

Advances in Cell Culture technology

The BioPark Hertfordshire, Welwyn Garden City, AL7 3AX: 15th April 2010

This meeting has CPD accreditation

- 9:00 – 9:45 **Registration**
- 9:45 – 10:00 **Introduction by the Chair:** *Dr John Davis*, University of Hertfordshire, UK
- 10:00 – 10:30 **Cell line misidentification**
Professor John Masters, UCL, UK
The failure to identify the species and individual from which a cell line originated has resulted in the use of misidentified cell lines and an enormous financial cost to the academic and pharmaceutical research community. Simple, cheap and reproducible quality control measures are available to distinguish species and to identify individual human, cow, horse and dog DNA. Currently, the ATCC is developing a standard for cell line identification which will be adopted as an American standard.
- 10:30 – 11:00 **Recent advances in cell culture methods as alternatives to animal testing**
Dr Carol Barker, XCellR8 Ltd, UK
The talk will summarise recent developments in cell culture methods as valuable alternatives to animal testing, and highlight some of the remaining key challenges. The main focus will be on human cell culture systems currently available for cosmetic testing and drug discovery applications, and how these systems can assist companies to comply with the 7th Amendment to the European Cosmetics Directive and REACH legislation
- 11:00 – 11:10 **Speakers' photo**
11:10 – 11:30 **Mid-morning break**
- 11:30 – 12:00 **Metabolomic profiling of mammalian cell lines – New approaches for understanding recombinant antibody production**
Dr Christopher A. Sellick, University of Manchester, UK
Mammalian cells (e.g. CHO or NS0 cells) are very important industrially for production of biopharmaceuticals due to the requirement for mammalian-type post-translational processing and appropriate folding. Metabolomics is proving to be a powerful tool for understanding responses of these cells to different medium formulations with and without the addition of feeds. Combined analysis of the fingerprint and footprint of cells under different growth conditions has enabled us to define key limiting metabolites that have been used to define simple rational feeds (containing only 5 metabolites) that improve antibody production by 100%.
- 12:00 – 12:30 **Remote Monitoring of Cell Culture - Splitting cells based on fact rather than tradition**
Dr Peter Djali, Essen Bioscience, Welwyn Garden City, UK
Cell culture is the first step of any cell based assay, yet quite often it is poorly controlled and practiced as an art rather than a science. Here we present a non-invasive technique allowing continuous QA monitoring, production of standard growth curves and record keeping for cell culture.
- 12:30–13:30 **Lunch**
- 13:30 – 14:00 **Harvest your cells without trypsin! Introducing cell releasing UpCell surface by temperature control**
Dr Masato Ishiwata, Thermo Fisher Scientific, UK
UpCell culture surface regulates cell adhesion and detachment by mere temperature control. UpCell surface is covalently bonded with a temperature-responsive polymer and is slightly hydrophobic at 37C, allowing cell adhesion and proliferation. However, the surface becomes extreme hydrophilic under 32C, prompting cell detachment without the use of cell-damaging proteolytic enzymes such as trypsin. Applications include immunoblotting and flow cytometry, and the detachment of strongly adherent macrophages or osteoclasts. When cells are cultured beyond confluence, the contiguous and scaffold-free cell sheet can be collected with deposited extracellular matrix, and can be used for regenerative medicine studies into, for example, corneal or cardiovascular indications.
- 14:00 – 14:30 **Development of Enabling Technology to Enhance the Growth and Function of Cultured Cells**
Dr Ross Carnachan, Durham University, UK
Scientists at Reinnervate have developed a novel 3-D cell culture system which it plans to produce and market in the near future. We have re-engineered the configuration of polystyrene, the growth substrate material that is currently used for the majority of existing cell culture applications, into a 3-D scaffold that has subsequently been adapted for cell culture applications. This offers several advantages to the user including an inexpensive simple un-wrap and use consumable technology, enabling reproducibility during routine use. The porosity of the polystyrene scaffold is

specially customized to within narrow tolerances during its manufacture. Optimisation of the growth medium and cell seeding density results in the growth of cells throughout the scaffold forming a 3-D block of tissue *in vitro*.

14:30 – 15:00 **Embryonic stem cell bioprocessing**

Dr Farlan Veraitch, UCL, UK

Embryonic stem cells (ESC) can proliferate indefinitely in culture whilst retaining the ability to differentiate into every adult lineage. There have been a number of studies showing that specific neuronal populations generated from ESC could represent new cellular therapies for the treatment of neurodegenerative diseases such as Parkinson's and Retinitis Pigmentosa. Delivering the safe and cost-effective ESC-based products will be highly dependent upon the development of efficient, reproducible and high-yield bioprocesses. One of the most inefficient steps in the majority of ESC processes is directing differentiation into specific target populations. Spontaneous differentiation into unwanted cell types results in the generation of low purity populations and little attention has been given to the low cellular yields associated with these processes. Studies in our laboratory have revealed that low oxygen tensions (2%) can significantly enhance the production of neuronal cells from mouse ESC, human ESC and human induced pluripotent stem cells (iPSC). Investigating the full spectrum of oxygen tensions revealed that further increases in the yield of neuronal cells can be achieved at higher oxygen tensions (4-10% O₂). In addition, steps up and down in oxygen tension at the mid-point of the differentiation protocol have demonstrated that dynamic control over the microenvironment represents a powerful tool for production of pure neuronal populations.

15:00 – 15:30 **Instant immortal cell cultures from *Cdkn2a* null mice: functional analysis of colour mutations using melanocyte lines**

Professor Dorothy C Bennett, Molecular and Metabolic Signalling Centre, Division of Basic Medical Sciences, St George's, University of London, UK

Normal somatic cells can undergo a limited number of divisions before undergoing the permanent growth arrest called cell senescence. Although an important tumour suppressor mechanism, senescence is an inconvenience for cell culturists. A key player is the *Cdkn2a* locus, encoding two powerful growth inhibitors and mediators of cell senescence, p16 and ARF. From mice with deletions at this locus, one can culture quite normal, functional cells, such as pigmented melanocytes, which do not senesce: they are immortal immediately. We have utilized this finding to study numerous pigmentary mutations in immortal cultured melanocytes, working with many other groups worldwide.

15:30 – 16:00 **Afternoon Tea/Coffee**

16:00 – 16:30 **Wnt signals in Prostate Cancer and Embryonal Carcinoma Cell Growth and Differentiation**

Dr Robert Kypta, Center for Cooperative Research in Biosciences CIC bioGUNE, Spain and Imperial College London, UK

Wnt signals play important roles in cell growth and differentiation and are aberrantly activated in many tumours. Our goal is to understand how Wnts, their antagonists and their effectors control cell growth and differentiation. We study these aspects in two contexts – prostate cancer (PCa) progression and neural differentiation. In addition, we are studying the differentiation of cancer and stem cells cultured in microgravity bioreactors.

16:30 - 17:00 **Developing *in vitro* 3D models of breast cancer**

Dr Debbie Holliday, Department of Pathology and Tumour Biology, Leeds Institute of Molecular Medicine, UK

A major challenge in studying cancer *in vitro* is the lack of relevant model systems which accurately represent human disease. This talk will describe the development and characterisation of a multi-cellular organotypic model of pre-invasive breast cancer. It will demonstrate that these models can be used to study cellular function and also as a potential tool to screen new drugs of interest.

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-elect 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), 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 UKCCR 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*. He is currently just completing work on *Animal Cell Culture* for the Methods Express series.

About the Speakers

Dot Bennett is Professor of Cell Biology at St George's, University of London, and past President of the International Federation of Pigment Cell Societies. Her research career, in London and California, has often focussed on novel cell culture techniques. In early research with Renato Dulbecco, she identified and cultured mammary epithelial stem cells. Later as an independent researcher she developed methods to immortalize normal human and mouse melanocytes and their precursors, isolating many lines from pigmentary mutant mice and analyzing the actions of the mutations and genes. Another interest is the relation of cell senescence to melanoma progression and therapy.

John Masters is Professor of Experimental Pathology and Head of the Research Department of Urology at University College London. His main research interest is in the area of prostate cancer. He is President of the European Tissue Culture Society, and editor of the 3rd edition of *Animal Cell Culture: A Practical Approach*.

Ross Carnachan gained a BSc(Hons) in Applied Chemistry at the University of Strathclyde in June 2000. He then completed his Ph.D at Durham University in the area of porous polymeric materials in June 2004 under the supervision of Professor Neil Cameron. He then took up the position of Scientific Officer for the North East Stem Cell Institute from 2004-2006 and is now currently a Postdoctoral Research Associate for Reinnervate Ltd/Durham University.

Chris Sellick has a PhD in biochemistry and molecular biology from the University of Manchester. His first post-doc was at the University of Manchester on a BBSRC funded project dissecting the mechanism of transcriptional activation of the GAL genetic switch in *S. cerevisiae*. He is currently working as a post-doc on a Bioprocessing Research Industry Club (BRIC) funded project that is using metabolite profiling to enhance our fundamental understanding of the molecular parameters that influence productivity in recombinant mammalian cell lines. This approach is also being used to improve cell line selection, optimise medium formulations and highlight areas for the engineering of cell lines to improve recombinant antibody production.

Debbie Holliday graduated from the University of Warwick with an honours degree in Chemistry with Medicinal Chemistry in 1996. After completing a Masters in Biochemical Pharmacology at the University of Southampton she spent 3 years gaining experience of the pharmaceutical industry working at AstraZeneca R+D Charnwood. During this time she developed an interest in 3-dimensional cell culture and organotypic models which continued as she undertook a PhD in Breast Pathology at the University of Leicester followed by a postdoctoral position at Bart's and the London. She currently works at the Leeds Institute of Molecular Medicine developing 3D models of breast cancer.

Peter Djali is a fellow of the Royal Microscopical Society and has a history of computer modelling and engineering, Peter has worked on bioimaging applications, including software and hardware development and is now European Sales Manager for Essen's InCuCyte. His PhD in molecular physiology was obtained at the University of Liverpool.

Masato Ishiwata is a department manager of business development department at CellSeed Inc, which is a late stage clinical developing regenerative medicine company in Japan. Since joining CellSeed in 2003, he has always been in a responsible position for marketing and business development activities across company's entire product portfolio ranging from regenerative medicine products to novel cell cultureware by capitalizing on strong marketing competency. He served as a senior marketing consultant at a major consultancy for several years before joining CellSeed. He earned his BA in law at Rikkyo Univ. Japan and an MBA at Edinburgh Business School, UK.

Carol Barker graduated from Sheffield University with an honours degree in Physiology and Pharmacology in 1993. After gaining experience in drug discovery with Glaxo Wellcome and Roche, she undertook a PhD at Nottingham, developing an *in vitro* model of human skin for use in cosmetic testing. In 2000 Carol joined US-based cell culture company Cascade Biologics, where she established and developed the company's successful European business until its acquisition by Invitrogen in 2007. Carol is now the Managing Director and co-founder of XCellR8 Ltd, a Manchester-based cell culture company providing products and specialist technical support services in cell culture technology.

Farlan S Veraitch has previously worked with Professor Mohamed Al-Rubeai who has been a pioneering figure in the development of robust, reproducible, high yield mammalian cell bioprocesses. Since joining UCL in 2005 his research has focused on the engineering challenges associated with stem cell processing. He has developed systems which control and monitor the oxygen tension during neuronal differentiation of mouse ESC (mESC) and has shown that physiological oxygen tensions can significantly enhance the yield and purity of the process. Farlan, in collaboration with Dr Andrew Pelling (Ontario), also works on the role of the mechanical microenvironment during stem cell differentiation.

Robert Kypta was an undergraduate at Oxford and did his PhD at EMBL in Heidelberg on Src family tyrosine kinases, He was then a postdoc at UCSF, where he studied neuronal cell adhesion proteins. In 1997 he joined the MRC Laboratory for Molecular Cell Biology at UCL as a Group Leader. In 2001 he moved to a Lectureship at Imperial College London and in 2005 he set up a new laboratory at CIC bioGUNE in Bilbao. His group focuses on how Wnt and GSK-3 signals regulate cancer and stem cell growth and differentiation.

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POSTERS

INCREASING ANTIBODY TITRES; MINIMAL PROCESS CHANGE MAXIMISES POTENTIAL.

Authors: E. Scotto, J. Thirlway, D. Simpson

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During recent years, there has been increasing pressure within the biopharmaceutical industry to produce recombinant products at high levels whilst maintaining critical quality attributes resulting in cost effective new medicines¹. Many recombinant proteins in the development pipeline are monoclonal antibodies which require complex post-translational modifications and are typically produced using mammalian cell culture systems. These systems have to be relatively easy to genetically engineer, transferable to large scale, robust, stable and produce a high concentration of recombinant protein.

The cell kinetics and productivity of CHO antibody producing minipools/clones was improved at shake flask scale through the use of a media screen combined with a feed strategy. This expression technology enabled improvement of the antibody yield by more than 50% only by selecting the best clone. This clone was transferred to 3L bioreactor scale for study of the effects of key parameters (pH and dissolved oxygen (DO)) on cell growth and antibody production. Bioreactors were run at pH 7.0, 7.2 and uncontrolled and although the highest cell density achieved was lower than in the shake flask experiments, the antibody yield increased by 25%. Changes in DO percentage did not have any significant impact on productivity. Bio-analysis studies were also performed during the runs to determine the effect of the fermentation parameters on the production/consumption of the main metabolites.

Reference:

1. Louise Almond and Jennifer L. Halsall, Pharmaceutical Technology Europe October 2008 60-63