were mainly concentrated towards the acidic zone of the gel while IFN4N$_{HEK}$ presented a more homogeneous glycoform distribution along the separation range. This result was in accordance with a 2-times higher sialic acid content of IFN4N$_{CHO}$ in comparison with that of the HEK derived protein (10.6 ± 0.2 vs 4.7 ± 0.2 mol sialic acid.mol$^{-1}$ IFN, respectively). Importantly, no antigenic glycans were found in IFN4N$_{HEK}$, while a 0.1% of Neu5Gc was detected for IFN4N$_{CHO}$.

Weak anion exchange chromatography of charged glycans revealed that IFN4N$_{HEK}$ exhibited a high proportion of neutral and mono-sialylated structures (65%), while IFN4N$_{CHO}$ glycans comprised mainly bi-, tri- and tetra-sialylated species (75.5%).

While no differences were detected between the antiviral specific biological activity (SBA) of CHO and HEK cells-derived IFN4N, the antiproliferative SBA of IFN4N$_{CHO}$ was significantly lower than the value obtained for IFN4N$_{HEK}$ (2.5 ± 0.2 U1.ng$^{-1}$ vs. 5.2 ± 0.6 U1.ng$^{-1}$, respectively).

Finally, IFN4N produced in CHO cells showed a better pharmacokinetic profile than the molecule obtained in HEK cells. IFN4N$_{CHO}$ reached a higher plasma concentration later than IFN4N$_{HEK}$ and was removed more slowly from circulation.

Our results show the importance of an appropriate host selection for the production of a therapeutic glycoprotein in which the glycosylation profile matches the desired properties.
CRE-MEDIATED CELLULAR MODIFICATION FOR ESTABLISHING PRODUCER CHO CELLS OF RECOMBINANT SCFV-FC

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Background and Novelty:
Recombinant biopharmaceutical proteins have been mainly produced using animal cells such as Chinese hamster ovary (CHO) cells as host cells. We previously developed an accumulative site-specific gene integration system (AGIS) using Cre recombinase and mutated loxPs for gene amplification of a target gene in the CHO cell genome [1-2]. Since the system enabled repeated integration of multiple transgenes into a pre-determined locus of a cell genome, we performed genomic integration of recombinant scFv-Fc gene using this system and succeeded in increasing productivity corresponding to the copy number of the expression cassette [3]. In the present study, we evaluated the effect of cis regulatory elements on recombinant scFv-Fc production through chromosomal environment of CHO cells.

Experimental Approach:
We constructed plasmids encoding an scFv-Fc expression unit flanked with or without insulator elements, which were derived from CHO cell genome. CHO/P1G containing EGFP gene flanked with a wild-type loxP and a mutated loxP were used as founder cells [4]. Recombinant protein producer cells were established using Cre/loxP system and confirmed by genomic PCR analysis for site-specific integration. The recombinant scFv-Fc productivity was assayed by ELISA methods.

Results and Discussion:
The insulator elements with various orientations were incorporated into scFv-Fc expression vectors. The expression vectors were introduced into founder cells with a Cre expression vector. Since a DsRed gene was placed upstream the scFv-Fc expression unit, we screened recombinant cells that showed the change of fluorescent color from green to red after Cre/loxP reaction. The transgene integration into the expected chromosomal site was confirmed by genomic PCR and sequencing of amplicons. When the expression unit was flanked with insulator elements, scFv-Fc productivity was enhanced 2.6-fold higher compared to that without insulator insertion. Transgene mRNA level exhibited a similar tendency of the protein productivity. The cells introduced the scFv-Fc expression units flanked by the insulator elements allowed more stable scFv-Fc productivity.

Bibliography. Acknowledgements:

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MIR-92A ENHANCES RECOMBINANT PROTEIN PRODUCTIVITY IN CHO CELLS BY INCREASING INTRACELLULAR CHOLESTEROL LEVELS

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Background and Novelty:
MicroRNAs (miRNAs) have emerged as promising targets for engineering of CHO cell factories to enhance recombinant protein productivity. Manipulation of miRNA levels in CHO cells have been shown to improve product yield by their effects on cellular processes such as increasing proliferation, resisting apoptosis and enhancing specific productivity (qP). We previously demonstrated increases in qP and titer of CHO-IgG cells by over-expressing miR-92a. Stably-transfected pools showed 27% increase in qP and 21% increase in titer compared to parental clone, without significant alteration in proliferation rates. The highest producing clone isolated from the miR-92a pool demonstrated 114% increase in qP and 88% increase in titer. However, the mechanisms by which miR-92a enhances qP in CHO cells are still uninvestigated. Here we report that miR-92a may enhance qP in CHO cells by increasing intracellular cholesterol levels.

Experimental Approach:
To understand possible pathways via which miR-92a enhances productivity in CHO cells, we carried out transcriptomics study on 2 high-producing miR-92a clones (~33pg/cell-day) and blank-transfected pool (16pg/cell-day) using microarray. Genes identified to be differentially expressed in both clones relative to the blank-transfected pool were analyzed for pathway/gene ontology enrichment. Cholesterol assays were also carried out on the cellular lipid extracts and the supernatant harvested from the miR-92a clones and blank-transfected pool.

Results and Discussion:
Genes involved in cholesterol synthesis and metabolism were found to be enriched among the differentially expressed genes identified by microarray. The intracellular cholesterol concentration of both miR-92a clones were significantly increased by ~30% compared to the blank-transfected pool. The extracellular cholesterol concentrations for the miR-92a clones were also ~2-3-fold higher than that of the blank-transfected pool. Knock-out of miR-92a has been reported to reduce systemic cholesterol levels in mice, concurrently with our observations that miR-92a over-expression increases cholesterol biosynthesis/secretion. Cholesterol is known to be essential for vesicular transport from the ER and Golgi. Our findings suggest that miR-92a may affect cholesterol metabolism, resulting in raised intracellular cholesterol levels and hence increased protein secretion.
RATIONALE HOST CELL SELECTION FOR SUPERIOR HOST CELL LINE GROWTH AND PROCESS PERFORMANCE

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Background and Novelty:
The selection of a suitable host cell is crucial for successful biopharmaceutical process development and production. Currently, mammalian production cell lines like CHO uses two main selection systems, either the dihydrofolate reductase (DHFR) or the glutamine synthetase (GS)-based selection, both of which have been in wide industrial use for many years. Although cell line development workflows, media and process development have essentially improved in the last years, one is still mainly working with the same CHO host cell lines. The differences in the hosts are not yet fully understood, but have a significant impact on the process development chain. Although the correlation of an “empty” host and the recombinant producer is not really known, host cell optimization is a leverage to obtain “desired” traits eg with respect to product quality profiles and/or metabolic behavior.

Experimental Approach:
Clonal CHO cell populations are phenotypically heterogeneous and this heterogeneity allows the selection of cells with improved performance under preferred conditions such as in a stirred-tank bioreactor. We have adapted adherent CHO-K1 cells to serum free suspension culture in CD/ACF media and sequentially optimized the cells to robust continuous growth by long-term passaging. Subsequently, high performance selection systems like GS and DHFR have been tested in these CHO-K1 cell lines. Applying a broad screening approach, several host cell clones were compared in a step wise approach. First, host cell lines were screened for optimal growth, metabolic and process performance. In parallel, a systems biotechnological approach was used by applying metabolic flux analysis before and after selection system integration to identify potential bottlenecks for media development already at the host cell level. Also, transcriptomics data were collected in different stages of the host cell optimization workflow. To finally identify high performance cell lines, several host cell lines were assessed for productivity and growth performance using a state-of-the-art cell line generation approach transfecting the host cells with a model IgG1 molecule. Advanced analytics were applied on the resulting recombinant clones for in-depth analysis of e.g. metabolic and product quality profiles.

Results and Discussion:
Extended passaging of suspension-adapted host CHO cells over more than 130 days resulted in IVC improvements up to 80 % when original and adapted cells were compared in head-to-head bioreactor cultivations. In line with the improved IVC, the best host cell candidates showed doubling times <16 h when cultured in our CD/ACF platform media during passaging cultivation. The new host cell lines showed up to 4x higher titers (up to 10g/L) compared to a standard host cell system expressing a IgG1 model molecule. Additionally, we could identify host cells expressing antibody with different product quality profiles and preferable metabolic behavior like lactic acid consumption.
IDENTIFICATION OF BOTTLENECKS IN ANTIBODY EXPRESSION USING TARGETED GENE INTEGRATION

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Background and Novelty:
Although different monoclonal antibodies may exhibit high similarity in the primary protein structure of their variable region they may exhibit pronounced differences in specific productivities in mammalian cell cultures. We expressed a panel of different monoclonal anti-HIV antibodies (2F5, 2G12, 4B3, 3D6) in CHO cells in different projects which led to the definition of low and high-expressing antibodies. We could observe a direct correlation of specific productivities and the protein sequence identity to the closest human germline genes. In order to investigate what mechanisms are responsible for the differential expression patterns, how specific productivities are correlated with biophysical properties of antibodies and how the product itself might influence the physiological state of cell cultures we constructed a CHO host capable for targeted gene integration using recombinase-mediated cassette exchange (RMCE). With this RMCE host cell line a defined antibody gene copy number and mRNA transcript level can be controlled to investigate the antibody expression levels of different antibody variants under isogenic conditions.

Experimental Approach:
The RMCE host is a CHO cell line with a single stably integrated gene copy of a reporter/selection marker flanked by two heterospecific recombinase recognition target sites. By co-transfection of the recombinase together with any gene of interest, e.g. antibody, flanked by the same set of target sites, it is possible to exchange the reporter/selection marker in a site-specific manner. Different transgenes were directed into the same chromosomal position and clones with identical mRNA transcript levels were generated by minimized screening effort.

Results and Discussion:
After controlling the gene copy number and mRNA transcript level as well as analysis of intracellular product content we could identify the bottleneck of the broadly neutralizing anti-HIV antibody 2F5 lying in the maturation /secretion pathway. With this gene targeting host we will be able to evaluate the influence of antibody productivity on cellular physiology or growth. Furthermore, we will investigate the biophysical properties and post-translational modifications of antibodies and correlate those with specific productivities of the generated production cell lines and their physiological properties.
PROFILING MIRNAS OF HIGH, LOW, AND NON-PRODUCING CHO CELLS DURING BIPHASIC FED-BATCH CULTIVATIONS UNRAVELS INTERESTING TARGETS FOR CELL LINE ENGINEERING

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Background and Novelty:
First identified as critical regulators of development in nematodes, miRNAs are known to play key roles in the coordination of almost every cellular process in eukaryotes, including proliferation, differentiation, apoptosis and development. MiRNAs are small RNAs, which function as regulators of posttranslational gene expression by binding to their mRNA targets. In difference to other interfering RNAs (RNAi), miRNAs can target many mRNAs, thus having an increased impact on regulation of gene expression. These properties of miRNAs makes them interesting and promising targets for biomarkers and cell line engineering. With next generation sequencing techniques, miRNA profiles of high, low and non-producing CHO cell lines were exploited during different culture phases and conditions of a fed-batch process. Additionally, the potential of differentially expressed miRNAs as targets for process optimization was investigated. This improved understanding of the role of miRNAs during a fed-batch cultivation and the pathways involved may pave the way to introduce a new layer of control for cell line engineering.

Experimental Approach:
MiRNA profiles of CHO cells during a biphasic fed-batch culture in 2L bioreactors were established using deep sequencing technology. The CHO-DG44 derived cell lines differs in their specific IgG productivity between high, low and non-producing. Fed-batch cultivations were conducted either as control runs with maintained temperature at 37°C during the complete cultivation or as biphasic runs where a temperature shift to 30°C was mediated during the exponential growth phase. For each of the processes, miRNA profiles at five time points throughout the cultivation in the bioreactors were analyzed. Differential expression, cluster- and pathway analysis were performed using different Perl based scripts and Bioconductor tools from R.

Results and Discussion:
Five miRNA profiles for each cell line and process condition including repetition resulted in 36 individual libraries for deep sequencing. Bioinformatic analysis of all of these libraries revealed new potential miRNAs for CHO. The differential expression of these new, together with annotated CHO miRNAs during monophasic and biphasic fed-batch cultivations including a temperature shift showed interesting variations of miRNA profiles during the bioreactor runs. The time resolution of our approach can discriminate between effects of the exponential, stationary or declining phase and the early and late response after temperature shift. Temperature shift had a high impact on the miRNA profile of CHO cells including many up- or down regulated miRNA. Most of these miRNA returned to levels before the temperature shift, underlining the role of miRNA as first adaptations responses of cells to new environment conditions. Furthermore, differential expression and pathway analysis between high, low and non-producing cells revealed interesting miRNAs involved in different pathways such as growth, apoptosis and IgG production. Taken together, these results improves our knowledge of role of miRNAs involvement during different process conditions and CHO cell lines with different specific IgG productivities.
TARGETING THE UBIQUITIN PATHWAY BY MIR-30 ENHANCES RECOMBINANT PROTEIN PRODUCTION IN CHINESE HAMSTER OVARY (CHO) CELLS

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Background and Novelty:
miRNAs (miRNAs) are small non-coding RNAs that post-transcriptionally regulate gene expression in mammalian cells. miRNAs have been demonstrated to be promising cell engineering targets for Chinese hamster ovary (CHO) production cells to improve bioprocess relevant cellular characteristics. miRNAs are capable of regulating entire physiological pathways by targeting hundreds of different genes concomitantly. Furthermore, forced expression of small non-coding RNAs does not add any translational burden to the cell thus maintaining maximum cellular capacity for transgene expression. The miR-30 miRNA family was previously reported to enhance recombinant protein expression in CHO cells. Here, we show the identification of downstream targets of miR-30 which are involved in the ubiquitin pathway. Engineering the ubiquitin pathways thus might be partly causative for observed productivity enhancing effects of miR-30 in recombinant CHO cells.

Experimental Approach:
Three different CHO cell lines expressing two different monoclonal antibodies (mAb) or the secreted alkaline phosphatase (SEAP) were transiently transfected with miR-30 mimics/antagomiRs and analyzed 72-96 h post-transfection for changes in recombinant protein expression, cell growth, apoptosis and necrosis. Bioinformatic target prediction and clustering was performed to identify putative target genes for the miR-30 family members. Down-regulation of messenger RNA (mRNA) and protein levels for predicted miR-30 target genes in CHO cells was carried out by quantitative reverse-transcription real-time PCR (qRT-PCR) and western blot analysis, respectively. Finally, the 3’-untranslated region (3’UTR) of the putative target genes were transferred to a dual-luciferase reporter vector and co-transfection of miR-30 mimics/antagomiRs with the reporter vector was carried out to confirm direct miRNA-mediated gene regulation.

Results and Discussion:
The entire miR-30 family comprising the five members miR-30a, miR-30b, miR-30c, miR-30d and miR-30e was found increase recombinant protein production in CHO-SEAP cells in a transient high-throughput miRNA screening approach. Transient validation experiments in two different mAb-producing CHO cell lines confirmed that the miR-30 family enhanced volumetric antibody productivity up to 7-fold compared to non-targeting miRNA transfected control cells. Furthermore, our results clearly point towards a cell line as well as product independent functionality of the miR-30 miRNA family in CHO cells. Notably, stable miR-30 overexpression in CHO-SEAP and CHO-mAb cell lines also increased recombinant protein production thereby supporting results of transient transfection experiments. To better understand the molecular mechanisms behind this improved phenotype mediated by miR-30 we performed a bioinformatic target prediction which revealed members of the ubiquitin pathway as putative target gene of miR-30. Forced expression of miR-30 miRNAs as well as knock-down of endogenous miR-30 family members in CHO cells significantly decreased and increased target mRNA levels, respectively. In addition, protein levels were also decreased following transient miR-30 mimics introduction for a selected target gene of the ubiquitin pathway. Luciferase reporter assay experiments could finally confirm the direct miRNA:mRNA target interaction. Our results support the hypothesis that the miR-30 miRNA family regulates parts of the ubiquitin pathway in CHO cells which might be responsible for the observed improvements in recombinant protein production.
CHOLESTEROL BIOSYNTHESIS ENGINEERING REVERTS SERUM DEPENDENCE IN RECOMBINANT RETROVIRUS PRODUCTION

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Background and Novelty:
The need of animal sera to support high infectious titers has challenged retroviral vector (RV) production. The problem extends to lentiviral vector (LV), although the transient nature of LV production attenuates this effect since the main impact of serum deprivation is felt upon long-term culture periods [1]. Previously, we demonstrated that cholesterol starvation was a major cause for the loss of infectious vector titers under serum deprivation conditions [1, 2]. Herein, we used functional genomics tools to investigate the metabolic impairments underlying cholesterol starvation in RV production and attempted to revert serum dependence by reactivating cholesterol biosynthesis. We describe the tailored design of viral vector producer cells in the pursuit of a serum-independent phenotype driven by functional genomics studies. By opposite to a more traditional trial-and-error approach, this work engages a systems biology concept on a broader understanding of the biological network to rationally design a phenotype. To the best of our knowledge, this is also the first gene-based metabolic engineering manipulation of cholesterologenesis in mammalian cell culture.

Experimental Approach:
The effects of serum deprivation on global gene expression were evaluated using whole-transcriptome microarray, combined with pathway analysis methods, by comparing standard serum supplementation to reduced serum conditions. Over-represented pathways were inspected to identify potential limiting genes. The gene targets identified were over-expressed in 293 FLEX RV producer cell line, alone or in combination, delivered through lentiviral vector transduction.

Results and Discussion:
Pathway analysis highlighted cholesterologenesis as the most significantly over-represented pathway under serum deprivation conditions, corroborating our previous findings [1, 2] and providing a transcriptome-phenotype relationship. Transcriptional profiling identified three potential gene bottlenecks in de novo cholesterol biosynthesis: mevalonate kinase (MVK), lanosterol synthase (LSS) and sterol regulatory element binding factor 2 (SREBF2). When individually over-expressing MVK or LSS, serum dependence was mildly alleviated. When in combination, it was totally reverted. The over-expression of SREBF2 alone, not only promoted the complete reversion of serum dependence, as it resulted in higher titers than those of standard serum supplementation conditions. This outcome was cholesterologenesis-specific since the over-expression of SREBF1, more related to fatty-acid biosynthesis, had no effect neither on viral titers nor in the capability of reverting serum dependence. Currently, the impact of these over-expressions is under study, particularly at the level of gene expression and cholesterol biosynthesis. Manipulated cells are also under evaluation for long-term RV production using serum-free media formulations and the strategy is being extended to stable production of LV.

The understanding and control of lipid metabolism is an important landmark to deliver improved cell lines and robust manufacturing processes for the production of RV and LV, important gene therapy viral vectors. Moreover, lipid metabolism is a transversal pathway for the production of complex biopharmaceuticals in mammalian cell lines, particularly relevant for the manufacturing of enveloped viruses of enormous biotechnological relevance including influenza, dengue or cytomegalovirus.

GLUCOSE AND LACTIC ACID CO-METABOLISM IN HEK293 CELLS: A MECHANISM FOR H⁺ DETOXIFICATION

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Background and Novelty:
Exponentially growing mammalian cells in glucose medium typically show an aerobic glycolysis resulting in high lactate production rates, which is consumed at late culture stages when glucose/glutamine are totally depleted.
We found out that HEK293 cells can be induced to simultaneously consume glucose and lactate by controlling the H⁺ concentration of the culture broth. After studying the culture conditions, we were able to trigger at will the co-metabolization of both substrates at very early culture stages without affecting cell growth or protein production, by means of adding exogenous Na-lactate and reducing the pH<6.85.

Experimental Approach:
Experimental runs were carried out either in 2L-bioreactors or in fully monitored (pH,pO₂,T⁰,OTR,CTR,RQ) shake flasks (Ramos-SFR platform,Khuner-Presens).
DoE was performed with the objective of estimating the dependencies between initial lactate/H⁺ concentrations and the metabolic shift.
Metabolic flux calculation was performed using a Recon-2 derived genome scale metabolic model adapted to HEK293 cells and further modified for the studied conditions. Metabolic flux distribution was obtained by maximization of ATP hydrolysis rate and determination of flux ranges around the optimal point.

Results and Discussion:
The extensively reported metabolism of HEK293 cells (unbalanced glycolysis with respect to TCA-cycle pathway), surprisingly switched when the pH value was kept below 6.85. Under these conditions a co-metabolism of extracellular glucose and lactate was observed, even when glutamine was present and lactate was far to reach cytotoxic concentrations. This metabolism was induced at early culture stages by adding exogenous Na-lactate and lowering the pH. However, HEK293 cells were unable to grow at that pH without lactate. Taking into account this observation together with the fact that when pH was controlled at 7.1 co-metabolism was never observed, it can be hypothesized that the co-metabolism of glucose and lactate was triggered as a H⁺ detoxification mechanism.
Statistical DoE analysis using different initial exogenous Na-lactate and H⁺ concentrations allowed to define a pH threshold (6.6≤pH≤6.85) where metabolic switch was observed. DoE results showed consistency with the detoxification hypothesis.
The H⁺ detoxification hypothesis involves that lactate and H⁺ transported into the cytosol are shuttled into the mitochondria, where lactate is oxidized and H⁺ are transferred to oxygen, obtaining CO₂ and water as final products. This pathway involves the existence of the mitochondrial lactate oxidation complex (mLOC) in HEK293 (mLOC was included in the Mito-Carta-list).
A metabolic flux analysis was included to identify the plausibility and consistency of the hypothesis with the available data. Thus while in exponentially growing cells nearly 20% of the carbon flux generated in glycolysis is metabolized into the mitochondria, in the co-metabolism case, it increases up to 80% (the rest has its origin in the lactate). The obtained results supported the proposed hypothesis and revealed the relevance of the mLOC pathway offering novel insights of HEK293 metabolism.
This study opens a door to re-direct genetic engineering of animal cells by modifying the lactate metabolism that may improve cell growth, and to develop novel culture strategies towards high cell density cultures.
RNAI MEDIATED KNOCKDOWN OF DNMT3A ENHANCES ANTIBODY TITER IN CHO CELLS UP TO 200 PERCENT

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Background and Novelty:
Increasing cell specific productivity is still an important challenge in today’s production of biopharmaceuticals in mammalian cell cultures. During the last two decades various efforts were made, e.g. by optimization of process strategies, media development or cell line and metabolic engineering to achieve higher yields. Nevertheless, some challenges remain for efficient and applicable high titer production processes at industrial scale: the stability of engineered cell lines and the complexity of stable high - titer production processes as well as the characterization for regulatory authorities and strategies for downstream processing. We found decreased transcript levels of the de novo methyltransferase 3a (Dnmt3a) in independent cultivation processes with increased productivity, namely in perfusions under glucose limitation and batch cultivation upon butyrate treatment. To prove the hypothesis that reduced transcription of Dnmt3a is responsible for the increase in productivity, we investigated the influence of RNAi-mediated knockdown of Dnmt3a in recombinant CHO cells on cell growth, viability and cellular and process productivity.

Experimental Approach:
We designed siRNA constructs to explore the influence of a decreased Dnmt3a level in recombinant CHO cell lines secreting monoclonal antibodies. Three different siRNAs were used for transient transfection via nucleofection in different CHO cell lines. Transfected cells were grown for two days in parallel with an untransfected and a scrambled control in chemically defined, animal-component free media. Samples for analysis of cell growth and viability, glucose, lactate and amino acid concentrations as well as for monoclonal antibody (IgG1) concentration were taken 24 h after transfection. RNA was extracted for qRT-PCR-measurement of Dnmt3a expression level; knockdown efficiency and cell specific productivity were calculated.

Results and Discussion:
Using RNAi mediated transient silencing of the de novo methyltransferase Dnmt3a, knockdown efficiencies up to 70 % were realized as shown by qRT - PCR. Similar to previously cultivations under glucose limitation or butyrate treatment, cell specific productivity was enhanced. Two of the three constructs resulted in a 7 - 16.7 fold increase in cell specific productivity 24 h after transfection of different CHO cell lines compared to control experiments. Although in some cases a strong decrease in cellular viability was observed, product titers were increased by 50 - 230 %. Our results confirm that the expression level of Dnmt3a has a strong impact on CHO cell productivity and can be an interesting target for cellular engineering of production cell lines. Currently, construction of stable transfected cell lines using a lentiviral system is ongoing. To minimize potential negative impact of some of the constructs on cellular viability and cell growth, an inducible promoter system is being used.
INVESTIGATION OF MICRORNA-MEDIATED ADAPTATION TO MILD HYPOTHERMIA UNCOVERS A UNIVERSAL MASTER REGULATOR ENHANCING PERFORMANCE OF MAMMALIAN PRODUCTION SYSTEMS

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Background and Novelty:
Mammalian cell systems still constitute the dominant choice for manufacturing of biopharmaceuticals. Besides CHO-based monoclonal antibody (mAb) productions, next-generation biomolecule formats such as viral vectors for gene therapy have been gaining increasing importance. With its groundbreaking clinical success, especially the demand for large-scale manufacturing of recombinant Adeno-associated virus (rAAV)-based vectors is inevitably raised. HeLa cell lines with stably integrated AAV rep, cap and vector represent a classical basis for rAAV production. However, attempts to increase vector yield in these systems have not been implemented yet. Mild hypothermia is causing differential gene and microRNA (miRNA) expression in mammalian cells and is frequently applied to increase cellular productivity in bi-phasic CHO-based production processes. miRNAs are small non-coding RNAs which regulate hundreds of different genes concomitantly and overexpression of specific miRNAs has been described to be highly useful for cell engineering. Discovering miRNAs and their target genes which are basically responsible for adaptation to mild hypothermia might help to understand beneficial effects on cell-specific productivity during low-temperature cultivation.

Experimental Approach:
Different mild hypothermic cultivation conditions were tested to increase productivity of rAAV vectors and mAbs in HeLa rep/cap and CHO-IgG producer cell lines, respectively. Microarray analysis was subsequently performed using the optimal cultivation conditions to identify differentially expressed miRNAs and mRNAs in response to temperature downshift in HeLa and CHO DG44 cells. Differentially expressed miRNAs were validated using quantitative reverse-transcription real-time PCR (qRT-PCR). Transient transfection approaches using miRNA mimics and antagoniRs in HeLa rep/cap and CHO-IgG producer cell lines at different temperatures were carried out to confirm functional effects of the identified miRNA. Following miRNA transfection, impacts on cell growth, viability, cell cycle and productivity as well as miRNA and mRNA levels were analyzed. Bioinformatic target prediction was performed to identify putative target genes for the identified miRNA.

Results and Discussion:
By taking advantage of optimized bi-phasic production processes, cellular productivity of rAAV vectors and mAbs were significantly increased in HeLa cells with stably integrated rep and cap genes and IgG producing CHO cells, respectively. In both corresponding wildtype cells, microarray analysis revealed differential expression of many miRNAs in response to these mild hypothermic cultivation conditions. Strikingly, one particular miRNA was shown to be most clearly up-regulated in both cell lines, which was confirmed by qRT-PCR. Comparison of bioinformatically predicted target mRNAs of this miRNA with mRNAs found to be actually down-regulated at mild hypothermic conditions resulted in pronounced consensus. In production processes using transient miRNA transfection, the yield of rAAV vectors and IgGs was significantly increased in the respective cell line.

Our findings provide evidence for the existence of a universal miRNA master regulator enhancing the yield of both next-generation as well as classical biomolecule formats in mammalian cell factories.
ENGINEERING GLUTATHIONE METABOLISM FOR THE PRODUCTION OF RECOMBINANT RETROVIRUS

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Background and Novelty:
Virus-based biopharmaceuticals – e.g. virus-like particles vaccines, oncolytic vectors and gene transfer vectors – have been progressively entering the market. Thus, increasing attention and investment are being directed to the development of robust and high-yielding hosts for the production of these complex products. In this context, metabolic manipulation of producer cell lines has already proven its efficiency in raising viral titers [1]. In previous work we have identified the metabolic networks and respective genes recruited for the production of recombinant retrovirus in human cell lines [2]. These findings enabled the rational design of gene engineering strategies in the pursuit of improved production phenotypes. Glutathione metabolism appeared as limiting the production of these viruses [2] and is also a metabolic pathway of great importance for animal cell culture bioprocess. Therefore, in this work, we conducted the rational manipulation of seven gene targets related to this pathway by using, as study model, a human cell line stably producing recombinant retrovirus. The genes and the number of engineered targets addressed herein are, to the best of our knowledge, the first and the largest attempt to manipulate glutathione metabolism to improve virus-based biopharmaceuticals productivity. Importantly, the approach conducted in this study enabled the understanding of the dynamic relationship between the expression levels of each manipulated gene target and virus productivity.

Experimental Approach:
Seven candidate genes of glutathione metabolism were over-expressed, in a human cell line stably producing recombinant retrovirus, at increasing DNA loads delivered through lentiviral vector transduction. The resulting populations were characterized in terms of growth, productivity, viral components and targeted gene expression and gene copy number.

Results and Discussion:
The manipulated populations raised specific productivity up to 10-fold. The manipulated genes and the level of over-expression induced different dynamics in the producer state. Depending on the metabolic gene, increasing levels of expression had different outcomes in titer: from upswing or downswing patterns, to defined and intermediate thresholds where productivity reaches a maximum. The existence of these patterns re-enforces the need of a fine tuned gene expression to achieve hyperproducing phenotypes. Also, high-titer yielding manipulations were associated to substantial amplification of viral components expression, which is now being investigated.
This work has a direct application in the field of retrovirus manufacture for vaccinology and gene therapy. Since retroviral vectors are a highly complex product, the metabolic gene engineering strategy performed herein, is likely to be also of relevance to other biopharmaceuticals produced in mammalian hosts, including complex proteins and other types of recombinant viruses that generally induce an increase in the cellular oxidative stress. Therefore, it will contribute to the understanding on how glutathione metabolism influences the manufacture of complex bioproducts in mammalian cell lines.

References:
FLEXIBLE INSECT CELL PLATFORMS FOR PRODUCTION OF PSEUDO-TYPED VLPS FOR DRUG AND VACCINE DEVELOPMENT

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Background and Novelty:
Virus-like-particles (VLPs) from enveloped viruses have been recently proposed as an efficient means to obtain correctly folded, active membrane proteins which are dragged along from producer cells during the budding process. In principle, the same VLP scaffold can be used to display at its surface i) conformationally-complex membrane receptors (major targets in current disease therapies) or ii) membrane protein epitopes from different viruses (allowing more efficient and flexible vaccine production). One of the main platforms used to produce enveloped VLPS is the baculovirus-insect cell system, yielding reasonable production levels in short time frames. However, a major drawback of this expression system is the negative impact of infection on cellular physiology and protein processing pathways. To avoid the lytic baculovirus infection cycle, stable insect cell lines using targeted gene integration based on the recombinase-mediated cassette exchange (RMCE) system have been developed by our group and can be considered as cost-effective candidates. The aim of this work is to extend this concept into optimal cell platforms directed for the production of enveloped VLPS pseudo-typed with membrane proteins of interest.

Experimental Approach:
The Influenza M1 and HIV Gag core proteins are used in an innovative RMCE-based strategy to obtain superior loci combining high expression with high recombination efficiency. Firstly, S9 and Hi5 genomic “hot-spots” supporting high expression of enveloped VLPs were tagged with eGFP-fused Gag or M1 proteins. A linker including a Flp recognition target (FRT) site was used to allow posterior removal of the marker gene from the particle through cassette exchange. This strategy allows to select from the beginning cells supporting consistent high expression of enveloped VLPS, avoiding expression and stability loss in traditional RMCE protocols. The resulting tagged populations were FACS-enriched with the highest eGFP-expressing cells and then submitted to targeted recombination with a second reporter cassette to select cells with high recombination efficiency.

Results and Discussion:
When comparing stable S9 and Hi5 populations expressing the fusion Gag- or M1-eGFP proteins, Hi5 cells seem to be better expressers of both core proteins. Gag localizes preferentially at the plasma membrane whereas M1 disperses within the cell. This was confirmed by significantly higher amounts of Gag-VLPs being accumulated in the culture supernatant in both cell hosts. Upon promoting Flp-mediated recombination in the tagging populations, cassette exchange was well-succeeded (showing that the FRT site composing the linker fusing the two genes does not impact recombination), allowing to recover cells tagged in loci supporting RMCE. We are currently evaluating the capability of both core proteins as scaffolds to display complex membrane proteins, such as GPCRs and Influenza HA proteins. Results of a strategy to improve M1 targeting to the plasma membrane and subsequent release of M1-VLPs will be presented as well. Overall, modular insect platforms are being constructed to be readily adaptable to produce a broad range of VLP-based vaccines and receptor display particles for drug screening or antibody discovery.

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DEVELOPMENT OF A NEW CELL LINE FOR STABLE AND CONTINUOUS LENTIVIRAL VECTOR PRODUCTION

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Background and Novelty:
Lentiviral vectors (LV) are an efficient tool for fundamental research and gene therapy since they integrate the
transgene on the target cell genome, allowing for long-term stable expression. In the past years, LV started to
be used in gene therapy clinical trials, slowly overtaking the γ-retroviral vectors. Its growing use is justified by
its unique ability to transduce both dividing and non-dividing cells and its safer integration pattern. Nowadays,
the major obstacle to the use of LV in gene therapy is their production at high titers. The transient production
systems are not ideal: are difficult to scale-up; the production is limited to 2-3 days; pose biosafety issues and
present batch to batch variability. A continuous production system, where a stable producer cell line allows for
the constitutive LV production for longer time period, may overcome the transient production disadvantages.
However, the development of this continuous production platform has been hampered by the cytotoxicity of
the LV protease.
Currently, only few truly LV packaging cell lines have been generated. However, the development of these cell
lines is very laborious and time-consuming, being the screening of a clone with a high Gag-Pro-Pol polyprotein
expression, one of the first limiting steps. In addition, these LV packaging cell lines are derived from a 2nd
generation LV packaging system and were generated by viral transduction of at least, one LV component,
raising safety concerns.
Within this context, our aim is to develop a new cell line for the stable production of LV with improved safety,
using a 3rd generation LV packaging system.

Experimental Approach:
Our strategy consisted in sequentially transfecting 293T cells with the four LV expression cassettes (Gag-
Pro-Pol, Rev, Envelope and Transgene). In order to avoid the LV protease cytotoxicity, a mutated and less
active protease was used. After each transfection, stable populations were selected, making use of antibiotic
resistance markers. Additionally, stable expression of the viral components was monitored by assessing the LV
productivity of the selected populations by transiently transfecting them with the remaining viral components.
The final population with all LV components will be screened with the single step cloning-titration method
(Rodrigues, A. F., et al (submitted)), a high-throughput screening tool enabling the identification of rare clones
with high productivity.

Results and Discussion
A stable population of 293T cells stably expressing the gag-pro-pol (with the less toxic protease) and rev
genes was successfully selected. Further optimization of gag-pro-pol expression cassette allowed the selection
of a population with a productivity of 1.7x10^6 I.P./ml, when transiently transfected with the remaining LV
components. We are now performing the transfections and population selections of the envelope and transgene
for further clone screening with the single step cloning-titration method.
This work shows that is possible to generate a cell line that stably expresses a LV gag-pro-pol gene without
using an inducible system or viral infection, increasing the safety standards of LV production.
CELLULAR SIGNALING PATHWAYS MODULATED BY INFLUENZA INFECTION IN HEK293 CELLS

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Background and Novelty:
The equivalent of 1500 influenza vaccine doses can be produced in a 1L bioreactor within 48 hours with HEK293 cells. The kinetics of production of H1N1 A/Puerto Rico/8/68 (H1N1 PR/8) in HEK293 cells has been previously characterized by our group. Two cycles of infection take place when cells are infected: a first round of virus is produced at 8 hours post-infection (hpi) which then infect cells again and lead to a second viral exit at 16hpi. Infection triggers a cascade of cellular responses which leads to cell death. Most studies focus on one pathway at a time, and aim at limiting the extent of the infection through the use of inhibitors. Our goal however is to increase viral yield and quality. The main objective is therefore to understand and manipulate the molecular events taking place at the cellular level upon influenza infection and replication, with particular attention to key time points corresponding to viral production and exit.

Experimental Approach:
Cells were infected with H1N1 PR/8 or H3N2 A/Aichi/8/68 (H3N2 Aichi). Samples were collected at 0.5 to 42 hpi, fixed, permeabilized and stored at -80\degree C until analysis. The levels of signalling molecules such as phospho-Akt (S473), phospho-mTOR (S2448) and phospho-Erk (Thr202-Tyr204) were measured using flow cytometry. Presence of intracellular influenza virus was assessed by staining hemagglutinin (HA), the main surface protein of influenza.

Results and Discussion:
Akt was highly activated and increased steadily between 15 and 24hpi with H1N1 PR/8, and to a lesser extent with H3N2 Aichi. The phosphorylation of mTOR was also increased during the first 24hpi but remained stable, and was similar for the two strains of influenza examined. Finally, ERK was initially activated by infection with H3N2 Aichi but not with H1N1 PR/8, and decreased at 24hpi with both strains. In all cases, kinase activation was the highest in HA-positive cells. These results suggest that signaling pathways are differentially modulated depending on the strain of influenza virus. The profile of activation of each kinase reflects different cellular events caused by viral infection. Akt was recently reported to be activated during viral replication and protein synthesis. Similarly, mTOR is solicited during protein synthesis. Erk is involved in export of viral ribonucleoproteins from the nucleus to the cytoplasm.

An understanding of the timing of these events will facilitate the selection of small molecules to control signalling pathways and we have first evidence that this will lead to an improved feeding strategy. For instance, viral yield was increased by one log when cells were treated with an Akt activator 15hpi. This time point was selected based on the profile of activation of Akt.

We have systematically investigated multiple signaling pathways using flow cytometry. Other important signaling molecules that will be examined include PKC, NFkB and P53. Based on the data thus obtained, a cocktail of small molecules can be prepared and added to the culture at the most appropriate time in order to improve vaccine production.
A POTENTIAL HDAC5 INHIBITING MIRNA ENHANCES PRODUCTIVITY IN CHO CELLS MAINTAINING PRODUCT QUALITY

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Valproic acid (VPA) acts as an unspecific inhibitor of histone deacetylases (HDACs) in mammalian cells. Treatment of biopharmaceutical production cells such as Chinese hamster ovary (CHO) cells with VPA has been demonstrated to enhance cell specific productivity. However, this effect is mediated by blocking cell proliferation as a result of a G1/S cell cycle arrest. Recently, it has been shown that MicroRNAs (miRNA) directly targets the histone deacetylase in mice, which may open a novel route to enhance productivity of recombinant cell lines bypassing the use of VPA. miRNAs are endogenous small non-coding RNAs that post-transcriptionally modulate gene expression. They are highly conserved across species. Due to the imperfect nature of miRNA target recognition a single miRNA is able to target multitudes of different genes thus controlling entire physiological pathways. In the present study we demonstrate that a specific miRNA might function as a modulator of histone deacetylase expression in CHO cells by specifically targeting HDAC5 and thereby enhancing productivity. The comparative approach re-evaluates effects of the unspecific HDAC inhibitor VPA.

Experimental Approach:
Suspension-adapted CHO DG44 cells stably expressing a secreted embryonic alkaline phosphatase (SEAP) or monoclonal antibody (mAb2) were routinely grown in SFM4CHO culture medium. Non-viral delivery of miRNA mimics or small interfering RNAs (siRNAs) was performed using ScreenFect®siRNA. Anti-mAb2 siRNA mix, CHO-specific cell death control siRNA as well as non-targeting, scrambled siRNA were used as controls. Analysis of soluble mAb-aggregates was performed by size exclusion HPLC. N-glycans were fluorescently labeled by reductive amination with 2-aminobenzamide and hydrophilic interaction chromatography was performed to investigate the glycosylation pattern. Cells were analyzed for cell cycle/ apoptosis by flow cytometry. RT-PCR was performed for mature miRNAs and for hdac5 expression analysis. Acetylation state of histone H3 was investigated by immunoblotting.

Results and Discussion:
A miRNA binding site is located in the coding sequence of mouse as well as of CHO HDAC5 mRNA. Employing a high content functional screening a miRNA was discovered to enhance recombinant expression of SEAP. Effects of miRNA mimics were comparable to anti-HDAC5-siRNA phenocopy including down regulation in HDAC5-mRNA and protein levels. miRNA-mediated HDAC knock-down as well as anti-HDAC5-siRNA or VPA treatment increased histone H3 acetylation. Stable overexpression of the miRNA in CHO induced a permanent HDAC5 down-regulation and increased product concentration in batch process. VPA also enhanced recombinant IgG expression in CHO-mAb2 due to cell cycle arrest and unspecific inhibition of HDAC. However, while miRNA did not alter product characteristics VPA treatment facilitated antibody aggregation accompanied by negatively affecting product quality in concentration dependent manner. The observed aggregation might be caused by intracellular mechanisms rather than direct VPA interaction with the antibody. While the VPA treatment induced alterations in the glycan profile of recombinant antibodies, overexpression of miRNA seemed to have no impact on N-glycosylation pattern of the expressed mAb. In contrast to VPA treatment or siRNA-mediated knockdown of HDAC5, ectopic expression of miRNA had no negative effects on cell growth or viability, highlighting the advantages of miRNA-mediated HDAC inhibition and supporting the potential of miRNAs for cell engineering.
EPIGENETIC REGULATION IN CHINESE HAMSTER OVARY CELL LINES

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Background and Novelty:
Chinese Hamster Ovary (CHO) cells are one of the main tools for recombinant protein production due to their “human like” glycosylation patterns. Beside genetic alterations, it is assumed that epigenetic regulation, such as histone modifications, can play a role in determining altered production characteristics. However, so far very little detailed data on epigenetic regulation in CHO cells is available.

Histone modifications are implicated in the regulation of gene expression, regulating transcriptional “on” or “off” states and influencing chromatin condensation and state. Modifications are mainly based on post-translational modifications occurring within the histone N-terminal tails and are responsible for structural changes in histones or their binding to DNA.

Experimental Approach:
Our main objective is the assessment of changes of histone modifications of CHO cells in culture. Due to the fact that antibodies are conserved between different species, we focus on the same six histone H3 modifications described in the International Human Epigenome Consortium. In humans, trimethylated H3K4 (H3K4me3) and acetylated H3K27 (H3K27ac) mark transcription start sites of actively transcribed genes, monomethylated H3K4 (H3K4me1) and H3K27ac mark active enhancers. Gene bodies of actively transcribed genes are associated with H3K36me3 and gene repression is associated with H3K9me3 and H3K27me3.

Chromatin immunoprecipitation (ChIP) is an established method to evaluate histone modifications. It uses a modification specific antibody to determine locations of a particular histone change. The different steps of the ChIP assay are cell fixation and crosslinking to stabilize protein-DNA interactions, chromatin shearing, immunoprecipitation, DNA purification and control of the immunoprecipitated DNA by Real Time PCR, followed by Next Generation Sequencing. Choice of genes to be used for control of successful precipitation is typically based on previous experiments and reports in the literature of results for the same cell type and culture conditions. As no previous experiments with CHO cell lines are available, we needed to explore patterns of histone modifications typical for CHO cells to enable verification of the protocol.

Results and Discussion:
We present an optimization of ChIP-seq for CHO cells, including a detailed protocol for optimal chromatin shearing conditions and selection of genes for quality control by Real Time PCR.

Using this protocol, dynamic changes in histone modifications were analysed in a CHO-K1 batch culture taking samples every 12 hours for an initial assessment of the contribution of epigenetic regulation on culture behavior of CHO cells.
ENGINEERING SELECTION STRINGENCY ON EXPRESSION VECTOR FOR THE PRODUCTION OF RECOMBINANT HUMAN ALPHA-1-ANTITRPYSIN USING CHINESE HAMSTER OVARY CELLS

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Background and Novelty:
We have previously shown that specific productivities can be improved when we increased selection stringency by destabilizing the selection marker through the addition of AU-rich elements (ARE) and murine ornithine decarboxylase (MODC) PEST region (Ng et al. 2007). Subsequently, an attenuated IRES element was used together with the PEST element to allow for high recombinant protein titer using stably amplified cell pools (Ng et al. 2012).
In this study, we evaluated the use of tandem PEST sequence, further attenuation of the IRES element, and codon-deoptimization of the dhfr selection marker, to further optimizing the strength of selection marker expression in CHO cells for the production of recombinant human Alphal-antitrypsin (rhA1AT), a serum protease inhibitor currently purified from human blood plasma as replacement therapy for patients who developed chronic obstructive pulmonary disease due to deficiency in the protein. Such vector combinations to attenuate translation initiation, protein elongation and protein stability for optimizing selection stringency have not been previously investigated. To our knowledge, there is also no report on high-titer production of rhA1AT in CHO cells, which is necessary for its manufacturability due to its high dosage requirement.

Experimental Approach:
7 expression vectors expressing rhA1AT were designed: The first vector set consists of pA1D, pA1Dp and pA1Dpp, which will allow us to validate the use of PEST element and tandem PEST elements in improving stable recombinant gene expression. The second vector set consists of pA1709Dp and pA1772Dp, which incorporated mutations described by Hoffman MA and Palmenberg AC (Hoffman and Palmenberg 1996) into the attenuated IRES (Bochkov and Palmenberg 2006; Gurtu et al. 1996). The third vector set comprised of pA1D* and pA1D*p, that incorporated a codon de-optimized dhfr selection marker to evaluate the use of codon deoptimization as a strategy to further reduce selection marker expression levels.

Results and Discussion:
pA1Dpp and pA1772Dp vectors gave further improvements in rhA1AT production when compared to pA1D and pA1Dp vectors, indicating that further selection marker attenuation can improve recombinant protein production. Using the pA1772Dp vector, we generated a cell pool that gave a maximum titer of 1.05 g/l of rhA1AT in an un-optimized shake flask batch culture using 2-step amplification till 50 nM MTX that took less than 3 months. Cell pools that gave maximum titers of 1.11 and 1.15 g/l from pA1772Dp and pA1Dpp vectors respectively, at 300 nM MTX were also generated.
Relative transcript copy numbers demonstrated that the transcription of rhA1AT and dhfr genes were correlated due to the IRES linkage, although the results also suggested that the protein expression were not solely dependent on transcript levels. Protein level analysis of dhfr showed that the strategies of further attenuating dhfr protein expression with the use of a mutated IRES and tandem PEST, but not codon deoptimization, were effective in reducing dhfr protein levels in these MTX amplified cell pools in suspension serum free culture.

References:
INCREASED ANTIBODY PRODUCTIVITY IN CHINESE HAMSTER OVARY CELLS THROUGH INDUCTION OF CHROMOSOMAL INSTABILITY BY CELL FUSION

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Background and Novelty:
Chromosomal instability is one of the key characteristics observed in Chinese Hamster Ovary (CHO) cells, which often occurs as a random event in an uncontrollable manner. This occasionally complicates the process of establishing stable and high-producing CHO cell lines. In our previous studies, we found that the abnormal increase of chromosome number linked to increased antibody production in adherent CHO DG44 cells. In this study, we analyzed the relationship between chromosomal variety and antibody production in CHO K1-derived IgG1-producing cells by inducing cell fusion in the attempt to artificially generate chromosomal variation within the cell line.

Experimental Approach:
Using CHO K1-derived IgG1-producing cells, we induced cell fusion via polyethylene glycol (PEG)-mediated method. PEG-treated cells were fused again for several times to increase the percentage of fused cells. Five cell lines were obtained from PEG-treated cells by single cell cloning using ClonePix 2. The chromosome number distribution of the cell lines was determined using fluorescent in situ hybridization (FISH). Specific growth and specific antibody production rates of each cell line were evaluated in batch cultures.

Results and Discussion:
The five cell lines obtained from PEG-treated cells showed distinct growth patterns and chromosome number distributions. Four out of five cell lines contain cells with high chromosome number (>41), three out of which showed final antibody concentration higher than unfused cells. The results revealed that the three high antibody-producing cell lines showed ambivalent chromosome numbers with high chromosome number (>41) and low chromosome number (<20) observed in the same cell line. This event was not observed in original unfused cells. The cell line which produced the highest antibody concentration showed the highest percentage of cells with low chromosome number (<20) while having cells with high chromosome number (>41). These results led to a new understanding of the association of chromosomal instability and antibody production. Cell fusion process is expected to contribute to the development of cell culture as well as construction of cell line in the future.

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EXPRESSION OF GLYCOPROTEINS WITH EXCELLENT GLYCOSYLATION PROFILE
AND SERUM-HALF LIFE FROM MODIFIED CAP CELLS

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Background and Novelty:
Due to their clinical importance, the development of therapeutic proteins has accelerated immensely over the past years. However, the development of recombinant therapeutic proteins, in particular for complex glycosylated proteins, is often held up by difficulties in obtaining glycosylation patterns similar to their counterparts extracted from human plasma. These differences in post translational modifications are often responsible for suboptimal pharmacological properties as for example reduced serum half-life, increased immunogenicity, or heterogeneity of the therapeutic proteins. We have found that the CAP system, based on immortalized human amniocytes, represents a protein production platform that can efficiently address some of these challenges. The system was specifically designed for successful production of complex glycosylated proteins with human-like post translational modifications.
C1 esterase Inhibitor (C1 Inh) belongs to the serpin superfamily. Its main function is the inhibition of the complement system to prevent spontaneous activation. The 500 aa protein is highly glycosylated with 7 predicted N-glycans and 8 predicted O-linked glycans. Plasma derived C1 Inhibitors (Berinert, CSL Behring and Cinryze, ViroPharma,) as well as recombinant C1 Inh derived from milk of transgenic rabbits (Ruconest, Pharming N.V.) are approved for the treatment of acute attacks in patients with hereditary angioedema (HAE). However, the recombinant product shows a dramatically reduced serum half-life in pharmacokinetic studies in comparison to the plasma derived counterparts. In this work we present our approach for the expression of recombinant glycoproteins, like human C1 Inhibitor (rhC1 Inh) with excellent pharmacological properties using modified CAP cells.

Experimental Approach:
We expressed rhC1 Inh from unmodified CAP cells as well as CAP-GO cells that have been specifically modified for the production of glyco-optimized proteins. PTMs were analyzed by immunological methods, protein chemistry, and MALDI-TOF. Finally serum half-life of CAP derived recombinant C1 Inh was determined by pharmacokinetic studies in rat.

Results and Discussion:
rhC1 Inh was expressed from all CAP cells with titers above 400 mg/L. Fully glycosylated full length protein without any signs of degradation was detected in all cases. However, rhC1 Inhibitor expressed from CAP-GO1 or GO2 cells showed more favorable glycan structures than protein expressed from unmodified CAP cells. More importantly, the serum half-life of rhC1 Inhibitor expressed by CAP-GO2 cells was significantly prolonged. Details on glycosylation and pharmacokinetic profiles will be presented. In conclusion, we have created a stable cell line expressing high level of recombinant human C1 Inhibitor with an excellent pharmacokinetic profile.
This new CAP-GO2 C1 Inh cell line will be of great value as for the first time it allows large scale production of C1 Inhibitor from a genetically modified cell line, ensuring economically priced unlimited supply of C1 Inh without the risk of transmission of human blood-borne infections.
GENERATION AND CHARACTERIZATION OF A SUPERIOR HOST
CELL LINE FOR BIOMANUFACTURING

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Background and Novelty:
Chinese hamster ovary (CHO) cells are the most widely used host for large scale production of recombinant therapeutic proteins exhibiting high productivities in the gram per liter range. Although these cells have been extensively characterized and optimized, a demand to further increase the performance towards higher productivity and clonal stability exists. Up to date, the clone selection process is mainly based on phenotypic screens like titer measurements and growth data instead of more reliable biomarkers. Recently, the genomes of Chinese hamster as well as of different CHO cell lines were sequenced, assembled and annotated. This new information gives the opportunity for functional analysis of the transcriptome and allows the identification of biomarkers for clone selection and targets for cell line engineering.

Experimental Approach:
Gene expression profiles of high and low monoclonal antibody producing CHO clones were analyzed. Based on these findings new cell lines were generated and their capability for cell line development and protein production was subsequently evaluated in depth.

Results and Discussion:
We discovered a unique gene pattern in transfected antibody producing CHO clones. New parental cell lines exhibiting such gene pattern showed increased productivities after transfection and significantly more high and stable producing clones were retrieved after single cell cloning, facilitating the screening efforts in order to obtain a comparable number of high and stable production clones.
DEVELOPING CELL LINES FOR INDUSTRIAL PROCESSES:
A SYSTEM BIOLOGY APPROACH

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Background and Novelty:
Early selection of that clones that better fit with the requirements for the future scale-up is a major technological challenge during development of mammalian cell culture processes, because the lack of validated predictors and straightforward methodologies that could render in a short time and with high efficiency cell lines that comply with all desired characteristics, i.e. high and stable expression level, ability to sustain high cell densities in protein free-medium, robustness to stress conditions as mechanical damages and limitations of some nutrients and oxygen. Development of screening strategies that integrate above mentioned characteristics has a great impact of further product development and scale-up.

Experimental Approach:
Several recombinant NS0 cell lines expressing different monoclonal antibodies and showing different pattern for growth and protein expression were studied.
Cell cloning was carried out by dilution method in 96-well plates. Expression stability studies were carried out in culture flasks by duplicates. Cell samples from each flask were seeded periodically by triplicate in 24-well cell culture plates and incubated for 10 days before to measure IgG concentration. Kinetic studies cultures were carried out by triplicate in spinner flasks. Samples were taken daily for cell counting by trypan blue exclusion method. IgG concentration in supernatant was tested by an anti-human IgG sandwich ELISA. Samples of cell population were fixed in 70% ethanol and relative level of intracellular antibody was determined using a FACScan flow cytometer using FITC-labeled mouse anti-human IgG.
Each of the best 30% of producing clones were transferred to six wells in 96-well culture plates and samples were taken at days 4th and 10th for testing antibody concentration and cell proliferation was determined using the AlamarBlue assay.

Results and Discussion:
To parameters obtained from characterization of several transfected NS0 cell lines with different growth and expression pattern the Principal Component Analysis and clustering methods were applied to allow the definition of key parameters for clone selection and the integral coefficient for selection (CIS). Moreover a methodology to select clones with high specific productivity or growth at 96-well plate scale was established. Following these criteria several clones of NS0 myeloma cell line expressing the anti-EGF monoclonal antibody Nimotuzumab were isolated in protein-free medium. The influence of different pre-selected phenotypes on overall process yields, measured as value of product integral, was evaluated by kinetic studies in spinners flasks and bench scale bioreactors, both in batch and continuous modes. During these studies growth parameters and metabolic yields for glucose, glutamine and lactate were determined and correlated with productivity. Further characterization was done attending stability of the expression using intracellular immunoglobulin measurement and robustness for nutrient deprivation conditions by the level of apoptosis induction. Clones with higher CIS showed also higher product integral when culture in bioreactor conditions, as was predicted from spinner flasks studies. This allowed us to propose an integrated selection methodology that includes specific expression levels, stability and cell growth pattern that could be implemented at bench scale and predict best clones in a shorter time schedule, compared with traditional approach.
CELL CULTURE MEDIA DEVELOPMENT FOR PERFUSION CULTURE OF RECOMBINANT NS0 CELL LINE USING A STATISTICAL DESIGN AND METABOLIC FLUX ANALYSIS

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Background and Novelty:
Culture medium optimization and characterization of cell metabolism had contributed to increase cell growth and specific productivity. In particular, strategies based on manipulation of signal pathways regulating cell growth and survival, energy-consumption and protein synthesis have been addressed. Use of continuous cell culture allows the establishment of well-defined steady states that is determinant to evaluate the relationship between the concentration of substances inside the bioreactor and biological reaction rates. Perfusion processes can render very high cell and product concentrations. However it’s necessary to keep a good physiological microenvironment by continuous addition of well nutrient balanced media and removal of by-products, allowing metabolic patterns that favor protein expression. In this way understanding mechanisms underlying enzymatic control of energetic pathways and metabolites transporters, could help to determine medium components that primarily influence in cell growth and specific productivity.

Experimental Approach:
A medium formulation capable to sustain protein and lipid free proliferation for NS0 myeloma cell lines expressing different recombinant antibodies was obtained. Screening of different medium components was done using a Plackett-Burman DOE approach. Kinetic studies of NS0 cell line expressing a humanized anti-EGFr Mab Nimotuzumab were done using stirred flasks and 2-4 L bioreactors in batch and continuous mode. Antibody concentration was quantified by sandwich ELISA. Intracellular IgG and apoptosis levels were detected by FACS. Glucose, glutamine and lactate concentrations were determined using an YSI analyzer. Levels of mTOR, Akt, eIF2α, cMYC, p53, TIGAR, p32, GLUT1, GLUT2, GLUT3, GLUT4, HK G6PDH, PFK, LDH, PYK, glutaminase and ALAT were detected by western blotting.

Results and Discussion:
Some aminoacids, one iron salt, four vitamins and ethanolamine were identified as important for cell growth and/or antibody production after several rounds of component screening. Specific consumption/production rates of aminoacids, glucose and byproducts were determined.
We decided to evaluate the supplement of insulin and different levels of glucose using continuous cell culture considering previous obtained results from our group, that show the relevance of insulin regulated pathways for this cell line under protein free medium conditions. Glucose concentrations in the feed ranged from 17 to 50 mmol/L. In glucose and insulin supplemented media specific production rate increased twice and fraction of apoptotic cells and eIF2α expression decreased. Insulin addition induces increase in glucose uptake, but not for lactate and LDH activity.
Insulin supplement also increases the expression of c-Myc and the activity of 3PGDH and PGK enzymes suggesting a more active glycolytic flux downstream 1,3-P glycerate and higher rate of serine generation for nucleotide and protein synthesis. An enhancement in glucose transporters p53, Akt, mTOR, G6PDH, PYK and ALAT was detected under these conditions. The presence of insulin induces an increase in the content of mitochondrial proteins such as ND1, COX IV, ATP synthase, p32 as well as the expression of PDH, GDH and CS. This relates to the increase in oxidative phosphorylation and contribution to a greater proportion of ATP synthesis.
A concentrated feed formulation was designed that allowed high density cell culture and productivity in perfusion cultures.
NOVEL CELL ENGINEERING OF THE UNFOLDED PROTEIN RESPONSE TO ACHIEVE EFFICIENT THERAPEUTIC PROTEIN PRODUCTION CELL LINE

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Background and Novelty:
Mammalian expression systems are currently the most widely accepted platform for the production of human therapeutics due to their ability to perform highly complex and human-like post-translational modifications. As with all manufacturing processes, an efficient production platform can significantly reduce time and lower manufacturing costs. A suitable production cell line exhibit physiological traits such as high specific productivity ($q_p$), rapid doubling time, high peak cell density and efficient metabolism. The emergence of a suitable production cell with all the aforementioned traits is an extremely rare event, as it requires all facets of the cells’ transcription, translation, secretion and metabolic efficiency are individually optimized and collectively synchronized into a system capable of high level protein expression. One of the major cellular bottlenecks thought to limit protein production in mammalian cells lies in the cellular translation and secretion capacity. The over-expression of complex recombinant proteins such as antibodies driven by strong viral promoters exert considerable burden in the Endoplasmic Reticulum (ER) and Golgi apparatus. The increase in ER stress triggers the Unfolded Protein Response (UPR) which can result in cell apoptosis and possibly eliminating cells with high uptake of the Gene of Interest (GOI). In this work, we sought to improve the probability of isolating suitable production cells through host cell engineering of the cellular translation and secretion machinery. We manipulated the ratio of XBP1 splice variants in CHO cells to overcome the negative regulatory effect of the unspliced XBP1 to produce a host cell line with increased expression of critical translation and secretion proteins.

Experimental Approach:
We created a plasmid encoding CHO spliced XBP1 followed by attenuated Internal Ribosomal Entry Site (aIRES) and GFP. The XBP1-GFP plasmid was stably transfected in suspension CHO cells and sorted using fluorescent activated cell sorting followed by confirmation of high spliced XBP1 ratio using qPCR. This host cell line was then used for comparative transient transfection studies with monoclonal antibody.

Results and Discussion:
The XBP1 host cell line exhibits increased expression of various chaperone and secretary vesicle proteins without increasing UPR associated apoptotic markers. It also demonstrated an 87.5% improvement in $q_p$ in transient production of monoclonal antibodies. Moreover, the improvement in $q_p$ was more profound with increasing amount of transfected antibody plasmid. The transfected XBP1 host cell also demonstrated an overall population shift towards being high producers, when compared to control non-engineered hosts. We observed 3-fold increase in the percentage of high producers with the XBP1 host cell line as determined using cell surface immunostaining combined with flow cytometry, this was also corroborated by the population medium shift from 1201 to 2902 Relative Fluorescent Units.
PRODUCTION OF AN ANTIBODY MOLECULE BY TRANSIENT GENE EXPRESSION IN INSECT CELLS

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Background:
Monoclonal antibodies and their fragments have been used for a variety of diagnostic and therapeutic applications. Novel antibody-based biologics are often selected from a large library of lead candidates. Hence, a high-throughput system for rapidly producing a large number of recombinant antibody molecules of high quality and in sufficient quantity is required. Insect cells have proven to be an excellent platform for the production of functional recombinant proteins. In the present study, the production of an antibody Fab fragment by transient gene expression in insect cells was investigated.

Materials and Methods:
The DNA fragments encoding the heavy chain (Hc; Fd fragment) and light chain (Lc) genes of an Fab fragment of a mouse anti-bovine RNase A antibody were separately cloned into the plasmid vector pHAneo. The pHAneo contained the Bombyx mori actin promoter downstream of the B. mori nucleopolyhedrovirus (BmNPV) IE-1 transactivator and the BmNPV HR3 enhancer for high-level expression. The Drosophila BiP signal sequence was employed upstream of the Hc and Lc genes. Trichoplusia ni BTI-TN-5B1-4 (High Five) cells were co-transfected with the resultant plasmid vectors using linear polyethyleneimine (PEI). When the transfection efficiency was checked, a plasmid vector encoding the enhanced green fluorescent protein (EGFP) gene was also co-transfected. After transfection, cells were incubated with a serum-free medium in static or shake-flask cultures.

Results and Discussion:
Transfection conditions were successfully optimized with flow cytometric analysis of EGFP expression in transfected cells. Western blot analysis and enzyme-linked immunosorbent assay (ELISA) of culture supernatants showed that transfected High Five cells secreted the Fab fragment with antigen-binding activity. Under optimal conditions, the production of more than 30 mg/L of Fab fragment was achieved in 6 days in a shake-flask culture. Transient gene expression in insect cells may offer a promising approach to the high-throughput production of recombinant antibody molecules.
CRISPR/Cas9 SCREEN OF CHO CELLS

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Background and Novelty:
Our ability to genetically precision engineer mammalian cells have improved dramatically over the past decade and in the past two years the price has dropped substantially largely due to the CRISPR/Cas9 technology. The CHO cell is currently being used to produce several highly valuable drugs, but current methods for establishing a good drug producing CHO cell line rely heavily on screening large numbers of clones to find candidates with both desired productivity, product quality, etc.
It would be of great benefit to start the drug producing cell line selection using a cell line engineered to exhibit certain desired phenotypes. Such engineering is likely possible by knocking out and/or over expressing a number of genes. Rational identification of such genes is possible to an extent using e.g. genome scale models or literature mining. But many cellular processes are only poorly or partially understood and thus difficult to rationally engineer.
To both improve our understanding of such processes and identify targets of interest have created a lentiviral guideRNA library against CHO genes.

Experimental Approach:
Using this guideRNA library we then subject the cells to various phenotype selection assays, harvest genomic DNA from the selected cells and perform targeted next generation sequencing to identify the guideRNA sequences which led to the improved phenotype.
The identified targets are then individually validated by creating knock out clones and performing the relevant phenotype assay on the individual clones.

Results and Discussion:
Targets identified in this way may then be recreated in a single cell line to create a much improved starting point for production cell lines.
A number of targets are specific for a specific drug, especially targets relating to quality, but several are universally useful or useful for a specific drug category.
CHARACTERIZATION OF HUMAN DIAMINE OXIDASE PRODUCED IN CHO

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Background and Novelty:
Human diamine oxidase (hDAO, E.C. 1.4.3.6), formerly termed histaminase and amiloride-binding protein 1 (ABP1), is encoded by the AOC1 gene and belongs to the group of copper-containing amine oxidases (CAOs). These enzymes are found in bacteria, yeast, plants and mammals and deaminate primary amines to the corresponding aldehydes, ammonia and hydrogen peroxide. Natural substrates of hDAO include histamine and polyamines like putrescine, cadaverine and spermidine. It is an important enzyme in the extracellular catabolism of exogenous or endogenous histamine. High expression levels of hDAO are detected in the gastrointestinal tract, kidney and placenta. In addition to increased absorption of histamine an impaired function, decreased production or competitive inhibition of hDAO are suggested to contribute to histamine intolerance. The enzyme is a homodimer of 170 kDa and is highly glycosylated. Each active monomer contains one Cu(II) ion, as well as a TPQ (topaque) cofactor. Each subunit carries two further cation-binding sites, presumably for Ca²⁺, with yet unknown function.

Experimental Approach:
So far, DAO from various sources, including human placenta, pregnancy plasma and porcine kidney, could only be obtained by cumbersome purification protocols. The heterologous expression of human DAO in insect cells, along with a detailed characterization of the enzyme was published by Elmore et al. in 2002. We present the heterologous expression of hDAO with human-like glycosylation in CHO cells and a three step purification protocol, comprising ammonium sulphate precipitation, hydrophobic interaction and size exclusion chromatography. With this, characterization of biochemical properties was performed, including ICP-MS analysis and phenylhydrazine titration to determine the metal content, as well as the content of reactive TPQ cofactor, respectively. Furthermore, MS analysis was conducted in order to verify sequence integrity of the protein, as well as to assess the glycosylation profiles. Circular dichroism spectra were obtained to characterize structural properties of the enzyme.

Results and Discussion:
Analysis of metal content using ICP-MS revealed 75% of active sites occupied by copper, the remaining mostly zinc. Phenylhydrazine titration confirmed that about 75% of DAO molecules contain reactive TPQ and are therefore active. Substrate specificity of recombinant DAO is in agreement with published literature. The amino acid sequence was found to be intact throughout the up- and downstream procedure. Three glycosylation sites were the highly complex type, with 2-4 antennae, and highly sialylated at the terminus. One site consistently was occupied by high-mannose type glycosylation.
Circular dichroism measurements at visible wavelengths resulted in a spectrum with two significant minima, typical for TPQ and copper bound to the enzyme. Analysis of secondary structures was performed by recording a CD spectrum in the far UV region. We found a 15.4, 24.3 and 60.3% distribution of alpha helices, beta strands and random coils, respectively. From the results obtained, we conclude that hDAO, recombinantly produced in CHO cells, is highly active, structurally intact and displays complex glycosylation patterns.
MEDIA AND FEED OPTIMISATIONS FOR CHO FED-BATCH PROCESSES USING A DESIGN OF EXPERIMENTS (DOE) APPROACH IN AUTOMATED HIGH-THROUGHPUT SINGLE USE BIOREACTORS

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Background and Novelty:
Chinese Hamster Ovary (CHO) derived cells are the most commonly used cell lines for the production of biopharmaceuticals. For the generation of high quality biotherapeutics in good yield, the selection of optimal growth media and the definition of suitable process parameters are important tasks and milestones. Still in this fast evolving market, time to production, flexibility and risk mitigation are capital elements for long term success. We have developed experimentally a plug and play, rapid and efficient method to systematically optimize process parameters and selection of optimal growth media and feed compositions.

Experimental Approach:
The experiments were comprised of a Mixtures Design DOE (Design of Experiments) approach using CHO cell lines in batch and fed-batch production processes. The optimization package described here includes a modular system that consists of employing defined media mixes, varying selected process parameters, and defining optimal feed compositions that were based on media and metabolite analysis. The DOE experiments were typically carried out in an automated single use stirred-tank bioreactor system with multiple closely monitored and controlled single-use bioreactors. We defined factors like media mixes and process parameters as well as responses like cell density and titer. The resulting mixture design, was imported in the DOE software, executed and analyzed. The DOE matrix was further performed with multiple design points to increase precision.

Results and Discussion:
The approach generated predictions for best medium to reach maximal peak cell density, protein production and quality attributes. A campaign carried out with this approach resulted in the definition of a unique medium tailored to a given CHO production cell line, a feed combination with a tested feeding strategy as well as optimized process parameters. The overall approach resulted finally in a more precise and transparent model and assisted in defining robust and efficient production processes.
SYSTEMS BIOTECHNOLOGY-DRIVEN GLYCOPROFILE ANALYSIS TO SUPPORT RATIONALE HOST CELL SELECTION

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Background and Novelty:
Within the last years we used different CHO host cell lines for biopharmaceutical process development. Comparison of product quality throughout several monoclonal antibody projects showed host cell specific variation of glyco-patterns between antibodies expressed by the two host subclones CHO-DG44-1 and CHO-DG44-2, respectively. Additionally, a newly established CHO-K1 host cell line showed differing glyco-patterns from the two CHO-DG44 cell lines. The molecular background in the hosts responsible for the product quality differences were not understood but had a significant impact on the process development chain. Recent sequencing and publication of several CHO genomes (Lewis, Liu et al. 2013) as well as the improvement of next generation sequencing and annotation tools as well as intracellular network construction tools now allows in-depth genomic/transcriptomic analysis and pathway understanding at the intracellular level.

Experimental Approach:
A global analysis was performed on glyco-pattern and gene expression level. Six different monoclonal antibody projects with over 550 analyses were reviewed concerning their glyco-pattern distribution based on ESI-MS and HPLC data. Additionally, nearly 200 RNAseq gene expression data were used for a pathway-oriented analysis of the glycosylation-associated transcripts according to Xu et al. (Xu, Nagarajan et al. 2011). Gene expression levels were compared between the three potential host cell lines as well as for host versus producing cell line.

Results and Discussion:
Global glycopattern analysis revealed a significant difference between three host cell lines when expressing mAb. Interestingly, the CHO-K1 and CHO-DG44-1 cell line seemed to be closer with respect to their glycoprofiles than the DG44 cell lineages. Global differences can be seen in the relative glyco-patterns. The phenotypic characterization of the host cell lines will allow to rationally select the optimal host for production of a given biologics in future projects. According to Xu (Xu, Nagarajan et al. 2011) 301 transcripts are correlated with glycosylation. We identified with our new transcriptomics pipeline 278 in our database. In contrast to Xu, 61% (vs 53 %) of these genes showed detectable expression in all hosts. Additionally, we saw significant gene expression for Mgtat3 (GNT-III), Mgtat4b (GNT-IV), Cmah (CMP-Neu5Ac Hydroxylase) and St6gal1 (Sialyltransferase 1). However, this expression was host cell specific and depended on whether a mAb was expressed or not. For example, Sialyltransferase 10 (St3gal6) and B4galnt6 (β 1,4-galactosyltransferase 6) could only be observed in the CHO-K1 while Cmah was only detectable in CHO-DG44. Interestingly, St6gal1 was switched-on in mAb producing CHO-DG44 cells but at a low level. This explains why normally only relatively low sialylation is observed with products produced in this cell line, and, since both the Sialyltransferase 10 and the CMP-Neu5Ac Hydroxylase activities are needed for constitution of with Neu5Ga c sialic acid glycosylated antibodies, by lacking of the St3gal6 (CHO-DG44 cells) or the Cmah gene (CHO-K1 cells) mainly the non-immunogenic Neu5Ac sialic acids are predominant in CHO cells. The used approach could help to identify new marker genes and targets for genetic engineering or generate predictive models for product quality by looking at pathways and networks.
ASSURING CLONALITY OF CELL LINES FOR BIOPHARMACEUTICAL PRODUCTION

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Background and Novelty:
Several guidelines relevant for biopharmaceutical production require that a production cell line be clonal, i.e. derived from one single cell precursor. Recently, the methods used to generate single cell clones in the process of development biopharmaceutical production cell lines, and the measures taken to document monoclonality of these cell lines, have come under intense scrutiny from health authorities, notably FDA. Since the efficiency of generating monoclonal cell lines varies strongly depending on the method used, and the settings applied, statistical approaches describing the probability of clonality, as well as appropriate control strategies, have become increasingly important for successful development of biopharmaceuticals. Here, we describe different approaches for generating single cell clones used throughout the biopharmaceutical industry, and their respective characteristics with respect to probabilities for generating single cell clones and the possibilities for monitoring of clonality. We discuss approaches for the validation of a state-of-the-art one-step cloning procedure which is capable of generating single cell clones with very high probability. Finally, we describe a control strategy applicable to cell banks of products at later development stages.

Experimental Approach:
A one-step cloning procedure, using flow cytometry for single cell deposition, followed by fluorescent and bright field imaging, allows for the generation and semi-automated monitoring of thousands of clones. Using two-color staining, the capability of this system to assure the deposition of single cells by doublet discrimination was evaluated. Also, the sensitivity of detection of non-clonal cell lines by imaging was validated. For existing cell banks, a control strategy consisting of extended genetic characterization of the production cell line at different cell ages and under different cultivation conditions can be applied. Use of very sensitive Southern blot methods with a well characterized level of detection, coupled to statistical evaluation of the likelihood to detect clonality and modelling of growth behavior of non-clonal populations, allows for the characterization of cell lines as clonal to a very high confidence level.

Results and Discussion:
The two color staining method allowed to demonstrate that typical doublet discrimination techniques robustly assure deposition of single cells, when used in combination. A well characterized set of parameters used for single cell deposition as well as appropriate sample handling can result in >99.95 % single cells. The use of imaging for the confirmation of clonality, especially in combination with optimized fluorescence staining and detection, further assures the detection of non-clonal cells. The genetic characterization of existing cell lines can also be used as a potential control strategy to assure clonality. Using very sensitive Southern blot techniques, non-clonal cell lines can be identified even when as little as 0.4% contamination is present. Coupled to the modelling of cell growth of mixed populations, and applying poisson statistics, this approach may allow for addressing concerns about the clonality of a given master cell bank.
IDENTIFICATION OF CHO HOST CELL LINES WITH DESIRABLE CHARACTERISTICS
VIA DIRECTED EVOLUTION

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Background and Novelty:
The keys to developing biomanufacturing processes which are efficient, robust and of high quality start during
cell line and process development. It is therefore imperative that the host cell line, vector and the process to
isolate and identify the best clonal cell line are of the highest quality. Here, we describe work undertaken at
FUJIFILM Diosynth Biotechnologies to develop host cell lines, with desirable characteristics, using a directed
evolution approach.

Experimental Approach:
Initially, a CHO host cell line was adapted from serum containing to chemically-defined, protein-free media.
The subsequent use of an improved sub-culture regime during continuous culture of the cells allowed a
reduction in doubling time from ~27 hours to ~21 hours.
Directed evolution approaches were then used to develop host cell lines with improved growth and productivity
characteristics. Such approaches are iterative and utilise the application of selective pressure to a system
to identify variants with desirable characteristics. Methods employed during this process at Fujifilm were:
continuous culture in altered subculture regimes; enrichment of cells with extended viability via flow cytometry
assisted sorting; cloning; and growth of cells in chemostat culture.

Results and Discussion:
During a series of assessments, the initial 77 host cell lines identified were reduced down to 25, then 5, 2 and
finally 1. Data from these assessments, which included studying parameters such as growth and metabolic
characteristics as well as MAb expression capability, will be presented. Observations from the assessment of the
25 cell lines included identification of cell lines which achieved up to a 3-fold increase in MAb expression from
transient transfection or a 2 fold increase in viable cell density at sub-culture when compared to the original
host. Interestingly, four of the top five ranked cell lines for this latter characteristic came from the chemostat
culture. However, when assessing the 25 cell lines for transient MAb expression, none of the chemostat origin
cell lines were ranked in the top five. This demonstrated the importance of assessing multiple characteristics in
the potential new host cell lines.
REDUCING BY-PRODUCTS ACCUMULATION IN FED-BATCH PROCESSES THROUGH METABOLIC ENGINEERING

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Background and Novelty:
The ability to generate high yield of therapeutics at lower cost remains a critical challenge for the biopharmaceutical industry, whether for supporting the development of new antibodies or for responding to market needs. The productivity of cell culture processes has been greatly enhanced through the development and application of fed-batch strategies. However, in such processes, lactate and ammonia accumulation in the culture medium over time can have detrimental impacts on cell growth and product quality. Continuous cell lines typically exhibit an inefficient metabolism whereby most of the pyruvate derived from glucose is diverted to lactate and only a small percentage is incorporated into the TCA cycle. The mitochondrial pyruvate carboxylase, which catalyzes the conversion of pyruvate into oxaloacetate, is one of the key enzymes at the junction between glycolysis and the TCA cycle. Previous metabolic flux analyses of CHO cells have revealed that only a minor fraction of the pyruvate pool is metabolized via this pathway. Thus, further process improvements can be expected by combining the application of rational fed-batch strategies with targeted metabolic engineering of cells to reduce waste metabolite accumulation.

Experimental Approach:
In this work, a recombinant CHO cell line producing an antibody was further genetically modified with the insertion of a cytoplasmic yeast pyruvate carboxylase (PYC2) gene. Following selection, individual clones were isolated by limiting dilution and screened for their cytoplasmic PYC2 expression levels. Positive-PYC2 clones were initially cultivated in shake flask to assess their growth, nutrients consumption, waste formation and antibody production kinetics. Isotopic tracer studies were also performed to characterize the efficient metabolism exhibited by the transformed cells. Cells were then cultivated in a 2 L bioreactor and a dynamic feeding strategy was applied using concentrated nutrient solutions.

Results and Discussion:
In contrast with the parental cell line, all the clones exhibiting strong PYC2 expression consumed lactate during the stationary phase until near depletion of this metabolite from the culture environment. This metabolic shift was found to occur consistently in all commercial culture media tested. The decreased lactate production was also shown to significantly delay the acidification of the medium in uncontrolled flask cultures. Interestingly, the expression of the PYC2 gene was also associated with at least a 30% reduction in final ammonia concentrations. Similar to shake flask cultures, PYC2 clones cultivated in fed-batch bioreactor show an alteration of the lactate metabolism, characterized by an increased consumption of lactate. As a result, PYC2 cells showed a prolonged exponential growth phase leading to a 2-fold increase in maximum cell concentration and an increase in final product titer.
INVESTIGATION OF FACTORS INFLUENCING RECOMBINANT HUMAN BMP2A EXPRESSION IN MAMMALIAN CELLS

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Background and Novelty:
Human growth factors have an enormous therapeutic potential. Several of them are in clinical use for years. We focus on one human growth factor, the bone morphogenetic protein-2 (BMP-2). Its capacity to induce de novo bone formation endows the protein a high therapeutic potential. This protein is synthesized in vivo as pre-pro-proteins, with the pre-sequences conferring translocation into the secretory ER. The recombinant expression of this growth factor was investigated in Chinese Hamster Ovary (CHO-K1) and Human Embryonic Kidney (HEK) cells after transient transfection as well as in stable clones established in CHO-K1 cells. In both cases, a bicistronic gene construct allowing for the concomitant expression of the hBMP2A and the enhanced Green Fluorescent Protein (eGFP) was used. First observations showed that growth rates and viabilities of the rhBMP2A-producing cells were similar to those of the parent cell line, while entry into the death phase of the culture was delayed in case of the recombinant cells. Additionally, our data suggest a possible influence of the expressed hBMP2A on the death phase of the BMP2A-expressing clone, showing a significant improvement in viability towards the end of the culture. Here, we discuss factors contributing to the low productivity observed for rhBMP2A in CHO-K1 cells focusing in particular on product and production stability as well as the effect of possible interactions between the product and the producing cells.

Experimental Approach:
CHO-K1 and HEK cells were transfected with the vector phBMP2A-IRES-EGFP using polyfection. Stably transfected clones were initially selected, screened by limited dilution and adapted to suspension culture. Cell number and viability were evaluated by trypan blue staining. Concentrations of rhBMP2A were determined by ELISA. The EGFP expression was assessed by fluorescence measurements. The mRNA expression were analysed by qRT-PCR and the intracellular development of the pre-pro-protein/pro-protein/mature protein was followed by western blot. The protein stability was investigated in vitro using commercially available recombinant hBMP2A produced in E. coli and in CHO. In that case, focus was put on pH and temperature as well as on proteases influences. For the latter, previously conditioned medium was also considered.

Results and Discussion:
Data presented in this contribution showed that for both cell lines, transiently or stably transfected, in spite of the bicistronic construct, molar expression ratios between rhBMP2A and EGFP were highly disproportionate. Moreover, while EF GFP was produced consistently throughout the cultivation, rhBMP2A concentrations in the culture supernatans peaked and then declined steeply. Possible reasons for these differences are discussed in terms of transcription/translation efficiency, mRNA/protein stability and processing. Our results showed that changes in the secretion pattern during the culture development as well as instability of the protein in the culture medium could be responsible for the drop in rhBMP2A titre observed. Finally, investigation of the putative interaction of the produced hBMP2A with the producer cells, which incidentally would also cause a “stripping” (adsorption / internalisation by the cells) of the product from the culture supernatant and thus further contribute to the low product titre are presented.
CATHEPSIN D IS NOT THE DOMINANT FACTOR MEDIATING ROS INDUCED APOPTOSIS IN A RECOMBINANT ENZYME PRODUCING CHO CELL LINE

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Background and Novelty:
Chinese hamster ovary (CHO) cells are used in the commercial production of recombinant lysosomal enzymes for enzyme replacement therapy. The production of such recombinant enzymes can disrupt normal lysosomal function eventually leading to lysosomal storage of incompletely processed substrates, similar to lysosomal storage phenotypes observed in human patient cells. Accumulation of storage material can cause lysosomal membrane permeability (LMP) leading to release of lysosomal enzymes into the cytosol. Cathepsin D, an aspartic lysosomal protease, when released into the cytosol due to LMP has been shown to induce apoptosis in primary cells and several cell lines. We set out to investigate if the cytosolic activity of cathepsin D plays a dominant role in inducing apoptosis in CHO cell lines. We treated cells with hydrogen peroxide (H$_2$O$_2$) as a controllable source of reactive oxygen species (ROS) to generate LMP in three related cell lines: the parental CHO cell line, a production cell line for a recombinant enzyme, and a null cell line transfected with the blank plasmid without the recombinant enzyme gene.

Experimental Approach:
We used double staining with PE Annexin V and 7-AAD to differentiate cell populations with apoptotic and necrotic phenotypes. The CHO production cell line and the parental cell line were stained at 2 hour intervals during treatment with H$_2$O$_2$ and analyzed using Flow Cytometry. Lactate Dehydrogenase (LDH) in the cell supernatant was measured to assess cell lysis. Immunofluorescent imaging using lysosome associated membrane protein 2 (LAMP 2) and cathepsin D antibody were used to detect LMP and the translocation of cathepsin D from the lysosomes to the cytosol at 1 hour intervals during H$_2$O$_2$ treatment. To inhibit cathepsin D activity in the cytosol, cells were pre-incubated with Pepstatin A before H$_2$O$_2$ treatment. The cultures were tested for viability by Trypan Blue and for apoptosis phenotypes using PE-Annexin V staining. Cathepsin D expression and inhibition were verified in cell lysates by Western Blotting and cathepsin D activity assay.

Results and Discussion:
Our results demonstrate that LMP in recombinant CHO cells can be induced by ROS, causing cathepsin D to be released into the cytosol. Additionally, while all three cell lines suffer H$_2$O$_2$ incubation dependent lysosomal membrane damage, the resistance of the three related cell lines to ROS is significantly different. Our study revealed that cytosolic cathepsin D activity was not the dominant factor inducing apoptosis in all CHO cell lines upon exposure to H$_2$O$_2$. It was only the null cell line which showed a cathepsin D dependent apoptotic phenotype. Western blotting revealed a difference in the activation of cathepsin D between the parental and production cell lines; in the production cell line cathepsin D exists predominantly in the pro enzyme form. Overall, our study revealed that there were significant differences in resistance to oxidative stress between three related cell lines. This finding implies that parental or null cell lines may not accurately predict characteristics of recombinant protein producing CHO cell lines.
CELL LINE DEVELOPMENT STRATEGY FOR MONOCLONAL ANTIBODIES ENGINEERED FOR SITE-SPECIFIC ANTIBODY DRUG CONJUGATES

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Background and Novelty:
Antibody drug conjugates (ADCs) have become very popular as new targeted cancer therapeutics. However, conventional ADCs, where lysines or cysteines are non-selectively used as conjugation sites, are heterogeneous. As a result, ADCs produced in this way are suboptimal in terms of efficacy and safety. Therefore, the site-specific antibody drug conjugates have been introduced to overcome drawbacks of the conventional ADCs. One of the approaches to generate homogeneous is to introduce conjugation sites into the antibody for site specific conjugation. These site-specific ADCs, using engineered antibodies, improve pharmacological properties and therapeutic index of drug products.

Experimental Approach:
Cell line development (CLD) for the engineered antibodies requires modification of the traditional CLD protocol because of additional feed of a supplement to media during the cell culture process. We have successfully developed a CLD procedure using ClonePix for such engineered antibodies.

Results and Discussion:
Our modified CLD method resulted in improvement in the production of these engineered antibodies. As a result we were able to generate cell lines with acceptable productivity, stable expression, and desirable product quality, which are critical for clinical and commercial application of the ADCs. Here we will share data from our development work and demonstrate how we improved productivity through cell line development.
ASSESSING CLONALITY OF PRODUCTION CELL LINES VIA HIGH-RESOLUTION IMAGING

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Background and Novelty:
Demonstrating that a production cell line originated from a single clonal progenitor is a regulatory requirement prior to commercial approval. Most approaches to meet this requirement rely on theoretical calculations, involving probability distributions, cell plating densities, and numbers of colonies observed. Based on the specifics of the subcloning procedure, however, a 2nd round of cloning is typically also needed to provide conservative assurance of clonality. Unfortunately, this 2nd round of cloning is laborious and adds up to three months onto cell line development timelines. In an attempt to streamline this process, we have evaluated the use of the Cell Metric to provide high-resolution, single cell images that can be used to prove clonality with just one round of subcloning.

Experimental Approach:
Initial tests using our CHO cell lines demonstrated that the Cell Metric yielded high quality images, capable of easily discriminating cells from debris present in both the culture and the plate. Subsequent optimization work involving inoculation density and culture media studies were performed to identify conditions favoring growth of a colony from a single cell in each well. In the course of this work, we observed a considerable amount of cell migration within each well on a day-to-day basis when using liquid media. As a result, we shifted our focus to plating in the cells in a semi-solid methylcellulose media.

Results and Discussion:
Using the Cell Metric with our optimized methylcellulose plating method, we were able to clearly identify single cells within a well and track their growth at the same location until the colony reached confluence. In this manner, the entire history of candidate production cell lines could be documented and traced back to a single clonal progenitor. Taken together, our evaluation strongly suggests the Cell Metric is capable of providing direct evidence of monoclonality with one round of subcloning, potentially reducing cell line development timeline by up to three months.
DEVELOPMENT OF A NOVEL, HIGH-YIELDING CHO EXPRESSION PLATFORM (APOLLO™)
THROUGH A SYSTEMATIC APPROACH WITH ACCELERATED CELL LINE DEVELOPMENT
AND HIGH-THROUGHPUT MEDIA AND FEED DEVELOPMENT

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Background and Novelty:
Chinese Hamster Ovary (CHO) cells are used extensively in the biopharmaceutical industry to produce
recombinant therapeutic proteins. Performance of CHO cell culture expression systems, including cell growth,
volumetric productivity, and product quality, have been dramatically improved through the rapid evolution
of emerging technologies. In this presentation, we will introduce a novel CHO expression platform, Apollo™,
which was recently established at Fujifilm Diosynth Biotechnologies. The Apollo™ platform includes a new
CHO DG44-derived host cell line, optimized DHFR expression vector and recombinant cell line development
(CLD) process, chemically-defined (CD) and protein-free (PF) platform basal medium and feed, and cell
culture bioreactor process. Using the Apollo™ CHO expression platform, we are able to achieve antibody titer
of ~ 4.4 ± 0.2 g/L on day 14 (n=7) and > 5 g/L with extended cell culture duration, with acceptable product
quality attributes related to glycan structures, aggregates, and charge variants.

Experimental Approach:
Through implementation of directed evolution approaches, we developed a CHO DG44-derived host cell
line with improved growth characteristics and expression capability. In addition, state-of-the-art screening
technologies have been introduced into the recombinant CLD process. These include the use of a fed-batch
shaken multi-well plate system to enable shortened timelines whilst allowing better decisions on which cell lines
to move forward for clone-selection confirmation in an ambr15™ microbioreactor system. To complement the
CLD process, we used a strategic, high-throughput, DOE-based development approach to develop Apollo™
platform basal medium and feed. BioMeck and TomTec robotic automation systems were used to maximize
study throughput in 96 deep-well plates (DWPs) covering thousands of media and feed testing conditions. With
DOE-based methodology, a sequential development strategy was designed to allow continuous optimization
based on Multi-Variant Analysis (MVA) by leveraging media formulation knowledge and Spent Media Analysis
(SMA) on key biochemical nutrients (i.e. amino acids, vitamins).

Results and Discussion:
With the Apollo™ CHO expression platform, we have achieved mAb titer of ~ 4.4 ± 0.2 g/L on day 14 (n=7)
and > 5 g/L in extended cell culture duration in bioreactor processes, with product quality within acceptable
ranges. In addition to the superior performance of the Apollo™ CHO expression platform, the efficient
and streamlined process flow of the Apollo™ platform, including recombinant CLD and upstream process
development, enables rapid delivery of Toxicology and Phase 1 clinical materials in approximately 15 months
(gene to Phase 1 GMP biomanufacturing).
A PART OF LONG NON-CODING RNA, WHICH IS ABNORMALLY OVEREXPRESSED IN HIGH-TITER CELLS, CAN IMPROVE CELL GROWTH AND HAS EFFICACY IN TRANSGENE CO-OVEREXPRESSION

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Background and Novelty:
Our CHO cell engineering strategy has shown great potential for improving the mAb titer of CHO cells. However, we believed that some important nuclear events that could be exploited to further improve CHO cells still existed because our studies revealed a gain in resilience in some mAb-producing CHO cells with respect to transgene overexpression.

Experimental Approach:
Therefore, we looked for a polyadenylated RNA transcript that was abnormally expressed in the high-titer cells. A Mouse Genome 430 2.0 array identified the transcript as a complementary sequence of the 3’ non-coding region of mouse NFKBIA mRNA. NFKBIA is an important regulator of the transcription factor NFKB, a positive regulator of cell growth. Since NFKBIA suppresses NFKB function, inhibition of NFKBIA by overexpression of the long non-coding RNA (lncRNA) might further enhance cell proliferation. Because we found a region in the lncRNA that was predicted to influence cell growth, we genetically modified distinct CHO cell lines to overexpress this part of the lncRNA.

Results and Discussion:
The resulting overexpression strains had increased proliferation, with the doubling time of the CHO-K1 host cell line reduced from 21.6 to 16.4 hours. Furthermore, overexpression of the partial sequence still functions as an antibody production enhancing sequence in mAb-producing DXB11 cell lines. In this presentation I will illustrate the efficacy of lncRNA. Although many unexpected functions probably still exist, overexpression of the partial sequence makes some proteins co-overexpressible.
CELL LINE GENERATION: RELYING ON TRICKS OR TOOLS OF THE TRADE?

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Background and Novelty:
Within the biopharmaceutical industry, early understanding and development in cell line generation offer leads to significant increase in product titer as we move down the manufacturing pipeline. However this process of forcing a cell to manufacture the protein of interest in large quantities is often a randomised process, almost a “black-box” approach. While industry know-how remains a critical aspect of this process, a number of tools have streamlined this “black-box” approach. The National Biologics Facility, situated in Brisbane, Australia, have developed its cell line generation platform to include the use of a ClonePix FL (Molecular Devices) to enhance current processes in cell line generation (CLG). The platform involves a single step method from transfection to clonal isolation.

Experimental Approach:
The investigation examines a comparison between the traditional approach to CLG; which is through the generation of a resistant cell pool followed by a clonal isolation method, and the modified cell line generation platform used by the facility; which combines selection and clonal isolation into a single-step process. Here we use a clinical relevant mAb as the protein of interest for this comparison in CLG. Briefly, an optimised method was used for the transfection of the CHO-XL99 cells, followed by a single recovery and selection step in a semi-solid growth environment. Clones from the semi-solid matrix are then isolated based on presence of mAb expression indicated by the level of a fluorescently labelled secondary antibody. The top 5 clones from both CLG approaches were evaluated using a standard fed-batch process.

Results and Discussion:
The single step approach was successful in identifying positive clones expressing the mAb of interest. In terms of clone survival or number of “pickable” colonies from the semi-solid matrix, the two methods were equal in survival rates. Following the clonal isolation process, expression study of top clones from both approaches were able to reach g/L quantities in volumetric productivity. By introducing semi-solid cloning at an early time point during the stages, there is a more defined and structured platform for CLG. The generation of a resistant pool or mini-pools represent a common practice in industry as the resistant pool would provide a basic understanding of the expression levels expected from the final clone. However with such complexity within the resistant pool, it is difficult to acquire any other information critical to process development such as cell growth, metabolism or clonal stability. Here we provide an alternative view is CLG without the need of deriving a resistant pool. As illustrated here, a more directed and robust approach with the right tools would achieve similar results with further reductions in timeline.
PROTEOMIC DIFFERENCES IN RECOMBINANT CHO CELLS PRODUCING TWO SIMILAR ANTIBODY FRAGMENTS

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Background and Novelty:
CHO cells were isolated in the late 50ies and have been extensively investigated ever since. They are commonly used to produce recombinant proteins and today they are the most frequently used mammalian hosts for the production of biopharmaceuticals. To overcome unfavourable features of CHO cells a lot of effort is put in cell engineering. ‘Omics studies investigating elevated growth rate and specific productivities as well as extracellular stimulus have already revealed many interesting engineering targets. However, it remains largely unknown how physicochemical properties of the recombinant product itself influence the host cell.

Experimental Approach:
In this study we used label-free proteomics to investigate product specific proteome differences in CHO cells producing two very similar antibody fragments. We established recombinant CHO cells producing the two antibody fragments 3D6- and 2F5-scFv-Fc. We applied three different vector strategies for transgene delivery, picked two clones each for label-free proteomic analysis and investigated three consecutively passaged cell pellets. Raw data of Nano LC-MS/MS were compared in several sample combinations to gain insight into different aspects of proteome changes caused by overexpression of two different heterologous proteins.

Results and Discussion:
We identified several proteins that were statistically significant differentially expressed between the two groups of 3D6- and 2F5-scFv-Fc producers, including proteins associated with folding and apoptosis. This study suggests that not only the levels of specific product secretion but as well the recombinant product itself has a large impact on the cell’s proteome.
FUNCTIONAL IMMORTALIZATION OF PRIMARY CELLS

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Background and Novelty:
A major limitation of current research is the shortage of physiologically relevant cells. To generate cells in sufficient numbers in vitro cell expansion is an attractive alternative. Cell expansion can be achieved upon expression of immortalizing genes. However, the establishment of new cell lines is unpredictable and conventional immortalization regimens lead very often to a drastic alteration of the cell physiology.

Experimental Approach:
We developed a defined immortalization technology allowing the efficient and reproducible establishment of novel cell lines. This approach was employed to establish cell lines from primary cells derived from seven different species. Importantly, novel cell lines were generated from different primary cell types within two to three months (e.g. endothelial cells, chondrocytes, fibroblasts, intestinal epithelial cells, macrophages).

Results and Discussion:
The resulting cell lines are immortalized as they can be cultivated for more than 100 cumulative population doublings, show a robust proliferation and can be frozen/thawed without any viability loss. Functional characterization demonstrated that the established cell lines retained the expression of cell type specific marker proteins as well as their specific functions. Importantly, this phenotype was stable throughout the whole cultivation period. We envision this immortalization system to provide physiologically relevant cells in sufficient numbers needed e.g. for drug discovery, ADME/Tox testing and in the long term for regenerative medicine approaches.
NEW TOOLS FOR THE ANALYSIS OF AMBR ONLINE DATA TO SUPPORT BIOREACTOR UPSCALING CAMPAIGNS

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Background and Novelty:
The ambr bioreactor, a new advanced bioreactor system, mimics the characteristics of classical bioreactors at microscale by using disposable micro-bioreactor vessels controlled by an automated workstation. These bioreactors can be run in parallel and can process and evaluate up to 48 bioreactor experiments simultaneously in a controlled manner. This ability to have higher throughput at a cost and manageability not considered before promises to revolutionize the way cell line development, process optimization and upscaling studies are performed. However, ambr bioreactors produce vast amounts of online and offline data (e.g. pH, O2, metabolic data) that need to be stored, tracked, aggregated, visualized, and statistically analyzed. Therefore, data analysis is recognized as a major bottleneck in systematically utilizing ambr data for bioreactor upscaling campaigns. In addition, there are challenges due to the large number of biomaterials to be tracked, and the requirement to integrate with relevant experimental context (e.g. fermentation protocols, media recipes, bioreactor control parameters).

Experimental Approach:
Here, we report on the development of a new system for analyzing ambr-based time-series data, together with relevant data on cell line history and experimental protocols. For this, we have developed new processing engines to gather, analyze, and visualize all relevant ambr online and offline data, based on a scalable database that stores all relevant data (e.g. expression condition, productivity, metabolic data, product quality such as aggregation, glycosylation profiles). A special emphasis of our work has been on the development of new tools for identifying critical correlations in bioreactor time series data.

Results and Discussion:
We will show how our system can be used for optimizing cell line development (e.g. selection and stability) and for identifying optimized media feed strategies and expression conditions (e.g. via DoE methods). Moreover, we will present analysis examples of bioreactor performance monitoring (i.e. fault detection), early assessment of productivity, and correlations with early cell line selection criteria. Finally, we will illustrate how the platform can be used to predict the behavior of cell lines and bioprocess protocols when upscaling to larger bioreactor volumes.
TOOLS FOR EXPRESSION SYSTEM OPTIMIZATION & BIOPROCESS DEVELOPMENT

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Background and Novelty:
The development of optimized protein expression systems and processes is key for the successful production of biologics for R&D purposes as well as for large-scale product manufacturing. Optimizing expression systems requires various disciplines and laboratories to work in a division-of-labor environment, exchanging biomaterials and data between molecular biology, cell science, engineering, assays and analytics, and bioprocess development groups.

Experimental Approach:
Here, we present a novel data management and analysis platform for vector development, cell line development, cell line engineering, protein expression optimization and bioprocess development. This package is part of a dedicated data management and analysis solution for biologics R&D, which is the result of multi-year development projects with leading biopharmaceutical corporations. A central database enables consistent storage and referencing of all relevant biomolecules (proteins and DNA, both antibodies and non-antibodies), biomaterials and product characterization data that arise from protein production activities. The system also includes unique in silico cloning tools that greatly facilitate vector design, and reporting and analysis tools for bioprocess development, among others.

Results and Discussion:
Here, we present concrete use cases including biomolecule registration, vector optimization, cell line development biomaterial registration and bioprocess development.
OPTIMAL SELECTION OF CELL LINES OF THERAPEUTIC ANTIBODIES
BY CONSIDERING N-LINKED GLYCOSYLATION

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Background and Novelty:
The large-scale manufacturing of therapeutic IgGs has had tremendous progress in recent years, but remains a significant challenge. Many suitable host cell lines for the production of IgG have been identified and are available today (e.g. CHO and NS/0). These cell lines grown in suspension, are able to produce large amounts of properly folded, assembled and post-translationally modified IgG. However, product yield has been found to be not the sole parameter in selecting the best production cell line. One important additional parameter is the glycosylation pattern of the Fc region which influences the therapeutics’ effector functions and thus, its potential therapeutic mode of action.

Experimental Approach:
We have developed some novel tools to systematically identify and compile the n-glycosylation profiles of panels of lead antibody candidates based on mass spectrometry. Enzymatic release of glycans followed by LC-MS analysis provides a powerful technique to identify glycans and estimate their relative abundances. These tools are coupled with a comprehensive workflow management system that track the individual cell line origins as well as all production protocols and conditions.

Results and Discussion:
We will show examples of how such tools can be used in efficiently and more accurately evaluating and identifying best producer cell lines taking into account n-linked glycosylation, both clonal variations and batch to batch variations, when performing and comparing expressions in batch and fed-batch production, including scale-up to thousand liter volumes.
SCALING UP DESIGN AND PRODUCTION OF NON-ANTIBODY TOOL PROTEINS

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Background and Novelty:
So much of the success of a drug discovery and development project depends on the availability and quality of tool proteins representing the drug targets and other protein reagents. The tool proteins typically produced by protein sciences and technology groups are supplied to small-molecule and large-molecule research groups, medicinal chemistry groups, etc. and used for various purposes such as high throughput screening, structural biology and crystallization studies or antigen materials.

Experimental Approach:
We formulated tools for the high-throughput design, engineering, validation and production of these important tool proteins. The system includes comprehensive tracking and annotation of proteins, their domain variants (e.g. truncated or mutated), vectors and cell lines, as well as expression and purification products, related analytics and quality data, together with sample inventory and warehousing information, and transfer and QC documentation (e.g. auto-generated Certificate-of-Analysis).

Results and Discussion:
We will present how our tools enable high-throughput, highly parallelized workflows and a systematic evaluation of protein production success.
DIFFERENTIAL RESPONSES TO INSULIN AND INSULIN-LIKE GROWTH FACTOR, AFTER CHRONIC ENDOPLASMIC RETICULUM STRESS, IN A RECOMBINANT ANTIBODY-PRODUCING CHO CELL LINE

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Background and Novelty:
The unfolded protein response (UPR) and mammalian target of rapamycin (mTOR) are two complex pathways that control protein expression. UPR activates upon endoplasmic reticulum (ER) stress, and can be activated by several chemicals. mTOR pathway is activated by several stimuli, such as mitogens, like insulin or insulin-like growth factor (IGF1). The crosstalk between these two components is beginning to be elucidated, and new evidence shows that mTOR can regulate UPR upstream or downstream, and chronic UPR can inhibit mTOR signaling. Chronic UPR can produce two outcomes, apoptosis or homeostasis, the latter generally as a result of increased mRNA degradation by UPR sensor IRE1α, misfolded protein degradation or increased chaperone expression. Given the recent approaches towards cell line engineering (mainly overexpression of mTOR or UPR components) more knowledge of UPR and mTOR crosstalk is needed in order to optimize these strategies. Here, we show that the effect of mTOR activators, insulin and insulin-like growth factor, on recombinant protein expression changes in response of chronic UPR activation.

Experimental Approach:
A CHOK1 clone expressing a chimeric antibody directed towards human CD20 was generated using standard procedures. Cells were treated with 0.3 μg/mL of tunicamycin (Tm), an UPR activator, or DMSO for 16 days and then counted and cultured in media without Tm, and supplemented with insulin (10 μg/mL) or IGF1 (20 ng/mL). After three days of culture, cells were counted and the expression of recombinant antibody on supernatants was analyzed by capture-ELISA.

Results and Discussion:
Untreated and treated cells showed different responses to insulin and IGF1. Cells treated with Tm showed a 30% increase of recombinant antibody production when treated also with IGF1 (p<0.05, one-way ANOVA), but no significant increase was observed when cells were treated with insulin (14%). Tm-untreated cells did not respond to insulin or IGF1 in terms of volumetric production and productivity. Insulin and IGF1, and their respective receptors are closely related, with extensive homology. Both receptor share many downstream signaling pathways, but mediate different cellular responses. Evidence shows that CHO cells tend to respond more to IGF1 than insulin as a result of a differential receptor expression, which, in part, could explain the results observed. By contrast, chronic UPR signaling can produce insulin resistance by inhibition of Akt activation, but in this case, there is an increase of IGF1 sensitivity, suggesting that IGF1 receptor can overcome this resistance or the effect can be mediated by other signaling pathway, independent of Akt activation or even mTOR involvement.

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ENGINEERING OXIDATIVE STRESS RESISTANCE IN CHO CELL FACTORIES

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Background and Novelty:
Oxidative stress is a phenomenon created by an imbalance in the amount of Reactive Oxygen Species (ROS) created within a cell, and the ability of its defence mechanisms to effectively deal with ROS. Oxidative stress is extremely deleterious to the cell, and is known to cause damage to DNA, proteins and lipids (Turrens, 2003). Mitochondria are the cell’s predominant producer of ROS (Murphy, 2009), although it has also been shown that increased protein folding in the Endoplasmic Reticulum (ER) results in an increase in ROS levels (Malhotra et al, 2008), an issue particularly pertinent as developers move towards hard-to-express proteins. As well as many enzymes dedicated to the eradication of ROS, such as caspases, peroxidases and superoxide dismutases (SODs) the cell maintains a glutathione pool to buffer the increase of ROS (Lu, 2009). In addition, there are several well known anti-oxidant compounds that can mitigate ROS production (Malhotra et al, 2008).

Experimental Approach:
A panel of commercially available anti- and pro-oxidants were screened for impact on cell growth and productivity using a high throughput, plate based system. Host and producer cell lines were compared, to better observe the effect once a cell line has been stably transfected.

Results and Discussion:
Oxidative stress has long been known as the cause of many diseases including atherosclerosis, cardiovascular diseases, chronic inflammation and stroke (Uttara et al, 2009) as well as being heavily implicated in the development of many cancers (Gorrini et al, 2013). Despite this, very little work has thus far been done to investigate the impact of oxidative stress on biologics production in mammalian cell lines. The results of this screen show a link between cell line performance and redox environments, providing targets both for media supplementation and cell line engineering.
MAMMALIAN SYSTEMS BIOTECHNOLOGY: AN INTEGRATIVE FRAMEWORK FOR COMBINING IN SILICO MODELING AND MULTI-OMICS DATASETS IN DIFFERENT CHO PARENTAL CELL LINES

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Background and Novelty:
The increasing availability of multi-omics data from Chinese hamster ovary (CHO) cell cultures entails both opportunity and challenges toward next generation cell culture engineering. Herein, we present a comprehensive and integrative framework to systematically combine transcriptome, proteome, metabolome and glycome datasets in conjunction with a genome-scale metabolic model of CHO cells. We then apply the framework to compare and contrast the metabolic characteristics of the three commonly used parental cell lines (CHO-K1, CHO-DUKXB11 and CHO-DG44) so that “global” attributes of the parental hosts (e.g. growth related characteristics, glycosylation patterns, etc.) could be highlighted.

Experimental Approach:
Suspension CHO-K1, CHO-DUKXB11 and CHO-DG44 cell lines were grown in triplicates in an in-house proprietary medium supplemented with soy hydrolysate. Samples for all analyses were collected at mid-exponential phase. Transcriptomics analysis was performed using a proprietary microarray (Affymetrix) encompassing about 15,000 unique CHO genes. Metabolomics analysis was performed using an ultra-performance liquid chromatography (UPLC) system coupled to a mass spectrometer (MS). Proteomic analysis was performed using TMT labelling (ThermoFisher) and LC-MS/MS. A CHO consensus genome-scale metabolic model was reconstructed from the available human models (recon1 and recon2) using their gene orthology.

Results and Discussion:
The integrative framework involves the use of the genome-scale metabolic model as a scaffold to map the multi-omics datasets. Such an analysis allows us to readily pinpoint the heterogeneity in cellular metabolism between the multiple conditions and/or cell lines tested, as well as their correlations. For example, the comparative analysis of omics datasets between the CHO parental cell lines indicated that K1 cells present the most active metabolism, which is also reflected in their best growth phenotype. Moreover, the correlation analysis of transcriptome and proteome for a given cell line revealed the plausible regulatory intracellular events that can be targeted for genetic engineering to achieve the enhanced productivity and quality of recombinant proteins in the context of bioprocessing. Interestingly, we identified many differences in the reactions associated with the N-glycan processing pathways for the various parental cell lines analyzed, which may be associated with different glycosylation capacity. Further investigation at the glycomics level may validate our hypothesis that choice of CHO hosts should be product-specific.
EVALUATION OF THE PRODUCTION OF EIAV LENTIVIRAL VECTORS IN SUSPENSION CELLS

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Background and Novelty:
Equine infectious anaemia virus (EIAV) lentiviral vectors are currently generated by transient co-transfection of adherent Human Embryonic Kidney 293T (HEK293T) cells with three plasmids that encode the necessary components for vector production (Vector genome, EIAV Gag/Pol, and a heterologous envelope, VSV-G). Manufacturing scale up is restricted by the adherent growth mode and therefore production would benefit from the use of a suspension cell line. The ultimate aim is to use producer cell lines to manufacture vector at scale in 200L bioreactors.

Oxford BioMedica has a HEK293T based EIAV packaging suspension cell line (PC120.2S). Other cell lines are being explored to determine if they could be a suitable alternative to a HEK293T based suspension cell line including CEVEC’s CAP-T cells which are a human amniocyte cell line, that grow in serum-free media, in suspension.

Experimental Approach:
The CAP-T cells were evaluated by transient co-transfection to assess their ability to produce EIAV lentiviral vectors. A protocol for the production of EIAV vector from CAP-T cells was developed by testing an array of transfection conditions. Transfection efficiency was assessed during vector production and the resulting vector was quantified using a vector titration GFP FACS assay. The process that produced the highest yield of vector from the CAP-T cells was further optimised and adapted for use in 500L bioreactors.

Results and Discussion:
Data will be presented on the optimisation and relative productivity of the CAP-T cells. CAP-T cells were able to produce high titre vector by transient transfection in shake flasks, following optimisation of the protocol and media. Furthermore, data will be presented on the successful scale up of vector production from CAP-T cells in 500ml bioreactors.
APPROACHES FOR RECOMBINANT HUMAN FACTOR IX PRODUCTION IN SERUM-FREE SUSPENSION CULTURES

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Background and Novelty:
Since the production of recombinant factor IX (rFIX) in mammalian cells emerged as an alternative therapy to the plasma-derived coagulation factors, some strategies to enable efficient large-scale production have been developed. Human cell lines have attracted great interest for this purpose, since they are capable of producing glycosylated proteins in a more similar way to native human proteins, reducing the potential for immune.

Experimental Approach:
Here we describe two approaches for rFIX production in serum-free conditions using the human cell line 293T: serum-free suspension cell adaptation of previously genetic modified cells (293T-FIX) and the genetic modification of cells already adapted to such conditions (293T-SF-FIX). The adaptation of 293T-FIX cells was made by gradual reduction of serum-containing media (D-MEM 10%). The best commercial serum-free media was selected based on doubling time (DT) and viability of the cells (FreeStyle, Life Technologies). The same process was performed with the wild-type 293T cells, which where then genetic modified (lentiviral mediated gene transfer) for rFIX production (293T-SF-FIX). Erlenmeyer flaks was used to maintain cells in suspension. Both cells (293T-FIX and 293T-SF-FIX) were then characterized regarding growth and protein expression kinetics.

Results and Discussion:
The adherent cell line 293T-FIX cultured in serum-containing media showed a maximum cell density (Xmax) of 7x10⁶ cells/mL and 54.84% of GFP positive cells. The production of rFIX was 0.742 μg /10⁶ cells (48 hours) and 1.256 μg /10⁶ cells (72 hours) with an activity of 0.050 and 0.110 U/mL /10⁶ cells in 48 and 72 hours respectively. FreeStyle media was selected during the adaptation process as the best serum-free media for 293T-FIX. In this condition, the adapted cells showed a Xmax of 21x10⁶ cells/mL, 3 times higher than the adherent one and maximum specific growth rate (μ_max) of 0.015h⁻¹. However, we observed a decrease in the amount of GFP positive cells (19.89%) and consequently a decrease in the production of rFIX: 0.071 and 0.138 μg/10⁶ cells in 48 and 72 hours respectively. After a cell sorting, the percentage of positive GFP cell was increased to 87.11% and, consequently, the production of rFIX also increased to 1.636 and 1.611 μg/10⁶ in 48 and 72 hours respectively. However, when chromogenic assay was carried out, no activity of rFIX was detected. Based on the hypothesis that the long and stressful adaptation process to serum-free condition could interfere in the active protein production, we also performed the genetic modification for rFIX expression of wild-type 293T cells already adapted to FreeStyle media and suspension culture (293T-SF-FIX). The kinetic characterization showed a Xmax of 23x10⁶ cells/mL, μ_max of 0.033h⁻¹ and 90.59% of GFP positive cells. The expression of rFIX was 2.135 and 1.431 μg/10⁶ cells in 48 and 72 hours respectively, and unlike the 293T-FIX, it was observed an activity of 0.100 and 0.310 U/mL /10⁶ cells. Taken together, the results indicate that the best approach to rFIX production in serum-free conditions involves the generation of the recombinant cell line from already adapted cells.
CHO QUASI-SPECIES: ON CLONING OF IMMORTALIZED CELL LINES AND THE GENOMIC DIVERSITY OF CHO PRODUCTION HOSTS - A DISCUSSION

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The genomic and phenotypic diversity of CHO cells (and other immortalized cell lines) is poorly understood and vastly underestimated. Knowledge about aspects of this diversity has accumulated for decades, but is buried in pre-internet publications rarely read or not accessible. Statements by regulatory agencies within the last few years, enhanced by available equipment for facilitation and image based verification of cell cloning, resulted in enforced demands for “CLONING” and “PROOF” of clonality. This is meant to provide the genetic and cellular foundation for reliable manufacture of recombinant proteins using recombinant cells. However, these assumptions - genetic / clonal identity among populations of cloned cells - are not supported by any data. More recent information, both from genomic sequence analysis and from phenotypic observations of hundreds of clonally derived cell lines enforce the notion that CHO cells adapt and modify their phenotype in cultured populations rapidly (in days to weeks). Most interestingly, clonally derived populations will respond efficiently towards “survival” while further enhancing their genetic and phenotypic diversity, each clone with a different response. It is important to note that any genetic modification (from point mutations to global chromosomal rearrangements) can / will occur within the timeframes of the 100-200 population doublings that are typically necessary, starting from a Master Cell Bank, for the manufacture of a given protein product.

The talk will summarize the unique and remarkable history of globally distributed CHO cells. The phenotypic and genotypic features of the more well known cell lines will be presented. Very recent genomic DNA sequence results and their interpretation will be added to this discussion. Based on this, a case is made to apply to CHO cells (and any other immortalized cell line) the term “quasi-species”. This term was established by Eigen and Schuster in 1977. It tries to describes genetically related populations of inheritable systems that undergo rapid evolution due to exceptionally high mutation rates. Population genetics theory and praxis predicts that such systems increase their genetic diversity when forced through narrow population bottlenecks – the cloning of cells being one of the most stringent. Other features of the quasi-species hypothesis fit very well also.

In conclusion, the talk will try to provide a better understanding of what CHO cells are, how they evolve, and what manufacturers and regulators together can do to enhance the robustness and quality of products derived from these remarkable cells.
STREAMLINING INSECT CELL LINE DEVELOPMENT THROUGH NOVEL TARGETED GENE INTEGRATION STRATEGIES

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Keywords:
RMCE / rational design / fast and flexible insect cell line technology / recombinant protein expression.

Background and Novelty:
Targeted integration of a gene-of-interest (GOI) in previously characterized genomic loci using site-directed integration allows predictable protein expression and avoids considerable clone-screening efforts associated with conventional cell line development. In a recombinase-mediated cassette exchange (RMCE) procedure, the integration site is marked by a “tag” consisting of two heterospecific recombination target sites flanking a selection marker. Provided on a donor vector, an analogous cassette encoding the GOI can replace the former cassette in a reaction mediated by a recombinase. We recently established a RMCE system in Sf9 cells using the yeast-derived Flipase (Fpl) recombinase expression. During this process, only a small fraction of the generated clones were tagged in a single locus amenable to Fpl-recombination. Herein, we propose a novel strategy to develop cell lines combining RMCE and FACS technologies, aiming for generation of cell clones tagged in a single high-expressing locus, with greater recombination efficiencies and in shorter time frames without extensive screening.

Experimental Approach:
Sf9 and Hi5 insect cells were stably transfected with a tagging cassette harboring a reporter icherry gene flanked by Fpl recognition target sites and then sorted for strongest fluorescent cells. These polyclonal populations were submitted to Fpl-RMCE by transfection with an eGFP-containing target cassette. Recombination efficiency was compared in two settings of Fpl delivery to the cells: i) by single transfection (incorporated in the target plasmid, leading to constitutive expression) and ii) standard co-transfection with an Fpl-containing plasmid (transient expression).

Results and Discussion:
The Sf9 and Hi5 tagging populations have similar icherry expression patterns as analyzed by flow cytometry and were successfully FACS-enriched with the highest expressing cells. To eliminate cells tagged in loci with low recombination efficiency, both populations were submitted to cassette exchange and subject to antibiotic selection. The resulting heterogeneous populations were composed by many cells which express both icherry and eGFP, suggesting the occurrence of multiple integrations of the tagging cassette. Pure eGFP-expressing cells were recovered by FACS, and subject to a second round of cassette exchange to demonstrate much faster selection with a much lower number of cells expressing two reporter proteins, thus a lower incidence of cells tagged in multiple loci. Overall, our approach consistently selected recombination clones and further improved the efficiency of Fpl-RMCE in insect cells, which dramatically speeds-up the capability to produce proteins of biomedical interest.

References:

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CELLULAR FUNCTIONS OF HSP27 IN IMPROVING RECOMBINANT MONOCLONAL ANTIBODY TITRE IN CHO CELL

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Background and Novelty:
Chinese Hamster Ovary (CHO) cells are the mammalian cell lines of choice for the production of approved recombinant biologics including recombinant monoclonal antibodies (mAb). With the increasing demands for recombinant biologics, there is an on-going need to improve the production yield. Due to emerging biosimilars market, together with constant development of innovator recombinant biologics, new production cell lines would have to be created, and hence the use of engineered CHO cell lines with improved production capability can be explored. Heat shock protein 27 (HSP27) was found to be down regulated toward the late-exponential and stationary phase of the fed-batch CHO culture. HSP27 are found to be highly expressed in human and mouse pathological tissues and cells where they are reported to possess anti-cytotoxic functions. This led to the hypothesis that overexpression of HSP27 in CHO cells can increase the production of recombinant biologics by extending the culture duration. However, knowledge of its cellular roles in CHO cells bioprocessing context is limited. Hence, elucidation of HSP27 cellular roles in CHO cells were explored.

Experimental Approach:
Stable HSP27 overexpressing CHO cells producing recombinant proteins were created and evaluated in fed-batch bioreactors. Concomitantly, its cellular roles in regulating CHO cells cellular processes under fed-batch bioreactor conditions were investigated using multi-omics technologies.

Results and Discussion:
We have demonstrated that HSP27 overexpression is able to increase titres of two different classes of recombinant biologics (cytokine and monoclonal antibody) produced in two different parental CHO cell lines. Additionally, glycosylation quality of both recombinant proteins are not affected. HSP27 was found to improve CHO cells fed-batch bioreactor cultures by delaying activation of caspases and interaction with several proteins involved in the apoptosis signaling pathways to suppress the onset of apoptosis. Subsequently, other potential cellular roles of HSP27 in CHO cells to improve culture performance in fed-batch bioreactor system were identified and a HSP27 interactome in CHO cells under fed-batch bioreactor conditions was proposed.
UTILIZATION OF RECOMBINASE MEDIATED CASSETTE EXCHANGE (RMCE) AND ALTERNATE START CODONS TO SELECT FOR GENOMIC “HOT-SPOT” INTEGRATION SITES

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Which makes the screening for a suitable cell clone very time-and labor intensive. The variable influence of random integration can be overcome by utilization of site-specific Recombinase Mediated Cassette Exchange (RMCE) whereby it’s possibility to transfer the GOI into pre-selected genomic locations with defined expression properties. This study aims at the identification of transcriptionally active genomic integration sites in Chinese Hamster Ovary (CHO) cells by the use of alternate start codons of a surface reporter protein in combination with RMCE.

Experimental Approach:
The sortable reporter gene CD4 with an alternate start codon, flanked by heterospecific FRT sites (Flippase recognition targets), was stably transfected into CHO cells. The alternate start codon (CTG or TTG) reduces translation efficiency of the CD4 reporter and allows sorting for CHO cells of highest transcription rates. Sorted cell pools were subjected to RMCE exchanging the CD4 expression cassette against the new expression cassette via co-transfection of a donor plasmid encoding the GOI (short lived GFP or Herceptin antibody genes) and a plasmid encoding a flippase recombinase, responsible for mediating cassette exchange at the FRT sites. Cells that have successfully performed cassette exchange were isolated via chemical selection and FACS sorting for top producers.

Results and Discussion:
We could show that the use of alternate start codons of a surface reporter protein enables selection of high-expressing cells. Sorted cell pools stably transfected with the CD4 reporter gene containing the alternate start codon CTG lead to higher GFP levels and antibody titers upon RMCE than the cell pools established with the usual ATG start codon. Two consecutive rounds of RMCE performed to select for integration sites showing reliable cassette exchange in addition to high transgene expression didn’t lead to improvements in recombination efficiency. Thus, instead of repeated cycles of RMCE we recommend to directly sort for the highest CD4 expression levels from multiple transfections. Repeated, highly stringent sorts for top producers, however, led to the selection of cells with a mutation of the alternate start codon to the usual ATG start codon, which necessitated a check for the correct start codon after each sorting step.
CHARACTERIZATION OF BIOPHARMACEUTICAL MOLECULES FOR
THE SELECTION OF THE BEST PRODUCER CELL LINE

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Background and Novelty:
Advancement in the characterization of biopharmaceutical molecules has been driven by improvements in variety of technologies, including mass spectrometry. Biotherapeutics require extensive testing to confirm that all critical quality attributes fall within acceptable tolerances. This occurs in many stages of the R&D workflow. The comprehensive capture, processing and reporting of these analytics is of paramount importance in ultimately identifying the lead molecule as well as the best production cell line.

Experimental Approach:
We developed a system that provides a complete traceability of the molecular engineering and bioprocessing history of a sample. Samples were prepared and mass spectrometry data were collected using standard laboratory procedures. The resulting native raw mass spectrometry files are processed through a workflow based software system to identify peptide features and annotate their identities. The system work mode can be fully automated, semi-automated, or even fully manual. Reports from the analysis can be generated automatically and can include full audit trail for documentation and distribution.

Results and Discussion:
Here we will show examples of streamlined workflows for PTM ide ntication, intact protein analysis, peptide mapping analysis, released glycan analysis and disulfide bond analysis and how the results are used to make informed decisions on developable molecules and the respective producer cell line.
INCREASED ANTIBODY YIELD DUE TO MODIFICATION OF LIGHT AND HEAVY CHAIN EXPRESSION BY GENE REGULATORY ELEMENTS

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Background and Novelty:
The amount of antibodies secreted from a CHO cell, is strongly dependent on the transcription and translation level of light (LC) and heavy chains (HC) as well as the ratio of both synthesized polypeptides inside the cell. In literature, it was shown that an excess of intracellular LC over HC improves the antibody production, whereas the opposite ratio results in decreased yields of product. In single-vector expression systems composed of two distinct LC and HC expression cassettes, the transcription of both genes and finally the polypeptide ratio and antibody production can be influenced by the arrangement and orientation of both units and promoters to each other, because of i.e. promoter interferences. Gene regulatory elements can prevent these interactions and could allow an adjustment of transcription levels and an increase of titers.

Experimental Approach:
Based on this assumption, an antibody expressing vector containing two expression units for LC and HC, was modified by insertion of insulators, S/MAR elements and non-functional, spacing sequences in a kind of flanking or separating both parts. Afterwards, the newly generated vectors were stably integrated in a previous tagged, high active genomic locus of CHO-K1 TurboCells™ by recombinase mediated cassette exchange (RMCE) technology. In contrast to random integration resulting in variable, locus dependent expression (position effect), targeted integration at a defined, characterized genomic locus allows a direct comparison of the vectors in terms of expression and genetic modification. Because of consistent chromatin environment around the identical integration site, the integrated vectors are influenced in equal measure. Differences in expression between vectors are solely related to their modifications. Targeted producer cells were analyzed in fed batch experiments for antibody production. Additionally, the amounts of LC and HC mRNA and polypeptides were quantified by qPCR and Western blot/ELISA, respectively.

Results and Discussion:
Dependent on the type of the inserted sequences and their positions inside the vector, the transcription of LC and HC was differently affected resulting in both different amounts and ratios of the transcripts. These variations, in turn, were associated with different amounts of LC and HC polypeptides and also different yields of secreted antibody. The majority of modifications inside the expression vector achieved an improved antibody expression in CHO cells. By insertions of gene regulatory elements between the LC and HC expression units, transcription of both mRNA was significantly enhanced resulting in an up to 2-fold increased titer.
**HIGHER TITER CLONES IN LESS TIME: OPTIMIZING THE CELL LINE GENERATION WORK FLOW TO SUPPORT ACCELERATED TIMELINES FOR BIOLOGICS DEVELOPMENT**

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**Background and Novelty:**
To support rapid timelines for biologics development, we aim to continuously improve our work flow for generating high producing CHO clones suitable for use in clinical manufacturing. This has included updating the processes for selection and sorting of stably transfected pools, implementing more stringent 96-well plate based screens, optimizing cell culture media, and devising new overall strategies for transfection initiation and for further development of lead candidate cell lines.

**Experimental Approach:**
All changes were applied to our existing cell line development platform, consisting of an animal derived component (ADC)-free CHO parental cell line, a chemically defined, ADC-free process, and an alternate start reporter expression system.

**Results and Discussion:**
The initial optimization work resulted in in higher titer clones being identified sooner, enabling earlier development and quality assessment of lead clones. In some cases, clonal cell lines were selected as development candidates in 15 to 16 weeks from transfection. In addition, cell lines generated using the optimized work flow achieved up to 2.5 g/L of mAb or Fc-fusion protein in unfed, unoptimized, small scale (10 to 30 mL) batch cultures. Subsequent optimization efforts have focused on cell sorting methods, media improvements, and novel strategies to enrich for high producing cells, all of which will help further reduce the development timeline. Data for several cell lines producing different recombinant proteins will be presented to illustrate platform improvements.
ENGINEERING THE KINOME: BETTER CELLS FOR BETTER PROCESSES

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Background and Novelty:
So-called “Bio betters” produced in novel cell lines are emerging to the market. Cevec’s Amniocyte Production (CAP\(^{®}\)) cells are among these upcoming workhorses due to their ethically derived human background with authentic human glycosylation combined with the ability to produce high titers of high-quality protein. We have set out to investigate the role of various kinases in CAP\(^{®}\) cells for multiple parameters in a recombinant protein production process. Kinases are among the key players in signal-transduction and present highly attractive targets for cell engineering.

Based on a functional RNAi screen setup that enabled us to evaluate proliferation, productivity and apoptosis in complex production media under typical batch cultivation conditions, we have identified a set of kinases that are crucial to the protein production process. These genes might serve as novel engineering targets for gene overexpression or gene knockout to generate CAP\(^{®}\) cells with enhanced phenotypical characteristics for a bioprocess.

Experimental Approach:
CAP\(^{®}\) cell lines expressing secreted embryonic alkaline Phosphatase (SEAP) or IgG were used in a high-content, high-throughput siRNA screen in complex production media employing a novel transfection agent ScreenFect\(^{®}\) A to evaluate process-relevant parameter. A library of 763 siRNAs against human kinases was tested for the modulation of proliferation, viability, productivity and apoptosis in a batch process in shaked multititer plates. We utilized a multi-parameter quantitative flow cytometry assay for cell-based parameters, enzyme activity assay for SEAP and a Protein A HPLC for IgG. INGENUITY\(^{®}\) pathway analysis was used to elucidate important pathways in a protein production process. This knowledge served as the basis for cell engineering of CAP\(^{®}\) cells by gene overexpression or gene knockout by CRISPR/Cas9 with enhanced phenotypical characteristics were established.

Results and Discussion:
A quarter of all siRNA induced kinase knockdowns resulted in a significant modulation of at least one process-relevant parameter in SEAP producing CAP\(^{®}\) cells. The strongest detrimental effects on proliferation and productivity were induced by knockdown of cell cycle kinases, members of the growth factor signaling pathway as well as genes involved in cellular assembly, organization and maintenance. Gene knockdown of proteins involved in the unfolded protein response in the endoplasmatic reticulum were found to decrease the productivity without affecting proliferation. A set of 128 siRNAs were re-evaluated in IgG producing CAP\(^{®}\) cells. Absence of effects observed in primary screening highlighted the importance of re-validation of screen data since these effects might be product and clone-specific.

The overexpression of such crucial kinases and the knockout of genes representing potential bottlenecks in the recombinant protein production process are feasible approaches for cell engineering.
IMPLEMENTATION OF NEW METHODS FOR SINGLE CELL SORTING AND PLATE IMAGING RESULTS IN AN IMPROVED CELL LINE GENERATION PLATFORM FOR BIOLOGICS DEVELOPMENT

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Background and Novelty:
Our cell line development process employs a fluorescence-based reporter system coupled with flow cytometry sorting to rapidly identify and isolate high producing cell lines as candidates for clinical manufacturing. In addition, high resolution imaging is utilized to provide clonality documentation of the candidate cell lines. Sorting methods were optimized, achieving improvements in several areas.

Experimental Approach:
Sorting methods were optimized, achieving improvements in several areas. These include post-sort cell viability, which has been improved up to 2-fold, sorting resolution, and cloning efficiency via automated single cell deposition to well plates. These improvements have not only resulted in an enhanced cell line generation platform but have also created exciting opportunities for novel sorting strategies, such as rapid enrichment to boost productivity with shortened timelines. Implementation of plate imaging into our process immediately after cell sorting has yielded rapid, high resolution (2 μm/pixel) images of single cells. These images, together with those showing subsequent growth of a single colony, are used to document that each final candidate clone is derived from a single cell progenitor. Improvements to the plate imaging process have led to better image quality and, in turn, an increase in the number of clones that can be further assessed as manufacturing candidates.

Results and Discussion:
Proof of concept and case studies will be presented to illustrate how the combined optimized methods have improved our overall platform for generating high producing recombinant cell lines. In addition, investigations of new sorting strategies will be presented, with a focus on those with the best potential to more rapidly achieve high producing pools and clones.
TECHNOLOGY TOOLBOX FOR CELL LINE DEVELOPMENT - NEXT GENERATION CELL LINE DEVELOPMENT TECHNOLOGIES

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Background and Novelty:
Chinese hamster ovary (CHO) cells are the most widely used host for large scale production of recombinant therapeutic proteins exhibiting high productivities in the gram per liter range. A novel toolbox of vector elements, selection marker and novel engineered CHO cell lines were developed which results in combination in significant increase of titer and improved product quality.

Experimental Approach:
We have evaluated novel vector elements and a new selection marker with a variety of antibody projects resulting in an increase of titer. Furthermore we have identified a key protein severely affecting the quality of non-antibody format therapeutic proteins. Subsequently the key protein was eliminated using Novartis propriety CHO cell line via novel targeted gene disruption tools. This resulted in a superior CHO cell line.

Results and Discussion:
The combination of novel vector elements and selection marker resulted in an increase of titers. Especially the combination of the recently published CHO genome in combination with screening methods and cell line engineering tools has enabled the development of a superior CHO cell.
EVALUATION OF THE DIFFERENTIAL EFFECT OF SPECIFIC GROWTH RATE AND CULTIVATION TEMPERATURE ON PRODUCTION OF RECOMBINANT PROTEINS BY CHEMOSTAT CULTURES OF CHO CELLS

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Background and Novelty:
CHO cell line is most widely used in the production of recombinant proteins in human therapy, due to its ability to properly perform posttranslational modifications necessary to give the protein required characteristics for use in humans.

One of the main strategies used to increase productivity, is the application of culture temperatures within the range of mild hypothermia (33-30°C). However, the temperature reduction entails a concomitant decrease in the specific cell growth rate. Recent research has showed a differential effect of culture temperature and specific growth rate on the production of the recombinant protein t-PA expressed in CHO cells, increasing the specific productivity of t-PA by reducing specific growth rate (0.012 h⁻¹), under mild hypothermia condition (MHC). However, it suggests that differences in the structure of the recombinant protein (long of polypeptide chain, glycosylation percentage, among others) could also influence the response observed against MHC and low specific growth rates.

Experimental Approach:
To verify this proposal, the production of recombinant GM-CSF protein (lower molecular weight and higher glycosylation %, regarding t-PA) was studied in chemostat cultures of CHO cells to analyze the effect of culture temperature and specific growth rate. Four set of chemostat cultures were carried out at two dilution rates (0.017 and 0.010 h⁻¹) and two culture temperatures (37 and 33°C). The cell response was evaluated in terms of the kinetic parameters, specific productivity of r-protein, and specific consumption and production of main metabolites. GM-CSF protein concentration was determined by immunoassay (Human GM-CSF ELISA KIT), concentration of glucose, lactate and glutamate; was determined by enzymatic assay using a biochemical analyzer YSI (Yellow Spring Instruments). Statistical analysis of the results was performed by ANOVA.

Results and Discussion:
At high specific growth rate (0.017 h⁻¹), MHC showed a positive effect on GM-CSF specific productivity, increasing twice, regarding 37°C. At lower rate of cell growth, no effect of MHC was observed. At 37°C the reduction in the specific growth rate showed no effect on specific productivity of r-protein, while under MHC, a negative effect on the specific productivity was observed, decreasing this twice.

Concerning metabolic behavior, MHC only caused a significant decrease in the consumption of glutamate, of 28% and 17% at high and low growth rate, respectively. While the decrease in the specific growth rate had a significant effect on the yield of glucose to lactate, reducing it from 1.3 to 0.3 (mol/mol) at 37°C and 1.3 to 0.6 (mol/mol) at 33°C, and on the specific consumption of glutamate which were reduced by 22% at 37°C and 11% at 33°C.

The results in the production of GM-CSF protein differ from those previously obtained with t-PA protein, at MHC the GM-CSF specific productivity is higher at high growth rate while the t-PA specific productivity is higher at low growth rate. However, the metabolic behavior of both cell lines responds similarly to the variables studied, efficient metabolism was observed in terms of glucose utilization, by reducing the rate of cell growth, affecting MHC only glutamate consumption.
EFFICIENT RMCE-BASED CELL LINE DEVELOPMENT IN CHO CELLS FACILITATES PREDICTABLE AND REPRODUCIBLE PROTEIN EXPRESSION

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Background and Novelty:
The first step in manufacturing recombinant glycosylated therapeutic proteins is the search for a high and stable expressing cell clone. In contrast to traditional approaches for cell line development Fraunhofer ITEM utilized an alternative strategy called recombinase-mediated cassette exchange (RMCE). Traditional concepts for the development of cell lines are based on random integration of the expression vector containing the gene-of-interest (GOI) which makes the screening for an appropriate cell clone very time and labor intensive. The application of the RMCE-strategy based on the site-directed integration of a single copy of the gene-of-interest into a transcriptional, exchangeable and known genomic locus dramatically shortens the timeline for the cell line development.

Experimental Approach:
The genomic locus of CHO-cells was initially marked by transfecting the cells with a lentiviral based Tagging vector containing a selection and a fluorescent marker flanked by two heterospecific recombination target sites. Master cell lines revealing stable reporter expression and bearing an exchangeable locus were identified. The following exchange of the Tagging versus the Targeting cassette, carrying the gene-of-interest (GOI), was catalysed by Flp-Recombinase. RMCE experiments with three fullsize antibodies, one antibody fragment and tPA have been performed. The performance of the targeted subclones was investigated in fed-batch cultivations.

Results and Discussion:
The transgene expression among the targeted subclones was homogenous and corresponding to the respective targeting-pool. However, the results do not indicate the universal applicability of a certain chromosomal locus for recombinant protein expression. They rather support the theory that some loci are appropriate for the expression of certain proteins, since the expression level among the full size antibodies was quite similar. The RMCE-derived clones demonstrated comparable growth and production characteristics within a defined design space in fed-batch processes.
ANALYSIS OF MOLECULAR MECHANISMS ASSOCIATED TO THE ADAPTATION OF NS0 MYELOMA CELL LINE TO PROTEIN-FREE MEDIA

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Background and Novelty:
The NS0 mouse myeloma cell line has become one of the most popular systems for large-scale heterologous protein expression. For reasons of regulatory compliance, cost, batch consistency, downstream processing, and material availability, industrial applications of NS0 have moved towards serum or protein-free medium platforms. For serum- or protein-free cultivation, the cell culture medium is often supplemented with lipids (derived from plant or synthetic sources) in addition to other protein supplements. NS0 cells are naturally cholesterol-dependent; not only is their growth greatly facilitated by lipid supplementation, but is also dependent on provision of cholesterol. Different mechanisms underlying a cholesterol-dependent phenotype could include the absence (or mutation) of a gene or a segment of gene along the cholesterol biosynthesis pathway. There could be changes in the expression level of some proteins of the pathway due to gene regulation or other control mechanisms. In addition to the specific gene expression alterations along the cholesterol and lipid metabolism pathways, cholesterol dependence could also be the result of insufficient precursor supply.

Experimental Approach:
A quantitative study of proteins with differential expression levels in four conditions (host NS0 cell line adapted and non-adapted to protein-free medium, and a monoclonal antibody (Mab) transfectedoma producer NS0 adapted and non-adapted to the same protein-free media) is reported. The study is based on the use of the combination of two-dimensional electrophoresis and mass spectrometry, and a novel quantitative proteomic approach, isobaric tagging for relative and absolute quantification (iTRAQ).

Results and Discussion:
The molecular mechanisms of host and recombinant NS0 cell lines that could be related to the adaptation to protein-free media are studied in this work. The metabolic study of these cell lines cultured in different nutrient conditions is also reported. Several proteins with differential expression profile were characterized and quantified. Changes in lactate production rate with respect to glucose consumption rate were observed according to the changes observed by proteomic. Carbohydrate metabolism, protein synthesis and membrane transport were the principal pathways that change after the adaptation by proteomic analysis. The same results were obtained using MFA and FBA in a murine metabolic network with selected medium conditions. Taking into account the proteomic results and metabolic analysis, a possible mechanism related to the adaptation of NS0 cell line to protein-free medium is proposed.
AN ADVANCED VECTOR GENERATION SUBSTANTIALLY ENHANCES EXPRESSION LEVELS OF MONOCLONAL ANTIBODIES

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Background and Novelty:
Modern approaches for generation of pharmaceutical cell lines combine the use of expression vectors enabling high and stable target gene expression with efficient selection and screening strategies. This typically results in producer clones expressing g/L of monoclonal antibodies. Hence, the optimal balance of heavy and light chain may be disturbed through different folding and processing kinetics. In such a situation a more gradual expression of the heavy and light chains is required. In some cases mRNA level can be limiting per se.

Experimental Approach:
Starting from a robust expression system where the antibody heavy and light chain genes are expressed from different cellular promoters on the same vector we evaluated alternatives:

1. An extended purely viral promoter driving both chains of the antibody
2. Screening for cellular noncoding DNA elements promoting long term high level expression and
3. Separate vectors for both antibody chains containing separate selection markers.

Vector variants were first analysed for long term performance in ProBioGen’s DG44 platform using gfp as a transgene. Preferred compositions were later compared side-by-side with the traditional system in multiple antibody cell line development projects.

Results and Discussion:
The new promoter increased average and peak expression levels and maintained activity in most clones for more than 50 population doublings without addition of selective agents. One of the identified noncoding DNA elements extended stable expression beyond 90 cell generations. Although separation of antibody chains and selection markers between two vectors required refinement of selection conditions, this composition contributed to better performance in particular for antibodies with impaired expression potential. In side-by-side comparison the advanced vector generation outperformed the traditional system. Applying the same selection/screening procedure and effort the pool expression and numbers of top performing clones increased providing more room for selection based on product quality parameters.
VITAMIN OPTIMIZATION OF A CHEMICALLY DEFINED GROWTH MEDIA FOR CHINESE HAMSTER OVARY CELLS

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Background and Novelty:
Celonic has developed a chemically defined cell culture media for cultivation of CHO cell lines. It has previously been shown that adaptation and cultivation of various CHO cell lines was easy and successful. To further improve the chemically defined media the concentrations of vitamins have been optimized by the use of DoE. The resulting CHO cell media shows improved growth and expression characteristics.

Results and Discussion:
Using Modde software and the original media as a starting point, 10 different media have been generated differing only in the concentrations of vitamins. A stable CHO K1 cell line has been adapted to all media for at least 6 passages. Glucose and L-Glutamine supplemented batch cultures in shake flask have been tested. Maximal viable cell density, IVCD and titer could be significantly increased for the best vitamin concentrations. In a second experiment the results from the first test could be reproduced and a theoretically optimized media regarding vitamin concentration was tested. Using optimized vitamin concentrations the maximal viable cell density and IVCD could be improved by a factor of 2 whereas the production titer increased by a factor of 3. Taken together the optimal supplementation of vitamins in chemically defined cell culture media is very important to achieve good growth and expression characteristics.

Topic:
Process development, stable cell lines.

Keywords:
Chemically defined Media. Titer. Vitamins.
TUBESPINS AS A SUITABLE SCALE-DOWN MODEL OF 2L HIGH CELL DENSITY BIOREACTORS FOR CHO CELL CULTURE

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High cell-density culture of Chinese Hamster Ovary (CHO) cells significantly increases cell growth, viability and volumetric productivity of recombinant therapeutic proteins. These improvements can reduce size or number of manufacturing cell culture vessels, decrease plant footprint and ultimately, the cost of goods.

Process development is typically performed in instrumented lab-scale bioreactors, which, while effective, require time and labor for set-up and operation. Is it attractive to develop alternate systems that provide representative results but without the effort of bioreactor operation. To this effect, we evaluated 50 mL Tubespins as a model to mimic high cell density cultures.

We tested 13 different CHO cell lines expressing recombinant proteins for 12 - 15 days concurrently in both 2L bioreactors and satellite 50 mL Tubespins. In general, Tubespins exhibited comparable cell viability and specific productivity to 2L bioreactors and with generally comparable high peak cell densities. Finally, product quality attributes such as galactosylation, afucosylation, and protein aggregation in Tubespins were similar to those in 2L bioreactors. Some differences were seen in protein charge heterogeneity and these could be attributed to the residence time differences between the systems. In summary, Tubespins can be used as an effective tool for CHO high cell density process development with 150-fold lower medium usage, no time-consuming or labor-intensive bioreactor preparation, and providing valuable and representative results of cell line growth, metabolism, productivity and product quality.
AUTOMATED HIGH THROUGHPUT CELL LINE DEVELOPMENT: HOW TO SOLVE THE HIGH VOLUME DATA CHALLENGE?

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Background and Novelty:
The biologics drug R&D process in pharmaceutical and biotech companies is characterized by division of labor across sites and high-throughput approaches. The integrated management of data from molecules, clones, materials and analytical experiments is a key challenge in this context. We have successfully implemented the Biologics Data Platform (BDP), a novel enterprise-IT solution based on Genedata Biologics software, for tailored support of our screening and protein production processes. Here, we describe the integration of BDP with our automation workstation in cell line development.

Experimental Approach:
We implemented an automated cell line development workstation for seeding, selection, incubation, passaging, analyzing, and cryo-conservation of cells. In parallel, we designed functionalities in BDP to track the full history of clones from the initial transfection up to their evaluation in bioreactor runs together with analytics data, in order to support the cell line development process.

Results and Discussion:
We demonstrate the advantages of comprehensive management in one IT system of cell line clones and fed-batch experiments together with molecule information such as primary sequences and experimental data. The system facilitates correlation analyses for clone selection during cell line development which will be discussed with examples.
AN INTENSIFIED PERFUSION INOCULUM PROCESS WITH HIGH DENSITY CELL BANKS

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Background and Novelty:
Process time is often a bottleneck in the throughput of biomanufacturing plants. This work describes how the use of high-density working cell banks (HDWCBs) and perfusion-based culture processes can help reduce operating times and simplify operations. A simplified approach was used for creating HDWCBs in a bioreactor perfusion culture. Cells were recovered from cryopreserved HDWCBs and used in a single-bioreactor inoculum process, creating enough cells to seed a 2000 L production bioreactor.

Experimental Approach:
Cell banks were created by growing cells in a 2 L culture in perfusion mode up to 50 x10^6 cells/mL in a disposable 10 L Cellbag™ bioreactor with a floating internal filter using the ReadyToProcess™ WAVE 25 system. For cryopreservation, chilled, fresh media with DMSO was added to the bioreactor, whereupon the culture was concentrated back to 50 x10^6 cells/mL before harvested, dispensed, and frozen in 4.5 mL cryovials. Cells from cryopreserved HDWCBs were recovered and directly transferred to a new 20 L Cellbag bioreactor at low volume. The volume was stepwise expanded to a final working volume of 10 L. The culture was continued in perfusion mode.

Results and Discussion:
HDWCBs with high viability (> 90%) after recovery were created and used for direct inoculation of a bioreactor culture. The used approach enabled expansion of cells to a concentration that allowed inoculation of a 2000 L production bioreactor in less than two weeks. Perfusion culturing enabled the entire inoculum expansion process, from vial recovery to a large-scale bioreactor, to be run in a single ReadyToProce WAVE 25 system, with few manual interactions and simple operations as a result.
INCREASED TITER AND REDUCED LACTATE ACCUMULATION IN RECOMBINANT RETROVIRUS PRODUCTION THROUGH THE DOWN-REGULATION OF HIF1 AND PDK


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Background and Novelty:
Many mammalian cell lines used in the manufacturing of virus-based biopharmaceuticals exhibit a Warburg effect-like (WE) phenotype with high glycolytic flux predominantly channelled to the production of lactate, hampering cell viability and decreasing final product titer and quality.

In previous works we identified that the reduction of the glycolytic metabolism and other metabolic networks are potential targets to improve viral titers [1,2]. Herein we report, for the first time, the stable down-regulation of hypoxia inducible factor 1 (HIF1) and pyruvate dehydrogenase kinase (PDK) - two key molecular effectors of the WE - in combination, and in the context of recombinant retrovirus production. PDK was chosen in order to increase the levels of active PDH, thereby increasing the entrance of pyruvate into the TCA; HIF1 was chosen as one of the master upstream inducers of WE. This work shows how silencing HIF1 and PDK can improve cell line productivity, reporting one of the highest fold-increase in specific productivity of infectious virus titers achieved by metabolic engineering. Importantly, it also shows that reduced lactate accumulation is not sufficient towards improved titer performances, highlighting the need of orchestrated changes in secondary pathways.

Experimental Approach:
Down-regulation of HIF1 and PDK was performed in a HEK 293 derived cell line producing recombinant gene therapy retroviral vectors – 293 FLEX – using lentiviral vector mediated short-hairpin RNA interference. The resulting populations and clones were characterized in terms of specific productivity, lactate production, transcriptome analysis, enzymatic assays and metabolite profiling.

Results and Discussion:
Specific productivity of infectious viral titers were increased by more than 20-fold for single gene knock-down (HIF1 or PDK) and more than 30-fold under combined down-regulation. Lactate production was reduced up to 4-fold. Interestingly, the reduction in lactate production alone was not sufficient to enhance the titer: high-titer clones also showed significant enrolment of metabolic routes not related to lactate production. Transcriptome analysis indicated activation of biological amines metabolism, detoxification routes, including glutathione metabolism, pentose phosphate pathway, glycogen biosynthesis and amino acid metabolism. The latter were validated by enzyme activity assays and metabolite profiling, respectively. High-titer clones also presented substantially increased transcript levels of the viral genes expression cassettes.

These results clearly reveal the impact of HIF1 and PDK down-regulation in the production performance of a mammalian cell line. Also, they provide insights on potential secondary pathways that can be further explored to pursue an improved metabolic status favouring a high-producing phenotype. Our approach constitutes a metabolic engineering tool of potential relevance to other complex biopharmaceuticals produced in mammalian hosts, including complex proteins and other types of recombinant viruses.

References:
HCES2: STUDIES ON THE ROLE OF GLYCOSYLATION FOR ENZYME ACTIVITY AND STABILITY

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Background and Novelty:
Carboxylesterases (CES) are a subset of esterases that catalyze the hydrolysis of esters, thioesters and amides and may be found in various mammalian tissues. These enzymes are responsible for detoxifying exogenous substrates like pesticides and environmental toxicants, and are also involved in metabolizing drugs and/or activating pro-drugs. In general, human carboxylesterases (hCES) are glycoproteins with one or more N-glycosylated sites, have four conserved cysteine residues, a signal sequence responsible for targeting to the Endoplasmic Reticulum (ER) and a C-terminal ER retention sequence. Human carboxylesterase 2 (hCES2), a glycosylated protein, is the major intestinal carboxylesterase and the knowledge of its structure would be relevant for the development of new drug substrates and inhibitors. However the 3D structure of hCES2 has not been deciphered yet, mostly because its crystallization has been a challenging task. Glycoproteins are frequently difficult to crystallize due to the heterogeneity of N-glycan structures and conformations at the surface of proteins and therefore we considered the production of non-glycosylated forms of hCES2 in order to obtain alternatives for crystallization and diffraction studies. Since these new forms would only be useful for structural studies if they remained active, the main goal of the present work was to evaluate the impact of glycosylation in hCES2 activity and stability.

Experimental Approach:
Soluble hCES2 10x-His was produced in HEK-293T cells. Partially deglycosylated or non-glycosylated forms of the enzyme were also obtained by three different approaches: i) enzymatic deglycosylation with peptide N-glycosidase F; ii) incubation with the inhibitor tunicamycin; ii) site directed mutagenesis of each or both N-glycosylation sites.

Results and Discussion:
Deglycosylated protein did not show a detectable decrease in enzyme activity. Tunicamycin led to decreased levels of secreted hCES2 that was still active. In agreement, mutation of each and both N-glycosylation sites led to decreased levels of secreted hCES2 but the enzyme remained active. However, the thermostability of the glycosylation mutants was decreased.

The results indicate that glycans are involved to some extent in protein folding in vivo, however, removal of glycans does not abrogate the activity of secreted hCES2, and therefore, non glycosylated forms are also suitable for functional and structural studies.

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TWO OPTIMIZED APPROACHES FOR THE EFFICIENT GENERATION
OF HIGH-PRODUCING STABLE CELL POOLS

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Background and Novelty:
The market for therapeutic proteins is steadily growing, resulting in an increased need to optimize methods for the generation of mammalian cell lines. In this context, important aspects are to express recombinant products at high levels in an efficient and cost-effective manner. In the present study, we focused on the generation of stable cell pools, one of the most critical steps in the course of the cell line development process. The aim was to reproducibly generate high producing pools with reduced workload and a shortened timeline by adjusting critical parameters in terms of the seeding, cultivation and selection process.

Experimental Approach:
Following transfection two main cell line development strategies are typically carried out, the ‘Large Pool’ and the ‘Mini Pool’ approach. ‘Large Pools’, consisting of about 1x10^9 cells, represent the faster and more effortless approach but will oftentimes lead to lower specific productivities, because of population dynamic effects. Low producing clones tend to have higher growth rates than high producing clones, because they do not have to use as much energy and other metabolic resources as the high producing clones for protein expression. This leads to an overgrowth of low producers. To avoid disadvantageous population dynamics, transfected cell pools can be divided into ‘Mini Pools’ the day after transfection. ‘Mini Pools’ consist of 2000 to 4000 cells. The ‘Mini Pool’ approach is laborious and time-consuming but usually results in stable pools with high productivities. The disadvantage of this approach is the prolonged expansion phase correlating with expanded timelines. A major aspect of this study was to combine the benefits of the two standard methods in terms of workload, timeline and productivity by fine-tuning specific parameters in the seeding, cultivation and selection process.

Results:
The implementation of well-balanced and synchronized parameters in the cell culture procedure cleared us the way to establish two improved approaches (‘Mid Pools’ and ‘Pooled Mini Pools’). Compared to the time consuming ‘Mini Pools’ the modified approaches harbor increased cell populations. This allows shortening the expansion phase up to two weeks thus providing significant benefits in terms of overall timelines. Concurrently, the novel techniques do not seem to exceed the critical population size ensuring the generation of stable pools with high productivities compared to the ‘Mini Pools’ approach. Furthermore, we demonstrate that a titer-based pre-selection during the ‘Pooled Mini Pools’ procedure is a powerful tool to further decrease workload compared to pools selected randomly. We selected top producing cell pools at an early stage of the cultivation process by analyzing their titers by ELISA. This approach not only tightens the timeline but also leads to clearly higher productivities (on average 50% increase compared to non-selected pools).
CELL BASED ASSAYS TO DRIVE DRUG DEVELOPMENT
MICROFLUIDIC 4-ORGAN-CHIP TO APPROACH ADMET PROFILES

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Background and Novelty:
Ensuring good absorption, distribution, metabolism, excretion and toxicity (ADMET) properties is crucial to analyse if a drug reached its intended target and had a therapeutic effect without causing unacceptable toxicities. However, only few drug companies test ADMET characteristics in the early stages of drug discovery and development. Current in vitro models are lacking a systemic approach and therefore fail to predict the interaction of metabolites between organ cultures. Here, we present a new multi-organ approach to overcome this problem.

Experimental Approach:
Four human organ equivalents were combined by a microfluidic flow in a bioreactor the size of a microscopic slide (Fig. 1). The 4-Organ-Chip consisted of two independent microfluidic circuits arranged on two levels, separated by a PET membrane. On the middle of the first, primary circuit, a primary human small intestinal model was inserted. The intestinal tissue was cultured in an integrated cell culture insert and provided a barrier function from the apical side of the intestine to the first circuit, allowing absorption. An on-chip micro-pump enabled the distribution from the basolateral side of the intestinal model to a liver equivalent, where potential substances could be metabolised. The microfluidic channel passed the bottom of the PET membrane, seeded with renal proximal tubule cells. This kidney model separated the first circuit from the second, excretory circuit. In this study, we combined this ADME approach with a skin biopsy to analyse toxicity, but this organ culture could also be replaced by any other organ equivalent, like neuronal tissue, lung tissue or others, depending on the target organ for toxicity.

Results and Discussion:
The combination of the four organs was cultured for up to 28 days and results showed a steady consumption of glucose and low LDH profiles during the complete culture period, providing evidence for a stable coexistence between the four tissues. The constitutive phase I and II enzymes were expressed in liver tissues and stayed constant on protein and mRNA level over the time cultured. The intestinal tissues expressed glucose transporters and its barrier function was proven by expression of tight junction proteins and stable, near to physiologic TEER values. Renal proximal tubule cells showed polarisation, a steady expression of tight junctions and metabolic activity. Thus, a new tool for subsystemic substance testing with a potential for ADMET profiling has been developed.
HT-29 AND CACO-2 REPORTER CELL LINES FOR FUNCTIONAL STUDIES OF NUCLEAR FACTOR KAPPA B ACTIVATION

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Background and Novelty:
The Nuclear Factor kappa B (NF-κB) is a transcription factor which plays a key role in regulating biological processes. In response to signals, NF-κB activation occurs via phosphorylation of its inhibitors IκBs, which dissociate from the NF-κB dimer allowing its translocation to the nucleus, inducing gene expression. Pro and antiinflammatory activities of different pathogenic organisms, probiotics and natural or synthetic compounds correlate directly with NF-κB modulation. In this way, NF-κB activation has direct screening applications for drug discovery for several therapeutic indications. Hence, pathway-specific reporter cell systems appear as useful tools to screen and unravel the mode of action of probiotics, natural and synthetic compounds. Here, we describe the generation, characterization and validation of human epithelial reporter cell lines for functional studies of NF-κB activation by different pro and anti-inflammatory agents.

Experimental Approach:
Two intestinal human epithelial cells were selected: HT-29 and Caco-2. Particularly, Caco-2 cells have the advantage of polarizing, acquiring similar characteristics, as the brush border in the apical membrane, of intestinal cells. Cell lines were stably transfected with a pNF-κB-hrGFP plasmid which contains the GFP gene under control of NF-κB binding elements. Hygromycin B resistant cells were stimulated with TNF-α and those responders, which express GFP, were cloned using a cell sorter. Flow cytometry was used as the main analyzing tool for the characterization and validation of HT-29-NF-κB-hrGFP and Caco-2-NF-κB-hrGFP clones. Firstly, time course kinetics was assayed with TNF-α (50ng/mL) at different time points (from 18 to 144h). The ability to activate NF-κB with different stimuli (TNF-α, IL-1β, and LPS) and the stability of the reporter cell clones were addressed. Finally, the validation of the reporter cell clones was evaluated with different immunomodulatory compounds and potential probiotic strains.

Results and Discussion:
Using the reporter gene strategy, we obtained three reliable and robust reporter cell clones that allow a simple and rapid examination of NF-κB activity regulation through the GFP expression estimation. Three proinflammatory cytokines (TNF-α, IL-1β, LPS) were able to activate the reporter cell systems in a dose-response manner, which corresponds to the activation of the NF-κB signaling pathway. Furthermore, NF-κB p65 subunit was detected in the nucleus by immunofluorescence indicating a direct correlation between NF-κB translocation and GFP expression in treated cells. The robustness of the selected clones was validated through their response to known activators, Lactic Acid Bacteria and a natural cyclic peptide. We observed a dose dependent stimulation of NF-κB in clones upon treatment with the pro-inflammatory cytokines TNF-α, IL-1β, and LPS. Furthermore, TNF-α and IL-1β were able to induce expression of the reporter gene also in polarized Caco-2-NF-κB-hrGFP cells, expanding the range of applications. Regarding the stability of the reporter cell clones, we recommend to use cells with a maximum of 8 passages. As a conclusion we can say that these new biological tools provide an alternative reporter system to the conventional existing ones and have direct screening applications for synthetic and natural compound libraries or potential probiotics discovery.

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HEMATOPOIETIC STEM CELL DIFFERENTIATION-INDUCTION EFFECTS OF POLYPHENOLIC COMPOUNDS OF OLEA EUROPAEA LEAVES

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Background and Novelty:
Hematopoietic stem cells of HSCs are blood cells derived from the mesoderm that give rise to other blood cells, both the myeloid and the lymphoid lineages. Transplantation of HSCs has recently become important in the treatment of blood cancers. HSCs are used to reestablish hematopoietic function in patients whose bone marrow or blood immune system is defective. The function of HSCs can be enhanced by some vitamins and nutrients, and recently, by certain flavonoids. This study evaluated the ability of the major flavonoids present in leaves of Olea europaea or olives to promote HSC differentiation.

Experimental Approach: Phytochemicals present in leaves of olives, oleuropein (Olp), apigenin 7-glucoside (Api7G), luteolin 7-glucoside (Lut7G), or their combination (Comb) were investigated for their potential as hematopoietic stem cells differentiation agents by evaluating their effects on the cell cycle and the differentiated cells identified using specific biomarkers.

Results and Discussion:
The compounds, individually or in combination had positive differentiation-induction effects on HSCs into the myelomonocytic cells and lymphocytes progenitors. Erythroid and megakaryocytic differentiation was confirmed by the expression of the erythropoiesis- and megakaryopoiesis-related genes and by the number of BFU-E and CFU-GEMM in methylcellulose cultures. This study is the first attempt to use major flavonoids present in leaves of Olea europaea or olives in the induction of HSC differentiation.
EMULATING THE HUMAN VASCULATURE IN A MULTI-ORGAN-CHIP PLATFORM

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Background and Novelty:
Our Multi-Organ-Chip (MOC) platform contributes to the ongoing development of \textit{in vitro} substance testing systems with the ultimate aim to replace animal models. At the scale of a microscope glass slide, the two-organ variant (20C) comprises two independent circuits each containing two separate cultivation cavities for any combination of 3D tissue constructs. These cavities are interconnected by microfluidic channels. The incorporated, on-chip micropumps provide pulsatile circulation at a microliter scale – enough to provide oxygen, nutrition to and deplete waste products from the cells. Each circuit contains only 600 µL of volume, enabling autocrine and paracrine crosstalk between the cultivation cavities through the enriched medium. The object of this work is to recreate a continuous endothelial barrier between a subjacent tissue and the medium or a potential blood surrogate. This would be needed for physiological-like interactions, regulation and, eventually, homeostasis within the chip.

Experimental Approach:
To recreate the human vasculature three major aspects had to be addressed – creating a physiological shear environment, enabling the 3D organisation of endothelial cells (ECs) in the entire platform and direct the ECs through cross-talk with other cell types or tissue. The dynamic flow was optimised using micro particle image velocimetry (µPIV). For this, we could establish the use of synthetic polymeric particles as well as human red blood cells (RBCs). Covering the microfluidics’ surfaces with human dermal microvascular ECs (HDMECs) was a first step towards a 3D alignment. Further, we used fibrin scaffolds to enable the self-organisation of ECs to microcapillaries within the cultivation cavities. Cell-cell interactions were created by the addition of adipose-derived stromal cells (ASCs).

Results and Discussion:
The velocimetry-optimised flow enabled physiological cell behaviour of the HDMECs within the microfluidic channels indicated by migration, proliferation and orientation of the cells with the direction of flow. Moreover, the cells exhibited a pronounced expression of actin and typical EC-markers like CD31, VE-Cadherin and vWF under dynamic in contrast to static cultivation conditions. The HDMECs could be cultivated for up to 40 days. Data of the colonisation and the vitality of the HDMEC layer will be presented. The co-cultivation of either HDMECs or human umbilical vein ECs (HUVECs) with ASCs inside a fibrin scaffold led to the formation of microcapillary-like structures. We will address issues to dynamic versus static cultivation environments, the stability of the fibrin hydrogel, along with the influence of the medium constituents on the cell behaviour. Here, we will present one possibility of the 3D recreation of basic structures and features of blood vessels. The results show the feasibility of a near physiological microenvironment in the MOC platform in general. This is a crucial prerequisite for the development to a subsystemic microbioreactor and for infinite tissue culture in future applications.
PERFUSED 2-ORGAN-CHIP SENSITIZATION ASSAY COMBINING PRIMARY DENDRITIC CELLS AND HUMAN EPIDERMIS

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Background and Novelty:
Multi-Organ-Chips provide high potential for the in vitro combination of different cell types and organoids to emulate their physiological in vivo crosstalk. To ensure consumer safety while avoiding animal testing, the cosmetic industry and academic partners strive for reliable and robust in vitro assays that correctly predict the skin-sensitizing properties of compounds. However, none of the existing assays is able to simulate the biological complexity. We used our 2-Organ-Chip as the basis to develop a new perfused skin sensitization assay that combines human keratinocytes and dendritic cells, two essential key players of skin sensitization. The 2-Organ-Chip is a microfluidic system consisting of a microchannel system which connects two culture compartments for cells or biopsies. A peristaltic on-chip micropump enables circulation of medium, allowing for a constant perfusion between the two compartments. Functionally different sensitizers were selected to investigate their effects in our model.

Experimental Approach:
First experiments were performed using a dendritic-cell-only approach in the 2-Organ-Chip. The activation of the cells in the new environment was analyzed based on the induction of co-stimulatory molecule CD86. In subsequent co-cultivation experiments ex vivo human epidermis and dendritic cells were cultivated each in one culture compartment connected by the microfluidic channels. The effects of substance application were comparatively analysed after systemically application (application into the cultivation circuit) and topical application onto EpiDerm™ models.
In addition, experiments were performed to establish an immune competent skin model with resident dendritic cells in combination with transient cells in the cultivation circuit of the 2-Organ-Chip to improve the former described set-up.

Results and Discussion:
Our data show a strong influence of pump pressure and pumping frequency on the activation of dendritic cells. Hence, we established an adequate set up by cultivating the dendritic cells in cell culture inserts, preventing cell activation due to shear stress. Compared to existing sensitization assays, the main advantage of the perfused 2-Organ-Chip sensitization assay is the presence of an epidermis equivalent, partially integrating important parameters such as metabolism and skin barrier function. We compared our data with reference CD86-values from the PBMD (peripheral blood monocyte derived dendritic cells) skin sensitization assay. For identical substances, we observed differences in dendritic cell activation between the PBMD assay and the 2-Organ-Chip perfused assay. Nevertheless, preliminary data on the immune competent skin model showed an optimization of the former separated cultivation of the epidermis and dendritic cells in the presented 2-Organ-Chip sensitization assay.
Furthermore, the first-time cultivation of primary dendritic cells on our microfluidic system is a promising enhancement to integrate immunological reactions on further multi-organ combinations.
IDENTIFICATION AND CHARACTERIZATION OF HUMAN INTERFERON-ALPHA ANTAGONIST COMPOUNDS THROUGH A WISH CELL LINE-BASED REPORTER GENE ASSAY

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Background and Novelty:
Interferons (IFNs) are essential glycoproteins in the immune defense. Nevertheless, different genetic alterations may lead to IFN-α overproduction in human autoimmune diseases, where the cytokine represents the etiology of these illnesses. Thus, blocking the IFN-α increment becomes noteworthy to control them. Consequently, this work proposes the identification and characterization of new antagonists of IFN-α.

The screening of compounds modulating the IFNs biological activity requires versatile, easy and robust methods. Then, a reporter gene assay (RGA), developed in our lab, was used to identify IFN-α activity modulator compounds using a WISH-Mx2/eGFP reporter cell line where the eGFP gene is driven by the specific human type I IFN (hIFN-I) inducible Mx2 promoter. Consequently, the eGFP percentage, induced by hIFN-I addition, is directly correlated with the cytokine. This RGA allowed the analysis of a complete synthetic chemical library, in a low throughput screening mode. Successfully, some compounds capable to decrease the hIFN-α activity were identified. Finally, twelve leads were selected and their specific properties were analyzed.

Experimental Approach:
The identification of new molecules that antagonize the IFN-α activity was carried out using the WISH cell-based RGA. Their characterization in terms of toxicity, effective dose, action on antiviral and antiproliferative activity (AVA and APA) of IFN, effect on cell cycle and evaluation of their combined action was accomplished.

Results and Discussion:
Initially, Z’ factor was measured to evaluate the RGA quality. This factor was estimated in 0.82 ± 0.07, assuring an excellent assay. The non toxic minimum concentration for each compound was calculated and further used for the RGA. Accordingly, twenty-seven compounds demonstrated an inhibitory action on hIFN-α2a activity and twelve out of them, considering their higher responses, were selected to be characterized. Therefore, their effect on AVA and APA was accomplished. These responses were properly correlated with those from the RGA with the exception of the APA where few compounds did not show effects. This result could be attributed to the selectivity of each compound to particularly modulate any of the IFN activities or, also, it depended on the sensitivity of each assay. Furthermore, their effect on cell cycle was studied. Two compounds stopped the cell cycle in G1 phase impeding the DNA synthesis whereas other two increased the S phase demonstrating an increase in DNA synthesis and cell proliferation.

Sixteen compounds combinations were also evaluated. All combinations showed higher inhibition of IFN activity than each compound by itself, demonstrating a cooperative effect. The inhibition of AVA and APA of IFN by the compound combinations correlated with that obtained by RGA. Therefore, the reliability of RGA was confirmed. Additional investigations showed their residual effects keeping their inhibitory action on IFN activity after the combinations were removed. Besides, all of them were able to reverse the activation pathway of the IFN activity.

Taken as a whole, this search for compounds which can block IFN-α activity shows a big potential in view of their therapeutic implications.
A NOVEL SCALABLE STRATEGY FOR IN VITRO RECAPITULATION OF TUMOUR MICROENVIRONMENT AND DISEASE PROGRESSION

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Background and Novelty:
Drug discovery for cancer therapy has faced tremendous inefficacy at advanced stages of clinical trials. The scientific community has been trying to tackle this issue by improving preclinical models for target validation, in which the complexity and heterogeneity found in human solid tumours can be recapitulated. In particular, the tumour microenvironment is known to influence tumour progression and drug resistance through tumour-stroma crosstalk and physicochemical oscillations. Three dimensional (3D) cell models allow maintenance of cell–cell and cell-extracellular matrix (ECM) interactions, which are key elements in tumour tissue signalling, modulating tumour responses to therapeutic agents. However, current 3D cell models are typically generated in non-scalable culture systems, with poor robustness, no control of the physicochemical parameters and use undefined, biologically active matrices.
In this work we present a robust and scalable 3D cell-based model system for long-term in vitro recapitulation of tumour-stroma cross-talk, suitable for investigation of disease progression and drug resistance mechanisms.

Experimental Approach:
MCF7 (human ER+ breast cancer) and H1650 (human NSCLC lung adenocarcinoma) cells were selected for the establishment of breast and lung cancer models, respectively. Tumour aggregates were microencapsulated in alginate together with fibroblasts as the stromal component. Cultures were performed in perfusion stirred tank bioreactors and physicochemical parameters (pO2, and pH) were set at tumour-specific values. Taking advantage of the non-destructive sampling offered this system, culture characterization was performed along the culture time.

Results and Discussion:
After an initial growth phase (up to day 5), cell numbers remained constant during 15 days of culture. Phenotypic characterization at day 5 indicated that, within capsules, fibroblasts surrounded MCF-7 tumour cell aggregates, which showed a luminal epithelial phenotype with partial polarization, recapitulating in vivo tissue organization. After 15 days of culture, deposition of fibers of collagen I secreted by fibroblasts was observed, along with altered cell phenotype within MCF-7 tumour aggregates: reduced membranous staining of E-cadherin, loss of apical ZO1, loss of cell polarity and increased cell migration. Moreover, tumour stroma cross-talk resulted in a differential pattern of cytokine secretion between mono and co-cultures that, together with the collagen accumulation, might be contributing to the described phenotypic alterations, which are typical hallmarks of tumour progression, into more invasive and aggressive stages of cancer.
In conclusion, we have developed a scalable, robust and versatile strategy for long-term in vitro recapitulation of tumour-stroma crosstalk and physicochemical tumour properties providing tools for characterization of disease progression mechanisms, target validation and drug response in different cancer types.

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IMMUNOGENICITY AS A CRITICAL PROPERTY OF THERAPEUTICS: EX VIVO
IMMUNOGENICITY ANALYSIS OF A LONG-LASTING HYPERGLYCOSYLATED
DERIVATIVE OF RHIFN-A2B

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Background and Novelty:
Immunogenicity is the ability of a drug to provoke an immune response and must be analyzed for the approval of biosimilars and new therapeutics. There is growing evidence that repeated dosing of rhIFN-α2b over several months induces neutralizing antibodies (NAb) against the therapeutic in up to 80% of patients, depending on the indication. Moreover, type I IFNs can both induce and unmask sub-clinical autoimmune diseases. In an attempt to prolong its plasma half-life, a hyperglycosylated derivative (rhIFN-α2b-4N) with remarkable pharmacokinetic profile was developed. The aim of this study was to investigate the immunogenicity of rhIFN-α2b-4N through a comparative ex vivo study that also included the endogenous O-glycosylated rhIFN-α2b (rhIFN-α2b-wt) and two commercial versions: rhIFN-α2b and pegylated rhIFN-α2b (rhIFN-α2b-PEG).

Experimental Approach:
Blood samples were taken from 26 healthy donors. Peripheral blood mononuclear cells (PBMC) were isolated from each donor by Ficoll-Hypaque density centrifugation. HLA-DR1 allotypes were determined by Luminex technology. Monocyte derived-dendritic cells (DCs) were generated and used to endocytose and process the IFN variants. Then, antigen-pulsed DCs were incubated with autologous T-cells. After 2 days of incubation, supernatants were collected and analyzed for IFN-γ and IL-4 productions by sandwich ELISA. A stimulation index (SI) criteria was defined as the ratio of cytokine concentrations from protein challenged treated PBMCs and unchallenged PMBCs (excipients). A geometric mean (GM) was then calculated. Positive responses were defined by donors who produced a SI≥GM.

Results and Discussion:
Comparison of the allotypes expressed in the cohort against those expressed in the world population revealed that all major HLA-DR1 alleles were well represented.

T-cell proliferation assays showed that all tested proteins induced both IFN-γ and IL-4 secretion, but at different levels depending on the protein and the donor. IFN-γ secretion by T-cells is characteristic of a Th1 profile and it is associated with an inflammatory response, whereas IL-4 production defines a Th2 phenotype and is associated with antibody secretion. Also, a comparative analysis revealed that 85% of the population developed a Th1 profile against rhIFN-α2b-4N. A similar proportion of responders was observed for rhIFN-α2b-wt. This result demonstrates that those glycosylation sites introduced into the molecule to generate rhIFN-α2b-4N did not increase the rhIFN-α2b immunogenicity in comparison with the cytokine produced by the human body. In addition, rhIFN-α2b and rhIFN-α2b-PEG induced a Th1 response in 45% and 28% of the population, respectively. Interestingly, a different behavior was observed when the Th2 profile was analyzed. In this case, rhIFN-α2b-4N induced the lowest response among all rhIFN-α2b variants (28%). Moreover, the commercial alternatives produced a positive response in a great proportion of the population (72% and 61% for rhIFN-α2b and rhIFN-α2b-PEG, respectively). Taking into account that a Th2 response is strongly correlated with in vivo Ab production, these results are in excellent agreement with the evidence that link anti-IFN-α2b antibody induction and autoimmune disease development with rhIFN-α2b or rhIFN-α2b-PEG therapy, highlighting rhIFN-α2b-4N as a promising candidate for clinical use.
INTEGRATING SKIN AND VASCULATURE IN A MULTI-ORGAN-CHIP PLATFORM

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Background and Novelty:
Tests for drug development require an almost perfect fit with the human (patho-) physiological microenvironment. The majority of skin equivalents currently commercially available are based on static culture systems emulating only human epidermis, or combining epidermis and dermis in so-called full thickness skin equivalents. None of the existing systems contain important elements, such as vasculature, skin appendices or an immune system. Therefore, current in vitro and animal tests are failing to accurately predict drug toxicity. Our Multi-Organ-Chip (MOC) platform is a micro scale bioreactor providing pulsatile dynamic perfusion for microscale organoids. Here, we combine skin equivalents with vasculature in our two-organ variant (2OC). This would be needed for physiological-like interactions, regulation and, eventually, homeostasis within the chip.

Experimental Approach:
The MOC platform provides a constant pulsatile flow of medium via its built-in micropump and ensures oxygen and nutrient supply. The system is filled with up to 600 µL of medium. No external reservoirs need to be attached that would otherwise dilute the enriched medium. Human dermal microvascular endothelial cells (HDMECs), isolated from human foreskin, were seeded into the microfluidic channel system using a syringe. After even cell infusion inside the circuit, the device was incubated in 5% CO2 at 37°C under static conditions for 3 h to allow the cells to attach to the channel walls. A frequency of 2 Hz was applied for continuous dynamic operation and, after 6 days of monoculture, the skin equivalent was added for co-cultivation for another 14 days.

Results and Discussion:
HDMECs exhibited a pronounced expression of actin and typical EC-markers like CD31, VE-Cadherin and vWF under dynamic cultivation conditions. Data of the vitality of the HDMEC layer will be presented. Additionally, in comparison to cultures utilizing conventional static conditions, skin equivalents cultivated in our perfused MOC system together with the endothelial cells showed improved consistency and vitality of most of their structures, rendering the MOC system a useful tool for long-term culture.
USE OF VALERIC ACID TO INDUCE THE CELL CYCLE ARREST AT G1 IN CHO CELL CULTURES: HARNESSING THE CELL CYCLE ARREST FOR THE IMPROVEMENT OF RECOMBINANT ANTIBODY PRODUCTION

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Background and Novelty:
For high-level expression of therapeutic proteins produced by CHO cell culture, cell proliferation is often controlled through inhibiting progression of the cell cycle, which can significantly increase the specific productivity (q_s). Chemical reagents such as sodium butyrate and valproic acid have been widely used to induce the cell cycle arrest in order to increase of q_s in a number of therapeutic proteins. However, these chemical reagents are cytotoxic and induce apoptotic cell death. In an effort to search for less harmful and more effective chemical reagents, cell cycle inhibitors including valeric acid were examined as an effective chemical reagent to induce cell cycle arrest with a concurrent increase of q_s with recombinant CHO (rCHO) cells producing a therapeutic antibody.

Experimental Approach:
Two recombinant CHO cell lines producing a monoclonal antibody (mAb), which were originated from CHO-K1 and DG-44, respectively, were cultured in shake flasks and bioreactors with pH and DO control. Various cell growth inhibitors (curcumin, colchicine, quercetin, DL-sulforaphane, thymidine, phenyl butyrate, lithium chloride, valeric acid, valproic acid) were added to the cultures to evaluate their effects on cell growth, mAb production, and mAb quality. Cell cycle analysis was performed using flow cytometer (BD LSR II) and expression level of cell cycle related proteins was measured using Western blot assay. The mAb quality was investigated with regard to its N-glycosylation, charge variant, and aggregation characteristics.

Results and Discussion:
Among the growth inhibitors tested, valeric acid showed the best production performance for both cell lines. The addition of valeric acid decreased the specific growth rate and maximum cell concentration in a dose dependent manner. However, its positive effect on specific mAb productivity resulted in two-fold enhancement in mAb titer at 1.5mM valeric acid. Valeric induced the cell cycle arrest at G1 phase by inhibiting histone deacetylase 1 (HDAC1). HDAC1 inhibition suppressed the dissociation of pRb from E2F, and blocked the transcription of the cyclins at S phase. Furthermore, valeric acid did not negatively affect the mAb quality in regard to N-glycosylation, charge variant, and aggregation characteristics. Taken together, valeric acid is an effective chemical reagent to increase mAb production of rCHO cells.
HUMAN HEPATIC 3D CELL MODELS WITH EXTENDED AND IMPROVED FUNCTIONALITY FOR LONG-TERM TOXICITY TESTING

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Background and Novelty:
The development of human cell models that can efficiently predict hepatic drug metabolism and cope with the reproducibility and scalability required for applications in preclinical development poses a significant effort in biotechnology. Primary cultures of human hepatocytes (HH), the preferred model for in vitro toxicity testing, are hampered by the short life span and phenotypic instability in two-dimensional cultures (2D). Herein, we apply strategies that resemble the liver microenvironment, such as three-dimensional (3D) culture and coculture with non-parenchymal cells to overcome some of these limitations.

Experimental Approach:
Hepatocytes isolated from resected human liver tissue were co-cultured with bone marrow-derived human mesenchymal stem cells (BM-MSC) as spheroids in stirred tank bioreactors (STB). A dual step inoculation strategy was used to allow the assembly of cell-cell contacts between parenchymal liver cells and, after micro tissue formation, the addition of BM-MSC. The cultures were maintained in automated STB, with controlled culture parameters and perfusion operation mode to minimize environmental shifts. The co-cultured spheroids were exposed to Acetaminophen (APAP) in a repeated dose regimen to evaluate long term toxicity testing.

Results and Discussion:
Extensive characterization indicated that the hepatocyte polarization and morphology, as well as the mesenchymal phenotype were maintained throughout the culture period. The secretion of collagen type I by MSC led to a collagen overlay around the spheroids, contributing to compact spheroids, as visualized by scanning electron microscopy. The viability and hepatocyte phenotypic stability were enhanced in co-cultures in comparison to spheroid mono-cultures and the biosynthetic hepatocellular functions (albumin and urea secretion) were not affected by the presence of MSC. The gene expression stability of CYP450 isosforms 1A2, 3A4 and 2C9 and also UGT1A1 was enhanced, CYP3A4 and CYP1A2 activity was inducible in the 2nd week of culture and the excretory activity was maintained in co-cultured spheroids, making them suitable to assess hepatotoxicity. Consistently, long term administration of APAP showed that the co-cultured spheroids are sensitive to APAP-induced toxicity. Moreover, the paracrine crosstalk of the co-cultures was depicted, indicating that the secreted factors such as IL-6 may contribute to improve hepatic functionality. In conclusion, the novel 3D strategy based on co-culture of HH and MSC results in a human hepatic cell model with extended and improved functionality, applicable for long-term toxicity testing. This strategy might be extended to other hepatic cell sources for the large-scale production of human cell models for prediction of liver toxicity in bioreactors, with the advantage of having an extensively characterized process for maintaining reproducibility in vitro. Moreover, this work may also be relevant to understand the interplay between BM-MSC and HH for possible therapeutic applications.
SCREENFLEX - TAILORED CELL LINES FOR BIOASSAYS

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Background and Novelty:
Stable cell lines expressing drug targets are major tools in primary drug screening. However, if the protein product (in its native form) is hard to detect and cannot serve to select for appropriate cells, conventional transduction protocols can be connected with insufficient expression characteristics. Drug screening campaigns take often advantage from reporter systems as reliable read-out. Therefore, the cell line of choice needs to co-express the reporter system along with the desired drug target. Furthermore, the use of endogenous or signaling specific promoters can be advantageous over classical promoter elements, since they offer a higher physiologically relevance. The classical selection of an appropriate cell line within a reasonable timeframe can be challenging.

Experimental Approach:
Our unique SCREENflex technology was developed exactly to meet these requirements – fast and reliable generation of functional cell lines with classical or endogenous promoters.
SCREENflex is based on “master cell lines”. We have shown that this technology can be adapted to various cell types, including HEK293, CHO, BHK and NIH3T3. Using a reporter expression cassette randomly tagged chromosomal integration sites were generated and screened for optimal expression characteristics. In a molecular “cut and paste” step the reporter cassette can easily be exchanged for any drug target of interest (GPCR, kinase, ion channel). In parallel we have co-integrated several reporter systems into the same locus in a single transduction step - thereby converting the master cell line to the final screening cell line. Furthermore we could show, that SCREENflex is also suitable for the generation of drug screening cell lines with endogenous promoters. As an example we generated a cell line harbouring a reporter cassette driven by a MX-promoter, which can serve as an infection model.

Results and Discussion:
Summarizing the presented results we show, that our SCREENflex technology can be used to establish various cell lines, ready for assay, within a guaranteed 4 weeks time-frame – without any screening efforts.
CELL-TO-CELL HETEROGENEITY IN ONSET OF TRANSGENE EXPRESSION FROM SYNTHETIC TETRACYCLINE-DEPENDENT PROMOTERS

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Background and Novelty:
Synthetic, tetracycline dependent promoters are frequently used in mammalian cells to restrict the expression of toxic proteins or to control the level or onset of gene expression. Gradual as well as positive feedback designs have been used for gradual or bimodal expression patterns, respectively. Routine applications of such synthetic expression systems are based on steady-state measurements while it has not been investigated how these steady-states are realised at the single-cell level. We focussed on the elucidation of the kinetics of doxycycline-controlled synthetic modules as a paradigm. The results reveal a so far non-appreciated heterogeneity in the onset of expression from Tetracycline-dependent promoters. Further, we show that posttranslational control of expression results in much more homogenous onset rates. Applying this method, a so far unknown regulatory mechanism in the interferon activation cascade could be identified on single cell level.

Experimental Approach:
Various classical as well as autoregulated synthetic Tet-driven transcripitional units driving fluorescent reporters were designed and introduced into NIH3T3 fibroblasts. As an alternative mechanism of transgene regulation, we employed the DHFR/Trimethoprim system to control proteosomal degradation of proteins. The kinetics of the onset of reporter expression was analysed in genetically identical stable cell clones on a single cell level by flow cytometry and time lapse microscopy.

Results and Discussion:
Following gene expression in single cells we observed a (gradual) increase of transgene expression within the first 48 h upon activation as determined by flow cytometry. Time-lapse microscopy revealed that the onset of transgene expression was highly variable in individual cells. While in some cells onset was visible as early as 3 hours after induction, others started gene expression as late as 48 hours. Interestingly, cassette design had a minor influence on this cell-to-cell heterogeneity. Virtual synchronisation was performed to investigate if the onset is correlated to the cell cycle. Statistical analysis revealed no significant correlation indicating that the onset is not restricted to particular cell states. In contrast, much higher synchronicity of transgene expression could be realised using a posttranslational regulation system that relies on ligand induced stabilisation of a target protein.

Together, the study highlights cell-to-cell heterogeneity in early onset of transgene expression in expression systems regulated on transcriptional level. Given the inherent heterogeneity of the Tet system kinetic data, correlation studies at the population level are prone to misinterpretation and thus should be critically evaluated. Thus, the inherent temporal variability of transcriptionally regulated synthetic transgene expression systems has to be considered for kinetic and correlative experimental applications.
ANTI-HOST CELL PROTEIN ASSAY DEVELOPMENT FOR THERAPEUTIC MONOCLONAL ANTIBODY BIOPROCESSING USING MAMMALIAN CELLS

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Background and Novelty:
Recombinant therapeutic proteins are usually produced by cell culture technology using genetically modified host cell lines. Besides the protein of interest, indigenous proteins so called host cell proteins (HCPs) are co-expressed. HCPs constitute a major subgroup of process-related impurities and the presence of HCPs in the drug product may generate potential clinical adverse effects. HCPs are typically quantified using immunoassays performed in an enzyme-linked immunosorbent assay (ELISA). Polyclonal anti-HCPs antibodies used in these assays are frequently generated by immunization of animals with the relevant expression cell line and standard cell culture conditions. Although generic ELISA kits are commercially available to quantify HCPs from different recombinant systems, a specific assay for biologicals using a mock cell line approach and the relevant upstream conditions, is required before drug registration and commercialization.

Experimental Approach:
The assay developed is cell line and process specific and can be likely used from Phase I throughout product registration. This is a valuable opportunity to save resources and time as the same assay can be used for various projects and various phases of development. Here we describe the first step of the development of a platform HCP-ELISA assay for UCB’s biopharmaceuticals produced in a platform Chinese Hamster Ovary (CHO) cell line, i.e. the production of mock material containing the HCPs using a null cell line (production cell line transfected with the expression vector containing a non-encoding product sequence). First, we assessed the null cell line growth and metabolic profiles at 2L scale. In these studies, the process was optimized to obtain comparable cell growth and metabolite profiles between the null cells and different producing cell lines using a design of experiment approach. Then, we performed a 80L scale fermentation using the best conditions developed at small scales and used the null cells to immunize animals. Different parameters have been monitored throughout the cultures at small and larger scales: viable cell density, cell viability, offline pH, offline dissolved CO₂ levels, glucose and lactate levels, glutamine and ammonia levels. In order to increase HCP coverage and limit variability, material for immunization was generated from the harvested clarified cell culture fluid (CCCF), the cell extract and concentrated harvested cell culture fluid. The concentrated harvested cell culture fluid was obtained from a single Ultrafiltration (UF) step with a low molecular weight cut-off membrane, followed by a diafiltration into an appropriate buffer. Moreover, a two dimensional difference gel electrophoresis (2D-DIGE) method was used to examine the HCP composition in the harvest stream of CHO cell cultures produced at different scales. Last but not least, HCP profiles from the null cell line and process performances have been compared to three different producing cell lines.

Results and Discussion:
After optimization, the 80L process performance and analytical results showed that the cell growth, metabolite and HCPs profiles were comparable between the null cell lines and the three producing cell lines. In addition, we demonstrated the scalability of the process as comparable growth, metabolite and HCPs profiles were obtained at the different scales tested.
CD MEDIA FOR CHO CELLS. HOW DO THEY COMPARE?

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Background and Novelty:
Several highly productive chemically defined (CD) media and feed platforms have become commercially available in CHO cell fed-batch culture to help reducing process development activities in preclinical phase and phase I. This shortens the time for startups or companies that do not have their in house media development department to reach commercially relevant product titres. Different media and feeds are available but a systematic comparison of these media with respect to growth, productivity and metabolism is still missing. The aim of this study is to systematically compare the existing culture platforms and to better understand the linkage between cell culture indicators and media/feed formulations in order to balance the cells’ need in further process development.

Experimental Approach:
We used 2 distinct behaving sub-clones (a good grower and a good producer) of CHO-K1 cells as model tools to systematically compare 4 media with 3 feed systems. Four commercially available CD media (OptiCHO™, FortiCHO™, ActiCHO™ and CD-CHO™) with several CD feed combinations (Efficient Feed™ A and B, EFA/B; Efficient Feed™ C, EFC; Acti feed™-A and Acti feed™-B, Acti a/b) are compared for fed-batch cultures. The daily bolus feeding was based on glucose concentration solely. The effect of using from lean to rich media and feed systems and the combination of them was compared and discussed. Culture performance was evaluated based on extracellular metabolite profiles, cell growth, culture longevity and monoclonal antibody (mAb) production. Furthermore, we evaluated essential amino acids’ (EAs) balancing of different feeding systems by comparing their abundances in feeds to their specific consumption rates during fed-batch cultures respectively.

Results and Discussion:
Different cell lines showed distinct preferences to media and feeds platforns. FortiCHO + EFA/B (a lean medium with a lean feed) showed the best result for the good growing clone while ActiCHO+Acti a/b (a rich medium with a rich feed) was preferred by the good producing clone. Feed EFA/B showed balanced amino acids levels with its glucose contents during fed-batch cultures. Actifeed system displayed overfeeding of most of detectable nutrients starting from culture stationary phase which is due to its rich content. However, it leaves more room for optimization. The EAs consumption rates of the good producing clone are generally 1.5 times higher than the good growing clone, however, the ratio of them were similar between two clones. Interestingly, the ratio of EAs consumption rate changed dramatically when culture progressed from growing to stationary phase. Considering the potential for further optimization, we propose glucose separate feeding strategy as well as differently tailored feeds to discriminate growing phase and stationary phase by using Actifeed systems.
EXPRESSIÓN DE ALGODON DE RABÍA GLICOPROTEÍNA UTILIZANDO EL SEMLIK FOREST VIRUS (SFV) EN 6 LÍneas CELULARES

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Background and Novelty:
The gene expression system derived from Semliki Forest Virus (SFV) has been widely used in recent times for expression in large quantities of many proteins, when compared with other systems. The aim of this study was to optimize the capacity of this viral vector to express proteins in different mammalian cell lines, using as target, the rabies virus glycoprotein (RVGP). To evaluate the expression of RVGP in different cell lines using the SFV system (SFV-RVGP), using the ELISA and indirect immunofluorescence (IFI); to determine the best conditions for cell culture and viral infection for the expression of the heterologous protein.

Experimental Approach:
Two different plasmids were used: an expression plasmid containing SFV genes coding for nonstructural proteins and the RVGP gene, and a helper plasmid containing SFV genes coding for structural proteins. In vitro transcription was performed and RNAs were co-transfected in BHK-21 cells, for generation of SFV-RVGP recombinant virus. They were then activated and used to infect BHK-21, Huh 7.0, Vero and L929 cells, and J774A-1 and IC-21 macrophages cells and induce the heterologous protein (RVGP). Expression evaluation was done by ELISA, 24 and 48 h after infection with MOI (Multiplicity of infection) 1, 10, 15 and 50. In IFI method, the production of RVGP was evaluated only at 24 h, with MOI 1 and 10.

Results and Discussion:
Using the SFV-RVGP method of expression, we evaluated the RVGP production in different cells (BHK-21; Huh-7; L929; Vero), using different MOIs. The experiments were performed in duplicate in 6 wells plate in CO₂ incubator at 37°C. The cell inoculum was of 7x1E5 cells/well with a working volume of 2 mL. Based on the results of the ELISA, BHK-21 cells showed the best rate expression RVGP, and the MOI 1 proved better than the others. A similar phenomenon occurred with Vero cells, but with lower production. However, in the process of infection of Huh-7 cells, and L929 cells, the expression of RVGP increased as the MOI was increased, being greater when used MOI 50. Another relevant fact is that the Huh-7 cells showed higher expression rate 48 h after infection, unlike the other tested strains obtained indices that best after 24h. When analyzed by IFI, the same patterns of expression were observed, especially for the high percentage of expression in BHK-21 lines, Huh-7 and HEK-293T. The both macrophages cells did not show RVGP expression and the quantification of SFV-RVGP in cell medium indicates no occurrence of virus entry.
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A METHOD TO DETERMINE AND SIMULATE THE PERMEATION THROUGH A GEL MATRIX IN A MULTI-ORGAN-CHIP

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Background and Novelty:
The Multi-Organ-Chip (MOC) is a new tool for modeling segments of human organism in a size range of a microscope slide. One future application of this system is the drug testing in vitro. In this study the permeation of different substances through the MOC is investigated in order to optimize a full skin model.

Experimental Approach:
A method was established in multiwell plates to determine the permeation/diffusion coefficient of a fluorescent tagged substance through a gel matrix in a transwell®-system (Corning, Lowell, MA) and then transferred on the MOC.
The permeation experiment was performed with fluoresceine sodium salt trough an agarose gel within the transwells®. It was executed in an incubator at 37 °C on a shaker, to mimic the cell culture cultivation conditions. The simulation program COMSOL Multiphysics was used to determine the diffusion coefficient. For this purpose the initial conditions and geometry of the permeation experiment was integrated in the simulation. The Chemical Reaction Engineering Module was used to simulate the diffusion process.
In the next step the simulation was transferred on the MOC. The diffusion coefficient of the previous calculation and the CFD Module was additionally used to determine the substance distribution through the channel in the next insert of the chip. Also a permeation experiment was done in the MOC. The simulation data was verified by further permeation experiments.

Results and Discussion:
With this demonstration it was shown that the simulated diffusion coefficient from an easy permeation experiment in a transwell®-system can be transferred on the MOC simulation. Permeation experiments in MOC prove the correctness of the simulation.
Actual experiments with a co-culture of fibroblasts and keratinocytes are carried out to show the feasibility of the full skin model.
VERIFICATION OF A NEW BIOCOMPATIBLE SINGLE-USE FILM FORMULATION WITH OPTIMIZED ADDITIVE CONTENT FOR MULTIPLE BIOPROCESS APPLICATIONS

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Background and Novelty:
Single-use bioreactors have gained rapid acceptance in biopharmaceutical manufacturing worldwide. Meanwhile, disposable bag technology is well established for culture media, buffer and intermediate storage and for the production of therapeutic antibodies, proteins and vaccines. However, one of the most cited disadvantages is the risk for release of potentially toxic or inhibitory substances, generated during gamma-irradiation, which can adversely affect cell growth and product titers [1]. It could be demonstrated that the poor cell growth observed in certain bags is due to bis (2,4-di-tert-butylphenyl)phosphate (bDTPP), a degradation product of a trisarylphosphite processing stabilizer tris (2,4-di-tert-butylphenyl)phosphate (TBPP, supplied under the trade name Irgafos 168 and other denominations as an antioxidant additive) present in many polyethylene (PE) based polymer films [2, 3]. In this study, we have focused on the in vitro detection of potentially cytotoxic leachables originating from the new polyethylene (PE) multilayer film called S80. This film was developed with an optimized additive content for multiple bioprocess applications, for example, storage of process fluids, mixing, and cell culture bioreactors.

Experimental Approach:
For the biological film qualification extractable studies were performed with a protein hydrolysate and animal-derived component free cell culture medium in gamma-sterilized (50 kGy) two-dimensional 0.8L bags. The extraction was performed for three days under “worst case” conditions (surface area to extraction volume ratio of 3 cm²/mL). The impact of critical film extrusion process parameters on cell growth was assessed in a DoE study. The influence of polymer aging on the biological performance was performed with an accelerated aging study. Furthermore, extended media extraction trials were carried out at cold room conditions (4-8°C). The detection of a potential release of cytotoxic leachables into the media was demonstrated by a standardized cell based assay with CHO-DG44 cells (Cellca GmbH, D) over 3 days.

Results and Discussion:
In contrast to a non-optimized film formulation (NC), our data demonstrate no cell grow inhibition with the optimized S80 film formulation under any of the investigated conditions, as shown for variation of interacting extrusion parameters in DoE study, an aging study, and for an extended media extraction trial. Thus, the film qualification was successfully verified in a sensitive standardized cell based assay for detecting toxic leachables.
THE INFLUENCE OF BISPHENOL A (BPA) ON MAMMALIAN CELL CULTIVATION

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Background and Novelty:
Bisphenol-A plays an important role for industry and is abundantly used as a primary raw material for the production of plastics and resins (e.g. Polycarbonates and epoxy resins). Polycarbonates (PC) have wide application in consumer products, medical devices, glazing applications, film and the electronics industry. The global BPA production is expected to exceed 5.4 million tons by 2015 according to in-demand report by Merchant Research & Consulting. BPA was determined to be moderately/slightly toxic to fish and invertebrates, however, it was not recognized as a mutagen. Recently it has been identified as a weakly estrogenic chemical that can modify natural endocrine functions and it can cause adverse effects on human health and wildlife. As BPA is commonly found in all kinds of aquatic and terrestrial environments because of its widespread use, it has become very important to elucidate its influence on mammalian cell cultivation as it has been shown that BPA can induce chromosome aberration in mammalian cells at a concentration range of 400-450 µM. There is an ongoing discussion if BPA-free products are needed for more efficient cell cultivation and better product quality. Therefore, we analysed systematically the effects of BPA on three different mammalian cell lines (CHO, CAP and hybridoma cells) by comparing BPA-free Poly-Ethylene Terephthalate Glycol (PETG) and PC shaking flasks.

Experimental Approach:
For comparison of PETG and PC we focused on the influence of BPA on cell count, viability and metabolic parameters such as glucose, lactose, ammonia and others. In addition, we determined the product concentration and aggregation behaviour of the target proteins expressed by these cell lines and the BPA concentration within the medium caused by leaching.

Results and Discussion:
We observed that BPA can be detected already in Milli-Q water and every medium used for cultivation. Highest BPA concentrations were observed in DMEM when used in PC flasks. Interestingly, the levels of BPA decreased with cultivation time most probably due to an uptake by the cells. However, our results indicate that the concentration of BPA caused by leaching over the time can be neglected as the observed concentrations were <7 µg/L. Within this concentration range no significant influence on cell viability, growth and product concentration as well as on aggregation could be observed. Additionally, we performed EC50 studies with up to 3 mM (solubility limit) to determine the toxic concentration of BPA for our cell lines. These experiments revealed that no toxicity could be observed up to concentrations of 300 µM BPA. We can exclude apoptosis as the Nicoletti assay showed no difference between cells of control and BPA group. On the other hand, a WST-1 assay showed that the metabolic activity of the cells was clearly decreased by mM concentrations of BPA. Hence, the use of BPA-free flasks is not beneficial compared to PC shaking flasks as the concentration of leached BPA in all flasks tested in our study was far below the critical concentration.
RAW MATERIAL CHARACTERIZATION: EFFECT OF TRACE METAL VARIABILITY ON PROTEIN GLYCOSYLATION

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Background:
Trace metal variability has been observed in cell culture media. SAFC’s raw material characterization program has identified that this trace metal variability is typically caused by raw materials used during manufacturing. The variability is seen at ppb concentrations for most trace metals. This low concentration can still potentially impact a biological system.

Methods:
Using an intact mass glycosylation assay, dose response, and Design of Experiment screening with an industry relevant CHO cell line, SAFC has shown the impact of 16 trace metals on our biological systems critical protein quality attributes. Statistical and multivariate analysis has been used to confirm the significance of this impact.

Results and Discussion:
This analysis has identified Fe and Mn at high concentrations can reduce the percent G0F and increase the G1F and G2F glycoforms. The effect of other trace metals on glycosylation was determined to not be significant using ANOVA analysis.
3D CULTURE CONDITIONS ENABLE RE-ESTABLISHMENT OF HEPATIC FUNCTIONS OF IN VITRO EXPANDED HEPATOCYTES

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Background and Novelty:
Hepatocytes are highly specialized cells that display a number of unique metabolic functions. These properties render hepatocyte cultures an attractive option for animal toxicology testing of compounds, screening for new drugs as well as regenerative approaches. Primary hepatocytes display a restricted expansion capacity \(\textit{in vitro}\) and a limited availability, while classical hepatic cell lines have lost the relevant functions, raising the need for improved in vitro models. We recently established hepatocyte cell lines by a novel immortalization strategy that preserves hepatic functions to a certain level. Here, we demonstrate that the properties of these cell lines are sensitive to culture conditions and can be increased by cultivation in the 3D culture conditions.

Experimental Approach:
A novel screening approach was used to identify gene combinations that enable the immortalization of primary hepatocytes. The generated cell lines were characterized in 2D and various 3D culture conditions as well as for their hepatic phenotype.

Results and Discussion
We generated novel hepatic cell lines based on five genes recently identified to support hepatic cell proliferation. While these cell lines display a certain level of hepatic markers we subjected the cells to various culture conditions to better mimic the physiological environment. We investigated if these cell lines have the capacity to self-assemble and form 3D structures in spinner flasks. Similar to primary hepatocytes, most of the newly established cell lines form 3D clusters under these conditions. In contrast, Hepal-6 cells failed to establish aggregates. Upon culturing the cells up to 10 days the hepatic phenotype significantly improved to levels comparable with primary hepatocytes cultured in spinner flasks. Moreover, histological analysis revealed that bile canaliculi were formed suggesting that these cells may have the potential for compound testing. Finally, we confirmed that the cells are capable to integrate into the liver parenchyma. We transplanted the immortalized cells into liver-diseased mice and could confirm the integration of the immortalized cells into the liver tissue. Together, this demonstrates that the in vitro expanded cell lines are capable of forming cell-cell contacts necessary to establish 3D structures \(\textit{in vitro}\) and \(\textit{in vivo}\) which correlate to the re-establishment of relevant hepatic function.
LINKING PROCESS ENGINEERING TO PRODUCT QUALITY
OPTIMIZATION OF PRODUCT QUALITY AND YIELD IN HIGH-PERFORMING FED-BATCH AND PERFUSION CHO CELL CULTURES

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Background and Novelty:
Improving productivity and controlling the product quality attributes can be a challenge during process development. In this presentation, we summarize results from studies on two human monoclonal antibodies (IgG1) produced in CHO cultures in fed-batch and perfusion modes using different bioreactor systems and technologies. Product quality was investigated with regard to molecular size, glycan profile, and charge variants. We show that there are means to improve both cell growth and production, while reducing product aggregation as well as charge and glycosylation heterogeneity of your product.

Experimental Approach:
High-cell density fed-batch culturing was performed in both conventional stirred-tank bioreactors and single-use WAVE Bioreactor™ systems. The process performance was evaluated in 5 L, 10 L, 25 L, and 100 L scales. The effect of temperature, feeding regime, and pH on IgG production and quality was investigated. Additionally, a perfusion process using two different filter-based cell retention systems was developed and evaluated at 1 L and 3 L scale.

Results and Discussion:
In fed-batch cultures, cell growth and viability as well as IgG production, glycan distribution, and aggregate levels were consistent and independent of bioreactor system or scale. However, an increase in acidic charge variants was observed over the fed-batch culture period. By reducing culture temperature, IgG production was increased by 50%, while acidic charge variants and aggregate levels could be reduced. The glycan profile could be altered by shifts in culture temperature or feed rate, whereas culture pH had no impact. In the perfusion cultures, acidic charge variants were lower compared with in fed-batch cultures. Both charge variants and glycan profile were consistent over the perfusion culture period. The studies summarized here increase the understanding of the impact of process conditions on cell culture performance and product quality.
AMINO ACID AND GLUCOSE METABOLISM IN FED-BATCH CHO CELL CULTURE AFFECTS ANTIBODY PRODUCTION AND GLYOSYLA TION

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Background and Novelty:
Fed-batch Chinese hamster ovary (CHO) cell culture is the most commonly used process for bioprocessing of IgG. To the best of our knowledge, this is the first study that presents an integrative understanding of cell growth, metabolism, IgG titer and glycosylation in correlation with the dynamics of glucose and amino acid consumption when optimization of fed-batch culture such as a change in media and feed occurs. This study provides us insight of obtaining higher IgG titer and better controlling of N-glycosylation.

Experimental Approach:
In this work, different fed-batch processes with two chemically defined proprietary media and feeds were studied using two IgG-producing cell lines.

Amino acid and glucose consumption, cell growth and metabolism, IgG titer and N-glycosylation patterns were analysed.

Result and Discussion:
Our results indicate that the balance of glucose and amino acid concentration in the culture is important for cell growth, IgG titer and N-glycosylation. The ideal fate of glucose and amino acids in the culture could be mainly towards energy and recombinant product, respectively. Accumulation of NH₄⁺ and lactate as a consequence of unbalanced nutrient supply to cell activities inhibits cell growth. Amino acids with the highest consumption rates correlate with the most abundant amino acids present in the produced IgG, and thus require sufficient availability during culture. In certain cases, the presence of Man5 glycan can be linked to limitation of UDP-GlcNAc biosynthesis as a result of insufficient extracellular Gln. However, under different culture conditions, high Man5 levels can also result from low α-1,3-mannosyl-glycoprotein 2-β-N-acetylglucosaminyltransferase (GnTI) and UDP-GlcNAc transporter activities, which may be attributed to high level of NH₄⁺ in the cell culture. Furthermore, galactosylation of the mAb Fc glycans was found to be limited by UDP-Gal biosynthesis, which was cell line and culture condition-dependent. Extracellular glucose and glutamine concentrations and uptake rates were positively correlated with intracellular UDP-Gal availability. All these findings are important for optimization of fed-batch culture for improving IgG production and directing glycosylation quality.
STIRRED TANK REACTOR EXPANSION OF hMSC ON MICROCARRIER IN CHEMICALLY DEFINED MEDIUM – CHALLENGES OF EXPANSION AND HARVEST ACCORDING TO GMP AND PAT

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Background:
Cell therapy has evolved into a distinct healthcare sector observable by an increasing number of clinical trials and FDA-approved commercial products. As stem cells are a complex and viable product, their manufacturing has to meet several requirements. Production in a Good Manufacturing Practice (GMP) environment and compliance with Process Analytical Technology (PAT) over the whole expansion process is challenging. One important cell source for clinical application are hMSCs (human mesenchymal stem cells). To enable their use as advanced therapy medicinal products (ATMP), high quantities of undifferentiated cells with high viability are required.

Experimental Approach:
Stem cell expansion systems using microcarrier are presented. Expansion processes up to 2.4 L were established using stirred tank glass reactors and a disposable bioreactor. Primary hMSC and a genetically modified cell line hMSC-TERT were expanded in a new chemically defined medium, harvesting was performed using recombinant trypsin variants and novel enzymes from non-animal origin. Stem cell quality was confirmed according to the criteria of the International Society for Cellular Therapy (ISCT).

Results and Discussion:
hMSC-TERT and prim. hMSC were successfully grown in stirred tank reactors. In both, glass and single-use system, cell concentration up to 4 x10^6 cells/L for hMSC-TERT and 2.9 x10^6 cells/L for prim. hMSCs were achieved. Cells were harvested with high viability; differentiation was successfully performed in osteoblasts, adipocytes and chondrocytes. Further stem cell fate was determined over surface markers (according to ISCT). The usage of a peptidase of non-animal origin showed promising results in detachment yield and viability especially for cell harvest from dynamic bioreactor systems. Cell growth after enzyme treatment was not influenced.
To ease the approval of hMSC as ATMP, an alternative to serum-containing media, a fully chemically defined medium is needed. A new developed growth medium showed promising results in hMSC attachment and growth on protein-coated surfaces.
New enzymes for cell harvest and new expansion media further promote the production of these cells in an ATMP production approved environment.
To go along with the quality by design (QbD) approach, online monitoring of viable cell mass in stirred bioreactor was performed with dielectric spectroscopy.
Performing the whole hMSC manufacturing process, including inoculation, expansion under GMP and PAT is challenging. Dynamic bioreactor systems offer a good opportunity to achieve large amounts of high-quality hMSCs under controlled and monitored process conditions. New enzymes for cell harvest and new expansion media further promote the production of these cells for clinical application.
PROTECTION OF BIOREACTOR CULTURE FROM VIRUS CONTAMINATION
BY USE OF A VIRUS BARRIER FILTER

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Background and Novelty:
Protection of bioreactors from viruses and mycoplasma remains a challenge. While the risk of these contaminations is known, and several recent high profile cases have raised awareness of this risk, many bioreactors remain unprotected. Currently available technologies, such as high-temperature short-time treatment and ultraviolet light treatment, can be difficult to implement due to footprint, efficacy, and media compatibility issues. Size exclusion nanofiltration may be a more ideal technology for virus and mycoplasma protection, potentially offering advantages in robustness, scalability, small footprint, and media compatibility. However, current virus removal filters, designed for monoclonal antibody purification, are generally poorly suited for cell culture media processes due to the large membrane surface areas needed to achieve adequate flow and capacity in a reasonable timeframe. Here we evaluate a novel virus barrier filter specifically developed for cell culture media applications.

Experimental Approach:
The new virus barrier filter was evaluated for retention of virus, bacteria and mycoplasma and for effects on cell culture growth and product quality. Flow rate and capacity were benchmarked against existing commercially available virus membranes in order to compare both the performance and economics for the filtration of cell culture media and feeds.
Retention of microorganisms was performed at constant pressure with both media and buffer utilizing seven microorganisms (viruses, bacteria and mycoplasma). Retention testing was performed in lab scale devices with typical membrane samples expected to give representative performance.
Media and feeds filtered with virus barrier filtration were compared to controls and used to culture recombinant mAb producing CHO cells under fed batch conditions. Cultures were evaluated for differences in cell growth, titer and product quality. 1H-NMR at 500 MHz and reverse-phase LC-MSMS were used to assess any effects of filtration on media components.

Results and Discussion:
Virus barrier filtration had high levels of retention for microorganisms. Full retention was achieved for bacteria and mycoplasma (>8.0 LRV - Log Reduction Value) while LRVs of approximately 5.0 were achieved for viruses. Over a six hour filtration process, the new virus barrier had a volumetric throughput approximately 3 to 30 times higher than the commercially available filters tested, with Viresolve® Pro being the second highest performing virus filter evaluated.
Multidimensional analysis of the performance of filtered media in recombinant mAb-producing cell cultures revealed no impact. Media and feed components were unaffected by filtration through the virus barrier filter as evaluated by 1H-NMR and LC-MSMS. Cultures showed no differences in cell growth or titer. Secreted antibody showed no differences aggregation profiles, charge variants or glycosylation patterns.
Evaluation of the virus barrier filter indicated that high levels of virus, bacteria and mycoplasma removal can be maintained while providing greatly improved flow rates and capacity for cell culture media and feeds. Virus barrier filtration had no impact on cell culture media and feed composition. Cell growth, titer, productivity, and protein quality were also unaffected. These data suggest that virus barrier filtration can be a practical and economical risk mitigation operation for the protection of bioreactor cultures.
THE EFFECT OF NUTRIENT FEEDING ON CELL GROWTH AND RECOMBINANT PROTEIN PRODUCTION IN CHO CELLS: MECHANISTIC PERSPECTIVES FROM METABOLITE PROFILING

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Background and Novelty:
Chinese Hamster Ovary (CHO) cells are the main expression platform for production of recombinant therapeutic proteins. It has been suggested that CHO cells have a partially truncated TCA cycle and improvement of the efficiency of the TCA cycle is hypothesized to improve the productivity of recombinant protein production. Improving the quantity and quality of protein production in CHO cells requires an understanding of how recombinant protein production is related to cell growth and metabolism and whether specific metabolic events predict the balance between cell growth and secreted recombinant protein production.

Experimental Approach:
This research examines metabolism related to growth and productivity of CHO cells. The relationship between growth, recombinant protein production and extracellular metabolite profile of CHO cells (assessed by methods established in our laboratory, Sellick et al [2011] Nature Protocols 6: 1241-1249) was examined in response to supplementation with nutrients (singly and in combination) that enter the TCA cycle (asparagine, aspartate, glutamate, pyruvate and glucose).

Results and Discussion:
The results showed that cells fed asparagine increased maximum cell density 1.5 fold and that the cells died earlier in batch culture than controls but there was no effect on overall recombinant protein yield. This observation was similar to the result of glucose addition where maximum cell density was increased and the cell died earlier without any effect on recombinant protein yield. In case of aspartate, glutamate and pyruvate additions, there were no effects on cell growth and recombinant protein production. Interestingly, the combined addition of asparagine and glucose had an additive effect on cell growth and recombinant protein production. The stationary phase of the cells was prolonged by four days and the recombinant protein production was increased two-fold. In terms of extracellular metabolite profiles, cells fed asparagine or asparagine plus glucose produced less lactate, glycerol and sorbitol than unfed cells and also showed enhanced production of alanine and use of amino acids in general. The accumulation of intermediates of the TCA cycle (citrate, isocitrate, fumarate and malate) increased in the medium but the combination of glucose with asparagine resulted in a greater accumulation of TCA cycle intermediates than asparagine alone. These observations did not occur on the cells supplemented with glucose alone. It is attractive to speculate that increased TCA cycle flux supported increased recombinant protein production. In conclusion, this study has shown a correlation between medium accumulation of increased TCA cycle intermediates and recombinant protein production. Moreover, decreased lactate, sorbitol and glycerol production may support the increased flow of carbon to the TCA cycle. Whilst a hypothesized truncation of the TCA cycle may have implications for the efficiency of CHO cell platforms, the data provided in our study indicate that modification of TCA cycle activity by gene engineering or media reformulation provides a valid target for increased productivity in CHO cell processes.
A CAMELID HEAVY CHAIN MONOCLONAL ANTIBODY DISPLAYS UNIQUE FcγRI BINDING PROPERTIES

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Background and Novelty:
Monoclonal antibodies (mAbs) are commonly used as biotherapeutic treatments in cancer and other autoimmune diseases. Engineering these mAbs to have a high affinity for their receptor is crucial for effective biotherapeutic use. One method to increase the affinity of the antibody to its receptor is by altering the N-linked glycosylation pattern in the Fc region (Asn297). This can be accomplished by using various glycoprotein processing inhibitors in the growth media. The inhibitors used in the media to grow an engineered 80 kDa chimeric antibody include kifunensine, swainsonine, and castanospermine. This heavy chain antibody (EG2-hFc) is designed with a camelid Fv that is attached to a human Fc region. This antibody’s small size makes it an ideal candidate for therapeutic use as it can probe areas too small for current IgG (150 kDa) biotherapeutics on the market.

Experimental Approach:
The effect of the N-glycan composition on the size distribution, secondary structure, and sedimentation rate of the antibody was analyzed via DLS (dynamic light scattering), CD (circular dichroism), and AUC (analytical ultracentrifugation), respectively. Furthermore, analysis of the effect of N-glycosylation on the binding of the antibody with FcγRI using SPR (surface plasmon resonance) was performed.

Results and Discussion:
It was found that upon alteration of the glycan by glycoprotein processing inhibitors, the secondary structure and sedimentation rate of the antibodies was not affected; however the degree of higher order oligomerization of EG2-hFc was increased. Comparing the effect of N-glycosylation on mAb binding to FcγRI for both EG2-hFc and a full IgG1, it was discovered that EG2-hFc has unique binding properties. Aside from a few differences observed in the correlation between the glycans attached and the affinity, removal of the glycan from Asn297 only reduced the affinity about 6 fold compared to a 118 fold decrease in IgG1.
OPTIMIZATION AND SCALE-UP OF CELL CULTURE AND PURIFICATION PROCESS FOR PRODUCTION OF AN ADENOVIRUS-VECTORED TUBERCULOSIS VACCINE CANDIDATE

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Background and Novelty:
Tuberculosis is the second leading cause of death by infectious disease worldwide. AdAg85A, an adenoviral vector expressing the mycobacterial protein Ag85A, is a new promising tuberculosis vaccine candidate. It has been extensively evaluated in pre-clinical studies with several animal models, and was shown to be effective against Mycobacterium tuberculosis infection. A phase I trial demonstrated that AdAg85A was safe and highly immunogenic.

To further evaluate the efficacy of this vaccine, a robust and cost-effective large-scale cell culture production process had to be developed for manufacturing large quantities of AdAg85A required for further clinical trials. Here we report our study on optimization of cell culture conditions, scale up of AdAg85A in 60L bioreactor and purification of the AdAg85A at different scales. The optimized conditions for AdAg85A production and purification were transferred to a GMP facility for manufacturing of AdAg85A to meet the needs of clinical trials.

Experimental Approach:
Four commercial serum-free media (SFM4Transfx-293, SFM4HEK293, Adenovirus Expression Medium and CD293) were evaluated for supporting the growth of HEK293SF-3F6 cell in suspension and the production of AdAg85A under various experimental conditions. The production of AdAg85A was then scaled up to 3L bioreactor under the optimized conditions, and further validated in 60L bioreactor.

Purification of AdAg85A was performed in sequential steps, including cell lysis, benzonase® treatment, anion exchange and size exclusion chromatography, and scaled up to 60L scale.

Results and discussion:
Three among four media supported growth of HEK293SF-3F6 up to 4x10⁶ cells/mL, however, the production yield of AdAg85A in the three media varied considerably. More than one log difference in viral titres has been observed. Although CD293 medium did not support cell growth, its use in the production phase improved virus production, indicating that HEK293 has different nutritional requirements during the cell growth and virus production phase. The highest titer (5x10⁹ total viral particles/mL) of AdAg85A was achieved by growing the cell in one of the 3 evaluated media to 4x10⁶ cells/mL and then diluting the culture with an equivalent volume of CD293 prior to viral infection. These optimized culture conditions eliminated the medium exchange step and the contamination risk associated with, and also contributed to reducing the cost of goods. The production of AdAg85A was successfully validated in 3L bioreactor runs, and then scaled up to 60L bioreactor production.

The experimental results showed that the bioreactor configuration might significantly affect the yield of AdAg85A.

The purification of AdAg85A was successfully scaled up to 60L. An additional step of freeze-thaw improved the release and recovery of the AdAg85A from the cell pellet. The recovery rate of total viral particles was more than 60% after anion exchange and size exclusion chromatography steps.

More than 99% of host cell protein was removed during the purification process. Purity of the AdAg85A bulk product was comparable to the ATCC VR-1516 Ad5 standard. The ratio of infectious viral particles to total viral particles in the purified material was approximately 10%.
ANTIBODY DISULFIDE BOND REDUCTION DURING PROCESS DEVELOPMENT: INSIGHTS USING A SCALE DOWN MODEL PROCESS

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Key words:
mAb reduction / disulfide bond scrambling / free thiols.

Background and Novelty:
During the development of the production process for a monoclonal antibody (mAb), we observed a significant increase in the reduction of interchain disulfide bonds following pilot scale protein A purification of the produced antibody (IgG1k). Different companies have reported the presence of fragmented IgG1 antibodies in the clarified cell culture fluid (CCCF) at manufacturing scale. Trexler-Schmidt et al, 2010 showed that enzymes of the thioredoxin system, released during the decline phase of the culture, were responsible for the interchain disulfide bond reduction. However, our observations were different as the majority of the interchain disulfide bonds were still oxidized in the CCCF. The massive fragmentation of interchain disulfide bonds occurred only after pilot scale protein A purification step. This study presents our insights in antibody reduction using scale-down models.

Experimental Approach:
At first, the mAb fragmentation phenomenon was reproduced at small scale by incubation of mAb containing CCCF with cell lysate followed by SDS-PAGE with and without NEM (N-ethylmaleimide). NEM is known to protect free thiol groups during sample preparation for SDS-PAGE (Liu et al, 2007). Secondly, a scale down model of the bioreactor process was established in order to understand which process parameters led to the massive reduction and hence fragmentation of the mAb during the pilot scale protein A purification process.

Results and Discussion:
We demonstrated first that the massive increase in mAb fragmentation can be replicated experimentally in small-scale when the antibody was subjected to protein denaturing conditions. Fully oxidized antibodies were observed in the CCCF using non-reducing SDS PAGE with NEM (protecting the free thiol groups), whereas without NEM, the interchain bonds of the antibodies were massively reduced. We conclude from these experiments that intradomain free thiols can reduce interchain disulfide bonds by disulfide scrambling when these are exposed to denaturing conditions during SDS-PAGE preparation or protein A at pilot scale. We also demonstrated that the accumulation of free thiols in the supernatant, observed at the start of the decline phase, led to the increase of free intradomain thiols in the mAb present in the CCCF at the end of the cultures. In parallel, the intracellular ratio GSH/GSSG showed a sharp increase from day 7 onwards, indicating a change in the intracellular redox potential in the process. One hypothesis is that these accumulated intracellular reductive forms were released in the supernatant as soon as the viability decreased, leading to a more reductive environment. The detailed mechanisms responsible for this intracellular change remains unclear.
CASCADING EFFECTS IN BIOPROCESSING: THE IMPACT OF CELL CULTURE ENVIRONMENT ON MAMMALIAN CELL BEHAVIOUR AND HOST CELL PROTEIN SPECIES

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Background and Novelty: 
Protein purification downstream of mammalian cell culture currently accounts for up to 80% of total production cost. One of the major challenges is to remove host cell proteins (HCPs) – they are immunogenic contaminants originating from the host cells. A Quality by Design strategy to overcome this purification challenge is to reduce the amount of HCPs entering the downstream train by tracing their source back to upstream culture. Previous studies have found that cell culture decisions, e.g. harvest time and culture temperature; impact HCP content at harvest significantly. However, this approach is currently constrained by our limited understanding of the dynamic host cell environment and complex physiological state. For example, we do not yet know how the host cell coordinates and regulates the molecular machinery under different culture environment, which results in different HCP profiles observed downstream.

Experimental Approach: 
Understanding the interplay between upstream cell behaviour and downstream purification requirements will open up the door for improvements in overall production efficiency. This study presents experimental results showcasing how culture temperature and harvest time impact the key process indicators, including the HCP content in CHO cell cultures. The study follows not only cell growth, but also cell cycle distribution and cellular wellbeing.

Results and Discussion: 
Cells appeared to be more robust under mild hypothermic conditions: (i) more than 90% of cells were maintained in healthy state until day 14, and (ii) the onset of apoptosis was less evident compared to the results for physiological temperature. Temperature shift, introduced on day 5, induced temporary cell cycle arrest in G0/G1 phase. However, this significantly reduced the rate of nutrients uptake, especially of glucose, which led to an overall reduction in recombinant protein productivity. Despite having robust cells, the HCP concentration in mild hypothermic culture was similar to that under physiological temperature on harvest day. Therefore, it is important to investigate the source of HCPs present in supernatant under these two culture conditions. Our current work includes identification of these HCP species using 1D LC-MS.
ON-LINE CELL CONCENTRATION AND VIABILITY MEASUREMENTS BASED ON HOLOGRAPHIC IMAGING WITHOUT STAINING

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Background and Novelty:
A new method based on holographic imaging has been evaluated to continuously measure cell concentration and viability on-line in a 2 litre bioreactor without using any type of dye to differentiate between viable and dead cells.
The advantage of working without staining enabled us to transfer the cell suspension back into the bioreactor immediately after analysis. Hence, there was no loss of culture volume caused by the cell concentration measurement.
As a consequence, we were able to increase the measurement frequency dramatically compared to other methods which consume culture volume with each analysis and obtained more detailed information on the culture process.

Experimental Approach:
In our experiments a membrane pump was used to transport the cell suspension through a silicon tube into a transparent flow-cell with a depth of 200μm, where holograms were generated. Afterwards, the suspension was transferred back into the bioreactor. Tubing and flow cell were mounted to the bioreactor and steam sterilized as one functional unit prior to the cultivation.
Right after the cultivation was started, the flow-cell was hooked into the optical unit which contained all components that were necessary to acquire the holographic images.
Within 20 minutes 25 holographic images are acquired and analysed with a unique image recognition software. Twice an hour statistically relevant cell concentration and cell viability values had been generated. The time course diagram of both parameters was updated and displayed on the graphical user interface.
The holograms used for the analysis carry phase information of the light passing an object, in our case a cell. The refractive indices of viable and dead cells are different, because cell membranes of dying cells become holey, cell components leave the cell and the surrounding liquid diffuses into it. This causes different phase shifts and varying interference patterns of viable and dead cells in the holographic image. This effect can be used to differentiate viable from dead cells.

Results and Discussion:
On-line data of different cultivation processes in 2 litre scale were collected with the holographic method and compared with proven standard methods, like manual cell counting using a hemocytometer, automated cell counting with the Cedex system (innovatis AG, Bielefeld, Germany) and the Casy TTC device (innovatis AG, Bielefeld, Germany). A comparison of cell concentration and viability data of all methods mentioned will be presented.
The experiments proved that both, cell concentration and viability data of the holographic method are equivalent to those generated with standard methods.
The fact that holograms carry more information than photographs which can be used e.g. to re-focus stored images solely based on holographic data while consuming no more disk space than an image, make this method a perfect tool for QbD and PAT.
RESOLUTION OF UNIQUE PROCESS AND SCALE TRANSLATION CHALLENGES FOR MAB PRODUCTION WITH A SENSITIVE CHO CELL LINE

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Background and Novelty:
Sensitivities of CHO (Chinese Hamster Ovary) cells to environmental and operational stress factors, such as hydrodynamic shear, bubble damage, and high metabolite or dissolved gas levels in production bioreactors, have been well characterized. The impact of these factors can usually be mitigated within relatively broad and extensively tested operational ranges. However, when a cell line does not conform to predicted behaviors in these “safe” ranges, profound changes to cellular growth, metabolism and productivity may arise. The problems are multiplied if the sensitivities are scale-dependent and are not easily observed during laboratory or pilot-scale investigations.

This presentation focuses on a production CHO cell line, for production of an IgG4 monoclonal antibody, which exhibited a uniquely high sensitivity to operational and culture conditions, particularly agitation, gas sparging rates, and trace metal concentrations, as well as to discrete shifts in dissolved oxygen, carbon dioxide, ammonia and lactate levels, that are typically considered safe for mammalian cell culture processes. For example, with a 3X increase in power/unit volume (20 to 60 W/m3) or superficial gas velocity during scale translation, a 50-70% decrease in titer was observed. Dissolved carbon dioxide levels over 60 mm Hg resulted in similar performance shifts, as did minute changes in media component and trace element concentrations, in the parts per billion range. Analysis of cell morphology revealed an unusual cell shape compared to the parental cell line, which affected viable cell density monitoring and thus the timing of several in-process control steps. The cell line sensitivity also resulted in amplified culture responses to medium lot to lot variability and higher susceptibility to potential antibody reduction.

Experimental Approach:
Novel approaches to process development and scale-down model development were required to meet these complex challenges. Accepted platform strategies and assumptions for feed media development and additive supplementation, based on specific component utilization, oxygen supply, and process control were re-evaluated and, in some cases, discarded to fit the specific and unusual requirements of this cell line. A rigorous multivariate experimental approach was employed to decouple, characterize and control the impact of the numerous scale-dependent operational parameters. Robust laboratory scale-down models were developed by modifying bioreactor design and control strategies, to mimic the cell line sensitivities and performance, observed in manufacturing, at the 1-2 L laboratory scale.

Results and Discussion:
From the analysis, it was determined that modification of dissolved oxygen setpoint (and in particular reduction of the upper control limit), manipulation of feed media and antifoam addition, timing and mode of delivery, as well as targeted nutrient over-feeding, could mitigate the issues observed with this sensitive cell line. Manufacturing performance could be restored with no impact to critical product quality attributes. This case study is a model for the rapid troubleshooting and resolution of the complex development challenges, incurred with a cell line impacted by slight changes in its environment and culture conditions, by revising and modifying typical CHO cell culture approaches.
NOVEL SMALL SCALE TFF CELL RETENTION DEVICE FOR PERFUSION
CELL CULTURE SYSTEMS

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Background and Novelty:
Perfusion processes have traditionally been used for the generation of unstable proteins in cell culture systems. The use of perfusion for production of stable proteins has been limited by low product concentration, media costs, and system complexity. With the advent of new single-use technology and high producing cell lines, perfusion processes are gaining increased attention from industry. Also, the enhanced productivity of perfusion bioreactor compared to fedbatch enables use of smaller single-use systems, both for clinical scale production as well as potentially for manufacturing scale. These new perfusion processes cannot only be used as a production platform but also for process intensification of fed-batch processes. Perfusion can be used to dramatically increase the cell density of the n-1 bioreactor to accelerate the production process and achieve gains in efficiency. The critical components of a perfusion system are the bioreactor controller, cell culture vessel, and cell retention device. The cell retention device or system is often used to define the type of perfusion system with gravity-based and filtration devices being the most common.
This study will show the application of a small-scale tangential flow microfiltration device (prototype small scale Prostak™) in a perfusion test platform. This prototype device was derived from Merck Millipore Prostak™ microfiltration family of products typically used in the primary clarification of cell culture.

Experimental Approach:
The performance of the prototype small scale Prostak™ devices were evaluated using a previously established baseline perfusion process which utilized a CHO-S cell line expressing a monoclonal antibody that achieved viable cell densities of 40-60 x 10⁶/ml using CellVento™ CHO-100 media at 2 vessel volumes per day (VVD). A variety of flow rates were tested (0.5-1.5 L/min) utilizing different pump types to determine their effect on cell culture health and fouling profiles. Pressure was monitored at critical points in the system. Performance was determined based on the measurement of viable cell density, culture viability, fouling profile, and titer.

Results and Discussion:
We will report the critical set-up parameters required for use of the prototype small scale Prostak™ in seed train or perfusion production applications. We will present data showing optimal processing conditions, which include recirculation flow rate, shear, permeate flux, and typical bioreactor process control parameters. Finally, the scalability of prototype to commercial scale device will be illustrated. With this knowledge, the utility of this novel device can be realized for perfusion process development applications.
DESIGN SPACE CONSIDERATIONS AND A QUALITY BY DESIGN APPROACH TO PROCESS CHARACTERIZATION AND VALIDATION

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Background and Novelty:
The goal of Quality by Design (QbD) is to achieve enhanced process and product understanding and develop manufacturing processes which deliver products with consistent and acceptable quality. This can be accomplished through the application of tools such as risk assessments and design of experiments (DOE) during process characterization and validation. Results of process characterization and validation studies determine acceptable process parameter ranges to ensure that the critical quality attribute (CQA) acceptable ranges are met. These ranges can then be proposed as part of a design space claim, where movement within an approved design space is not considered a change as defined by ICH guidance. Although a “process-wide” design space may provide maximum flexibility, there may be drivers for unit operation specific design spaces that only include a subset of process parameters depending on process capability and desired operational flexibility.

Experimental Approach:
A QbD approach was applied to characterize a monoclonal antibody (MAb) cell culture production process. First, study factors were selected through a risk ranking assessment based on historical development data, manufacturing history and scientific understanding. Statistically designed, multifactor process characterization studies were performed in scale-down bioreactor models to build understanding of parameter impacts on CQAs and key performance indicators (KPIs).

Results and Discussion:
This case study will describe the multi-stage approach to characterizing the cell culture production step. After an initial screening DOE, a higher resolution DOE was performed to resolve interactions between process parameters and refine acceptable operating ranges. Model-predicted worst case conditions were subsequently tested to verify the statistical models. Challenges associated with wide observed process output ranges and limited model predictability for several CQAs will be discussed. The process capability of each unit operation was taken into account in considering the proposed design space claim for this MAb process. Additionally, the desired flexibility to move process targets to modulate CQA levels or meet facility transfer requirements in the future was considered. An assessment of each of these aspects will be discussed.
A NOVEL LOW-PARAMETER COMPUTATIONAL MODEL TO AID IN-SILICO GLYCOENGINEERING

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Background and Novelty:
Glycosylation is a key post-translational modification that can affect critical properties of proteins produced in biopharmaceutical manufacturing, such as stability, therapeutic efficacy or immunogenicity. However, unlike a protein’s amino acid sequence, glycosylation is hard to engineer since it does not follow any direct equivalent of a genetic code. Instead, its complex biogenesis in the Golgi apparatus integrates a variety of influencing factors most of which are only incompletely understood. Various attempts have been undertaken so far to computationally model the process of glycosylation, but due to the high parametric demand of most of these models, practical leverage for glycoengineering purposes has proven challenging. Consequently, industrial glycoengineering is still largely carried out using costly and time-consuming trial-and-error strategies and could greatly benefit from computational models that would better meet the requirements for industrial utilization. Here, we introduce a novel approach combining constraints-based and stochastic techniques to derive a computational model that allows predicting the effects of gene knockouts on protein glycoprofiles while requiring only minimal a-priori parameter input.

Computational Approach:
We use the COBRA toolbox to generate an in-silico representation of the N-glycosylation network. The transition of glycans through this reaction network is modeled as a Markov chain stochastic process. This way, probabilistic glycoprofile predictions can be achieved that outperform predictions made from pure constraints-based modeling. In particular, the user can submit an experimentally derived glycoprofile on a specific protein (for instance, obtained from a cell culture grown under standard conditions) which is used to automatically fit model parameters to that specific glycoprotein of interest. Subsequently, the model allows one to simulate how knockout or overexpression of enzymes involved in the glycosylation process affect the glycoprofile under study. Thus, in contrast to previous approaches, our model offers straightforward predictions without the need for manual reconciliation of a vast number of kinetic input parameters.

Results and Discussion:
Our model is capable of creating N-glycosylation reaction networks that are complex enough to cover typical glycoprofiles found in biopharmaceutical manufacturing including tetra-antennary, highly sialylated or polyactosamine carrying glycans. Tests on both simulated as well as published glycoprofiles show that the model derives sound predictions of glycoprofile change upon genetic modification which are in good congruence with published knockout experiments. Thus, the model has the potential to provide a cheap and fast guidance tool to help find host conditions that can yield a desired glycoprofile, thus providing an important step towards the in-silico process of glycoengineering. So far, the reaction network considered is specific for CHO cells but can be easily modified to include reactions occurring in other hosts. In addition, as this glycosylation model is integrated into a whole-cell metabolic model, a comprehensive in-silico representation of the host cell in its growth medium would be attained that would allow one to simulate how the glycoprofile would respond to a wide range of modifications to the growth conditions.
FOLLOWING PRODUCT QUALITY AND PROCESS PERFORMANCE IN FED-BATCH FERMENTATIONS VIA 2D FLUORESCENCE SPECTROSCOPY

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Background and Novelty:
Increasing interest is directed towards various process analytical technology tools and their application as process analyzers and later on as process control tools in upstream processing. 2D fluorescence spectroscopy (2DFS) is one of these tools, which enables fast and reliable data acquisition throughout the whole fermentation process based on intrinsic fluorescence of the cell culture and media components. At present, literature refers to it mainly as a process analyzer tool where large volumes of data can be generated and processed by chemometric modeling in order to gain information about substrate and product concentration or cell culture state. Among possible process strategies, fed-batch fermentations with continuous feed addition are considerably challenging approaches for chemometric modeling. Fluorescent components typically involved in cell growth and metabolism will not only dynamically change during the process, but will be also supplemented to the culture depending on process strategy and feed composition. These process manipulations due to feeding alter the appearance of the 2D excitation/emission matrices (EEM) recorded during fermentation and conceal the original fluorescence information. Hence, for specific target parameters the particular information may not be necessarily represented by the most prominent signal alterations, therefore relevant signals have to be identified and preprocessed. In this study, PLS and PARAFAC as multivariate data analysis tool were evaluated towards their ability to provide reliable models for the prediction of cell culture parameters during fed-batch fermentation with a special focus on product quality issues such as product aggregation.

Experimental Approach:
Fed-batch fermentations were carried out in a 2L bioreactor (Sartorius) cultivating a CHO cell line producing a monoclonal antibody. Glucose and glutamine concentrations were kept constant via continuous feed addition and a temperature shift was carried out to prolong the process time. SE- and Protein A-HPLC were used as reference methods for the determination of protein concentration and aggregation levels. EEM were recorded with the BioView® (Delta) sensor every 15 min during fermentation. PLS and PARAFAC models were build using MATLAB and the PLS-Eigenvector toolbox. These models were evaluated for their ability to enable reliable real-time monitoring of cell culture parameters and product aggregation.

Results and Discussion:
Continuous addition of complex feed medium during fed-batch interfered with the fluorescence signals originated by growing cells. Its impact on the EEMs and thus on resulting regression models were visible in the scores plots of PLS models. Nevertheless, the resulting calibration models were able to predict common target parameters such as metabolites, substrates and product concentration. Due to a careful evaluation of possible data preprocessing and multivariate data analysis methods such as PLS and PARAFAC, it was also possible to extract the relevant signals within the EEMs for more complex process parameters such as product aggregation. The evaluation of resulting calibration models towards their ability to predict target parameters led to the assumption, that process control via 2DFS should be possible.
SPECIAL APPLICATION MEDIA DESIGN FOR TRANSFECTION  
AND SINGLE CELL CULTIVATION

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Background and Novelty:
State-of-the-art medium and feed development has proved its potential for increasing bioprocess efficiency many times. By application of such advanced chemically defined, animal component-free formulations combined with recent cell lines, the yield of mammalian cell cultures was pushed to the two-figure gram-per-liter level. For establishing high producing cell lines, one method for clone selection and ensuring uniformity is limited dilution. In the context of medium development, this application has its specific challenges which differ from those encountered for production media, namely to enable cell growth without the presence of serum at very low cell densities. The same is true for other special applications of cell culture media such as transient transfection. The significance of transient gene expression in mammalian cells is continuously growing. Applications of this fast and flexible approach range from purposes in R&D and production of preclinical material to applications in personalized medicine or vaccine production. To streamline such applications and to simplify upscaling, a bifunctional medium supporting both transfection and cell growth for high productivity is required.

Experimental Approach:
In this work, the current progress in developing above-named special application media for transient gene expression and single cell growth presented. The work on a chemically defined and animal component-free medium for single cell growth was performed in 96 well plates in comparison to serum containing medium. A proof of concept for clone selection was performed with different CHO cells. For transient transfection, the potential of the developed formulations was demonstrated using various mammalian cell lines (human and CHO). For that, the transfection efficiency was evaluated via transfection by polycationic reagent of a GFP expression plasmid. Additionally, the expression of an IgG1 antibody was investigated for a HEK cell line by performing shaker batch, fed-batch and pseudo-perfusion cultivations after transient transfection in fresh medium.

Results and Discussion:
The potential of the single cell medium was shown by a successful proof of concept. Different single cells were expanded from 96, 24 and 6 well plates and to shaking flasks. The differences in final growth performance and productivity of the selected clones exhibited the heterogeneity within the original population which was used for limited dilution. This was demonstrated in one exemplary experiment, where protein activity of some clones was up to ten times higher, compared to the original culture. Referring to transient transfection, using fresh medium at the time of gene delivery, efficiencies in the range between 70-90% for HEK cell lines and 60-80% for CHO cell lines were achieved. Batch cell densities of 6-9·10⁶ cells/mL with high viabilities throughout the cultivations were achieved for different HEK cell lines. Regarding productivity, titers of 50-100 mg/L were measured with a commercially available reference, whereas application of our medium resulted in elevated titers of more than 500 mg/L. Further, DOE based optimization of the protocol with addition of a specialized feed increased the yield to 680 mg/L. In parallel, small scale pseudo-perfusion cultivation enabled high volumetric productivity of about 400 mg/(L·d).
A MODEL FRAMEWORK LINKING THE EXTRACELLULAR ENVIRONMENT TO ANTIBODY GLYCOSYLATION

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Background and Novelty:
Glycoproteins make up the bulk of biologically-derived medicines, and are taking up an ever increasing share of total prescription pharmaceuticals. As opposed to chemically defined small drug molecules, glycoproteins are large complex molecules with heterogeneity arising from a multitude of glycan moieties. Glycans are complex post-translation modifications, which arise in a number of enzymatic reactions in the ER and Golgi, and play an important role in pharmacokinetics such as drug safety, efficacy and half-life. It has been shown that the process of protein glycosylation can be partially driven by the intracellular availability of nucleotide sugar donors (NSD), which are the co-substrates to the glycosylation reactions. It is also known that the availability of the NSDs can be affected by a number of process conditions such as culture mode, temperature, dissolved oxygen, nutrient availability and feeding of precursor molecules. In this work a mathematical model platform is presented to quantify the impact of nutrient availability and feeding strategies onto the glycosylation process with the aim to enable the design of strategies to optimise the product glycoform.

Experimental Approach:
As part of this work a modelling platform was developed to link the extracellular environment, through the availability of intracellular metabolites in the cytoplasm and the Golgi apparatus, to the glycosylation of conserved glycosylation site of a monoclonal antibody. The model platform comprises four parts, which are interlinked through dynamic fluxes and metabolite concentrations: 1) a modified Monod kinetics cell growth model, 2) a semi-structured purine and pyrimidine synthesis network, 3) a structured and mechanistic representation of the NSD synthesis pathway, 4) the del Val model (del Val et al., 2011, Biotechnology Progress) describing the N-linked glycosylation process of the antibody heavy chain. The main focus of the work has been the bottom-up mechanistic in silico reconstruction of the NSD synthesis network. The 34 species that make up the metabolic network were represented by means of mass balances that are connected through a network of 60 reactions, which were modelled as saturation rate kinetics based on reaction mechanisms found from literature. The model platform is able to reproduce cell growth dynamics, extracellular nutrient availability, dynamic intracellular NSD data, product titer and the antibody product glycoform.

Results and Discussion:
The model was trained using dynamic experimental data from hybridoma as well as CHO cell culture. The output shows good agreement for batch and fed-batch data. Furthermore the model is able to capture the dynamic impact of hexose and nucleotide precursor additions to culture media and their impact on NSD concentrations and product glycoform. The framework is a step towards assessing the impact of feeding strategies and ultimately the in silico optimisation of bioprocess conditions in order to maximise product safety and quality with respect to glycan heterogeneity. Lastly the modular structure of the model allows it to be coupled with other dynamic models by means of NSD flux or concentrations such that it can easily be translated to other expression systems, operation modes and culture conditions.
DEVELOPMENT OF AN INTEGRATED CONTINUOUS BIOMANUFACTURING PROCESS

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Background and Novelty:
The recent improvements of continuous up- and downstream processes in the production of therapeutic proteins suggest their final integration to a single process stream. The physical integration of the upstream production with a downstream capture has previously been reported. Besides general benefits of continuous manufacturing, such as reduced equipment size, enhanced cost efficiency and high volumetric productivity, the steady state operation favors constant or even improved product quality.

Experimental Approach:
A stable perfusion culture at different viable cell density set points has been developed comparing two different filter-based cell retention devices, namely tangential flow filtration (TFF) and alternating tangential flow filtration (ATF). Thorough characterization of physical properties, such as hydrodynamic stress and gas liquid mass transfer, as well as cellular growth, productivity and product quality have been carried out. The primary modulation of quality attributes, in particular mAb glycosylation, was achieved and can be readily predicted applying a mechanistic model for continuous culture. The in-group developed capture SMB process was modified to continuously process fresh harvest from the bioreactor. Operating parameters are set according to a mechanistic model, forecasting entire process performance.

Results and Discussion:
Finally, the perfusion bioreactor has been directly connected to a continuous capture step. Using a mAb producing CHO cell line, stable operation has been achieved for several steady states. The adaption of the downstream process to multiple viable cell density set points is shown. Model predictive control has been implemented using at-line monitoring of the product concentration and adjusting the downstream capture step operation parameters.

In contrast to previous work, desired product attributes such as mAb glycosylation, fragments and charge variants can be tuned in steady state operation of the integrated process. The control and monitoring of the perfusion culture as well as the adaption of subsequent downstream processes will be shown. This study illustrates, how to build product quality into future biomanufacturing processes.

Literature:
MECHANISTIC MODELING MEETS HIGH-THROUGHPUT EXPERIMENTS – CASE STUDIES IN UPSTREAM AND DOWNSTREAM TO IMPROVE PRODUCT QUALITY

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Background and Novelty:
Mammalian cells represent the state-of-the-art expression system for many therapeutic proteins such as monoclonal antibodies (mAbs) and fusion proteins due to their ability to perform posttranslational modifications. Protein modifications including N-linked glycosylation, charge variants, clipped forms and aggregates can play a crucial role in terms of bioavailability, efficacy as well as safety of therapeutic proteins and are therefore considered as major quality attributes. The growing awareness of the importance of these protein quality attributes during the last decades has pushed the focus in biopharmaceutical production from simple yield improvement towards a product quality driven multi-objective optimization.

Experimental Approach:
Although high-throughput development methods have helped to meet the growing demand to control product quality, mechanistic modeling can further add to the understanding of many processes and thus represents a valuable tool for Quality by Design. This is illustrated with two mechanistic mathematical models of an up and downstream process, where experimental data obtained from high-throughput devices were used to simulate the entirety of the design space. So called “sweet spots” were identified for both models in order to obtain desired process performance with respect to product quality.

Results and Discussion:
Case study I: High-throughput micro-bioreactor experiments with working volumes of 10-15 ml were carried out to relate N-linked glycosylation patterns to cell culture conditions. Analysis of basic metabolites in combination with high-throughput measurements of N-linked glycosylation and intracellular nucleotide sugars1 were included in a dynamic and mechanistic mathematical model2 describing the N-linked glycosylation pattern as a function of trace elements, sugar supplementation, culture pH and ammonia. The mathematical model is able to predict the overall N-linked glycosylation pattern as well as the time evolution throughout the culture. In addition, the ratio between individual glycosylation structures can be adjusted with optimized feeding strategies.

Case study II: The design of chromatographic polishing steps that remove clipped forms and aggregates with high yield is becoming a challenging and time consuming task considering decreasing acceptable levels of these undesired forms. Experimental results from high-throughput downstream experiments were used to fit the parameters of a mechanistic model based on van der Waals and electrostatic interactions (DLVO theory)3. The design of the polishing step of several mAbs and fusion proteins is presented on both CEX and AEX materials where high mAb purity and process yield can be found.

Literature:
AUTOMATED AND NON-INVASIVE MONITORING OF ADHERENT CELL CULTURES

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Background and Novelty:
Monitoring adherent cell cultures with Differential Digital Holographic Microscopy (DDHM) gives information not only on cell number and confluence, but also on morphological parameters of the cells, at a single cell level, directly in the culture vessel. DDHM captures intensity and optical phase information of the sample and combines these into a digital hologram, offering 4D (3D+time) imaging capabilities. The typical depth of investigation in holography is about 100x larger compared to classic light microscopy (same objective) and remove the need to focus prior imaging: through computational means objects in a hologram can be refocused post-acquisition. The optical phase information detects changes in the optical path with a nanometric accuracy, offering a precise quantitative tool to assay the local composition changes in transparent objects.

OVIZIO’s iLine-M microscope uses the DDHM technology and has been designed for adherent cell monitoring in either single or multilayer cell culture vessels. The iLine-M application range covers multi-well plate screening but also multilayer vessels like Cell Factory System (ThermoFisher) and CellSTACK products (Corning). Screening is automated and does not require detaching the cells for sampling and staining leaving those available for further analysis or for time-lapse experiments.
The iLine-M is equipped with the OsOne software for data acquisition and analysis. Cell confluence and cell count are reported in nearly real-time, applying a label-free approach (based on the holographic fingerprint of cells). Cell confluence is indicated as the percentage of surface covered with cells and for a more in-depth monitoring, each cell can be observed and analyzed individually. A simple click on a cell in the image or in the dot plot shows all data linked to that particular cell, offering a convenient tool to validate the results generated by the algorithms in the software.

Experimental Approach:
In this study, we compared the results generated by the iLine-M with classic cell counting methods, requiring cell detachment, sampling and staining. Several industrially-relevant, adherent cell lines were diluted at five different cell densities, inoculated in triplicate in 48-wells plates and cultivated on 3 subsequent days. Daily analysis was performed on the iLine-M, then several wells were trypsinized for Trypan-blue cell counting.

Results and Discussions:
Results indicate that the triplicate cell counts were reproducible over 3 days. The values were linear and fitted well with the expected values, except for highest cell densities, where they were slightly below expectations. All cells in the well were properly identified and counted as the whole well is imaged. Other assays report the applicability with multilayer vessels, replacing results based on estimations with data while also improving on reproducibility.

Using DDHM technology combined with OsOne generates a large amount of data with specifics for each cells (e.g. thickness, skewness, etc.). These data could be used to perform several statistical analyses on the cell population, in order to define and control critical parameters of the cell cultures process, also envisioning the role of this technology in a PAT initiative.
A MATHEMATICAL DESCRIPTION OF THE CHANGES IN MAB GLYCOSYLATION DURING CHO CELL CULTURE

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Background and Novelty:  
Controlling the glycosylation profile of monoclonal antibodies (mAbs) has become a priority in the field of pharmaceutical bioprocessing because the relative abundance and monosaccharide composition of mAb-bound carbohydrates (glycans) determines the therapeutic mechanism, serum half-life and immunogenicity of these therapeutic proteins. Furthermore, several bioprocess conditions, including nutrient availability, metabolite accumulation, temperature and pH, have been linked to changes in the glycan profiles of mAbs. In order for glycosylation control to be achieved, further understanding of the interaction between bioprocess conditions, cellular metabolism and the intracellular process of glycosylation is required. To better understand how the process of glycosylation evolves over culture time, we have analysed CHO cell culture data using a bespoke dynamic mathematical model.

Experimental and Modelling Approach:  
Experimentally, an in-house CHO DG44 cell line producing mAb A was cultured under fed-batch conditions where different degrees of glucose starvation were induced at stationary growth phase. Temperature was shifted on day 5 of culture from 37 to 33.5°C. Data for cell density, glucose, glutamine, lactate and ammonia were collected every two days. Intracellular availability of nucleotide sugars (NSs) and mAb glycan distribution was determined for days 5, 9, 12 and 14 of culture.  
The modelling strategy consisted in coupling a Monod-based model for cell culture dynamics (CCDyN) with a previously described model for mAb glycosylation [1]. The CCDyN model considers glucose and glutamine as the growth-limiting substrates and includes simultaneous lactate secretion and consumption. Two separate stages of growth, each associated with conditions before and after the temperature shift, were considered. The $q_s$ calculated by the CCDyN model was used as the input for the mAb glycosylation model. The parameters required for the mAb glycosylation model were obtained as described previously [1]. With these estimated parameters, the coupled model was simulated to assess how the mAb glycosylation profile would change over the culture period. All parameter estimation, optimisation and model simulations were performed using the gPROMS modelling software.

Results and Discussion:  
For this cell line and the employed culture conditions, the Man5 glycoform was observed to accumulate heavily and in a time-dependent manner. Similarly, an increase in immature glycoforms (A1G0F and G0F) and a decrease in galactosylated glycans was observed as culture progressed. Interestingly, this decrease in glycan complexity was found to be inversely correlated with the degree of glucose starvation. By analysing these results using the mAb glycosylation modelling platform, we found that the likely cause of the mAb glycan under processing was the increase in mAb $q_s$, possibly associated with the temperature shift. These results indicate that glycosylation may be limited by the availability of some glycosyltransferase enzymes relative to mAb $q_s$. The overall strategy presented in this work shows that synergy between models and experiments is useful to further describe the mAb glycosylation process and may also help in identifying possible genetic engineering targets to produce optimally glycosylated mAbs.

IMPLEMENTATION OF A QUALITY BY DESIGN APPROACH INTO CELL LINE AND PROCESS DEVELOPMENT FOR RECOMBINANT PROTEIN PRODUCTION

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Background and Novelty:
Cellca is a recognized leader in cell line and upstream process development for large-scale protein production of biopharmaceuticals (e.g. antibodies) in mammalian cells and offers a unique platform technology which is characterized by high efficiency regarding productivity, scalability, and protein quality. As the market for therapeutic proteins is steadily growing, the need to optimize methods for the generation of mammalian cell lines is steadily increasing. In this context, important aspects are to express recombinant proteins not only at high levels and in an efficient cost-effective manner, but also with the desired protein quality attributes such as the glycoprofile. This study aims to contribute to a better understanding of how quality in terms of expressing proteins with pre-defined glycoprofiles can be built into a cell line and process development process.

Experimental Approach:
Different cell clones expressing a monoclonal IgG1 antibody were generated using Cellca’s proprietary cell line development technology and subsequently evaluated in a platform fed-batch process in shake flask scale for their producer cell line potential. Quality analyses of the thereby produced material allowed selection of the clone with the most desired quality profile. Based on Cellca’s know-how in media and process design, a set of different conditions, including media components and fed-batch process parameters, was applied in shake flask studies to actively influence the glycopattern of the IgG1 antibody. A final fed-batch process was transferred from small scale shake flask level into a 1000 L single-use bioreactor to confirm scalability of cell growth, productivity, and protein quality attributes.

Results and Discussion:
In this study, we show that the galactosylation profile can be influenced, both towards higher and lower galactosylation levels, by media and process variation. The thereby optimized production process is scalable from shake flask up to 1000 L bioreactor levels regarding growth, productivity and protein quality. Implementing the quality by design approach into cell line and process development can enable to successfully target a desired protein quality profile.
MULTIVARIATE PROFILING AS A PIVOTAL TOOL IN IDENTIFYING CRITICAL RAW MATERIALS THAT MODULATE N-LINKED GLYCOSYLATION PROFILES

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Background and Novelty:
Over the past decade, notable advances in the characterization and control of post translational modifications (PTMs) to enhance therapeutic properties have continued to drive the development of many next-generation biopharmaceuticals. It has been widely reported that the quality of secreted therapeutic proteins is dependent on the consistency of attached glycan moieties. Insufficient glycosylation potentially impairs efficacy and safety. Our particular interest lies in identifying critical raw materials that modulate N-linked glycosylation profiles of therapeutic proteins from selected high-producing clones. This study represents an important step towards establishing cGMP ready chemically defined supplements that significantly and reproducibly adjust glycan moieties. In addition, the multivariate profiling process described here represents a pivotal tool in the upstream optimization of biopharmaceutical platforms.

Experimental Approach:
CHOZN-GS−, CHO-S, and DuxB11 high-producing clones were screened with 40 nutritionally diverse chemically defined SAFC feeds to establish variable culture responses. A high throughput analytical method using intact protein accurate mass data was employed to perform glyco-profiling. Multivariate analysis was applied to the extensive data set to identify critical raw materials correlated to cell culture performance. Novel chemically defined supplements were optimized to directionally adjust glycan moieties. Data represents average relative glycoform distributions of purified protein sampled 14 days post-inoculation of fed-batch cultures. Growth, productivity, and metabolite production were studied using standard analyses.

Results and Discussion:
To address the significant unmet need in the biopharmaceutical industry for a practical method for improving glycosylation profiles, SAFC performed an extensive multivariate analysis of critical raw materials to identify those that have an optimum influence on glycan attributes. The newly developed protein quality supplements provide twofold to fourfold directional shifts in the relative G0, G0F, G1F and G2F distributions for a wide range of bioproduction cell origins, including CHOZN-GS−, CHO-S, and DuxB11. In addition, the protein quality supplements did not have a negative effect on desirable process outputs. In all three high-producing cell lines, identical or higher cell densities and volumetric productivities were obtained. The ease of operation and simplicity of the supplementation process enables the rapid transfer of biologics into clinical manufacturing while ensuring quality and reducing costs.
CONTROLLING PRODUCT QUALITY VARIABILITY IN LARGE SCALE PERFUSION CULTURES BY ADDRESSING MICROSERGER SUSCEPTIBILITY TO FOULING EVENTS

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Background and Novelty:
Due to high cell densities and short vessel geometries of large scale perfusion cultures, microspargers are often used to meet \( k_a \) requirements. Over the course of a perfusion culture lasting months, significant fouling of these microspargers can occur. This fouling reduces the surface area available for aeration and increases superficial gas velocities. Partial Least Squares regression of process data resolved a family of parameters that suggested a relationship between superficial gas velocities of microspargers and cell death. This coincided with product quality variability of the drug substance, and this relationship was found to be consistent between multiple facilities and at different operating volumes. As a result, it was hypothesized that microsparger-mediated cell death released cytosolic enzymes into the culture, resulting in variable product quality of the drug substance.

Experimental Approach:
The relationship between superficial gas velocities to cell death and product quality was evaluated by directing gas flow to one or two microspargers in small scale perfusion bioreactors that were operated at a constant viable cell density. This ensured consistent total gas flow rates between conditions, and consistent non-microsparger associated rates of cell death. Given that the most significant contributor to gas velocity variability in large scale cultures is microsparger fouling, this also provided the impetus to assess fouling susceptibility of various microsparger designs. Microspargers were evaluated by sparging at pre-determined flow rates in high density cell culture broths. The microsparger configuration that coincided with the least fouling susceptibility was then evaluated in large scale perfusion bioreactors to determine its impact to product quality.

Results and Discussion:
When gassing was restricted to a single microsparger (high gas velocity), product quality decreased and coincided with an increase in LDH and cytosolic enzymes. This effect was reversed after gas velocities were halved, thereby confirming the causative relationship between gas velocities and product quality. Upon evaluating various microspargers, larger pores were found to exhibit less fouling and lower death rates than small pores. As expected, bubbles formed from large pore microspargers were larger and reduced the effective surface area available for mass transfer. \( k_a \) measurements were made between the small and large pore microspargers in large scale bioreactors, and no significant difference was observed at low gas flow rates, while at high gas flow rates, large pore microspargers had higher \( k_a \).
After over 75 days of continuous cell culture in a large scale bioreactor, no significant fouling events were observed when the large pore microsparger was used. In comparison, 90% of the vessels that had used a small pore microsparger experienced fouling events severe enough to require intervention. Equipment specifications for our process have since been modified to require large pore microspargers. Since implementation of such microspargers, product quality variability our drug substance has decreased 6-fold. Based on this study, taking into consideration pore size is a relatively simple solution that ensures mass transfer needs are met, while minimizing any potential impact of microsparger fouling to process performance and product quality.
QUALITY COMPLIANCE IN THE DEVELOPMENT OF CELL-BASED MEDICINES IN NON-PHARMA ENVIRONMENT

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Background and Novelty:
Academic institutions and transfusion centres currently lead early-stage clinical development of cell-based therapeutics seeking its future marketing authorisation application. However, the requirement of quality management structures to comply with good scientific practice regulations (GxP) that were originally designed for product development in corporate environments represents a major challenge.

Experimental Approach:
Adaptation of existing either general (i.e. ISO9001) or specific (i.e. JACIE or NetCord) quality assurance programs into GLP/GMP may appear as a substantial effort. However, many of these processes can suit up to some extent several of the aspects covered by the GLP/GMP.
Another point to consider was the design of specific assays for the assessment of pharmacodynamics, pharmacokinetics and toxicology endpoints when using cell-based therapeutics as opposed to traditional small molecule drugs or biologicals.

Results and Discussion:
GLP/GMP standards were implemented gradually along the development of cell-based therapeutics based on the use of MSC aiming at articular cartilage and bone regeneration.
We will present and discuss:
1) the difficulties that non-pharmaceutical institutions need to overcome for the successful translation of cell-based products into the clinics from the quality compliance standpoint;
2) the compatibility of current voluntary accreditation schemes (such as JACIE, FACT, NetCord and others) with mandatory GxP required for cell-based product development in any Investigational New Drug (IND, in the US)/Investigational Medicinal Product Dossier (IMPD, in Europe); and
3) the analysis of a case-study of a cell-based medicinal product development for the treatment of osteoarthritis in a non-profit institution.
A NOVEL MEASURING CHAMBER AND AUTOMATION PLATFORM
FOR MAMMALIAN CELL CULTURE PROCESSES

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Background and Novelty:
One of the biggest aims of biotechnological production is the implementation of PAT to obtain a highly automated process. Therefore the implementation of suitable analytical sensors has to be addressed at the earliest opportunity. This implementation faces different problems when modern reactor concepts are used, e.g. disposable systems, and often optimizations are run in small scale glass reactors. These systems are not optimized for well-established analytics like Ingold-port mounted spectrometers or in situ microscopes designed for production scale processes.
A new measuring chamber, together with an automation platform was constructed and tested in different mammalian cell culture processes. These modular chambers enable the possibility to include standard Ingold-Port analytics, as well as PG 13.5 probes as a bypass measurement system. The automation platform uses a new approach for sample taking, which withdraws a volume from the reactor, into the chambers, analyzes it and afterwards either transports it back to the reactor or stores it in a temperature controlled sample collector.

Experimental Approach:
The measuring chamber, together with the automation platform were used within several processes, e.g. a 2L perfusion process and a 15L fed batch process. Every 4 hours a sample was drawn from the reactor and stored in a temperature controlled sample collector. Furthermore, a 2D fluorescence spectrometer and an in situ microscope were mounted into the measuring chambers and integrated into the cultivations.

Results and Discussion
With this system, it was possible to monitor several cultivations, in some cases for around 14 days, without any contamination. The obtained data from a 2D fluorescence spectrometer and an in situ microscope are comparable to the standard inline mounted operation mode. Thus a scale-up from measuring within the chamber to the inline measurements is possible. With the use of multivariate data analysis it was possible to do a qualitative comparison of cultivations from 2 L- and 15 L-scale, which showed very similar behavior. Furthermore a calculated model from bypass and inline operation mode could be used to predict cell count in a different inline monitored process.

Furthermore the automation-platform is able to go one step further and use the obtained data from inline- or bypass-probes to directly create a closed-loop controller. A glucose prediction model, based on MIR-spectroscopy, was used to provide the recent glucose-concentration, which then was interpreted by the automation-system and used for triggering a feed pump. During this controlled fed-batch a steady glucose-concentration could be maintained for more than 40 hours.

This technology will enable new approaches during process development as a larger range of devices can be tested in early stages. Other applications are seen in continuous cultivation, where such chambers can be mounted between different unit operations, e.g. monitoring the protein titer or remaining impurities between purification steps. In addition to this, the automation platform simplifies the operation of a bioprocess, especially the time-consuming sample taking for building reliable chemometric models.
THE CHEMISTRY AND STABILITY OF POLYAMINES IN CHEMICALLY DEFINED
CELL CULTURE MEDIA AND SUPPLEMENTS

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Background and Novelty:
Polyamines (spermine, spermidine, putrescine and cadaverine) are naturally occurring nitrogenous compounds found in eukaryotic organisms. These biogenic amines are involved in various biological processes such as cell proliferation and maintenance of cell viabilities. Spermine and putrescine are common in commercially available cell culture media and supplements while spermidine and cadaverine are slowly finding their way into cell culture applications. These polyamines are added to cell culture media and supplements in form of hydrochloride salts for ease of handling and solubility. Throughout our work with these polyamines in cell culture applications, we have observed stability issues in chemically defined (CD) supplements at accelerated conditions used during stability studies. The purpose of this study was to investigate and identify chemical reaction(s) that could result into reduced concentrations of certain polyamines in a complex system such as cell culture media and supplements.

Experimental Approach:
Cell culture media and supplements are manufactured in liquid (aqueous) or powder formats. Therefore, chemical reactions taking place in CD media and supplements must be favored in aqueous and solid state or neat conditions. Reactions between polyamines and selected CD components, such as vitamins and carbohydrates, were investigated by heating mixtures of polyamines and these components in water to 100°C over two hours or in a mixture of methanol/water for 1-2 hours at 65°C. The reaction mixtures were then cooled down; recrystallized if required and analyzed using HPLC. Polyamines were separated by ion-pairing chromatography and detected by post-column derivatization with ortho-phthaldehyde (OPA) to generate fluorescent molecules. Reaction and/or degradation products were identified using LC-MS and/or HPLC by comparing to authentic positive control samples. Aqueous reactions were further confirmed by incubating a powdered mixture of polyamines and the selected components in a stability chamber set at 35°C and 55% relative humidity for 1-2 weeks. The powders were rehydrated in water after incubation and analyzed using the OPA HPLC method.

Results and Discussions:
The results of the study demonstrated that polyamines selectively react with certain CD media/supplements’ components, thereby leading to reduced concentrations of the polyamines in the media/supplements. These nitrogenous compounds were found to react with reducing sugars such as dextrose in a Maillard type reaction in powder formulations, the rate of which was increased under accelerated conditions. In addition, spermine and spermidine are oxidized in the presence of riboflavin resulting in the degradation of spermine and spermidine and the generation of other polyamine compounds. Spermine was shown to oxidize, resulting in the generation of spermidine and 1, 3-diaminopropane, whereas spermidine oxidation in the presence of riboflavin resulted in the generation of putrescine and 1, 3-diaminopropane. These findings have reinforced our interest in understanding the chemistry and biochemistry of polyamines in cell culture media and supplements.
SPECTROSCOPIC TOOLS FOR AN AUTOMATED SUSPENSION CELL CULTURE SCREENING SYSTEM

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Background and Novelty:
We developed an automated, multiwell plate (MWP) based screening system for suspension cell culture which is now routinely used in cell culture process development. It is characterized by a fully automated workflow with integrated analytical instrumentation and uses shaken 6-24 well plates as bioreactors which can be run in batch and fed-batch mode with a capacity of up to 384 reactors in parallel. A wide ranging analytical portfolio is available to monitor cell culture processes and also cooperation with internal HT analytic groups to characterize product quality.

Experimental Approach:
Current work focuses on expanding the analytical portfolio to develop control mechanisms for automated cell culture processes. Besides setting up a robust method for pH measurement we evaluated different spectroscopic techniques like Raman or infrared as fast and powerful analytical tools.

Results and Discussion:
A fully automated, multiwell plate based pH measurement assay and a pH control mechanism was developed for the screening system. It is based on pH sensitive absorption and fluorescent dyes. Comparable pH profiles, cell culture performance and product titer could be shown for multiwell plates and bioreactors using this strategy for two late stage project cell lines. Additionally we could successfully show the implementation of a pH shift in multiwell plates. Infrared spectroscopy is a powerful tool for the simultaneous detection and quantification of several key components in cell culture processes. Non-invasive and near real-time measurement make it an ideal tool for small scale high throughput systems. We could quantify different metabolites and nutrients, i.e. glucose, ammonia and glutamine using this technique. In addition we could control the glucose concentration based on mid-infrared spectroscopy and chemometric models.
LOW VIABILITY AND SLOW GROWTH IN INITIAL SEED TRAIN CULTURES: A TROUBLESHOOTING EFFORT

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Background and Novelty:
While starting cell processing from a cell bank thaw step into bioreactors using standard procedures for a CHO cell line, challenges were encountered where multiple seed trains had to be discontinued due to low viability and slow growth within the first few passages after thaw. When investigating the potential root cause it was determined that the observed wide range of post thaw viability for this cell bank was likely attributed to the occurrence of an unintended thaw (or partial thaw) and re-freeze event during one of the physical moves that occurred in this cell bank’s storage history.

Experimental Approach:
Experiments with cell bank ampoules were performed to re-create the conditions that potentially led to the low post-thaw viability phenotype. In these studies, the effect of exposing cell bank ampoules to room temperature for different time durations followed by non-rate-controlled freezing was tested. Additionally, studies were conducted to potentially identify factors that can improve thaw recovery. Initial scale-down troubleshooting experiments using a shake flask system showed rapid recovery in both viability and growth rates as opposed to their 2L bioreactor counterparts. Follow-up studies were conducted to examine differences in the two small-scale systems that would result in different thaw recovery rates, including pH control and levels of agitation and aeration.

Results and Discussion:
Ampoule studies revealed that the eutectic point potentially serves as the threshold below which partial thaws can be tolerated without affecting cell viability. Significant impact to post-thaw cell viability was found to occur with 30-45 minute room temperature exposures of ampoules, followed by non-rate-controlled freezing. It is hypothesized that this is due to ice crystal formation inside the cells during fast freezing, which leads to cell damage.
Among the factors tested to identify why low viability thaws recovered more rapidly in shake flasks, culture pH was shown to have the most significant impact, where a higher pH setpoint resulted in better performance in the thaw and early seed train passages as measured by cell growth and viability. Based on these findings, thaw performance characterization studies may be useful to conduct on specific cell lines to better understand the optimal operating ranges for cell line thaw and early seed train passages.
SCALE-UP TO 500 L COMMERCIAL MANUFACTURING SCALE OF VERY HIGH CELL DENSITY CHO CULTURES (>200 mln cells/mL) IN SINGLE USE STIRRED-TANK BIOREACTORS EXPERIENCES FROM THE HIGHLY INTENSIFIED XD® CELL CULTURE TECHNOLOGY AND THE INTEGRATED CLARIFICATION AND PURIFICATION RHOBUST® EBA TECHNOLOGY

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Background and Novelty:
Over the past decade DSM (now Patheon) Biologics has developed a number of breakthrough technologies in the field of mammalian cell culture process intensification and in the field of downstream process integration. With XD® cell culture routinely around 150 million cells per mL can be obtained in a ~2 week process with CHO and other cell lines. Due to the resulting high IVCs, typically a 5 to 10 fold titer boost is achieved compared to classical Fed-Batch operation with the same process length. Record titers of up to 27 g/L overall in the cell culture broth (and 40 g/L in the supernatant) have been reported. This in turn enables the use of relatively small (single use) bioreactors, typically 500 – 1000 L scale, for commercial scale production.

To deal with the high cell density XD® harvests, a next generation expanded-bed technology (Rhobust®) is has been developed. This technology is capable of dealing with viscous and highly particle loaded feed streams, such as yeast-, E.coli-, and also mammalian XD® cell culture broths. These technologies have now been scaled-up to commercial manufacturing scale in 500 L bioreactors in Patheon’s Brisbane Australia site.

Experimental Approach:
A step-wise approach was taken to the development of the XD® and Rhobust® technologies to commercial manufacturing scale. Identification of key process parameters and definition of control strategies was performed at 2 L bioreactor and 1-2 cm column diameter scale. XD® cell culture scale-up was performed step-wise in single-use bioreactors to respectively 50, 200 and 500 L scale. To deal with the extremely high cell densities, specific SUB and controllers were selected and customized. Also a customized cell retention system was designed. The Robust® Expanded-Bed performance was scaled-up from 2 to 10, 30 (and 60) cm column diameter. Customized large-scale EBA columns and skids were also designed.

Results and Discussion:
Results from successful scale-up and tech transfer at commercial scale of these cultures are presented. Furthermore typical examples from productions of e.g. IgGs as well as other recombinant proteins are presented, including biosimilars. Finally examples of continuous manufacturing are shared where continuous XD® cultures are followed by Rhobust® Protein A EBA clarification and purification.
THE CHALLENGES DURING SCALE UP AND TECH TRANSFER OF A HIGH CELL DENSITY CHO PERFUSION CELL CULTURE PROCESS IN SINGLE USE STIRRED-TANK BIOREACTORS AT 200L AND 2000L SCALES

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Key words:  
Single-use bioreactor / scale up / cho cells / perfusion.  

Background and Novelty:  
Single-use bioreactors are commonly used in the manufacturing of biologics because of the advantages of reduced facility complexity and increased operation flexibility. To support the production of therapeutic proteins, a fully disposable perfusion stirred tank bioreactor system has been developed at Shire. The system consists of a disposable stirred tank bioreactor and a disposable cell retention device. A high cell density perfusion cell culture process utilizing a CHO cell line was developed in a 10L laboratory scale bioreactor. The process was scaled up to 200L and 2000L single use bioreactors to enable the pre-clinical and clinical production of a recombinant protein. Challenges encountered during the development, scale up and tech transfer, such as cell retention device, mass transfer, mixing, shear stress, and centrifuge perfusion capacity will be discussed. The approaches to address these scale-up related challenges, and process performance across different scales (10L, 200L and 2000L) including cell growth, metabolite profiles, process yield, and product quality will be presented.

Experimental Approach:  
The scale-up strategy included two parts, one for the bioreactor and another for cell retention device (CentriTech® centrifuge). Bioreactor configurations including different options of sparger and impeller, as well as KLa and mixing studies at 10L, 200L and 2000L scales were used to finalize the bioreactor scale-up strategy. Five CentriTech® centrifuge operation parameters were evaluated to ensure the success of scale-up of the centrifuge, these included: rotor speed, separation pump flow rate, separate time, discharge pump flow rate and discharge time.

Results and Discussion:  
The CentriTech® centrifuge was used as a cell retention device and successfully support the perfusion cell culture of 30 – 40 × 10^6 cells/mL. A bleeding process, which is critical to the cell culture performance, was developed with the aid of ABER Biomass probe. Comparable process performance and product quality across scales were observed. Impacts of scale-dependent parameters on process performance and scale-up strategy will be presented.
DEVELOPING A HIGH-PERFORMANCE CHO PERFUSION PROCESS BY USING A DESIGN OF EXPERIMENTS STRATEGY

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Background and Novelty:
Continuous biomanufacturing has recently come into focus for both upstream and downstream unit operations. The advantages of continuous manufacturing include high volumetric productivity, reduced equipment size, steady state operations, and consistent product quality. Compared with media for fed-batch processing, however, perfusion culturing poses different challenges on the cell culture medium. High-performing cell culture media that are optimized for perfusion are required to achieve low perfusion rates, high cell densities, and high product titers in the perfusate. Here, we present a methodology based on a design of experiments (DoE) strategy for the development of a high-cell density perfusion process for antibody-producing Chinese hamster ovary (CHO) cells. The starting point for the optimization was commercially available ActiCHO™ medium and feeds. Productivity and product quality of the perfusion process were compared with a standard fed-batch culture process.

Experimental Approach:
ActiCHO P base medium was supplemented with ActiCHO Feed A and ActiCHO Feed B in various concentrations. The combination of medium and feeds were optimized in batch cultures using a DoE approach. The selected ratio of medium to feeds was evaluated in small-scale perfusion cultures using the single-use ReadyToProcess WAVE™ 25 rocking bioreactor system. The performance of the perfusion process was further validated in lab scale using single-use, stirred-tank bioreactor systems.

Results and Discussion:
With the DoE methodology used, a suitable combination of ActiCHO medium and feeds were quickly identified. The best combination of medium and feed that was identified in this study resulted in a process with a cell-specific perfusion rate (CSPR) of about 20 pL/cell/d and a cell-specific productivity of about 30 pg/cell/d. When compared with the reference perfusion process in this study, the perfusion rate decreased by more than 70%, while the cell-specific productivity remained constant. The selected ratio of medium and feeds were shown to be well-suited for use in a perfusion process. We conclude that DoE methodology can be adapted and used as a generic tool to optimize cell culture media to improve the performance of perfusion processes.
MONITORIZATION OF PH AND OTR USING A MULTIPLE SHAKE FLASK PLATFORM: A TOOL FOR METABOLISM AND CELL GROWTH ASSESSMENT IN MAMMALIAN CELL CULTURES

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Background and Novelty:
From 1989 the number of bioprocesses based on mammalian cell systems has been continuously increasing from 33% of the total number of new drug approvals (up to 1989) to 60% of that number (period 2010-2014)1. Due to the high costs of these bioprocesses and the increasing competitiveness in the field, the good characterization and optimization of cell growth and product obtention at laboratory scale have become highly necessary. Moreover, PAT initiative has grown in importance in recent years in the pharmaceutical industry. In this line of thinking, big efforts are directed to develop systems at laboratory scale to obtain real-time cell culture monitoring in a non-invasive manner to avoid cell culture disturbance.

In the present communication, the shake flask reader (SFR) from Presens was used in combination with the RAMOS-System2 adapted to disposable shake flask, for pH and pO2 monitoring and OTR, CTR and RQ determination. The comparison of those measurements with off-line metabolite and cell growth assessment, along with further analysis of the variables, led to (1) determine a good correlation between pH evolution and HEK293 metabolic behaviour, (2) define an OTR profile corresponding to cell growth evolution and cell activity and (3) get information of cell culture differences under distinct physicochemical circumstances.

Experimental Approach:
HEK293 cell batch cultures were performed in 250mL disposable shake flasks attached to the RAMOS-System and to SFR to get on-line measurements of pH and dissolved oxygen (DO). An initial batch culture with SFMTransFx-293 media 5%FBS and 10%CBS (80g/L) supplemented was performed. This media has been already tested for the obtention of high cell density cultures (up to 18x106 cells/mL)3. Then, two media modifications were carried out in order to grow the cells in a non-desired environment and detect differences if any- on pH evolution, OTR profile, cell growth, glucose consumption, lactate production and GFP production. These modifications consisted on: (1) acidification of media to pH=6.6 and (2) acidification of media to pH=6.6 and addition of sodium lactate (12 mM).

Results and Conclusions:
For the three conditions tested, pH profiles correlated good with lactate evolution: pH value decreased when lactate was produced and it increased when lactate was consumed. Moreover, pH value was stable when lactate was nor secreted neither consumed from media. In regards OTR profile, it was found out that its evolution perfectly fitted cell growth during exponential phase. In addition, a decrement of OTR slope was detected before a viable cell number drop was noticed. Accordingly, the linearization of OTR values showed shorter linear phase in comparison to the viable number cells linearization.

Taken all this into account, RAMOS-System in combination with SFR is an instrument with high potential for mammalian cell culture characterization as it provides reliable data of cell metabolism, growth and state. Furthermore, all this data is taken on line in a non-invasive manner and offers continuous measurements. Altogether would meet GMP constraints of a given bioprocess.
A NOVEL FULLY AUTOMATED PLATFORM FOR HIGH-THROUGHPUT CELL LINE SCREENING & DEVELOPMENT

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Background and Novelty:
Cell line development (CLD) of mammalian cells to produce biopharmaceuticals remains a challenging and costly exercise. Speed in CLD is of utmost importance, especially for generating early phase clinical material, where process development and provision of first GMP material is part of the critical path. Here, we present a newly developed automation and informatics infrastructure to parallelize the development of stably expressing cell lines. The platform can be applied to both antibodies (IgGs, novel formats) as well as therapeutic proteins (e.g. engineered FVIII variants, fusion proteins).

Experimental Approach:
We will show how Bayer Global Biologics has streamlined the generation and assessment of mammalian production cell lines by using this platform - shortening the development timelines campaign by up to 6 months and thereby significantly reducing costs.

Results and Discussion:
Parallel handling of up to six clone selection campaigns is achieved through a robotics system for high-throughput clone screening and selection. The increased throughput requires a systematic tracking of all cell lines, both host and developed, including seeding and incubation conditions, transfection and selection pressure protocols, clone parent-child relationships, online monitoring of growth characteristics, passaging, cryo-conservation (thawing/freezing cycles), genetic stability and hit selection criteria. As a fully integrated platform, the CLD database directly integrates with all instruments, such as steering of plating operations and cryovial preparation. Moreover, we present our workflow for downstream assessment of hit clones using mini-bioreactors (the ambr system), which emulates more realistic fermentation manufacturing conditions, and supports the incremental upscaling to the 100L scale and beyond. To control product properties and quality, our CLD platform also tracks all key analytics data, such as N-glycan profiles related to the process conditions. We will report on our experiences with this new CLD platform and provide an outlook on current development activities to further extend its capabilities and throughput.
DEVELOPMENT OF A SCALE-DOWN MODEL FOR A CHO FED-BATCH PROCESS USING THE MICRO-SCALE BIOREACTOR SYSTEM (AMBR15™) AND THE STATISTICAL SOFTWARE JMP 11.0

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Key words:
Ambr / jmp / scale-down / cell culture / process development.

Background and Novelty:
The identification of optimal upstream process parameters is a time, material and labor intensive task. Reliable scale-down models allowing very good process control like benchtop-scale bioreactors are limited in throughput, while high-throughput systems like tubespín® bioreactors or shake flasks allow only poor prediction of the larger scale. A new high-throughput microbioreactor system called the Ambr15™ (TAP biosystems) offers a good compromise between process control and throughput.

Design of experiment (DoE) is used in the development and optimization of products and processes, as it allows a greater understanding of the relation between factors (input values) and responses (output values), with minimal experimental effort. The statistical software JMP (SAS Institute, Inc., current version 11.0) is a very useful tool for the design of well-powered DoEs that allow the building of significant models.

Experimental Approach:
The combination of the high-throughput microbioreactor system Ambr15™ with DoEs (D-optimal, custom design) designed using the statistical software JMP 11.0 (SAS Institute, Inc) allowed the building of significant and reliable models looking at the impact of cell culture parameters on cell culture performance (viable cell density, viability, titer and product quality). The robustness of the scale-down model and the optimized conditions were assessed by comparing the process in stirred tank bioreactors at different scales (10L, 50L and 250L).

Results and Discussions:
The use of JMP allowed us to identify optimized culture parameters in the Ambr15™. Results will be presented showing the comparability of these parameters in stirred tank bioreactors at different scales (10L, 50L, 250L). These analyses were performed for different stable cell lines expressing therapeutic antibody candidates.
THE IMPACT OF MANUFACTURING PROCESS IN THE BIOSIMILARS DEVELOPMENT

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Background and Novelty:
In order to obtain the approval from the regulatory agencies, biosimilars must go through a rigorous development process and have to demonstrate no clinically significant differences in safety and potency when compared with the reference product. Considering this complex process, it is important to understand how biosimilars are developed.

Experimental Approach:
The biosimilars have to follow the same manufacturing process to their reference biologic product, which is very expensive and time-consuming. However, there are potential time and cost savings when comparing the biosimilar development to that of the reference product, since some steps are not necessary in the generation of a similar biologic product. Developers of biosimilars usually do not have access to the details of the manufacturing process and active ingredients used for the reference product development. Although following almost the same steps, the inherent variability of the biologic system used and the manufacturing process will not result in a biological product identical to their respective reference product. The only characteristic that will be a copy of the reference product is the amino acid sequence. A comparison of the structural and functional characteristics and the product and process-related impurities of the biosimilar and their reference product will be necessary. Any difference found must be justified with regard to the potential impact on the clinical performance of the biosimilar. Details in the production of any biologic product vary from batch to batch and with any manufacturing change that can occur for different reasons, inherent to the process itself or resulting of changes in equipment or improvement in any part of the process, including finishing. The available comparability protocols allow these changes to occur, and in the same way, this concept provides support for the biosimilars evaluation. The biosimilars manufacturers are developing and validating powerful analytical tools to compare their products with its originators. An interesting point is that these improved analytical methods that allow the detection of even small changes, are also revealing variability between lots of reference products currently on the market. The use of different expression systems in the development of biosimilars compared to their references may change the post-translational modifications such as the glycosylation profile of the protein, which in turn could affect the safety or effectiveness of the product. Variations in the glycosylation protein pattern can alter the immunogenicity or clearance of the final product.

Results and Discussion:
The development of biosimilars to replace the biopharmaceuticals for which the patents has expired or are about to expire led to new concepts in the manufacturing process. The production facilities for biosimilars can adopt the use of single-use technologies, reducing the initial costs while relying on multipurpose plants, aspects sought after by biosimilars industry.
SCALING-UP STEM CELL MANUFACTURING: IMPACT OF ALLOGENEIC STEM CELL MANUFACTURING DECISIONS ON COST OF GOODS AND PROCESS ROBUSTNESS

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Background and Novelty:
Cell-based therapies are gearing up to have extensive impact on the healthcare field in the coming years. In order to capitalize on the potential of these therapies, efficient methods for growing the adherent stem cells at larger scales are required. This sets the stage for the development of technologies that combine safety, reproducibility and GMP compliance while keeping the cost of goods under tight control. The portfolio of technologies currently available in the market include the use of microcarrier systems in suspension and Xpansion® (Pall Life Sciences), a controlled and closed system with the capacity of up to 122,400 cm². The novelty of the work described herein lies in the use of an advanced process economics and optimization model to identify (a) the most cost-effective expansion technology for different scales, (b) upstream and downstream “technology gaps,” and hence, where additional development is required to meet target scales, (c) the robustness of these technologies to process variations and (d) cost of goods targets for different reimbursement scenarios.

Experimental Approach:
This work describes a detailed cost analysis of the commercial manufacture of allogeneic mesenchymal stromal cells (MSCs). A case study is presented that compares single-use planar technologies to microcarrier-based bioreactors across a range of commercial scales and doses to examine how their ranking changes in different industrial scenarios. The economic feasibility of these technologies was evaluated using a decisional tool developed at University College London that integrates bioprocess economics with optimization. The key assumptions used in this study were attained through comprehensive a literature review in combination with interviews with cell therapy experts.

Results and Discussion:
The analysis identified the current limitations of both upstream and downstream technologies for commercial-scale manufacture of MSCs. The tool outputs predicted the optimal technology for MSC cell expansion for different demands and lot sizes. A detailed set of sensitivity analyses was carried out to identify the critical performance levels of microcarrier systems to be the most cost-effective option for commercial scale manufacture of cell therapy products at each combination of lot size and demand.

The analysis was extended by exploring the impact of technical uncertainties and failure rates on the feasibility of the strategies using Monte Carlo simulation. The risk analysis results allowed the economic robustness of the technologies to be explored. These studies, coupled with an analysis of possible market penetration uncertainties and different reimbursement strategies, provide insight into the overall profitability of different cell therapy products and the optimal manufacturing strategy to be applied in different scenarios.
EFFECT OF MEDIA COMPONENTS ON THE INCREASE IN THE INCORPORATION OF THE IMMUNOGENIC N-GLYCOLYLNEURAMINIC ACID

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Background and Novelty:
Glycosylation of recombinant proteins is known to play a critical role in the clinical properties of the final glycoprotein such as stability, solubility, secretion, in vivo clearance and immunogenicity. Thus, the post-translational machinery present in mammalian cells has become valuable for the production of recombinant therapeutics. However, murine-produced glycoproteins can contain non-human glycan epitopes that can result in immunogenic reactions (e.g. terminal Gal 1-3Gal and N-glycolylneuraminic acid) if used in vivo as a therapeutic agents. The objective of this study was to determine which media components can enhance the incorporation of N-glycolyneuraminic acid into glycoproteins.

Experimental Approach:
For this purpose, NS0 cells were cultured in media containing different combinations and concentrations of manganese chloride (MnCl₂), uridine and galactose. Cell growth, viability and substrate consumption were monitored daily. The glycosylation profile was analyzed using hydrophilic interaction liquid chromatography (HILIC) and N-glycolyneuraminic acid (NGNA) and N-acetyleneuraminic acid (NANA) levels were determined by reverse-phase (RP) chromatography.

Results and Discussion:
Using HILIC we showed that the addition of MnCl₂ (7μM) increased the galactosylation index (GI) of IgG produced by NS0 cells from 0.58 to 0.64. Further increases were observed when MnCl₂ was combined with uridine (GI=0.73) or galactose (GI=0.78). In addition, the percentage of sialylation was incremented in all media supplements. The highest increase in sialylation (34%) was observed in media containing both MnCl₂ and galactose. RP chromatography allowed the quantification of the proportion of the sialic acids, NGNA and NANA under the described conditions. We also detected an inverse correlation between the galactosylation index and the fucosylation index when MnCl₂ plus galactose were incorporated into the media. Thus, the fucosylation index decreased from 0.85 to 0.69. Cell growth and viability were not affected by MnCl₂ alone or when combined with uridine or galactose. However, there was a slight decrease in growth observed in cultures containing a combination of MnCl₂, uridine and galactose along with a decrease in cell viability.
METABOLIC PHASE DETERMINATION AND MODELING USING STATISTICAL TOOLS: APPLICATION TO CHO CELLS PRODUCING A MONOCLONAL ANTIBODY

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Background and Novelty:
Mathematical modeling of the cell metabolism can be applied in order to characterize the physiological behavior of the cells during biopharmaceutical production. However, throughout the bioreactor production, the cells adapt to the changing extracellular conditions resulting in the development of different metabolic phases and related metabolic shifts. This makes modeling of the cell metabolism for all phases of cultivation more difficult. In order to deal with this complexity, one can identify metabolic phases, divide the cell culture process into several phases and perform separate analysis which allow applying many mathematical simplifications. So far the identification of these phases rely on expert judgment. To determine and characterize metabolic phases, the concept of metabolic flux ratio has been extended to relation between the specific consumption/production rates of each metabolite i (v_i) and the growth rate. We propose different systematic methods for the identification of metabolic phases. We applied and compared: (a) segmented regression, (b) recursive partitioning and (c) hierarchical clustering methods.

Experimental Approach:
The model developed in this study assumes that, within each metabolic phase, a linear relationship between each (v_i) and the growth rate can be observed. Breakpoints of the slopes defined the metabolic shift between each metabolic phase and are used as a metabolic phase selection criteria. Three mathematical tools, i.e. (a) segmented regression, (b) recursive partitioning and (c) hierarchical clustering, have been successfully tested to select the breakpoints in a systematic manner. The accuracy, precision and limit of the methods were first tested on generated data with increasing noises. Then, the methods were applied to a fed-batch process using Chinese ovary hamster (CHO) cells producing a monoclonal antibody (Mab). To get various growth profiles and different experimental conditions, three amino acids have been modulated in 2 liters bioreactors. Metabolites analyzed were all amino acids, glucose, lactate, ammonium and Mab.

Results and Discussion:
We present a new methodology to determine and characterize in a systematic manner metabolic phases in fed-batch processes. Three metabolic phases have been successfully determined as a function of the growth rate. High precision and accuracy were observed for the three methods used. For each phase, all v_i correlated linearly with the growth rate. A simplification of the interpretation of cell culture data and a better understanding of the interconnection between metabolites were achieved. Being able to use only one fixed stoichiometric model for each metabolic phase facilitates modeling of cell cultivation to a large extent. Moreover, the impact of the three amino acids tested on each v_i was observed by the three methods. Finally, a methodology to select and characterize metabolic phases has been developed combining the three methods: (a) segmented regression, (b) recursive partitioning and (c) hierarchical clustering.
CHEMICALLY-DEFINED MEDIA IMPLEMENTATION ACTIVITIES TO MINIMIZE MEDIA VARIABILITY IMPACT TO CELL CULTURE PERFORMANCE AND PRODUCT QUALITY

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Background and Novelty:
Chemically-defined media have been developed for CHO cell culture platform processes. During implementation of the media at multiple scales and sites globally, additional activities were required to seamlessly balance practical considerations with desired process and product quality performance. Three main focus areas were further investigated and optimized to enable the platform. These focus areas included: 1) media preparation, filtration, and post-filtration stability; 2) media and supplement viral barrier compatibility; and 3) and raw material variability considerations. Overall, the effort provided an outlined approach for media implementation in the global network including scale and site-specific case studies where novel options were required.

Experimental Approach:
First, liquid media preparation, filtration, and post-filtration stability were evaluated against desired acceptance criteria. Media preparation was performed at multiple scales, including relevant manufacturing scales and resulting media were tested with a model cell line. Filtration capacity was evaluated using relevant scale-down filtration devices. Post-filtration media stability was evaluated extensively over a range of variables and data was gathered that included the effect of handling practices and storage environment. Second, viral barrier compatibility via high-temperature-short-time (HTST) treatment was assessed for chemically-defined media via analytical and cell culture tests. And, other viral barrier methods were assessed for smaller volume media supplements. Third, the risk of negative impact from specific raw material impurity variability was assessed.

Results and Discussion:
The data generated supported successful implementation of chemically-defined platform media at different global sites within our network. Manufacturing-scale media preparations met acceptance criteria and resulted in comparable cell culture performance and product quality to controls. Practical challenges were identified and risk mitigation efforts initiated. The challenges were primarily related to poorly-defined media preparation mixing environments as a result of variable equipment availability and use. Filtration capacity acceptance criteria were met in the scale-down model and large-scale filtration events were successful. HTST-compatibility studies showed that HTST-treated media performed comparably to control media in cell culture performance and product quality. Post-filtration media stability investigations led to the identification of key risk factors including temperature and agitation variation during storage, nucleation and media destabilization as a result of specific handling practices, and the impact of specific process-contacting materials. Options for mitigating the media stability risks and enabling successful implementation were developed and showed no negative impact for the application. Finally, platform media modifications were developed to mitigate raw material variation and product quality challenges. The overall evolution of the media platform to ensure flexibility and successful implementation will be summarized.
IDENTIFICATION OF PERFORMANCE DRIVERS FOR AN ANTIBODY PRODUCING
CHO-S CELL LINE CULTURE IN THE XRS20 SINGLE USE BIOREACTOR
UTILIZING HISTORICAL DATA

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Background and Novelty:
During the development of processes for high production CHO fed batch cultures, there is usually insufficient
time for thorough process optimization. Once defined, these processes often have ranges rather than specific
values for many parameters, resulting in minor variations in the runs. These small process variations, combined
with resultant differences in performance, can be used to further optimize the process. Over the course of one
year, eleven XRS20 fed batch cultures of an antibody producing CHO-S cell line were generated. In addition
to process variation, small process changes occurred due to method optimization efforts or culture timing
requirements. An analysis of the combined historical data was undertaken to better understand the specific
culture performance drivers, possibly leading to further improvement in culture outputs, specifically final
antibody concentration and maximum viable cell density (VCD).

Experimental Approach:
Two different XRS20 bioreactors were used for the fed batch runs. CHO-S mAb cell line was grown in CD
FortiCHO® media supplemented with Efficient Feed Cā (five consecutive days) and glucose (as needed). In
cases the final expansion was done in the XRS20 biocatalyst. The culture variables analyzed: passage
number; initial and final post expansion seed density; mixing speed; VCD at first feed; viability at harvest; and
total days in culture.
In addition to almost daily sampling for cell count, viability, metabolite/nutrient, gases and pH measures, the
final harvest was assayed for mAb, host cell protein, and DNA concentrations. The data from these runs was
compiled and analyzed for trends linking the variations in run parameters with the critical outputs of maximum
viable cell density and mAb titer.

Results and Discussion:
The XRS20 single use bioreactor proved quite reproducible, allowing for a holistic analysis of relatively small
changes in process conditions and the resultant small differences in performance. In three separate paired runs,
the first being identical and the second two differing by 30 vs. 35 cycles per minute, the replicate run results are
extremely similar, highlighting the overall reproducibility of the system. This bioreactor reproducibility makes
it possible to review historical data to identify performance drivers that were missed in limited optimization
testing done at smaller scale prior to the 20L runs. The VCD at the time of the first feed addition proved very
important for overall mAb yield. Although this was expected, the optimal range was somewhat tighter than
previously believed. Nutrient feed timing can be easily modified as there is very good understanding of the
growth curves for this CHO-S cell line in the XRS20 bioreactor. This approach can be expanded to other cell
line / media combinations in any culture system having sufficient reproducibility to distinguish relatively small
changes in process conditions and the resultant small differences in performance.
FROM CLONE SELECTION TO 80L BIOREACTOR PRODUCTION:
BETTER, FASTER, LEANER

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Background and Novelty:
Bio-pharmaceutical industries face an increasing demand to accelerate process development and reduce costs. Indeed as technologies mature, pressure on cost and timelines is greater for delivering scalable and robust processes. In this poster, we present a novel method in order to speed up and interconnect clone selection with cell culture process development and scale up to 80L scale.

Experimental Approach:
In one minibioreactors run different feeding strategies were assessed on 4 pre-selected lead clones for selecting the lead clone and the fed-batch feeding strategy all at once. In the Design of Experiment (DoE) 16 conditions were run in triplicate. Triplicates were pooled on harvest day for Product Quality Attributes analysis. The clone and feeding strategies were selected based on the maximization of monoclonal antibody (mAb) titer, target product quality profile and process robustness. We then scaled up directly to 80L manufacturing production.

Results and Discussion:
High throughput technologies enable us to interconnect clone and process development thus reducing the risks associated with early-stage process development while reducing timelines and enabling us to achieve higher process robustness in a leaner manner. Indeed selection of the lead clone together with the optimal feeding strategy was performed in one single ambr™ run and 16 conditions were tested in triplicate at the time of clone selection, compared to conventional approaches relying on 1L-10L stirred tank bioreactor experiments run on a single lead clone. Scale up was achieved successfully thus enabling early material production for downstream, formulation and analytical development. Similar cell growth profiles and mAb titers were obtained at ambr™ scale and 80L scale for the selected condition (lead clone and feeding strategy). Percentages of monomers and aggregates obtained at harvest day were comparable between scales. Process robustness was shown by performing a minimum number of 3 batches at 80L scale. In conclusion, miniaturized bioreactors enabled us to interconnect clone and process design space selection and enabled immediate 80L production.
TOWARDS MITIGATING THE IMPACT OF POLOXAMER P188 VARIABILITY ON COMMERCIAL BIOLOGICS MANUFACTURING PROCESSES

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**Background and Novelty:**
Multiple protein-based therapeutics are produced in Roche’s commercial drug substance manufacturing facilities every year, which provide remedies for several life-threatening diseases. These biologics medicines are produced at large-scale in fed batch mammalian cell culture processes utilizing suspension-adapted Chinese hamster ovary-based cell lines. Kolliphor® P188 (also known as Poloxamer P188), a water-soluble synthetic copolymer with hydrophilic as well as lipophilic properties, is used in the cell culture media formulation as a cell shear protectant.

**Experimental Approach:**
Poor cell growth and/or lower product yield has been recently observed at commercial scales in more than one manufacturing process within Roche network when specific lots of P188 were used. An investigation is being conducted to unearth the root cause of “poor-performing” P188 lots and to identify means to mitigate the product supply risks. P188 raw material is sourced from a single vendor with additional supply constraints and risks associated with the impending change in the vendor manufacturing facility and location. These factors further exacerbate the supply risks for the drugs produced by Roche.

**Results and Discussion:**
A multi-pronged approach has been undertaken. A small-scale cell-culture based screening assay was developed and deployed to proactively identify poor-performing P188 lots. The screening tool aids in securing adequate inventory of good-performing P188 lots and mitigate the associated supply risks. To discern the root cause for the lot-specific loss of P188 functionality, analytical fingerprinting methods have been used to test different hypotheses including presence of impurities as well as structural differences. A novel treatment method was also uncovered and optimized which can improve the functionality (i.e. cell culture performance) of a suspect P188 lot. The presentation will highlight the risk-mitigation activities pursued for this crucial raw material ubiquitously used in cell culture manufacturing processes.
OVERCOMING THE CLARIFICATION CHALLENGES
OF HIGH CELL DENSITY CULTURE

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Background and Novelty:
Cell line engineering and process intensification efforts have led to high performing fed-batch cell culture processes where cell density can reach >30x10^6 cells/mL. These high performance processes also come with new challenges, in particular developing efficient and scalable primary recovery processes. Centrifugation coupled with depth filtration is a standard approach for clarification of mammalian cell culture broth. However, with high cell density cultures, it can become challenging to obtain low turbidity clarified cell culture fluid (CCCF), and the filtration surface area required for clarification may become limiting when scaling up.
We explored novel clarification strategies with the goal of removing more impurities during harvest and increasing the robustness of this process step while opening up possibilities for the reduction of the filtration surfaces. In this poster we will present the evaluation of the addition of a flocculation agent at the end of the bioreactor production step and prior to disc stack centrifugation and filtration.

Experimental Approach:
Small scale screening studies were designed to determine the most suitable flocculation agent and the optimal flocculation conditions for a CHO cell culture broth producing a monoclonal antibody. The most promising flocculant concentration and agitation speed during flocculation were then selected to be scaled-up in a 80L bioreactor culture.
At the end of a 14-day fed batch, the cell culture broth was treated with the flocculation agent. Standard harvest operations were then performed on the feed stream. These include disc-stack centrifugation, depth filtration and membrane filtration.
The impact of flocculation on antibody recovery and quality attributes was evaluated as well as on impurity clearance.
Clarification performance was assessed by measuring turbidity of the feed stream after each clarification step and by evaluating its filterability.

Results and Discussion:
The small scale screening studies enabled to select a flocculation agent which was shown not to affect antibody recovery and quality attributes. Moreover, the analysis of the CCCF showed that the use of the flocculation agent enabled to reduce significantly the level of impurities prior to the purification process.
The optimal flocculation agent concentration and agitation speed selected at small scale were then successfully scaled-up to 80L culture and the flocculated broth was processed through disc-stack centrifugation and depth filtration.
Performance of the harvest process was significantly improved. A 5-fold reduction of CCCF turbidity was observed and processability of the feed stream through filters was significantly increased.
Especially, filtration capacity of CCCF treated with flocculation agent through 0.2μm filter was increased more than 10 fold compared to control conditions. Filtration through 0.1μm filter, which was impossible with untreated CCCF, was enabled.
The effect of flocculation on impurity removal observed during screening studies was confirmed at 80L scale. Overall, the results we obtained suggest that the introduction of a flocculation step would improve the efficiency and scalability of the harvest process, by reducing impurity levels and required filter surface area, and therefore ease the future implementation of our high cell density process in a large scale facility for commercial manufacturing.
PROCESS RAMAN SPECTROSCOPY FOR IN-LINE MONITORING
OF MAMMALIAN CELL CULTURES IN REAL TIME

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Background and Novelty:
Mammalian cell cultures are complex processes where cells are cultivated under highly controlled conditions using media with a very high number of components. Current effort is focused on obtaining a better understanding of mammalian cell cultures by cultivating predominantly CHO cells for therapeutic protein production. To ensure a healthy progression of the cell culture, it is important to understand and monitor the stages of the biologic manufacturing.
In order to build quality into a process a primary step is to analyze the process, understand what the critical quality attributes are, and monitor or rather control those factors. Consequently, there is a significant interest and value in techniques that provide instantaneous response for monitoring and analyzing biopharmaceutical processes. Molecular spectroscopy techniques are widely used for PAT applications, because they provide in-situ information in real-time.

Experimental Approach:
Raman spectroscopy is non-destructive and doesn’t require sampling. It works very well in aqueous systems and spectra contain chemical and physical information. The spectrometer allows to run 4 probes sequentially and the use of in-line or non-contact optics eliminate the contamination risk due to manual sampling. The measurement time is usually 10 to 15 min for cell culture processes and the instrument design is made toward production which grants simple transferability among up- and downscale.
Off-line reference standards and in-line Raman data can be used to create specific or universal partial least square (PLS) regression models. Calibration models can be initially developed from around 20 reference samples, but building a larger data matrix - by simply adding more data to the model - will increase its robustness and precision.

Results and Discussion:
Real-time measurements within Biopharma are achieved for Glucose, Glutamine, Glutamate, Lactate, Ammonium, Viable Cell Density, Total Cell Density, Osmolality, and Viability. The analyzer software enables a completely integrated bioprocess management and the spectrometer can be used for various applications, because it allows a very high flexibility in terms of sampling since the immersion or non-contact optics are compatible with all industry standard housings and available in various focal length or areas.

Self-biography: I am a Life-Science Engineer, worked at Max Planck Institute for Molecular Genetics in Berlin as a Biologic-Technical Assistant, at Procter&Gamble Brussels Innovation Centre as a Process Development Engineer, and at Bayer Technology Services in Berkeley as a PAT-Biologics Engineer. I recently joined Kaiser as an Applications Scientist to analyze data, create chemometric models and to look for new applications.
INFLUENCE OF CELL CULTURE MEDIA AND FEED SUPPLEMENTS ON CELL METABOLISM AND QUALITY OF IgG PRODUCED IN CHO K1, CHO-S, AND CHO DG44

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Background:
Since the first use of Chinese hamster ovary (CHO) cells for recombinant protein expression, production processes have steadily improved through numerous advances. During the last years, the field of medium development has advanced in particular and several chemically defined CHO specific culture media have become available, sometimes also media systems that combine basal medium and feeds. Although the portfolio of media and feed systems is continuously expanding, the lack of published data examining how cell metabolism, antibody production and quality in batch or fed-batch cultures are affected by different combinations of commercial basal media and feeds, encouraged us to test such combinations. Further, we aimed to understand how these attributes would change if the same antibody was expressed by different CHO cell lines in different media combinations.

Experimental:
In this study, chemically defined CHO cell culture media and feed substrates from different vendors were investigated regarding their influence on cell metabolism as well as product titer and quality. Special emphasis was put on elucidating how these attributes change with the use of different CHO host cell lines. Three recombinant CHO cell lines, CHO K1, CHO-DG44, and CHO-S, each producing the same IgG antibody, were adapted to ActiCHO™ P and CD CHO medium. All three cell lines were grown both in batch and in fed-batch cultures using the manufacturer’s specific concentrated feed supplements. The impact of the different media and feeds was investigated by analyzing antibody production, cell growth, cell-specific nutrient consumption, and by-product formation throughout the process.

Results and Discussion:
During batch cultivation, the cell-specific growth rate differed among the recombinant CHO K1, CHO DG44, and CHO-S cell lines (0.4, 0.5, and 0.6 d⁻¹, respectively) and was up to 20% higher in ActiCHO P compared with CD CHO medium. The combination of basal medium and cell line greatly influenced the viable cell integral, which differed up to two-fold between combinations. Accordingly, titers ranging from 56 to 391 mg/L were achieved in the batch cultures. Combination of basal medium and feed also had a substantial effect on growth rates during fed-batch culture. While all cell lines grew at comparable rates of about 0.5 d⁻¹ during the exponential growth phase in ActiCHO P, up to 40% lower values were observed for cultures grown in CD CHO. In ActiCHO P also higher peak cell concentrations were achieved, resulting in up to a five-fold difference in the viable cell integral. Due to additional effects on the cell-specific productivity, up to six-fold differences in IgG titers were found between cells grown in the two media. To correlate cell-specific productivities with intracellular mRNA transcript levels, antibody production was monitored at different time points throughout the fed-batch culture. Additionally, the expression of light and heavy chain polypeptides was quantified in the three CHO cell lines. To determine the effect of the different cell culture media on antibody quality, IgG from all cultures was affinity-purified and analyzed in-depth for product quality by SDS-PAGE, Western blotting, size exclusion chromatography, microcalorimetry, distribution of charge variants, and antibody glycosylation.
TITER IMPROVEMENT OF MEDIUM WHILST MAINTAINING PRODUCT QUALITY IN BIOSIMILAR DEVELOPMENT

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Background and Novelty:
During continuous process optimization different supplements and feed components have been tested in order to increase titer of a biosimilar project. It has been shown that by using single components only a minor effect on titer or process performance could be observed. By combining specific supplements a significant effect on specific productivity was observed.
Mammalian cell metabolic engineering holds the potential to develop high-producing hosts for the manufacture of complex biopharmaceuticals. In order to achieve this, all the relevant components need to be available in sufficient concentration.
To be highly productive it is important that the correct supplements are available but it is even more important that the correct combination of components is present.

Experimental Approach:
Specific supplements alone and in combination were tested to examine their effect on titer enhancement and/or growth performance. One component was an amino acid mix. Amino acids serve as the primary nitrogen source for cells; they are a very important element in protein biosynthesis and have to be available in sufficient amounts. We tested several feeds in order to increase productivity. According to literature altering the mixture of amino acids enhances the glucose uptake of CHO cells, a crucial component of building up complex proteins. There are other alternative carbon sources used in fed batch culture and although their impact has been extensively studied alone, combined effects have not been systematically investigated. Alternatives can even be favored during specific stages of cultivation. Additional factors that improve intermediate metabolic flux have also been tested and in some, moderate effects were observed. The novel part of these experiments was the combination of adding additional nitrogen and carbon sources with a metabolic enhancer.
Therefore, these components mentioned above were tested in a DoE-based approach.

Results and Discussion:
We found that individually none of our components tested greatly improved outcome but in combination they showed a very good and significant increase in titer. Importantly product quality was maintained within defined ranges in order to remain comparable to the reference molecule. These results show the positive synergistic effect of adding a specific combination of supplements to a culture process instead of adding single components and the concomitant maintenance of product quality.
UPSTREAM PROCESS DEVELOPMENT FOR GBR 1302, A BISPECIFIC ANTIBODY BASED ON GLENMARK’S PROPRIETARY BEAT® (BISPECIFIC ENGAGEMENT BY ANTIBODIES BASED ON T CELL RECEPTOR) FORMAT

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Key words:
BEAT®, bispecific antibody, CHO cells, heterotrimerization level, scale-up.

Background and Novelty:
GBR 1302 is a therapeutic antibody of the Glenmark proprietary BEAT® (Bispecific Engagement by Antibodies based on T cell receptor) format, targeting simultaneously the T-cell marker, CD3 and a receptor overexpressed in different cancer types, HER2 (Erbb2). This antibody has a unique mechanism of action. Its mechanism is to redirect effector T cells towards cancer cells, which subsequently are efficiently lysed.
GBR 1302 is composed of three different subunits (HC, scFv-Fc and LC) that are expressed in recombinant CHO-S cells. The goal of this study was to develop an upstream fed-batch process in 5L scale benchtop stirred-tank bioreactors and to scale-up the process to 250 L while maintaining industrially-relevant productivity levels.

Experimental Approach:
The feeding strategy was developed in shake flasks using a custom D-optimal experimental design created by the statistical software JMP 11.0 (SAS Institute, NC). A model was built based on the optimal process conditions for performance. The conditions identified were used as a basis for a process implemented in a 5L stirred-tank benchtop bioreactor. After optimization of the gassing strategy, the process was scaled to 50L and 250L.
Product quality attributes were assessed at several time points during the bioreactor run. While GBR 1302 is a heterotrimer composed of 3 subunits of different size, the cells could also produce minor contaminants of heterogeneous composition. The assembly of GBR 1302 was identified by non-reducing CGE, the aggregation level was determined by SEC, the glycosylation was analyzed by CE-LIF, charged variants by CEX and the accumulated titer in the supernatant was determined by both PA-HPLC and PG-HPLC.

Results and Discussion:
After minor modifications in the gassing strategy the benchtop scale stirred-tank bioreactor showed similar titers to those determined during the small-scale optimization runs. This process was successfully scaled up to 50L and 250L scale, without loss in performance.
The major product’s quality attributes were not affected by the scale of production. The level of heterotrimerization, fragmentation, aggregation and charge variants remained within the same range. The glycosylation pattern of GBR 1302 was found to be within the range of that found with IgG antibodies produced using the same host cell.
Samples analyzed at different time points during the same process also showed a constant heterotrimerization and aggregation level throughout the length of the culture. Only the glycosylation pattern changed towards less mature forms at the end of the culture, which is a well-known phenomenon of CHO culture.
These data show that the GBR 1302 BEAT antibody has characteristics in upstream process development that are very similar to a standard IgG antibody. The assembly of GBR 1302 appears to be very robust and is not subject to modifications during different phases of a bioreactor fermentation or with the bioreactor process run at different scales.
APPRAISAL OF THE MIXING PERFORMANCE OF A SHAKEN BIOREACTOR WITH CONICAL BOTTOM

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Background and Novelty:
The pharmaceutical industry is at the forefront of the production of antibodies using mammalian cell-based cultures. Mammalian cells are usually preferred to other host cells because of their favourable folding characteristics, assembly and post-translational abilities. At laboratory scale cells are often grown in shaken bioreactors, which are largely employed in the early stages of bioprocess development. Once the process is optimised at small scale, it is implemented in stirred tank reactors (STRs), which is bioreactor type most commonly used at production level. It should be emphasised however that cell growth and environmental conditions vary significantly between shaken cultures and those encountered in large STRs. This often becomes a bottleneck in bioprocess development as cells respond differently to the different geometries and mixing mechanisms taking place in the two scales and types of bioreactors. Such differences have prompted the industry to look at production scale of Orbitally Shaken Bioreactors, OSRs. OSRs employ the agitation principle of shaken flasks and microwell plates, thus facilitating scaling-up and simplifying regulatory approval.

Experimental Approach:
The aim of the work is to characterize the mixing and flow dynamics in a cylindrical orbitally shaken bioreactor with conical bottoms of different heights. The rationale for a conical bottom is to ease the suspension of microcarriers, which are used for the cultivation of cells not yet adapted for suspend culture, such as legacy cell lines and stem cells. This study builds upon previous works of the research group (Weheliye et al 2013, Rodriguez et al. 2013, Rodriguez et al. 2014,) for flat bottom reactors, where increases in Froude number were found to determine a mean flow transition and to increase the turbulence levels. The major objective of the current work is to determine the performances of shaken bioreactors with conical bottom, and to assess to what extent the mean flow and flow regime transitions already identified for a flat bottom are affected by the geometry variation.
Particle Image Velocimetry, PIV, and Dual Indicator System for Mixing Time, DISMT, were employed to assess the mixing performances in the bioreactor with a conical bottom. DISMT consists in a colorimetric method where two pH indicators are used to visualise the level of mixing reached after insertion of an acid solution.

Results and Discussion:
Preliminary results indicate that the toroidal vortices characterizing the flow for in-phase conditions are more stable as the inclination of the conical bottom is increased, with the vortices reaching to the bottom of the reactor at a lower Froude number to that identified for a flat bottom reactor. Nevertheless the Froude number associated to the laminar to turbulence flow regime transition does not change with variation of geometry, indicating that a conical shaped bottom might be preferred for suspension of cells and microcarriers at low speed, when the system is in phase.
The findings of the current study provides insight into the flow of a shaken bioreactor and offers a novel approach to design the next generation of products and improve scaling methodologies.
THE SPECIFIC POWER INPUT AND ITS SPATIAL DISTRIBUTION AS IMPORTANT PARAMETERS FOR SCALE-UP AND SCALE-DOWN IN ORBITALLY SHAKEN BAG BIOREACTORS

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Background and Novelty:
Orbitally shaken bag bioreactors can be considered to be an economic alternative to standard stirred bioreactors. If a shaking incubator (Multitron Cell by INFORS HT) is already installed in a laboratory, low expenditure is required in order to increase the culture volume from several hundreds of millilitre in shake flasks up to a size of 10 L. Successful cultivation of mammalian, insect [1] and plant [2] cell suspension cultures demonstrated the wide range of possibly application of orbitally shaken bag bioreactors. Recently, the mixing time and the oxygen mass transfer have been measured for water [1] and non-Newtonian fluids [2] to even mimic the fluid properties of plant cell suspensions.

Experimental Approach:
Within this presentation main attention is given on specific power input investigations, which are very important in order to fully characterize the fluid flow behaviour in mixing devices. The data were determined experimentally and by numerical methods using Computational Fluid Dynamics (OpenFOAM).

Results and Discussion:
Specific power inputs of more than 200 W m⁻³ were achieved for 2 L and 20 L bags. Both the numerical and the experimental data correlated well. Furthermore, very good correlation was found with data from the investigations in which mixing time and oxygen mass transfer were determined [2]. The out-of-phase phenomenon, in particular (previously identified for certain operating parameters) could be confirmed by measuring the specific power input. When operating parameters change during the growth phase, due to changes in volume resulting from feeding or because of variations in broth viscosity, fluid flow may shift to an out-of-phase condition. Therefore, the characterization is essential to avoid out-of-phase which will lead to de-mixing and a decreased mass transfer.

The suitability of this approach to scale-up processes will be proven by application examples with insect cell (SF-9) and plant suspension cell (Helianthus annuus) cultures.

IDENTIFICATION OF EXPRESSION BOTTLENECKS OF RECOMBINANT POLYMERIC ANTIBODY PRODUCTION

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Background and Novelty:
The generation of efficient production systems for recombinant proteins such as antibodies typically involves the screening of an extravagant number of clones in order to finally select a stable and high-producing cell line. Thereby, the underlying principles of a powerful protein machinery, but also potential expression limitations often remain poorly understood. To shed more light on this topic we applied several different techniques to investigate a previously generated cell line (4B3-IgA), which expressed recombinant immunoglobulin A (IgA) with an unusually low specific productivity.

Experimental Approach:
The low-producing cell line (4B3-IgA) was extensively characterized regarding growth rate and IgA productivity in long-term culture as well as by qPCR to determine gene copy numbers (GCN) and mRNA transgene levels and compared to the well-producing cell line (3D6-IgA) and host cell line. Further, flow cytometry and immunofluorescence microscopy were applied to determine the content and possible bottlenecks of intracellular antibody polypeptide processing. Intra- and extracellular product of light chain and heavy chain polypeptides were visualized by Western blotting to characterize misfolded and wrongly processed polypeptides.

Results and Discussion:
The low specific productivity of clone 4B3-IgA could not be explained by GCN or mRNA levels, but insufficiencies in protein maturation and/or secretion were determined. Despite the presence of free light chain polypeptides they occasionally failed to associate with their heavy chain partners. Consequently, heavy chains were misassembled and accumulated to form intracellular aggregates, so-called Russell bodies. These protein deposits evoked the expression of increased amounts of ER resident chaperones to combat the induced stress. Despite bottlenecks in protein processing the cells’ quality checkpoints remained intact and predominantly correctly processed IgA was exported into the culture medium. The results of our study demonstrated that recombinant protein expression was impaired by heavy chain aggregation despite the presence of disposable light chain and revealed elevated chaperone formation in combination with limited antibody assembly. Our studies suggest that the primary amino acid sequence and consequently the resulting structure of an expressed protein needs to be considered as a factor influencing a cell’s productivity.
METHOD DEVELOPMENT AND APPLICATION OF GROWTH PHASE SPECIFIC RECOMBINANT PROTEIN GLYCOSYLATION ANALYSIS

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Background and Novelty:
Heterogeneity in recombinant protein quality (e.g. glycosylation) can result from a) changes in cultivation conditions during a fermentation process, b) product degradation due to differing residence times of product molecules in the supernatant, c) heterogeneities in intracellular PTM machinery, or from a combination thereof. To minimize heterogeneity in product quality it is of importance to evaluate the impact of these different mechanisms.
Therefore we developed and applied a protocol, which can be used for analysis of a spectrum of different recombinant proteins and production cell lines aiming to provide a tool that enables growth phase related product analytics, for monitoring variations of the product during different phases of a cultivation process.

Experimental Approach:
Important for growth phase specific product analysis is the possibility to separate recombinant proteins produced during different growth phases. Exchanging media without changing its composition regarding nutrient and waste products, should result in growth characteristics comparable to a cultivation without media exchange.
Two CHO K1 derived production cell lines were cultivated in parallel with the host cell line in batch culture in chemically defined, animal component-free CHO growth media for different cultivation periods. At certain time points a media exchange was done, by replacing the supernatant of the half of the production cell line batches, which contained the product, with supernatant of the host cell line batches. Afterwards cultivation was continued until viability dropped below 70%. Cultivations with and without media exchange were compared according to viable cell density, viability, glucose and lactate levels as well as amino acid concentrations. Secreted recombinant proteins were analyzed regarding quantity and quality. In particular the N-glycosylation pattern was determined by HPAEC-PAD and MALDI-TOF. In further studies other cell lines (e.g. AGE.hn) producing different recombinant pharmaceuticals were analyzed.

Results and Discussion:
No changes of neither growth behavior nor glucose, lactate and amino acid metabolism could be observed after media exchange confirming the applicability of the chosen approach.
On this basis we investigated whether product heterogeneity in terms of different glycosylation patterns is growth phase dependent or related to the residence time of the product in a supernatant. Therefore glycosylation analyses of different products from different growth phases were performed and data will be presented. These results will help to identify the reasons for heterogeneity in recombinant protein production and develop optimized process for production of more homogeneous products.
EVALUATION OF THE INTERACTION OF PLURONIC F-68 AND BOVINE SERUM ALBUMIN AS SHEAR PROTECTANTS IN INDUSTRIAL NS0 CELL CULTURE

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Background and Novelty:
NS0 cells are a mouse myeloma cell line commonly used for therapeutic Monoclonal Antibody production. NS0 cells require cholesterol provision using a synthetic cholesterol carrier, cyclodextrin. A number of shear protective additives are added to NS0 culture media; including Pluronic F-68 and Bovine Serum Albumin (BSA). Pluronic is a non-ionic surfactant which protects cells from bubble bursting. Pluronic is a heterogeneous additive; therefore there is batch to batch variability in the shear protection capability of different lots of Pluronic. The protective effect of Pluronic has also been shown to be limited at high cell concentrations experienced in large scale bioreactors. BSA is required to protect some NS0 cell lines from bioreactor shear stresses. BSA has a number of functions in blood, e.g. nutrient carrier (including cholesterol). Limited research has been published to support the shear protective methods of action of BSA. It has been suggested that Pluronic and BSA could protect cells similarly, however with different mechanisms of action. Regulatory constraints make it unfavourable to add BSA to cell culture media for production of human therapeutic Monoclonal Antibodies. The hypothesis of this work is that BSA and Pluronic work synergistically to protect NS0 cells from shear stresses in large scale bioreactors. This research is novel, as it attempts to identify the fundamental mechanisms by which BSA protects cells from shear damage.

Experimental Approach:
Baffled shake flasks were used to expose NS0 cells to hydrodynamic shear conditions to investigate the shear protective effect of Pluronic and BSA. The concentration of Pluronic was optimised to expose the protective effect of BSA in the small scale models. The physical or biological protective nature of BSA was tested by growing NS0 cells With BSA and exposing them to shear Without BSA, and also growing NS0 cells Without BSA and exposing them to shear With BSA. The effect of BSA on the plasma membrane fluidity was tested using flow cytometry. Cell membrane integrity was also tested using LDH activity assays. The plasma membrane cholesterol content was quantified for cells grown in the presence and absence of BSA. The amount of cholesterol bound to BSA in NS0 cell media was also quantified.

Results and Discussion:
Two models were devised to show the protective effect of Pluronic and BSA.
1. Moderate shear conditions. Under these moderate conditions cells grown With BSA showed an 80% increase in growth.
2. Extreme shear conditions. Using this model cells cultured With BSA showed 58% less cell death.

The protective effect of Pluronic and BSA were shown to be dose dependent. Low concentrations of Pluronic (0.025g/L) were required to expose the shear protective effect of BSA. This suggests that BSA and Pluronic work synergistically to protect NS0 cells. The protective effect for Pluronic and BSA were shown to be physical (or fast acting biological) due to the shear protection being afforded instantly. These experiments focus on the cellular changes caused by the presence of BSA and Pluronic in the cell culture media.
WHAT’S HIDING BEHIND THE CURTAIN: EXPANDING PROCESS UNDERSTANDING TO INCLUDE RAW MATERIAL VARIABILITY

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Background and Novelty:
Late stage small scale process characterization yields a detailed understanding of controlled process parameters that can be used to affect a process at manufacturing scale. In this case study for process characterization, bench scale bioreactors were utilized to evaluate a wide range of process parameters.

Experimental Approach:
Briefly, a response surface model was generated from process characterization experiments including inputs such as pH and temperature and outputs such as titer and product quality. While moderate changes in process inputs did not significantly change product quality, we were able to identify parameters that do affect product quality at extreme input values. These extreme conditions represent inputs well outside the expected performance in manufacturing. In parallel to bench-scale studies, a large number of batches were executed at manufacturing scale and produced similar product quality as expected from the process characterization work. However, as manufacturing experience grew, variation in product quality was eventually observed outside of the response surface model expectations.

Results and Discussion:
Analysis of data from multiple scales and manufacturing sites determined that this variation was correlated with additional inputs not originally included in the response surface model generation. Specifically, two independent chemically defined raw materials were identified to impact two independent product quality attributes. In one case, an orthogonal enzymatic assay was created to a priori predict product quality impact from the raw material. In the second case, a raw material impurity was identified. Small scale studies demonstrated clear dose responses between the impurity concentration and product quality. Consequently additional controls were placed on this raw material in order to ensure process consistency. While process characterization had been completed prior to understanding the impact of the raw materials, raw material understanding has been combined with process characterization studies to produce an even more consistent process.
OPTIMIZATION OF CHO-S CELL CULTURE MEDIUM BY SUPPLEMENTATION WITH NON-ANIMAL DERIVED COMPONENTS USING DoE

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Background and Novelty:
Chinese hamster ovary (CHO) cells are most commonly cultured with ADCF media for the production of biopharmaceuticals. Improvement of existing CD and ADCF media can still be obtained by supplementation with specific components. This work addresses the optimization of cell culture media supplementation using the methodology of Design of Experiments.

Experimental Approach:
Three different commercial media (ActiCHO, ProCHO5 and FreeStyleCHO) and eight non-animal derived components (r-insulin, r-transferrin, r-albumin, selenium, tocopherol acetate, tween 80, synthetic cholesterol and fatty acids) were used for media supplementation in order to increase maximum cell density by means of design of experiments methodologies.
First, Plackett-Burman design was used to screen the supplements with an important effect on cell growth but as this statistical technique is not useful to analyse interactions between supplements, the number of experimental runs was doubled in order to identify the most evident ones. After selecting the components with a positive effect on cell growth, a Box-Behnken design was performed to determine the optimal concentration of each of these supplements so as to increase maximum cell density.

Results and Discussion:
FreeStyleCHO was selected for subsequent optimization. The experimental series for Plackett-Burman analysis consisted in 24 experiments in duplicate. Out of the results, r-transferrin had the greatest positive effect on cell growth. Selenium, r-insulin and tween 80 also showed a positive effect on cell growth, whereas fatty acids and synthetic cholesterol had no effect and r-albumin and tocopherol acetate had a negative effect. The factors with a positive effect on cell growth were selected for subsequent optimization, they included r-transferrin, r-insulin and selenium. Since lipids were not relevant for cell growth, tween 80, a hydrophilic nonionic surfactant mainly used for emulsification and dispersion of lipidic substances, was not selected.
The results were analyzed by the Box-Behnken design methodology and a model was generated. The number of experiments for Box-Behnken analysis consisted in 15 experiments in duplicate. The model predicted optimum concentrations of 26.1 mg/L, 20.2 mg/L and 1 μg/L for r-insulin, r-transferrin and selenium in FreeStyleCHO medium, respectively. These optimal conditions were further validated experimentally by triplicate with very good agreement.
Using this optimized supplementation, a significant 20% improvement was finally obtained for CHO-S cell line growing in FreeStyleCHO medium.
THE POTENTIAL OF SMALL MOLECULES TO MODULATE GLYCOSYLATION BY MEDIA DESIGN

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Background and Novelty:
A large number of recent publications demonstrate the effect of cell culture media on post-translational modifications of recombinant proteins\(^1\). This study aims to extend the toolbox of media design beyond the commonly known media components. We identified and tested a large variety of cell culture compatible chemical components such as pigments and sugar derivatives in industrial relevant Chinese hamster ovary cell lines (CHO) expressing recombinant antibodies.

Experimental Approach:
The cells were cultivated in fed-batch mode cultures using shaking 96-deepwell plates, and process performance such as viable cell density, viability and product titer were monitored. Supernatants of each culture were purified and N-glycan analysis was performed by CGE-LIF. The findings were confirmed in 30 mL fed-batch shake tube cultures and 15 mL micro-scale bioreactors (ambr15\(^\text{TM}\) system) operated at controlled pH and pCO\(_2\).

Results and Discussion:
The addition of the components at the beginning of the culture exhibited significant changes of the glycosylation profile of the expressed protein. Furthermore, the adjustment of the levels and the supplement addition in the feed instead of the media allowed to fine-tune the effect of the components on glycosylation profile. Finally, the use of some of the tested supplements increased peak cell density to levels above 20 mio viable cells/mL and product titer up to 1.5-fold, while maintaining high viability throughout the culture. These results show that media design alone is sufficient to specifically modulate some of the essential protein quality attributes and to increase productivity, which circumvents the need of modifying the gene expression of the cell line.

\(^1\) D. Brühlmann et al., Tailoring Recombinant Protein Quality by Rational Media Design, Review Paper submitted.
**BIOTHERAPEUTIC PROCESS CHARACTERIZATION, BUILDING ROBUSTNESS TO CONTROL PRODUCT QUALITY**

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**Background and Novelty:**
Through the advent of Quality by Design (QbD), the development of a biopharmaceutical manufacturing process requires more extensive understanding of process parameter influence on product quality attributes. This concept results in identification of critical quality attributes and the process parameters that influence them. As a result, late stage biopharmaceutical processes are developed and expected to operate within a narrow range around a given parameter set-point. The identification of critical process parameters can be understood through the execution of process characterization studies to determine the acceptable operating range of a process without impacting product quality.

**Experimental Approach:**
Process characterization studies can be guided by the assessment of parameter risk through failure mode effects analysis (FMEA). This case study represents the generation of process characterization data for a perfusion process producing a complex recombinant biotherapeutic. The execution of FMEA identified three parameters, viable cell density, temperature and pH with highest potential risk on product quality for the perfusion process in question and allowed for prioritizing the study design. Utilizing design of experiment, a response surface design was generated testing five points per parameter through execution of eighteen bioreactors to provide a robust assessment of the influence of the individual parameters and the potential interactions of those parameters.

**Results and Discussion:**
The data generated during this study identified specific influences of the viable cell density, temperature and pH on product quality attributes such as host cell protein, residual DNA and glycosylation, as well as overall process performance. Additionally this study has determined the setpoints and normal operating ranges of the developed process allow for robust process operation away from edges of failure that would result in adverse product quality. The utilization of risk analysis to define process characterization studies and execution of those studies allows for building process repeatability and controlling product quality. The execution of process characterization allows for achieving compliance and greater process understanding to aid in long term manufacturability.
GLYCOEXPRESS™: A TOOLBOX FOR THE HIGH YIELD PRODUCTION OF GLYCOOPTIMIZED FULLY HUMAN BIOPHARMACEUTICALS IN PERFUSION BIOREACTORS AT DIFFERENT SCALES

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Background and Novelty:
Glycosylation is one of the major post-translational modifications of biotherapeutics important for bioactivity, bioavailability, immunogenicity and patient coverage. By establishment of the GlycoExpress™ toolbox (GEX™) we have generated a set of glycoengineered human cell lines for the high yield production of fully human glycopolypeptides to optimize the glycosylation of antibodies and non-antibody biotherapeutics for improvement of the clinical efficacy and side effects. The system is biotechnologically superior in quality, reproducibility and yield compared to other including conventional production systems. All four clinical products derived from GlycoExpress cells are produced using a perfusion bioreactor system in order to assure highest possible product quality and reproducibility combined with high yield production.

Experimental Approach:
GlycoExpress cells producing mAb are cultivated with perfusion bioreactor systems applying different cell retention mechanisms such as centrifugation (centriTech) or alternating tangential flow (ATF) filtration at different scales. Growth, product yield and product quality, especially glycosylation, are evaluated during the cultivation.

Results and Discussion:
Perfusions culture helps to keep cells in the optimal growing and production phase over the production process which leads to highly stable product quality allowing a flexible duration of the run in one batch size in combination with stable high productivity of the cells over time. Furthermore the product qualities produced in different scales ranging from 10 mL to 1000 L cultivation vessels are highly comparable.
EXON SKIPPING IN CHO-K1 CELL-LINE AND METHOD FOR SPECIFIC DETECTION OF THESE VARIANTS DURING A FED-BATCH PRODUCTION PROCESS

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Background and Novelty:
Alternative splicing is a process by which the exons of the RNA produced by transcription of a gene are joined in multiple ways during RNA splicing. Among the different forms of alternative splicing, exon skipping is the most common one, usually leading the “exon-skip-over” mRNA variants translated into different protein isoforms.
Amyloid precursor protein (APP) is an integral membrane protein expressed in different cells/tissues in many organisms. It has been previously reported that Chinese hamster ovary (CHO) cells express full length APP. The biological function of APP in CHO cell-line remains unclear. In addition, no exon skipping isoforms of APP have previously been detected in CHO cells.

Experimental Approach:
In this study, CHO cell samples have been collected at different time points from a fed-batch monoclonal antibody production process. For specific exon skipping detection, a novel method based on amplifying and sequencing APP mRNA isolated from these cell samples has been developed.

Results and Discussion:
Described is the identification of at least 8 different splicing variants of the APP mRNA in CHO-K1 cells, with 5 of them occurring mainly through skipping one or more full exons within the APP gene. This method allows for improved detection limits of spliced RNA variants in cell samples particularly from a fed-batch process used for monoclonal antibody production.
TECHNOLOGICAL AND METHODICAL PROCESS INTEGRATION IN MANUFACTURING OF MONOCLONAL ANTIBODIES

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Background and Novelty:
Recent advances in cell culture technology result in a significantly increased product titer as well as in changed compositions of process depending impurities like host cell proteins (HCP). The optimization process of DSP has to deal with this new separation task. The increase in titer and this change of impurities result in limitations in capacity and throughput of downstream processing, e.g. for Protein A chromatography [1]. Possible solutions consist in extensions of capacity but also in establishing new or optimized unit operations [2]. In this work, new technological approaches as well as a new methodical approach are presented. The technological approach consists in a substitution of a centrifuge, Protein A and a second chromatography step by aqueous two-phase extraction (ATPE) and precipitation. The methodical approach includes a parallel optimization of cultivation conditions in USP and of process parameter optimization in DSP regarding HCP and IgG/protein as additional optimization foci.

Experimental Approach:
By changing cell culture media compositions according to DoE screening designs, influences of media components on the concentration and composition of HCP are identified. A media optimization is carried out regarding not only high product titers and cell growth but also concentration and composition of HCP. By these experiments, different fermentation broths are produced containing different impurity spectra. These broths are used in subsequent development of ATPE and precipitation. A DoE based screening is carried out to identify the influences of following parameters: system composition, NaCl, cell number, viability, pH, product concentration, precipitant concentration, temperature, precipitation environment.

Results and Discussion:
A new medium is designed regarding the additional criteria of HCP concentration and composition. Experiments including varying fermentations broths confirm the high influence of impurities on the separation efficiency of different unit operations in DSP. ATPE can be used as cell harvest method. It separates more than 95 % of cells from the broth without significant product losses. The product is concentrated within the target phase. At the same time, a first purification step is carried out removing HCP, DNA and media components. A subsequent precipitation removes the majority of remaining HCP and other impurities. The integration of ATP and precipitation can result into a significant reduction of costs and efforts. An optimization of the overall manufacturing process can be advanced by regarding process depending impurities resulting from the fermentation process. The optimization of USP towards relatively high product titers as well as an impurity spectrum which is easy to separate from the product can improve the manufacturing process concerning the total process costs. A combination of an integration of upstream and downstream processing regarding process depending impurities as well as an establishment and integration of alternative separation mechanisms can present an interesting solution to purify high concentrated antibodies [3].

Literature:
EVALUATION OF PROTEIN PRODUCTION AND TRANSCRIPT LEVELS UNDER DIFFERENT CHO CELL CULTIVATION CONDITIONS

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Background and Novelty:
In the past years, the interest in continuous processes has been increasing due to several advantages. Besides the high volumetric productivities, new cell retention technologies and advances in both cell culture media and process control have enabled perfusion cultivations with very high cell densities, allowing an increase in product titers. Previous process development works of our group was aimed at investigating a CHO cell-based perfusion process for production of a labile recombinant protein. This work focuses on the effects of cell cultivation at sub-physiological temperatures and addition of short-chain fatty acids as productivity enhancers. Since high productivities were obtained in lower temperature conditions, quantitative real-time PCR (qPCR) experiments were performed to verify if the increase in production was related to changes in product transcript levels.

Experimental Approach:
A CHO cell line producing a recombinant protein was cultivated in a customized chemically defined animal component-free medium (Xell AG, Germany). Batch runs were carried out in shake flasks at 5% CO2, 185 rpm and 5 cm shaker orbit. Perfusion cultures were carried out in stirred bioreactors at 40% air saturation and pH 7.1, using a gravity settler as cell retention device. To investigate the effect of low temperature and fatty acid supplementation, cultivations under different conditions were studied: 37°C, 31°C and 31°C with 1 mM valeric acid. Changes in protein production were monitored by measuring product concentration by ELISA and product biological activity in the supernatant. Product transcript levels were compared at different time points by qPCR using four different housekeeping genes (HKGs). These also allowed the detection of gene expression variability under the different culture conditions.

Results and Discussion:
In batch shake flask experiments, the hypothermic conditions yielded lower specific growth rates, but higher specific productivity (qp). The addition of valeric acid further increased the qp. In perfusion runs, qp and volumetric productivities were higher at 31°C compared to 37°C (control). The qp related to ELISA at both conditions at 31°C was approximately 2.3 times higher than the control. Interestingly, the qp in terms of biological activity was 2.9 times higher at 31°C with valeric acid comparing to 37°C while at 31°C it was 1.6. The volumetric productivities at low temperature conditions varied from 1.5 to 2 times for ELISA and product activity comparing the low temperature conditions to the control. The HKGs were differently expressed under the different culture conditions. Regarding the expression of the gene of interest in perfusion reactors, the transcript level at 31°C was up-regulated by factor 2 compared to 37°C. Assays were also performed to determine the stability of the protein at the two temperatures and the results showed no difference in product stability in the period of time corresponding to the residence time inside the bioreactor. The mechanisms responsible for higher transcript levels and higher productivities at the culture conditions investigated are still unknown and should be further investigated.
HIGH-TITER EXPRESSION OF RECOMBINANT ANTIBODIES BY TRANSIENTLY TRANSFECTED HEK 293-6E CELL CULTURES

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Background and Novelty:
HEK 293-6E cells (1) expressing constitutively a truncated version of EBNA-1 and originally developed at the NRC-BRI in Montreal, Canada have been already shown to be an excellent tool for transient transfection and subsequent high-titer production of recombinant proteins. We substantially improved the existing transfection protocol by introducing a number of additional or alternative steps during and after transfection finally resulting in a high-titer production protocol suitable for routine production of mg to gram quantities of desired proteins.

Experimental Approach:
A recombinant antibody construct introduced into an optimised plasmid vector was used a model protein for all experiments (2). The original transfection and protein production protocol was improved step by step introducing alternative or additional steps of media supplementation. This includes protein hydrolysates other than tryptone N1, doubling of the culture volume by using a second cell culture medium (SMIF8 2x) different to the culture medium which showed best results for the transfection process itself (FreeStyle F17), and addition of productivity enhancers such as the HDAC inhibitors sodium valproate and sodium butyrate and applying specific feed supplements. Optimization experiments were performed in either shaker flasks, bench-top bioreactor systems (which could be operated also in continuous perfusion mode) or the BioLector 48-well microbioreactor (m2p-labs). Transfection efficiency was monitored by co-expressed eGFP using a flow cytometer (as well as on-line by fluorescence measurement in the BioLector), antibody production by biolayer interferometry (Octet RED) and the concentration of various metabolites in the supernatant photometrically (Gallery).

Results and Discussion:
Antibody yields were significantly increased by this protocol, thus either reducing the total amount plasmid DNA and culture volume required for the transient transfection of HEK 293-6E cells and subsequent production of a certain amount of proteins or the other way around increasing the yield of recombinant protein which could be obtained from a certain quantity of plasmid DNA by at least a factor of 4.

EVALUATION OF SCALE-UP PARAMETERS FOR SINGLE-USE BIOREACTORS USED FOR THE PRODUCTION OF BIOPHARMACEUTICALS

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Background and Novelty:
Nowadays stirred single-use bioreactors up to 2000 L are widely accepted for the development and production of therapeutic proteins. For a successful scale-up of a production process from lab into large production scale a high knowledge of biochemical engineering aspects are beneficial for efficient process development. The key parameters in biochemical engineering are the mixing time and the volumetric oxygen mass transfer coefficient (k_\text{a}). These two parameters were determined in stirred bioreactors of 10L (glass), 100L, 250L and 1000L (single-use) working volume. The aim was the definition of optimum parameter settings (e.g. tip speeds) for a successful scale-up in the different bioreactor scales from minimum to maximum working volume in order to reach equivalent mixing times and k_\text{a} and therefore an efficient process transfer from scale to scale.

Experimental Approach, Results and Discussion:
Two different methods for the determination of mixing times were used and compared in the 10L and 100L bioreactors: the decolorization of iodine and measuring the electric conductivity. Both methods delivered comparable data. For the decolorization method the bioreactor bag has to be transparent or at least an inspection window is necessary. This is not given for the 250L and 1000L bioreactors whereas only the electronic conductivity was used. The volumetric oxygen mass transfer coefficient (k_\text{a}) value was determined using the gassing-out method using conventional optical oxygen sensors. The influence of parameters such as working volume, tip speed, gas flow, and addition of supplements (e.g. defoaming agents) was also evaluated.

Using the results, it is now possible to identify and specify the optimal parameters for a successful scale-up: For example with a tip speed of 0.5 m/s at half working volume a mixing time of 13.7 s was determined. In order to reach the same mixing time in the 100L single-use bioreactor a tip speed of 0.81 m/s has to be set.

The evaluation of the engineering parameters for the 10L, 100L, 250L and 1000L stirred bioreactors gives the opportunity for successful scale-ups and therefore more efficient process development in terms of time and cost.
SECOND GENERATION PROCESS DEVELOPMENT: INCREASING PROCESS ROBUSTNESS, PRODUCTIVITY, AND PRODUCT QUALITY CONTROL

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Background and Novelty:
Second generation or Cycle 2 process development offers an opportunity to increase productivity and process robustness by implementing process changes and new control schemes. Here, we will describe a case study in second generation process development for a CHO cell line producing a therapeutic IgG protein. Business demands required this work to increase scale up robustness and manufacturing fit, implement a chemically defined medium and feed strategy, increase productivity, and if possible, demonstrate a method to reduce product quality concerns by advanced process control.

Experimental Approach:
Increased understanding of metabolite requirements and cell line sensitivities allowed for optimizing the seed train and production process for increased robustness. DOE practices were used to define optimized process parameters, increasing productivity through increased cell density and prolonged viability. In parallel to the primary development work, Raman spectroscopy based analytics were used to develop an online glucose monitoring tool to mitigate concerns over percent glycated product.

Results and Discussion:
Overall, process optimization and intensification resulted in increased productivity with decreased risk from scale up and raw material variability while advanced process analytical technology enabled the controlled mitigation of product quality concerns.

MANUFACTURING OF LENTIVIRUS GENE THERAPY VECTORS: DEVELOPMENT OF A SCALABLE PROCESS IN DISPOSABLE BIOREACTORS

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a) Lentiviral vectors are increasingly used to treat genetic diseases and cancer via ex-vivo transduction of haematopoietic stem cells or T-lymphocytes. The production of lentivirus vectors relies on the simultaneous expression of viral proteins gag, pol, rev, a pseudotyped envelope protein and the transgene RNA to be packaged into the vector particles. The most commonly used manufacturing process consists of transfecting 4 plasmids in adherent cells grown in cell factories using calcium phosphate. However, this method is cumbersome, prone to variability and shows limited scalability.
b) To address those drawbacks, we have developed a transfection process in suspension cells in disposable stirred tank bioreactors. The cell culture is performed in a chemically defined medium to eliminate the potential variability and virus/TSE risks associated with bovine serum.
c) Today, the efficiency of the process has been demonstrated at scale from 2-10L (glass) up to 50L (disposable) using several vector constructs. The full process is already a robust alternative to the cell factory system and will be scaled up to 200L soon.
REVISITING THE DETERMINATION OF HYDROMECHANICAL STRESSES ENCOUNTERED BY MICROCARriers IN STEM CELL CULTURE BIOREACTORS

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Background and Novelty:
Expansion of mesenchymal stromal cells (MSC) is one of the key step for their use in tissue engineering or cell therapies. Today, expansion processes are mainly based on the use of microcarriers to allow large interfacial adherence areas. However, this culture technology is known to be practically limited to low agitation intensity and microcarrier concentrations due to possible cell damage arising from particle hydromechanical stress or collisions between microcarriers. However the description of the relationship between bioreactor hydrodynamics, microcarrier suspension, and occurrence of collisions is neither established in the case of stem cell cultures, nor based on a local description of the bioreactor hydrodynamics heterogeneity. Thus, in the present study it is proposed to realize numerical simulations to describe not only the liquid phase but also the microcarrier dispersion and the hydromechanical stress encountered along the particles pathways.

Experimental Approach:
Computational Fluid Dynamics (CFD) was used to simulate the hydrodynamics inside a mini-bioreactor (250 mL). Agitation was ensured using Rushton turbines, ear-elephant or marine propellers. Surface aeration was sufficient to allow oxygenation of human MSC cultures, thus no gas sparging was needed. Concentrations of Cytodex-1 microcarriers varying from 1 to 10 g L⁻¹ were considered to study the impact of this concentration on the microcarrier just-suspended agitation rate $N_p$. A two-fluid granular Euler-Euler model was used to simulate both the liquid and solid (microcarrier) movement inside the bioreactor. This approach was never used in literature to simulate microcarrier dispersion inside culture vessels. The agitation rate $N_p$ was also experimentally determined using visual observations to validate the CFD simulations.

Results and Discussion:
From the results, it could be shown that the choice of optimal mixing conditions (impeller design and agitation rate) to get the best compromise between good mixing capacities and non-damaging temporal hydromechanical stress distributions is critical. Indeed, for instance, in given mixing conditions, our simulations revealed that dramatic differences may be observed between the distribution of hydromechanical calculated for the liquid phase and the stresses really encountered by the microcarriers, due to partial suspension of microcarriers or due to microcarrier concentration heterogeneities. Similar observations could be also made concerning the intensity and the frequency of collisions between microcarriers, which were sensibly impacted by the gradients of microcarrier concentration inside the turbulent liquid flow field.
CONTROL OF CELL HEALTH AND PRODUCT QUALITY OF A MAB CHO CELL CULTURE USING AN AUTOMATED CLOSED LOOP FEEDING SYSTEM

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Background and Novelty:
The majority of fed-batch processes rely on the use of pre-determined feeding protocols based on nutrient requirement estimates, or the use of regular sampling and off-line assay of culture media in order to determine the concentration of key components. Both of these techniques can lead to non-optimum feeding, with depletion and large swings in nutrient concentration, potentially leading to inconsistent product quality and lower process productivity.

An alternative to these approaches is to monitor the metabolic activity which is a function of nutrient concentration. For the majority of the cell types used in upstream bioprocesses an increase in substrate concentration results in an increase in activity until a maximum metabolic rate is reached (known as the 'Monod relationship'). It is therefore possible to use a decrease in metabolic activity as an indicator for reduced nutrient concentration, and a trigger for feed additions.

Experimental Approach:
Using the Ranger system, developed by Stratophase, we have applied an Automated Closed Loop Feeding Control to a CHO cell culture process in order to automatically adapt to the various stages of the process and provide nutrient feed addition on demand.

Results and Discussion:
The growth and production phases of the culture were shown to exhibit notably different feeding control characteristics, and the resulting feeding regime was shown to promote cell growth, maintain cell health and support strong product evolution without the need for user intervention. The use of AFC and the resulting optimisation of feed rate and minimisation of nutrient concentration variation has led to an elevated final product titre and a consistently high degree of cell health throughout the process.
GALACTOSE AND LACTATE CO-METABOLISM IN CONTINUOUS CULTURE OF CHO CELL

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Background and Novelty:
In highly proliferative organism, like Chinese hamster ovary cell, glucose is very high. This high flux leads to the production of undesirable and toxic metabolite for the cell: lactate. It has been reported the ability of CHO cells to co-metabolize galactose and lactate (Altamirano et al 2006; Wilkens et al 2011) in bath culture, leading to benefits associated as avoid high lactate accumulation and cell growth inhibition. In this work aims to evaluate the co-metabolism of galactose-lactate in the modality of continuous cultures of CHO cells producing tissue plasminogen activator (rh-tpa) on metabolic behavior and productivity.

Experimental Approach:
Two substrates concentration levels were used in the feed flux: Gal 10mM/Lac 5mM (Low concentration culture, LC) and Gal 20mM/Lac10mM (High concentration culture, HC). The basal medium was Bioprol (Lonza, Belgium) supplemented with 6 mM of glutamate. The dilution rate was 0.015 h⁻¹, in 250-mL spinner flasks specially conditioned for continuous cultures, and using 150 mL of medium. Cultures were carried out in an incubator with controlled atmosphere 95% R.H., 5% CO2 and 37°C. The metabolites measured were: galactose, glutamate and lactate using a biochemical analyzer (YSI2700) and amino acid by HPLC. t-PA was measured by ELISA (Imulyse t-PA, Biorepol). After 3 times of residence we considered stationary state and we take samples of cells and supernatants.

Results and Discussion:
In both conditions the number of viable cells was similar (8.2 ± 0.47 y 8.9 ± 0.45, LC and HC respectively). About lactate concentrations, it was observed that after reaching steady state (t=340hr approximately) were lower than feed concentration (3.5 ± 0.22 y 7.43 ± 0.12, LC y HC, respectively) indicating that in net terms this compound is consumed in parallel with galactose. Presenting an specific uptake rate of 13.2±±0.11 nmol/h·10⁶ cell in LC culture and 18.7±±0.18 nmol/h·10⁶ cell in HC culture. On the other hand the galactose specific uptake rate was 22.2 ± 0.09 nmol/h·10⁶ cell and 57.6 ± 0.05 nmol/h·10⁶ cell in the cultures LC y HC, respectively. The lactate uptake can be metabolized via the mitochondrial lactate dehydrogenase (Lou et al., 2011) which enable its oxidation in TCA cycle or through of a no-oxidative pathway (ketone bodies synthesis) (Altamirano et al 2006). To evaluate the carbon fluxes fate involved in co-metabolizing galactose/lactate at different condition studies, the metabolic flux analysis (MFA) was performed. Our results show that the lactate catabolism can be explained through its degradation by an oxidative pathway (TCA) and a non-oxidative pathway, which take place simultaneously.

References:

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CONTINUOUS SUSPENSION CELL CULTURE MONITORING IN BIOREACTORS

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Background and Novelty:
Monitoring of suspension cell cultures often relies on sampling followed by a staining procedure. Estimations of the cell count and cell viability are traditionally performed once a day using Trypan-Blue cell exclusion as a method of choice. Stained samples are destroyed afterwards creating toxic wastes. Sampling a bioreactor and counting cells involve manual operations and weekend work is regularly needed.

Differential Digital Holographic Microscopy (DDHM) is a new quantitative imaging technique that allows cell counting as well as cell viability monitoring in a continuous, label-free set-up. No need for sampling (eliminating the risk of contamination), staining and waiting for the results generated by an off-line counter: results are available in nearly real-time over the whole run.

Compared to classical light microscopy, Differential Digital Holographic Microscopy offers:
- A greater depth of focus: 100x (applying the same magnification),
- The ability to refocus images post acquisition,
- The collection of quantitative phase information (optical density), covering the shape and density of an object. This quantitative phase parameter (not captured by the human eye) is the key advantage in numerous applications developed at OVIZIO.

DDHM helps the operator to track the total cell density and the cell viability at any time, while the OsOne software plots the cell growth curve, live on the screen. Moreover, OsOne also shows real-time images of the cells, offering the experienced operator a particularly convenient tool to check about the condition of the cell culture.

Experimental Approach:
In this study, we compared the results generated by the iLine-F with off-lines methods applying sampling and Trypan-Blue staining. OVIZIO’s iLine-F was benchmarked versus the Vi-Cell XR (Beckman Coulter). A bioreactor equipped with a BioConnect (OVIZIO’s continuous, close-loop, sampling device) plugged into an iLine-F was inoculated with CHO cells at 0.3x10^6 viable cells/mL in CD-CHO medium (Life Technologies) for a final volume of 2L. The culture was sampled daily via the usual sampling port for Vi-Cell cell count. The iLine-F was set to generate 2 cell counts per hour. The culture was left to grow in batch mode for a total of 13 days. Thus we were able to also capture the decrease in cell viability at the end of the culture.

Results and Discussion:
A correlation factor R^2 of 0.93 was obtained for the viable cell density demonstrating that the results achieved with the label-free DDHM method are in line with current methods applying Trypan-Blue staining.

Furthermore, the iLine-F shows the benefit of having the full trend on the culture which can be more relevant than a single point, on a single sample, once a day. The availability of full data at the single cell level, for the whole experiment, allows to envision the use of iLine F for PAT approach. Indeed the large amount of data produced can be used to perform various statistical analysis on the cell population in order to define and control critical parameters of the cell culture process.
MODEL-BASED DESIGN OF A DOE FOR FED-BATCH CULTURE OF A CHO CELL LINE

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Background and Novelty:
Experimental process-development and optimization is expensive and time-consuming. Real optimization by means of design of experiments involves data generation before optimization can be aimed for. This can make the way from process development to process establishment even harder, since academia or start-up research facilities might not have the possibility to generate these data. Furthermore, bioprocesses involving mammalian cells deal with many critical variables; processes are not only carried out batchwise, but increasingly in fed-batch mode with optimized feeding profiles.

The use of DoE tools in combination with an appropriate growth model might be a valuable tool to develop and to test fed-batch strategies in silico, before experiments are carried out in the laboratory.

Experimental Approach:
In our work, an unstructured model for mammalian cell culture was used. After short shake-flask experiments for kinetic parameter determination of a CHO cell line (CHO XM 111-10 (CCOS 837)), the model was tested for data generation on common fed-batch strategies. By means of design of experiments strategies, relevant conditions were selected and experimentally tested.

Results and Discussion:
In this way, suitable fed-batch strategies for mammalian cell lines are evaluated in silico before bioreactor experiments are to be performed. This results in a significant reduction in the number of experiments during process development for mammalian cell culture.
CONSIDERATIONS FOR CELL PASSAGING IN CELL CULTURE SEED TRAINS

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Background and Novelty:
The production of biopharmaceuticals for diagnostic and therapeutic applications based on suspension cell culture in bioreactor scales from a few hundred liters up to 20 m³ is state of the art. However, the generation of an adequate number of cells for the inoculation of a production bioreactor is time- and cost-intensive. From volumes used for cell thawing or cell line maintenance the cell number has to be increased while usually passaging into larger cultivation systems. Examples are T-flasks, roller bottles or shake flasks, small scale bioreactor systems and subsequently larger bioreactors. In order to reduce the number of passages within the seed train also systems are used which are inoculated at a partly filled state and culture volume is increased afterwards by medium addition.

Nevertheless, seed trains last for significant periods of time and generate corresponding costs, e.g. a seed train from a 5 mL-scale until inoculation of a 3 m³-scale lasts in the range of 3 weeks.

There are various options for the optimization of existing seed trains in biotechnological plants or for the design of new seed trains that can be exploited.

Experimental Approach:
A software tool has been developed which provides possibilities for simulation and analysis of seed trains based on cell line, medium and seed train vessel properties. This enables seed train analysis, layout and optimization. The tool includes multiple strategies for the determination of optimal points in time for cell passaging from one scale into another.

Results and Discussion:
The concept and the seed train software tool will be shortly presented followed by considerations on different criteria for cell passaging from one seed train scale into the next. Experimental results of two different seed train examples with two different cell lines at lab scale are presented on a second poster (Kern et al. “Model-based strategy for cell culture seed train layout verified at lab scale”).
MODEL-BASED STRATEGY FOR CELL CULTURE SEED TRAIN LAYOUT VERIFIED AT LAB SCALE

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Background and Novelty:
The production of biopharmaceuticals for therapeutic and diagnostic applications with suspension cells in bioreactors requires a seed train up to production scale. For the first steps, the transitions between T-flasks, spinner tubes, roller bottles, shake flasks, stirred bioreactors or single-use reactors are difficult to design, as here often the same scale-up steps cannot be realized. The experimental effort to lay-out these steps is correspondingly high. At the same time it is known that the first cultivation steps have a significant impact on the success or failure on production scale.

Experimental Approach:
A software tool has been developed which provides possibilities for simulation and analysis of seed trains based on cell line, medium and seed train vessel properties. (compare: Hernández Rodríguez et al. “Considerations for cell passaging in cell culture seed trains”). In this work the concept was evaluated experimentally.

Results and Discussion:
In the present work, for two suspendable cell lines (AGE1.HN, ProBioGen AG and CHO-K1) basic kinetic information for cell growth and death, substrate uptake and metabolite production were generated in shake flask batches using different initial substrate and metabolite concentrations. Based on batch data, a Nelder-Mead-algorithm has been applied to determine the model parameters of an unstructured kinetic model. Using a MATLAB simulation based on this model, optimal points in time or viable cell concentrations respectively for harvesting during a seed train from spinner tubes over shake flask up to a stirred bioreactor (5 L) were determined and subsequently verified experimentally. Model prediction for optimization and experiment agreed very well.

Without such time consuming lab work, the tool has delivered the same optimized seed train only based on data of two batches. The concept offers a simple and inexpensive strategy for design of the first scale-up steps.

The bioreactor (Labfors 5 Cell) was kindly provided by the company Infors AG, the cell line AGE1.HN by ProBioGen AG.
DECISION TREE FOR SELECTION OF SUITABLE CULTIVATION PARAMETERS
FOR MAMMALIAN CELL CULTURE PROCESSES

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Background and Novelty:
Development of bioprocesses for animal cells has to deal with different bioreactor types and scales. Bioreactors might be intended for seed train and production, research, process development, validation or transfer purposes. During these activities, not only the problem of up- and downscaling might lead to failure of reproducibility, but also the use of different bioreactor geometries and operation conditions. In such cases, the criteria for bioreactor design and process transfer should be re-evaluated in order to avoid an erroneous transfer of cultivation parameters.

Experimental Approach:
For selection of process conditions several questions can be asked:
- Type and scale of the intended cultivation system.
- Which data are required (cell specific parameters, specific data for the cultivation system.
- Are appropriate data e.g. for cell growth, substrate uptake, medium composition available?
- For which cultivation systems have these data been determined?
- Are data on power input, mixing time, oxygen transfer etc. available?
- Which methods can be used to determine or estimate the above mentioned parameters?

Results and Discussion:
For selection and evaluation of suitable cultivation parameters a decision tree has been formulated to provide a guideline for design of mammalian cell culture processes. The suitability of this approach will be discussed in view of on experimental and literature data.
LARGE SCALE PERFUSION CULTIVATION WITH EXTERNAL ROTOFILTERS

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Background:
Fedbatch fermentation is dominating the cell culture landscape for industrial manufacturing of monoclonal antibodies and glycoproteins. However, perfusion fermentation is nowadays recovering popularity based on its higher volumetric productivity. Among perfusion devices, stainless steel rotating filters were popular years ago, but data from large scale use of these devices is still missed. The Center of Molecular Immunology, in Cuba, has scaled-up perfusion fermentation up to 2000 liters using external rotofilters for the manufacturing of antibodies. In the present work we discuss our recent achievements in understanding the principles of the dynamic separation of cells using rotofilters for perfusion fermentation. Up to our knowledge, this is the first report of industrial perfusion cultivation with rotofilters for antibody manufacturing at a scale bigger than 1000 liters.

Experimental Approach:
Operational and design parameters were investigated including filter-mesh pore diameter and structure, rotational speed, recirculation rate, and perfusion rate. Cell concentration, viability, product concentration and filtration volume were used to investigate the effect of operational variables.

Results and Discussion:
We demonstrate that stable cell concentration with high viability could be achieved using filter-meshes with pores bigger than cell diameter. This result is discussed in light of our previous findings of particle migration in vortex-filtration using Computational Fluid Dynamic studies. Separation efficiency was strongly influenced by the rotational speed of the rotofilter but less affected by recirculation or perfusion flow rates. Rotofilter capacity (filtration volume) could be significantly increased by modification of the filter-mesh construction (wire-orientation). No product retention inside the bioreactor was observed during perfusions with the rotofilters, in contrast to the common problem reported with the tangential-membrane filters, like ATF.
CAN WE REALLY MAKE ANTIBODIES AFFORDABLE FOR PATIENTS?

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Background:
Immunotherapy is moving more and more in the direction of chronic use of antibodies to treat cancer, leading to a demand of several (could reach 10) grams per patient per year. Current pharmacy prices for most of therapeutic antibodies are in the range of 1000 US$/gram. Following these economic figures one can only predict that chronic use of antibodies will not be affordable for the majority of cancer patients, especially in the developing world. This high price scenario is based on manufacturing costs in the range of 100 US$/gram, with a high fraction of fixed costs, that can only be reduced at product output of hundreds or thousands of kilograms per year, as it is the case of large multinational companies. Small and medium size local companies, with less market access, cannot manufacture affordable antibodies under current bioprocess and cost paradigm. Technologies with costs well below 100 US$/gram are needed if we want immunotherapy to reach cancer patients in the developing countries. In the present work we explore the potentials of intensified bioprocess strategies to overcome the cost limitations for antibody manufacturing in medium-size business players.

Experimental Approach:
The Center of Molecular Immunology (CIM) in Havana, Cuba, is manufacturing antibodies at large scale using perfusion cultivation for several years. Based on real life economical and process data from CIM (a mid-size manufacturer), we develop an empirical cost model useful to explore the economic implications of new business scenarios and process intensification strategies.

Results and Discussion:
Operational costs were found to be strongly dependent on liquid volume handling in upstream area and product mass per purification batch in downstream. Less effect, but significant, was observed for fermentation time and facility utilization in downstream rooms. Process intensification guided to increase in cell density, medium exchange rate and product specific productivity resulted in a significant reduction of operational costs, but an asymptotic effect was observed indicating that bioprocess strategies alone cannot make antibodies affordable. The influence of business factors like market size, facility fixed costs, administrative costs were also evaluated in the cost model. Based on the insights from these calculations we make a proposals of optimization to look for an affordable antibody manufacturing technology.
CONTAMINATION RISK MITIGATION IN CELL CULTURE MEDIA PREPARATION

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Background and Novelty:
The contamination of bioreactors with various infective entities like bacteria, mycoplasma and virus is a great risk for the biopharmaceutical industry. Whereas contamination with bacteria and mycoplasma are effectively prevented with various methods, the contamination risk with small non enveloped viruses poses a greater challenge due to the properties of these contaminants. Virus filtration of cell culture media and HTST are two methods to mitigate this risk.

Experimental Approach:
This talk focusses on application of virus filters for upstream operation. The performance of particular filters, namely a typical downstream filter and a newly developed filter dedicated for upstream, is considered in terms of throughput and retention (bacteriophage PP7). These results are interpreted in their meaning for the application. The requirement for the level of retention in the upstream operation of virus filters can be compared to sterile filtration. Similarities and differences between sterile filtration and virus contamination mitigation are used for an empirical risk evaluation.

Results and Discussion:
The actual operation of virus retentive filters in upstream is sketched: Media preparation from powder, prefiltration and virus filtration are presented with underlying data for a newly developed upstream filter as an example. Interactions of these sub-operations on the whole process are shown. A particular operational variant of serial and parallel usage of virus retentive filter elements demonstrates how the filters are used to maximize the risk mitigation effect.
FINE-TUNING GLYCANS IN CHO CELLS

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Background and Novelty:
Glycosylation is one of the most complex and critical quality attributes of biotherapeutics produced by
mammalian cell culture. Glycans modulate binding characteristics and often participate in the molecular
interaction with other proteins, mask immunogenic epitopes or hydrophobic surfaces and contribute to structural
stability. For antibodies, the Fc glycan modulates effector functions: reduced fucosylation and, to some degree,
increased galactosylation enhance Antibody Dependent Cellular Cytotoxicity (ADCC) while galactosylation is
also critical for complement activation (CDC). While an optimal and consistent glycan pattern is desired for
novel biologics reproduction of the original pattern is of utmost importance in development of Biosimilars.
To generate affilicosylated glycans, the fucosyltransferase Fut-8 is knocked out or, alternatively, fucose de novo
synthesis is shut down and the cytosolic sugar nucleotide pool of GDP-L-Fucose is depleted via expression of
the Pseudomonas enzyme RMD (GlymaxX®). Taking the advantage of an intact salvage pathway of fucose
synthesis and normal core fucosylation only the GlymaxX® technology allows to set specific fucosylation levels
by fucose feeding during production. Similarly, the degree of galactosylation can be adjusted by heterologous
expression of galactosyltransferase combined with feeding sugars, cofactors and pathway modulators.

Experimental Approach:
For directed fucosylation adjustment, increasing concentrations of fucose in the range between 0.01 – 1000 µM
were applied in fed-batch processes for GlymaxX®-modified antibody producer clones.
Resulting antibodies were quantified and assessed in respect to their ADCC activity and the degree of
fucosylation was determined chromatographically. To change the degree of galactosylation DG44 cells with
variable levels of galactosyltransferase were generated, tested for expression stability and subjected to a feeding
regime at variable concentrations of Manganese and sugar precursors.

Results and Discussion:
A clear relationship between the fucose concentration in the culture medium and the degree of core fucosylation
was observed. Although the salvage pathway is only a minor contributor to the fucose pool in unmodified cells,
a fucose concentration of 200 µM was able to return core-fucosylation in GlymaxX®-modified cells to almost
100%. The fucosylation degree clearly reflected in the ADCC efficiency, being the lowest at fully fucosylated
and the highest at the almost non-fucosylated state.
Neither RMD expression nor high level fucose supplementation had an impact on process parameters or
productivity. The study will determine how individual glycan attributes can be changed independently of
each other. In summary, a combination of approaches that allow manufacture of antibodies with preset glycan
attributes to support Biosimilar development as well as applications where intermediate effector functions are
desired.
SPARGER DESIGN FOR IMPROVED BUBBLE-LIQUID HYDRODYNAMICS IN BIOREACTORS

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Background:
Air and oxygen sparging is a commonly used strategy to maintain desirable dissolved oxygen (DO) and carbon dioxide (dCO2) levels in mammalian cell culture bioreactors. However, sparging can result in cell damage through high gas entrance velocity and bubble rupture as well as foam accumulation. As a result, sparger design and aeration strategies are of critical importance for bioreactor operation since they directly affect bubble formation and bubble size distribution. Despite this criticality, there has been relatively little research done in the area of sparger design.

Experimental Approach:
Various drilled-hole sparger designs have been tested over a range of gas entrance velocities (GEV) to evaluate the bubble formation patterns and dynamic behaviors. Initial tests occurred within a 2D bubble-liquid cold flow reactor using a high speed camera, and further studies were carried out in pilot scale bioreactors using a submersible video camera. Second order moment (SOM) bubble-liquid two-phase turbulent modeling was used to simulate the effect of sparger design on key hydrodynamic parameters.

Results and Discussion:
Bubble size distribution and mass transfer capabilities were compared, with experimental and simulated results indicating that desired bubble properties can be obtained through carefully designed drill-hole spargers and aeration schemes. By controlling these parameters, the operating range can be expanded while reducing cell damage in order to support high density cell culture processes. The positive results observed in pilot scale runs utilizing the optimized sparger will be presented.
HIGH-THROUGHPUT SYSTEM FOR CELL CULTURE (HTS-CC) COMPARABILITY: GENERATING DATA TO GUIDE 2L SMALL-SCALE EXPERIMENTS

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Background and Novelty:
HTS-CC (High-throughput System for Cell Culture) has been an enhancement to Late Stage Cell Culture (LSCC) development at Genentech. HTS-CC is an important tool for LSCC, allowing for large experiment spaces to be explored that offer a wide range of high-throughput production experiments that result in anticipated and scalable production of monoclonal antibodies. Data generated from HTS-CC 50-mL tubespins enables large screening experiments that can effectively guide 2L small-scale experiments from clone screening through pilot-scale manufacturing. Optimization and routine use of the HTS-CC system has improved the comparability of cell culture metrics from 50mL to 400L, resulting in predictable titer and product quality attributes.

Experimental Approach:
The HTS-CC project began in 2006 as a tool for media development. The first system came online in 2008 and was used extensively for media development and proof-of-concept studies for clone screening and process development. A second system was built in 2009 to increase our capacity. So far, about 26,000 reactors have been run on the systems. The system is based on 50mL TubeSpin shaken bioreactors. The original TubeSpin design had 0.2μm filters in the cap for gas exchange. A septum was added to this to allow a robotic liquid-handler to access the tube contents without removing the cap. The tubes spend most of an experiment in a Kühner shaking CO₂ incubator. When a sample or feed is required, the tubes are carried from the incubator onto the robot, and the robot performs the operations. The HTS-CC robot also analyzes the samples. pH is measured both online in the incubator, using PreSens fluorescence-based sensors and their ITR 96-tube reader, and offline, using a custom-built robotic tool with small pH electrodes. Metabolites such as Glucose, Lactate, and Ammonia are measured using Cobas reagents run in a 384-well plate. Cell counts and viability samples are prepared and then measured in a Cellavista wellplate imager. Antibody concentration is measured on a ForteBio Octet. Since the TubeSpins have a relatively generous 30mL working volume, multiple 1mL samples are taken for HPLC titer and product quality assays performed by Genentech’s Analytical Operations group.

Results and Discussion:
A monoclonal antibody project was used as a case study to identify the clones that would produce the most protein. If the study were performed in 2L bioreactors, the researcher would have only been able to test each clone using a single, platform process. Using the HTS-CC, we were able to assess the potential of each clone under several process conditions with the goal of maximizing antibody production. These conditions included pH setpoint, feed strategy, and temperature, in a full-factorial design. Top clones had different optimal conditions that were readily identified. Follow-up testing at the 2L and 400L scales confirmed that performance above the platform process was achieved based on the process conditions identified via the HTS-CC study.
THE IMPACT OF CRE ACTIVITY AND CO-INFECTION ON ADENOVIRUS PROPAGATION: OPTIMIZING CANINE HELPER-DEPENDENT VECTOR MANUFACTURING

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Background and Novelty
The production of adenoviral helper-dependent vectors (HDVs) is commonly based in the Cre/loxP system to minimize helper vector (HV) contamination. HDV producer cells express Cre-recombinase and are generally maintained in culture for longer periods of time as they are needed for the serial amplification steps of the vector until sufficient titers are generated. However, Cre-recombinase induces toxicity which impairs cell-line performance. Therefore, the evaluation of cell-line stability in the scope of HDV production becomes critical.

Experimental Approach:
In this work, Cre-expressing cells stability, infection conditions and their relation to adenovirus amplification and HV contamination were evaluated during the development of a production protocol for HD canine adenovirus type 2 (CAV-2) vectors in serum-free medium.

Results and Discussion:
Despite presenting similar cell growth profile with increasing culture passages, the long-term Cre expression in MDCK-E1-Cre cells after 20 passages reduced the adenovirus production up to 7-fold. Furthermore, a slight increase in non-infectious particles was obtained with MDCK-E1-Cre cells. The best conditions to produce HD CAV-2 followed a high HDV/HV MOI ratio (5:0.1), where HV contamination levels were the lowest. Curiously, we found that such MOI ratio can effectively reduce HV contamination even when using cells without Cre recombinase. This raises the possibility of producing HDVs without Cre-expressing cells when optimal infection conditions are used. This work shows how Cre and MOI ratio can impact HD CAV-2 production, identifying key aspects to be considered when designing a production of gutless adenoviral vector with better yields and quality.

References:
AUTOMATED NUTRIENT MONITORING AND CONTINUOUS FEED PLATFORM
FOR PRECISE NUTRIENT FEED CONTROL

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Fed-batch culture processes are commonly used for the production of various biotherapeutic and other industrial commodities. A widely employed nutrient feed control strategy for both cell culture and microbial fermentation processes is direct feedback control, in which the nutrient feed is directly controlled by the nutrient, i.e., glucose, concentration. However, various iterations of this feeding approach exist which may not be conducive to culture growth and productivity. In order to optimize culture performance, the means by which the substrate is fed to the culture is just as, if not more, important as maintaining the desired media substrate levels.

An automated feedback control platform, which employs a continuous feed strategy based on the culture’s real-time nutrient consumption rate, has been developed. The integrated platform consists of the Seg-Flow® automated on-line sampling system, a nutrient analyzer, and a feed pump. This system was evaluated for its ability to precisely control media glucose concentrations in both low and high consumption rate culture simulation models in 3 different working volumes. Hourly analysis performed by the SegFlow system provided comprehensive glucose concentration profile for each trial. Consumption was recalculated after every sample to ensure setpoint is maintained between cycles.

The results showed that the integrated Seg-Flow system was able to precisely control media glucose concentrations at the prescribed levels through real-time glucose consumption rate calculation and continuous feeding. The continuous feed platform allowed quantitative monitoring of consumption rate at each sampling.
OVERCOMING CHALLENGES AND ENHANCING PRODUCTION OF ANTIBODIES FOR SITE-SPECIFIC ANTIBODY-DRUG CONJUGATES

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Background and Novelty:
Antibody-drug conjugates (ADCs) have been emerging as a new class of anticancer therapeutics, in which monoclonal antibodies are designed to deliver a cytotoxic drug selectively to antigen expressing cells. Traditional lysine- or cysteine-based conjugation technologies generate heterogeneous ADC molecules, exhibiting different pharmacology and presenting challenges to controlling safety, as well as manufacturing and analytical characterization. The site-specific ADC technology we are using results in ADCs with a homogenous drug-antibody ratio. This technology incorporates non-native amino acids which have selectively linkable and chemical and enables the precise and site-specific modification of therapeutic mAbs.

Experimental Approach:
In this work, we made efforts in improving cell lines, optimizing cell culture process, and developing new media, in order to enhance production of antibodies. Cell line development (CLD) for the engineered antibodies requires modification of the traditional CLD protocol. We developed a new CLD procedure using ClonePix for such engineered antibodies by implementing gene amplification prior to subcloning. Through systematic DOE studies, we discovered that feeding of non-native amino acid interacts with temperature shift and optimization of temperature shift and the feeding of the non-native amino acid is critical for the titer improvement. In addition, we developed a proprietary chemically-defined feed medium by balancing concentrations of nutrients including amino acids, trace metals, vitamins.

Results:
Our modified CLD method resulted in >2-fold improvement in the production of such engineered antibodies. The cell lines demonstrate a good stability to support clinical production at large-scale. Applying the novel feed medium we developed and combining it with process optimization (nutrient feed, non-native amino acid, temperature shift) lead to an additional 2-fold increase in antibody productivity. These titer enhancements for the engineered mAbs make it possible for clinical and commercial application of the ADCs which are generated using the novel site-specific conjugation technology.
DATA BASED CONCEPT TO IMPROVE THE PH CONTROL FOR CELL CULTURE PROCESSES

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Background and Novelty:
The culture pH is an important parameter for mammalian cell culture processes as it can not only significantly affect process performance but first and foremost product quality. Therefore, precise and accurate control of the culture pH is crucial for the majority of biopharmaceutical processes. As common standard, pH probes are installed in the bioreactors to enable pH on-line monitoring. Contrary to other on-line probes like temperature or dissolved oxygen (DO), it is known that pH probes may drift during lifetime. In consequence, a strategy for the adjustment of pH online probes should be established using an orthogonal pH measurement method as control.

Therefore, fermentation samples are usually taken at defined time points and on-line probes are adjusted on basis of an at-line reading, if the difference between the pH on-line value and the at-line value - the so-called ΔpH - exceeds a certain limit. This procedure comes along with several drawbacks, if no upper limit for the ΔpH is defined and the root cause for an increased ΔpH is not investigated:

- Incorrect at-line readings may lead to a wrong adjustment of the on-line probe.
- A pronounced drift of the on-line probe may remain undetected.

Efforts to improve the control concept led to the establishment of the ΔpH criterion with the following requirements: The limit should be strict enough to detect and challenge unusually high ΔpH values, but it should still cover the usually observed ΔpH range to reflect the variability that was present during process development/characterization and clinical manufacturing in the past. It was decided to define this ΔpH criterion on basis of historical data since an appropriate experimental design that accounts for all random possible differences (e.g. ΔpH extend, time, frequency…) is barely possible.

Experimental Approach:
Historical data of four processes served as basis for the definition of the ΔpH criterion. The data derived from pre-stage and main-stage fermentations were performed in development (n = 4670) and manufacturing (n = 928) and represent the different scales and systems in an appropriate way. For the data evaluation, a 3-step approach was applied:

1) Calculation of the ΔpH for every single measurement.
2) Statistical comparison of the median ΔpH values for development and manufacturing (Kruskal-Wallis Test).
3) Final definition of the ΔpH as 99% percentile on basis of the complete available data set.

Results and Discussion:
As result, a generic ΔpH criterion was defined on basis of approx. 5600 data pairs from development and manufacturing, with proven transferability between the two areas, different scales, processes and process steps. Due to the implemented criterion, unusually high ΔpH values are challenged ensuring an improved process control and reduced risk with regard to process performance and product quality. In addition, an implemented root cause investigation for ΔpH above the upper limit allows an understanding of the sources and enables constant improvement.
BIOSIMILAR DEVELOPMENT ENHANCED THROUGH THE USE OF AMBRTM 15

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Background and Novelty:
It has become increasingly important in bioprocess development to identify and characterize the effect of critical process parameters as early in the development cycle as possible. This quality by design (Qbd) approach is not only important for developing processes for novel molecules entering the clinic but also, and perhaps more readily applied to, the process development for biosimilar manufacturing. While sometimes it may be difficult to know what the optimal product quality attributes will be for a novel molecule, the exact target attributes are known for a biosimilar. In addition of the importance for the molecule to meet the quality attributes of the innovator, so too is it important that a commercially viable process is developed in an timely manner such that the timing of product launch will match as closely with the expiration of patent exclusivity as possible. To accomplish both of these goals we have chosen to use the Ambr™ 15 system for high throughput process development using DOE methodology. This high throughput bioreactor system is typically used during the clone selection phase or for media development however we have employed the Ambr™ 15 as a scale down model for process development as well. In the current work, we demonstrate the use of the Ambr™ 15 for the upstream process development of a biosimilar produced in CHO cells using Qbd principles.

Experimental Approach:
As mentioned above, it is desired to begin the identification of key parameters as early in the development cycle as possible. This could be applied to the clone selection phase or earlier, however, the scope of work for this project began after the clone was selected. As such, the work described begins with media and feed screening as well as media/feed component identification for the purpose of maximizing titers as well as the identification of media types and or components which impact the critical quality attributes. Following media and feed selection, the influence of upstream process parameters was also investigated these include pH, temperature shift, seeding density and feed strategy among others. Throughout the individual studies, samples were analyzed for titer and product quality profiles including charge variants and the glycoprofile.

Results and Discussion:
Through the screening and response surface studies several key process parameters and media components that affect product quality attributes such as charged variants and glycol profile were identified. In addition, ranges over which acceptable levels could be achieved were also identified. Here we show the effectiveness of the Ambr™ 15 as a scale down model for media development, process development and key process parameter identification. Through this rapid development we were able to achieve a comparable glycoprofile to the innovator as well as identify parameters that impact charge variants such that a closer match to the innovator was possible at harvest. The Ambr™ 15 proved to be an invaluable tool for key component and process parameter identification in the upstream process for the manufacture of a biosimilar.
EMERGING TECHNOLOGIES
NOVEL TYROSINE AND CYSTEINE DERIVATIVES TO SIMPLIFY CELL BIO FED-BATCH PROCESSES

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Background and Novelty:
Fed-batch culture bioprocesses are currently used predominantly for the production of recombinant proteins, especially monoclonal antibodies. In these cultures, concentrated feeds are added during cultivation to prevent nutrient depletion, thus extending the cellular growth phase and increasing product concentrations. One limitation in these bioprocesses arises from the low solubility or stability of some compounds at high concentrations, in particular amino acids. This study describes the synthesis and evaluation of a phosphotyrosine disodium salt as a tyrosine source and cysteine derivatives in fed-batch processes. These molecules are highly soluble in concentrated feeds at neutral pH. Mechanistic studies demonstrated that e.g the phosphotyrosine is cleaved in the cell culture supernatant after processing by released phosphatases, leading to phosphate and free L-tyrosine which can be taken up by the cells. No intact phosphotyrosine was detected intracellularly or incorporated into the sequence of the monoclonal antibody. Similar findings will be presented for several Cysteine derivates as novel ingredients in cell culture media. The use of these new molecules allows the simplification of fed-batch processes in large scale manufacturing under GMP via the implementation of neutral pH, highly concentrated feeds.

Experimental Procedure:
We have tested first the solubility and stability of the amino acid derivatives in concentrated media and feed at neutral pH. Afterwards the novel molecules where applied into a simplified fed-batch CHO process to produce a monoclonal antibody. Besides viable cell density, viability and titer a comprehensive characterization of the expressed molecule was done to secure that none of the modifications end up in the final molecule.

Results and Discussion:
The usage of the novel molecules was tested in different scales in 30 mL small scale in shaker tubes and 1.2L bioreactor scale. The simplified bioprocess was benchmarked vs. the dual feed regime where besides the main amino acid feed at neutral pH the caustic Tyr/Cys feed at pH >11 is used. Without leveraging the full potential of process development it is shown that the simplified fed-batch process resulted in doubling the specific productivity using a CHO K1 bioprocess.
RECENT DEVELOPMENTS IN SCALING DOWN AND USING CAPACITANCE PROBES FOR MEASURING THE LIVE CELL CONCENTRATION IN SINGLE USE BIOREACTORS

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Background and Novelty:
Real-time bioprocess monitoring is fundamental for maximizing yield, improving efficiency and process reproducibility, minimizing costs, optimizing product quality, and full understanding of how a system works. Bioreactors that are monitored continuously and in real-time offer the advantage of meeting current and future supply demands with biological product of the utmost quality and safety, achieved at the lowest overall cost and with least risk. This paper will focus on the latest developments in dielectric spectroscopy for live cell concentration measurement and how the technology has been scaled down allowing single use bioreactors to be monitored in real time. The poster will also focus on how several approaches can be taken to use dielectric spectroscopy in single use bioreactors.

Experimental Approach:
The development of the first single use probes produced for dielectric spectroscopy focused on a design that replicates the probes used in conventional cGMP production bioreactors. The electrode geometry and platinum material that is exposed to the cell culture in the single use probe is identical to geometry and material used in the robust 25mm diameter steam sterilizable probes with four flat, parallel flush electrodes typically used in the larger cGMP production bioreactors. These single use probes have been proven to be ideal for the larger single rocking motion bags and stirred tank bioreactors and are now commercially available. For the smaller bags, like those required in stem cell production and typically less than 1 litre working volume, a different design was required and the poster describes the challenges of the probe scale down. Particular attention is given to the way electrode polarization was addressed on the new style of probe; the polarization is an artefact at lower measuring frequencies that increases as the electrode size is reduced. The connecting dielectric spectroscopy electronics are also required to be assembled in a much smaller design space and again the poster shows how these were modified to connect to the small single use probes. An alternative approach was also taken to allow a conventional 25mm diameter steam sterilizable probe to be inserted into a specially designed flow through cell that can connect to the silicone tubing on a recirculation loop or seeding line to a single use bioreactor.

Results and Discussion:
The scaled down single use probes for use in smaller bioreactors were shown to have a comparable performance to the capacitance and conductivity readings obtained with conventional steam sterilizable probes with annular or flush electrode arrangements. For the 25mm steam sterilizable probe, this required a special calibration due to the close proximity of the electrodes to the base of the flow through cell. Once recalibrated, then the system performed with identical results to a reference capacitance probe placed in the headplate of a bioreactor.
COMPACTION OF CELL CULTURE MEDIA – A NEW TECHNOLOGY TO LEVERAGE THE ADVANTAGES OF DRY POWDER FORMULATIONS

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Background and Novelty:
Dry powder cell culture media (CCM) formulations provide many advantages with respect to shipping and storage because of their reduced volume and increased stability. However, to leverage these benefits, they need to be highly soluble, homogeneous and convenient to handle. An improvement of dry powder media properties can be achieved by granulation, agglomerating fine CCM powders to larger particles. In contrast to the well-established wet granulation technology, the presented roller compaction technology simply uses compression force to form granules not adding any water or other additive to the CCM. Being well-established in pharma formulation, the roller compaction is new to the field of CCM, offering an alternative to existing granulation methods.

Experimental Approach:
Multiple CCM formulations were produced as homogeneous dry powder to evaluate different roller compaction setups regarding the resulting granules’ quality. Hence, three groups of analytical methods were applied in this study to classify the different production setups: (1) Physicochemical parameters such as abrasion, hardness, bulk density reduction and flowability as well as porosity, water content, vitamin and amino acid stability were used to evaluate the shipping ability and stability of the compacted media. (2) Using a nephelometer aerosol monitor, conductivity and focused beam reflectance measurement probes as well as visual observation, the handling of the compacted CCM was evaluated regarding dust formation and solubilization speed. (3) As a final proof of applicability, the performance of the compacted CCM was compared to powder CCM in batch and fed-batch cell culture experiments.

Results and Discussion:
The feasibility of applying roller compaction in the processing of dry powder CCM was subject to the research work presented in this contribution. Starting from a homogeneous dry powder medium, the roller compaction technology is able to fix this homogeneity in larger granule particles (> 1000 μm) so that de-mixing can be prevented. Data presented will outline that this technology leads to stable compacted particles with enhanced flowability properties and consistent amino acid and vitamin composition compared to the dry powder. But the key advancements of using compacted media are the up to 2-fold increase in bulk density, the strongly reduced dust formation and the up to 3-fold accelerated solubilization speed shown in this presentation. Additionally, compacted CCM are proven to deliver the same cell culture performance in batch and fed-batch cultivation experiments as powder media, underlining the technology’s applicability for cell culture media production. Finally, a correlation of this broad analytical data with the media formulation and powder properties can guide the selection of the most suitable compaction setup for the respective CCM formulation. Summarizing the results, it will be shown that compacted powder media make the handling of CCM more efficient and convenient while delivering the identical performance as free powder CCM.
TEMPORAL REGULATION OF CHO CELLULAR PHENOTYPES IN PRODUCTION BIOREACTORS USING NOVEL RNAI TECHNOLOGY

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Background and Novelty:
Conventional culture process development employs a combination of tools including medium optimization/supplementation, bioreactor operations, and sophisticated feed control schemes to both maximize protein yield and optimize product quality. Here we report the demonstration of inducing specific RNA interference during fed-batch production cultures using the proprietary technology from Alnylam Pharmaceuticals. Unlike the traditional knock-in/knock-out cell line engineering approach, the transient siRNA transfections allow the modulation of undesirable cellular phenotypes at the production stage without incurring longer timeline needed for stable clone selection. The suppression of target gene expression can also be prolonged with repeated transfection without compromising cell growth or productivity.

Experimental Approach:
As a proof of concept study, three gene targets were selected to address different areas of common bioprocessing challenges: lactate accumulation, glycan control, and protease activity. Cells were cultivated in bench scale bioreactors using in-house platform media formulations and process conditions. At pre-determined optimal time points, siRNA duplexes were delivered to the bioreactors to quench target gene expressions. Daily samples were taken to monitor culture performance, target gene expression levels, as well as their corresponding phenotypes.

Results and Discussion:
It was shown that LDHa silenced 1L bioreactor cultures displayed lower lactate dehydrogenase activities and eventually less lactate accumulation in culture. As a result specific productivities of the cell lines were improved. In similar types of experiment set up, terminal galactosylation and cathepsin D protease activity exhibited dosage-dependent correlation behavior to siRNA transfections, demonstrating the ability to fine-tune relevant quality attributes instead of an all-or-none response. These positive experimental outcomes are supportive of applying the RNAi technology in a biologics manufacturing setting. Furthermore, the feasibility of this technology also introduces a new dimension in bioprocessing control by demonstrating practical use of CHO genomics data in culture process development.
THE DIFFERENTIAL POLARIZABILITY OF CHO CELLS CAN BE USED TO MONITOR CHANGES IN METABOLISM

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Background and Novelty:
The structure of individual cells allows them to hold an electrical charge, which can be polarized in an applied electrical field with an alternating current in the radiofrequency range. This is the principle for cell measurements based on dielectrophoresis. We have developed a prototype cytometer which measures the dielectrophoretic properties of individual cells. This tracks the trajectory of individual cells as they pass through a bank of electrodes designed to differentially perturb the cells according to their polarizability. Cells are analyzed in the instrument (DEP cytometer) by their displacement in an electrical field with a sensitivity of 0.1 μm and at a rate of 5 cells per second.

Experimental Approach:
Cell samples taken from a batch bioreactor run of CHO cells were analyzed by the DEP cytometer at regular intervals with a sample size of around 600 cells. The changing polarizability of the cells was measured by a force index (FI) which was related to the electrical displacement of the cells which changed as cells lost viability. The cell samples were also monitored by image analysis, capacitance and multiple fluorescent stains in a Guava flow cytometer. Comparison were made between these methods.
In a second experiment the time course of metabolic changes in CHO cells were observed by dielectric response in the DEP cytometer following addition of oligomycin which was used to inhibit mitochondrial ATP production. Oligomycin is known to inhibit the ATP synthase by blocking the proton channel on the mitochondria transmembrane within minutes of treatment. Monitoring the cell response within the first hours of the treatment with oligomycin reveals important information on the ion regulations and the impact of mitochondrial ATP synthase inhibition.

Results and Discussion:
The dielectrophoretic state of cells as measured by the cytometer could be correlated to the metabolic state of the cells as they passed through the various stages of apoptosis. Samples taken from the stationary to decline phase of the bioreactor culture showed a gradual change of FI which could be related to the sub-populations of cells revealed by fluorescent apoptotic assays. Early changes in the measured FI of cells correlated with the Nexin V fluorescent assay, which was associated with early phase apoptosis and a change in the cell membrane. Oligomycin treatment caused the rapid change in the dielectric response of the cells due to loss of mitochondrial ATP production. This work demonstrates that the inhibition of mitochondrial ATP synthase causes a decrease in cytoplasmic conductivity that can be monitored by the DEP cytometer.
We conclude that the DEP cytometer offers a sensitive method of monitoring the metabolic state of CHO cells during a bioreactor run or following treatment with a metabolic inhibitor.
IMPROVED SELECTION OF HIGH-PRODUCING CHO CELL POPULATIONS WITH HI-CATCHER, AN HIGHLY EFFICIENT AFFINITY BASED SYSTEM IMPLEMENTED INTO CELL LINE DEVELOPMENT

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Background and Novelty:
Chinese hamster ovary (CHO) cells are still the major mammalian cell line for the production of biopharmaceuticals due to high viabilities and cell densities achieved in suspension cultures during fermentation processes. However, screening procedures for isolation and development of high-producing cell lines needed for demanding manufacturing processes as required in the pharmaceutical business remain an extreme time-and labor-intensive challenge, with the main bottleneck being the identification of stable high-producer clones. The aim of this study was to investigate the implementation of an improved selection system for high-producing cells into CHO cell line development. The highly efficient Hi-Catcher system presented herein is based on a specific affinity matrix simultaneously binding a surface antigen on CHO cells and the Fd-part of IgG1 antibodies, capturing recombinant antibodies secreted by CHO cells on their cell surface, thereby eliminating the need for non-selective biotinylation of cell populations. Subsequent labeling with fluorescent detection conjugates creates a specific cell surface signal correlating with captured amounts of antibody, allowing identification and isolation of high-producing cells via flow cytometry and cell sorting.

Experimental Approach:
High-producer selection was initiated with three different stable transfectant pools exhibiting low producer rates and antibody productivities. Both producer rates as well as antibody productivities were increased with the Hi-Catcher system, initially with magnetic enrichment using anti-PE microbeads and subsequently with FACS sorting, selecting targeted producer cells with the highest fluorescence signal on their cell surface. After adaptation of the sorted producer cell pools to chemically defined CHOMACS CD medium and cultivation in an orbital shaken reactor, gene amplification was started by stepwise increases of recombinogenic MTX agent. The second amplification cycle was followed by another Hi-Catcher-supported FACS sort, again selecting producer cells with the brightest fluorescence intensities. In addition to the generation of homogeneous high-producer pools, the Hi-Catcher system was also applied to the identification and isolation of high-producing single clones mediated by FACS sorting. According to sorting of the Hi-Catcher-subjected cell pools, single cells exhibiting the brightest surface fluorescence staining were sorted into wells of microtiterplates followed by automated semi-solid high-producer clone screening, expansion, and productivity assessment.

Results and Discussion:
The homogeneous cell pools obtained by sorting Hi-Catcher-subjected cell pools comprised high producer rates and concomitantly, antibody productivities could be increased up to 136-fold compared to non-selected, heterogeneous pools reaching only 2-25-fold increases in productivity after MTX amplification. Furthermore, high-producing clones could also be efficiently isolated on the single cell level.
To summarize, the results of this study demonstrate the advantages of applying the Hi-Catcher system at different cell line development stages, either by generation of homogeneous producer cell pools before and after successive MTX amplification cycles, and/or final isolation of high-producing clones by single cell sorting. The presented Hi-Catcher system efficiently generates high-quality cell populations regarding homogeneity and antibody productivity, substantially reducing screening efforts during CHO cell line development.
REAL-TIME MONITORING OF THE PHYSIOLOGICAL STATE OF CHO CELLS IN BIOREACTOR BY USING A DUAL SPECTROSCOPIC STRATEGY

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Background and Novelty:
Bioprocesses of mammalian cell culture have become essential in pharmaceutical fields for the production of recombinant therapeutic proteins such as monoclonal antibodies or for tissue therapy. Following the FDA's recommendation to apply Process Analytical Technology (PAT) approach as the mean to control production processes and ensure the quality of the end-product for patients, Near Infra Red (NIR) and dielectric spectroscopic technologies have gained great attention over the past decade as online tools for cellular bioprocesses monitoring. In such processes, cells are one of the most critical parameter, because their physiological state directly impacts the final product titer through their productivity as well as the product quality. Animal cells are typically classified into viable or dead entities via trypan blue exclusion technique, but lysed cells are not accounted for, although they could represent a significant proportion of the cell population in bioreactors. Therefore, they should be considered for optimal control of processes. In this work, we propose the first online strategy combining NIR and dielectric spectroscopies for in-depth characterization of cellular growth and physiology of CHO (Chinese Hamster Ovary) cells cultivated in bioreactors.

Experimental Approach:
Cells were cultivated in 2 L bench-top bioreactors under various operating conditions (glucose and/or glutamine feeding and temperature shift at 32°C). A dielectric probe (Fogale Biomass System) was used to measure the overall permittivity and conductivity of cultures submitted to an alternating electric field. A sterilisable NIR probe served for spectra acquisition of medium molecules vibrations due to photon absorption between 4,000 and 10,000 cm⁻¹. NIR spectra with a 8cm⁻¹ resolution were pre-treated with first derivative, SNV and mean-centered.

Results and Discussion:
The combined use of NIR and di-electric spectroscopies allowed to determine in real time throughout culture the densities of the different cellular sub-populations: viable cells (VCD), dead cells with permeable membranes (DCD) and lysed cells (LCD). NIR spectroscopy calibration models were generated with partial least square (PLS) for LCD through on-line monitoring of extracellularly released lactate dehydrogenase (LDH) upon cell membrane disruption. In parallel, on-line measurements via dielectric spectroscopy of permittivity was confirmed to be a good monitoring parameter for VCD with R² greater than 0.87, while conductivity proved to be an innovative and effective way to monitor DCD (R² > 0.9) potentially resulting from a gradual alteration of membrane permeability. Moreover, the specific rates of cell growth, substrate consumption and metabolites production could be determined throughout culture without the need for culture medium sampling.
These results demonstrate the strong potential for a dual spectroscopy strategy to enhance real-time monitoring towards better understanding and control of bioprocesses. In particular, it will allow to identify harmful culture conditions that could result in direct cell lysis. Work is in progress to complete this approach by combining also on-line Raman spectroscopy measurements.
SEQUENCING THE CHO DXB11 GENOME REVEALS REGIONAL VARIATIONS IN GENOMIC STABILITY AND HAPLOIDY

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Background and Novelty:
The DHFR negative CHO DXB11 cell line (also known as DUX-B11 and DUKX) was historically the first CHO cell line to be used for large scale production of heterologous proteins and is still used for production of a number of complex proteins. Here we present the genomic sequence of the CHO DXB11 cell line as well as provide a detailed analysis of the copy number variations seen in this genome in addition to the genomes of the currently sequenced CHO cell lines.

Experimental Approach:
Genomic DNA was extracted from adherently growing CHO DXB11 cells and sequenced using the Illumina HiSeq 2000 platform yielding 0.5 bn paired reads. The reads were aligned to the Chinese hamster genome analyzing specifically the sequencing depth at each gene in the genome.

Results and Discussion:
Based on the number of single nucleotide polymorphisms (SNP’s) detected in the genome, a significant genomic drift was seen favoring Gc->AT point mutations in line with the chemical mutagenesis strategy used for generation of the cell line. The sequencing depth for each gene in the genome revealed distinct peaks at sequencing depths of 0x, 16x, 33x and 49x coverage corresponding to a copy number in the genome of 0, 1, 2 and 3 copies. The data indicate that 17% of the genes are haploid revealing a large number of genes which can be knocked out with relative ease. This tendency of haploidy was furthermore shown to be present in eight additional analyzed CHO genomes (15-20% haploidy) but not in the genome of the Chinese hamster. The dhfr gene is confirmed to be haploid in CHO DXB11; transcriptionally active and the remaining allele contains a G410C point mutation causing a Thr137Arg missense mutation. We find ~2.5 million SNP’s, 44 gene deletions in the CHO DXB11 genome and 9357 SNP’s, which interfere with the coding regions of 3458 genes. Copy number variations for nine CHO genomes were mapped to the chromosomes of the Chinese hamster showing unique signatures for each chromosome. The data indicate that chromosome one and four appear to be more stable over the course of the CHO evolution compared to the other chromosomes thus might presenting the most attractive landing platforms for knock-ins of heterologous genes.
MULTI-OMIC PROFILING OF EPO PRODUCING CHO CELL PANEL REVEALS METABOLIC ADAPTATION TO HETEROLOGOUS PROTEIN PRODUCTION

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Background and Novelty:
The Chinese hamster ovary (CHO) cell line is the predominant mammalian cell factory for production of therapeutic glycoproteins. Overexpression of a heterologous glycoprotein increases the trafficking through the secretory pathway to the limit of the protein processing capacity leading to productivity bottlenecks. In this work, we aimed to study potential bottlenecks in the secretory pathway associated with the production of human erythropoietin (EPO) in CHO cells. In connection to this, we discovered indications of metabolic adaptation in favor of heterologous protein production.

Experimental Approach:
We established a panel of stably EPO expressing CHO-K1 clones spanning a 25-fold productivity range and characterized the clones in batch and chemostat cultures. For this, we employed a multi-omic physiological characterization including NMR-based metabolic footprinting (exo-metabolome) of amino acids, LC-MS based metabolite fingerprinting (endo-metabolome) of glycolytic intermediates, NAD(P)H/NAD(P)⁺ and adenosine nucleotide phosphates (ANPs). Quantitative PCR (qPCR), quantitative reverse transcription PCR (qRT-PCR), western blots (WB) and Affymetrix CHO microarrays were used to assess EPO gene copy numbers, EPO gene expression, intracellular protein levels and genome-wide gene expression analysis of differentially expressed genes functionally related to secretory protein processing, respectively. For the analysis of metabolic adaptation, we generated a network reconstruction of the relevant metabolic pathways in CHO cells based on legacy knowledge from mouse and performed manual model curation using genome annotation and available literature. The reconstruction was utilized as a platform for interpretation of differential gene expression data in a biological meaningful manner.

Results and Discussion:
Initially, the EPO producing clones and a parental clone serving as control were physiologically characterized in duplicate batch cultivations in bioreactors. Next, we performed a quantitative characterization of intracellular metabolites related to glucose catabolism (i.e. specific glycolytic intermediates, NAD(P)H/NAD(P)⁺ and ANP’s) across the EPO producing clones. To identify bottlenecks in the protein secretory pathway, we compared EPO gene copy numbers, EPO gene expression levels, intracellular EPO retention and extracellular EPO levels for a low, medium and high producing clone during steady state in chemostat culture. Furrthermore, we performed a global gene expression analysis and investigated genes related to secretory protein processing. Finally, when inspecting the gene expression landscape of the relevant metabolic pathways, we observed an apparent adaptation in favor of EPO production.
IMPROVED PROPERTIES OF HUMAN INTERFERON-ALPHA CONFERRED BY O-GLYCOSYLATION IN MAMMALIAN CELLS

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Background and Novelty:
Human interferon (hIFN)-α family consists of small proteins that exert antiviral, antiproliferative and immunomodulatory activities. Recombinant hIFN-α2b (rhIFN-α2b) is the current treatment for chronic hepatitis and some types of cancers; however, therapy outcomes have not been completely satisfactory. The short serum half-life and rapid clearance of the cytokine account for its low in vivo biological activity. For this reason, frequent administration of IFN is needed in order to achieve the desired therapeutic action, leading to patient inconvenience and producing the well-known IFN-α side effects. Therefore, applying strategies to improve the pharmacokinetic properties of this protein is relevant, aiming to increase its in vivo biological activity and, consequently, its clinical efficacy.

Experimental Approach:
In this work, an O-glycoengineering approach was carried out fusing the carboxi-terminal peptide (CTP, derived from the hCG β-subunit) to the N-terminal end (CTP-IFN), the C-terminal end (IFN-CTP) or both ends (CTP-IFN-CTP) of hIFN-α2b. In this way, IFN analogs containing 4 to 8 O-glycosylation potential sites were obtained in CHO cells and a physicochemical, biological and pharmacokinetic characterization was performed.

Results and Discussion:
The success of CTP incorporation and the consequent O-glycosylation was demonstrated by the higher molecular mass and the heterogeneous electrophoretic profile of CTP-modified variants compared to the non-modified cytokine (wt-IFN). Nevertheless, CTP-IFN-CTP exhibited the highest molecular mass, showing an increment of approximately 1.4-fold and 2-fold regarding singly-modified CTP-IFNs and wt-IFN, respectively. Isoelectric focusing analysis of the three variants showed a higher number of acidic isoforms as regards wt-IFN, which was consistent with a higher sialic acid content conferred by the new O-glycosyl moieties. In relation to the in vitro antiviral specific biological activity (SBA), CTP-IFN-CTP retained 68% and 77% of the SBA corresponding to CTP-IFN and IFN-CTP, while its antiproliferative SBA was 45% and 33%, respectively. Differences in the antiproliferative and antiviral activities confirmed that distinct molecular regions of the cytokine are responsible of the events that exert both sort of actions. Pharmacokinetic assays in rats demonstrated that CTP-IFN-CTP reached its maximum plasma concentration 4 h later than the singly-modified CTP-IFNs and was removed more slowly from circulation. Thus, CTP-IFN-CTP showed a significant increment in the area under curve (AUC) which translated in a decrease in plasma clearance in comparison with CTP-IFN, IFN-CTP and wt-IFN (23 ml.h⁻¹ vs 47 ml.h⁻¹, 51 ml.h⁻¹ and 68 ml.h⁻¹, respectively). The higher molecular size and negative charge of CTP-IFN-CTP, provided by the attached O-glycans, might explain the improvement of the mentioned parameters. Finally, stability assays showed that the heavily glycosylated IFN variant exhibited a substantially increased thermal resistance, preserving higher residual antiviral activity (60%) in comparison with the non-glycosylated cytokine (8%) at temperatures above 65°C. In addition, O-glycosylation increased the stability of CTP-IFN-CTP against in vitro protease inactivation.
In conclusion, rhIFN-α2b properties which are beneficial for increasing its in vivo biological activity were successfully modified by O-glycoengineering.
EARLY DETECTION OF SEQUENCE VARIANTS DURING CELL LINE DEVELOPMENT USING NEXT GENERATION SEQUENCING

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Background and Novelty:
The Next-Generation Sequencing (NGS) revolution has had a profound effect on the manner in which we approach the fields of biology and medicine, but the effects are not limited to these fields alone. With the process of collecting and analyzing large datasets becoming more seamless, a growing number of disciplines are incorporating NGS technologies into their workflow. Biotherapeutic development is one such field that is beginning to benefit from the comprehensive and rich datasets that NGS can provide. A straightforward use of NGS technology would be to verify that the biotherapeutic mRNA sequence is free of mutations during clone screening. We developed a method that takes advantage the multiplexing and high-throughput capability of NGS to sequence confirm the mRNA from multiple antibody producing clones in one experiment. Results from these NGS experiments are combined with product quality and productivity data points to inform clone decisions. This method is now an integral part of our clone screening workflow and has opened the door to utilizing NGS capabilities for different aspects of biotherapeutic development.

Experimental Approach:
A NGS method was developed to screen the heavy and light chain mRNA based on amplicon sequencing. Total RNA was used to create cDNA from each candidate production clone. Heavy and light chain amplicons were generated using specific primer sets for each transcript. The resulting PCR products are then fragmented and loaded onto the NGS instrument. For informatics, we are assisted by our computational biology group, which will assess the quality of reads generated by the sequencer, align them to our heavy and light chain reference, and then format results for evaluation. To determine the limit of detection (LOD), we created a point mutation in the heavy chain expression plasmid and performed a dosing study with various amounts of this mutated plasmid mixed with correct plasmid. After mixing, the exact same protocol developed for production clone analysis was employed in this dosing study. The results of this experiment confirmed that a mutation can be detected at various percentages down to 1-2%, which is the error-rate for most NGS experiments.

Results and Discussion:
Using the multiplexing capability of RNA-Seq, we are able to process and sequence-confirm heavy and light chain mRNA from 30-50 antibody producing clones per experiment. Previously, sequence verification did not occur until the production lead and backup clones were analyzed using low throughput mass spectrometry techniques. This early screening method has identified clones with synonymous and non-synonymous mutations at the clone screening stage in four programs to date. Antibody sequencing also offers us an advantage in workflows that aim to generate toxicology material from uncloned pools in place of isolated clones. With this method in place, we have confidence in the sequence integrity of uncloned pools moving forward in this process. In summary, we have successfully implemented a NGS application to verify production clones sequences, adding more quality to clones moving forward in our cell development workflow without losing time.
CHARACTERIZATION OF ALTERNATIVE PROMOTERS TO STAGGER AND CONTROL PROTEIN EXPRESSION IN THE BACULOVIRUS INSECT CELL SYSTEM

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Background and Novelty:
The ease of use and versatility of the Baculovirus Expression Vector System (BEVS) has made it one of the most widely used systems for recombinant protein production. This has led to considerable progress in improving the utility of the system, including more efficient methods of baculovirus production, better quantification methods, new vectors for the introduction of genes into baculovirus and an improved understanding of the dynamics of protein production within this system. Furthermore, single baculoviruses can be engineered for simultaneous expression from multiple genes guaranteeing expression of the different proteins within a single cell for production of products such as virus like particles (VLPs) that can only be formed by producing multiple proteins simultaneously. However, co-expression systems currently in use mainly make use of the very strong very late p10 and polyhedron (polh) promoters to drive expression of foreign genes, which does not provide much scope for tailoring gene product ratios within the cell.

Experimental Approach:
This work demonstrates the use of different Autographa california multicapsid nucleopolyhedrovirus (AcMNPV) promoters to control the timing and expression of proteins within a two protein system in BEVS. The first part of the work involves expressing two simple fluorescent proteins, a green fluorescent protein (eGFP) and a red fluorescent protein (DsRed2) under the control of different promoters to investigate whether the expression of one gene can affect the expression of the other, and if the fine control of gene expression can be achieved. A further extension of this work uses the same constructs with differing promoter control, except that in this case the two genes are influenza virus hemagglutinin (HA) and matrix (M1) proteins which have been genetically linked to eGFP and DsRed2 proteins respectively. This allows us to individually track the expression of each of the proteins, to track their localization and relative expression levels within the insect cell over time, and to count and determine relative levels of HA and M1 proteins within VLPs produced from each of the constructs.

Results and Discussion:
The results show that the expression levels of genes can be easily controlled using this strategy, and it is hoped that this work will serve as a template for the idea of making “designed” expression systems in which the timing and expression level of different proteins can be carefully modulated by a pre-determined genetic program. In addition, the effect of “competition” between genes for cell resources has been examined and shows that there exists only a limited pool of nutrients and protein production resources within the cell, from which only a limited amount of protein can be produced. Therefore, there can be a very real improvement in complex protein production if protein expression ratios are modified to eliminate non-essential production.
NOVEL APPROACHES OF INTRACELLULAR METABOLOMICS FOR QUANTIFYING COMPARTMENT-SPECIFIC METABOLIC LEVELS AND FOR MIRRORING ANTIBODY DEGRADATION IN CHO

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Metabolomics have evolved into a highly valuable tool for analyzing and qualifying cells such as mammalian producer cell lines in biopharmaceutical processes. The knowhow of intracellular metabolite patterns may serve as a pillar for evaluating cell lines and process strategies.
To exploit this potential further research studies at IBVT concentrated on so far not yet addressed fields of metabolomics application – namely
1. the compartment-specific analysis of intracellular metabolite levels and
2. the estimation of intracellular degradation rates focusing on monoclonal antibodies (mAB)
in CHO producer cell lines.

Ad (i): A novel protocol will be presented enabling the separation of intact mitochondrion from cytosol thus allowing selective metabolome analysis in the compartments. The application of the approach will be shown studying IgG1 producing CHO in lab-scale fed-batch cultures. Special emphasis will be given to the cellular energy management facing multiple limitations during the course of cultivation. Metabolite and nucleotide dynamics will be revealed showing significant differences between cytosolic and mitochondrial levels during the course of cultivations.

Ad (ii): The intracellular degradation of IgG1 is in the center of complementary studies. Applying 13C-labeling techniques the fate of the labeling signal is mirrored in intracellular peptide fragments of the mAB. Using straightforward modelling approaches intracellular degradation rates are quantitatively estimated showing that IgG1 is already degraded in exponentially growing (and producing) CHO.
STUDYING THE IMPACT OF ADENOVIRUS INFECTION ON CELL METABOLISM USING 13C-LABELLED GLUCOSE AND GLUTAMINE

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Background and Novelty:
Adenoviral vectors constitute powerful vehicles for gene transfer with potential clinical applications in vaccination and treatment of cancer or many monogenic and acquired diseases. Human adenovirus type 5 (Ad5) vectors have been mainly produced in HEK293 cell cultures, but new human cell lines have recently been used (such as the amniocyte-derived 1G3 cell line), avoiding the emergence of replication-competent adenoviruses associated with HEK293. Furthermore, alternative canine adenoviral vectors (such as CAV2), have been proposed to skip the pre-existing immunity against Ad5. To meet market demand, more efficient and economical production processes are required. Relevant knowledge on the mechanisms through which adenoviruses manipulate the metabolic network of the host cell to provide the energy and macromolecule components necessary for their replication and assembly, is still lacking. The main focus of this work was to study how adenoviruses reprogram cell metabolism to assist their replication in both human and canine infection systems.

Experimental Approach:
1G3 and MDCK cells were mock-infected or infected in exponential growth or growth arrest conditions. For each scenario, parallel labeling cultures were applied using two isotopic tracers, [1,2-13C]glucose and [U-13C]glutamine, at 4 hours post-infection. The 13C-labeling profiles of intra- and extracellular metabolites were measured by gas chromatography-mass spectrometry (GC-MS). A comprehensive analysis of the transport rates across the plasma membrane was also performed by 1H-NMR. The resulting data will be contextualized into a metabolic network model to provide an integrated global view of fluxes in each condition.

Results and Discussion:
For both human and canine cell lines, the labeling patterns produced in lactate and alanine (M+1 and M+2 isotomers) in cultures supplemented with [1,2-13C]glucose indicates glucose was mostly metabolized through the glycolytic pathway, with less than 5% being processed through the oxidative branch of the pentose phosphate pathway. Interestingly, intracellular serine became enriched with 13C coming from glucose despite being provided in the culture media, indicating its de novo synthesis. By turn, the intracellular fate of [U-13C]glutamine was significantly different in each cell line: carbon backbones of TCA cycle intermediates derived from glutamine were over 60% and 80% in MDCK and 1G3 cells, respectively. Furthermore, MDCK cells processed over 10% of the glutamine through the reductive carboxylation pathway as suggested by the M+5 citrate isotope, a feature not observed in 1G3 cells. For cultures performed with adenovirus infection, a general increase in the uptake rates of glucose and essential amino acids was observed, highlighting the demands for energy and building blocks required for viral replication. However, the labelling patterns described above were mostly maintained in these conditions. Further insights will be gained by integrating transport rates and 13C-labeling patterns in metabolic models allowing to map cellular fluxes conferring different adenovirus productivities. This work contributes to advancing biological knowledge on adenovirus-host cell interactions and should lead to improvements in infective adenoviral titers.

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SYNTHETIC PEPTIDE MATRICES AS SUPPORT FOR STEM CELLS CULTURE

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Background and Novelty:
In vivo, most cells are surrounded by an extracellular matrix (ECM), complex macromolecules network, which possesses many functions, e.g. cell adhesion, migration, metabolic regulation and structural support. More specifically, ECM-composing peptide sequences regulate many cell functions. In vitro, cell culture supports should ideally ensure the same roles. Initially, cell culture methods included animal-derived ECM proteins. However, these molecules present several bottlenecks, i.e. biosafety, for therapeutic applications. Synthetic self-assembling peptides networks are developed to mimic ECM physico-biochemical properties, to display bioactivity, biomimicry and biodegradability and to improve biosafety for clinical applications. This work aims at assessing synthetic self-assembling peptides adsorption on polymer surfaces and their capacity to interact with stem cells and induce their adhesion and growth. The selected peptides contain bioactive sequences derived from collagen, fibronectin and laminin, as molecular cues for cell recognition.

Experimental Approach:
Self-assembling peptides were synthesized and characterized analytically (HPLC, RMN) by Peptisynthia. Physico-chemical characterization of adsorbed peptides was carried out on polystyrene substrates, using XPS (surface chemical composition), AFM (surface topography), SEM (surface observation), ToF-SIMS (amino-acids surface occurrence) and HPLC on working coating solutions before and after coating. Coating efficiency depending on (i) polymer hydrophilization method, (ii) working solutions concentrations, (iii) initial salts concentrations, (iv) coatings ageing and (v) γ-irradiation, was studied by cell adsorption tests. Human Adipose tissue Derived Stem Cells (ADSC) were incubated in a serum-free medium for 6h and 24h. Media were then exchanged for serum containing medium and cells were cultured till 5 days. Cell adhesion efficiency was followed after different durations using microscopic observations. At 5 days, cells were fixed with 4% formaldehyde, nuclei stained with DAPI and cell densities estimated by fluorescence microscopy (NIS software analysis). Peptide coatings were compared with natural proteins.

Results and Discussion:
First of all, peptides coatings stability and coated surfaces topography were studied. XPS and ToF-SIMS analyses showed that peptides remained at the polymer surfaces after coating and washings. AFM and SEM observations evidenced the variable topography of resulting networks. HPLC dosage estimated peptides adsorption from working solutions and verified adsorption stability upon storage in physiological solutions. Moreover, the effect of different physico-chemical parameters on networks efficiency was studied. It was shown that peptides coatings promoted cell adhesion and growth. Peptides efficiency varied depending on bioactive sequences and polymer hydrophilization method. However, very small differences were globally observed upon varying studied parameters and in comparison with controls.
In conclusion, self-assembling peptides stably adsorb on hydrophilized polymer surfaces, are able to induce cell adhesion and growth and are interesting avenues for entirely synthetic culture systems, improving therefore the biosafety of culture conditions for clinical uses.

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ROBUST EXPANSION OF HUMAN PLURIPOTENT STEM CELLS: INTEGRATION OF BIOPROCESS DESIGN WITH TRANSCRIPTOMIC AND METABOLOMATIC CHARACTERIZATION

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Background and Novelty:
Human pluripotent stem cells (hPSC), including human embryonic stem cells (hESC) and induced pluripotent stem cells (iPSC), have an enormous potential as source for cell replacement therapies, tissue engineering and in vitro toxicology applications. The lack of standardized, robust and scalable protocols for cell production under well-defined conditions, compliant with good manufacturing practices has hindered the transfer of hPSC and their derivatives to biomedical and clinical settings [1]. In this work we developed protocols for the expansion of undifferentiated hESC using environmentally-controlled stirred-tank bioreactors and evaluated the impact of the culture strategy on cell phenotype using transcriptomic and metabolomic tools.

Experimental Approach:
This study was performed using two phenotypically different hESC lines with different growth characteristics (feeder-dependent and feeder-free lines) in order to evaluate their expansion on different xeno-free Synthemax II microcarriers in stirred-tank bioreactors. In all cultures, cell proliferation and viability were monitored daily and, after cell expansion, the undifferentiated phenotype and pluripotency were evaluated by immunofluorescence microscopy, flow cytometry, RT-qPCR and in vitro pluripotency assays. Moreover, the transcriptional and metabolic profiles of hESC cultured in stirred tank bioreactors and in 2-dimensional (2D) monolayer systems were followed during the expansion process and compared to better understand the biological changes induced by the culture system.

Results and Discussion:
Our results demonstrated that Synthemax II-polystyrene microcarriers were the most effective beads for the expansion of both hESC lines in stirred-tank bioreactors, enabling high fold increase in cell concentration and high viable cell recovery yields after harvesting from microcarriers. Importantly, cells maintained the expression of undifferentiated hESC markers (Oct-4, TRA-1-60, SSEA-4 and SSEA-5) throughout culture time and showed high and similar pluripotency scores (according to PluriTest bioinformatic platform) when compared to 2D cultures, confirming the effectiveness of established bioprocess in maintaining the hESC pluripotency. Genome-wide transcriptome profiling revealed a transcriptional phenotype convergence between both hESC lines along the expansion in microcarrier-based stirred culture systems, providing strong evidence on the robustness of the cultivation bioprocess to homogenize cellular phenotype. The most significant changes steering this convergence, were found at the level of central carbon metabolism and cytoskeleton and extracellular matrix rearrangements. Under low oxygen tensions, results showed a metabolic shift with the up-regulation of the glycolytic machinery favoring an anaerobic glycolysis Warburg-effect like phenotype, with no evidence of hypoxic stress response, in contrast to 2D culture [2].

This study offers valuable insights on the metabolic hallmarks of hPSC expansion under fully defined conditions, which can help to guide process design and media optimization towards improved cell quality and higher cell quantities.

References:
COMPREHENSIVE VIEW OF CHO CELLS METABOLISM USING KEY $^{13}\text{C}$-SUBSTRATES

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Background and Novelty:
Optimization of culture conditions through trial-and-error has been responsible for much of the past progress on animal cell culture. However, a renewed account of the underlying biologies through a “systems” view should provide a powerful vehicle not only to a deeper knowledge of cellular physiology but also to reengineer desirable states of increased culture performance.

Experimental Approach:
In this work, we took a comprehensive view of cellular metabolism by extensive profiling extracellular metabolite concentrations and intracellular flux distributions after $^{13}\text{C}$-label supplementation in parallel labelling cultures using [1,2-$^{13}\text{C}$]Glucose, [U-$^{13}\text{C}$,$^{15}\text{N}$]Asn, [U-$^{13}\text{C}$,$^{15}\text{N}$]Ser and [1-$^{13}\text{C}$]Pyruvate as isotopic tracers. This integrative approach was used to trace metabolic rearrangements in different scenarios of asparagine and serine availability in glutamine synthetase (GS)-CHO cells.

Results and Discussion:
The absence of asparagine in the medium caused growth arrest, and was associated with a dramatic increase in pyruvate uptake, a higher ratio of pyruvate carboxylation to dehydrogenation and an inability for de novo asparagine synthesis. The release of ammonia and amino acids such as aspartate, glutamate and alanine were deeply impacted. This confirms asparagine to be essential for these GS-CHO cells as the main source of intracellular nitrogen as well as having an important anaplerotic role in TCA cycle activity. In turn, serine unavailability also negatively affected culture growth while triggering its de novo synthesis, confirmed by label incorporation coming from pyruvate, and reduced glycine and formate secretion congruent with its role as a precursor in the metabolism of one-carbon units. The results obtained suggest that feeding schemes of asparagine or serine should be tightly tuned to minimize by-product formation while assuring biosynthetic needs. Ongoing is the contextualization of the data from the parallel labelling cultures into a metabolic network model to provide an integrated global view of fluxes in each condition, allowing to map cellular fluxomes conferring different productivities. Overall, this work contributes to unfold important insights into GS-CHO cells metabolism, and can be used as a basis for exploring bioprocess optimization strategies.

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GENERATING HIGH ISOTOPIC SUBSTITUTION OF $^{13}$C AND $^{15}$N SPECIFICALLY LABELED AMINO ACIDS IN THERAPEUTIC TARGET PROTEINS USING BIIC BACULOVIRUS

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Background and Novelty:
Recent advances in isotopic labeling strategies have unleashed a broad range of valuable NMR spectroscopy tools to enable the elucidation of molecular dynamic and structural properties of many therapeutic protein targets. The use of BIIC (Baculovirus Infected Insect Cells) method as an emerging technology for amino acid specific labeling provides consistently high isotopic substitution (>90%) using $^{13}$C and $^{15}$N labeled amino acids. The synergistic combination of BIIC technology and NMR is transforming NMR’s impact in structural biology across a wide range of protein targets.

High levels of isotopic amino acid specific incorporation afforded by the BIIC method provides the substrate upon which multiple investigative structural analyses can be elucidated. These include DFG -in/out determination for inhibitor design strategy, allosteric binding site determination and characterization, NMR fragment based screening, along with mechanistic and molecular profiling of compound binding and ligand interactions for compound selectivity and lead generation.

Experimental Approach:
Here we outline a novel amino acid specific labeling strategy featuring the BIIC method for the production of soluble recombinant target proteins using the baculovirus expression insect cell system. The stepwise protocol describes methods for BIIC preparation, infection kinetics, harvest time optimization, amino acid depletion and isotopic label addition. As a test case, we will apply the BIIC isotopic labeling method to express a seven-helix transmembrane receptor GPCR, outlining specific steps:

* The BIIC Infection and 40 hr Growth Period.
* The Depletion Step.
* The Isotopic Labeling Step.

Results and Discussion:
As transmembrane proteins, GPCRs can be difficult to express. Isotopically labeling one and expressing it in significant quantities to be purified and characterized by LC-MS for NMR presents a major challenge. The many advantages of BIIC technology and the identification of critical factors for isotopic protein labeling success are highlighted.

* The BIIC Labeling Method Enables High Isotopic Substitution of Specifically Labeled Amino Acids across Multiple Platforms.
* The advantages of using BIIC technology for isotopic labeling are manifold:
  - BIIC - a stable source of virus stock over time, as BIIC are stored in liquid nitrogen. Eliminates need for virus amplification and titering.
  - Greatly improves expression consistency and reproducibility batch after batch. Significantly reduces turn around time and resources.
  - Simple BIIC preparation method.
* Identifying “Critical Factors” insures isotopic labeling success:
  - Use of BIIC for Infection.
  - 40 hr growth and infection period.
  - 4 hr depletion step.
  - 200ml/L label addition.
  - Optimized harvest time.
* High amino acid specific isotopic substitution enables multiple NMR structural analyses across the drug discovery portfolio.
DEVELOPMENT AND APPLICATION OF GLYCOENZYMES FOR IN VITRO GLYCOENGINEERING – USEFUL TOOLS FOR BIOPHARMA INDUSTRY

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Background and Novelty:
Investigation of glycan structure – function relationship of therapeutic proteins becomes more and more of interest. Introducing changes to the glycan profile of proteins, e.g. monoclonal antibodies, by cell line engineering or through the bioprocess is limited and time consuming. In this talk the in vitro glycoengineering technology will be introduced, a technology which has not been applied widely in the past since the raw materials, glycoenzymes and activated sugars, have not been reliably available.

Experimental Approach:
Development and analytical characterization of glycosyltransferases will be addressed as well as their application to investigate glycan structure – function relationship of IgGs. Sample preparation workflow, analytical methods and results for in vitro sialylation and galactosylation of IgG1 Fc glycans and its effect on the Mode-of-Action and pharmacokinetic properties will be addressed by different case studies.

Results and Discussion:
The developed glycoenzymes were found to be highly active and target specific. Glycosylation and disulfide bridges of the enzymes were identified.
In structure-function analysis of IgG1s, we found that galactose contributed positively to FcyRIIIa binding and ADCC activity, whereas sialic acid did not have an impact. The technology can be applied to increase specific molecule knowledge as well as to optimize molecular properties for clinical candidates.
INCORPORATING PRIMARY HUMAN RENAL PROXIMAL TUBULE CELLS INTO A HOLLOW FIBRE BIOREACTOR IN THE DEVELOPMENT OF AN *IN VITRO* MODEL FOR PHARMACEUTICAL RESEARCH

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**Background and Novelty:**
Current *in vitro* cellular methods utilised in drug metabolism and pharmacokinetic (DMPK) studies during drug development do not provide the 3D structure and functions of organs found *in vivo*, such that resulting *in vitro-in vivo* extrapolation (IVIVE) may not always accurately reflect clinical outcome. This highlights the potential need for the development of new dynamic *in vitro* cell models to aid improvement of IVIVE. The aim of the project is to incorporate characterised primary renal cells within a hollow fibre bioreactor for use in DMPK studies investigating renal clearance.

**Experimental Approach:**
Fluorescent cell based assays were developed in cell lines overexpressing P-glycoprotein (P-gp), Breast cancer resistance protein (BCRP) (efflux) and Organic cation transporter 2 (OCT2) (uptake) to detect transport functionality. The fluorescent substrates and inhibitors used were: Calcein-AM in the presence of Verapamil and Ketoconazole (P-gp), Hoescht 33342 in the presence of Novobiocin and Ko143 (BCRP) and ASP in the presence and absence of Ipratropium and Imipramine (OCT2). These assays were applied to primary human renal proximal tubule cells (RPTECs) to assess the functionality of transporters seeded at increasing population doublings. The expression of P-gp, BCRP, OCT2, Multidrug and toxin extrusion protein 1 (MATE1) at these doublings was also assessed via PCR and immunostaining (with the addition of Multidrug resistance-associated protein 2 (MRP2) and Organic anion transporter 3 (OAT3)).
4 polysulfone (PSF) based porous membranes ((PSF, PSF + Polvinyllpyrrolidone (PVP), PSF + Malonic acid (MA), PSF + Glutaric acid (GA)) were developed for renal cell attachment and characterised physically and in terms of short term cell attachment before producing hollow fibres from the best performing blend.
Hollow fibres were assembled into single fibre bioreactors and cultured with RPTECs to assess cell performance under flow conditions.

**Results and Discussion:**
The fluorescent assays were developed successfully in the cell lines employed, showing an increase in fluorescence in the presence of inhibitors for the efflux transporters and a decrease in the uptake transporter. When applied to RPTECs P-gp functionality initially decreased before increasing significantly although gene expression and visualisation was shown to decrease through the doublings. BCRP functionality and visualisation also decreased over time and all transporters visually decreased in expression except OAT3. OCT2 functionality could not be assessed in RPTECs due to the loss of cells through the assay. The membranes produced showed similar structures through all 4 blends, with a skin layer formed on the outer surfaces and large finger-like macrovoids in the centre with smaller pores throughout. Surface chemistries concurred with the predicted formation for PSF+PVP but showed no discernable acid binding for PSF + MA and PSF + GA and hydrophilicity remained constant across all 4 blends. Short term cell attachment was similar across all the membranes with good viability demonstrated (75-85%). Hollow fibre production and single fibre bioreactors have been successfully assembled, sterilised and utilised for cell culture. Overall this work will provide proof of concept data to develop this device into a valuable *in vitro* 3D model of the proximal tubule for pharmacological applications.
HIGH-TITER MONOCLONAL ANTIBODY PRODUCTION IN A FED-BATCH PROCESS USING SINGLE-USE STIRRED-TANK AND ROCKING BIOREACTOR SYSTEMS

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Background and Novelty:
Today, single-use technology is used in many different upstream applications and disposable bioreactors are available in volumes up to 2000 L. Two of the most commonly used bioreactor formats are based on stirred-tank and rocking technology for mixing and aeration. In this presentation, we show the feasibility of using the single-use ReadyToProcess WAVE™ 25 rocking bioreactor system in seed train culturing for a monoclonal antibody (MAb) production in a fed-batch process using the single-use Xcellerex™ XDR-200 stirred-tank bioreactor system. The XDR-200 process was also scaled down to a ReadyToProcess WAVE 25 satellite bioreactor to study process performance in parallel cultures in the two bioreactor formats.

Experimental Approach:
Cell expansion was performed in a 15 L culture using the ReadyToProcess WAVE 25 system to seed a 200 L culture in the XDR-200 stirred-tank bioreactor. From the 200 L stirred-tank culture, 7 L was transferred to a satellite ReadyToProcess WAVE 25 system. Cell growth, IgG production, metabolite profile, osmolality, and partial CO2 pressure were monitored daily. Key product quality attributes such as protein charge heterogeneity, relative aggregation, and glycosylation were analyzed post harvest.

Results and Discussions:
The process was shown to be scalable between the rocking and stirred-tank bioreactor systems. Comparable cell growth and productivity were observed in the two bioreactor formats. Also, the metabolite profile and product quality were similar between the bioreactor cultures. Although having a different vessel geometry, the ReadyToProcess WAVE 25 gave a representative reflection of the process at larger scale using the XDR-200 system. Our results show that single-use bioreactors with a design based on different technologies can provide a similar output if operated under comparable conditions.
A SIGNIFICANT INCREASE OF PRODUCTIVITY OF CHO CELLS EXPRESSING HYFc-FUSION PROTEIN BY THE APPLICATION OF NEW CD MEDIA AND SUPPLEMENT

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Background and Novelty:
One of major challenges for the development of biopharmaceutical drugs is the high production cost due to a relatively low productivity of recombinant proteins when using conventional media. In order to get much better productivity compared to other commercial media, we have applied New chemically-defined ASF CHO medium (ASF CHO CDM) and chemically-defined feed medium (ASF Feed) to hyFc-fusion proteins. These media were designed by Ajinomoto’s medium optimization strategy including the elaborate DOE screening to control both chemical and metabolic interactions among each medium component and such interactions contributed to achieve high performance and medium stability. As a consequence, these media worked very well for major CHO cell lines such as DG44, K1, and DXB11.

Experimental Approach:
Two CHO cell lines expressing hyFc-hGH, a recombinant human growth hormone fused to hyFc (hyFc) and hyFc-G-CSF, a recombinant G-CSF fused to hyFc respectively were tested in this study. The hyFc (hybrid Fc) consisting of a portion of the human immunoglobulins (Ig) D and IgG4 was designed to allow a target protein to provide a longer half-life in the human body. The IgD portion allows for the hinge region to be relatively more flexible and the half-life is more increased by the IgG4 moiety. The overall performance of CHO cell expressing the hyFc fusion proteins such as viable cell density, viability, productivity, and purity in ASF CHO CDM was evaluated and compared with that in the commercial media in which CHO cells were cultivated in a scale of 15L or 200L bioreactor with a Fed-batch mode. The cells were seeded with 5 x10^6 cells/ml and feeds were periodically added 3–5 days after the inoculation. The viable cell density and viability were measured by an automatic cell counter. Glucose and other metabolites were detected by a biochemistry analyzer. The productivity and purity were calculated using a HPLC.

Results and Discussion:
Compared to established culture conditions using commercially available media and feed, the productivity when using ASF CHO CDM and ASF feed were increased more than 2 and 4 fold and maximum cell density were increased 1.6 and 2.3 fold for hyFc-hGH and hyFc-G-CSF respectively while purity and cell viability were similarly maintained within the acceptance criteria.
In conclusion, when applying ASF CDM and ASF Feed the productivity and viable cell density were highly increased compared to those when using well-known commercial media.
A NEW AUTOMATED CELL COUNTER FOR MAMMALIAN CELL CULTURE ASSESSMENT

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Background and Novelty:
Performing accurate sample analyses during the culture of mammalian cells is vital in order to track the number and viability of cells present. This allows operators to recognise when reagents may need to be added to maximise yield, harvest cells appropriately and to identify when a targeted cell concentration has been reached. This is commonly performed via manual cell counts. Cell stains, such as Trypan blue are classically utilised for ‘dye-exclusion’ assessment of viability, whereby dead cells are stained the colour of the dye and live cells remain colourless. However, this method is accompanied by many potential sources of human error and subjectivity as well as being a highly time consuming, labour-intensive mode of sample analysis. Various technologies introduced to the market in recent years automate this process in order to remove some human error and subjectivity associated with manual cell counts. However, the industry has not fully accepted these technologies, as the dye exclusion method is still the familiar and standard mode for assessing viability.

Experimental Approach:
This paper focuses on a new automated cell counter, the Countstar®, which uses brightfield image analysis and the trypan blue dye-exclusion method. This has the advantage of automating the process to remove human error and subjectivity, while retaining the standard trypan blue dye-exclusion method. It uses disposable five chamber slides, and associated software to analyse 20 µl samples. Like many of the existing automated call counters it provides the live cell count, the % viability, average cell size and a stored image of the cells. It also provides an aggregate and cell size histogram and a circularity index. In this poster an in depth assessment is made on comparing the manual counts and viability performed with the haemocytometer with those obtained from the Countstar.

Results and Discussion:
The following cells lines were investigated during the study: CHO, MCF7, HEPG2, MDCK and VERO. Excellent correlation coefficients were obtained for all cell lines with the cell count was as high as 0.9967 and for the viability as high as 0.9937. The study also showed that the disposable slides with five chambers enabled the overall time for analysis time to be significantly lower than conventional automated cell counting devices found in cell culture.
CHO-DHFR CELL LINE DEVELOPMENT PLATFORM: APPLICATION OF CLONEPIX
AND AMBR TECHNOLOGIES TO MEET ACCELERATED TIMELINES

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Background:
New and emerging technologies like Clonepix and Automated Mini Bioreactors (AMBR) have become an
integral part of stable cell line generation platform, however, the methodologies on how and when they are
applied vary widely among users. In this study, we describe methods used for successful development of a
robust cell line development platform resulting in high-yielding stable cell lines using in-house VRC-DG44-
CHO cells and expression vectors.

Experimental Approach:
The study was focused on a) evaluating early incorporation of Clonepix so as to maintain maximal transfectants
pool heterogeneity, b) identify Clonepix fluorescence attributes that increase the probability of isolating high
producing clones c) evaluating and optimizing operating conditions in AMBR to achieve process attributes
similar to large scale bioreactors and apply them for early clone screening.

Results and Discussion:
Our data indicate that Clonepix attributes such as External Fluorescent area (EFA), White light area (WLA) and
normalized EFA to WLA are predictive of productivity at 96 well stage and beyond. Early AMBR optimization
studies indicate that down draft agitation can maintain the dissolved oxygen (DO) concentration at higher cell
density than updraft. Using downdraft to be able to maintain DO in the range of 50± 5% resulted in clones
with improved titers that are comparable to 3L bioreactors. Overall, our first generation integrated platform
which includes pre-adapting host cell line to production medium prior to transfection, introducing ClonePix
technology very early in the process flow (48 hour post-transfection) and screening clones in AMBR for down-
selection of top 3 to 5 clones to 3 L bioreactor evaluation enabled us to generate stable clones with high
productivities (2.5 to 3 g/L) in a 14 day fed batch process at AMBR stage. The integrated approach reduced
the CLD timeline to 5 months from vector generation to final clone selection. Work is in progress to further
optimize the platform by enhancing transfection efficiency, selecting a better culture medium for cloning,
impovring operating conditions and feeding strategies for AMBR and fine-tuning sub cloning and DHFR
amplification methodologies to further improve productivity (>5 g/L) at 3 L bioreactor-scale.
MALDI-TOF MASS SPECTROMETRY BIOTYPING - AT-LINE MONITORING OF RECOMBINANT CHO CELL LINES

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Background and Novelty: 
The cultivation of mammalian cells, especially Chinese Hamster Ovary (CHO), has found widespread acceptance for the production of biopharmaceuticals. Process monitoring - enforced by the PAT initiative of the FDA - underwent major changes from examining the bioreactor/culture as a “black-box” towards integrated methods, helping to gain deeper process understanding by assessing the cells themselves. Here, we present the novel application of Intact Cell MALDI-TOF Mass Spectrometry (ICM MS) biotyping, a method originally used in clinical and environmental microbiology that only recently found application in the context of animal cells, in order to detect early signs of cellular stress and apoptosis.

Experimental Approach: 
Previously, we presented first results of CHO batch cultures assessed by ICM MS in to monitor cell stress and early apoptosis [1, 2]. Now, we broadened its application and addressed additional CHO cell lines (DXB11 and CHOK1), different cultivation-formats (uncontrolled Erlenmeyer flasks vs. lab scale bioreactors) and operation modes (Batch vs. Perfusion). Furthermore, we transferred the method to a compact, “low-priced” mass spectrometer (MicroFlex, Bruker Daltonik). The experimental aim was to establish characteristic MS patterns indicative for different apoptotic states with emphasis on detection of early apoptosis ubiquitously applicable independent of CHO-subline, cultivation-format, -mode or instrumental equipment (MS).

Results and Discussion: 
We report the successful scalability (Erlenmeyer flask vs. lab-scale bioreactor) and operation mode independent applicability of ICM MS. Reproducible stress related changes in m/z signal intensities were identified that allowed the prediction of upcoming cell viability changes up to 24 h earlier than standard monitoring methods. Furthermore, this approach was successfully applied to in total four CHO cell lines; three suspension adapted CHOK1 and one DXB11 cell line. Based on condensed cell line specific subsets of m/z values (39 – 55 m/z values, depending on individual cell lines) or even based on a “CHO-general” subset of 9 m/z values, we built classification models allowing for a reliable discrimination of unknown samples regarding their state (viable, early and late apoptotic). The method was successfully transferred from a high-performance mass spectrometer (AutoFlex III, Bruker Daltonik) to a more compact and lower-priced spectrometer (MicroFlex; Bruker Daltonik). This adaptability of the method might be of impact for potential users in the field. Although we focussed on apoptosis induction, in the future it might be possible to expand the method towards detection of other pattern associated cellular states. The fast, robust and automated acquisition of cell state specific MS signatures together with simple, label free sample preparation could become a promising tool for at-line CHO culture monitoring.
UTILITY OF HYBRID PERFUSION/FED-BATCH OPERATIONAL MODE TO ESCAPE THE LIMITATIONS OF CONVENTIONAL FED-BATCH CULTURE

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Background and Novelty:
Peak cell densities in fed-batch cell culture are primarily constrained by two factors, availability of sufficient nutrients, and rate of accumulation of cell-growth inhibiting cellular byproducts. It has generally been a straightforward matter to supply cells with the necessary nutrients for growth and high productivity, but inhibitors quickly accumulate and limit cell growth both due to high osmolyte accumulation and also potent cell-cycle inhibitory effects. Continuous perfusion simultaneously solves the issue of nutrient delivery and byproduct removal, but adds significant complexity and cost to operations. The volumes of medium required are often tenfold what might be used in a similar productivity fed batch and since cell viabilities are often lower, methods to retain cells can prove problematic at large scale.

Building upon earlier work [1] [2] and new insights into cell growth inhibitors [3] our recent work has combined a short duration of continuous perfusion with a carefully timed transition to fed-batch culture allowing us to achieve significantly higher cell densities than a fed-batch, without the operational complexities of continuous long duration perfusion.

Experimental Approach:
Experiments were performed using CHO cell lines producing an IgG molecule. 1-3 liter working volume bioreactors were fitted with external hollow fiber microfiltration units and perfused for four to five days at increasing rates as the cell density of the cultures reached about 40x106 cells/ml. Bioreactors were then placed in fed-batch operational mode and fed a highly concentrated nutrient medium solution for another six to eight days.

Results and Discussion:
Short duration perfusion has been combined with a standard fed-batch operating paradigm leading to dramatic increases in cell density and productivity. Because the perfusion effectively flushes out cell-growth inhibitors at the near optimal time point, cell division continues longer, leading to peak cell densities approximately twice those achieved in a standard fed-batch. The batch can still be completed in a standard fed-batch timeslot and provide more than a doubling in final titers when compared to an optimized fed-batch. We will present data showing peak cell densities as high as 80x106 cells/ml with titers at nearly 10 grams/liter antibody achieved in a 12-day fed-batch operating window using as little as three reactor volumes of perfusion medium.


MANUFACTURING ULTRA-CONCENTRATED LIQUID FEEDS: 
TRANSITIONING THE AQUEOUS SOLUBILITY BARRIER OF THE FEED AMINO 
ACIDS CYSTEINE AND TYROSINE

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Background and Novelty:
The volume in a bioreactor is limited and feeds must be added to continuously supply cells with essential nutrients. The down side is that feeds add significant volume since e.g. the amino acids tyrosine and cysteine are poorly water soluble. Over several days large volumes of water are unavoidably added resulting in dilution of target product concentration and physically filling up the bioreactor to max. volume. Currently the only alternative is to start with smaller working volumes thereby significantly reducing total product yield per batch and the process economics.

Experimental Procedure:
It is general practice to increase the solubility of tyrosine and cysteine by decreasing or increasing the pH of the solution. The increase in solubility is enormous but nevertheless still limited and significant volumes of acid/alkali need to be added for neutralization purposes adding to volume. Addition of acids and alkali have the further disadvantage of being aggressive/caustic and cause cell lysis / death with release of proteins which can cause foaming problems.

Results and Discussion:
Ideally, these amino acids should be added in a pure state as well as in liquid form due to handling conveniences. Use of solvents to increase solubility is of limited advantage since they are not desirable additives. The presentation covers alternative novel methods for the preparation of liquid amino acid solutions.
HIGH-PURIFIED SERICIN AS MEDIUM SUPPLEMENT FOR MAMMALIAN CELL CULTURE

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Background and Novelty:
Although mammalian cells are extensively cultured in the presence of mammal-derived factors such as FBS and BSA, mammal-derived factors should not be used in cell culture for regenerative medicine because they are expensive and have the concern about the risk of contamination with viruses, abnormal prions and so on. Therefore, alternatives are strongly required and we have studied silk protein sericin hydrolysates as culture supplement. Previously, we reported that sericin hydrolysates promoted the proliferation of several cell lines. And its mitogenic effect was enhanced by excluding materials with low molecular weight (< 10 kDa), and named high-purified sericin.

In this study, we aimed to improve the culture of human induced pluripotent stem cells (hiPSCs), which could be expanded limitless and developed into target cells. We also tried to reveal the mechanism how sericin hydrolysates improve the proliferation of cells and inhibitor assay were performed.

Experimental Approach:
With high-purified sericin, we tested to replace conventional additives, Knockout Serum Replacement (KSR) and Fibroblast Growth Factor 2 (FGF2). 20187 and 253G1 hiPS cell lines were cultured with sericin (Wako, Japan), FGF2 and KSR (Invitrogen, USA), and evaluated by staining alkaline phosphatase.
In order to reveal the signaling from sericin, cells were cultured for several days in the presence of sericin hydrolysates and inhibitors such as U0126 against MEK. Cell density was measured by counting using the trypan blue dye exclusion method.

Results and Discussion:
Replacement of KSR. High-purified sericin improved the proliferation of hiPSCs cells in the absent of KSR. But its optimal effect was as much as 2.5% KSR, suggesting its effect was not enough because the standard concentration of KSR was 20% and the effect of sericin was quite lower than 20% KSR. Sericin might replace only a small part of KSR constituents.
Replacement of FGF2. High-purified sericin improved the proliferation of hiPSCs in the absent of FGF2. Without FGF2, high-purified sericin prolonged the culture period. After 7 passages (36 days), the hiPS cells with sericin had higher alkaline phosphatase activity than those with FGF2.
Sericin improved the proliferation of hiPSCs in the absence of FGF2 or KSR, suggesting that sericin could be used as additive in hiPSCs culture.
UTILIZING FLUORESCENCE STAINING METHOD CAN MAKE HIGH ANTIBODY-PRODUCING CHO CELLS SELECTED MORE EFFECTIVELY

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**Background and Novelty:**
It is important to select high antibody-producing CHO cells for manufacturing of biopharmaceuticals. Since there are many laborious steps for selection of high antibody-producing cells, improvement of procedure is demanded. For example, it takes too much time and labor to observe the colony formation of cells with microscopes after a gene of interest is integrated into host cells. In addition, high producer could only be selected among a lot of low or even non producer after measuring the titers of all the colonies by assays with binding affinity. This study shows that utilizing our fluorescence staining method can make high antibody-producing CHO cells selected more effectively compared to conventional methods.

**Experimental Approach:**
To investigate feasibility of this method, a plasmid including a gene of an antibody was integrated into host cells, the cells were seeded into 384-well plates with a liquid selection medium including FITC-labeled anti-IgG antibody, and fluorescence signals were measured with a fluorescence detector 7 days later. Then, the titers were measured by assays with binding affinity, and the correlation between the titer and fluorescence signal was examined.

Also, to confirm that high antibody-producing cells can be selected with this method as expected, it was investigated whether or not detected signal levels were different between high and low producer which had already been established.

**Results and Discussion:**
When this method was used, it took shorter time of less than one-sixth and less labor of less than half to observe the colony formation of cells after a gene of interest was integrated into host cells compared to conventional method. Also, it was shown that most cells with high signals gave higher titer compared to other cells and many low or no antibody-producing cells could be excluded by this method, which would reduce the number of cells for titer measurement.

Furthermore, it was confirmed by using two types of cells with different antibody producing levels that high antibody-producing cells gave high signal level whereas low producer gave low signal level.

These results showed the difference of titer of antibody-producing cells could be distinguished with the difference of signal levels.
INCREASED RELIABILITY OF CELL COUNTING SYSTEMS 
BY USING COATED SAMPLE CUPS

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Background and Novelty:  
Adsorption of cells to single use cups during cell count measurement is a widely known problem that results in measurement deviations in all kind of cell counting systems that use sample cups. When cell counting systems are applied for process control in GMP, reliable results could be crucial and selfmade workarounds such as BSA coating of the cups are not applicable. Therefore, ultra low binding cups were developed for avoidance of cell adsorption cooperatively with a company with expertise in covalent coating of single use materials.

Experimental Approach:  
Several Cell lines and cell culture media were tested in order to identify a worst-case combination with regard to cell adsorption to the sample cup surface. A system where up to 75% adsorption was observed was used for evaluation of the ability of coated sample cups to avoid this adsorption.

Results and Discussion:  
Ultra low binding cups were confirmed to successfully avoid adsorption of cells to sample cups and therefore ensure measurement reliability. In addition, a quality control of the coating was implemented in order to assure integrity of the coating. Albeit this strongly increases expenses for this single use material, the use of coated cups is recommended for cell culture systems with a high ability for adsorption. The risk of erroneous measurements in a GMP or scientific environment and potential efforts for selfmade workarounds equal out the increase of expenses.

PROCESS SCALE-UP BASED ON CONSTANT TIP SPEED FOR SHEAR SENSITIVE CHO CELLS

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Background and Novelty:  
The development of a CHO fermentation process for commercialization requires several manufacturing scale up steps during the course of development as the project moves through the clinical phases with ever increasing material demand.  
A commonly used and usually quite successful approach is to keep the power input constant across all scales. However, this approach is not necessarily applicable for shear sensitive cells. The shear stress is greatest near the impeller tip and the tip speed increases with scale when keeping the power input constant.

Experimental Approach:  
In this case study we demonstrate that a constant tip speed as scale up criterion delivers better and more consistent performance across scales. Afterwards, optimization of the shear protectant type and concentration lead to an improved and robust process performance.

Results and Discussion:  
Ultimately, the product titer and the cell growth could be increased by 40% and maintained across scales between 1L and 1,000L by implementing constant tip speed as scale-up parameter and Pluronic F-68 as shear protectant.
SPLIT-GFP ENABLING A FAST AND RELIABLE EXPRESSION SCREENING IN EUKARYOTIC HOSTS

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Background and Novelty:
Protein expression frequently presents a bottleneck in structural biology. Especially for difficult-to-express proteins numerous constructs have to be generated. Often prokaryotic expression systems fail for these proteins and other expression hosts need to be engaged. Thus eukaryotic alternatives like insect cells with their high productivity but also simple and homogenous glycosylation pattern can be employed. However testing multiple constructs for expressibility in the Baculovirus Expression Vector System (BEVS), is time, labor and cost intense since recombinant baculovirus needs to be generated and amplified. Therefore, miniaturization in conjunction with the fast plasmid based transient transfection method would be a vast improvement for initial screening in insect cells.
To enable such a screening system it was required to compensate the rather weak viral independent plasmid based expression with the very sensitive Split-GFP detection system. We established the Micro-Split-GFP system (originally described by Cabantous and Waldo in 2006 in E.Coli) for high throughput insect cell expression screening.

Experimental Approach:
In our screening system the viral independent OpIE2 promoter drives the expression of the target gene which either N- or C-terminally carries a GFP11 beta strand peptide. Since this GFP11 peptide merely consists of 15 amino acids it does not interfere significantly with the target protein solubility. The target constructs fused to GFP11 were individually coexpressed with the truncated and inactive GFP1-10 in High 5 insect cells. If the target protein is successfully expressed a fully functional stable GFP is autonomously assembled and green fluorescence can be detected. To achieve a miniaturized high throughput system with a direct online readout, the BioLector Microfermentation system (M2P Labs) was applied, which allowed simultaneous comparison of up to 48 individual cultures.

Results and Discussion:
Our Micro-Split-GFP screening system was able to show successfully differences in soluble expression of several constructs of various proteins. Eventually the GFP quantities of our Micro-Split-GFP screening system were verified to strongly correlate to the expression yields in the baculoviral production system. Consequently our Micro-Split-GFP system presents a very sensitive and reliable novel method to show expression of constructs in insect cells based on the very fast transient plasmid transfection.
CHEMICALLY MODIFIED CYSTEINE IN FED-BATCH PROCESSES AND IMPACT ON CHO SPECIFIC PRODUCTIVITY

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Background and Novelty:
Industrial fed-batch cultivation of mammalian cells is used for the production of therapeutic proteins such as monoclonal antibodies. Beside medium ensuring initial growth, feeding is necessary to improve growth, viability and antibody production. Established commercial systems include a slight acidic concentrated main feed and a separate alkaline amino acid feed containing L-tyrosine and L-cysteine. Since L-cysteine is not stable due to its dimerization to cystine in the presence of air and metal catalysts, a stable L-cysteine derivative is needed to include all amino acids in neutral pH feeds. Those single feed systems are favored to simplify feeding schemes and improve the overall process robustness through stabilization of both pH and DO signals. Here, we suggest the use of a chemically modified cysteine in combination with phosphotyrosine disodium salt in an industrial single feed fed-batch process applicable at small scale as well as in bioreactors.

Experimental Approaches:
Cell culture experiments were carried out either in spin tubes or bioreactors with a CHO suspension cell line expressing a human monoclonal antibody. Viable cell density and viability were measured using an automatic cell counting device. Spent media analysis of supernatants was carried out for amino acids after pre-column derivatization and UPLC analysis and for vitamins using LC-MS/MS. Metabolite measurements were performed with Cedex Bio HT relying on photometric and turbidometric methods. Characterization of the monoclonal antibody was performed using 2-AB labeling for glycan analyses, cIEF for charge variants analyses and LC-MS/MS for peptide mapping experiments.

Results and Discussion:
Stability studies of the feed containing the modified cysteine derivative showed that the molecule was stable and that no L-cysteine or L-cystine was released over three months when stored at room temperature or 4°C. Moreover, no change in the color of the feed was observed over time. Small scale batch experiments where L-cysteine was replaced by the same amount of chemically modified cysteine indicated no change in growth or viability profiles. Use of the modified cysteine derivative in small scale fed-batch processes indicated comparable maximum viable cell density, prolonged viability and increased titer compared to the established two feed system. Bioreactor experiments confirmed the increase in specific productivity described at small scale when the single feed strategy was compared to the two feed strategy. In depth characterization of the monoclonal antibody indicated no change in the glycosylation, or charge variant pattern whereas peptide mapping experiments were not able to detect any integration of the modified amino acid in the sequence of the monoclonal antibody.
IDENTIFICATION OF CHEMICALLY DEFINED DRY POWDER CELL CULTURE MEDIA AND FEEDS WITH RAMAN SPECTROSCOPY: SENSITIVITY LIMITATIONS AND POLYMORPHISM

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Background and Novelty:
Identification of cell culture media samples by traditional LC methods, such as amino acid or vitamin analysis, has high costs, and requires significant analytical expertise and laboratory space. Raman spectroscopy offers many potential benefits, such as low cost, portability, and potentially limited skill required to operate the instruments.

Experimental Approach:
A comprehensive analysis of raw material spectral contributions to dry powder media spectra was performed in order to evaluate the capabilities of Raman spectroscopy for dry powder media identification and raw material quantitation. Spectra of pure solid raw materials were obtained and used for classical least squares (CLS) analysis of chemically defined dry powder media spectra.

Results and Discussion:
The analysis reveals that sensitivity to low mass percent raw materials and raw material polymorphism limits the capability of Raman spectroscopy for identification of complex chemically defined dry powder media. Using the predictive power of the CLS model, simple feeds where Raman spectroscopy was predicted to be an adequate quantitation and identification analysis tool were selected. Data supporting the application of a CLS model, and recommended feeds for application of Raman spectroscopy will be discussed.
A SIMPLIFIED IMPLEMENTATION OF THE O.U.R. STATIONARY LIQUID MASS
BALANCE ESTIMATION METHOD FOR ON-LINE MONITORING
IN ANIMAL CELL PRODUCTION PROCESSES

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Background and Novelty:
Many efforts have been invested in the development of culture strategies for animal cell culture process. Several
strategies have been studied: batch, fed-batch and perfusion cultures. For implementation of those strategies,
a monitoring system for automated, controlled and optimised processes based on simple measurement could
be of great interest.
Oxygen is a key substrate in animal cell metabolism and its consumption is thus a parameter of great interest
for bioprocess monitoring and control. The application of the OUR (Oxygen uptake rate) is investigate here.
The main advantages of OUR is that correlates well with the physiological state of cells and also for the
prediction of viable cell concentration.
Different methods for the oxygen uptake rate (OUR) determination in animal cell cultivation have been
developed: Dynamic estimation in the liquid phase, the global mass balance in the gas phase, and the Stationary
liquid mass balance. Dynamic estimation has a considerable disadvantage because of disturbances suffered by
the growing cells because of the necessary variations of the DO concentration. Gas phase balancing has several
advantages; knowledge of the $k$,$\alpha$ value is not necessary, and yields a higher density of accurate data. However,
it has not historically been widely used due to the need for complex and expensive instrumentation like mass
spectrometers and extremely accurate DO control systems. The Stationary liquid mass balance method offers
minimum cell stress and great estimation accuracy, but still needs for a significant investment in mass flow
controllers as well as some additional instrumentation to determine the oxygen’s molar fraction in the gas
phase.

Experimental Approach:
In this communication we present a simplified embodiment of the stationary liquid mass balance method for
the continuous OUR estimation by means of the use of inexpensive proportional valves and the monitoring of
their control signals. In this way, there is no need for knowledge of the inlet gas composition to obtain a high
data density OUR record.

Results and Discussion:
Such method was used to study the growth kinetics of a 293HEK cell line producing a human therapeutic
protein. With the aid of this method it was possible to find a correlation between the oxygen uptake rate
(OUR), the cell concentration and the glucose consumption whilst the first phase of the culture. In a second
phase, lactate was metabolized, and a significant change in the OUR was detected. The ability of 293HEK
cells to consume endogenous lactate opens up the possibility of defining different culture strategies (ie diauxic
strategies). Moreover, the results obtained can be applied to the optimization of strategies like fed-batch or
continuous cultivation in which the proposed method can be applied.
DEVELOPMENT AND APPLICATION OF A SERIES OF CHEMICALLY DEFINED AND COMPLEX FEED SUPPLEMENTS FOR EXTENDED GROWTH AND ENHANCED PRODUCTIVITY IN CHO-K1 AND CHO DG44 CULTURES

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Background and Novelty:
The improvement of large scale CHO bioreactor processes to optimize recombinant protein production is an area of huge interest in the biotechnology industry. The cell culture processes utilized by industry are batch culture, fed batch culture and perfusion. Batch and fed batch culture have a number of advantages including tight process control, ease of operation, improved glycosylation properties and a high concentration of desired product. Kerry’s custom designed products and services not only provide manufacturers with enhanced upstream process performance but have the potential to reduce process timelines as well as time to market. This study describes two complex animal component free feed system’s, Sheff-Pulse I and Sheff-Pulse II, which exploit existing synergies among plant derived hydrolysates, yeast extracts and recombinant proteins while focusing on the specific nutritive needs of common production CHO cell lines. This system can be applied to both protein containing and Protein free production systems. The balanced feeding of nutrients throughout a fed batch process can result in increased and prolonged cell growth, viability and titer of the cultures. With regard to media formulations, there is an ever increasing trend away from the use of complex or animal based components and towards chemically defined media. The design of such a feed formulation can be both expensive and time consuming for a company. In response to customer requirements, Kerry has also developed the Sheff-Pulse CD feed system, a truly chemically defined fed-batch supplement.

Experimental Approach:
In order to evaluate the applicability of the complex feed system’s and the chemically defined feed system in CHO cell lines, the Sheff-Pulse systems were screened in two different IgG expressing CHO DG44 dhfr- lines and one CHO-K1 line expressing recombinant secreted embryonic alkaline phosphatase (SEAP). The feed systems were supplemented into both a popular commercially available chemically defined CHO media and Kerry’s Sheff-CHO CD complete media. Multiple rounds of fed-batch bioreactor experiments were performed in the various CHO models. Viable cell density, viability and nutrient profiles of both the complex Sheff-Pulse and the Sheff-CHO CD supplemented 1-liter bioreactor cultures were generated and compared to cultures supplemented with a commercially available competitor feed supplement. Supernatant was collected to assess the effect of each feed formulation on either IgG or SEAP production.

Results and Discussion:
When supplemented into a commercially available chemically defined basal media and Kerry’s chemically defined CHO media Sheff-CHO CD complete, the Sheff-Pulse feed system’s demonstrated an equivalent or enhanced ability to improve both CHO-K1 and CHO-DG44 culture performance as compared to a chemically defined competitor feed and an un-supplemented control. It was determined that the complex supplements gave the most improvement in titer when compared to the chemically defined supplements. These results demonstrate that both the complex and chemically defined Sheff-Pulse systems are an effective feed option for various CHO cell lines.
DEVELOPMENT OF CHEMICALLY DEFINED MEDIA FOR EXTENDED GROWTH AND ENHANCED PRODUCTIVITY IN CHO-K1 AND CHO DG44 CULTURES

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Background and Novelty:
Chinese Hamster Ovary (CHO) cells are widely used in the biopharmaceutical industry for the production of recombinant proteins. The current strict regulatory norms for the biopharmaceutical industry has led to the wide use of serum-free culture processes including chemically defined (CD) media. A significant amount of time and investment is required on the part of a business to develop an optimal CD media, which fits the needs of both the process and the company. In response to customer requirements, Kerry has developed Sheff-CHO CD complete medium for extended growth and enhanced recombinant protein production. The Sheff-CHO CD complete chemically defined media is specifically designed to work in conjunction with Kerry’s complex supplements such as the Sheff-CHO series of supplement systems or individual soy, wheat, rice and cotton hydrolysates and cell culture grade yeast extracts.

Experimental Approach:
Sheff-CHO CD complete is a powdered, animal component free, peptide/protein free, complete chemically defined medium that has been demonstrated with multiple CHO cell lines to considerably extend the growth and enhance product titers. The developmental formulation of the Sheff-CHO CD complete medium was screened in two different IgG expressing CHO DG44 dhfr- cell lines and one CHO-K1 expressing recombinant secreted embryonic alkaline phosphatase (SEAP). The viable cell density, viability and nutrient profiles of Sheff-CHO CD complete medium were compared to commercially available CD medium in multiple experiments in shake flasks. To analyze the scalability of the Sheff-CHO CD complete medium, similar comparison experiments were performed in 1-liter bioreactors. The supernatants were collected to assess the effect of the media on the production of IgG or SEAP. A range of Kerry’s complex supplements also were dosed into Sheff-CHO CD complete at various concentrations in the CHO cell lines mentioned above in bioreactors. The effect of the complex supplements was also measured in terms of Cell growth, viability and titer.

Results and Discussion:
The use of Sheff-CHO CD complete medium resulted in an enhanced performance in terms of cell density and recombinant protein productivity for both CHO DG44 and CHO-K1 cell lines. The Sheff-CHO CD complete medium supplemented with our complex supplements also demonstrated an enhanced performance. These results demonstrate that Sheff-CHO CD complete medium is a viable option as a complete chemically defined medium for a variety of CHO cells lines.
A GENOME-SCALE SYSTEMS BIOLOGY APPROACH TO
MODELING GLYCOSYLATION

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Background and Novelty:
Glycosylation serves essential functions on many proteins produced in biopharmaceutical manufacturing, making it mandatory to thoroughly consider its biogenesis during the production process. Thus, glycoengineering efforts have aimed to rationally design glycosylation by adjusting culturing conditions or genetically modifying host cell lines. Computational models have been developed to aid this process, aiming to offer cheaper and faster alternatives to costly screening strategies. However, it has been difficult to account for genetic and environmental factors in the prediction of glycan synthesis.

Experimental Approach:
Here we have developed a framework to build computational models of metabolism and glycosylation for specific CHO cell lines using RNA-Seq data, whole genome resequencing, and metabolomics. Using this framework, we are able to couple all media components that have direct or indirect influences on glycan synthesis, and account for transcriptional differences between cell lines. The models were then used to analyze the cell-line specific effects of mutations and variations in media formulation on metabolism and protein glycosylation in CHO-K1 and CHO-S cells.

Results and Discussion:
Through these analyses we demonstrate that large systems biology models can integrate genomics, transcriptomics, and metabolomics to gain detailed insights into how processes external to glycosyltransferase activity ultimately may influence glycan diversity. Furthermore, these approaches show incredible promise for informing cell line development and glycoengineering, in an effort to better control biotherapeutic safety, efficacy, and affordability.
UPSTREAM EXPANSION SOLUTIONS FOR STEM CELLS

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Background:
The long-term view of regenerative medicine therapies predicts an increased need for expansion solutions that ease scalability, utilize animal origin-free materials and are compatible with limited downstream processing steps. As more stem cell therapeutics progress through clinical testing, current in vitro culture methods in 2D vessels are proving cumbersome to scale. Moreover, the concurrent decreased demands for serum from the recombinant protein and vaccines markets may result in a shortage of serum as clinical cell therapy programs are successful.

Approach:
We have developed an approach for selecting media and microcarriers, using adipose-derived MSCs as a model cell line. Media were screened in 2D culture, followed by small-scale microcarrier evaluation in both static and stirred platforms, and finally in the Mobius® CellReady 3L bioreactor.

Results:
We identified that the interplay of culture surface and media formulation and harvest solutions can contribute to the success of the expansion and recovery system. Next, an evaluation of animal-free media supplementation and cellular detachment solutions was performed. Human platelet lysate was assessed in comparison to fetal bovine serum while cellular detachment was optimized using animal-free enzymes. Platelet lysate supported growth of MSCs in a variety of expansion paradigms, providing a xeno-free system when combined with animal-free detachment. Because cellular therapeutic manufacturing processes are further complicated by the requirement to separate cells from microcarriers whilst retaining cell yield, viability and target phenotypic and functional characteristics, the importance of acknowledging downstream effects while establishing upstream parameters must be considered.
BIOREACTOR BASED STEM CELL EXPANSION IS SUPPORTED BY ADVANCED ANALYTICS

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Background:
As more cell therapeutics approach the commercialization phase, a gap in analytical equipment within traditional labs has been identified. Measuring glucose and lactate levels during bioreactor expansion can be used as an orthogonal method for assessing stem cell growth and optimizing feed protocols. Use of the Nova BioProfile FLEX® analyzer is standard for measuring nutrients and metabolites in traditional biopharma manufacturing settings. For potential application to cell therapy manufacturing, this study compared the standard nutrient and metabolite analytics to measurements with handheld glucose and lactate single-test analyzers and a compact blood gas and chemistry analyzer.

Approach:
Multiple assessments of control solutions were performed in order to characterize the variability of the standard method and the alternative equipment. Additionally, samples of from stirred tank bioreactors were taken and measured on all five instruments. Some of the instruments measure glucose and lactate while others are single analyte analyzers.

Results:
The working range for each of the instruments was established and each had less than 10% variation. In the samples from bioreactors, the alternative instruments reliably measured the glucose and lactate levels. We found the Nova equipment can be a portable, low-cost solution that provides a high level of comparability to the standard instrumentation. Additionally, the blood gas-chemistry analyzer can be used to measure process attributes including pH and dissolved oxygen, making it particularly useful in both the setup and calibration phase as well as the expansion phase.
AN ULTRA SCALE-DOWN STUDY TO ASSESS THE IMPACT OF SHEAR ON VACCINIA VIRUS AND CV-1 CELLS DURING CROSSFLOW MICROFiltrATION FOR A CANCER VACCINE THERAPY

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Background and Novelty:
Oncolytic virotherapy is an emerging therapy for cancer treatment. The use of virus as a therapeutic vaccine to selectively infect and replicate and therefore destroy cancer cells is promising, as conventional cancer treatments are ineffective. Vaccinia virus (VV) previously used as a prophylactic vaccine against smallpox, possesses various attributes such as its natural tropism to cancer cells that contribute to its potential as an oncolytic virus. During the smallpox eradication programme 10⁶ PFU/dose of vaccinia virus was sufficient, however as an oncolytic virus more than 10⁹ PFU/dose is required. The high dosage of vaccinia virus for a cancer vaccine presents an obstacle for the manufacturing process as potentially 10⁹ fold more VV needs to be produced. Moreover, maintaining the infectivity of VV during processing is equally important in contributing to the process yield and to an effective cancer vaccine.
The current research focuses on the recovery stage of the vaccinia virus vaccine bioprocess, consisting of the cell recovery, cell lysis and virus recovery steps. The main aim during recovery was to release and recover intracellular VV from green African monkey kidney fibroblast (CV-1) cells using crossflow microfiltration, while studying the shear sensitivity of these mammalian cells and VV.

Experimental Approach:
VV is majorly intracellular and was expressed in green African monkey kidney fibroblast (CV-1) cells. Crossflow microfiltration was chosen as the unit operation to aid in the recovery of VV. Use of an ultra scale-down membrane shear device to predict the shear experienced at the membrane surface during crossflow microfiltration, provided a rapid insight into the shear sensitivity of these mammalian cells during the cell recovery and disruption steps as well as the shear stability of VV during the virus recovery step. Hypotonic buffer was used in combination with crossflow microfiltration for the cell disruption of CV-1 cells to release the virus. An LDH assay was used to quantify the percentage of cell disruption after shearing and osmotic shock. Additionally the infectious virus yield was obtained through the use of 50 % tissue culture infective dose (TCID₅₀) assay.

Results and Discussion:
During the cell recovery stage, the membrane shear device has been used to decipher the shear sensitivity of VV infected CV-1 cells when concentrating the cells (prior to adding the hypotonic buffer for cell disruption). VV infected CV-1 cells maintained cell integrity at low shear conditions, compared to a high shear environment in which more than 70 % cell disruption and therefore viral release occurred. High shear combined with hypotonic buffer to weaken the cells demonstrated a high infectious VV release. Additionally, optimal microfiltration performance conditions were sought and the critical fluxes identified for each step. In this study the shear sensitivity of VV during microfiltration at various shear conditions has also been evaluated during the virus recovery step.
DEVELOPMENT OF A NOVEL THERMOPLASTIC TUBING, FP-FLEX™, AND SINGLE-USE FREEZING BAG FOR WORKING CELL BANKS ENABLING CLOSED-SYSTEM PROCESSING TO CRYOGENIC TEMPERATURES (-196°C)

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Background:
Working cell banks (WCB’s) are commonly applied to initiate cell culture manufacturing campaigns for production of therapeutic proteins. Campaigns typically begin with inoculation of cells previously cryopreserved in cryovials. The baseline process proceeds from thaw of cell bank cryovials to scaling up through seed train and inoculum train, followed by fed-batch production. Cryovials are typically used for development of WCB’s and initiation of manufacturing campaigns, but aren’t optimal for the growing demands of commercial production. Cryovials are small and filling/removal is performed through an open cap. This process leads to numerous manual operations and culture vessels, resulting in contamination risks and campaign-to-campaign variability. Single-use bags have been investigated recently as a possible solution to minimize open steps and shorten seed train scale-up. Although, wide-spread adoption of single-use bags for WCB applications hasn’t been observed as current available tubing doesn’t hold up to the demands (break; can’t be welded) when stored/transported at cryogenic (-196°C) temperatures.
To overcome these challenges, novel thermoplastic tubing was developed to balance the robustness demands of cryogenic storage and tube welding characteristics necessary for sterile closed-system processing. The new FP-FLEX™ tubing can be frozen, maintained at cryogenic temperatures, thawed and sterile welded to other thermoplastic tubing (ex. C-Flex).

Approach:
A selection of thermoplastic elastomer (TPE) combinations were investigated to discover optimal blends of materials required to support the robustness requirements of frozen and sterile welding needs for processing. A novel manufacturing method was also developed enabling welding of the FP-FLEX™ tubing directly to single-use bags. This was critical to support a completely unitized design. Studies were performed to determine durability and functional utility of the FP-FLEX™ tubing for frozen storage and processing applications. Testing was accomplished using 500mL bags having (1/4” OD x 1/8” ID) FP-FLEX lines and compared to bags having PVC tubing (standard). For LN2 studies, bags were filled to 140mL (+/- 5mL) with water, placed into aluminum cassettes and stored with tubing attached. Drop tests were performed to assess durability. Bags were pulled from LN2 storage, dropped horizontally 4X from 1ft, thawed and evaluated. 10 of 10 bags with FP-FLEX™ were intact while 9 out of 10 bags with PVC tubing were broken. A single drop from 2ft resulted in similar results. Testing was also performed to evaluate sterile welding capabilities of FP-FLEX™ tubing post-thaw. Tubing was capable of welding directly to C-Flex using standard welders with flow rates up to 1L/min achieved successfully. Simulated frozen transportation studies are ongoing but results are not currently available.

Discussion:
WCB’s are commonly used for seed train manufacturing of therapeutic products. Traditionally cryovials and bags can’t accommodate the increased production and processing demands. The new FP-FLEX™ tubing has been designed and shown herein to meet the critical processing requirements for WCB’s. The FP-FLEX™ tubing and Freeze-Pak™ bag represent a closed-system solution enabling frozen storage, sterile connection and ultimately reduced scale-up time for therapeutic production.
COMPARISON OF CHO CELL CULTIVATION AND MAB PRODUCTION IN PALL XRS 20 AND CONVENTIONAL ROCKER TYPE SINGLE-USE BIOREACTORS

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Background and Novelty:
The Pall XRS 20 Bioreactor System is a new single-use bioreactor system suitable for applications ranging from general life sciences research to seed train operations as well as small scale production at the 2 to 20 liter scale. It features a 3D rectangular biocontainer that is rocked across two independent axes perpendicular to one another. These features allow for significant improvements in mixing and k_La when compared with a conventional rocking bioreactor that features a 2D “pillow” biocontainer that rocks across a single axis. The Pall XRS 20 controller is able to control agitation of the biocontainer over a wide range of conditions, from gentle agitation required for shear sensitive cultures, to more vigorous conditions generating higher oxygen transfer rates and decreased mixing times needed for the high producing processes seen in the biopharmaceutical industry today. Control of pH and DO is fully automated by the optical sensors supplied with the biocontainer. Gassing strategy is managed by three independent mass flow controllers and fluid additions can be made via three integrated pumps. All operations are managed through a user friendly touchscreen interface.

Experimental Approach:
In this poster we demonstrate increased performance of the Pall XRS 20 System compared to a conventional single axis rocking bioreactor, when culturing a CHO cell line producing a monoclonal antibody, in fed-batch culture. It has been established in previous optimization studies that this cell line favors conditions where the mixing time is low and the k_La is high. A conventional rocking type bioreactor was set up in parallel and operated to give mixing times and oxygen transfer rates as close as possible to the Pall XRS 20 System. All tests were performed using commercially available, chemically defined media. Samples were taken throughout each bioprocess to determine cell growth, antibody production, metabolite profiles and product quality.

Results and Discussion:
Cultures from both the conventional rocking bioreactor and the Pall XRS 20 system grew at a similar rate up until the conventional bioreactor entered the stationary phase. Cell growth continued in the XRS 20 for a further 24 hours, resulting in an average increase in viable cell density of 16% over the control average. The conventional bioreactor began to steadily decrease shortly after entering the stationary phase, however in the XRS 20 bioreactor, high culture viability was maintained steadily for close to 100 hours. This shows that when operating both bioreactor systems at similar operating conditions there is an increase in both the maximum cell density and culture duration with the Pall XRS 20 System. We have demonstrated that with this process, the Pall XRS 20 system is able to produce more viable cells for a longer duration. This is reflected in the final monoclonal antibody concentration from each reactor, with an average 67% increase in target antibody production in the Pall XRS 20 system over the conventional bioreactor. This increase in performance is accompanied by similar product charged variants and glycosylation profiles.
CHARACTERIZATION OF THE PERFORMANCE OF THE ALLEGRO™ STR 1000
SINGLE-USE STIRRED TANK BIOREACTOR SYSTEM

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Background and Novelty:
The benefits of single-use technologies in both upstream and downstream operations are now widely acknowledged by the biopharmaceutical industry, and have resulted in radical changes to the design and operation of many pharmaceutical processes. In mammalian cell culture, multi-use, cleanable glass or stainless steel stirred tank reactors (STRs) have been used successfully for growth of suspended cell lines for both small and large scale systems. However, to achieve the same or better performance from a single-use bioreactor presents a significant challenge to product designers and developers.

Experimental Approach:
This poster describes the studies aimed at characterizing the fluid dynamics of the Allegro STR 1000 by measurement and modelling of specific physical parameters known to be critical for mammalian cell culture. More specifically, oxygen transfer rates, mixing times, carbon dioxide stripping rate and temperature control were assessed using in house developed protocols. An in depth characterization using computational fluid dynamics complement these measurements.

Results and Discussion:
Our studies have shown that, the Allegro STR 1000 single-use bioreactor can be engineered suitably to provide a practical alternative to stainless steel bioreactors. By evaluating the hydrodynamics of the Allegro STR 1000, we established that the direct drive large impeller could provide power input for fluid volumes up to 1000 L that can support the growth of mammalian cells. This power transfer in conjunction with a customised cubical biocontainer with integrated baffles produced homogeneous mixing. User experience is also enhanced by providing features that make the installation and use of the bioreactor intuitive and easy.
CHARACTERIZATION AND ENGINEERING PERFORMANCE OF THE ALLEGRO™ STR 200 SINGLE-USE STIRRED TANK BIOREACTOR SYSTEM

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Background and Novelty:
Currently, stirred tank reactors (STRs) represent the gold standard for the large scale growth of suspension cell lines. Culture performance is strongly influenced by the efficiency of mixing, as measured by the mass transfer coefficient and mixing time. The success of traditional stainless steel or glass STR systems lies in their impeller-driven agitation that efficiently mixes large volumes of culture fluid. However, the transition from stainless and glass STR vessels to single use STR systems still remains a challenge, particularly in terms of replicating efficient mixing. Many single use STRs uses magnetically coupled driving mechanisms to eliminate the risks of bioccontainer leaks from rotating mechanical seals. While this design significantly simplifies the manufacturing of the bioccontainer, insufficient magnet strength limits the power that can be transmitted to the culture fluid, and often results in poor mixing and mass transfer performance.

Experimental Methods:
The Allegro STR 200 design encompasses key features aimed at maximizing the bioreactor performance: a direct drive agitation mechanism coupled with a large bottom-mounted impeller able to deliver a wide range of power input per unit volume of fluid, a cuboid shaped bioccontainer with natural baffling effects, as well as integrated baffles.

Results and Discussion:
The benefits of this design were assessed by measurement of critical physical parameters including oxygen transfer rates, mixing times, carbon dioxide stripping rate and temperature control. This experimental approach was completed by an assessment of fluid dynamics within the whole bioreactor using a computational fluid dynamic model.

Data shows that the bioreactor design features allow for:
- A wide range of power input per unit volume of fluid.
- Mixing times below 10 seconds.
- Oxygen transfer rate (k,a) as high as 33 h⁻¹ using a ring sparger and air.
- Efficient CO₂ stripping.
- Precise and homogenous temperature control.
USE OF THE PALL MICRO24 MICROREACTOR SYSTEM AS A CROSS PLATFORM SCALE DOWN TOOL FOR BIOPHARMACEUTICAL PROCESS RESEARCH

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Background and Novelty:
In recent years a number of small scale bioreactor systems with increasing levels of control and automation have been commercialised and these are frequently used in the development of processes for the production of biopharmaceutical molecules. Both mammalian and microbial processes are widely used in such processes but the specific limitations and requirements of each host organism type usually require specifically configured bioreactors meaning different equipment is required depending on the host type.
The Micro24 Microreactor system (Pall Corporation) is a scale down system consisting of 24 x 15mL parallel bioreactors in a plate based format. Several different plate types are available for use with the Micro24 system enabling sparged or surface gassed bioreactors to be operated using the same hardware. This flexibility combined with pH and DO control allow the operation of a wide range of bioreactor conditions and therefore the ability to model both mammalian and microbial bioprocesses.

Experimental Approach:
In this work we have investigated the production of biopharmaceutical molecules from three different host types in the Micro24 Microreactor system and compared the data to that from conventional systems. We have successfully demonstrated monoclonal antibody production from CHO cells, antibody fragment production from E.coli and recombinant protein production from SF9 insect cells at the ~15mL scale.

Results and Discussion:
Comparison of the small scale data obtained in this work to larger scale bioreactor data for each process showed that meaningful data for each host type process was achieved in the Micro24 Microreactor system. Overall this work has demonstrated the utility of the Micro24 Microreactor system as a cross platform scale down system and the potential for its integration into mammalian, microbial and insect cell process development.
AMBR™ MINIBioreactor as a High-Throughput Tool for Culture Process Development to Accelerate Transfer to Stainless Steel Bioreactor at Manufacturing Scales

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Background and Novelty:
Enhancing throughput of bioprocess development has become increasingly important to rapidly screen and optimize cell culture process parameters. With increasing timeline pressures to get therapeutic candidates into the clinic, resource intensive approaches such as the use of shake flasks and bench-top bioreactors may limit the design space for experimentation to yield highly productive processes. The need to conduct large numbers of experiments has resulted in the use of miniaturized high-throughput (HT) technology for bioprocess development. One such high-throughput system is the ambr™ platform, a robotically driven, microbioreactor system developed by TAP-Sartorius.

Experimental Approach:
In this study we assessed and compared the performance parameters of ambr™ minibioreactor run to 2L, 80L and 400L stainless steel bioreactors using a CHO cell line producing a recombinant monoclonal antibody. The daily parameters monitored were cell growth and cell viability, offline pH and dissolved oxygen, metabolite profiles (glucose, lactate and ammonia) and monoclonal antibody titer. In addition, we compared the product quality attributes of the clarified cell culture fluid post Protein-A elution generated in the microbioreactor run (high molecular weight and low molecular weight species, charges variants) to the larger scales.

Results:
The scale down model in ambr™ was established taking into account different parameters: the feeding strategy, the mode of feed addition, the current culture volume, the aeration strategy, the agitation speed. In addition, the daily sampling was adapted to the low bioreactor volume in the ambr™ system. Our results demonstrated comparable cell growth, cell viability, metabolite profile and monoclonal antibody titer within scales. In addition, the product quality attribute results of the clarified cell culture fluid post Protein-A were also comparable. Hence, this study shows the scalability of the ambr™ minibioreactor platform up to 400L manufacturing scale.
CHALLENGE OF PROCESS TRANSFER FROM SINGLE-USE PRODUCTION TO SCREENING BIOREACTOR

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Background and Novelty:
Screening systems are the base of a good development procedure in modern mammalian cell culture.
A variety of processes parameters is tested in screening systems to define a first version of the production process. The process transfer between a screening system and production scale is a challenging step. The agitation principle of screening systems most often differs from conventional production bioreactors that are commonly designed in accordance to stirred tank reactors, well-defined and characterized. To facilitate a direct process transfer from screening to production scale a complete single-use bioreactor family from 0.25 to 2,000 L with comparable geometrically rations and impeller design was developed.

Experimental Approach:
The applied CHO fed-batch process in this trial is established in scales from 2 - 1,000 L. The aim was to transfer this process to a new stirred single-use 0.25 L screening bioreactor. A complete process engineering characterization was performed for all used systems. The critical process parameters like mixing time, k_a and shear stress were considered and scaling criteria were defined with a design space approach. As a reference system a multi-use 5 L glass bioreactor was used.

Results and Discussion:
Based on the oxygen transfer, the mixing behavior and shear stress the process parameters were defined in order to fit the design space for all scales. Cell densities of up to 32 x 10^6 cells/mL and product titers of up to 8 g/L were reached. Overall, the process was established successfully in all scales and similar results were achieved. All results were comparable to prior performed cultivations in reference bioreactors. The archived results represent a major step in terms of process development in single-use bioreactors. In the future this new screening bioreactor system makes it easy to transfer new processes from screening to production scale.
SUPERIOR SCALABILITY OF SINGLE-USE BIOREACTORS

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Background and Novelty:
Single-use bioreactors are an attractive technology for the biopharmaceutical industry. They are excessively used for mammalian cell cultivations e.g. for the expression of vaccines or monoclonal antibodies. This is motivated by several advantages of these bioreactors like a reduced risk of cross contaminations or short lead times. Commonly, single-use bioreactors differ in terms of shape, agitation principle and gassing strategy. Hence, a direct process transfer or scale-up is challenging. Consequently, re-usable bioreactors are still regarded as gold standard due to their well-defined geometrical properties and the extensive process engineering knowledge.

Experimental Approach:
Based on this knowledge a stirred single-use bioreactor family was developed with similar geometrical ratios. These bioreactors include a small scale system for screening and process optimization and large scale systems for production. To follow a Quality by Design (QbD) approach the bioreactors evaluated here are characterized by using process engineering methods. For the definition of a control space the specific power input per volume, mixing times and k_{L}a-values are determined for scales from 0.25 to 2000 L.

Results and Discussion:
The achieved data are compared to an established glass bioreactor. This comparison shows that these systems are suitable for cultivations of mammalian cells. Based on the data scale-up/down and process transfers are optimal within this bioreactor family by using general scale-up criteria. Therefore, the presented stirred bioreactors are a major progress for single-use technology.
APPLICATION OF COMPUTATIONAL FLUID DYNAMICS (CFD) AS A QUALITY BY DESIGN (QBD) TOOL FOR FAST-TRACK PROCESS DEVELOPMENT

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Background and Novelty:
The demand for complex therapeutic proteins and antibodies for treatment of various diseases has increased continuously in the last decades, prompting the continuous development of new processes. For the development of these novel processes, the Quality by design (QbD) approach has gained popularity since the FDA’s PAT-publication (1). However, this practice requires a thorough understanding of a product and especially of the manufacturing process. Having evolved from a rather complex fluid mechanic application, Computational Fluid Dynamics (CFD) is currently an easily employed tool for the improvement of bioreactor design and modeling of local hydrodynamic conditions, including local turbulence, gas-fluid mixing and mass transfer in stirred tank bioreactors (STR). Therefore, CFD can be readily used for characterization of manufacturing equipment.

Experimental Approach:
Following up on an approach from (2) (Rathore, 2011), an application of CFD for the set-up of a process design space for an acceptable range of the volumetric oxygen mass transfer coefficient (kLa) is demonstrated in this study. Based on agitation, aeration and working volume as input parameters a DOE study is conducted to characterize the influence of these parameters on the kLa in an aerated 2L stirred benchtop bioreactor.

Results and Discussion:
For two phase simulations, instead of the previously applied Euler-Euler approach, the physically more accurate Euler-Lagrange model along with the standard k-epsilon turbulence model was used for simulation of the dispersed phase. Bubble breakup and coalescence were considered using the stochastic secondary droplet (SSD) model natively included in the Fluent software (Fluent 15.07, ANSYS Inc., Canonsburg, USA) and the kLa was calculated based on Higbie’s penetration model. Validation experiments were then conducted at the boundaries of the model to validate the identified design space. This experimental set-up substantiated the usefulness of CFD in process development and can help to speed up process development and scale-up, thereby saving valuable experimental time.

SYSTEMS BIOTECHNOLOGICAL INVESTIGATION OF MAMMALIAN CELL CULTURE PERFORMANCE IN CONTINUOUS BIOPROCESSES

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Background:
Commercial production of biopharmaceuticals such as monoclonal antibodies (mAb) is dependent on high performance bioprocesses using mammalian cells e.g. Chinese hamster ovary (CHO) cells. Expansion of mammalian cells, usually doubling once per day, for production scale fed-batch processes requires time and cost intensive seed trains that have an important impact on production-stage performance, product quality and often limit facility outputs. In this field, continuous bioprocesses, such as perfusion cultures, offer several advantages e.g. regarding volumetric productivity, overall process time and investment costs.

Experimental Approach:
We will present ongoing research at Boehringer Ingelheim Biopharma targeting advanced bioprocess concepts for perfusion based expansion of high performance mAb producing BI-HEX cells. The influence of bioprocess parameters as well as mammalian cell metabolism on the overall process performance is evaluated for novel process formats using systems biotechnological tools such as gene expression profiling. For this purpose, small-scale models of perfusion based cultures are used to investigate (i) cell culture performance during seed train and fed-batch production culture and (ii) lead parameters influencing cellular metabolism during transfer from N-1 to N-stage and lag phase.

Results and Discussion:
Results comprise both macroscopic process data, such as extracellular rates and yield coefficients, as well as in depth cell-level based analysis by expression data using advanced omics approaches. Results are discussed with respect to rational process design applications. This allows for targeted bioprocess optimization focusing on production-stage intensification and robustness based on an improved process understanding.
PAIA ASSAYS: A NEW BEAD-BASED ASSAY SYSTEM FOR HIGH THROUGHPUT PROTEIN QUANTIFICATION

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Key words: Protein quantification / cell line development / high producer screening / high throughput.

Background and Novelty: The patent pending PAIA technology provides a novel platform for the quantification of proteins, e.g. monoclonal antibodies. PAIA assays consist of functionalized beads to capture the protein of interest (the analyte) and a fluorescent marker to detect the analyte. In contrast to other bead-based assays, which measure the fluorescence intensity of the beads, PAIA assays measure the fluorescence of unbound marker remaining in solution. We use 3D structures on the bottom of the microplate that separate captured analyte-marker complexes from unbound fluorescent marker. Therefore, no washing steps are needed and the assay can be performed in an automated 384-well plate format. The readout is obtained with either a plate reader or a fluorescence microscope in less than five minutes per plate. The time to result, the amount of sample required, and the hands-on time are substantially reduced compared to other immunoassay formats. Hence, this novel approach is particularly suitable for high throughput analysis of protein containing samples like supernatants of antibody producing cells.

Experimental Approach: The PAIA platform is based on 384-well plates and assays are usually performed in a volume of 60 μL. Depending on the expected analyte concentration, as little as 2 μL of sample is sufficient for quantification. The sample is diluted in reaction buffer and added to the microplate which contains dried beads and fluorescent marker. PAIA assays offer the possibility to choose a marker against the specific epitope of the analyte that shall be detected. For example, in a PAIA assay to quantify human IgG1, Protein A beads capture the Fc part of IgG and the fluorescent marker can be directed either against the whole antibody or against specific regions like the Fab fragment or the light chain. After addition of buffer and sample, the microplate is incubated for about 30 minutes under agitation at room temperature and subsequently allowed to stand for about 10 minutes. The 3D structure on the microplate bottom ensures that the beads are settling outside the detection area. Hence, only the supernatant containing unbound fluorescent marker is detected.

Results and Discussion: This presentation highlights assay formats for different proteins, like human IgG1 and His-tagged proteins. The PAIA assays work in aqueous buffers as well as common cell culture media. We outline the workflow of a typical PAIA assay and the analysis of the obtained data using the PAIA-software. This tool rapidly generates calibration curves and QC parameters as well as an overview of the results, e.g. for identification of high producers. We believe that the high throughput, the small sample volume, and the low overall costs represent substantial advantages over existing methods. In future, we will expand the range of assays to other proteins to fully exploit the potential of this versatile technique.
A COMMUNITY GENOME-SCALE MODEL OF CHO CELL METABOLISM FOR MULTIOMIC DATA ANALYSIS AND BIOPROCESS DESIGN

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Background and Novelty:
Genome-scale models of metabolism have successfully been employed in many microbial and eukaryotic metabolic engineering efforts by guiding pathway engineering and media optimization. They have also been used to explore the genotype-phenotype relationship in mammalian cells. With the recent publication of the genomic sequence for Chinese hamster ovary cells, these metabolic modeling tools can now be used to explore similar questions in CHO-based recombinant protein production.

Approach:
Here we have developed a genome-scale, manually curated, community metabolic network reconstruction of the CHO-K1 cell line. The metabolic model is capable of integrating proteomic, transcriptomic, and metabolomic data.

Results and Discussion:
The model can accurately simulate experimentally measured growth and production phenotypes. We have also used the model to assess the metabolic limitations on recombinant protein producing lines subject to different stressors and found that common culture conditions have a theoretical maximum specific production rate 10-fold higher than has been reported. Thus, there are many opportunities to rationally engineer CHO cell lines for higher yields in biotherapeutic protein production.
PERFORMANCE CONSISTENCY OF PLATFORM FED-BATCH CULTURES ACROSS MULTIPLE SYSTEMS USED IN INDUSTRIAL PROCESS DEVELOPMENT

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Background and Availability.
Each stage of cell culture process development requires fit for purpose tools. The selection of a fed-batch cultivation system is often based on throughput and cost. However, the process knowledge derived from different systems and scales is not necessarily identical. Hence, a careful evaluation of systems which are already established or newly implemented is essential. We recently introduced a novel high throughput fed-batch screening system (1) and the objective of this study was to provide data on how it compares with other systems used in early and late stage cell culture process development.

Experimental Approach:
We describe the performance of 10 different recombinant CHO cell lines expressing the same antibody in fed-batch culture systems ranging from a few hundred microliters to lab scale. The 10 cell lines were selected based on distinct phenotypes covering a range which can be expected in typical industrial process development projects. The cell lines were cultivated using the same expansion and fed-batch protocol (proprietary fed-batch system). The following cultivation systems were evaluated: shaking 96-deepwell plates, 50 mL vented shake tubes, micro-scale bioreactors (ambr15™ system) and lab-scale bioreactors (3L).

Results and Discussion:
The results of this study show both the limitations and the potential of each cultivation system and their suitability for process development, process characterization and scale-up. The shaking systems offer unprecedented parallel throughput but are limited with respect to culture control (e.g. lack of pH and pO₂ control). Despite their limitations, they are expected to be used in the future as important tools for early process development and for the improvement of fed-batch platform processes. On the other hand, the data obtained from this study show that micro- and lab-scale bioreactors represent ideal tools for the confirmation of process consistency. Both micro- and lab-scale systems will be extensively used to support tech transfers and perform process characterization studies.

Bibliography:
RAPID PROCESS DEVELOPMENT USING THE AMBR™ 15 FROM CLONE SELECTION TO PHASE I CLINICAL PRODUCTION

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Background and Novelty:
Multiple tools are now available to decrease the time from molecule discovery to clinical trials. Platform approaches are one way to decrease timelines, however, sole reliance on a platform process risks sacrificing potential productivity gains in order to achieve this reduction. Often, specific media/feed requirements not provided for by the platform process may erroneously weed out a top performing clone where, if the correct combination of nutrients is provided, a given clone could be a top producer. In addition, clones with an optimal pH different from the platform process may also be unnecessarily ruled out. Completely altering a platform process may not be desired or even feasible given the desire for reduced timelines, however, high throughput technology and automation may have the answer. In this study we employ the use of the ambr™ 15 to rapidly select the top performing clone using a representative fed-batch process with several commercially available media and feed options. Following the selection the ambr™15 was also used to investigate the effect of various process parameters on process performance.

Experimental Approach:
Clones were initially adapted to several commercially available basal media and cultured in a representative fed-batch process including several different feed media. Once the top two media and feed combinations for the top clones were identified, a subsequent run was performed to ensure process related parameters such as feed amount and pH were not limiting the performance of the clones and a top clone was selected. Finally, a third round of ambr was performed with the top clone in a response surface DOE to ensure optimal process parameters were identified. These conditions were then confirmed at larger scale.

Results and Discussion:
While some clones exhibit rapid growth to high densities and subsequent rapid declines others yield lower peak densities but overall higher integral viable cell densities during fedbatch culture. Each cell line has different nutritional requirements and a platform medium may not optimally suite either. Through the use of the media/feed screen early during the clone selection stage, we were able to achieve enhanced productivity over the platform medium and ultimately select a clone/media combination with higher sustained productivity. The ambr system was an integral part in this endeavor, first by providing the bioreactor like controlled conditions which allowed for the extended productivity period to be realized during the clone selection and second by providing the platform for high throughput process development where 48 conditions could be run in parallel. This allowed for drastically reduced timelines and an overall greater confidence in the robustness of the process. In total, 144 mini-bioreactors were run over a period of 8 weeks resulting in a high producing clone and significant understanding of the effect of controlled process parameters. While the implementation of such an approach may be prohibitive in traditional stirred tank reactors due to resource constraints, the ambr™15 alleviates that constraint and in fact allows for a further reduction in time line.
AN INTEGRATED DATA MANAGEMENT PLATFORM
FOR BIOPHARMACEUTICAL R&D

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Background and Novelty:
The discovery and development of biopharmaceuticals is a complex and time-consuming endeavor. The management and interpretation of data generated in the biologics R&D process are key bottlenecks in making biopharmaceutical R&D more efficient.

Experimental Approach:
We developed an enterprise software platform that supports the entire biologics R&D process including antibody screening, protein engineering, as well as biologics expression, purification, and analytics. The focus of the platform is to support scientists in their daily R&D operations. The system helps to simplify and streamline laborious, manual processes such as cloning or instrument operation, resulting in increased efficiency and throughput, as well as in improved quality of results through reduction of ad-hoc and error-prone sample and data handling. Genedata Biologics is the result of a concerted multi-year development program in close collaboration with leading international players in biologics R&D.

Results and Discussion:
Here, we present concrete use cases including antibody phage-display, screening automation, affinity maturation, design and evaluation of engineered molecules, as well as high-throughput expression, purification, and analytics of novel biologics candidates.
DEVELOPMENT OF A NOVEL LAMINAR SHEAR SYSTEM FOR INVESTIGATION INTO THE PROTECTIVE EFFECTS OF BOVINE SERUM ALBUMIN ON MAMMALIAN CELLS DURING CONTINUOUS SUSPENSION CULTURE

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Background and Novelty:
Bioreactors are typically used in industrial applications to culture mammalian cells which have been genetically modified to produce monoclonal antibodies (mAbs) during suspension culture. These cells are subjected to fluid shear stress (FSS) from various sources within the bioreactor environment that can result in membrane damage and decreased mAB production. Bovine serum albumin (BSA) is an animal-derived additive which mitigates FSS-induced cellular damage, but the mechanism by which this occurs has not been well-characterised. There is a need to understand this so that BSA can be replaced with a more regulated chemically-defined substitute.

Experimental Approach:
To investigate the protective effect of BSA on mammalian cells, a novel shear device was developed. This utilises a combination of concentric cylinder and cone and plate geometries to produce Couette flow. The shear device was constructed using stainless steel grade 316L to ensure corrosion-resistance and suitability for autoclaving between test cycles. The system was further modified to incorporate the development of a cell culture apparatus in series with the shear device which allows the user to grow cells while continuously circulating them through the shear device.

Results and Discussion:
The shear device can be used to subject cells in suspension culture to well-defined laminar FSS in the range of 0 - 2.5Pa. Unique aspects of the system are that it can be maintained completely sterile, produces conditions conducive to cell growth and has a large capacity (50ml) which allows for the aseptic shearing of bulk quantities of cells in continuous culture. It is proposed that the system can be used to subject cells to FSS with and without BSA present in the culture medium in order to characterise the protection afforded to cells by BSA and to determine if this protection is dependent upon time and/or shear-magnitude. It is also suggested that the system produces a more representative model of the long-term effects of shear on cells in bioreactor culture and their mAb production levels than previously described Couette flow devices.
LAB-AUTOMATION: INTEGRATED FRAMEWORK FOR SEAMLESS PROCESS DEVELOPMENT - A VACCINE CASE-STUDY

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Background and Novelty:
Vaccine production and process development are rendered more complex due to the variety of expression systems including adherent cell lines, pathogenic strains, complexity in characterization (viral particles), hence biosafety considerations and the need for dedicated laboratories and equipment. Here we describe multipurpose cell and bacterial culture, parallel automated bioreactors and downscaling demonstration versus current industrial manufacturing. Performance of the approach is supported by specific, customized data management methodology.

Experimental Approach:
Downscaling methodology and assessment consider bacterial and animal strains in comparison to process productivity, biomass, cell counts, product, metabolic profiling at up to 1000L scale, testing by design of experiments (DOE) of defined set points, ranges.

Results and Discussions:
Time saving of up to 50% can be achieved for both cell culture and bacterial project, while achieving sound prediction of process performance when reaching industrial scale. As a result of speed process operations, data management becomes a bottleneck in the automation value chain which has been addressed through a specific software allowing for fast comparison and iteration for the next round of improvement.
IN LINE MAMMALIAN CELL CONCENTRATION MEASUREMENT WITH A BIO CELL VITALITY ANALYSER

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Background and Novelty:
The Seq uip Bio Cell Vitality Analyzer was used to monitor the viable cell concentration of a mammalian cell line during a 19 day cultivation in a 30L stainless steel stirred bioreactor. The L.E.S (Light Emitting Source) beam of the Seq uip Bio Cell Vitality Analyzer shows a different reflection pattern on living and dead cells due to differences in cell membrane reflectivity. Therapeutic glycoproteins and other recombinant proteins, are produced in mammalian cell cultures, because of their ability to secrete the target proteins with the correct glycosylation pattern. Here, a CHO (Chinese Hamster Ovary) Cell was used. These Cells are frequently used in biotechnological and pharmaceutical production processes and they have the ability to stay in the stationary phase for a relatively long time before they start starving. Cell death usually leads to a loss in product quality, which makes the measurement of the living cell concentration during a mammalian cell fermentation process indispensable. The Seq uip Bio Cell Vitality Analyzer offers the opportunity to measure biomass concentrations under in situ/inline conditions and distinguishes between living and dead cells.

Experimental Approach:
This in situ technique was compared with two automatic offline cell analyzers which requires sampling: the Cedex and the Casy system. The Cedex System distinguishes between viable and dead cells in a microscopic picture based on the trypan blue exclusion method and reliably measures cell concentrations. The Casy system is a particle counter, which determines the cell diameter and total cell concentration by impedance measurement under offline conditions.

Results and Discussion:
The cultivation process can be divided into three phases. Phase I shows a good correlation between all measurement techniques. At cell concentrations above 5*10^5 Cells/ml cell aggregation starts and effects the different cell concentration measurements (phase II). Since the Casy system is not able to distinguish precisely aggregates from single cells, the measured cell concentration is too low compared to the other cell count techniques. Both the Seq uip in situ system and the Cedex system are able to estimate the number of cells within the cell aggregates; however, the actual cell concentrations are considerably higher for both instruments if the cell sample is trypsinized to dissolve cell aggregates and to separate the cells. This proves that, the Cedex System, can analyze aggregates to a certain extent only. Remarkably in phase III of the cultivation the formed aggregates the formed aggregates start to disintegrate. Even though viability is still high in this late phase of the cultivation.

The Seq uip sensor is able to illustrate this phenomenon more exactly, whereas the Casy and the Cedex system are not. Moreover, the Seq uip sensor is able to detect alterations of the CHO cell membrane throughout the whole cultivation process, which can be seen in the permanent changing of the obscuration factor.

In conclusion, the offline Casy and Cedex and the in situ Seq uip System are able to analyze reliably single CHO cells during a fermentation process.
RECONCILING PILLARS OF TRANSIENT GENE EXPRESSION: FROM DNA PREP VIA MEDIA, REAGENT AND CELL LINE DEVELOPMENT TO HOLISTIC PROCESS OPTIMIZATION

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Background and Novelty:
As a contract manufacturer of recombinant proteins and antibodies for research, diagnostic and preclinical applications, InVivo BioTech Services has implemented a novel technology for efficient transient transfection and expression in HEK cells during the last years. In cooperation with emp Biotech, InVivo BioTech Services developed a transfection reagent with very low cytotoxicity. A culture medium that can be used for transfection and production was designed in cooperation with Xell AG. The establishment of a TGE optimized HEK cell line and a method for large scale plasmid preparation helped us to install a production platform for HTS approaches and large scale transfection for the production of gram quantities IgG within days.

Experimental Approach:
Transfections were performed according to standard protocols described in the literature. Briefly, 5x10E6 cells/mL were transfected with 2 pg DNA/cell and INVect or 25 kDa PEI. Cultivations were carried out using shake flasks under standard conditions. Transfection efficiency was determined via flow cytometry and yields were quantified by SEAP assay or protein-A affinity chromatography. Bulk-sorts were performed using a Bio-Rad S3 cell sorter and DoE was used for process optimization.

Results and Discussion:
Several E.coli strains and media were screened for high productivity, high quality and flexibility for DNA preparation in comparison to commercial kits. Afterwards a purification process was implemented using a weak anion exchanger. Up-scaling this process to 50 L culture volume results in approx. 250 mg purified plasmid DNA with low endotoxin level. However, to our surprise, endotoxin levels up to 50,000 EU/mL do neither influence viability, transfection efficiency nor productivity. Starting from a simple basal medium we were able to generate a novel medium, which supports transient transfection and high titer transient gene expression. Improvements were achieved by stepwise and/or block wise screening and optimization of media ingredients due to higher transfection efficiency and/or productivity. To generate an optimized host cell line for TGE processes we utilized FACS-assisted evolution, which results in a threefold increase in IgG productivity.

In combination with the recently developed transfection reagent INVect, it was possible to generate the first high-yielding “pseudo” perfusion TGE production process. This enables space time yields exceeding 200 mg IgG per L and day. Because of the low toxicity of INVect, transfection and cultivation at ultra-high densities (about 1-4x10E7 cells per ml) and extreme-high densities (about 8x10E7 cells per ml) was possible. This performance is highly desirable for the production of labile products like vaccines. Furthermore we work on a simplified procedure of the production process using concentrated feed supplement. First screenings in which VCD, amount of DNA, feed volume and transfection enhancer were varied showed promising results with titers up to 680 mg/L.
DEVELOPMENT OF IGGS PURIFICATION PROCESS USING MONOLITHIC COLUMNS: A TECHNOLOGICAL PLATFORM

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Background and Novelty:
The NS0 mouse myeloma cell line has become one of the most popular systems for large-scale heterologous protein expression, especially recombinant proteins and monoclonal antibodies. The Center of Molecular Immunology is one of the most important Cuban centers specialized in the production of monoclonal antibodies (Mabs) for therapeutic use in cancer patients. IgG purification process usually consists in a capture step by affinity chromatography followed by anionic and cationic exchange chromatography, in order to obtain pure molecules with high recovery.

Experimental Approach:
CIM protein A, QA and SO3 disks monolithic column and different mobile phases were examined during the development of purification process. Three different monoclonal antibodies were used during the study. The removal of contaminants like DNA, protein A and HCP were followed during the processes. Also, the purity and aggregation presence were tested in the study.

Results and Discussion:
The quantification of IgG in supernatant was established and the characterization of final product was performed. The purity of product and DNA removal were monitored with SDS-PAGE (presence of aggregation or degradation and presence of proteic contaminants) and picoGreen assay (DNA removal).
APPROACHES TO IMPROVING PRODUCT QUALITY AND SAFETY USING NEXT GENERATION SEQUENCING

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Background and Novelty:
From cell line development and engineering to biological safety - Next generation sequencing (NGS) technologies are being applied across the product life cycle from initial clone selection, manufacturing scale-up to QC release of drug product. This presentation considers various applications of NGS and how such technologies are improving product quality and safety.

Experimental Approach:
The testing approaches discussed within this presentation are based on experimental data generated using Roche 454 Life Science FLX/FLX+ and the Illumina MiSeq Platforms.

Results and Discussion:
For the production of recombinant protein therapies, stable expression and maintaining product quality are important requirements and therefore clone selection and cell bank characterisation are critical aspects of product development and manufacture. There are trends towards applying NGS to clone selection prior to MCB production to provide valuable information on genomic integration loci, copy number and mRNA transgene expression, thereby allowing faster identification of high producing clones and generating valuable data to support future validation. As clones are selected and cell banks are produced, NGS is finding a role in GMP genetic characterisation and stability comparisons.
As modern technologies and scientific advancements change the landscape of biologics development and manufacture, along with it we must consider how we look at safety. From characterisation of novel cell substrates to screening of raw materials and final products, NGS methods have demonstrated unbiased and sensitive detection of contaminating agents and are likely to play an increasing role in evaluating safety of biological products in the future.
NOVEL TECHNOLOGIES FOR THE DETECTION OF ADVENTITIOUS GENTS IN BIOLOGICS PRODUCTS

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Background and Novelty:
The advent of next generation (e.g. massively parallel) sequencing technologies has revolutionised the field of bio-safety testing. This technology has become an adjunct to traditional nucleic acid based safety testing, supporting vaccine and therapeutic development by providing rapid and unbiased means to evaluate critical processing materials for a range of adventitious agents that otherwise would remain undetected. The use of this technology in assessment of contamination of vaccines and biologics by adventitious a viruses has been gathering interest as an alternate system for assessment of such materials used throughout the biological product lifecycle.

Experimental Approach:
Examination of traditional in vitro technologies and also next generation sequencing methods in the context of bio-safety testing of biological products has been assessed and experiments have been performed to enhance the sensitivity and specificity of such tests. These experiments have challenged known viral agents which have been implicated as contaminants of host cell systems and used varying concentrations of agent to report on sensitivity of the tests methods.

Results and Discussion:
The results from studies utilising alternate methods for detection of adventitious agents; classical in vitro methods and molecular based sequencing methods will be experimentally challenged and data generated from limit of detection and specificity experiments will be reported. The use of such systems, with a view to providing a proven quality approach coupled with the ability to reduce time to market for biologics products will be presented.
PERFUSION PROCESS OF HUMAN MYOGENIC STEM CELLS IN ELECTROSPUN NANOFIBER SCAFFOLD-BASED MINI-BIOREACTOR

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Background and Novelty:
Stem cells bear an enormous promise for future therapy and have already shown their efficacy in numerous clinical trials. Today’s research in stem cell-based therapy focuses on identifying the appropriate cells with the targeted properties of biological efficacy, differentiation, phenotype and safety. The state-of-the-art methods for stem cells expanding and differentiation rely on 2D static culture protocols, which are highly labour consuming, inefficient and lacking reproducibility. To meet the demand of health care addressing life-threatening diseases by cell therapy, new methods and equipment to enlarge the manufacturing capability of these cells under controlled conditions are urgently needed.

Our ultimate goal is to create a new perfusion bioreactor supporting the culture of human stem cells adhering on electrospun nanofiber scaffold (ENF) of biocompatible and biodegradable polymer. In the present study, we aim at developing scale-down mini-bioreactors, and use them to develop and optimize a perfusion process of human stem cells with myogenic progenitor potential grown in ENF.

Experimental Approach:
A perfusion process sustaining human myogenic stem cell culture is developed. The cells are monitored by immunocytochemistry and RT-PCR, and their metabolism, i.e. metabolite concentrations, by analyser Bioprofile FLEX.

Results and Discussion:
Cell-supporting ENF scaffolds are developed and screened for optimal materials and structure properties. Eight mini-bioreactors are created and used in parallel for the development and optimization of a perfusion process sustaining human myogenic stem cell expansion. The process is optimized for several parameters like the medium recirculation rate, the recirculation direction, the perfusion rate, pH, DO, etc. Various immunocytochemistry analyses and other assays are studied for the cell quantification in the scaffolds during the cultivation and/or at end-point.

Acknowledgement:
This project is supported by FP7-Health-2013-601700 program, acronym HESUB.
CHARACTERIZATION AND SCALABILITY OF THE MOBIUS® CELLREADY SINGLE-USE BIOREACTORS FROM BENCH-SCALE TO CLINICAL SCALE

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Background and Novelty:
With the increased adoption of single-use bioreactor platforms in upstream biomanufacturing processes, there is a need to expand single-use offerings to include larger production equipment to complement existing smaller bench and pilot scales systems. In order to implement successfully a large scale single-use bioreactor platform, its ability to obtain equivalent performance, i.e., scalability, with smaller sized bioreactors across the platform must be considered during its design and ultimately demonstrated. In addition to the Mobius® CellReady bench-scale (3 L), small-scale (50 L) and pilot-scale (200 L) platform of bioreactors targeting mammalian cell growth and recombinant protein production applications, EMD Millipore has recently developed a single-use clinical scale (2000 L) bioreactor. This 2000 L single-use bioreactor system has been designed to deliver scalability within the Mobius® CellReady platform while enhancing the manufacturing process volume range.

Experimental Approach:
One approach used for the successful scale-up of a biomanufacturing process across a bioreactor platform is to maintain geometric similarity between the vessels, such as tank aspect ratio, impeller placement and design, sparger type and location as well as the inclusion of an appropriate baffle system. However, it has been shown that even when two geometrically similar vessels are used it is not always possible to simultaneously maintain key bioreactor performance parameters such as shear, mixing time, and k, a identical in both the large and small vessels. Therefore, successfully scale-up of a biomanufacturing process also must consider the effects of critical process parameters such as gas flow rates and impeller agitation speed on the cell culture environment across the multi-volumetric bioreactor systems. As such, the performance design space of the entire Mobius® CellReady bioreactor platform was characterized using several key engineering parameters including oxygen mass transfer coefficient (k, a), power per unit volume, Reynolds number (Re), mixing time and tip speed. Based on a detailed understanding of the dynamic performance capabilities of each bioreactor system across the platform, appropriate process parameters can be selected to achieve scalable performance.

Results and Discussion:
The results of this study define the performance design space of the Mobius® CellReady 2000 L bioreactor system and demonstrate the capability to achieve the expected cell culture performance results across scales. The work demonstrates that a scale-up approach based upon geometric scaling and established design conventions is often insufficient to insure the required scalable performance. Therefore, the approach should also employ sufficient iterative testing and design refinement during system development to insure that performance targets can be met. This detailed examination of the cell culture environment from an applications perspective thus demonstrates the scalability of the family of Mobius® CellReady bioreactor systems that have been developed using this approach.
BIOINFORMATICS ANALYSIS OF THE CHO PROTEOME

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Background and Novelty:
Chinese hamster ovary (CHO) cells are the host cell line of choice for production of a variety of biologicals including multiple antibody formats. The endogenous CHO proteins can play key roles in the bioprocessing of these proteins. In order to characterize the function and properties of the endogenous proteins an intensive bioinformatics analysis was performed on the previously published CHO cell proteomics data.

Experimental Approach:
The proteomics data, used in this study, is based on analysis of different fractions from CHO-K1 cultures and its subjection to an in-solution digestion followed by LC-LC/MS/MS analysis. Grouped proteins were identified and in order to elucidate the functional characteristics of these proteins, multiple bioinformatics tools were applied including WoLF PSORT and TMHMM.

Results and Discussion:
The identified and characterized proteins included those that play roles in cell growth, proliferation, and folding as well as those involved in degradation and removal of other proteins. As part of this effort, we also created a publically accessible web-based tool called GO-CHO to functionally annotate the proteins in the three GO categories of CHO cells. This work and database will enable the CHO community to rapidly functionally categorize proteins in their cultures in order to facilitate biopharmaceutical processing efforts in the future.
iTRAQ COUPLED PROTEOMICS REVEALS THE BIOMARKERS AND MECHANISM OF LOW PRODUCTIVITY AND INSTABILITY OF BISPECIFIC ANTIBODY PRODUCTION IN CHO CELLS

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Background and Novelty:
The generation of stable and high-producing cell lines is required for the delivery of affordable drugs to patients. However, the complexity of bispecific antibodies and new biologics formats may pose manufacturing challenges for expression and protein quality as well as long-term stability in production cell lines. The comparison of both mRNA and protein levels of host cell genes is beginning to provide a better understanding of key bottlenecks in CHO secretory and physiological pathways.

Experimental Approach:
Recent developments in the mass spec-based technologies provide enhanced detection of cellular proteins for identification and relative quantification purposes. In this study, comparative proteomics and transcriptomics have been used to quantify the expression levels of thousands of genes.

Results and Discussion:
Coupling these two omics strategies has allowed us to monitor the expression of 12,000 genes in different bispecific expressing CHO cell lines. In order to investigate potential mechanisms for low productivity and instability of these cell lines, we studied the influence of culture conditions, clonal and passage differences by using Illumina sequencing and iTRAQ coupled proteomics for understanding the differences in host cell proteins. This work has revealed potential dysregulation of CHO physiological pathways provides insight into identifying biomarkers of instability and suggests potential strategies to improve production and stability through host cell engineering approaches.
REINVENTING CULTURE MEDIA THROUGH CELL FUNCTIONAL ENVIROMICS

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Background and Novelty:
Cell functional enviromics [1,2] may be defined as the systematic analysis of the effect of the totality of environmental factors on the totality of cellular functions. In this work, it will be presented how cell functional enviromics can be applied to design the composition of culture media, which otherwise has been an empirical discipline. While traditional trial and error experiments and even DoE are done very inefficiently, cell functional enviromics elucidates the role of medium ingredients in terms of up- or down-regulation of cellular functions, providing the knowledge that ultimately enables the “engineering” of novel culture media formulations tailored to boost growth and productivity.

Experimental Approach:
The platform is built upon a high performance basal formulation, a set of metabolically optimized supplements, and a bioinformatics toolbox for multi-objective composition optimization. Our bioinformatics toolbox uses information of the target protein and of physical process constraints to design a customized formula for the target protein and process.

Results and Discussion:
The broad applicability of this platform will be illustrated with examples of animal CHO cells as well as the yeast Pichia pastoris. Basal media formulations for each organism were developed and successfully benchmarked against relevant commercial competitors on both small scale shake flask experiments and bioreactor validation runs up to 50 L. Initial model-driven optimization was based on detailed metabolic descriptions of each cell type, allowing to design a set of supplements to the basal medium tailored to explore the boundaries of the biological system in terms of growth and productivity. Our bioinformatics toolbox further organizes metabolic and process data into functional enviromics maps describing the individual effect of medium ingredients on cellular functions, offering a faster and more efficient way to achieve thorough medium optimization and customization.

References:
NEW THERAPEUTIC APPROACHES BASED ON ADVANCES IN CELL BIOLOGY AND MEDICINE
OFF-THE-SHELF MESENCHYMAL STROMAL CELLS DERIVED FROM UMBILICAL CORD TISSUE

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Background and Novelty:
Human Mesenchymal Stromal Cells (MSC) are multipotent cells residing in all support and structural tissues of the organism capable of extensive expansion in vitro while preserving their identity, potency and genetic stability.
Umbilical cord-derived MSC (UC-MSC) are more primitive progenitor cells than those isolated from bone marrow or lipoaspirates and this makes them candidates for the generation of a cell bank for later use in the allogeneic treatment context as off-the-shelf product.

Experimental Approach:
In the present study, a simple method for UC-MSC derivation from umbilical cord tissue was developed and a two-tiered system of Master Cell Bank and Working Cell Bank (compatible with the Good Manufacturing Practice-compliant production environment) is proposed.
UC-MSC derived following this method were extensively characterised as follows: morphology, cell growth and metabolic profile, immunophenotype, multipotentiality, immunomodulation capacity, genetic stability, in vivo subchronic toxicity assessment, in vivo biodistribution.

Results and Discussion:
Adherent cells resulting from the mechanical disruption of source tissue were subsequently expanded using uncoated plastic yielding $\geq5 \times 10^6$ UC-MSC within 17.3 $\pm$ 3.4 days from the start of the procedure with a success rate of 92% (n=12). Parameters affecting such success rate included weight and length of the tissue fragment and time from birth to tissue processing. UC-MSC derived following our protocol were 97.5%±2.3% CD90, 0.9%±0.4%CD45, 97.9%±1.6%CD73, 0.7%±0.3 CD31, 97.6%±1.3 CD105, and 0.5%±0.3 HLA-DR (n=6).
UC-MSC’s immunophenotype was maintained over prolonged cell culture expansion (up to 40 cell population doublings), which is required for the generation of clinical grade Master Cell Bank, Working Cell Bank and Drug Product.
No genetic abnormalities were detected by G-banding karyotype and no toxicity was observed after either intravenous or intrathecal administration of UC-MSC, as determined in a subchronic model of safety assessment in immunodeficient mice.
UC-MSC produced following the method described in this work displayed immunomodulatory properties that could be exploited in clinical settings.
DEVELOPMENT OF A TOOL BOX FOR MESENCHYMAL STEM CELL SECRETOME ANALYSIS

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Background and Novelty:
The development of suitable cultivation strategies of MSC is one major field in the area of tissue engineering and cell based therapies. Since the physiological “home” of stem cells is represented by dynamic 3D environment these conditions shall be applied when expanding and differentiating MSC in vitro. This also includes the application of physiological oxygen concentration (e.g. 3-8% for most tissues). A lot of research work is dedicated to the analysis of distinct signaling pathways through cytokine expression profiles and growth factor effects.

Experimental Approach:
In our study we investigate the effect of different cultivation conditions towards the composition of the secretome of MSC. Therefore a proteomics approach was applied addressing the following questions:

1. characterization and detection of changes in the secretome of mesenchymal stem cells
2. analysis of low abundant secretome proteins after removal of high-abundance proteins from the cell medium (affinity enrichment and depletion) and
3. comparison of the secretome after normoxic and hypoxic cultivation using a DIGE approach.

Results and Discussion:
The first step was the evaluation of the method for protein precipitation and 2D-GE had to be established. Sample preparation revealed to be highly reproducible, but the detection to visualization of low-abundance proteins remained difficult. Therefore, high-abundance serum proteins had to be significantly removed without losing valuable secreted proteins. An effective depletion of serum proteins and thus reduction of high-abundance proteins was observed resulting in a reduced number of protein bands in 1D and a lower number of spots in 2D PAGE was achieved after utilizing Top12 depletion spin columns. The high possibility of co-depletion of low abundant proteins was considered to be very likely. Therefore the combinatorial peptide library was also tested, but showed a lower efficiency for serum protein removal. Serum albumin was successfully removed to small concentrations. Finally DIGE analysis was introduced to point out proteome differences between the normoxic and hypoxic secretome of mesenchymal stem cells. Overall it can be said that both methods efficiently reduced high abundance serum proteins giving access to the interesting secretome of mesenchymal stem cells. Future work will focus on the identification of differentially expressed proteins by mass spectrometry.
MAGNETIC POLYCAUTION-GRAFTED CORE-SHELL-CORONA NANO-PARTICLES AS TOOLS FOR STUDYING EVENTS IN NON-VIRAL GENE DELIVERY

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Background and Novelty:
The transfer of genetic information into mammalian cells is a crucial step in genetic engineering and recombinant protein production. Over the last decade, polycations have become promising candidates for non-viral gene delivery, in particular since such polymers can be produced with high control over composition and architecture. As polycations, these agents readily form stable polyelectrolyte complexes (“polyplexes”) with polyanions such as pDNA, which can be shown to enter most mammalian cells upon contact with the cellular membrane. However, the exact mechanism(s) of cellular uptake and even more so of the subsequent steps and sequences of intracellular trafficking, pDNA release, nuclear entry, etc. are still under considerable debate. In the past, we have introduced “Nano-Stars”, i.e. PDMAEMA-based Core–Shell–Corona nano-particles, as superior gene delivery vehicles compared to the traditional PEI-based polycationic transfection agents. In one manifestation, these Nano-Stars were based on a paramagnetic maghemite (γ-Fe₂O₃) core (Majewski et al., 2012, 2013). These agents have since proven themselves as excellent tools for the investigation of the mechanisms of non-viral gene delivery, as they allow, e.g., the study of the nanoparticle internalization kinetics and the analysis of cell components bound to the polymers via magnetic pull down. For an in depth study of the structure-function relationship, a library of highly homogeneous magnetic Nano-stars with varied arm length and grafting density was subsequently synthesized.

Experimental Approach:
Based on this library, gene delivery experiments were carried out with different fibroblast cell lines (CHO-K1, L929), using a pEGFP-N1 based transfection vector (enhanced green fluorescent protein as reporter for successfully transfected cells). Transfection efficiencies and cytotoxicity (via propidium iodide counterstaining) were determined by flow cytometry. Magnetic sorting of the transfected cells was performed using commercially available systems.

Results and Discussion:
We observed a high variance in the transfection efficiency and toxicity of the Nano-stars dependent on their structure. The best performing Nano-star showed a high efficiency of gene delivery with > 70% transfected cells together with a high viability of the cells (> 95%). Initial polyplex internalization happened quickly (< 15 min), but a minimum of 180 min contact time was necessary to reach maximal transfection efficiency. We then used magnetic cell sorting to separate magnetic and non-magnetic cells. Interestingly, transfection efficiency did not correlate to the percentage of macroscopically “magnetic” cells.

EX VIVO ERYTHROID DIFFERENTIATION IN CULTURE OF HUMAN RECOMBINANT HEMATOPOIETIC STEM CELLS

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Background and Novelty:
The in vitro generation of red blood cells (RBCs) from human hematopoietic stem cells (HSCs) could represent an alternative to classic transfusion products. Despite the advances in multiple technological approaches, problems related with large-scale production and cost effectiveness have to be overcome in order to allow clinical availability. Culture medium is excessively expensive, with growth factors representing almost 60% of the price, and human erythropoietin (hEPO), representing 33% of the total price. The aim of this work was to genetically modify HSCs to produce recombinant hEPO, and to analyze its in vitro effect over cell proliferation and differentiation towards erythroid cells. This strategy might lead to a reduction in the amount of hEPO to be added to the medium.

Experimental Approach:
CD34+ HSCs were obtained from peripheral blood and umbilical cord blood through density gradient centrifugation and immunomagnetic separation. In order to generate the recombinant hEPO-producing cells (E-HSCs), CD34+ HSCs were transduced using self-inactivating lentiviral vectors containing the hEPO sequence, after a pre-stimulation with a cytokine cocktail. A negative control of non-modified HSCs was used. In each case the M.O.I was 4 TU/cell.
Proliferation and erythroid differentiation capabilities were assessed through semisolid culture in medium StemMACS HSC-CFU basic, supplemented with hIL-3 and hSCF, both in presence and in absence of exogenous hEPO. Colony counting and classification was performed and hEPO concentration in the culture supernatant was determined by sandwich ELISA.
In addition, suspension cultures of E-HSCs in absence of exogenous hEPO, and non-modified HSCs in presence of hEPO were executed. After 15 days-cultures cell morphology was examined by May Grünwald- Giemsa staining.

Results and Discussion:
For peripheral blood derived-HSCs the results showed that, the development of erythroid colonies was higher for the E-HSCs, cultured both in presence and in absence of hEPO (62% and 70%, respectively). Meanwhile, the non-modified cells developed colonies only in the medium supplemented with hEPO (16% of erythroid colonies).
The increased erythroid differentiation in E-HSCs cultures is related with the higher concentrations of hEPO determined in supernatants. In this regard, 5.2 ng/ml of hEPO were detected in the supernatant of E-HSCs cultured in absence of hEPO. The protein concentration was too low to be quantified in non-modified HSCs cultures.
In the case of umbilical cord blood-derived HSCs, the development of erythroid colonies was 20% both in E-HSCs cultures and in non-modified HSCs cultures supplemented with hEPO.
Likewise, in suspension cultures, the non-modified HSCs supplemented with hEPO and the E-HSCs cultured in absence of hEPO, proliferated and reached cell populations mainly composed by orthochromatic normoblasts and reticulocytes.
Altogether, these results demonstrate that it is possible to develop culture systems in which recombinant HSCs are total or partial self-suppliers of hEPO, leading to an important cost reduction of the culture medium. This strategy may be of interest in biotechnological processes intended for the in vitro production of RBCs, which are currently in development and optimization stages.
INTEGRATED STRATEGIES FOR THE PRODUCTION AND STORAGE OF FUNCTIONAL CARDIOMYOCYTES DERIVED FROM HUMAN PLURIPOTENT STEM CELLS

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Background and Novelty:
The ability of human pluripotent stem cells (hPSC) to self-renew indefinitely in culture and to differentiate into any somatic cell type makes them a powerful unlimited source of cardiomyocytes (CM) suitable for regeneration therapies, disease modeling and cardiotoxicity testing [1]. However, the complex nets of signaling pathways involved in cardiomyogenensis as well as the line-to-line variability compromise the effectiveness of the existing differentiation protocols to reproducibly produce high-quality CM from multiple hPSC lines. Moreover, the applicability of these cells in the clinical/industrial settings is highly dependent on the development of novel and efficient methods that allow worldwide shipment and long-term banking of the produced CM until later use. In this study we aim to overcome these hurdles by devising an integrated strategy for scalable production and storage of functional CM derived from distinct hPSC lines.

Experimental Approach:
hPSC were cultured as aggregates in environmentally controlled bioreactors, where the necessary conditions to control stem cell fate are tightly tuned [2]. Dissolved oxygen and hydrodynamic forces (promoted by intermittent stirring or a wave induced agitation) were manipulated in order to improve CM differentiation yields [3]. The differentiation process was monitored using fluorescence microscopy, flow cytometry, quantitative RT-PCR, transcriptomic and metabolomic tools. Novel strategies for cryopreservation and hypothermic storage of either 2D monolayers of CM or 3D cardiospheres were also explored. After storage the ultrastructure and functionality of the CM were assessed by TEM and electrophysiology analyses, respectively.

Results and Discussion:
Our results showed that hypoxia and hydrodynamics forces are key parameters in the bioprocessing of hPSC, affecting cell differentiation towards functional CM. The bioreactor protocol herein described (i.e. the controlled hypoxic and specific hydrodynamic environment) improved hPSC differentiation towards CM by enhancing culture homogeneity, process reproducibility, CM yields and productivities. Cell characterization and functional analysis confirmed that the produced CM presented typical cardiac morphology, calcium transients, electrophysiological profiles and drug responsiveness. Metabolomic and transcriptomic analyses are being applied along the differentiation process to disclose which pathways are differentially activated and/or repressed in low versus high yielding bioprocess conditions.
Furthermore, efficient methods for cryopreservation of pure CM were developed. Using xeno-free cryopreservation formulations and a controlled slow freezing rate protocol, significantly higher cell viabilities and recoveries were obtained after thawing. In addition, we showed that monolayers of CM and cardiospheres can be stored up to 7 days at hypothermic conditions without compromising cell viability, morphology and functionality.
In summary, this work describes significant advances towards mass production of hPSC-derived CM and their short- and long-term storage, meeting some of the currently needs of the cardiac regenerative medicine market and industrial field.

References:
NEW NANOMATERIALS THAT MIMICK PEI FOR DELIVERY INTO CELLS

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Background and Novelty:
The controlled delivery of genetic material or drugs into eukaryotic cells has been the focus of interdisciplinary scientific activities during the last two decades. Beside evolutionary qualified and very efficient viral transfection, non-viral delivery is of high interest, reflected in the large number of e.g. non-viral transfection agents being proposed. Thereby, the cationic polymer poly (ethylene imine) (PEI) represents the “gold standard” for in vitro applications. Several studies show that the polymer architecture and the overall molar mass have major impact on the transfection efficiency.\cite{1,2} Beside, the influence of buffer capacity and complexation strength of polymers has been studied to understand the superiority of branched PEI, containing primary, secondary and tertiary amines.\cite{3}

Experimental Approach:
In this study we show the synthesis of new linear primary, secondary and tertiary amines containing methacrylates using the reversible addition-fragmentation chain transfer- (RAFT) polymerization technique. For this purpose, 2-aminoethylmethacrylate (AEMA), N-methyl-2-aminoethylmethacrylate (MAEMA), N-tert-butyl-2-aminoethylmethacrylate (tBAEMA) and the well-studied N-dimethyl-2-aminoethylmethacrylate (DMAEMA) were synthesized as homo-, di- and terpolymers (block/statistical) and tested for encapsulating and complexation as well as transfection of DNA.

Results and Discussion:
Copolymerized with hydrophobic groups the materials show superior drug release behavior depending on the pH value. Using only cationic monomers, genetic materials can be transferred. Therefore the transfection efficiency will be compared to the ratio of primary, secondary, and tertiary amines as part of this work. Furthermore the materials are investigated regarding the influence of DNA complexation versus buffer capacity, providing also an indication of the thin line between transfection efficiency and cytotoxicity.

Bibliography and Acknowledgments:
TAILOR-MADE POLYMERS AS BUILDING BLOCKS FOR NANOCARRIERS WITH SUPERIOR DELIVERY POTENTIAL


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Background and Novelties:
One vision of the Nobel Prize winner and father of the modern polymer chemistry Hermann Staudinger was that scientists of different disciplines work together to exploit the potential of polymers, in particular for biology and medicine. Nowadays, polymers can be synthesized in a controlled manner and are able to form nanoparticles or micelles via self-assembly. These nano-scaled objects are in the focus of our research, from synthesis and characterization to applications. In this study we will show the tremendously developments on the side of material science and how this can be applied in biological systems and nanomedicine. Therefore, different synthetic polymers were used.

Experimental Approach:
As first example multicompartiment micelles from stimuli-responsive triblock terpolymers is presented as non-viral delivery agent.1 The surface of the micelle reminiscent to those of viruses because of their patchy shell that showed a strong pH-dependency (Scheme 1). Superior transfection efficiencies were observed for both adherent and suspension cells. Furthermore, detailed investigations on the transfection mechanism were performed. The concept of enhanced cellular interactions because of different charged surface of nanocarriers is investigated in detail in a further example. Here, we used a versatile ABC triblock terpolymer platform with different functionalization of the B segment for introduction of hydroxyl, amino, and carboxy groups.2 The prepared sub-30 nm micelles are based on a hydrophobic core for drug delivery applications, a shell with different functionalization, and a PEG corona preventing undesired interactions. Additionally, co-assembly of differently functionalized polymers is investigated. This allows the design of nanocarriers with a mixed shell and adjustable features.

As highlight, the targeted and efficient delivery of siRNA by highly specific nanoparticles in vivo based on a platform technology is presented.3 As targeting moiety a near infrared fluorescent polymethine dye were used, coupled to biodegradable polymers and expressed on the surface of nanoparticles. The dye mimics a ligand for transporters in hepatocytes leading to fast and efficient uptake of the dye (single or coupled). Body distribution, hepatocyte uptake and excretion into bile were investigated by intravital microscopy or even non-invasively by multispectral optoacoustic tomography. A reduction of cholesterol levels was observed in vivo, when using HMG-CoA reductase siRNA encapsulated into the nanoparticles.

Results and Discussion:
These examples will provide an interesting overview of the potential of polymers for the delivery of active components as plasmid DNA, small molecule drugs or siRNA into cells, also interesting for biotechnological applications.

Bibliography:
APPLICATION OF FILTRATION METHODOLOGIES FOR THE DOWNSTREAM PROCESSING OF HUMAN MESENCHYMAL STEM CELLS

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Background and Novelty:
Currently human mesenchymal stem cells (hMSC) are expanded using planar technologies or microcarrier-based stirred culture systems from one to hundreds of liters of culture volume, to guarantee the required cell numbers to be delivered to the clinic. Such culture volumes need to be concentrated and washed without compromising the cells’ characteristics. Tangential flow filtration (TFF) is a well established technology that due to its several advantages (e.g. integration, flexibility, automation, GMP compliant), arises as an attractive solution for cell therapy downstream processing (DSP). Nevertheless, the impact of TFF process parameters on cells’ characteristics and quality needs assessment.

Experimental Approach:
The aim of this work was to develop a scalable integrated strategy for the concentration and washing of hMSC using TFF technology. More specifically, we have evaluated the impact of i) several TFF’s process parameters (e.g. membrane material and pore size, shear rate and permeate flux) and ii) two operation modes (continuous and discontinuous) on cells’ quality (i.e. cell morphology, viability, identity and potency) and recovery yield. Two-dimensional gel electrophoresis and mass spectrometry-based proteomic approaches were also used to characterize hMSC’s total proteome profile during processing.

Results and Discussion:
Focusing on the cell concentration step, our results show that hMSC could be successfully concentrated up to a factor of 20 while maintaining their identity, potency and high cell viability, allowing for the recovery of over 80% of viable cells; polysulfone membranes with pore sizes higher than 0.45 μm were identified to be key conditions to obtain such concentration factors; shear rate and permeate flux were also shown to impact the cells’ recovery yields, viability, cell adhesion and proliferation capacity. Furthermore, a mathematical model was developed for predicting the mass balance throughout time in both discontinuous and continuous TFF processes. The operation mode did not impact cell recovery yield (80%) and cell viability (95%); however, higher cell concentrations were achieved faster when operating TFF in a continuous mode, as validated experimentally.

Regarding cell washing, we have integrated a diafiltration step, and two operation modes (discontinuous and continuous) were evaluated; the number of diafiltration volumes necessary to wash out protein impurities from the concentrated cell suspension was assessed. Our results showed that continuous diafiltration operation mode allowed a higher clearance of protein impurities (98%) and recovery yields of viable cells (80%), than discontinuous diafiltration (93% and 60%, respectively). At the end of the integrated DSP strategy, hMSC maintained their morphology, viability, proliferation capacity and immunophenotype, and presented multilineage differentiation potential.

Overall, this work shows that TFF is an efficient methodology for the concentration and diafiltration of hMSC that can be incorporated in the biomanufacturing workflow of cell-based therapies. Moreover, by knowing the effects of each of the parameters on the process and on cells’ quality, more rational approaches can be developed to further increase the concentration factor up to 50. The described DSP strategy will have applicability to other stem cell types (e.g. human pluripotent stem cells) relevant for the cell therapy industry.
EXPLORING ANALYTICAL PROTEOMICS PLATFORMS TO DEFINE THE ROLE OF HUMAN CARDIAC STEM CELLS IN MYOCARDIUM REPAIR

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Background and Novelty:
Cardiovascular diseases (CVDs) are the leading cause of death worldwide. Myocardial infarction (AMI) severely affects patients’ heart muscle and microvasculature, critically decreasing the number of functional cardiomyocytes (CM). Stem cell and protein based therapies became promising cardiac repair strategies since it was found that under pathological stress, resident cardiac stem cells (CSC) of the adult myocardium are activated by growth factors (GF) secreted by the surviving CM. Consequently, an auto/paracrine loop is triggered to maintain GF production, which enhances CSC activation and differentiation into new CM, endothelial and smooth muscle cells contributing to the repair of damaged myocardium [1]. Since this repopulation of the myocardium is neither robust nor durable enough to have significant beneficial physiological/anatomical impact in severe and acute myocardial losses, local administration of GF has been shown to be efficient in enhancing CSC activation, improving cardiac output post-AMI through the formation of new vascularized and functional autologous myocardium [2, 3]. Aiming at supporting the development of allogeneic cell-based therapies and providing new insights about molecules/pathways involved in cardiac repair, we investigated hCSCs receptome and secretome profiles.

Experimental Approach:
hCSC cultivated in environmentally controlled stirred tank bioreactors were characterized regarding cell viability, metabolism, phenotype, GF secretion and differentiation potential. A high-throughput proteomics workflow was implemented, enabling identification of low abundance (receptors and GF) and highly hydrophobic proteins (membrane proteins-receptors). Enrichment of plasma membrane proteins was performed prior to nanoLC-MS (Orbitrap-Elite) for receptorome analysis. For secretome characterization, conditioned medium of hCSC cultures was collected. Samples were run in a SDS-PAGE gel, each lane sliced in several fractions, individually digested and fractionated by nanoLC. The entire run was collected and spotted for further MS analysis (MALDI-TOF/TOF).

Results and Discussion:
hCSC cultured in bioreactors remained phenotypically and functionally similar to cells cultured in static culture systems. Receptome analyses lead to the identification of more than 2000 proteins/replicate, several hundred with numerous predicted transmembrane domains (e.g. ATPase, Ca++ transporting, cardiac muscle slow twitch-2; Connexin-43), from which around 100 were plasma membrane receptors (e.g. insulin-like growth factor 2 receptor; Interleukin-6 receptor subunit beta). Cardiovascular system development and function was the top hit of functional analysis by IPA software. Secretome data analysis is on-going, with about 300 human proteins identified up to now (e.g. Retinol-binding protein-4; IGF-binding protein-7). Proteomics approaches implemented allowed the identification of a wide-ranging list of receptors and secreted factors that are currently being further investigated. Furthermore, to better mimic the ischemia/reperfusion situation we are using controlled culture systems to simulate an AMI in vitro, aiming at understanding the regulatory cascades underneath hCSC activation and GF secretion.

References:

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EX VIVO PRODUCTION OF RED BLOOD CELLS FROM HUMAN CORD BLOOD

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Background and Novelty:
Blood transfusion is the only therapeutic approach that is clinically effective for treating oxygen transport deficits (i.e. blood loss in surgical interventions and anaemia). However an imbalance between the supply and demand of blood is predicted for the near future due to aging population, an increase in the transmission of infectious diseases, limited compatibility of stored stocks and the requirement for rare blood groups. This situation has a direct impact in Public Health and it has consequently spurred the development of novel technologies for blood substitutes. The candidate products for human use should be safe, display adequate profiles for the uptake, transport and delivery of oxygen, a prolonged half life in the bloodstream, stability at room temperature that would facilitate cost-effective storage and it must be manufactured under GMP quality standards. Given the potential of stem/progenitor cells to become erythrocytes under controlled in vitro conditions, we developed a bioprocess for producing blood ex vivo.

Experimental Approach:
The expansion strategy lasted 21 days and it was divided in three phases: 1) CD34+ isolation from a fresh cord blood unit using magnetic beads, 2) CD45+ progenitors expansion using EPO/SCF/IL3/hydrocortisone-supplemented media and 3) a subsequent maturation and enucleation of erythroblasts into erythrocytes (CD45+ , CD36+, CD235+ and CD71+) using EPO/SCF-supplemented media. Nucleated cells were then removed from the final product using leukodepletion filters. The final product was characterized by cytometry, HPLC (haemoglobin content) and microscopy.

Results and Discussion:
We developed a two-stage bioprocess in 250 mL stirred bioreactors and using CD34+ hematopoietic stem cells isolated from cord blood as starting material. Biconcave morphology of in vitro-produced erythrocytes was confirmed and haemoglobin content resulted in 53±12% foetal Hb and 26±8% adult Hb.
Further work will focus on bioprocess optimisation and scale up to a clinically significant dose.
DYNAMIC CULTIVATION OF HUMAN STEM CELLS UNDER PHYSIOLOGICAL CONDITIONS

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Background and Novelty:
The development of stable tissue-engineered autologous bone grafts in the field of regenerative medicine is still a challenge. Perfusion bioreactors not only provide continuous nutrition supply and waste removal, but are also suitable for the controlled application of mechanical forces like fluid shear stress. Mechanical loading is known to cause mechanotransductive effects like the induction of differentiation, resulting in enhanced deposition of extracellular matrix.

Experimental Approach:
In our study, we determined the optimal flow rate for the osteogenic differentiation of human adipose-derived mesenchymal stem cells (MSCs) by applying fluid shear stress that mimics the physiological environment normally experienced by bone progenitor cells in vivo. For this, we first analyzed the porosity of cell substrates with nanofocus-computed tomography as well as their specific permeability at different flow rates. To investigate the effect of controlled application of physiologic fluid shear stress a flow rate of 0.3 ml/min was used to cultivate MSCs in a self-developed perfusion bioreactor. Cells were seeded on a three-dimensional macro-porous zirconium dioxide ceramic scaffold and cultivated in expansion or osteogenic media under normoxic (21% O2) or hypoxic (5% O2) conditions.

Results and Discussion:
Matrix deposition (i.e. extracellular calcium and phosphate) was observed in both osteogenic and expansion media without any osteoinductive additives. The viability of cells cultivated under perfusion was considerably higher (6-fold or higher) than under static conditions. Our results show that application of physiologic fluid shear stress supports the osteogenic differentiation and viability of adipose-derived MSCs.
HIGH THROUGHPUT DEVELOPMENT AND SCALE-UP OF NON-REPLICATIVE LENTIVIRAL VECTOR PRODUCTION PROCESS BY TRANSIENT TRANSFECTION

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Background and Novelty:
The use of viral vector applications has significantly increased over the past years in various therapeutic areas such as gene therapy, cell therapy and vaccines. To answer this growing demand, viral vector production processes should gain robustness and scalability. Thus a high throughput development method has been implemented to allow fast track process optimization and scale up of non-replicative lentiviral vector production by transient transfection.

Experimental Approach:
A state-of-the-art process development strategy was put in place to develop robust and highly-productive lentiviral vector production platforms by transient transfection of cell suspension in serum-free conditions. Labscale automated representative models are developed allowing performance of large number experiments at the same time. Designs of experiments are applied to identify interaction between the optimized parameters. With the perspective of fast implementation into cGMP, disposable solutions are implemented all along the process to decrease development timelines. High throughput process monitoring tests were also developed to support associated increase in sample generation, needs for on / at line analysis

Results and Discussion:
Starting from serum-containing-adherent HEK 293T cells benchmark in CF10, a robust and efficient Serum Free process has been developed in less than 12 months. Lentiviral vector was improved up to 10 times in comparison with serum-content benchmark. Consistent results are observed from 15 ml labscale model to 200L single-use bioreactor scale.
INTRACELLULAR TRAFFICKING PATHWAYS FOR PLASMID DNA COMPLEXED WITH HIGHLY EFFICIENT ENDOsome ESCAPE POLYMERS

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Background and Novelty:
Non-viral gene delivery vectors are widely used for the delivery of genetic materials into mammalian cells. Currently, there is a need to develop cheap and efficient transfection agents for use in production of recombinant proteins via transient gene expression. There are several barriers that non-viral vectors must overcome for successful transfection, these include cellular internalisation, endosome escape, protection of DNA and delivery of DNA into the nucleus. The ability to escape the endosome and gain entry to the nucleus are of the two primary barriers to successful transfection. The processes involved in the pathways for cellular uptake, intracellular trafficking, and nuclear entry are still not fully understood. More detailed understanding of the pathways involved in transfection is needed in order to develop highly efficient transfection agents.

Experimental Approach:
This work investigates the use of three series of cationic diblock copolymers as transfection agents for the production of recombinant proteins, as well as examining the pathways the polymers used for delivery of DNA into the nucleus. The diblock copolymers were synthesised using ‘living’ radical polymerization techniques, with each series using the same first block poly(2-dimethylaminoethyl acrylate) (PDMAEA). This polymer can self-degrade through a self-catalysed hydrolysis mechanism to a negatively charge and nontoxic poly(acrylic acid) in a time-dependent manner. The second block consists of N-(3-(1H-imidazol-1-yl)propyl) acrylamide (ImPAA) or butyl acrylate (BA) or a combination of both. The three series of polymers were first tested for their ability to bind/release and protect pDNA before transfection studies occurred. Transfection studies were performed in Human Embryonic Kidney (HEK293) cells where internalisation pathways into the cell, endosomal escape and nuclear entry were investigated before recombinant protein yields determined.

Results and Discussion:
Polymer A-C3, with the second block copolymer of both the ImPAA and BA not only showed the best protection against DNase I with a timed-release mechanism between 24-48 h, but also achieved the highest level of transfection efficiency with 95% of HEK293 cells testing positive for gene expression. To understand the pathways involved in the delivery of pDNA within the cell and the nucleus several different chemical inhibitors were employed. The main internalisation pathway into the cell was determined to be clathrin-mediated endocytosis. Through the addition of chloroquine, a chemical known to swell and burst endosomes, our results show that polymer A-C3 is efficient at endosomal escape as no increase in transfection efficiency was seen. Nuclear entry of the pDNA is thought to occur either through the nuclear pores or during mitosis. To determine how the pDNA enters the nucleus, wheat germ agglutinin (which is known to block the nuclear pores) was added before transfection. Our results demonstrate that entry occurs primarily though the nuclear pores.

The results presented here attempts to improve our understanding of the pathways involved in the successful delivery of pDNA. The ability to rationally design cationic polymers to overcome the barriers to successful transfection could result in the next generation of highly efficient transfection agents used in transient gene expression systems.
HIGH THROUGHPUT PROCESS MONITORING TOOLS FOR EFFICIENT PROCESS DEVELOPMENT OF A LENTIVIRAL VECTOR

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Background and Novelty:
Gene therapy has regained opportunities for safe treatment and answers to unmet medical needs. In order to rapidly develop a manufacturing process for a gene therapy product based on a lentiviral vector platform, high throughput process monitoring methods have been developed.

Experimental Approach:
Different monitoring methods have been implemented to assess process productivity, efficiency and product quality. First, several product quantification methods have been compared so that only the best methods in terms of product attribute vs throughput were selected. Following method selection, automated solutions and new technologies have been used in order to gain throughput and fasten process development, namely nucleic acid in 96-well format and digital droplet polymerase chain reaction.

Results and Discussion:
Altogether, these adjustments enabled to increase throughput, method capability and test duration, thus providing monitoring tools adapted to high-throughput process development. Finally, the ability to test large design spaces thanks to high throughput monitoring tools has increased process knowledge, which will be an asset in process scale-up and transfer.
ESSENTIAL OIL OF MEDITERRANEAN *ROSMARINUS OFFICINALIS* L. SHOWS EFFECTS ON NEUROTRANSMITTER AND HORMONAL SECRETION BY INDUCING NEURONAL CELL DIFFERENTIATION

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Background and Novelty:
Background and Novelty:Chronic stress is one of the causes of several neuropathological illness such as depression and anxiety. According the current hypothesis concerning mechanism of pathogenesis of depression “neurogenesis hypothesis”, exposure to the stress activates the secretion level of glucocorticoids in the body, then it causes the suppression of production of neurotrophic factors such as neuronal growth factor (NGF). These situations decrease the frequency of neurogenesis in the brain which triggers several psychotic disorders. As a prescription for these disorders, anti-depressants or anxiolytics would be used, however these medicines have been reported to show serious side effects such as drug-dependence, anterograde amnesia and withdrawal symptoms. To avoid unwanted side effects, alternative medical treatments are needed. For these problems, essential oils (EOs) derived from several kinds of plants have been used as the remedy for stress or controlling mood using aromatherapy. Recently, EOs have been reported to show anti-stress effect(1) and anxiolytic effect(2). *Rosmarinus officinalis* L. (RO) has been used as folk medicine with beneficial effects on mood improvement. Ethanolic extract of Mediterranean RO and its polyphenolic compounds were reported to show anti-depressant-like effect(3) and enhancement of cholinergic activity(4). The effects of EO of RO (ROEO) on neuronal function, however, have not been revealed. In this study, the effects of Mediterranean ROEO on neuronal function and differentiation were evaluated.

Experimental Approach:
The effect of ROEO on body and neuronal function was evaluated in vivo with animals subjected to inhalation of EO and subjecting them to behavioral test. After the behavioral test, the level of hormone and neurotransmitter were evaluated. The effect of ROEO on neuronal differentiation was evaluated in vitro using PC12, a model cell line. Effect of main components of ROEO on neuronal differentiation was also evaluated.

Results and Discussion:
As shown by the results, inhalation of ROEO decreased stress-related behavior in behavioral test and modulation of the levels of hormones and neurotransmitters. *In vitro*, ROEO showed effects on neuronal outgrowth elongation and neurotransmitter secretion pathway. ROEO component, camphor showed significant promotion of a neurotransmitter secretion activity. Considering these results, promotion of neuronal cell differentiation caused by ROEO affected the function of neuronal system including neurotransmitter and hormone synthesis in the brain. For this point, a downstream factor of NGF pathway, tyrosine hydroxylase (TH) is known to be activated by neuronal differentiation, and also, TH is the rate-limiting enzyme of catecholamine synthesis. For the future study, effect on TH activity by ROEO would be evaluated.

References:
INVESTIGATION OF RECOMBINANT GENE EXPRESSION IN MAMMALIAN CELLS
AS A FUNCTION OF THE PROMOTER TYPE

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Background and Novelty:
The basis of recombinant protein production in mammalian cells is the introduction of DNA sequences into the cells’ genome (“transfection”). Successful transfection involves the efficient protection, delivery and release of pDNA up to and into the nucleus. Cationic polymers can be used as non-viral delivery agents, although transfection efficiency and biocompatibility highly depend on the polymer chemistry and structure. In recent years, we showed that PDMAEMA-based star-shaped nanoparticles (“Nano-stars”) synthesized from an inorganic (PDMAEMA230/20) or a polybutadiene core (B290D245) show high potential for the transfection of several difficult-to-transfect cell types [Schallon et al., 2012, Raup et al. 2015]. However, the mechanism(s) responsible for the outstanding transfection efficiency are still unknown and in particular the lack of generically applicable transfection protocols for these and other cationic gene delivery agents remains disturbing. One additional aspect adding to the heterogeneity of the cellular performance in a given transfection protocol is the chosen promoter, since different promoters are known to have variable strengths and kinetic profiles in the different cell lines. Success of a given combination of polycation, promoter, gene and transfection protocol in one cell type says little in regard to the potential of this combination to transfect another. Optimal transgene expression may therefore very well require an adaptation of the promoter.

Experimental Approach:
Commercially available cell lines (CHO-K1, Jurkat (human leukemia T cells), L929 (murine fibroblast,) and HEK-293) were maintained as recommended by the supplier. Transfection of these cell lines was performed, using two standard protocols, subdivided for adherent and suspension cells and based on our past transfection experiences [Raup et al. 2015]. The relative EGFP fluorescence and viability of the cells was assessed via flow cytometry.

Results and Discussion:
We present a systematic investigation of the influence of the promoter sequence on the transfection outcome under otherwise identical experimental conditions. Here, we observed differences up to 50% depending on the tested cell line, using the similar PDMAEMA-based “Nano-star” for gene delivery. Furthermore, the transgene expression (reporter gene: enhanced green fluorescent proteins EGFP) was analyzed under the control of one cellular (elongation factor 1 alpha, EF-1a) and three viral (CMV, SV40, SV40/enhancer) promoters in various cell lines. Finally, co-expression levels of two types of fluorescent proteins (eGFP and YFP) under the control of different promoters were investigated and discussed. Detailed results on transfection efficiencies and transgene expression intensity are presented.


FLUID FLOW IN A POROUS SCAFFOLD TO IMPROVE CO-CULTIVATION OF HUMAN MESENCHYMAL STROMAL CELLS AND HUMAN DERMAL MICROVASCULAR ENDOTHELIAL CELLS FOR BONE TISSUE ENGINEERING

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Background and Novelty:
Bone tissue engineering is a promising application for overcoming the drawbacks of current clinical bone replacement treatments (e.g. allografts or autografts). However, the success of bone regeneration depends strongly on the revascularisation of the bone tissue. For this reason, co-cultures of endothelial cells and human mesenchymal stem cells (hMSCs) are of increasing interest. Until now, co-cultures have been grown in static 2-dimensional (2D)-cultivation systems. However, the usage of 2D-systems is accompanied by an accumulation of metabolic cell products and the formation of nutrient gradients that impair cell expansion and differentiation. Perfusion-based, 3-dimensional (3D)-bioreactor systems operating with porous scaffolds and oscillating fluid flows have proven themselves to be a promising alternative. The flow-induced shear stress (WSS, FSS) in these 3D-cultivation systems may support cell differentiation and the formation of vascular structures, as has recently been published.

Experimental Approach:
In order to find the optimum cultivation conditions for the two cell types in the co-culture, an oscillating fluid flow in a porous hydroxyapatite scaffold (d = 8; h = 3.5 mm) was simulated (ANSYS Fluent v 15.0). For this purpose, a cylinder (d = 7 mm; h = 7.5 mm) that represented the region of interest (ROI) and included a pipe-like inlet and outlet was considered and discretized into approximately 4 mio. control volumes (CV).

Results and Discussion:
The numerical investigations indicated significantly higher fluid velocities in the smallest scaffold pores (up to 13 mm s⁻¹) and at the centre of the scaffold. Moreover, a clear dependency between the two different inlet directions was also recognizable, which resulted in 20-times higher fluid velocities than at the scaffold inlet. Based on these higher fluid velocities, the highest wall shear stresses (up to 0.27 Pa) were predicted at the inlet of the scaffold and at the edges of the smaller pores. There was also an obvious reduction in cell growth at these locations in the subsequently performed cultivation studies. Our findings demonstrate the importance of shear stress investigations in co-cultivations for the purposes of bone tissue engineering.
SERUM-FREE MEDIA FOR MESENCHYMAL STEM CELLS
EXPANSION ON MICROCARRIERS

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Background and Novelty:
Expansion of mesenchymal stromal cells (MSC) is one of the key step for their use in tissue engineering or cell therapies. One of the major obstacles to obtain a reliable manufacturing process is that most of MSC cultivation methods still rely on media being supplemented by a significant volume of fetal calf serum. While efforts have been made to develop serum-free (SF) media for MSC expansion, they were systematically designed for planar plastic cultivation systems. The aim was to select and to compare commercial and in-house SF media with the specific needs of 3D cultures in sight, in particular the dynamic control of cultures and the cell adhesion to another material than plastic in 3D.

Experimental Approach:
We performed batch cultures in 2D (static culture plates) and 3D (microcarriers) of human bone-marrow and adipose-derived MSCs. On the basis of cell growth monitoring, 2D cultures first allowed us to screen well-performing SF commercial media and more than a dozen of usual cell culture supplements using an automated microscopic follow-up with the Cellscreen® device (Innovatis AG). In a second time, 3D cultures on microcarriers at small-scale were performed. Cell adherence was first assessed on GE Healthcare® and Solohill® microcarriers, and only the best conditions were brought to cell expansion in shake flasks allowing us to monitor cell growth on microcarriers.

Results and Discussion:
Commercial SF media performed as well as the control group in 2D cultures (performed with alpha-MEM supplemented with 10% SVF) but required a full renewing of the medium volume regularly and the necessity to use proprietary expensive cell coating reagents. We provide evidence that adherence factors can also be directly provided by supplementing the media with gelatin and laminin, but surprisingly, not fibronectin. The use of adequate pre-coated microcarriers was also investigated (i.e. Cytodex 3, GE Healthcare®). Moreover, most SF media still contain animal/human derivatives. In the present study, we tested several of them independently in comparison with plant and yeast derivatives. With a drastic negative effect on MSC expansion, these latter will probably not be a viable alternative to serum or platelet lysate. We conclude that MSC, as primary culture that are not adaptable, are likely to require recombinant protein supplementations if a truly defined culture medium formulation is necessary for regulatory purposes.
EXTRACELLULAR MATRIX SCAFFOLDS DERIVED FROM PLURIPOTENT
STEM CELL AGGREGATES REGULATE STEM CELL
PROLIFERATION AND DIFFERENTIATION

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Background and Novelty:
Pluripotent stem cells (PSCs), including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), emerge as promising sources for tissues engineering, drug screening, and disease modeling due to their unique proliferation ability and differentiation potential. At various developmental stages, PSCs and their progeny secrete large amounts of extracellular matrices (ECM), which could interact with regulatory growth factors to modulate the microenvironment of stem cells. In this study, it is hypothesized that PSC-derived ECM can be used as a novel type of scaffolds to mediate stem cell proliferation and differentiation.

Experimental Approach:
To create different biological cues, ECMs from undifferentiated aggregates, spontaneous embryoid bodies, or PSC-derived neural progenitor cell (NPC) aggregates were decellularized. The ECMs from PSC at different developmental stages were characterized by confocal microscopy and in situ ELISA. In addition, the effect of crosslinking on the ECMs derived from PSCs on the scaffold stability and biomechanical properties was investigated by scanning electron microscopy, atomic force microscopy, and dynamic mechanical analyzer for measurement of topography and elasticity. To interrogate the biological cues of ECM scaffolds, undifferentiated PSCs or PSC-derived NPC aggregates were reseeded on different ECM scaffolds and the proliferation and the differentiation of reseeded cells were characterized.

Results and Discussion:
It is observed that ECM expression pattern is dynamically remodeled during expansion and differentiation. These results indicate that various culture conditions enable to generate different ESC-derived structures composed of specific ECM molecules composition. Moreover, it is found that ECM scaffolds are able to influence ESC proliferation and the three germ layer differentiation by direct interactions with the cells and by influencing the signaling functions of the regulatory macromolecules such as retinoic acid. The crosslinking of the ECM scaffolds using genipin or glutaraldehyde significantly enhances the scaffold stability and changes their biomechanical properties. In addition, it is observed that crosslinking of PSC-derived ECM scaffolds affects proliferation and neural differentiation of reseeded PSC-derived NPC aggregates. Together, the results indicate that various PSC-derived ECM scaffolds affect stem cell expansion and differentiation through intrinsic biological cues and biophysical properties and have potential for in vitro cell culture applications and in vivo delivery.
GENERATION OF A NOVEL MICROCARRIER FOR EXPANSION
OF HUMAN MESENCHYMAL STEM CELLS

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Background and Novelty:
Multipotent stem cells have been isolated from multiple tissue sources including, bone marrow, adipose tissue, placenta and umbilical cord and cardiac tissue. It is predicted that large numbers of therapeutically active cells isolated from these tissue sources will be required to treat patients afflicted with various disorders. Experimental evidence suggests that these various cell types can exhibit distinct characteristics depending upon tissue source and method of expansion. Differential expression of cellular markers is sometimes detected, doubling times and expansion limits can differ and physical differences that influence the ability of cells to adhere to various synthetic surfaces are observed. We have generated a novel prototype microcarrier that promotes rapid attachment and growth of multiple cell types in stirred-tank reactors. These desirable attributes manifest in both serum-containing and animal component free medium formulations.

Experimental Approach:
We designed and developed a spherical microcarrier with a chemistry that promotes rapid attachment and superior growth of multiple cell types under a variety of environmental conditions. Microcarrier chemistry was optimized through an iterative process using these parameters to guide development. Cell attachment and growth in medium containing high concentrations of serum was quantified and microcarriers that provided the best substrate for attachment and growth were selected for further analysis. Once an optimal surface chemistry was achieved, the ability to expand human bone marrow-derived mesenchymal stem cells in stirred reactors was tested. Initial growth and optimization studies were performed in small scale spinners. Cell densities, doubling times and maintenance of identity and function were ascertained after culture in stirred tank culture. Favorable conditions identified at small scale were then examined in environmentally controlled bioreactors. Cell harvest efficiencies at small scale and bioreactor levels were optimized.

Results and Discussion:
Human bone marrow-derived mesenchymal stem cells expanded on this microcarrier type reach acceptable cell densities in spinner cultures under variety environmental conditions. Harvest efficiencies achieved in small scale cultures are excellent and cell identity is maintained. Conditions optimized in small scale spinners have been successfully employed in environmentally-controlled bioreactor and cell harvesting optimization studies at larger scale are underway. Current results indicate that this novel microcarrier type will provide a superior substrate for large-scale propagation of cells under various environmental conditions.
PROMISING POLYMER LIBRARY FOR NON-VIRAL GENE DELIVERY

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Background and Novelty:
Nowadays, nanomaterials become more and more important in medicine, material science, production processes and our daily life. Thereby, the delivery of active components into cells is in the focus of interdisciplinary scientists. As an alternative to viral transfection approaches, the use of non-viral gene delivery vectors achieved a significant high attention reflected in the large number of non-viral transfection agents being proposed. Synthetic polymers have an immense potential for non-viral gene delivery since they offer the advantages of large scale production, simple storage conditions, and varying architectures or modifications. Cationic polymers play a crucial role within the field of gene delivery due to their good interaction with negatively charged genetic material leading to high transfection efficiencies but also to a high cytotoxicity. Linear poly(ethylene imine) (PEI) prepared from 2-oxazoline-based polymers is the most studied cationic polymer for that application. To overcome the drawback of undesired interactions a combination of non-ionic and cationic copolymers are often used.

Experimental Approach:
Here, we demonstrate how a screening of a 18-membered library of cationic 2-oxazoline-based polymers was used to investigate the influence of the polymer side chain hydrophobicity and the type and content of amino groups on the pDNA condensation, the transfection efficiency, the cytotoxicity, the cellular membrane interaction as well as the size, charge, and stability of the polyplexes. To further examine the impact of molar mass, high molar mass poly(2-oxazoline) based polymers were modified to obtain copolymers of PEI, PEtOx and monomers functionalized with primary amines. These polymers, differing in their degree of hydrolysis and amine content, were investigated as described above (Scheme 1).

Results and Discussion:
We will demonstrate that cationic poly(2-oxazoline)s with short side chains and primary amines reveal a good balance between toxicity and transfection efficiency making them a promising polymer platform for biological applications. In detail, by screening the library, a cationic 2-oxazoline-based polymer with primary amines and an amine content of at least 40% could be identified as efficient transfection agent. A further improvement of the transfection efficiency was obtained by using higher molar masses.

Bibliography:
NUMERICAL STUDY OF HYDRODYNAMICS IN WAVE BIOREACTORS

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Background and Novelty:
Culture of mammalian/human cells in wave bioreactors is nowadays widely used for cell expansion or biological production. These systems are used for cells in suspension or adherent to micro-carriers. Stem cell culture in wave bioreactor is being recognized as an attractive option. The optimal operation conditions however have not been systematically investigated. In particular, the speed and rocking angle are set by empirical approaches. The purpose of this study is to achieve a better understanding of the hydrodynamics in these systems and provide tools for optimal operation setting.

Experimental Approach:
Computation Fluid Dynamics (CFD) is considered an inexpensive and efficient tool for predicting the fluid behavior. In this study, we perform detailed numerical simulations employing Ansys-FLUENT to characterize the flow conditions in 10L cellbag of a wave bioreactor. Three-dimensional and two-dimensional simulations are carried out to describe the flow motions and fluid structures in the cellbag. In order to study the influence of the rocking motion intensity, we examine three rocking angles and three rocking speeds typical of real applications.

Results and Discussion:
The liquid-gas interface and fluid velocity are investigated at different stages during the rocking cycle. We calculate the shear stress from the velocity fields obtained from unsteady numerical simulations. Moreover, we document the spatial variations of the shear stress levels at different operating conditions. Interestingly, we find particular operating conditions of angle and speed generating both higher shear stress and higher dissolved oxygen rate (KLa) than intuitively expected. This can be explained by the fact that complicated fluid structures induce large shear stresses that is accompanied by improved mixing of dissolved oxygen. The agreement between our simulation results of shear stress and our experimental results of KLa measurements provide a good validation for our numerical simulations. Our detailed results extend the knowledge of shear stress and oxygen transfer in wave bioreactors. This can help to select operation settings generating favorable hydrodynamic conditions of oxygen transfer while minimizing the shear damage.
TRANSIENT PRODUCTION OF RECOMBINANT ADENO-ASSOCIATED VIRUS (AAV) VECTORS FOR GENE THERAPY APPLICATIONS USING SUSPENSION-ADAPTED HEK 293 CELLS IN ORBITAL SHAKEN BIOREACTORS

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Background and Novelty:
Adeno-associated virus (AAV) is one of the most popular vectors for gene therapy applications. AAV mediated gene therapy approaches offer the advantages of safety, a broad tissue tropism and limited host immune response. Today one commonly used AAV production platform for pre-clinical and clinical use is based on transient transfection of adherent HEK 293 cells grown in serum containing media. This production method is difficult to scale-up and labor intensive, therefore cost-effective and scalable protocols are needed. Recent research validated the use of orbital shaking technology for the cultivation of HEK 293 up to a 1,000 Liter scale in disposable vessels [1].

Experimental Approach:
Here we describe the production of recombinant AAV serotypes 6 and 9 using polyethyleneimine (PEI) based transfection of suspension adapted HEK 293 cells. A two- plasmid system was used to produce the rAAV serotypes in three different suspension-adapted HEK 293 cell lines and one adherent HEK 293 standard. Suspension cells were cultivated in serum-free medium in disposable, orbital shaken bioreactors (OSR) of different scales from 5 ml to 500 ml. Optimization of transfection parameters like cell density at the time of transfection, the ratio of helper to donor plasmid and total plasmid to PEI was conducted for each individual cell line. Purification of AAV was performed according to established protocols [2]. Lysates and supernatants were processed separately using gradient centrifugation and affinity chromatography. Purified virus stocks were then analyzed and titrated using methods to determine total viral particles, viral genomes and infectious titers.

Results and Discussion:
Preliminary results indicate that rAAV particles produced with suspension cells yield infectious titers equal or higher than particles produced by standard methods based on adherent cells. Further work is ongoing to analyze and implement this OSR platform. The presented process offers a novel method to produce rAAV for pre-clinical and clinical trials compliant to GMP, single-use and scalable.

EVALUATION OF THE CAPABILITY OF A REAL-TIME PCR ASSAY TO DETECT MYCOPLASMA IN ADVANCED THERAPY MEDICINAL PRODUCTS

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Background and Novelty:
Mycoplasmas are among the world’s smallest bacteria capable of independent reproduction. They belong to the class of Mollicutes, have a very slow and parasitic growth and can cause human infections. Mycoplasma contaminations of ATMPs can arise from unsterile source material of different human origin, starting materials or the complex manufacturing process. The traditional growth-based detection method requires a cultivation time of at least 28 days before a contamination can be ruled out with certainty. But shelf lives of final ATMPs are often extremely short compared to classical drugs (24 - 48 h, sometimes only a few hours). That is why the official culture method or indicator cell method are not suitable. Furthermore ATMP sample amounts are usually limited and of great “value”. Nucleic Acid Amplification Techniques (NAT) allow reducing time to result to just hours and only small sample amount are necessary to generate highly sensitive results.

Experimental Approach:
A mycoplasma Real-time PCR kit was designed especially for the detection of Mollicutes (Mycoplasma, Acholeplasma, Spiroplasma) contamination in ATMPs and cell cultures by using the cells itself, cell culture supernatant or a defined mixture as test material. An acceptable sample volume in respect of the expensive, unique and limited sample is processed for mycoplasma detection. In this study the robustness of the kit was demonstrated by testing 20 randomly selected Advanced Therapy Medicinal Products from samples submitted by customers for mycoplasma detection. These tests were done as part of the kit validation. All selected ATMPs were tested negative for mycoplasma and could therefore be used as spiking matrices for the intended studies. These 20 different products were spiked with 10 CFU/ml of Mycoplasma fermentans. Each spiked ATMP matrix was then subjected to a DNA isolation process and tested in duplicate by Real-time PCR.

Results:
The Real-time PCR used is a multiplex assay which is able to detect Mollicutes with a FAM-labeled probe and an internal control DNA plasmid with a ROX-labeled probe. The ROX channel is used to check whether the reaction is inhibited due to matrix effects for example. A positive signal in the ROX channel indicates the absence of inhibitory effects and a successful amplification reaction. All 20 randomly selected ATMPs out of 20 in total were tested positive in duplicate and fulfilled the acceptance criterion (Ct values < 40). None of the matrices showed inhibitory effects in the ROX channel. Mean Ct values were 32.3 ± 0.9 for FAM and 33.0 ± 0.7 for ROX.

These studies provided detailed information about the suitability and robustness of the kit by reflecting influences of the sample matrices frequently used for manufacturing of ATMPs and in cell culture technology in general. M. fermentans was easily detectable at the spiked concentration of 10 CFU/ml even in highly complex sample matrices.
CELL CULTURE BASED VACCINES
PRODUCTION OF VIRUS-LIKE PARTICLES IN HIGH DENSITY CELL CULTURES AND SERUM FREE MEDIA. A PROMISING RABIES VACCINE CANDIDATE

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Background:
Rabies is a fatal virus disease without efficacious treatment. Vaccination is the main intervention for post-exposure prophylaxis in humans and vaccination of dogs is recommended by WHO. Nowadays there is a real need of an efficacious, cheaper and biosecure vaccine for rabies. Virus-like particles (VLPs) are excellent candidates for developing vaccines for human and veterinary infections.
Our group has developed rabies virus-like particles (RV-VLPs) expressed in adherent mammalian cell lines. RV-VLPs are spherical, enveloped, empty particles containing the rabies virus glycoprotein in its surface. In this work, we show the production of RV-VLPs in high density suspension cultures with serum free medium (SFM) in continuous cultures bioreactors.

Experimental Approach:
In a previous work, RV-VLPs expressed in HEK293 cells were biochemically and physicochemically characterized. In this work, RV-VLPs expressed in adherent cell lines were used in immunization protocols. Total antibody titer was measured by indirect specific ELISA and neutralizing antibodies measured in an in vitro test. Further, protection studies were achieved performing NHI potency test for rabies vaccine. Once the immunogenicity features of this vaccine candidate was confirmed, the recombinant HEK293 cell line was cloned. The chosen clone (adhP2E5) was adapted to grow in suspension conditions with serum free medium (SFM; EXCELL™ 293, SAFC). RV-VLPs expressed in SFM were concentrated and analyzed in a NHI potency test for rabies vaccine. In this case, VLPs were formulated in the stabilizer recommended for veterinary vaccine, with aluminum hydroxide gel as an adjuvant. To compare and analyze the effect of the adjuvant in the immune response triggered, VLPs were also injected without adjuvant in a stabilization buffer for RV-VLPs.
Finally, this clone was cultured in a 5 L bioreactor. Cells were cultured in perfusion mode reaching densities higher than 1.6x10^7 cells.mL^{-1}. Perfusion rate varied between 0.2 and 0.8 reactor volumes per day as it was necessary.

Results:
The results obtained in mice showed that RV-VLPs, expressed in adherent conditions, trigger a specific neutralizing antibody response. Further, the results obtained in the NIH potency test showed that the immune response induced by these VLPs is able to protect vaccinated animals in a rabies virus challenge assay. Later, a VLPs producing clone (adhP2E5) was adapted to grow in SFM (sP2E5). In batch conditions, sP2E5 reaches cell densities of 8x10^6 cells.mL^{-1}. RV-VLPs produced in SFM and injected without adjuvant showed an activity of 1.3 IU.ml^{-1} and purified RV-VLPs with the addition of adjuvant showed a specific protection activity of 1.5 IU.ml^{-1}. These results showed that RV-VLPs produced in SFM are highly immunogenic and trigger an immune response able to protect mice against virus challenge without the need of adjuvant addition. Finally, RV-VLPs were continuously produced in a 5 L bioreactor for 20 days and the final harvest was analyzed with the NIH potency test obtaining approximately 26,000 doses of veterinary rabies vaccine. Thus, RV-VLPs produced at high cell density continuous culture in SFM represents a novel platform for the production of a biosecure recombinant rabies vaccine.
IMPAIRED ANTIVIRAL RESPONSE OF HUMAN DESIGNER CELL LINES SUPPORTS VIRAL VACCINE PRODUCTION

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Background:
Influenza viruses are a major threat of human health and cause worldwide 3 to 5 million cases of severe illness every year. To prevent morbidity and mortality caused by influenza virus epidemics and pandemics, annual vaccination is required. Since the global demand for influenza vaccines is continuously increasing, mammalian cell cultures are an attractive alternative to egg-based production systems due to better flexibility and scalability. Different human-derived production cell lines (e.g. PER.C6 (Crucell), HEK293SF, AGE1.HN (ProBioGen AG)) are currently tested as substrate for the cultivation of influenza viruses. Compared to other vaccine production cell lines such as Vero and MDCK, human-derived cells have the advantage to produce viruses carrying authentic post-translational modifications, e.g. glycosylation patterns, which might have advantages regarding their immunogenic properties. Cell culture-based virus production can be restricted by cellular defense mechanisms and virus-induced apoptosis of host cells. These mechanisms interfere with virus replication and shorten the productive life span of infected cells. Hence, it was our aim to study the impact of the innate immune response on influenza virus production in human designer cell lines in more detail.

Experimental approach:
Infection experiments were performed with two common adherent cell lines (A549, HEK293) and two designer cell lines (HEK293SF, AGE1.HN) growing in suspension. In addition, cells were directly stimulated using high doses of recombinant human IFN-β. The activation of the innate immune response was analyzed by quantitative real-time PCR and virus yields of influenza A virus were determined by the hemagglutination assay.

Results and Discussion:
During infection as well as after stimulation with recombinant IFN-β only A549 cells were able to express antiviral genes. In contrast, HEK293, HEK293SF and AGE1.HN cells showed an impaired induction of their antiviral defense. Obviously, inhibition of virus replication by IFN signaling only occurred in A549 cells while this IFN pathway had no effect on virus growth in all other analyzed human cell lines. The observed phenotype can be explained by the different cell immortalisation histories. In contrast to A549 cells, which were isolated from carcinoma tissue, HEK293, its subclone HEK293SF and AGE1.HN cells were deliberately immortalised by the expression of the adenoviral proteins E1A and E1B. Using specific siRNAs to down-regulate E1A and E1B protein expression, the antiviral response could be partially restored by E1A knockdown. Furthermore, IFN-sensitive viruses such as vesicular stomatitis virus (VSV) and influenza delNS1 (lacking the IFN antagonist NS1) were able to replicate in HEK293, HEK293SF and AGE1.HN cells even after IFN-β stimulation. Therefore, adenoviral transformed cells lend themselves particularly well to the production of viruses. These new findings broaden the application spectrum of adenoviral transformed designer cell lines and could also support optimization of vaccine production processes.

Keywords:
Immune response, influenza vaccine production, designer cell lines.
SINGLE CELL ANALYSIS OF INFLUENZA A VIRUS-INFECTED CELLS FOR THE OPTIMIZATION OF CELL CULTURE-BASED VACCINE MANUFACTURING PROCESSES

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Background and Novelty:
In comparison to the conventional production of influenza vaccines in embryonated chicken eggs, cell culture-based vaccine manufacturing is more flexible, scalable and reliable. Besides the design of efficient process platforms, the optimization of the smallest production unit, the single infected cell, has an enormous potential to increase yields.
Single cells within a population display an extraordinary heterogeneity. For instance, cells possess different sizes, cell cycle stages, pools of cellular resources or induction states (referred to as cellular determinants). Therefore, population average measurement data is not representative for individual cells. In contrast, single cell analysis enables to reveal subpopulations of cells with diverse characteristics or cellular behavior.
The most important source of cellular variability is stochastic gene expression. Two factors, cellular determinants and the inherently stochastic nature of biochemical reactions (noise), contribute to heterogeneity in gene expression.
In the present work, we exploit technological achievements of single cell analytics to elucidate what exactly defines a high- or low-productive virus-infected cell.

Experimental Approach:
Madin-Darby canine kidney (MDCK) cells were infected with influenza A virus (IAV). Afterwards, we isolated and cultivated cells in 384-well plates, and wells containing single cells were identified by microscopy.
Increase in the virus yield in the supernatant was determined by using the plaque assay and intracellular viral RNAs (vRNA) were detected by reverse transcription quantitative PCR (RT-qPCR). Both absolute quantitative methods could be performed simultaneously along with microscopic analyses, which allowed us to conduct multi-parametric correlation studies on single cells.

Results and Discussion:
We found a very large heterogeneity in the productivity of single infected cells with almost 1000-fold difference in virus yield. Surprisingly, we could not find any dependency of productivity and cell size. Abundances of genomic vRNAs spanned about two to three orders of magnitude indicating a vast variability in intracellular virus replication as well.
Measurement of vRNAs levels of several segments together with virus yields of single cells enabled us to perform correlation studies. First, our investigations reveal the existence of noise in virus replication. Second, we conclude that both noise and cellular determinants affect virus replication heterogeneity. Third, we demonstrate that both factors impact the productivity of single cells. Finally, and most strikingly, we found that low-productive cells exclusively contain a very high and disproportional level of segment 7 vRNA as compared to the other genome segments. This genome segment encodes for IAV matrix protein 1 (M1), which is believed to serve as a negative regulator of virus replication. Hence, it seems to down-regulate replication resulting in a low-productive cell type.
Taken together, we were able to identify one trait which we can now use to differentiate between high- and low-productive cells. These findings serve as a starting point towards a better understanding of cell culture-derived virus production platforms and, in the future, single cell analytics may enable us to find strategies to increase process yields.
SEPARATION OF ENTEROVIRUS 71 VIRUS LIKE PARTICLES FROM BACULOVIRUS BY CROSSFLOW FILTRATION

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Background and Novelty:
Hand, foot and mouth disease (HFMD) is a highly infectious viral disease causing skin inflammations and oral ulcers, which usually resolve without special medical treatment. In rare cases, however, HFMD patients develop very serious neurological complications creating the need for a protective vaccine. One recent approach for the development of a HFMD vaccine is the use of enterovirus 71 (EV71) virus like particles (VLPs), which induce immunogenicity similar to the causative virus (EV71). One popular system for VLP production is the baculovirus expression vector system (BEVS), where an insect cell line is infected with a recombinant baculovirus to express VLP proteins. Besides optimization of VLP production, purification of these nanoparticles is a challenge at industrial scale. This is in particular due to the co-amplification of BEVS during the expression of VLPs, which results in a significant amount of contaminating baculovirus in the cell harvest. For an approval of a VLP based EV 71 vaccine, this baculovirus contamination has to be removed to ensure the purity and safety of the product.

Experimental Approach:
A crossflow filtration (CFF) process was evaluated to separate the EV71 VLPs from the baculovirus contaminants. For screening different filter membranes, 30 nm gold nanoparticles and the E. coli phage ΦX174 were used as a substitute to the VLP. The membranes were tested for their suitability to retain baculovirus and recover the VLP model particles in the permeate. To quantify ΦX174 phages and baculovirus in these filtration experiments, a qPCR method was implemented and optimized. In addition, a design of experiment (DOE) approach was used to investigate the effect of process conditions (pH, transmembrane pressure and crossflow rate) on ΦX174 recovery, baculovirus depletion and permeate flow.

Results and Discussion:
Screening with gold nanoparticles as well as with ΦX174 phages showed a clear benefit of microfiltration membranes compared to an ultrafiltration membrane. About 90% of the model particles were recovered with 0.1 μm and 0.08 μm microfilters, whereas the 750 kDa ultrafiltration membrane showed a higher retention for the phages, leading to a low recovery of only 50%. Additionally over 98% of the baculovirus was depleted from permeate with microfiltration membranes. With a 0.08 μm filter membrane for the DOE experiments over 75% of ΦX174 phages were recovered. In addition, 99% of the baculovirus contamination was removed. Finally, the optimal process conditions predicted by the DOE model were used to run a CFF process for the removal of baculovirus from an EV71 VLP containing cell culture supernatant. With a depletion of baculovirus higher 98%, results were comparable to the model system. However, additional contaminations or different properties of the VLPs compared to phages led to a massive membrane fouling. As a result only 73% of the VLP passed through the membrane. The remaining VLP remained in the retentate and were not separated from the baculovirus. Despite this drawback the results show a promising method for separation of Baculovirus from small VLPs using new filter membrane technology in a crossflow filtration process.
PHASE I CLINICAL TRIALS OF SAFETY AND IMMUNOGENICITY OF LIVE CULTURAL INFLUENZA VACCINE “VECTOR-FLU”


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Background and Novelty:
Live influenza vaccines trigger all major components of the anti-flu immune response machinery. Key advantages of live vaccines include a feasibility of intranasal administration, opportunity to rapidly scale up production of the viral substance, simplicity of vaccination, and robust protection against antigenic drift variants of the virus. Use of certified cell cultures for the cultivation of seasonal strains of influenza along with biodegradable materials for constructing delivery vehicles is considered one of the mainstream approaches to the development of new generations of flu vaccines.
We developed a live cultural influenza vaccine called “Vector-Flu”, which is based on the cold-adapted virus strain A/17/California/2009/38 (H1N1) and MDCK cell line obtained from the certified cell culture depository. Preclinical studies have demonstrated safety and high immunogenicity of Vector-Flu in a ferret model.

Experimental Approach:
Phase I of clinical trials was conducted on healthy volunteers in the Medical Unit #163 in Koltsovo, Russia. The trial pursued the following goals:
1) Evaluation of safety and tolerability.
2) Evaluation of the humoral and adaptive immune response using HI, ELISA and microneutralization assay.
3) Evaluation of the cellular immune response, as measured by the cytokine release level in response to the ex vivo stimulation of blood lymphocytes by the influenza virus.
Phase I of clinical trials included 3 arms:
Arm 1 (n=20): a treatment group. Volunteers were vaccinated using a single dose of the Vector-Flu vaccine containing 10^6 EID₅₀ of the influenza virus.
Arm 2 (n=20): a treatment group. Volunteers were vaccinated twice over a course of 10 days using Vector-Flu vaccine containing 10^6 EID₅₀ of the influenza virus.
Arm 3 (n=20): a placebo control group. Volunteers were injected twice over a course of 10 days using sterile sodium chloride.

Results and Discussion:
Our findings show that the Vector-Flu has high tolerability and no significant side effects. Virus was not detectable in nasal mucus and blood sera of the healthy volunteers after a single and double injections as early as at day 1, demonstrating a rapid clearance of the live virus from the vaccination sites. Additionally, no indications of infection generalizations were observed.
A seroconversion level was detected at 45% after a single injection, as measured using HI assay. After the second injection, peaks of immunogenic activity were recorded at 2 and 3 weeks, and seroconversion level rose up to 80%.
Activation of the Th2 immune response was measured in whole blood cells. Post-vaccination cytokine indexes were high and stable: 100% for IL-10 and TNFα, and 75% for IL-6.
The Ministry of Health of Russia has granted a permission to advance to the phase II trials for the Vector-Flu vaccine.
CONTINUOUS AND SEMI-CONTINUOUS PRODUCTION OF CELL CULTURE-DERIVED MODIFIED VACCINIA ANKARA VIRUS IN TWO-STAGE BIOREACTOR SYSTEMS

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Background:
One challenge for the coming decades in animal cell culture technology will be the implementation of continuous processes. They could be particularly efficient for production of biologicals that are required in large amounts such as viral vaccines. One virus that has received much clinical attention is Modified Vaccinia Ankara virus (MVA). It is a potential platform for expression of recombinant proteins and can be used as a vector in gene therapy (Verheest et al. 2012, Vaccine 30:2623). Recently, a new MVA virus strain has been successfully propagated at high yields in avian suspension cells (Jordan et al. 2013, Viruses 5:321). MVA is a lytic virus and therefore continuous production strategies can only be implemented using multi-stage bioreactor systems where cell growth and virus propagation occur in separated vessels. However, practical limitations for the study of multi-stage systems are their complexity and process time that greatly limit optimization. In this work, continuous production of MVA virus in a two-stage bioreactor set-up with two 1 L stirred tank bioreactors was evaluated. Subsequently, the set-up was scaled down to a non-instrumented semi-continuous cultivation system as approximation to a continuous cultivation (Westgate and Emery 1990, Biotech&Bioeng 35:437) that would facilitate screening of the two-stage bioreactor.

Experimental Approach:
The virus strain MVA-CR19 and the duck cell line AGE1.CR.pIX (both from ProBioGen, Berlin) were used. The continuous system involved a 1 L bioreactor for cell growth (uninfected) and a second bioreactor in series for virus propagation (infected) with set-up and dilution rates described previously (Frensing et al. 2013, PLOS ONE 8(9):e72288). The small-scale system consisted of two shaker flasks, one for cell growth (120 mL working volume) and another for virus propagation (different working volumes). Harvest, cell transfer from uninfected to infected shaker, and addition of fresh medium were done manually twice a day.

Results and Discussion:
Continuous production of MVA-CR19 was maintained for 18 days with the continuous system. Virus titers reached stable values after 7 days with a total production of 7.1 L at a rate of 2E10 viruses/day (1E10 – 2E11 viruses/day estimated for batch cultivations). The space-time yield (STY) of the continuous system was 3E6 viruses/(mL*day) which is low compared to 1E7 – 1E8 viruses/(mL*day) in batch cultivation. The process at small scale resulted in stable production of uninfected cells for up to 14 days with virus titers in the infected shake flask of up to 1E9 viruses/mL that approached the dynamics and values of the continuous system. Additional cultivations at small scale showed that different residence times in the infected bioreactor could influence STY. Finally, it was demonstrated that continuous production of MVA-CR19 virus in a continuous two-stage bioreactor system is feasible. Also, a small scale two-stage semi-continuous cultivation was successfully established as a tool for screening virus production in two-stage continuous systems before scale-up.
EVALUATION OF INSECT CELLS EXPRESSION PLATFORM FOR PRODUCTION OF HEPATITIS C VACCINE CANDIDATES

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Background and Novelty:
Hepatitis C virus (HCV) infection is a major public health problem, causing more than 350,000 deaths every year, according to the World Health Organization. Currently, there is no Hepatitis C vaccine and the standard treatment for acute HCV infections has several limitations, including its side effects and high costs. There is thus a clear need for the development of a vaccine with both preventive and therapeutic roles. Virus like particles (VLPs) presents a great potential as vaccine candidates due to the capacity to induce stronger immune response as compared to the use of isolated antigens, and due to the lack of genetic material representing a safer alternative to the traditional attenuated vaccines. Particularly, retrovirus like particles (rVLP) represents a versatile platform with tremendous potential for vaccine design, since it is possible to pseudotype these particles with different proteins such as the HCV envelope proteins.

The insect cell production platform has attractive features for recombinant protein production, particularly its potential to achieve high productivities, coupled with easy scale-up. In this work, we aim at evaluating the potential of Sf9 cells and Hi5 cells for the production of HCV pseudotyped rVLP and compare its performance to that of the traditional cell substrate for retrovirus production, the HEK293 cells.

Experimental Approach:
Retrovirus like particles were produced transiently in Sf9 and Hi5 cells using de Baculovirus expression vector system (BEVS) to express Gag protein from Moloney murine leukemia virus (MLV) and HCV envelope proteins (E1 and E2). Different infection parameters were tested and optimized for each cell line and a codon optimization is being tested to obtain a better production.

For rVLP-HCV production in human cells, a MLV Gag-Pol expressing HEK293 cell line was stably transfected with a plasmid expressing HCV-E1/E2. The particles produced in the best conditions in each cell platform were purified from culture supernatants and characterized.

Results and Discussion:
MLV Gag and HCV-E1/E2 expression was readily detected in both Sf9 and HEK293 cells by western-blot analysis. Hi5 cells were also evaluated for VLP-HCV production. Gag and HCV-E1 expression was observed, whereas HCV-E2 was not detected.

Gag concentration in pure VLP-HCV samples was quantified by densitometry using a Gag standard. HEK293 cells productivity was 5-fold higher than that of Sf9 and 9-Fold higher than that of Hi5. Nevertheless, the particles produced in Sf9 present higher incorporation of HCV envelopes, which can be relevant for the induction of specific HCV immune responses. Moreover, it is anticipated that codon optimization of Gag for expression in Sf9 would result in higher Gag productivity. Due to importance for the immune response, the glycosylation profile of HCV-E1E2 is being assessed and compared with the glycosylation profile of HCV-E1E2 expressed in HEK293.

Our data shows that insect cells are promising as an alternative substrate to produce retrovirus based vaccines.
DEVELOPMENT OF CANCER VACCINE BASED ON HER-1 EXTRACELLULAR DOMAIN
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Background and Novelty:
The epidermal growth factor receptor (EGFR) belongs to the erbB family of 4 closely related cell membrane receptors, also known as the Type I receptor tyrosine kinase family (Her1). Although expressed in nonmalignant cells, the EGFR can be found overexpressed or mutated in many human epithelial tumors such as breast, lung, prostate, vulva and ovarian tumors. EGFR has been targeted by different therapeutic products, including immunotherapy mainly employing monoclonal antibodies. A new approach is being evaluated at CIM based on the formulation of extracellular domain of Her-1 receptor (Her1-ECD) with a proprietary adjuvant (VSSP) and Montanide as cancer vaccine.

Experimental Approach:
Human Her1-ECD is a glycoprotein with a molecular weight of 105 kDa and has 11 potential sites for N-glycosylation. BALB/c mice were immunized with murine EGFR-ECD and human Her 1-ECD as proof of the principle of vaccine. Both the murine and human ECD glycoproteins were obtained from culture supernatant of stable recombinant HEK-293 using a protein free culture media. Different cell culture media were tested and bioreactor operation conditions were screened at 3-5 L scale. Murine EGFR-ECD and human Her 1-ECD were purified by immunoaffinity procedure using an anti-EGFR monoclonal antibodies as ligand, followed by a sequence of ion exchange and size exclusion chromatography for intermediated and polishing steps. A detailed physico-chemical and biological characterization of the obtained Her 1-ECD protein was accomplished and the effect of different cell culture media on protein characteristics was assessed using SDS-PAGE, SEC-HPLC, IEF, peptide mapping, mass spectrometry, size and Z potential distribution. Biological activity of the vaccine was evaluated by ELISA, flow cytometry and western blotting, both at antibody and cellular response level.

Results and Discussion:
The mEGFR-ECD is immunogenic in mice and autologous vaccination in VSSP adjuvant induces antimetastatic effect. Also autologous vaccination based on HER1-ECD in VSSP adjuvant induces humoral response in mice with PAb that binds EGFR+ human tumor cell lines, inhibit their growth and show a potent anti-metastatic effect. Developed pilot scale production process yields a product with high quality attributes and productivity levels that meets the requirements for clinical use. Purification protocol was optimized, employing a high-throughput method. Product obtained showed purity values over 95 % measured as well as DNA, pyrogen and other contaminant levels below those accepted by regulations. Product purified from different cell culture media showed differences related to IEF band intensity that was associated with a decrease in sialylation. Moreover differences in G0/G1 profiles were detected by mass spectroscopy. However the biological activity (measured as Mabs titer and recognition) is similar for the products obtained in different conditions. The autologous HER-1/VSSP vaccine induces specific antibodies without any observed adverse event both in preclinical studies and in Phase I Clinical Trial. Vaccine preparations based Her1-ECD have demonstrated, both in vitro and in vivo, a potent antimetastatic effect on EGFR + Lewis lung carcinoma model, while associated side effects were absent. Results of preliminary safety studies in a Phase I clinical study will be also showed.
DEVELOPMENT AND OPTIMIZATION OF A RETROVLP-BASED HCV VACCINE CANDIDATE

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Background and Novelty:
Virus-like particles (VLPs) are ideal alternatives to attenuated and inactivated vaccines due to their high immunogenicity and safety. Retrovirus-like particles (retroVLPs) are a particular subset of VLPs widely used in virtue of their ability to incorporate heterologous membrane proteins. In this work HCV vaccine candidates based on retroVLPs pseudotyped with HCV envelope proteins E1 and E2 were developed as an alternative to HCV-derived particles as the latter show low production yields and are still poorly characterized. The aim of this work is to compare the quality of HCV retroVLPs produced in different expression systems and evaluate the immunogenicity of HCV antigens displayed.

Experimental Approach:
HCV-retroVLPs were produced in different HEK293 derived cells stably expressing both Murine Leukemia Virus structural proteins and HCV antigens. HCV antigens were expressed in these cells either by using the recombinase mediated cassette exchange [1] or by using standard transfection and clonal selection methods. Moreover HEK293 derived cells silenced for CD81 tetraspanin [2] were also used to evaluate the impact of CD81 on the overall immunogenicity of retroVLPs and on the immunogenicity of HCV antigens. The different production systems were characterized for overall productivity using NanoSight NS500 and particle quality by evaluation of both retroVLPs structural integrity and antigens incorporation. Structural integrity of retroVLPs was determined using transmission electron microscopy (TEM) and the incorporation of different HCV and host-derived antigens was determined by western blot, ELISA and MALDI-TOF/TOF spectrometry. Immunogenicity of the different retroVLPs was evaluated by subcutaneous immunization of female BALB/c mice.

Results and Discussion:
HCV-retroVLPs production yields were maintained across production systems, with a global production of over 1x10⁹ particles per milliliter of culture. Furthermore the glycosylation profile of HCV antigens derived from different expression systems was essentially identical. Notwithstanding similar productivities and glycosylation, different production systems induced different incorporation levels of HCV antigens which impacted HCV-retroVLPs immunogenicity. In summary, this work highlights the influence of host-derived proteins on retroVLPs immunogenicity while developing a stable and continuous system for the production of a retroVLP-based HCV vaccine candidate.

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References:
GENE EXPRESSION USING SEMLIKI FOREST VIRUS SYSTEM IN BHK-21 CELLS 
IN SERUM FREE MEDIUM CULTURE

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Background and Novelty: 
Rabies is a zoonotic viral disease caused by a virus of the genus Lyssavirus that affects several species of 
mammals. Rabies remains a global public health threat that kills more than 61000 people per year mainly in 
Africa and Asia. The rabies virus envelope is composed of a glycoprotein, known as a unique antigen capable 
of conferring immune response against the rabies, for this, is the focus of research for development an efficient 
and safe recombinant vaccine based on expression of this viral antigen. Viral vectors have been recognized as 
the most efficient tool for the production of proteins recombinant in eukaryotic cells since they allow a high 
efficiency of infection and, therefore, introducing the gene into the desired cell line. The Semliki Forest Virus 
(SFV) system has gained increasing interest due to its efficiency in heterologous protein expression. An ideal 
production cell line is able to grow in serum-free media (SFM). The usage of serum is not preferred due to the 
high costs, lot-to-lot variation and risk of contamination with viruses, mycoplasmas and prions. Additionally, 
SFM growth simplifies the purification process. For these reasons, current biotechnological approaches of cell 
culture need to avoid the use of serum. Our aim was to express rabies virus glycoprotein (RVGP) using an 
expression system based on SFV in BHK-21 cells in serum free culture.

Experimental Approach: 
In this work, BHK-21 cells were adapted to 4 commercial SFM: VP-SFM, Hybridoma-SFM, MAb medium 
and CHO-S-SFM II. Recombinant SFV-RVGPs were obtained by electroporation and quantified by qRT-PCR. 
Infection trials were carried out with different ratios between SFV-RVGP and cells (1; 10; 20; 50). Samples 
were collected 24 hours after infection to analyze RVGP expression by ELISA.

Results and Discussion: 
Virus titration (copies RNA SFV/ μL) were 15.2×10^2; 15.8×10^2; 0.5×10^2; 3.8×10^2; 45×10^2; 7.31×10^3 for 
Hybridoma, MAb, CHO-S-SFM II, VP-SFM and Control (DMEM with 10% of FBS), respectively. The virus 
SFV-RVGP-Hybridoma and SFV-RVG-MAb were 2 times higher than SFV-RVGP-DMEM and SFV-RVGPCHO was 6 times higher than SFV-RVGP-DMEM. RVGP expression reached 4.8 μg/ 10^6 cells in cells growth 
in Hybridoma, 2 μg/ 10^6 cells in VP and 0.6 μg/ 10^6 cells in MAb medium. BHK-21 cells adapted in SFM were 
able to produce recombinant SFV and also to expression of RVGP.

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DEVELOPMENT AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES AGAINST GREEN TEA POLYPHENOL EGCG BINDING REGION OF THE 67-KDA LAMININ RECEPTOR

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Background and Novelty:
The 67-kDa laminin receptor (67LR) was identified as the cell-surface receptor conferring the major green tea polyphenol (−)-epigallocatechin-3-O-gallate (EGCG) responsiveness to cancer cells. Recently, the 10 amino acid sequence, IPCNNKGAHS, was identified as the functional domain responsible for the anti-cancer activity of EGCG. In this study, we generated anti-67LR monoclonal antibodies specific to EGCG binding region and characterized their activities against the anti-proliferation and/or pro-apoptotic action of EGCG, for further investigation of the underlying mechanism for interaction between EGCG and 67LR.

Experimental Approach:
We synthesized the 10 amino acid peptide corresponding to the 161-170 region of human 67LR encoding a 297-amino acid, and conjugated it to carrier protein, bovine serum albumin (BSA), via Cys residue by MBS method. BALB/c and ddY mice were immunized with this peptide-BSA conjugate. Spleen cells were obtained from immunized mice, and fused with myeloma cells (Sp2/O-Ag14) using PEG method. These cells were cloned by limited dilution. We measured the binding activities of monoclonal antibodies from supernatants by ELISA and western blotting.

Results and Discussion:
We established sixteen hybridoma clones specific to the EGCG binding motif peptide-BSA conjugate. In the culture of the established hybridoma clones, the remarkable decreased viabilities even under low cell densities were observed as compared with the control clones. Viabilities of these hybridoma clones were recovered by removal of the specific antibodies from the media by substitution to the conditioned media from the control clones. Moreover, the higher expressions of 67LR dimer form in these hybridoma clones were confirmed by the western blot analysis. These results suggest a possibility that the hybridoma clones established in this study have caused cell death by the activities of the antibodies which themselves secreted. We proposed that the anti-67LR antibodies generated in this study might have agonistic activity against the anti-proliferation and/or pro-apoptotic action of EGCG.

References:
IMPACT OF VIRUS REDUCTION STEP ON UP-STREAM PROCESS (USP) RAW MATERIAL PERFORMANCE

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Background and Novelty:
A risk analysis on adventitious virus contamination was conducted on a vaccine Up Stream Process. Despite the absence of potential contamination resulting from the use of animal-origin-free raw material, potential virus entrance points have been identified in the manufacturing environment, storage and shipment of some of these raw materials. Consequently, an action plan including additional precautionary measures has been implemented to prevent potential adventitious virus contamination.

Experimental Approach:
Several techniques aimed at mitigating those risks have been identified, among which autoclaving, short-time high temperature, nanofiltration and UV-C. They have been studied and compared in terms of virus removal efficiency, impact on medium components, cost of implementation and speed of technique.

Results and Discussion:
The impact of these additional precautionary measures was assessed, first with regards to their impact on medium components and then with regards to cell growth performance and virus production. The selected treatment was then assessed for viral removal using a spiking study with virus models and was implemented for cGMP manufacturing.

EVALUATION OF SINGLE USE BIOREACTOR FOR CELL CULTURE IN SUSPENSION AND ADHERENCE

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Background and Novelty:
Single use bioreactors are now widely used for cell culture and viral production in order to increase the development time and to work more cost-effectively in cell culture based processes. However, having flexible equipment allowing the production of different cell lines at different scales is still a challenge.

Experimental Approach:
In this study, we evaluated single used bioreactors both for adherent and suspension cell lines at different scales. First, a study was led to determine optimal mixing speed regarding microcarriers allowing homogeneity with a minimal shear stress. The model allowed determining mixing conditions which were implemented on a cell amplification process of an adherent cell line. Moreover, cultures with different types of cell lines growing in suspension were performed. The mixing speed and gazing strategy were adapted.

Results and Discussion:
This study allowed determining cell culture conditions of different cell lines both in adherence and suspension. The culture volumes ranged from 2L to 200L scale with comparable cell growth kinetics.
BIOPROCESS DEVELOPMENT OF DROSOPHILA S2 CELLS AND SEMILIKI FOREST VIRUS FOR THE EXPRESSION OF RABIES VIRUS GLYCOPROTEIN

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Background and Novelty:
Here we report bioprocess development using Drosophila melanogaster Schneider 2 (S2) cells and Semiliki Forest Virus (SFV) to produce the rabies virus glycoprotein (RVGP). The S2 cell system offers suitable bioprocess conditions for generating high-level expression of functional membrane proteins. Acquired knowledge on biology and engineering of S2 cells opens well based conditions for production of recombinant proteins. The SFV carrying the mRNA coding for RVGP offers the possibility not only to express the RVGP in cell cultures but also its use as a vaccine to generate suitable humoral and cellular immune response.

Experimental Approach:
We have constructed gene vectors with the hygromycin selection gene in which the RVGP gene was inserted under the control of the metallothionein promoter. After transfection, recombinant S2 cell populations were selected. The expression of RVGP was evaluated by qRT-PCR, flow cytometry, ELISA and western-blotting. Protocols for cell cultures in bioreactors were developed and batches of RVGP were produced and purified. The SFV expression system is based on a positive single-strand RNA virus carrying the gene of interest under the control of a viral promoter (SFV-RVGP). After infection, a great amount of heterologous mRNA is produced leading to a high expression of the recombinant protein. The recombinant SFV performs a single round of infection, does not integrates in cell DNA. The ability of S2 cells derived RVGP as well as the SFV-RVGP to induce immune response and protect mice against an experimental rabies virus challenge were investigated.

Results and Discussion:
High RVGP expression level could be detected in both S2 cell populations (~ 52 % of RVGP positive cells with ~ 4 micrograms of RVGP per 1E7 cells). RVGP mRNA kinetic analysis by qRT-PCR enlightened the relationship between S2 cell growth and specific productivity, showing a peak of RVGP mRNA and RVGP synthesis at the transition to the stationary cell growth phase. A protocol of RVGP purification was developed based on His-tag affinity chromatography after membrane preparations by ultracentrifugation and solubilizing with OG. The SFV-RVGP was used for cell infection and the RVGP mRNA and rRVGP were determined by qRT-PCR and ELISA. A methodology based on qRT-PCR was developed for viral vector titration. Upon S2 RVGP and SFV-RVGP immunization high levels of antibodies against RVGP (~ 4 EU/mL) were found in mice (3 weekly doses of 3 micrograms of RVGP each). SFV-RVGP led to high cell immune response as evaluated by IgG1a/IgG2, IFN gamma and IL2. Preliminary data show that RVGP immunization was capable of inducing protection against rabies experimental challenge (~ 90 % of mice survived a rabies virus challenge).
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INSECT CELL CULTURE IN SINGLE USE FIXED-BED icELLis® BIOREACTOR. 
PRODUCTIVITY AND SCALING UP PERSPECTIVES

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Background and Novelty:
Disposable bioreactors play a key role in the development and application of animal cell biotechnology. The iCELLis bioreactors (Pall Life Sciences) are a scalable line of single-use bioreactors, providing up to 500 m² of growth surface area in a reduced footprint. By combining the advantages of single-use technologies with the benefits of a fixed-bed system, iCELLis bioreactors have been shown to be an important advance in terms of high productivity combined with ready to use bioreactor for academic and industrial use. Our aim has been to establish optimized bioprocesses for the culture of suspension insect cells in the iCELLis fixed bed bioreactor. Lepidoptera insect cells (Sf9 cells) are largely used by laboratories and industries for production of recombinant proteins for diagnostic kits as well as for vaccine production (VLP). Diptera insect cells (S2 cells) are the subject of intense studies for bioprocesses involving synthesis of recombinant proteins and also vaccines. The system is based on cell populations that have been transfected and integrated the heterologous gene. Sf9 and S2 cells represent an outstanding tool for laboratories and industries. These cells are typically semi-adherent cells and usually multiply in suspension.

Experimental Approach:
Cells were cultivated in 50 mL shake flasks in SF900-II or IPL-41 medium and inoculated into bioreactors at 1 to 2x1E6 cells/mL. Cell cultures were performed in a 1 L stirred tank bioreactor at 90 rpm and in a iCELLis nano bioreactor with a fixed bed (containing macrocarriers, hereafter MC) of 200 mL/1.5 compaction with 4 m² available surface at 700 to 1000 rpm and with a 800 mL medium volume and a 6 cm falling film. Comparable temperature (28 °C) and dissolved oxygen (30%) parameters were used.

Results and Discussion:
By using our cell culture protocol, we have shown that insect cells could grow in the iCELLis fixed bed bioreactor achieving high cell densities (55 to 80x1E6 cells/mL of fixed bed). Our bioprocess protocol allowed these cells to be “entrapped” in the iCELLis fixed bed bioreactor. Once the cultures reached the desired high cell densities we could harvest 85 to 90% of the cells from the fixed bed in very good physiological conditions. It allowed both the recovery and extraction of cell associated recombinant protein (2.2 mg/1E7 cells) and the use of harvested cells for further cell culture bioprocesses or applications. In such conditions, one iCELLis nano bioreactor (4 m² – 1.5/200 mL) bioreactor would produce approximately 2,500 doses of recombinant rabies vaccine. Moreover, In view of the cell entrapment in the iCELLis fixed bed bioreactor, the immobilized cells are submitted to low hydrodynamic stress, as comparable to stirred tank or wave systems allowing the production of recombinant proteins with a higher degree of quality assurance. Our data show that comparable S2 cell densities in stirred tank and fixed bed iCELLis bioreactors led to higher biological active recombinant protein synthesis in the fixed bed system.
VETERINARY VACCINE PRODUCTION USING SU BIOREACTORS: SCALE UP STUDY FROM LABORATORY & PD UP TO COMMERCIAL PRODUCTION

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Background and Novelty:
Vaccination has proven itself as the most effective tool to control and prevent the disease and to facilitate the safe trade of live animals.
Viral vaccine manufacturing processes present some specific constraints as compared to other biotech products linked to the cell substrate used and to the viral production.
Multiple cell lines are used for productions such as VERO, MDCK, MRC5, BHK, and CHO cells.
As the feasibility of transferring the BHK-21 cells growth in microcarriers from a conventional bioreactor to single-use bioreactor (SU) was demonstrated, actually was time to scale-up and verify the flexibility and ease of use of these bioreactors enable rapid scale-up without any loss in product quality.
Identification of optimal parameters values, testing at intermediary bioreactors scales (2 – 50 L) and application of the new process settings at industrial bioreactor scale of 200 liters were performed. Taking into account that a critical feature of microcarrier-based cell culture is the homogeneity of cell adhesion to the beads, mixing and aeration performance evaluation remain mandatory to ensure robust scale-up of cell culture processes.
The goal of this study was to evaluate the possibility to produce the antigens for animal vaccines manufacturing in a bioreactor SU as alternative to roller bottles without any loss in product quality.

Experimental Approach:
Selection of appropriate culture conditions can be important to achieve consistent cell culture and virus production across sites and scales. Because characteristics like tank geometry and hardware are not subject to change during scale-up, the scalability from 2L to 200L in the BIOSTAT® STR bioreactor was an easy strategy for our production process.
Several studies were conducted to compare the growth of BHK-21 cells in microcarriers.
Identification of optimal parameters values and testing at intermediary bioreactors scales (2L – 50L). Application of the new process settings at industrial fermentor scale (200L).
Cultures were regularly sampled to monitor the in-process parameters such as final cell concentration and product yield (TCID<sub>50</sub>/ml).
Taking into account that for vaccine formulation microcarriers must be eliminated from the viral suspension, filtration through Sartopure PP2 cartridges was performed.

Results and Discussion:
The most important process criterion for evaluating the performance of each size of bioreactor is their ability to support the same level of viral production as in RB. To evaluate this point, antigen production for a bovine vaccine was produced in the different bioreactors.
Comparable results between 2L, 10L, 50L and 200L concerning cell density, Viability, Productivity and Product quality. Bioreactor system allows even equivalent cell growth profiles and equivalent cell population homogeneity on microcarriers.
Viral production obtained with large scale bioreactors were equivalent to the one obtained with the 2L bioreactor.
Assays to eliminate microcarriers retaining the cellular debris filtrating through Sartopure PP2 or PP3 cartridges indicated no significant drop in virus titer.
As a conclusion, Bovine antigen with satisfactory yields can be obtained using this technology by culturing BHK-21 in a 200L bioreactor.
SUBioreactor technology is a good alternative to RB and is a suitable system for propagation of bovine viruses.
TOWARDS YELLOW FEVER VIRUS PRODUCTION IN SUSPENSION CELLS

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Background and Motivation:
Live attenuated Yellow fever (YF) virus as vaccine provides the only effective method to control and prevent endemic outbreaks. YF virus is a well-characterized member of the mosquito-borne flaviviruses and is utilized as vector to develop recombinant chimeric vaccines against genus-relating diseases such as Dengue, West Nile, and Japanese encephalitis. Increasing demand to produce YF virus and its chimeric forms may prove challenging with current egg-based production processes that have remained essentially unchanged over the past 60 years. Moving towards cell-culture-based production approaches in bioreactors can overcome limiting drawbacks of current manufacturing processes and allow to identify options for simple and efficient scale-up. Due to its extraordinary safety profile the Vero cell lineage, derived from kidney tissues of an African green monkey is a good candidate for a cell substrate. Its potential to produce YF virus under anchorage-dependent conditions has been shown in different studies but adaptation of Vero cells to growth in suspension remains an open problem.

Experimental Approach:
Different adaptation approaches were investigated for the adaptation of adherent Vero cells to suspension culture under serum-free conditions. The stepwise exchange of fetal calf serum containing medium to serum-free medium was performed by either multiple passages into new T-flasks with fresh medium or by replacement of spent medium with conditioned medium while cells were further cultivated in the same T-flask maintaining maximal confluency. YF infection studies with Vero suspension cells obtained under these conditions were performed in siliconized spinner flasks and ultra-low attachment well-plates. Virus quantification was carried out by plaque assay described by De Madrid and Porterfield (1969).

Results and Discussion:
The present work shows the successful adaptation of anchorage-dependent Vero cells (WHO Seed ECACC 134(9) to proliferate in suspension under serum-free (PEM) or chemically defined (Smi8, CD_U3) conditions. Adaptation of cells was achieved within 10 weeks, and cells grew either as single cell or in small aggregates with maximum 10 cells, depending on the medium. Stable cell cultures could be maintained in low-attachment T-flasks, however, growth rates were decreased in the absence of serum. The transfer from static culture into stirred systems showed high shear sensitivity and the tendency to form larger cell clumps leading towards an instable cell line. Thus, further adaptation is currently in progress. Initial infection studies of suspension cells with the egg-derived YFV-17D-RKI virus (one passage in adherent Vero cells) showed titers of 9.8 x 10⁶ Pfu/mL in Smi8 and 2.6 x 10⁶ Pfu/mL in PEM. Use of CD_U3 as virus production medium was not successful. Neither virus adsorption by reduced media volume nor freeze-thaw cycles for virus release could increase virus yields. However, it can be assumed that yields may be improved by virus adaptation over multiple passages (Beasley et al., 2013). In addition, to evaluate the productivity of Vero suspension cells, permissiveness and multiplicity of YF virus is currently investigated in a range of other cell lines. This includes adherent cells (insect cell C6/36, human derived A-549) and stable suspension cells (BHK, MDCK, etc.).
OPTIMIZED PRODUCTION OF HIV-1 VIRUS-LIKE PARTICLES BY CAP-T CELLS

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Background and Novelty:
Upon expression, the Gag polyprotein of HIV-1 spontaneously assembles in the vicinity of the plasma membrane and is released by a budding process producing enveloped particles that resemble immature HIV-1 virions. These particulate immunogens have proven to be potent stimulators of both cellular and humoral immune responses in animal models. In addition, they do not contain a viral genome minimizing biosafety concerns. Animal cells are the preferred system for production of this kind of vaccines candidates due to their complexity. So far, HEK293 and S9 cell lines have been used for production of HIV-1 VLP. The CAP-T cell line is a new platform for vaccine production with the ability to grow up to high cell densities in serum free and chemically-defined media, and to express complex recombinant proteins with human post-translational modifications. Furthermore, this cell line is easily transfected with PEI, which offers the flexibility to rapidly generate and screen a number of candidate variants in preclinical studies.

Experimental Approach:
Generation of fluorescent VLPs was carried out by transient transfection of CAP-T cells using a plasmid coding for the Gag polyprotein fused to GFP and PEI Max as transfection reagent. CAP-T cells were expanded in PEm medium. As the latter is not compatible with PEI transfection, cells were concentrated in FreeStyle293 medium prior to transfection. Five hours after transfection, PEm was added to the concentrated transfected cell culture. A DoE approach was used to optimize the transfection protocol. An in-house developed quantitation method based on fluorescence was used to assess VLP titers. VLPs were characterized by fluorescence confocal microscopy, transmission electron microscopy (TEM) and nanoparticle tracking analysis (NTA).

Results and Discussion:
The CAP-T cell line was evaluated for Gag-GFP VLP production. Gag-GFP accumulation at the cell membrane was observed by confocal fluorescence microscopy after transfection. Spherical particles surrounded by a lipid membrane with a size consistent with immature HIV virions (130 nm) were observed by TEM and NTA. Following a standard transfection protocol, supernatants containing 3×10^10 VLPs/mL were harvested in batch culture 72 hours post-transfection. Several key steps of the production protocol were studied to establish the best transfection conditions both in terms of VLP yield and protocol simplicity. It was determined that for optimal production cells need to be growing at mid-exponential phase, need to be added PEm medium post-transfection and can be transfected by independent addition of DNA and PEI with no prior complexation. A Box-Behnken experimental design was used to optimize cell density at time of transfection and DNA/cell and PEI/cell ratios. For optimal production, cells were transfected at a density of 5.24E+06 cells/mL with 0.5 pg of DNA/cell and 3.51 pg of PEI Max/cell. Using the optimized protocol titers of 6×10^10 VLP/mL were achieved. Further improvement of VLP production was achieved by implementing an Extended Gene Expression (EGE) strategy previously developed in our laboratory to improve VLP production in HEK293 cells. To conclude, CAP-T is a suitable and promising cell line for the production of HIV-1 VLPs and potentially other complex viral-based biotherapeutics.
PURIFICATION OF DIFFERENT SEROTYPES OF ADENO-ASSOCIATED VECTORED VACCINE AGAINST HEPATITIS E VIRUS

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Background and Novelty:
Hepatitis E virus (HEV) infection is the major cause of acute hepatitis in Southeast and Central Asia and the second most important cause in the Middle East and North Africa. Recently a HEV vaccine was licensed in China for local use. The vaccine named Hecolin is composed of a truncated HEV capsid protein, p239, as the sole antigen. The vaccine is adjuvanted and administered via the intramuscular route. In this work, we investigated the development of a novel candidate vaccine against hepatitis E infection using adeno-associated virus (AAV) as a vector expressing the gene of the truncated capsid protein of HEV (aa 112-aa 660) that will be delivered in a less invasive way, via the nasal route. rAAVs were produced in Sf9 cells using the baculovirus expression vector system. In this study we focused on the purification of rAAVs using different chromatographic techniques, with the aim to determine the optimal purification protocol of each serotype that results in a high overall yield and functional particles of rAAVs.

Experimental Approach:
Sf9 cells were infected by recombinant baculoviruses (BacRep, BacCap or BacCap/Rep and BacITRHEVORF2). Production of the different serotypes of rAAVs (2, 5 and 6) were conducted in shake flasks under optimal conditions previously determined in our laboratory. Cultures were stopped at ~96 hours post-infection. Culture supernatants were collected by centrifugation, the pellet containing infected-cell was treated to recover intracellular rAAVs. The Purification was conducted by affinity chromatography using the AVB Sepharose column, cation exchange and anion exchange columns. Collected fractions were analyzed by qPCR to determine rAAV titer; fractions were also analyzed by SDS-PAGE and western blot.

Results and Discussion:
Each harvest of the different serotypes of AAV was purified by affinity chromatography, anion exchange and cation exchange. AVB Sepharose column had resulted in an overall yield varying between 45% to 100%, depending on the serotype. The highest yield was obtained for rAAV5. Cation exchange purification of rAAVs on Hitrap SP HP column showed a yield in the range of 5% to 99%; the best yield was reached for AAV2. However anion exchange purification on Hitrap Q Sepharose column resulted in poor yields (less than 10%) for all the serotypes. The best purity profile was seen for the affinity chromatography. Further purification step will be conducted on Superdex 200 gel filtration column to improve the purity of rAAVs fractions collected through the cation exchange purification step.
EVALUATION OF THE USE OF SHEFF-VAX PRODUCTS FOR MRC-5 CELLS
GROWTH AND MEASLES VIRUS REPLICATION

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Background and Novelty:
Animal sera are essential components that are routinely used for the production of biologicals in mammalian cells. They are required for vigorous growth of animal cells used as substrate for virus propagation. They were shown to have several essential functions in culture. They are a source of nutrients, hormones, growth factors and protease inhibitors. However, besides these growth-promoting properties, animal sera have some major disadvantages (source of adventice contamination, lot variability, cost, etc.). Many attempts have been made to replace serum in order to design serum free media; plant hydrolysates are commonly used in serum free formulations to replace those from animal-origin. In this work we investigated the use of Sheff-Vax products developed by Kerry from different plants, in the cultivation of MRC-5 under serum reduced medium. These products will be also tested during the replication of measles virus in MRC-5 cells.

Experimental Approach:
The effect of four products Sheff-Vax ACF, Sheff-Vax ACF Plus, Sheff-Vax PF ACF and Sheff-Vax Plus PF ACF VP were investigated in T-flask cultures. MRC-5 cells were first grown in MEM+5% FCS, and then infected at an MOI of 0.001 with the AIK-C measles virus strain. Cells were infected in M199 supplemented with the Sheff-Vax product to be tested at different concentrations. In a second step MRC-5 cells were gradually adapted to the serum reduced medium DMEM +1.5% FCS+ 2.5 g/L Sheff-Vax Plus PF ACF+10 mg/l insulin. Finally the effect of the different Sheff-Vax products was investigated on measles virus replication in MRC-5 cells previously adapted to the serum reduced medium.

Results and Discussion:
The effect Sheff-Vax products on measles virus production in MRC-5 cells previously grown in MEM+5%FCS depends on the product to be tested and its concentration. The maximal measles virus titer was around 1.3x10^7 TCID50/ml which was slightly higher than the level obtained in the control medium M199+0.5% albumin (5.6x10^6 TCID50/ml). The highest virus productivity was reached when Sheff-Vax ACF, Sheff Vax PF ACF and Sheff Vax PF ACF VP were added to M199 medium during cell infection. Nevertheless the enhancement effect of these supplements varies according to their concentrations. The use of 2 g/l of these products improved virus productivity by 1.7, 2.3 and 1.8-fold, respectively, when compared to the control.
MRC-5 cells were adapted to grow in serum medium (DMEM+1.5% FCS+ 2.5 g/L Sheff-Vax Plus PF ACF+10 mg/l insulin) by gradual decrease of FCS in the medium. However cell growth in this medium showed lower growth performance in terms of cell density.
The effect of the different supplements on measles virus production in MRC-5 cells previously grown in serum reduced medium is currently under investigation.
MICROCARRIER-BASED CELL CULTIVATION IN ORBITALLY SHAKEN BIOREACTORS

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Background and Novelty:
Microcarriers have proven to be useful for a range of different applications in the cultivation of anchorage-dependent cells including virus vaccine production and the expansion of undifferentiated stem cells. In this study, an investigation of microcarriers for mammalian cell cultivation in orbitally shaken bioreactors (OSRs) was performed. The low mean specific dissipation rates, high gas transfer rates, and efficient mixing observed for OSRs were expected to provide a suitable environment for the cultivation of cells on microcarriers.

Experimental Approach:
CHO-K1 cells were cultured on different types of microcarriers at different concentrations in various OSRs. A scale-up study in OSR and a comparative study culturing cells in the commonly used tissue culture flask and in OSRs at two different scales, with volumes from 10 to 200 mL were performed. Viable cell counting, phase contrast microscopy, were used to monitor cell growth. pH, DO, and dCO₂, Glucose, Glutamine and Lactate during the culture were also recorded. In addition to CHO-K1 cells, Vero cells were also cultured using the same protocol.

Results and Discussion:
High cell densities, and homogeneously confluent microcarriers were observed. In addition, it was demonstrated the feasibility and scalability of continuous cell propagation in OSRs. The results indicated that OSRs are a good alternative to stirred-tank bioreactors and spinner flasks for the cultivation of mammalian cells on microcarriers and for the development of anchorage-dependent cells-based vaccines.
OPTIMIZATION OF MICROCARRIER-BASED VIRUS PRODUCTION IN A SINGLE-USE ROCKING BIOREACTOR SYSTEM

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Background and Novelty:
A majority of cell lines commonly used for viral vaccine production are anchorage-dependent and require expansion on solid surfaces. Since the 80s, microcarriers have been used to provide growth support for adherent cells in vaccine biomanufacturing processes in scales up to 6000 L. Today, however, most vaccines are manufactured in smaller scales. For such productions, single-use technology offers an attractive option. The combination of microcarriers and single-use bioreactors has been studied in both stirred-tank and rocking bioreactor formats. The outcome of these studies has shown that bioreactor design and operating parameters need to be carefully chosen to avoid excessive shear, which can be detrimental for the cell growth and virus production. In addition, microcarriers need to be maintained in suspension and the culture kept well aerated. These prerequisites can limit the operating conditions, for example, culture volume, under which microcarriers can be used. Here we show that by carefully selecting operating parameters, high cell growth and virus yields can be achieved in a single-use bioreactor system.

Experimental approach:
In this work, Vero cells were grown to high cell density on Cytodex™ microcarriers in serum-free cultivation medium using the ReadyToProcess WAVE™ 25 bioreactor system. To maximize the window of operation, the effects of bioreactor rocking speed, angle, and motion on cell growth and viability were investigated in different culture volumes. Using the tilt function of the rocker, microcarriers were allowed to settle to enable removal of the supernatant prior to infection of the cells with influenza virus. The rocker tilt function allowed medium exchange with minimal microcarrier loss, omitting the need for additional filters or sieves. Virus yields were determined as the levels of infectious virus particles and hemagglutinin in the culture supernatants at different time points. Furthermore, the metabolic profiles of the cultures were analyzed under different rocking conditions.

Results and discussion:
The results show the importance of using optimized bioreactor rocking conditions for microcarrier-based cultures. By adjusting the operating parameters, different cultivation volumes could be used with maintained high virus yields. Also, cell growth and viability remained high throughout the growth phase. These results offer valuable information to facilitate design-in of single-use rocking bioreactor systems to enable flexible future vaccine productions in smaller scales.
ADAPTATION OF ADHERENT MDCK CELLS TO SUSPENSION CULTURE IN ANIMAL COMPONENT-FREE, CHEMICALLY-DEFINED MEDIUM FOR INFLUENZA VIRUS PRODUCTION

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Background and Novelty:
Influenza related illnesses have caused an estimated over million cases of severe illness, and about hundred thousands of deaths worldwide annually. In response to rapid antigenic drift in influenza viruses, vaccination is considered as the most effective intervention. Traditionally these vaccines are produced in chicken embryonated eggs. However, in the case of a pandemic outbreak, this egg-based production system may not be quickly enough to meet the surge demand. The limitations associated with egg-based vaccines are reliable egg supplies, prolonged cultivation periods, and cumbersome operations and these limitations resulted in spurred exploration of alternatives. MDCK cells are widely being considered as an alternative host to embryonated eggs for influenza virus propagation. Although MDCK cells were considered to be suitable for the virus production, their inability to grow in suspension still limits the process of scale up and their production capability.

Experimental Approach:
In this study, a previously characterized adherent MDCK (aMDCK) cell line (Bioreliance, U.K.), were cultured and adapted in an optimized animal component-free (ACF), chemically-defined (CD) media prototype through two-step processes. Experiments were conducted to evaluate the adapted cell growth kinetics and its productivity of culturing avian influenza viruses, measured by Hemagglutinin (HA) titer and Tissue Culture Infectious Dose (TCID₅₀/ml) in spinner flasks compared to its parent cells in microcarrier-based cultures.

Results and Discussion:
The suspension MDCK (sMDCK) cells up to 2 × 10⁶ cells/ml after 96 hrs were obtained and the doubling time was very similar to the aMDCK cells cultivated on microcarriers (5g/L). In addition, no medium exchange appears to be necessary in sMDCK cell culture during the cell growth stage. The H7N9 virus strain (NIBRG-268, China) was used and infected to sMDCK and aMDCK cells with low multiplicity of infection. The harvest of viruses was collected on day three post infection. The HA titers in sMDCK and aMDCK cells were reached to 803.6 and 472 HA units/100ul, respectively. The HA titer of sMDCK cultured in serum-free medium increased nearly 70% compared to the aMDCK cells cultured.. The peak viral titer in sMDCK cells was 8.5 log TCID₅₀/ml which was also one log higher than that in aMDCK cells. A repeated study using H5N1 strain demonstrated similar observations. Small-scale bioreactors are being currently used to further evaluate and confirm our findings in sMDCK.

In summary, a new adapted sMDCK cell line was developed. This sMDCK cultured using ACF, CD medium is able to remain the similar growth rates as aMDCK cells and also showed higher viral titers in the tested influenza strains. This new combination of sMDCK with specifically-optimized ACF, CD medium provides a new solution for establishing large-scale culture of MDCK cells as subsequent well as large-scale influenza vaccine production.
HEK293 AS A RECOMBINANT PROTEIN FACTORY: THREE DIFFERENT APPROACHES FOR PROTEIN PRODUCTION

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Background and Novelty:
While initially employed for adenoviral vector production HEK293 became also one of the preferred cell lines for transient or stable protein expression. This is mainly due to its high transfection efficiency. Meanwhile genetic manipulation also includes new techniques for directing the integration of the foreign gene to highly expressed chromosomal sites e.g. by RMCE.

In this work, three approaches for recombinant protein production in HEK293 cells have been compared: (1) rAdV production expressing protein of interest, (2) stable cell line establishment by random gene transfection or (3) site-specific integration using RMCE technology. As protein of interest we used the Cap protein from the capsid of porcine circovirus serotype 2 (Cap-PCV2). This virus is related to post weaning multisystemic wasting syndrome (PMWS), which has major implications for the pig industry worldwide.

The work presented here provides comparison of several expression systems for the production of a candidate vaccine anti-PCV2.

Experimental Approach:
1. **CapPCV2 recombinant Adenovirus.**
   Viral DNA was isolated from field and the gene of the capsid (CapPCV2) was cloned into Adh5 genome using AdEasyXL kit. Virus and protein production were performed by infecting HEK293-F6 suspension cells.

2. **HEK293 stable cell line by random integration.**
   CapPCV2 gene sequence was codon optimized for mammalian cell expression. The obtained sequence was cloned into pIRESpuro3 bicistronic vector. HEK293-F6 suspension cells were transfected (PEI-DNA method) and positive cell pool was selected by puromycin addition to the media.

3. **HEK293 stable cell line by RMCE.**
   Three targeting vectors encoding for different promoters were transfected by electroporation to three different HEK293 master cell lines. Positive clones were selected by neomycin and ganciclovir addition to media. Clones were isolated by single colony pick up. Upon the selection of the best promoter, RMCE was applied to 293T cell line. Positive clones were selected by puromycin addition to media and single colony isolation was carried out. The selected clone was finally adapted to grow in suspension.

Results and Conclusions:
We generated transient CAP-PCV2 producer cells by infection of the HEK293 cell line with a recombinant adenovirus (rAdV-Cap). This approach gave a specific production (qₚ) of 2.79e-3pg/cell, which is considerably low. In contrast, a significant increase in production was achieved upon random integration of a CapPCV2 encoding plasmid which resulted in a 50-fold increment of qₚ (0.14pg/cell). The best results were obtained upon RMCE based strategy, targeting an expression cassette into previously identified chromosomal hotspots which resulted in an 100-fold increment (0.27 pg/cell).

The main conclusion of this work is that a bioprocess based on site-integrated stable suspension cell line would be the preferred one not only because the highest qₚ but also due to the easy characterization of the process (in comparison to adenovirus infection), the repeatability of batch productions (in comparison to the other approaches) and the possibility of rapid substitution of the protein of interest.
PROCESS INSTENSIFICATION BEYOND THE CELL DENSITY EFFECT: STABLE CELL-SPECIFIC MVA VIRUS PRODUCTION AT 60×10⁶ CELLS ML⁻¹

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Background and Novelty:
One approach to increase virus titers in viral vaccine production is to perform infections in high cell-density (HCD) cultures. However, this strategy usually results in reduction of cell-specific yields (cell density effect) compared to low cell density (LCD) cultivations [1, 2]. Here, the assessment of several medium-feeding strategies for propagation of Modified Vaccinia Ankara (MVA) virus in HCD cultivations using the suspension cell line AGE1.CR.pIX (ProBioGen AG, Berlin) is presented. Depending on the strategy, increases in cell-specific virus yield (CSY) and space-time yield (STY) towards the reference process at LCD [3] were observed. Finally, hybrid modeling was used to identify key factors that had an influence in successful strategies.

Experimental Approach:
AGE1.CR.pIX cells were cultivated in the chemically defined medium CD-U3 in 250 mL baffled-shake flasks. After a 2.5-day batch phase, semi-perfusion (periodic centrifugation and medium exchange) was performed based on cell growth and glucose consumption rates until more than 60×10⁶ cells mL⁻¹ were reached. Then, various medium feeding/exchange strategies at time of infection (toi) and during virus propagation were performed, according to the following table.

<table>
<thead>
<tr>
<th>Mode</th>
<th>Medium exchange at toi [%]</th>
<th>Medium feeding during virus propagation</th>
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<tr>
<td>0-FB</td>
<td>0</td>
<td>Fed batch (10-fold Glc &amp; Gin-concentrated medium)</td>
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<tr>
<td>50-FB</td>
<td>50</td>
<td>Single pulse at 24 hpi (10-fold Glc &amp; Gin-concentrated medium)</td>
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<td>Volume-expanded fed-batch [4, 5]</td>
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<tr>
<td>DME</td>
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<td>Daily medium exchange (90%)</td>
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<td>V+D</td>
<td>100</td>
<td>0 -24 hpi VEF, 36-120 hpi DME</td>
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Results and Discussion:
Semi-perfusion at a rate of 0.05 nL cell⁻¹ d⁻¹ was suitable to propagate AGE1.CR.pIX cells above 60×10⁶ cells mL⁻¹ with neither limitation nor overload of nutrients. Complete medium replenishment at toi accelerated accumulation of infective virus particles. The volume-expanded fed-batch (VEF) strategy influenced mainly the CSY, showing a 2-fold increase with respect to the LCD process. In contrast, the daily medium exchange (DME) strategy resulted in a 3-fold increase of the STY. Finally, combining VEF with DME (V+D) led to a 2-fold increase in CSY and a 4-fold in the STY. Based on the hybrid grey-box model the impact of medium exchange rates and feeding strategies on virus yields could be captured.

Conclusions:
Shake flasks are suitable systems to design and optimize infection experiments of AGE1.CR.pIX cells at HCD. Potential culture-derived factors that may limit or interfere with virus propagation were eliminated by complete medium replenishment at toi. Based on an optimal medium feeding strategy virus yields at HCD were increased significantly. Hybrid modeling could support scale-up into a controlled bioreactor using an appropriate perfusion system.

References
INFLUENZA VACCINE TITRE DETERMINATION USING BIOLAYER INTERFEROMETRY (BLI)

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Background and Novelty:
Fast, accurate determination of vaccine titre during influenza vaccine manufacture is important in understanding process performance and correctly scaling each process step. Traditionally Single Radial Immunodiffusion (SRID) assays have been used as the ‘gold standard’ but the assay requires very skilled operators to obtain reproducible results and is relatively low throughput. ELISAs have also been used to determine titre but have lower precision and dynamic range. Biolayer Interferometry (BLI) combines the high throughput characteristics of a 96-well plate based ELISA assay in conjunction with improvements in accuracy and repeatability derived from a simpler direct measurement of mass transfer on binding.

Experimental Approach:
The assay is based on the binding of the vaccine to polyclonal antibodies that recognise the influenza epitopes presented by the vaccine. The polyclonal antibody is bound to a protein G or protein A derivatised biosensor, depending on the animal source of the antibody. This configuration gives increased flexibility by allowing swift changes between vaccines derived from different viral strains by simply binding the paired Antibody for the new strain to a biosensor without the need for derivatisation. Hence the assay is suitable for the rapid changes in the viral strains represented in a vaccine. A robust assay, capable of determining vaccine titre from various process stages has been developed. A standard curve was acquired for each virus antigen to be tested and in-process samples were then assayed against this, the antigen concentration being derived from the binding rate of the antigen to the antibody bound to the biosensor. Samples and antibodies were diluted using process derived buffer solutions or Fortebio Sample diluent. Testing was carried out on a Fortebio Red 384 BLI system.

Results and Discussion:
Assay times on the Octet are less than 3 hours including sample preparation time and result reporting. Typically a linear range in the region of 5 to 100 µg/mL for attenuated and recombinant derived vaccines is achieved, although for recombinant hemagglutinin samples the range for certain antigens could be increased to over 100 µg/mL. The assay has been shown to be applicable to both live attenuated and recombinant vaccines. Data has been successfully gained for in process samples and different antigen species, recombinant hemagglutinins H1, H3, BMa, BBris, and attenuated viruses H1N1 and H5N1. The developed assay does not require a skilled analyst to run samples and ultimately the assay will be transferred to the ForteBio Blitz platform so that sampling can occur at-line by process development scientists.
EVALUATION OF THE USE OF SHEFF-VAX PRODUCTS FOR VERO CELLS GROWTH AND RABIES VIRUS REPLICATION

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Background and Novelty:
Since the beginning of the development of the cultivation of animal cells, animal serum was used in culture media to supply the cells with growth factors, hormones, adhesion proteins and transport factors. However, the use of serum has several drawbacks, therefore the development of serum-free media is interesting for the production of biopharmaceuticals, since it alleviates the risk contamination by infectious animal agents.
To eliminate the serum supplementation of culture media for animal cells, it is fundamental to choose an appropriate basal culture medium, as well as supplements that can provide the nutritional compounds necessary for cell growth. Among the nutritional supplements possible, hydrolysates of plant origin are gaining importance, since no animal components are present and such supplements provide peptides and amino acids that can have a positive impact on the growth of different strains of animal cells.
The proposed study aims to investigate the use of Sheff-Vax products developed by Kerry from different plants, in the cultivation of Vero under serum-free media formulation. These products will be also tested during the replication of rabies virus in Vero cells.

Experimental Approach:
The effect of four products Sheff-Vax ACF, Sheff-Vax ACF Plus, Sheff-Vax PF ACF and Sheff-Vax Plus PF ACF virus production were investigated in T-flask cultures. Vero cells were first grown in MEM+10% FCS, and then infected at an MOI of 0.1 with the LP2061 rabies virus strain. Cells were infected in M199 supplemented with the Sheff-Vax product to be tested at different concentrations. In a second step Vero cells were gradually adapted to the serum free medium DMEM + 2 g/L Sheff-Vax Plus PF ACF+10 mg/l insulin. Finally the effect of the different Sheff-Vax products was investigated on rabies virus replication in Vero cells previously adapted to serum free medium growth.

Results and Discussion:
Infection of Vero cells previously grown in MEM+10%FCS, showed that all the Sheff Vax products enhanced rabies virus production as compared to the control medium (M199+0.2% albumin). Depending on the Sheff Vax product, its level and the time of harvest, the highest rabies virus titer was varying between 7.7x10^4 to 1.2x10^7 FFU/ml whereas for the control medium this level did not exceed 3x10^6 FFU/ml. M199 supplementation with Sheff Vax Plus ACF and Sheff Vax PF ACF at 6 g/l, had resulted in the highest virus productivity which was enhanced by 5.6 and 4.1-fold, respectively.
Vero cells were successfully adapted to DMEM+2 g/L. Sheff-Vax Plus PF ACF + 10 mg/l insulin. Cell growth performances were comparable to those reached in DMEM+5%FCS. The effect of the different supplements on virus production in Vero cells grown in serum free medium is currently under investigation.
AN EMPIRICAL AND THEORETICAL BASED APPROACH TO DEFINING AGITATION REQUIREMENTS FOR MICROCARRIER PROCESSES IN SINGLE-USE BIOREACTORS

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Background and Novelty:
Recent advancements in adherent cell culture technologies such as stem cell and cell-based vaccine production are driving the need for improved process efficiencies. Scale-up using traditional 2D methods has proven to be cumbersome and expensive. Microcarrier processes in stirred-tank bioreactors are becoming a viable alternative to achieve the production levels required to meet the growing demand of these applications. In parallel, the biomanufacturing industry is rapidly adopting single-use technologies in order to reduce costs and increase flexibility. Microcarrier processes in single-use bioreactors represent a new paradigm for the production of adherent cells in 3D culture while presenting unique challenges for process optimization and scaling.

During microcarrier processes, it is desirable to maintain a homogeneous state of suspension to insure a uniform growth environment throughout the entire vessel. However, since the sensitivity of cells to hydrodynamic shear forces is increased when cells are grown on microcarriers, microcarrier users develop their processes with agitation rates that are as low as practically possible. Balancing this against the need to keep the microcarriers adequately suspended while also maintaining sufficient bulk liquid mixing to insure appropriate aeration of the culture can make the selection of process conditions challenging.

Experimental Approach:
Traditionally, the criterion for suspension of particles in a stirred-tank vessel has been to maintain an off-bottom state, such that no particle remains on the bottom of the vessel for more than 1-2 seconds. The impeller speed that maintains this state is called the just suspended speed ($N_{j}$) and can be calculated using the Zwietering correlation (Chem. Eng. Sci., 8, 244, 1958). While this empirically-defined correlation has been used to help predict agitation requirements in traditional stainless steel bioreactors, it cannot be applied directly to the vast majority of single-use systems because values for the geometric constant within the equation do not exist for the unique geometries of these vessels.

An empirical approach was taken to define agitation requirements of a microcarrier-based cell culture process using the Mobius® CellReady single-use bioreactor platform as a model system. Microcarrier suspension was characterized in the range of practical agitation rates and liquid working volumes. Qualitative assessment of flow and settling patterns and quantitative analysis of suspension gradients were used to determine agitation requirements. These empirical determinations were then used in conjunction with the Zwietering correlation to make predictions across varying microcarrier concentrations and vessel sizes, and the results were verified by experimental analysis.

Results and Discussion:
This study demonstrates the compatibility of microcarrier processes with the Mobius® CellReady single-use bioreactors and describes a methodology for determining agitation requirements that is applicable to any microcarrier/stirred-tank bioreactor system. By taking a combined empirical and mathematical approach, it is possible to characterize the agitation requirements in a stirred-tank bioreactor system for which empirical Zwietering constants do not exist. This approach enables the use of scalable single-use bioreactor platforms for adherent cell culture processes and minimizes the amount of necessary experimental work, allowing process developers to take full advantage of the speed and flexibility that single-use technology offers.
TRACKING DNA/PEI COMPLEXES ENTRANCE IN HEK 293 CELL LINE AFTER PEI TRANSIENT TRANSFECTION

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Background and Novelty:
Transient Gene Expression is a widely used technique to introduce foreign DNA into mammalian cells. It is very suitable to produce enough quantities of biomaterial to perform pre-clinical studies and the scalability of the process has been demonstrated in recent years. Even though it is a very widespread technique, less is known about the transfection process at its cellular level. The aim of this work is to study the process by which DNA crosses the cell membrane and reaches the nucleus after transfection using PEI as transfection reagent in a process in which Gag-GFP VLPs are produced.

Experimental Approach:
Suspension HEK 293 cells were grown in Freestyle medium with non-animal derived additives. Cultures were transfected with 25-kDa linear PEI when cells reached 2×10^6 cells/mL using 1 μg of plasmid DNA/mL of cell culture and a DNA:PEI ratio 1:2.
Flow cytometry analysis: After transfection, samples were taken and centrifuged at 300 g during 5 minutes. Then, cells were seeded in ultra low binding wells with fresh medium. Samples were taken at 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 60, 90, and 120 minutes post transfection. This allows having cultures that have been in contact with the DNA/PEI complexes during different times. At 24 hpt, the transfection efficiency was analyzed by flow cytometry and at 48 hpt, the fluorescence accumulated in the supernatant (VLP production) was analyzed by fluorometry.
Confocal microscope analysis: Plasmid DNA pGag-EGFP plasmid was labeled using Label IT® Tracker™ Cy™3 (red fluorescent color) and the transfection process was followed by Flouview® FV100 confocal microscopy. HEK293 cells were dyed with CellMask™, which stains the plasma membrane in a deep red color, and with Hoechst, which stains the nuclear DNA in blue cyan color. Samples were placed in glass bottom dishes for visualization under the microscope. Images were taken every 5 minutes in the first half hour, and afterwards, more spared images were analyzed until 5 hpt. Images were processed with LAS AF™ software.

Results and Discussion:
In the flow cytometry assay it can be observed that DNA:PEI complexes start entering the cells from the very beginning and a linear increase in transfection efficiency is observed until 60 minutes of contact between the cells and the complexes. No change in transfection efficiency is obtained afterwards. The same profile is observed when fluorescence in the supernatant is analyzed. In the confocal assay, it can be observed the DNA/PEI complexes seem to be attached to the plasma membrane since the moment that the culture is transfected. No significant change is observed during 2 hours and no complexes are observed inside the cell. After 2 hpt complexes start to be detected in the cytoplasm of the cells and reach the nucleus around 5 hours post transfection.
These results are in concordance with the obtained in the flow cytometry assay as the increase in transfection efficiency during the first hour post transfection can be due to the increase of interaction between the complexes and the cell membrane.
SELECTION AND OPTIMIZATION OF ADDITIVES FOR THE ENHANCEMENT OF HIV1 GAG-GFP VIRUS LIKE PARTICLES PRODUCTION IN HEK293 CELLS

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Background and Novelty:
Transient Gene Expression has been used at small and medium scale for the production of biological material in sufficient quantities to perform pre-clinical and characterization studies. Several additives have been tested and proven as effective enhancers of protein production by TGE in mammalian cell culture. The capacity of Trichostatin A, Valproic acid, Sodium Butyrate, DMSO, Lithium Acetate, Caffeine, Hydroxiurea and Nocodazole to improve the production of Gag-GFP VLPs using a suspension HEK 293 cell line is explored in this work.

Experimental Approach:
Suspension HEK 293 cells were grown in Freestyle medium with non-animal derived additives. Cultures were transfected with 25-kDa linear PEI. Transfection was typically carried out when cells reached 2×10^6 cells/mL using 1 μg of plasmid DNA/mL of cell culture and a DNA:PEI ratio 1:3. Design of experiments (DoE) was used, first to screen which supplements had a positive effect on VLP production and second to find the optimal concentrations of each supplement to maximize VLP production. Cell viability is considered as well a key factor, together with VLP production, since it is considered that low cell viabilities at the point of VLP harvest will increase the percentage of non-assembled free Gag protein.

Results and Discussion:
Using a Plackett-Burman assay, Valproic acid, Sodium Butyrate, Caffeine were found to have a positive effect Gag-GFP VLP production. Lithium acetate was also selected, as it showed a positive effect in transfection efficiency and there is a possibility of interaction with the other components leading to an overall improvement in Gag-GFP VLP production. Using a Box-Behnken design, optimal concentrations of Lithium Acetate, Valproic Acid, Butyric Acid and Caffeine, were determined to be 6.3 mM, 0.88 mM, 5 mM and 0 mM, respectively. Taking into account the negative interactions of butyric acid with the rest of compounds studied and the low viability observed when butyric acid is used, two different possibilities were studied: a) using only Butyric acid at optimal concentration of 6mM b) using a combination of Lithium Acetate, Valproic Acid and Caffeine. In the second case, the optimal of Lithium Acetate, Valproic Acid and Caffeine, were determined to be 20 mM, 3 mM and 3.5 mM, respectively. A validation experiment was carried out using these two strategies. The results in VLP production using the optimal concentrations of Lithium Acetate, Valproic Acid and Caffeine were slightly lower than when only Butyric acid is used, but cell viability is the highest of all conditions tested, even higher than when no additives are added to the culture. When VLP production is compared with the negative control, the increase with the combination of Lithium Acetate, Valproic Acid and Caffeine is 3.79-fold, with final viability of 94%. Alternatively, the use of only butyric acid allows to obtain a 4.87 fold increase, however with final viability of 70%.
## LIST OF PARTICIPANTS

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