

The 2012 London Regenerative Medicine Event

Thursday, 16 February 2012

The Penridge Suite, 470 Bowes Road, London N11 1NL

Continued improvement in the nation's health depends upon the development of affordable and effective medicines and new therapeutic treatments. The last 20 years has seen the growth of a global healthcare industry based on human proteins produced in transformed mammalian cell lines, with a current market value of £30 billion/year. There is now an opportunity to replicate this growth in new industries using human stem cells in pharmaceutical and regenerative medicine applications. This meeting will focus on exploring the biological and engineering challenges which lie ahead before cell based therapies can become an everyday reality.

Meeting Chair: *Professor Christopher J. Hewitt*
CBiol CEng CSci FIBiol FICHEM, Loughborough University, UK

This event has CPD accreditation and will have a discussion 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:** *Professor Christopher J. Hewitt*, Loughborough University, UK
- 10:00 – 10:30 **The future potential of cellular immunotherapy's**
Dr Mark Cobbold, The University of Birmingham, UK
- 10:30 – 11:00 **Skeletal Stem Cells – Bridging the Gap in Bone Regeneration from Cell Biology to Clinical Translation**
Professor Richard OC Oreffo, University of Southampton, UK
The application of selected skeletal or mesenchymal progenitor cells and appropriate scaffolds/ growth factors in regenerative strategies is currently one of the most exciting and promising areas for disease treatment and reparative medicine. Work to be presented is centered on harnessing the potential of skeletal stem cells and translational studies and will include information on a nanomaterials approach for stem cell maintenance and lineage a novel clay-gel based strategy for the delivery and application of growth factors without the need for complex chemical modifications and, finally, translational studies aimed for clinical application, to examine the efficacy of human skeletal populations using impaction bone grafting as an exemplar. Interdisciplinary strategies across the life science and clinical science interface are set to play a vital role in the field of skeletal repair in an increasingly ageing population.
- 11:00 – 11:30 **Translating Research Into Viable Clinical Treatments. How to build on 60 years of patient focused clinical delivery**
Dr Simon Ellison, National Blood Service, UK
The criticality of managing the process from consent to patient.
 - Utilising open innovation partnerships can deliver treatments and revenue.
 - How to generate patient focused manufacturing and scale up.
 - Accessing validated national cold supply chains
- 11:30 – 11:50 **Speakers' photo then mid-morning break and trade show**
- 11:50 – 12:20 **Scaling Red Blood Cell Manufacture from Cord Blood and Placenta Derived CD34+ Haematopoietic Stem Cells**
Dr Robert Thomas, Senior Lecturer, Associate Director EPSRC Centre for Innovative Manufacturing in Regenerative Medicine
The technology to expand and differentiate stem cells from human umbilical cord blood will potentially underpin the manufacture of a number of therapeutic cellular products, including erythrocytes and platelets. Such technology will overcome the logistical limitations associated with sourcing short shelf-life and disease screened voluntary blood donations. However, these cell products pose significant challenges in the development of economically scalable production systems and processes that are capable of consistent delivery of clinical quality cells.
As part of an international and multidisciplinary consortium of engineers and cell biologists led by Celgene Cellular Therapeutics, we have established key challenges and mitigating strategies for the production of erythrocytes at large scale from umbilical cord and placenta derived CD34+ cells. We have demonstrated a multi-stage expansion and differentiation process that integrates both established and novel technologies and cytokine supplementation schedules to consistently achieve high production densities of erythroid cells. We have further employed a new millilitre scale precision process development platform (AMBR, TAP Biosystems) to rigorously characterise critical elements of the production environment and identify factors determining cell expansion and quality (assessed

through development of multiple erythroid markers including CD71, CD235a and Haemoglobin) and to reduce process cost.

In the near future the consortium anticipates further optimisation of the bioprocess and automated integration of downstream processing to facilitate clinical-scale production of a therapeutically effective red blood cell like product. The process development and control learning, and the key parameter ranges established, are likely to be transferable to other development programmes for regenerative cell products.

12:20 – 12:50

The role of bioreactor technology in regenerative medicine

Professor Julian B Chaudhuri, Professor of Biochemical & Biomedical Engineering, Centre for Regenerative Medicine, Department of Chemical Engineering, University of Bath, UK

Stem cells and their differentiated progeny have the potential to be used for a number of clinical applications, eg cellular therapies and tissue engineering. Most stem cell expansion and differentiation studies have been performed on a 'laboratory' scale i.e., using small culture volumes, typically less than 10 mL. These studies have highlighted the fact that current 2-dimensional (2D) static culturing techniques are inadequate for large-scale production, and 3D solutions are required. In situations where differentiated cell progeny will ultimately be used for cell-based therapy or tissue engineering, clinical numbers of cells will be required and these will need to be generated in an efficient, cost-effective and reproducible manner. In this presentation we will describe our approach to address this challenge, which has been to develop 3-dimensional (3D) dynamic systems that support effective proliferation of pluripotent adult and embryonic stem cells which overcome the restrictions of 2D systems.

12:50 – 13:20

Improving the yield of pluripotent stem cell differentiation processes

Dr Farlan Veraitch, Department of Biochemical Engineering, University College London, UK

Stem cells can self-renew in vitro whilst retaining their ability to differentiate into multiple adult cell types. These properties suggest that stem cells will have a number of potential applications including the generation of adult tissue for regenerative medicine, drug discovery, drug development and whole cell delivery of gene therapies. One of the major technical challenges will be the development of scalable, cost effective, reproducible and safe whole bioprocesses. Farlan Veraitch's talk will focus on his research group's work on the development of the process with particular focus on the production of retinal lineages from pluripotent stem cells.

13:20 – 14:15

Lunch and trade show

14:15 – 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

Cardiac regeneration, a role for stem cells

Dr Annette Meeson, Institute of Human Genetics, Newcastle University, International Centre for Life, UK

The promise of cardiac regeneration using cellular approaches seems to be moving ever closer. However, many questions remain unanswered, such as what cells should be used and ideally where should they be isolated from, what are they doing in vivo and why under normal situations do they fail to repair the injured heart? While studies on the transplantation of stem cells into animal models of cardiac injury are encouraging transferring this knowledge to treatment of human patients comes with unique challenges. Our research focuses on the role of CSC during cardiac development and in cardiac injury/disease.

15:30 - 16:00

Afternoon Tea/Coffee and trade show

16:00 – 16:30

Label Free Kinetic Morphological Analysis

Mr Kevin McCormack, Chip-Man Technologies Ltd, UK

The use of The Cell IQ System to quantitate morphological changes in cells over extended incubation periods to generating kinetic profiles / analysis. Applications include stem cell growth / differentiation, regenerative medicine, migration and cell tracking.

16:30 – 17:00

Regulation of Regenerative Medicine Products

Ms Alison Wilson, CellData Services, UK

- Outline of the Advanced Therapy Medicinal Products Regulation
- Other relevant legislation
- Major regulatory issues in progressing from the lab to the clinic

17:00

Chairman's summing up

About the Chair

Chris graduated with a first class honours degree in Microbiology from Royal Holloway College, University of London in 1990. He then went to the University of Birmingham to study for his Ph.D. in Biochemical Engineering where he worked on the synthesis of alpha-amylase by *Bacillus amyloliquefaciens* under the supervision of Professor Gerald Solomons. Chris stayed at Birmingham for a further 13 years first as Lecturer then Senior Lecturer, developing his research work at the Life Science/Engineering interface. In October 2006, Chris came to Loughborough University to take up a new chair in Biological Engineering and is now busy establishing Loughborough's Centre for Biological Engineering (CBE), of which he is co-founder.

About the Speakers

Alison Wilson is a consultant providing specialist regulatory affairs advice for advanced therapy medicinal products (ATMPs). Previously regulatory affairs manager for Smith & Nephew Wound Management, she has >20 years experience of regulatory affairs in medicinal products, medical devices, and human tissue-based products. She provides strategic regulatory and development advice for a range of UK, EU and US clients seeking to commercialise ATMPs in the EU. Alison has close links with the ATMP industry and is a member of several UK groups active in developing guidance and standards for tissue-based products. She is a nominated UK expert for ISO (International Standards Organisation) TC150/WG 11 - Tissue Engineered Medical Products and a member of BSI Technical Panel RGM/1 – Regenerative Medicine. Her current activities include being a Module Advisor for TOPRA MSc in Medical Technology Regulatory Affairs (Module 6 – Combination Products and Other Technologies) validated by Cranfield University and a Member of Topic Selection Panel for the MHRA Medical Device Technology Forum. She was a member of the MHRA (Medicines and Healthcare Products Regulatory Agency) (then MDA) working group responsible for production of the Code of Practice for Production of Human-Derived Therapeutic Products, 2002, and is the technical author for the BSI publication PAS 93: Characterisation of human cells for clinical application, published August 2011.

Annette Meeson obtained a PhD in Neurobiology from Imperial College, London, before moving to the USA where she became interested in skeletal and cardiac regeneration and the role of stem/progenitor cells in these processes (Department of Cardiology, UT Southwestern Medical Ctr. at Dallas). Dr Meeson was then recruited to Newcastle University (UK) in 2005 and established an independent research group whose research interests not only focus on the role of stem cells in development and regeneration but also in cancer progression.

Simon Ellison is developing strategies that are enabling the National Blood Service to utilise its technical skills, GMP facilities, and clinical contacts to provide contract manufacturing services to the growing cellular therapy field, under the brand of Clinical Translation Partnerships (CTP). Simon has an MSc in Environmental Science from Newcastle University and subsequently an MBaA from Oxford Books University focusing on the management of innovative collaborations. Simon's career started with Sartorius, managing both national and international commercial channels, and launching new products into emerging markets. He has since worked in a variety of bio-pharmaceutical markets ranging from antibodies to ultra-pure water, delivering novel strategies to take companies forward. Simon now brings these commercial skills into the not-for-profit sector, initially as Commercial Director for the National Pharmacy Association, managing a partnership based turnover of £7m and developing innovative partnerships with Santander and Learn Direct. He now sits on the BIA's Cellular Therapy & RegenMed Industry Group Advisory Committee, and works within the National Blood Service driving strategies to utilise their clean rooms, skills, knowledge and logistics to help regenerative medicine companies translate their research into commercially viable treatments. Clinical Trial Partnerships (CTP) enables companies, academics and clinicians to develop their production into GMP systems, optimise the processes, and develop viable cold supply chains in partnership with the National Blood Service. This gives the cellular therapy market access to a unique skill set built within the NHS and currently delivering over 2 million cellular therapies annually.

Kevin McCormack has had extensive industrial experience in imaging and cellular analysis and has worked for several companies in various countries around the world including Amersham International and GE Healthcare.

Richard Oreffo leads the tissue engineering programme at Southampton. He has extensive expertise in skeletal biology and the mechanisms involved in skeletal stem cell differentiation. In 2001 he was recognised with the Maxime Hanns award for collaborative research in Bone Tissue Engineering, appointed to a Senior Lectureship in 2002 and to a Readership and Personal chair in 2004. Research of the group is primarily centred on i) development of unique tissue engineering approaches for new cartilage and bone formation for orthopaedic application using human skeletal stem cells and, ii) elucidating the role of fetal programming as a consequence of maternal nutritional challenge on skeletal stem cell differentiation, activity, potential and bone function with age. The group is currently developing strategies to couple stem cell technology with biomimetic scaffolds in close collaboration with biomaterials and tissue engineering groups in the UK as well as international collaborations in Germany and the USA.

Robert Thomas is a Senior Lecturer at Loughborough University and also a registered pharmacist. His early research was on liver tissue engineering models. He currently holds a research council fellowship focussed on developing the process science and manufacturing capability for cell based regenerative medicine therapies, an area in which he has published a number of novel processes. He is associate director of the new EPSRC centre for innovative manufacturing for Regenerative medicine led from Loughborough. In the past three years he has won the IChemE international award for innovation and excellence in bioprocessing and been highly commended in the DaVinci Healthcare Engineering awards.

Farlan Veraitch gained his PhD from the University of Birmingham where his research focused on the optimisation of mammalian cell culture processes. He then moved to UCL where he worked as a Post Doctoral Research Assistant on the automation of embryonic stem cell processing. Since gaining his Lectureship in 2006 Farlan has helped to establish the UCL's Cell Therapy Bioprocessing programme which has been applying ultra scale-down, bioprocess modelling and a 'whole bioprocess' vision to the development of robust stem cell production processes.

Julian Chaudhuri holds degrees in biochemical engineering from University College London and the University of Reading. He is currently Professor of Biochemical and Biomedical Engineering at the University of Bath where he is carrying out research in aspects of regenerative medicine. His interests include bioreactor design for tissue engineering, bioprocessing of stem cells, biomaterials for three-dimensional scaffolds and mathematical modelling. He was a co-founder, in 2003, of the University's Centre for Regenerative Medicine.

Registration Web Site: **www.regonline.co.uk/regen2012**

Key words: *iPSC, hepatocyte, liver, CYP p450, translation, supply chain, delivery, GMP, manufacturing, drug, pluripotent stem cell, hepatocyte, pancreas, liver; beta-cell, Stable Karyotype, High-throughput efficiency, Embryonic Stem cells, Induced pluripotency, Nanog, neural stem cell, glioblastoma, DNA methylation, reprogramming, iPS cells, reprogramming, Pluripotency, STEMCCA, ESGRO 2i, Pluripotency; reprogramming; chromatin signatures; DNA replication timing; histone acetyltransferase p300, Blood, Haematopoietic Stem Cells, CD34+, Manufacture, Process, pluripotent stem cells, bioprocessing, Cardiac Side Population Cells, ABCG2, Islet1, fetal, embryonic, Morphological Analysis, kinetic imaging*

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POSTERS

UNDERSTANDING THE HUMAN TISSUE INTERACTIONS WITH EXTRACELLULAR MATRIX-DERIVED BIOLOGICAL SCAFFOLDS

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Introduction

Implantation of natural biomaterials derived from the decellularisation of tissues into animal models has shown recellularisation of the material by host cells and the formation of organised tissue thus, indicating clear clinical potential in soft tissue reconstruction. Despite this, the interactions between human tissue and extracellular matrix (ECM)-derived biological scaffolds are poorly understood. By establishing a novel culture system these studies aimed to investigate the interactions at the interface between an ECM-derived biological scaffold and fresh human stromal tissue to better understand the mode of regeneration of an implanted biomaterial.

Experimental approach and results

An organotypic culture system was developed in which a decellularised porcine bladder matrix was maintained in close apposition to surgically-excised human urinary tract stromal tissue sourced with REC approval from consenting patients. Histological examination of the constructs showed migration of cells from the tissue into the biological scaffold over an 11 day culture period. Fluorescent microscopy showed a significant ($P < 0.05$) increase in cells immunolabelled for the macrophage scavenger receptor, CD163 at the biological scaffold-tissue interface over the 11 day time course. Treatment of primary human monocyte-derived macrophages with the glucocorticoid receptor (GCR) agonist, dexamethasone or the anti-inflammatory cytokine Interleukin (IL)-10 showed increased expression of CD163. Immunoperoxidase labelling for GCR in the tissue:biological scaffold construct showed intense nuclear GCR labelling in the cells at the biological scaffold-tissue interface throughout the culture period.

Discussion

CD163 is up-regulated by anti-inflammatory mediators and has been associated with the anti-inflammatory 'M2' macrophage phenotype. The presence of CD163⁺ macrophages at a tissue-biological scaffold interface may be driven by a default tissue response to resolve inflammation following wounding which may be mediated through the GCR pathway. Understanding these interactions will reveal new directions for enhancing biomaterial integration into human tissue.

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EXPLOITING THE HETEROGENEITY WITHIN STEM CELL POPULATIONS: LOOKING TO INCREASE DIFFERENTIATION EFFICIENCIES

Kate Fynes, Dr. Diana Hernandez, Professor Chris Mason and Dr. Farlan Veraitch.

Pluripotent embryonic stem cells (ESCs) have the potential to form all cell types within the body, and represent a potentially unlimited supply of cells for therapeutic use, developmental studies and toxicity testing.

Current protocols for differentiation are, in the main, long and inefficient. For these processes to become clinically viable, it is necessary to increase their efficiency, making them a more robust and reproducible option.

Our understanding of the nature of stem cell populations has progressed, with evidence now suggesting that cultures are heterogeneous and contain subpopulations of cells characterised by gene expression. Therefore, we have hypothesised that ESCs are generally a heterogeneous population of cells, with only certain subpopulations appropriately primed and capable of differentiating into the required final cell type.

In this study we have picked and expanded 35 clonal murine ESC lines from the 46C cell line, which is transfected with a green fluorescent protein (GFP) under the promoter of SOX1, the first neural gene to be expressed. Each resulting clone was differentiated (1) spontaneously into cells from all the three germ layers using an Embryoid Body protocol and (2) into neuronal cells using a directed differentiation protocol.

For the Embryoid Body assay we have looked at Sox17 and Brachyury expression for Endoderm and Mesodermal differentiation respectively; Sox1, b3-Tubulin and Pax6 expression for Ectodermal differentiation; and finally Oct4 expression for residual pluripotency.

For neural directed differentiation, we assayed GFP expression at day 6 of the differentiation protocol for each of the clones.

These results demonstrate the clonal heterogeneity within mouse ESC populations, and highlight how this can be exploited for selecting the appropriate high producing clones for required production processes rather than starting with mixed populations.

DESIGN, DEVELOPMENT AND DETAILED EXAMINATION OF AN EXPANDED BED REACTOR FOR THE SEEDING OF EMBRYONIC STEM CELLS TO MICROCARRIERS.

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The aim of this study was to design, develop and examine a novel bioreactor system; 'The Expanded Bed Reactor' for the improved seeding of embryonic stem cells to microcarriers. The principle of expanded bed absorption or chromatography is used; the relatively low shear, plug flow expansion of a resin bed against gravity. Cytodex 3 (GE) microcarriers are the resin used here; a dextran bead coated in porcine gelatin, with a diameter range of 133 μm (d_{50}) – 215 μm (d_{95}) and a reported density of 1.04 g/mL. Therefore the Cytodex 3 microcarrier shares similar properties of more typically used expanded bed absorption resins. Here it is shown to perform within the accepted empirical models of typical resins expansion profile (or resin height versus flowrate/velocity). During flow the bed height is regulated and the microcarriers are geostationary, however with the flexibility to rotate on an axis.

Proof of concept for the successful cell attachment to microcarriers in the Expanded Bed Reactor under continuous flow was first demonstrated using mouse embryonic fibroblasts. Subsequently it was found that mouse embryonic stem cells would require intermittent flow to allow for cell attachment. Mouse embryonic stem cells (Oct-4, E14 & CCE Cell Lines) were grown on gelatine treated, tissue culture plastic (T25 Flasks), in Knock-Out media with 15% foetal bovine serum and Lif supplementation for maintenance. The Expanded Bed Reactor was inoculated with 10,000 cells per cm^2 with a total microcarrier surface area available of 270 cm^2 . After 12 hours all microcarriers were harvested and fixed in Paraformaldehyde 4% for 10mins, DAPI stained and manually counted for cell attachment using a microscope. Intervals of flow examined were 0 i.e. no flow in 12 hours (control), 1 minute per 30, 1 minute per 60, 1 minute per 120, 1 minute per 240.

The results presented show that the Expanded Bed Reactor has improved the seeding of stem cells to Cytodex 3 microcarriers; increasing the seeding rate on average (number of microcarriers with cells attached) from 35% (control) to 70% for the E14 cell line. Additionally the distribution of the cells on the microcarrier surface is improved. Similar results are expected with Oct 4 and CCE cell lines. This method is believed to be extremely beneficial for future use due to the potential of embryonic cells to be differentiated by shear intervention alone.