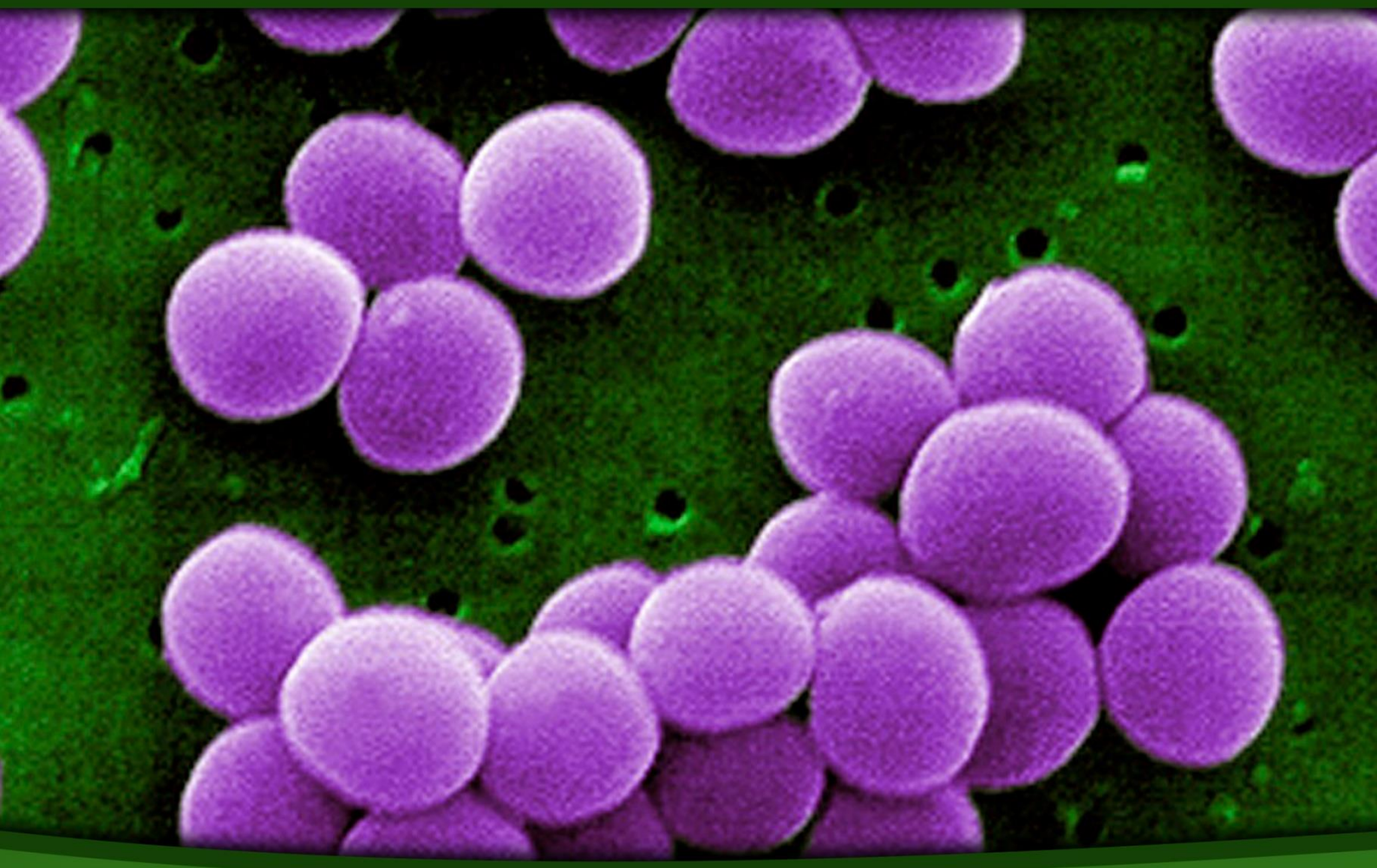
Cell Culture 2016

ABSTRACTS



2nd - 4th February 2016
London, UK

EuroSciCon

This three-day international summit 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. Cell culture has matured to become the pivotal technology in biopharmaceutical research, development and production, and its use in this and other areas continues to expand rapidly.

The enormous potential of cell culture technology in the fields of stem cell and regenerative medicine is being realized; and its utility in research grows ever greater. This event will bring together leading researchers from many backgrounds in an informal setting, with plenty of opportunity for discussion and debate.

This event has CPD accreditation

This abstract book will be finalised two weeks before the event

www.CellCulture2016.com

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Invited Speakers Abstracts

3D culture of osteoblasts in microbioreactors: critical role of dynamic fluid loading for cell morphogenesis

Dr Brigitte Altmann, PhD, University Medical Center Freiburg, Center for Dental Medicine, Department of Prosthodontic Dentistry, Freiburg im Breisgau, Germany

Mechanical loads represent a critical factor in modulating bone cell functions and therefore play an important role in bone formation and/or remodeling. In this context, information on bone cell mechanosensitivity in physiologically orientated 3D-in vitro microenvironments is still sparse. Hence, we established a novel osteogenic chip-based 3D-culture model that provides unique features for mechanobiological studies under physiologically relevant conditions. This novel system revealed discriminative osteoblast morphogenesis under static and microfluidic growth conditions. As proved by time-lapse microscopy, multicellular osteoblast aggregates reorganized quickly within 24h upon fluid flow application, thereby reflecting a very early reaction in response to biomechanical cues.

Applications of a thin layer angiogenesis assay

Dr David Bishop-Bailey, Royal Veterinary College University of London, Royal College Street, London, United Kingdom

Angiogenesis, the development of new blood vessels from old, underlies many physiological and pathophysiological processes. It is common to study this process in cells in culture using 2d and 3d matrices. We will describe a simple thin layer assay for angiogenesis that has advantages not only of cost, but also of utility. Since the assay is thin, imaging of the events at the cellular is vastly improved. Moreover, this simplified assay allows for direct RNA extraction, meaning rather than complex cell digestion or implicating findings from monolayers, events can be studied directly in the cells themselves.

Biomimetic poly(glycerol sebacate)-based elastomeric scaffolds for soft tissue engineering

Dr Biqiong Chen, Department of Materials Science and Engineering, University of Sheffield, Sheffield, United Kingdom

tretchable and biodegradable polymer scaffolds are highly interesting for soft tissue engineering. In this talk, I shall introduce our recent work on the design and development of tissue scaffolds, which mimic the mechanical properties of the extra-cellular matrices of soft tissues, using poly(glycerol sebacate) blends and co-polymers. Synthesis of the polymers as well as the fabrication and characterisation of the scaffolds will be discussed. In vitro cell viability and proliferation with these scaffolds will also be briefly covered to demonstrate their potential in soft tissue engineering.

Modelling the human nervous system in a dish

Professor Patrizia Ferretti, UCL Institute of Child Health, London, United Kingdom

The results of animal studies on central nervous system (CNS) damage and neuroprotective agents have often proven poor indicators of success rate of clinical trials. Hence there is much need for developing systems in which human CNS cells behave in a physiologically relevant manner and better mimic the situation in vivo. The development of human models for studying responses to neural injury, and in particular of 3-dimensional (3D) systems, will be discussed.

Biospray approaches for regenerative biology/medicine and theraputics

Dr Suwan Jayasinghe, BioPhysics Group, University College London, Department of Mechanical Engineering, London, United Kingdom

The ability to manipulate and distribute living mammalian cells with control presents fascinating possibilities for a plethora of applications in our healthcare. These imply several possibilities in tissue engineering and regenerative biology/medicine, to those of a therapeutic nature. The physical sciences are increasingly playing a pivotal role in this endeavour by both advancing existing cell engineering technology and pioneering new protocols for the creation of biologically viable structures. The talk will introduce the leading technologies, which have been fully validated from a physical, chemical and biological stand point for completely demonstrating their inertness for directly handling the most intricate advanced material known to humankind. Hence, each protocol's advantages and disadvantages will be clearly identified, whilst recognizing their future biological/medical and engineering challenges. In conclusion, a few selected biotechnological applications will be presented where these protocols could undergo focused exploration. Successful development of these bio-protocols sees the emergence of unique future strategies within both a laboratory and a clinical environment having far-reaching consequences for our healthcare.

From Systems Engineering to Systems Biology: How mathematical models can help us improve cell culture processes.

Dr Alexandros Kiparissides, Department of Biochemical Engineering, University College London, London, UK

Despite the outstanding research developments in biotechnology, the sophisticated mathematical toolset that lead to the explosive growth of manufacturing capacity in the traditional chemical industries, known as Process Systems Engineering (PSE), has not been widely applied to the bio-industry. The multi-layered and complex nature of cellular function and regulation has dictated a paradigm of research in highly specialised fields. This has led to the generation of in-depth, yet disjoint, knowledge. What is presently lacking are novel engineering approaches able to integrate, organize and guide experimental information across multiple scales of complexity, all the way from strain design to bioprocess optimization.

A versatile miniature bioreactor platform for antibody production in fed-batch mammalian and microbial cell cultures

Dr Farlan Veraitch, University College london, Department of Biochemical Engineering, London, United Kingdom

Antibodies and their variants (fragments, fusions etc) are the most important class of biopharmaceutical products on the market to date. New cellular expression systems and advances in antibody engineering are driving manufacturing strategies away from established platform processes based on production of whole antibodies in mammalian cells. This demands versatile process development strategies that can handle a range of antibody products produced in a variety of expression systems. This presentation will describe the engineering characterisation and applications of a shaken, 24-well miniature bioreactor system for early stage bioprocess development. It will address the production of antibody products in fed-batch cultures of both mammalian (CHO) and microbial (E. coli) expression systems for both cell line selection and prediction of larger scale stirred-bioreactor performance.

Precise Engineering of Biomimetic 3D Environment

Dr Alvaro Mata, School of Engineering and Materials Science, Queen Mary University of London, London, United Kingdom

The talk will focus on three novel techniques that enable the fabrication of 3D biomimetic environments. The first relies on molecular self-assembly to realize 3D gels that use extracellular matrix proteins as structural and functional building blocks. The second technique relies on a dynamic self-assembling system capable of creating tubular hydrogels that can be grown to acquire different geometrical shapes while incorporating specific bioactive sequences. Finally, the third technique uses electric fields to print bioactive molecules within 3D hydrogels in order to create molecular patterns and subsequently anisotropic hydrogels that can recreate specific biological environments with high precision and tuneability.

Understanding the injury dynamics of kidney fibrosis in vitro through 3D co-culture system of kidney proximal tubular cells and fibroblast

Dr. Bramasta Nugraha, D-BSSE ETH Zurich & Roche Innovation Center Basel, Basel, Switzerland

Halting the progression of human kidney fibrosis is challenging because the lack of understanding of the precise mechanisms involved in the injury. By creating an in vitro 3D co-culture system of human proximal tubular cells and fibroblast, we try to understand an aspect of the epithelial/mesenchymal crosstalks in the fibrosis progression. In this platform, we track the injuries in different aspects and try to modulate and inhibit the fibrosis to progress

Building artificial nervous system tissues in vitro

Dr James Phillips, University College London, London, UK

Tissue engineering technology provides a useful approach for the construction of artificial nervous system tissues in vitro. These can be used as replacement tissues in regenerative medicine, or maintained in vitro as model systems for neuroscience research. This talk will focus on (1) the development of engineered neural tissue for use in peripheral nerve repair and then (2) will introduce some aspects of using artificial tissues as 3D culture models in CNS and peripheral nerve research.

2D and 3D culture of neural stem cells: implications and future prospectives for neural stem cell transplantation research

Professor Peter Ponsaerts, University in Antwerp, Wilrijk, Antwerp, Belgium

Although transplantation of neural stem cells in injured brain tissue is widely studied as a potential treatment for various neurological pathologies, effective cell replacement therapy will rely on the capacity of cellular grafts to overcome specific hypoxic and immunological barriers after transplantation. In this presentation, I will present my groups contribution to current understandings of the in vivo cellular fate of grafted single cell suspensions of neural stem cells in the mouse brain, as well as our recent efforts to the development of 3D

neural structures on decellularized mouse brain, being an alternative in drug screening and cell/tissue transplantation research.

3D cultures of estrogen-responsive primary human breast epithelial cells offer novel insights into differential regulation of target genes in healthy vs. malignant cells

Dr Afshin Raouf, Department of Immunology, University of Manitoba, Winnipeg, Canada

Dr. Raouf obtained his PhD at the University of Toronto, and specialized in the application of normal and cancer stem cell concepts to the study of breast cancer research under the tutelage of Dr. Connie Eaves. Dr. Raouf holds a number of awards from including the American Association for Cancer Research Junior Scientist awards, and has published peer reviewed articles including journals such as the Cell Stem Cells, and Proceedings of the National Academy of Sciences. He is currently the group leader for the Manitoba Breast Cancer Research group and member of the Regenerative Medicine Program in University of Manitoba.

A new 3D cell-based engineered intestinal in vitro model to explore intestinal absorption at earlier stage of drug delivery

Dr Bruno Sarmento, Instituto de Engenharia Biomédica, Porto, Portugal

3D culture models encompass a great potential to build up a bridge between the cell culture models and in vivo animal models [2]. The present work extended a traditional intestinal model (Caco-2 cell monolayer) to the deeper layers of the small intestine, in particular the intestinal mucosa, that includes a broad variety of stromal cells. The model comprises CC18-Co intestinal myofibroblasts embedded in extracellular matrix, Caco-2 and HT29-MTX epithelial cells and Raji B lymphocytes, as an instrument in initiating mucosal permeability and a portal explored for the delivery of macromolecules and nanoparticles due to its high transcytotic capacity. The 3D model was successfully established reproducing somewhat the native intestinal mucosa in which stromal cells play a pivotal role in the establishment of intestinal architecture.

Widening the analytical bottleneck for stem cell culture by combining microfluidic approaches with novel analytical methods

Professor Nicolas Szita, Department of Biochemical Engineering, University College London, London, UK

The clinical and commercial use of pluripotent cells is highly reliant on the development of tightly controlled culture processes. Microfluidic stem cell culture devices offer precise control over the soluble, physical and mechanical microenvironment of the cells. We developed a multiplexed microfabricated culture device for the culture of embryonic stem cells, which incorporates cutting edge microfluidic and analytical methods. In our device, confluency and dissolved oxygen tension levels are monitored on-line and in situ, yielding time profiles of the two and the ability to correlate oxygen consumption with growth data.

Controlling Single Cell Geometry in 3D

Dr Huabing Yin, University of Glasgow, Division of Biomedical Engineering, School of Engineering, Glasgow, United Kingdom

Cells in the body are surrounded by complex, 3D microenvironments that modulate cell shape and their functions. Currently, standard in vitro cell culture is on 2D surfaces, which often causes the loss of cell phenotypic functions. Therefore, developing microenvironments that resemble the multiplex cues found in vivo, provides a promising avenue to address these challenges. Here, I will introduce a novel culture system for modulating single cell geometry in such 3D microenvironments. This approach not only allows the successful control of chondrocyte dedifferentiation in vitro, but also offers a versatile platform in the search for niches that lead to either self-renewal or targeted differentiation of pluripotent cells.

Using Cell-Culture -Based LDL Uptake Model for Screening PCSK9 inhibitors

Dr Weiming Xu, London Biotech and University of Sheffield, UK

PCSK9 is a hot genetic validated target for the reduction of LDLc. Several anti-PCSK9 antibodies have been passing Phase III trails and on the way to the direct clinic for patients with hypercholesterolemia who are difficult to treat with statin only. However, developing novel compounds that inhibit PCSK9 function is pharmaceutically preferred over both antibody and siRNA routes. We have recently developed a cell-based, functional assay incorporating recombinant PCSK9 protein for high-throughput screening of human liver cell HepG2. One of the lead compounds, colchicine, has been further validated. Colchicine is a prescribing drug for the treatment of acute gout flares. Our research highlights the potential of the novel use of this drug for cardiovascular disease treatment.

Integration of the ambr platform into mammalian process development

Dr Steve Warr, GSK Medicine Research Centre, GlaxoSmithKline, Stevenage, UK

This talk will describe how the ambr platform is beig integrated into early process development for the production of biopharmaceutical molecules from CHO cells. Case studies will highlight specific applications of the ambr15 and ambr250 platforms to streamline the progression of molecules through early process development to clinical manufacture and to increase platform process understanding.

Accelerating strain development workflows with a novel single-use bioreactor system

Dr. Barney Zoro, Sartorius Stedim Biotech, Royston, United Kingdom

n collaboration with Biopharm industry partners, a new platform has been developed for small scale microbial cell culture, supporting high density fed-batch cultures in a high throughput single-use bioreactor format. Technical system data and industrial biopharm process results demonstrate the potential of this new bioreactor technology to enable a step change in the capacity and productivity of bioreactor operations in microbial cell culture strain development workflows.

Day 1:

Oral Presentation Abstracts

Oral presentations will be added after the submission deadline

PERFORMANCE OF PROFESSIONALIZED 3D NANOFIBROUS SCAFFOLDS IN CELL CULTURE AND ORGANIC BUILDING

L. Vysloužilová, K. Vodseďálková, L. Vejsadová, J. Erben and L. Berezkinová
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Nanofibers are unique materials with very fine fibers and low area weight. These structures have high specific surface area, i.e., there are fibers with a diameter ranging from 100 nm to 1 µm and with high porosity but with very small inter-fiber pores. Their structure reminiscent of the extracellular matrix. Due to these specific properties, nanofibrous structures have an excellent usage in a medical field as a replacement of a damaged tissue. They can be used as nanofibrous scaffolds enabling a support of cell proliferation and a creation of the new tissue. The ideal solution for an appropriate support of the new tissue formation is a three-dimensional (3D) structured scaffold. This 3D structure allows enough space for cell proliferation, migration and growth through the whole structure of the scaffold.

Nanopharma developed professionalized 3D nanofibrous scaffolds from Polycaprolactone (PCL), Polylactide acid (PLA) and Poly(L-lactide-co-caprolactone-co-glycolide) (PLCG) using the electrospinning method. They are biocompatible and biodegradable polymer materials suitable for using in tissue engineering. Electrospinning is a process that employs electric forces to produce ultra fine fibers. Polymer solution is delivered through electrically charged spinning electrode, this is drawn and elongated while applying a high electric field and nanofibers are collected on the oppositely charged collector. Homogeneous and uniform nanofibrous structures from PCL, PLA and PLCG were produced for using in tissue engineering.

Cell affinity and biocompatibility were evaluated in vitro by seeding each scaffold with human dermal fibroblasts (HDF), human adipose-derived stem cells (ASC), rat aortic smooth muscle cells (HSB) and human dermal keratinocytes (HaCaT). The metabolic activity of the cells was investigated using The CellTiter 96® Aqueous One Solution Cell Proliferation Assay (MTS). Polystyrene (PS, the bottom of the cultivation well plates) was used as a comparative control for MTS. The Cell density and morphology were evaluated using a confocal microscopy. The cell density was increased with the time of cultivation of the cells. The 14th day after the seeding, the cells reached of confluence, that means they were distributed over the whole surface of the sample. Tests showed, developed nanofibrous scaffolds are suitable for cell applications with different cells lines, for example for the creation of a thymus.

Key words: nanofibers, 3D scaffolds, tissue engineering, cell culture

Day 2:

Oral Presentation Abstracts

RAPID EXPANSION OF HUMAN EPITHELIAL STEM CELLS FOR AIRWAY TISSUE ENGINEERING

Robert E. Hynds, Colin R. Butler, Kate H. C. Gowers, James M. Brown, Dani Do Hyang Lee, Claire Crowley, Vitor H. Teixeira, Claire M. Smith, Nicholas J. Hamilton, Ricky M. Thakrar, Helen L. Booth, Martin A. Birchall, Paolo De Coppi, Adam Giangreco, Christopher O'Callaghan and Sam M. Janes*

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Stem cell-based tracheal replacement represents an emerging therapeutic option for patients with otherwise untreatable airway diseases including long-segment congenital tracheal stenosis and upper airway tumors. Clinical experience suggests an urgent need to restore mucociliary function in the lungs following transplantation of tissue-engineered grafts while pre-clinical studies show that seeding scaffolds with autologous epithelial cells improves regeneration. Recent data suggest the importance of high epithelial cell seeding density and existing cell culture techniques are inadequate to achieve coverage of clinically suitable grafts. We aimed to develop a scalable airway epithelial cell culture system to deliver high quality epithelial cells to clinical grafts.

Human airway epithelial cells derived from endobronchial biopsies were cultured in either bronchial epithelial growth media or using a combination of immortalized 3T3 fibroblast feeder cells and Rho-kinase (ROCK) inhibition (3T3+Y). Epithelial cells were analyzed using immunofluorescence, qPCR and flow cytometry to assess their expression of airway stem cell markers. Differentiation capacity was assessed in air-liquid interface cultures. Ciliary structure and function was assessed using electron and high-speed video microscopy.

3T3+Y results in rapid and sustained expansion of airway basal epithelial stem cells in comparison to conventional culture techniques. These cells are capable of multipotent airway differentiation in vitro, forming epithelia containing both ciliated and goblet cells. Ciliated cells are functional with a normal ciliary beat pattern.

3T3+Y represents a method to achieve the required quantity and quality of airway basal epithelial cells in the time window demanded by clinical transplantation.

EX-VIVO EXPANSION OF HUMAN CARDIAC BIOPSY-DERIVED STEM CELLS IN PHYSICO-CHEMICALLY DEFINED NUTRIENT MEDIA

S Bardelli, S Duarte Jorge, B De Jesus Da Cruz Monteiro, F Messi, T Moccetti, M Moccetti.

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Physico-chemically defined media are fully synthetic nutrient mixtures, exclusively made of small molecules characterized by their CAS and EINECS numbers. Such media are made of non-animal derived ingredients only. The advantage of using physico-chemically defined media is the maximum control over cell metabolic functions, and the reduced variability in product synthesis and quality. The objective of the present study is to design a specific physico-chemically defined cell culture medium for the growth of human Cardiac Stem Cells (hCSCs) for therapeutic use by: 1. Selection of the basic culture medium; 2. Reduction of Fetal Bovine Serum (FBS) content; 3. Identification of specific adhesion substrates; 4. Selection of specific growth factors. Unfractionated and MACS-enriched cells from human biopsies were grown in selected physico-chemically defined cell nutrient media at 10% FBS. The sequential decrease of serum concentration was evaluated for all the culture media. In favorable physico-chemically defined media, such as HAM'S F12, further reduction of FBS concentration below 5% was not tolerated. Therefore, hCSC growth at lower serum concentrations was supplemented by a comprehensive approach including specific cell adhesion surfaces and a specific combination of growth factors. In order to select adhesion substrates specific for our cells, the natural composition of the cardiac extracellular matrix was considered as a model system. To further optimize our cell therapy product in serum-free condition, specific GMP-grade growth factors were included in the study. These data support the hypothesis that CSCs obtained from human cardiac biopsies can be extensively grown in vitro in physico-chemically defined nutrient media at reduced serum concentration by preserving their safety profile for future therapeutic use.

Day 3:

Oral Presentation Abstracts

Oral presentations will be added after the submission deadline

TOWARDS PERSONALISED HEALTHCARE ENGINEERING: A NEW PARADIGM IN BLOOD DISORDER TREATMENT

A. Mantalaris
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Personalized medicine is a medical model that proposes the customization of healthcare, with decisions and practices being tailored to the individual patient by use of patient-specific information (initially genetics information) and/or the application of patientspecific cell-based therapies. The BioBlood project aims to deliver personalised healthcare through a "step change" in the clinical field of haemato-oncology. BioBlood represents an engineered bioinspired integrated experimental/modelling platform for normal and abnormal haematopoiesis that receives disease & patient input (patient primary cells & patient/disease-specific data) and will produce cellular (red blood cell product) and drug (optimal drug treatment) therapies as its output. Herein, we will present the experimental platform, which is a novel three-domensional hollow fibre bioreactor capable of culturing normal and abnormal haematopoietic cells in the absence of exogenous growth factors by mimicking the structure and function of the bone marrow, alongside a population balance model (PBM) that is able to capture cellular heterogenity and in particular leukaemia heterogenity. The PBM, which is able to extract patient- and disease-specific information is linked to a pharmacokinetic/pharmacodynamic (PD/PD) model, which is the used to optimise chemotherapy treatment in a personalised manner.

A PREDICTIVE MODEL FOR ENERGY METABOLISM IN MAMMALIAN CELLS.

A. Quiroga M. M. Papathanasiou E. N. Pistikopoulos A. Mantalaris
Dept. of Chemical Engineering, Centre for Process Systems Engineering (CPSE), Imperial College London SW7 2AZ, London, U.K

Monoclonal antibodies (mAb) exhibit remarkable properties such as homogeneity, high specificity, affinity and long elimination half-life in the human body, consequently, mAbs have been used in a wide range of applications in diagnosis and therapy. Mammalian cell systems have been extensively used as manufacturing platforms to produce mAb due to their capacity for posttranslational modifications, which are known to be essential for MAb biological function and pharmacokinetics [3]. The implementation of animal cell cultures requires optimization of the yield, growth and medium expenditure to reduce the operational cost. Manual testing is labour intensive, time consuming, the results are susceptible to human error and costly. Model-based techniques have been developed to reduce unnecessary experimentation such as modelbased optimization strategies that identify feeding regimes to maximize mAb titer in GS-NS0 cultures [2]. However, existing feeding strategies depend mainly on glucose and glutamate supply neglecting the exhaustion of other essential amino acids and the energy requirements of the cell not only for the proliferation and maintenance of cells but also for mAb production.

In this work, cell and product compositions, and energy requirements for proliferation, maintenance and production, have been considered in the development of a novel dynamic predictive model for GS-NS0 cells producing cB72.3 MAb. Model development follows the framework described in [1] and it describes growth kinetics, metabolism, mAb secretion and the production/consumption of ATP based on glucose/amino acids energy metabolic networks. The full range of the model's parameters was subjected to Global Sensitivity Analysis (GSA) to identify the significant parameters that were then estimated from specifically-designed batch experiments. Finally, the validity of the model was tested against a set of four independent batch experiments, each of them under different initial conditions. The model successfully predicts the number of viable, apoptotic and dead cells, and the concentrations of ATP, glucose, 19 amino acids and lactate. The close alignment of the experimentally derived ATP concentration and the calculated ATP concentration based on the metabolic network prove the remarkable performance of the model. The successful coupling of growth kinetics equations and stoichiometric balances and the in vitro/in silico approach has enabled us to develop the first dynamic model that predicts the ATP content in mammalian cell cultures. The next step is to include the ATP concentration effect in the viable cells balance and implement a model-based optimization to design a feeding strategy that maintains cellular metabolism in energy-efficient pathways to maximize the mAb titer.

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THE ROLE OF CARBON DIOXIDE IN CHINESE HAMSTER OVARY (CHO) CELL KINETICS

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Since the commercialization of the first therapeutic monoclonal antibody product in 1986, this class of biopharmaceutical products has grown significantly. This increased market has led to a need for increased production volumes and a drive to improve process efficiency. It was found that the combination of two common scale translation parameters, mixing time (<5 s) and energy dissipation rate (P/V = 10 $W\ m-3$), were more capable of defining the operating conditions at which the microtiter plates (24-SRW MTPs) and the shake flasks (SFs) should be running to be used as a scale-down model of the CHO-CY01 cell line culture in stirred tank bioreactor (5L-STR). Culture pH, temperature, dissolved oxygen and carbon dioxide levels, and nutrient concentrations have a significant impact on cell growth, metabolism, and product synthesis. Carbon dioxide, CO2, is known to play an essential role in mammalian cell culture in vitro not only to maintain the culture pH at the normally optimum range but also to regulate many cellular activities. With PreSens Precision Sensing (Regensburg, Germany) technology a high-throughput online multi-parametric analysis method of mammalian cell cellular activity was developed. From varying the carbon dioxide (CO2) concentration inside the CO2 incubator, it was possible to generate multiple pH profiles. A culture pH higher than pH 7.50 caused an increase in lactate production rate and so increased glucose consumption rate. CHO-CY01 cells at pH higher than 7.00 did not produce IgG4. Cell culture pH between pH 6.80 and pH 7.00 provoked an arrest in the ammonium production. While, culture pHs between pH 6.70 and pH 6.80 inhibited the cell growth, glucose consumption and lactate production. Ammonium production rate increased at pHs between 6.0 and 6.70. Below pH 6.60, there was immediate cell death through apoptosis. These pH ranges were validated with CHO-CY01 cell line cultures at different pHs in a 5L-STR. A specific IgG4 production rate of 106.06mg 10−9 $cells\ day$−1 (1.8-fold increase) was reached at pH 6.8.

Keywords: CHO cells, carbon dioxide, pH, 24-SRW MTPs, high-throughput, PreSens® sensor technology

TOWARDS THE INTENSIFICATION OF MAMMALIAN CELL CULTURE SYSTEMS: AN INTEGRATED IN-SILICO/IN-VITRO APPROACH

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Monoclonal antibodies (mAbs) form an increasingly growing market, with projected, combined worldwide sales of approximately $125 billion [1]. However, market competition (such as the appearance of biosimilars) dictates significant improvements in the biomanufacturing of mAbs that will lead to reduced-cost end products of decreased time-to-market [2, 3]. Therefore, to answer to global trends, there has been a paradigm shift towards the development of continuous/quasi-continuous operations, aiming to reduce capital and operational costs [4]. The shift from the current state of the art to continuous processing is nonetheless a challenging task that is coupled with various technical, as well as implementation challenges.

As a result, there is eminent need for advanced computational and monitoring tools that will facilitate the shift to continuous processing. The development of such tools will provide the basis for reduced-cost simulations of various operating scenarios that will pave the way towards the realization of optimal, continuous operation. Moreover, computational tools can be used to assist experimentation through design of experiments, leading to tailor-made, targeted experiments that provide the essential information with minimal labor time and cost. Additionally, rigorous, dynamic, mathematical models can be used as tools to compliment experiments in the system understanding, as well as to provide an insight into the system response to disturbances.

In this work we present the development of an advanced, integrated, in-silico/in-vitro platform for the optimization and control of mammalian cell culture systems. In particular, following our previously presented framework [5] we develop a dynamic, mathematical model for a GS-NS0 cell culture system that we validate through various sets of experimental conditions, as well as experimental data available in the literature [6]. The model is used for the development of advanced, model-based optimization and control strategies, aiming to the maximization of the culture productivity, following the PAROC framework and software platform [7, 8]. The performance of the controller is assessed in a 'closed-loop', in silico fashion, against the high-fidelity model for both normal culture conditions, as well as under the occurrence of disturbances. The results of this work demonstrate prolonged cell culture time, as well as maximization of the culture productivity. Moreover, the continuous feeding that is designed by the controller testifies the efficiency and advantages of continuous processing.

Acknowledgements
Financial support from the European Commission (OPTICO/G.A. No.280813 & SyMBiosys/H2020-MSCA-ITN-2015-675585), EPSRC (EP/M027856/1) and Texas A&M University is gratefully acknowledged.

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Poster Presentation Abstracts

Poster abstracts will be finalised weeks before the event

IMPORTANCE OF MULTIPLE CELLULAR ENDPOINT ANALYSIS- CYTOTOXIC EFFECTS OF THE MYCOTOXIN DON ON DIFFERENTIATED PORCINE INTESTINAL EPITHELIAL CELLS

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A stable *in vitro* cell culture platform was established in order to examine cytotoxic effects of the mycotoxin deoxynivalenol (DON) on the porcine intestinal epithelial cell line IPEC-J2. The mycotoxin deoxynivalenol (DON) was used as model toxin, as it is one of the most prevalent food- and feed-associated mycotoxins in cereals and cereal-derived products. Intestinal impairments following mycotoxin contaminated food and feed consumption may partially be due to the fact that intestinal epithelial cells are exposed to high luminal concentrations for a substantial period of time. The effects of DON [5-100 µM] on differentiated IPEC-J2 were therefore assessed after 24 h, comparing different cellular endpoints (neutral red (NR) assay for lysosomal activity, sulforhodamine B (SRB) assay for total protein content (both Aniara), LDH assay for membrane integrity (Thermo Scientific), WST-1 and MTT assay for mitochondrial metabolism (Roche and Life Technologies, respectively), CellTiter-Glo® (CTG) for ATP synthesis (Promega) in four independent experiments. Furthermore, IC50 values were calculated when possible.

The highest sensitivity was observed using the SRB, followed by the NR assay. Viability of IPEC-J2 was decreased significantly starting at 10 µM for the SRB assay and at 50 µM according to the NR assay. At 100 µM DON, viability was reduced by 76.7% and 57.55%, respectively. The calculated IC50 values (± SD) were 35.97 µM (11.85) for the SRB and 64.07 µM (10.8) (24 h) for the NR assay, thus, making the SRB assay 1.78 times more sensitive than the NR assay. The LDH assay, showed even lower sensitivity at the tested concentrations. After 24 h, only 100 µM showed a significant LDH release (> 2.1 times compared to the negative control). Interestingly, assays, targeting the mitochondria, such as CTG, WST-1, and MTT, did not reduce but slightly induce (~10-54% (24 h)) absorbance at lower concentrations (5-50 µM) compared to the cell control.

Depending on the selected endpoint, DON affected the viability of IPEC-J2 in a differential manner. The SRB assay, measuring protein content was the most sensitive, followed by the NR and LDH assay. Assays targeting mitochondrial metabolism or ATP synthesis however, did not reduce cell viability at the tested concentrations. Therefore, multiple cellular endpoint analysis is of great importance, to reflect the actual state of the cell and to get insight in the toxin's effects. This information could therefore significantly contribute to increase the quality of *in vitro* data to avoid under- and overestimation of cytotoxicity.

ARTIFICIAL ELECTROSPUN MATRICES FOR 3D CELL CULTURE AND GENERATING HUMAN THYMIC ORGANOID

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Compared to other nanofiber preparation processes, electrospinning is versatile and superior in production and construction of ordered or more complex nanofibrous assemblies. Besides traditional two-dimensional (2D) nanofibrous structures, electrospinning is an efficient tool in engineering and production of three-dimensional (3D) fibrous macrostructures, especially for tissue engineering applications. For this reason, 3D nanostructures with a significant thickness are increasingly being investigated for applications requiring a third dimension, such as cell culture, organoid development and tissue engineering in general. 3D scaffolds can provide a better link between single cells and organs than traditional 2D cultures because the 3D scaffold offers another direction for cell–cell interactions, cell migration and cell morphogenesis, which is an important aspect in regulation of the cell cycle and tissue functions.

As our starting point, we focused on the scaffolds development for the generation of an OP9-DL1-based organoid, which have been extensively modified to optimally support the output of committed T cell progenitors. Three different techniques were employed for preparation of 3D tissue engineering scaffolds that can support cells attachment, survival and differentiation. Firstly, a special technique called AC spinning was used for preparation of 3D scaffolds. Secondly, a combination of electrospinning method and linearized air flow was employed in order to obtain voluminous structure with the required porosity. Thirdly, a combination of electrospinning and technology which enabling the formation of a layer containing both microfibers and

nanofibers while guaranteeing good mechanical properties of the 3D scaffolds. NanoMatrix3D standardized scaffolds were also tested in order to prepare scaffolds that can support cells attachment, survival and differentiation.

Biocompatibility and adhesive properties of the scaffolds were determined by seeding each scaffold with OP9-DL1 cells, mouse bone marrow stromal cells transduced to express green fluorescent protein (GFP). Cell proliferation was then quantified using MTS assay, and cell density and morphology were assessed by confocal microscopy. Samples were analyzed by laser scanning confocal microscopy on day 6. Cell images were captured from the most representative area of the fiber mat. Confocal microscopy images showed good cell adhesion and persistent cell growth for all samples. In order to assess host biomaterial interactions such as inflammatory responses, fibrous encapsulation, as well as vascularization, scaffolds were implanted subcutaneously into C57BL/6 mice and the host-biomaterial interactions indicate excellent biocompatibility. Immunohistochemical analysis of the endothelial cell marker CD31 revealed that all scaffold types were properly vascularized by day 22 after implantation.

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BIOLOGICAL CONSIDERATIONS FOR THE CHOICE OF CELL CULTURE MEDIA

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Success of any in vitro experiment critically depends on the choice of cell model, as well as cell culture conditions, including culture medium. There is a number of established synthetic media that is validated to support survival and differentiation of cells of different lineage. Commonly used media include Media 199, developed in 1950s by Morgan and co-workers; RPMI-1640 developed by Moore and co-workers in 1960s and Minimum Essential Medium (MEM), developed in 1970s by Harry Eagle, which also has widely used modifications such as Dulbecco's Modified Eagle's medium (DMEM). In this study we compared the reported ion concentrations of sodium, potassium, chloride, calcium, magnesium, phosphate, bicarbonate and sulphate in MEM, DMEM, RPMI, Media 199 as well as Hank's and Earle's balanced salt solutions to those found in normal human plasma. We have found that none of these widely used culture media demonstrated perfect correspondence of ion concentrations to those in plasma, with Media 199 being the closest and RPMI being most different from plasma. Our data demonstrate that the choice of culture medium requires more serious considerations than generally given and suggest that media formulations need to be revisited with the goal of close simulation of conditions found in the body rather than best maintenance of cell growth and differentiation.

ESTABLISHMENT OF CULTURE CONDITIONS FOR USE IN DEVELOPING AN ARTIFICIAL SALIVARY GLAND

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Introduction
At present it is not possible to fully investigate the development of a number of salivary gland disorders such as tumour development, Sjogren's syndrome or viral-associated infections. Radiotherapy for head and neck cancer, and chemotherapy for non-oral cancers, can lead to salivary gland damage, reduced salivary flow, xerostomia and a worst case scenario of severe, debilitating mucositis with potentially life-threatening systemic infections. The aim of this project was to establish in vitro models of human salivary glands, to investigate the pathogenesis of salivary gland disease and to further understand the development of salivary glands for regenerative studies.

Methods
The submandibular, mucoepidermoid carcinoma cell line (HTB-41) was used as a control and cultured alongside human tissue isolated from sublingual glands. The cells were grown on Alvetex scaffolds and PTFE transwells coated with various extracellular matrices (ECM), both submerged in tissue culture medium (non-ALI) and at an Air Liquid Interface (ALI), and under either dynamic or static culture conditions. Haematoxylin and eosin stained sections of the cultures were used to visualise growth and RT-PCR was used to determine the differential expression of cell type-specific markers.

Results
HTB-41 cells grew more effectively as multilayers under ALI conditions than when fully submerged. Interestingly the most effective growth conditions for the PFTE inserts was ALI under dynamic conditions whilst those on the Alvetex scaffold, where cells grow into the scaffold, preferred ALI under static conditions. In comparison the normal sublingual cells preferred non-ALI, dynamic conditions on the PTFE inserts and preferred non-ALI, static conditions in the Alvetex scaffold.
For both cell types collagen provided the best matrix for cell growth on PTFE and interestingly the HTB-41 cells also preferred collagen-coating of Alvetex scaffolds whilst matrigel was a better matrix in the Alvetex scaffolds for the sublingual cells.
RT-PCR was used to confirm the expression of cell type-specific markers. HTB-41 expressed Nanog, amylase, E-cadherin, ZO-1 and claudin-1 but not smooth muscle actin or c-kit. In comparison the human sublingual cells did express smooth muscle actin and c-kit and also Nanog, amylase, ZO-1 and CD10.

Conclusion
Our current data indicates that salivary gland cell phenotype is dependant upon the scaffold, the ECM and culture conditions used. Our results also indicate important differences between the cell type-specific markers expressed by our cancer cell line (HTB-41) and the normal human salivary gland cells. This provides important information for future studies involving the culture of primary acinar-like cells from human salivary glands.

USING HUMAN NEURAL STEM CELLS TO MODEL CNS PATHOLOGY IN 2D AND 3D CULTURES

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The central nervous system (CNS) is the most complex tissue in the human body, for which there is currently an unmet need to develop reliable and accurate in vitro models. Conventional monolayer culture systems have allowed for the isolation and investigation of human neural stem cells (hNSCs), neurons and glia, in order to study their physiology and pathology at a cellular and molecular level. With the aim to better mimic in vivo CNS tissue structure, we have here demonstrated the use of a 3D hydrogel-scaffold cell culture system.

Fetal brain-derived hNSCs have been isolated and cultured in 3D hydrogels consisting of collagen I/Matrigel, alongside conventional 2D monolayers. hNSCs cultured in the 3D hydrogels were shown to display distinct expression of stem, neural and glial markers, as compared to those cultured in 2D. These initial findings indicate the importance of ensuring a physiologically relevant architecture when modelling the CNS.

Using hNSCs and hNSC-derived neurons, the present study further aims to demonstrate the potential of this 3D culture system to model pathologies of neural development or death. Thapsigargin, a sarco/endoplasmic reticulum calcium ATPase (SERCA) inhibitor, has been used to induce intracellular Ca2+ release in hNSCs and neurons, ultimately resulting in apoptosis. This system is now being used to test putative neuroprotective pharmacological interventions for Ca2+ induced- and hypoxic-ischemic induced cell death.