Developments in cell culture technology to enhance cell growth in vitro

The BioPark Hertfordshire, Welwyn Garden City, AL7 3AX: 2nd May 2008

9:00 – 9:40 **Registration**

9:40 – 9:50 **Introduction by the Chair:** *Dr Stefan Przyborski,* School of Biological and Biomedical Science, Durham University, UK

9:50 – 10:20 **Animal Cell Culture: a brief history and basic principles**

Dr Ian Freshney, CRUK Beatson Laboratories, UK

Cell culture has evolved from simple explant techniques to sophisticated selective culture of specialised cells over the past 100 years utilising development of the correct culture environment, including media, matrix, and cell interaction to generate a normal phenotype

10:20 – 10:50 **Silane Modified Tissue Engineering Substrates Induce and Maintain Human Mesenchymal Stem Cell Differentiation**

Dr Judith Curran, UK Centre for Tissue Engineering, The University of Liverpool, UK

Utilising material/surface parameters to control a cell response is undoubtedly an exciting, and necessary area of current tissue engineering research. The ability to dictate protein adsorption, cellular adhesion, and subsequent function on a surface has ramifications in all aspects of tissue engineering.

PLLA, PCL and glass were modified with $-CH_3$, $-NH_2$, -OH and –COOH groups and their ability to control human mesenchymal stem cell (hMSC) differentiation, both in the presence and absence of exogenously supplemented factors *in vitro* was evaluated. The potential of these materials to control MSC differentiation, in the absence of exogenous biological supplements was proven.

10:50 – 11:00 **Speakers photo**

11:00 – 11:15 **Mid-morning break**

11:15 – 11:45 **Development of Technology for the Routine Growth of Cells in Three Dimensions**

Dr Stefan Przyborski Director and Chief Scientific Officer, ReInnervate Limited, UK

ReInnervate has developed a novel 3-D cell culture system which it plans to produce and market in the near future. In brief, scientists in Durham 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. The scaffold is inert and can be supplied pre-fabricated, sterile and ready to use. This offers several advantages to the user including an inexpensive simple un-wrap and use consumable technology, enabling reproducibility during routine use, robustness, stability and less preparation time. The porosity of the polystyrene scaffold is specially customized to within narrow tolerances during its manufacture. This is an important feature to create a consistent and suitable environment for 3-D cell growth. Engineering the scaffold into a thin membrane (e.g. 200µm thick) enables the entry of cells into the interior of its structure (and out again for cell retrieval) and it is of suitable thickness to allow cells sufficient exchange of gases and nutrients. Cell culture work using this material has shown that the scaffold provides vertical space to support the growth of cells to form complex 3-D interactions with their neighbours, in a way resembling the structure of real tissues. 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. Cell viability is maintained at high levels in these cultures and cells are not exposed to the un-natural geometric stresses experienced in cells grown on flat surfaces. Furthermore, cells grown within polystyrene scaffolds show enhanced ability to differentiate and respond to biochemical agents in a manner resembling the activity of their native counterparts in vivo.

11:45 – 12:15 **Three dimensional perfused cell culture using microbioreactors**

Professor Z F Cui, Donald Pollock Professor of Chemical Engineering, Oxford University, UK

Optimisation of culture conditions for stem cells, testing of drug efficacy and chemical toxicity, and study of cell functions all need proper cell culture apparatus, which can conduct cell culture in parallel with reasonably throughput and with small number of cells. Further it becomes more and more convincing that perfused three dimensional cell culture offers distinctive advantages over conventional static monolayer culture. In this presentation, multiple parallel microbioreactors, TissueFlex™, will be described and experimental results on cell physiology study and using stem cells for drug testing will be presented to demonstrate the advantage of these microbioreactors and why three dimensional perfused culture is more desirable

12:15 – 12:30 **Biopark introduction talk**

12:30 – 13:15 **Lunch and Poster Viewing**

13:15 – 13.45 **Effects of altering oxygen tension**

Professor Patrick Maxwell, Imperial College London, UK

13.45 – 14.15 **Controlling cell attachment and development using extracellular matrix motifs presented by biomimetic surfaces**

Mr Michael Cooke, Durham University, UK

Cell culture is an artificial system, cells are removed from their natural environment and placed into simplified laboratory conditions. Multiple factors act upon cells in vivo to control their behaviour and efforts have been made to mimic the in vivo environment more faithfully in vitro. One such factor is cell-extracellular matrix (ECM) interactions. Coating the culture surface with ECM molecules is time consuming, uncontrollable and not reproducible. Here we use a controllable biomimetic surface to present peptide motifs from laminin, collagen and fibronectin to control the attachment of neurite behaviour of PC12 cells. Furthermore we demonstrate our surfaces can be used to control the neuronal differentiation of neural stem/progenitor cells (NSPCs). Hence we propose a controllable system for the presentation of peptide motifs to control cell behaviour.

14:15 – 14.45 **Hollow Fiber Cell Culture, A Better Way to Grow Cells**

John J.S. Cadwell, President and CEO, FiberCell Systems Inc, USA

Hollow fiber bioreactors will be discussed in detail with particular emphasis on the manners in which they can control the micro-environment of cells. Topics covered include the control of cyokines, effect of cell density, effects of matrix,physical effects such as shear stress, cellular co-cultivation, effects of medium composition and their use for monoclonal antibody and protein production.

14.45 – 15.00 **Spontaneus calcification process in a primary culture of renal cells**

F Mezzabotta,, Via Giustiniani 2, 35128 Padova, Italy

In primary cultures, spontaneous transdifferentiation, i.e. the ability of differentiated cells to take on a new identity by switching off one set of lineage specific genes and activating genes of an other differentiated cell type, occurs very rarely. One example of this phenomenon is represented by vascular pericytes that may spontaneously differentiate in osteoblast-like cells. Here we reported an unusual case of spontaneous transdifferentiation process that we observed during primary culture of human renal cells. After two passages, cells showed the tendency to overlap and to aggregate in calcifying nodules with a behaviour similar to that described for pericytes. If this process was related to the differentiation of stem cells or to the transdifferentiation of mature renal cells has to be ruled out

15:00 – 15:30 **Afternoon Tea/Coffee and Last Poster Viewing**

15:30 – 16:00 **Application of Cell Culture for Screening in Drug Discovery Research**

Darren Cawkill, Senior Principal Scientist, Pfizer Global R&D

Cell-based assays are an integral part of screening for modulators of biological target function within drug discovery. Screening in the early phase of research requires large compound numbers to be tested in the given assay system hence a flexible, sustainable supply of a large quantity of cells is paramount. Over recent years, this practical requirement has focussed the industry on meeting the challenge of constructing and supplying cell-based reagents that can meet the demands of high throughput screening. However the trade off to enable this logistically difficult operation has often been a shift away from physiologically relevant cell types. This talk will use examples from Pfizer UK to outline the current status of cell culture and cell-based reagents in drug discovery projects and outline trends for future development of cell culture technologies.

16:00 – 16:30 ***Stem Cell Technology: From Resource to Platforms***

Tim Alsopp, Stem Cell Sciences, UK

Stem cells are a cellular resource providing unprecedented potential for biological discovery, drug development & future cellular medicines. Significant challenges are presented to researchers seeking to provide stem cells of a quantity & quality for these applications. For example defining appropriate expansion conditions to maintain indefinite self-renewal, designing animal component free culture and the development of a production process with minimal cell manipulation. Stem cells have tremendous utility in discovery to explore, for example the genetics of disease susceptibility, cell dysfunction, tissue physiology & the development of small molecule disease intervention strategies. Use of embryonic stem cells can increase the amount and quality of biological information obtained from discovery screens, despite the controversies surrounding their provenance. Human stem cells are difficult to control in culture with many cell types requiring convoluted systems to maintain indefinite expansion without unintentional differentiation. Thus few human tissue stem cell types have been described in which the 'platform technology' adequately & faithfully supports the inherent biology and in a manner rigorous for disparate laboratories.

16:30 – 17:00 **Chairman's summing up**

18:00 **Soiree at *The Best Western Homestead Court Hotel for all the participants**

About the Meeting Chair

Dr Stefan Przyborski, School of Biological and Biomedical Science, Durham University, UK **-** As part of his postdoctoral training, Dr Przyborski worked at the Jackson Laboratory (USA) and in Professor Andrews laboratory (University of Sheffield) where he developed his interest in stem cell biology and developmental neuroscience. He subsequently established an independent research programme at Durham University where he developed technology to produce populations of neural derivatives from human stem cells. These systems are currently being used to investigate the mechanisms of how cells commit toward the neural lineage and how to specify the formation of certain neural subtypes during cell differentiation. A significant amount of effort has been devoted toward validating these culture-based models to ensure that they provide appropriate and informative data in a manner that closely resembles the behaviour of cells in vivo. Current work, for example, examines physical factors that are often taken for granted during cell culture, including the topography on which cells grow and the concentration of oxygen in the culture medium. Both of these factors have been found to markedly influence cell differentiation in vitro. These cell systems are also proving useful to identify biomarkers to track the behaviour of neural cells in both health and disease and the development of novel growth reagents. Some of the technology emanating from Dr Przyborski's laboratory currently being commercialised through the University spin-out company, ReInnervate.

About the Speakers

Dr Ian Freshney, CRUK Beatson Laboratories, UK
B.Sc. (Zoology) Glasgow University 1960, PhD (biochemistry) 1964. Worked with Dr Robert Auerbach in Madison Wisconsin, 1964 – 1965, on differentiation of embryonic mouse liver. Beatson Institute for Cancer Research 1965 − 1981, then senior lecturer in Medical Oncology at Glasgow University working latterly on the effects of glucocorticoids on paracrine control of differentiation. Retired in 1998 but retains Honorary Senior Research Fellowship. Teaches on a number of international basic and specialised cell culture courses; author of Culture of Animal Cells, a Manual of Basic Technique, has co-edited ten other books on specialised cell culture, and has written numerous reviews and original articles in the areas of cell culture, cytotoxicity assay, and induction of differentiation in vitro

Mike Cooke studied molecular biology and biochemistry as an undergraduate at Durham University. He is now a third year PhD student studying in Dr Stefan Przyborski's laboratory at Durham University. His project is a collaboration between Durham University and Professor Jeremy Lakey's (Newcastle University) spin out company - Orla Protein Technologies. His current project focuses on controlling cellular behaviour in vitro using extracellular matrix motifs. A recent collaboration with Professor Molly Shoichet of Toronto University has enabled Mike to test his surfaces using a neural stem/progenitor cell system. He is currently looking to further his career with a post-doctorial position

Federica Mezzabotta graduated in Biological Sciences in 1998 at the University of Padua. She's now a third year PhD student at Doctoral School of "Medical, Clinical and Experimental Sciences, Nephrology Course", University of Padua. She's working at the Laboratory of Histomorphology and Molecular Biology of the Kidney, Department of Medical and Surgical Sciences, attending to her project focused on molecular and cellular mechanism of nephrocalcinosis and Randall's plaque. Her project entitled "Nephrocalcinosis and Randall's plaque: is it an ectopic calcification process at the renal level?" was funded by the Italian Ministry of Education, University and Research.

Dr Judith Curran's research background has origins in material science and especially biomaterials, the evolvement of the research field naturally progressed towards tissue engineering, focusing on developing in vitro models which could be used to investigate cell-surface interactions in a controlled environment. This area of research encompassed inflammatory, progenitor and differentiated cell types and naturally progressed towards investigating how surface/material factors could be used to control a cellular response. Currently the major underlying theme is to develop smart materials that can be directly implanted into the body and control a host response without prior ex vivo manipulation.

Dr Darren Cawkill has been at Pfizer in the UK for 9 years. On completing his PhD in 1998, he joined Pfizer from the University of Leeds and has since worked in the fields of protein biochemistry, cell biology and *in vitro* screen development. His current role is that of leading a group developing and supplying cell based reagents for compound screening at Sandwich, an area of particular current interest is investigating more physiologically relevant model systems for biological targets that are amenable to screening

Dr Tim Allsopp graduated in 1984 from UCL and completed his PhD in 1988 at KCL. Post-doctoral research at the Max Planck Institute for Developmental Biology, Germany & a number of UK research institutes, including the Fujisawa Institute for Neuroscience at Edinburgh. He has contributed to the fields of developmental neurobiology, programmed cell death and studies of CNS diseases. He joined the company as Principal Scientist & now, as CSO, leads the research & product development teams for the Company. Tim is a member of the Company's Scientific Advisory Board, the Society for Medicines Research and acts as a consultant to a number of UK and international research & technology support organizations

Professor Zhanfeng Cui was educated to be a Chemical Engineer in China and got a PhD in 1988, did a postdoc in Strathclyde University (88-91) and joined Edinburgh University as a Lecturer in Chemical Engineering. Moved to Oxford as a University Lecturer in Engineering Science in 1994, and elected to the Donald Pollock Chair of Chemical Engineering in 2000. Research interests include bioreactor technology, bioseparation, monitoring and cryopreservation.

*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. The event was hosted by **'BioPark'**(www.biopark.co.uk), a research and development centre in Welwyn Garden City providing specialist facilities and support for bioscience and health technology businesses to grow, and to develop new products and technologies*

*To book your accommodation at **BEST WESTERN HOMESTEAD COURT HOTEL** and any travel arrangements please download the **booking form** or contact us with your requirements to accommodationandtravel@euroscicon.com / + 44 (0) 1926 888027. We will negotiate the best rates for you