

Cell culture technology: Recent advances, future prospects

5th Annual event Friday, 09 March 2012
The Penridge Suite, 470 Bowes Road, London N11 1NL

In the last 4 decades mammalian cell culture has matured from being merely a research tool into being one of the foundations of the biopharmaceutical industry, and its use is continuing to expand rapidly. In vitro models are replacing animals in many tests and assays; its enormous potential in the fields of stem cell and regenerative medicine has hardly started to be realized; and its utility in research grows ever faster".

Meeting Chair: Dr John Davis, Chairman of the UK Branch of the European Society for Animal Cell Technology.

This conference will examine some of the latest applications of cell culture technology, some that are still "over the horizon", and some of the problems that must be solved before it can reach its full potential.

This event has CPD accreditation and will have a troubleshooting panel session.

On registration you will be able to submit your questions to the panel that will be asked by the chair on the day of the event

- 9:00 – 9:45 Registration
- 9:45 – 10:00 **Introduction by the Chair:** *Dr John Davis*, Chairman of the UK Branch of the European Society for Animal Cell Technology
- 10:00 – 10:30 **Polymeric matrix construction**
Professor Alexander M. Seifalian, University College London, UK
- 10:30 – 11:00 **A discussion of the systems used to measure autophagy in vitro**
Katy Petherick, University of Bristol, UK
Autophagy is a rapidly growing field of research. Methods to analyse autophagy are constantly advancing. The complexity of the process makes it important to have accurate readouts of autophagic activity. This talk will discuss a range of methods for measuring autophagy and important points to consider when using these techniques.
- 11:00 – 11:30 **Clinical grade human embryonic stem cells**
Dr Dusko Ilic, Kings College London School of Medicine
hES cells are undifferentiated cells derived from an early embryo that can grow in vitro indefinitely, while retaining their capability to differentiate into specialized somatic cell types. Their use in therapy and regenerative medicine as well as in toxicity screening and drug development is widely anticipated. However, even if we pay no attention to ethical, religious and political issues that relate to hES cells, there are still a number of obstacles to be resolved before these cells can be broadly used for cell-based therapy. The talk provides an overview of the current status and the future perspective of the field from the point of view of the standard level of patient safety and efficacy for the healthcare industry.
- 11:30 – 12:00 **Speakers' photo then mid-morning break and trade show**
- 12:00 – 12:30 **Single use bioreactor for clinical-grade production of stem cells**
Dr. Thorsten Adams, Sartorius Stedim Biotech GmbH, Germany
As the field of regenerative medicine further develops, there is an increasing need for larger scale generation of stem cell derived therapeutics for clinical applications. Stem cells are frequently cultured in simple devices such as petri dishes and static bags. However, the main drawback of these technologies is the lack of scalability and process control. Single use bioreactors are well suited for this application. They offer a controlled environment for hypoxic cultivations and the possibility for process automation. This talk will address the technology, first results and the challenges that need to be overcome in order to optimize the single use bioreactor technology for the regenerative medicine market.
- 12:30 – 13:00 **Automated Approaches to the Optimisation of Stem Cell Expansion and Differentiation**
Professor Gary Lye, University College London, United Kingdom
Stem cell culture is a largely manual process, with major challenges to address in the methods used regarding scalability and variability. Process automation can be of great benefit to reduce operator-dependent variation, therefore improving cell yield and quality. This would be beneficial for production of defined cells for high throughput screening or definition of a robust cGMP process suitable for scaled-out production of cells for clinical application. This work describes the use of a custom assembled Tecan platform for the hands-free expansion and directed differentiation of a range of stem cells.
Key bioprocess variables were initially optimised to develop a Standard Operating Procedure (SOP) for the expansion and differentiation steps. Comparisons between the manual and automated process over eight sequential passages were then performed. Automated culture was shown to improve the consistency of cell yield up to 3-fold.

Using the platform's ability to control oxygen tension, stem cells were further differentiated into neural precursors at 2% oxygen and results compared to manual differentiation at 2% and 20% oxygen. Use of the enclosed automated platform avoided changes in oxygen tension during media changes, as occurs in manual culture. Up to a 16-fold increase in gene expression in the differentiated cell lines were obtained.

13:00– 14:00 **Lunch and trade show**

14:00 – 15:00 **Question and Answer Session**

Delegates will be asked to submit questions to a panel of experts. Questions can be submitted before the event or on the day

15:00 – 15:30 **Dry Powder Mammalian Cell Culture media production: From Easy going to sophistication – a case study DMEM / F12 production**

Dr. Jörg von Hagen, PMP, Head of Process Development / Launch Management, Pharm Chemicals Solutions, Merck Millipore Division, Germany

This talk will discuss Dry Powder Mammalian Cell Culture media production-From Easy going to sophistication - a case study DMEM / F12 production. Points will include :

- Physico chemical characterisation of DMEM F12
- Batch to batch consistency: the grail in media production and supply
- Impact on cellular performance of Chinese Hamster Ovary cells
- Media testing beyond cell growth
- Perspectives in future media preparation

15:30– 16:00 **Afternoon Tea/Coffee and trade show**

16:00 – 16:30 **Recent advances in photoporation technology for cell injection and transfection**

Dr Maria Torres, University of St Andrews, Scotland, UK

The injection of exogenous materials into mammalian cells is an important and ubiquitous procedure in cell biology. Recently, the use of laser was realized as an effective means to transiently permeabilise the cell membrane allowing intracellular delivery of a variety of substances such as plasmid DNA, siRNA, mRNA or nanoparticles. This technique called photoporation is a gentle, robust and highly efficient method for cell transfection. In this presentation, the recent progress in this field and the development of the photoporation technology will be discussed with the end users in mind.

16:30 – 17:00 **Remodelling of mRNA translation in the cold during mammalian cell bioprocessing**

Professor Mark Smales, University of Kent, UK

Reduced temperature cultivation of mammalian cells is often used during both stable and transient expression of recombinant proteins to improve the yield and product quality. Upon such cold shock, mammalian cells activate a number of cellular responses including remodelling of mRNA translation. Here we will describe our understanding of this remodelling and the implications for bioprocessing.

17:00 Chairman's summing up

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About the Chair

John Davis is Visiting Lecturer in Biotechnology at the University of Hertfordshire, and Chairman of the UK Branch of the European Society for Animal Cell Technology (ESACT-UK). After a degree in Biochemistry at Sheffield, he moved in 1974 to Renato Dulbecco's laboratory where he was initiated into the art of cell culture. Following PhD studies in Leicester, he moved to Switzerland, working with both Norman Iscove and Georges Köhler, the latter starting him on his many years of research into the use of monoclonal antibodies, particularly in therapy. After a further postdoctoral position, at the University of Cambridge where he worked on the early stages of the development of Campath (Alemtuzumab; now also known as Lemtrada), he made the transition to industry, working first for PA Technology and then (for nearly 20 years) for the Bio-Products Laboratory. In 2007 he made the transition back to academia. In addition to undergraduate and postgraduate teaching, he now runs open courses on Basic Cell Culture and Intermediate/Advanced Cell Culture. He has served on both the UKCCCR subcommittee on the Use of Cell Lines in Cancer Research, and the EC task force on Good Cell Culture Practice. In addition he has edited a number of books on cell culture, including Basic Cell Culture: A Practical Approach, and (with Glyn Stacey) Medicines from Animal Cell Culture. His most recent book is Animal Cell Culture: Essential Methods, which was published in March 2011 by Wiley-Blackwell

About the Speakers

Maria Torres is currently a postdoctoral research fellow in Biophotonics at the University of St. Andrews, Scotland. She did her PhD studies in the same university, having obtained a prestigious Scottish University Physics Alliance (SUPA) prize studentship for her PhD. She works in collaboration with biologists in the field of neuroscience, breast cancer and embryology among many others to use the novel technique optical transfection for advance biological applications. Her expertise is on using a variety of lasers and microscopy systems to perform optical manipulation of single cells and developing embryo.

Katy Petherick is in her final year of a PhD investigating the role of autophagy in colorectal cancer at the University of Bristol, supervised by Professor Chris Paraskeva in the Tumour Biology group. Prior to commencing her PhD, Katy completed her degree at the University of Birmingham, which involved a year in industry at Cambridge Antibody Technology (now MedImmune).

Gary Lye is Professor of Biochemical Engineering within the Advanced Centre for Biochemical Engineering at University College London (UCL). He is Deputy Head of Department and Director of the Industrial Doctoral Training Centre (IDTC) in Bioprocess Engineering Leadership. He received his PhD in Biotechnology from the University of Reading in 1992. Between 1993 and 1996 he was successively a Research Fellow and then Lecturer in Chemical Engineering at Imperial College London and the University of Edinburgh. He joined UCL in 1996. He has broad research interests on the application of microscale and automation techniques to rapid bioprocess design, optimisation and scale-up.

Thorsten Adams, molecular biologist, is product manager fermentation technologies at Sartorius Stedim Biotech. He was educated at the University of California, Berkeley and the University of Goettingen, where he obtained his Ph.D. from the department of microbiology and genetics in 2005. He worked as a scientist in technology development in several positions, latest at Morphosys in Munich, Germany. He developed cell culture expression and screening systems and is experienced in cell culture development using several production systems to large scale bioreactors. Dr. Adams was amongst the first users of single use upstream technology in the industry. He authored several papers, book chapters and patents. In 2007, he joined Sartorius Stedim Biotech, where he is responsible for product management of single use bioreactors. He is an expert for the implementation of single use bioreactor technology in upstream processes.

Jörg von Hagen is head of Merck Millipore Process Development R&D in Darmstadt (Germany). Having studied biotechnology and signal transduction in Giessen and Darmstadt, he received his academic degree with an award-winning thesis in 2001. Dr. von Hagen has more than 20 years of practical expertise in biotechnology, especially in molecular cell biology and proteomics.

Dusko Ilic, obtained his MD degree and BSci in Molecular Biology at the University of Belgrade, PhD at the Tokyo University, Japan, and postdoctoral training at the University of California in San Francisco. Before joining King's College School of Medicine in London as a Senior Lecturer in Stem Cell Science, he held positions of Adjunct Associate Professor at the University of California San Francisco, Consultant at the Veteran Affairs Medical Center, San Francisco, and the Director of R&D at StemLifeLine, a California-licensed stem cell company. His current research interest lies in human embryonic stem (hES) cells, induced pluripotent stem (iPS) cells, cancer stem cells, and regenerative medicine.

Mark Smales work focusses upon furthering our understanding the cellular processes underpinning recombinant protein synthesis and quality from mammalian cells. He is currently Professor of Biotechnology at the University of Kent and the Director of the Center for Molecular Processing at Kent.

Keywords: cell, transfection, lasers, microscopy, Autophagy, Puncta, p62, Flux, cell, transfection, lasers, microscopy, dry powder, mammalian cell culture media, batch to batch consistency, human embryonic stem cells, clinical grade, cGMP, bioreactor, process development, large scale, upstream processes, recombinant protein production; cold-shock; CHO cells; cell engineering

INTEGRIN EXPRESSION AND ACTIN CONFORMATION IN CHO SUSPENSION CELLS

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Biopharmaceutical manufacturing is predominantly based upon the use of mammalian cell lines capable of proliferation as single cells in suspension in a chemically-defined synthetic environment. This environment lacks exogenous growth factors, cell-matrix and cell-cell contacts – all of which usually contribute to proliferation and survival of fibroblastic cell types such as Chinese hamster ovary (CHO) cells. CHO cells are routinely used as production vehicles *in vitro*; hence a lengthy “adaptation” process which successively selects clonal derivatives able to proliferate as single cells in suspension is a pre-requisite for successful bioprocess development. Virtually nothing is known of the underlying biological adaptations that permit particular cell clones to survive and proliferate in suspension. In this project we test the hypothesis that suspension adaptation requires changes to the complement of functional proteins at the CHO cell surface. We specifically target those involved in cell-matrix interactions, integrins. For fibroblastic cell types cultured *in vitro* disruption of integrin-mediated attachment to the extracellular matrix initiates apoptosis (termed anoikis in this context); integrins are also known to potentiate growth factor receptor (tyrosine kinase) signalling cascades, interact extensively with the cytoskeleton, mediate cell-cell cohesion and coordinate mechano-transduction, all of which are highly relevant to CHO cell performance *in vitro*.

Integrins in adherent cells are usually expressed in clusters at points of focal adhesions. Integrin expression levels and conformation were analysed using antibody specific probes against integrin $\alpha 1$, integrin $\alpha 4$ and integrin $\alpha 1$. We found increased abundance of those integrins in suspension cells in a regular, clustered distribution indicative of an active conformation despite absence of cell-matrix interaction. Analysis of the actin cytoskeleton revealed re-organisation of the actin cytoskeleton from a typical fibrillar morphology in adherent cells to a thick spherical sub-cortical sheath in suspension cells. The demonstrated re-enforcement of the spherical actin conformation by up-regulated integrin clusters on the cell surface of suspension adapted CHO cells could be a requirement to resist shear stress as experienced by stirring during suspension growth in a bioreactor. The spherical actin structure is also found in the unadapted adherent cell line when cultured in suspension but these cells lack the regular distribution of integrin clusters on the cell surface. Hence re-enforcement of the spherical actin conformation by integrin clusters on the cell surface could be necessary for CHO cells to grow successfully in suspension.

Our data suggest that adaptation to suspension growth requires conservation, and even enhancement of integrins, both with respect to (i) their role as cell structural elements anchoring the plasma membrane to a sub-cortical actin sheath and (ii) constitutive activation of signalling pathways that promote proliferation via clustering.

MICROSCALE CHARACTERISATION OF A MANUFACTURING ROUTE FOR LENTIVIRAL VECTORS

HM Guy, KA Mitrophanous, GJ Lye, TK Mukhopadhyay

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In order to generate large quantities of lentiviral vectors (LVs) for clinical purposes, the development of producer cell-lines that are amenable to growth in suspension is highly desirable. Towards this objective, a number of studies have reported the successful creation of human embryonic kidney 293 (HEK293) or HEK293T derived LV producer cell lines. However, delivering sufficient titres once these cells have been adapted to growth in suspension can present a significant challenge. To address this issue the present study employed a strategy combining statistical experimental design (DoE) with microscale cell cultures to rapidly characterise key process parameters influencing LV production from suspension-adapted producer cells. ProSavin[®], a LV engineered for treatment of Parkinson's disease for which producer cell lines have been previously developed, was used as a model system. Three rounds of DoE (central composite designs) were employed to characterise the effect of three factors known to influence ProSavin[®] titre. These factors were: post-induction period, liquid fill volume, and concentration of inducer (doxycycline). Key insights into the relationship between functional titre, cell growth and total particle production were obtained and the information presented here will be useful for researchers looking to develop a scalable production process for LVs.

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This meeting was organised by Euroscicon (www.euroscicon.com), a team of dedicated professionals working for the continuous improvement of technical knowledge transfer to all scientists. Euroscicon believe that they can make a positive difference to the quality of science by providing cutting edge information on new technological advancements to the scientific community. This is provided via our exceptional services to individual scientists, research institutions and industry.

EXPANSION AND MAINTENANCE OF HUMAN EMBRYONIC STEM CELLS ON AN AUTOMATED MICROWELL PLATFORM

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Human embryonic stem (hES) cell culture is currently a largely manual process, with major challenges to address in the methods used regarding scalability and variability. Process automation can be of great benefit to reduce operator-dependent variation, therefore improving cell yield and quality, and to enable scaled-out manufacture. It can also be critical in the helping define a robust cGMP process suitable for production of cells for clinical application. The work described here aims to develop a Standard Operating Procedure (SOP) for the passaging of hES cells on an automated microwell platform, providing a more consistent method for cell maintenance and expansion.

Mechanical passaging of hES cells can introduce variability during a process and furthermore is not suitable for the automated microwell platform. Two new cell lines were derived from mechanically passaged hES cells. Both Shef-3 and Shef-6 cell lines were adapted to enzymatic passaging using a recombinant trypsin replacement enzyme, TrypLE Express™. This process took place over two months, resulting in a karyotypically abnormal (Shef-3) and normal (Shef-6) cell line.

Once cell lines were established, optimum growth conditions for each line were evaluated. Particularly the feeder and hES inoculation cell density (ICD) were found to be critical during cell culture. Design of Experiments (DoE) was used to evaluate these factors and their interactions. Results indicated that both TrypLE-adapted lines were capable of growing on a feeder layer ICD as low as 3,125 cells.cm⁻². hES ICDs between the two lines vary greatly, with that of the Shef-6 line almost double the ICD of the Shef-3 line. This was as expected, with the karyotypically abnormal line passaged at higher split ratios.

An SOP for passaging of Shef-3 TrypLE-adapted cells in 24-well microplates was established and optimised through a series of dissociation experiments. The dissociation step was found to be critical in maintaining cell yield over multiple passages on the platform. This step was improved by using a non gelatin-coated tissue culture surface and increasing the dissociation time to 30 min. Cells were passaged every 3 days over 5 consecutive passages. Cell yield remained stable at approximately 2 x 10⁵ cells.well⁻¹ and cell viability did not drop below 98%. Cells were shown to express pluripotency marker Oct-4 throughout all 5 passages. Pluripotency was further confirmed by high expression of SSEA-4 (97.86% and 98.03%) and TRA-1-60 (95.35% and 82.34%) before and after processing respectively. Future work will involve passaging of the karyotypically normal Shef-6 line on the automated microwell platform.

Registration Web Site:

www.regonline.co.uk/cellculture2012

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