CELLS, PROTEINS *and* BIOPROCESSING

ABSTRACTS

17th-19th June 2014
London, UK

EuroSciCon

This three day event will discuss three main topics

- Cell Culture Technology Event: Recent advances, future prospect
- Therapeutic proteins and antibodies: from design to function
- Bioprocess development: Discussing facilitation of industrial uptake

With plenty of opportunity for networking and debate, this informal international meeting will bring you up to date with current research and thinking.

This event has CPD accreditation

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Day 1:

Invited Speakers Abstracts

If you think ignorance is bliss, you're more stupid than you look.

Dr John M. Davis, School of Life & Medical Sciences, University Of Hertfordshire, Hatfield, AL10 9AB.

Skeletons lurk in the cell culture cupboard. Some have been there for more than 50 years. Ignoring them has cost millions, ruined reputations, and damaged many areas of research including the search for cancer therapies. NOW is the time to set our house in order.

This talk will review these 'skeletons' and introduce a new piece of assistance to help remove them from, or prevent their appearance in, YOUR laboratory.

Real time, label-free characterisation of live cells in monolayer culture using high resolution, multi-modal light microscopy

Dr Melissa Mather, Principal Research Fellow, Institute of Biophysics, Imaging and Optical Science, University of Nottingham, UK

The clinical implementation of stem cell therapies requires methods capable of discriminating distinct differentiation stages of cells, and characterisation of their purity, viability and proliferation in culture. Here a novel multi-modal light microscope for non-invasive, label-free characterisation of live cells is reported. This instrument incorporates proximity microscopy which produces high contrast, low artefact images of the cell 'footprint' showing sub-micron features. It also automatically changes between other imaging modes (bright field, dark field and quantitative phase contrast) enabling the 3D morphology of cells to be reconstructed. Further, images of single cells and cell populations can be obtained simultaneously.

New method to identified amino acids, peptides and transjunctional proteins: Co-culture transwell system and mass spectrometry.

Dr María D. Mayán, Biomedical Research Center (INIBIC), Universitario A Coruña, Spain

Neurons, hepatocytes, cardiocytes, chondrocytes and almost all cell types form gap junction's (GJ) channels that are critical for cell function and tissue homeostasis. GJ channels provide a selective signalling route by the direct exchange of potent signalling molecules such as cAMP, second messengers, electrical signals, nutrients, ions and several other molecules that regulate cell survival, growth and metabolism. Using several methods, researchers have identified specific transjunctional molecules that helped to better understanding cellular communication and its influence in several molecular mechanisms in health and disease. However the techniques are limited to the measure of dye transfer or to the identification of small molecules using radioactivity for labelling endogenous transjunctional molecules. Here, we have development a new method to identify and quantify transjunctional molecules combining stable isotope labelling of amino acids in cell culture (SILAC) and Transwell co-culture system with mass spectrometry (MS). Transjunctional free amino acids were quantified using EZ:faast method combined with Orbitrap mass spectrometry. Transjuntional peptides and proteins were analysed directly by MS. The method provides a rapid and valuable system to study the functionality of GJ channels and to study the effect of the direct exchange of specific peptides and proteins between neighbouring cells that probably differs from the uptake of peptides or proteins from the surrounding medium.

Human stem cells for disease modelling and tissue reconstruction - promises and challenges

Dr Patrizia Ferretti, Group Leader, UCL Institute of Child Health, Stem Cell and Regenerative Medicine Section

Different stem cell sources for studying human tissue biology and tissue repair will be discussed. Focus will be on the use of human somatic stem cells derived from embryonic brain and paediatric adipose tissue for modeling tissue injury in vitro and for tissue reconstruction.

Introducing a Novel liquid 3 Dimensional Scaffold for Culturing Cells in 3D

Dr Anthony Mitchell Davies, Director of INCHSA, Dept Clinical Medicine Trinity College Dublin, Ireland

3D Cell Cultures & New Reagents for Improved Cellular Viability Quantitation

Dr Lucy Wheatley, Field Application Specialist, Promega UK

In comparsion to traditional 2D cell culture formats 3D cultures represent significant challenges when it comes to cell viability assessment following compound or drug treatment. Factors which need to be addressed include whether the reagent used can effectively lyse the 3D microtissue, allowing the necessary components to penetrate to the core of the 3D structure and generate a robust signal which is not quenched by the mass of cells present. This talk will focus on recent developments in respect of a new 3D culture-optimised viability reagent based around quantitation of the live-cell metabolic biomarker ATP.

Biofabrication technologies for 3D cell culture systems

Dr Lorenzo Moroni, Associate Professor, University of Twente, Tissue Regeneration Department, The Netherlands

Cells in a tissue, organ or organism are exposed to complex biological environments. Conversely to classic culture systems, these environments are not flat, but three-dimensional (3D). Consequently, two-dimensional (2D) tissue culture models used to translate a new therapy to the clinics are far from accurate. Despite numerous attempts to create 3D culture systems better mimicking the environmental conditions that cells face in vivo, cell culture and the understanding of biological phenomena are still extensively performed in 2D substrates. These surfaces favour a loss of the original cell phenotype (dedifferentiation) during culture, resulting in a different cell behaviour evidenced by an abnormal cell activity. Furthermore, a rational understanding of the mechanisms behind cellular processes after in vitro culturing on 2D surfaces is still lacking. These problems contribute to the poor correlation between in vitro and in vivo observations. Here, we present a number of alternative cell culture systems that enable to culture cells in 3D in clinically relevant volumes and compare cell activity in these systems to conventional 2D cultures.

3D-tetraculture system mimicking the cellular organization at the alveolar barrier.

Dr Arno C. Gutleb, Chef de projet, Centre de Recherche Public - Gabriel Lippmann, Luxembourg

3D in vitro model of a functional epidermal permeability barrier from hESC and iPSC

Dr Anastasia Petrova, Research Fellow, Immunobiology Unit, UCL Institute of Child Health/ Assisted Conception Unit, King's College London, UK

Cornification and epidermal barrier defects are associated with a number of clinically diverse skin disorders. However, a suitable in vitro model for studying normal barrier function and barrier defects is still lacking. Here we demonstrate the generation from human embryonic and induced pluripotent stem cells (hESC and iPSC) of the first human epidermal equivalent (HEE), structurally similar to native epidermis, with a functional permeability barrier. Such HEE generated from disease-specific Ipsc will be an invaluable tool not only for dissection of molecular mechanisms leading to epidermal barrier defects but also be instrumental for drug development and screening.

Poster Presentation Abstracts

EXTRACELLULAR MATRIX COATED CERAMIC SCAFFOLD ENHANCE OSTEOBLAST DIFFERENTIATION

Pullisaar H.[*1], Verket A.[*1], Szöke K.[2], Tiainen H.[1], Haugen H.J.[1], Brinchmann J.E.[2, 3], Reseland J.E.[1], Østrup E.[1, 3]

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The aim of the study was to assess the osteogenic performance of a hybrid construct consisting of ceramic titanium dioxide (TiO_2) scaffold, seeded with either human adipose tissue-derived mesenchymal stem cells (hAD-MSCs) or primary human osteoblasts (hOBs), and subsequently coated with alginate hydrogel containing extracellular matrix (ECM) components (Enamel Matrix Derivative, EMD). The constructs were assessed in terms of cell survival and osteogenic differentiation.

An evenly distributed ECM-like environment was created around hAD-MSCs and hOBs seeded on TiO_2 scaffolds without inducing a cytotoxic response evaluated by lactate dehydrogenase activity and live/dead staining (acridine orange/ethidium bromide). EMD coating did not enhance the secretion or expression of

osteogenesis-related factors (collagen type I alpha 1 (COL1A1), osteoprotegerin (OPG), osteopontin (OPN), alkaline phosphatase (ALP), osteocalcin (OC)) in hAD-MSCs compared to control. However, EMD induced an enhanced secretion and expression of bone markers like COL1A1, OPG and OC in hOBs cultured on scaffolds.

The potential of providing an ECM-like environment for hAD-MSCs and hOBs using an alginate hydrogel coating on a pre-cell-seeded TiO_2 scaffold was demonstrated. The local delivery of EMD in alginate enhanced differentiation of osteoblasts, and the coating procedure may have a potential for local delivery of other ECM components or stimulatory factors for use in stem cell-based bone tissue engineering.

* Contributed equally

OPTIMIZING ANTIBODY MICROARRAY FOR COMPARING HUMORAL IMMUNITY IN SMOKERS AND NON-SMOKERS SUBJECTS

Nesrin Tarbiah, Lucy Fairclough, Patrick Tighe and Ian Todd
School of Life Sciences, MBTI Research group, the University of Nottingham

Chronic obstructive pulmonary disease (COPD) can be defined as the permanent damage of the airways in the lung. It has been estimated that COPD affects approximately three million people in UK. Although not all smokers are susceptible to COPD, it is predominately caused by smoking.

The immunological effects of cigarette smoking have been investigated and can provide essential information about inflammation and infection in smokers. To better understand the alteration of the immune response associated with smoking, research can be conducted on the basic immune response in healthy and COPD participants. The aim of this study is to determine whether immunoglobulin levels are different in their concentrations between healthy individuals (smokers and non-smokers) and COPD patients in serum and saliva specimens.

In order to determine Ig isotype levels, microarray assays have been established and calibrated for determining sample concentrations of IgM, IgG, IgA and IgD.

If cigarette smoke is found to influence the serum and salivary levels of Ig isotypes, then the mechanisms underlying these effects will be investigated through studying the effects of cigarette smoke on B-Cell differentiation.

Day 2:

Invited Speakers Abstracts

Therapeutic IgE antibodies: enhanced effector functions for cancer therapy

Dr Sophia Karagiannis, Senior Research Fellow in Translational Cancer Immunology, Head of Cancer Antibody Discovery and Immunotherapy, King's College London School of Medicine, UK

Therapeutic antibodies with enhanced effector functions are now emerging and these properties are expected to enhance their clinical utility for the treatment of cancer. Our group has demonstrated that antibodies engineered with Fc regions of the IgE class targeting tumour-associated antigens can confer superior effector functions against tumours when compared to antibodies with IgG Fc regions. IgE class antibodies, known for potent immune activatory functions in tissues and high affinity for cognate receptors on frequently tumour-resident effector cells may be redirected to mediate protection against solid tumours. An antibody against the tumor antigen Folate Receptor α serves as a paradigm of this concept and this talk will explore the design and functional disease-relevant model systems interrogated to elucidate the potency and mechanisms of action of this antibody class. The pathway to translation of this agent towards first-in-man clinical testing will be discussed.

Developing of long acting glycoprotein hormones using gene fusion and gene transfer: from bench to clinics

Professor. Fuad Fares, University of Haifa, Israel

One major issue regarding the clinical use of many peptides is their short half-life due to the rapid clearance from the circulation. The major strategies for overcoming this problem by pharmaceutical companies are based on chemical techniques. To overcome this problem, we used genetic engineering techniques that have been found successful for designing long acting hormones

Quantum dot- conjugated antibodies as diagnostic and therapeutic tools for cancer imaging and treatment

Dr Weiming Xu, CEO, London Biotech Ltd, UK

Therapeutic monoclonal antibodies have enormous potential for the treatment of cancer. The conjugates of monoclonal antibodies and nanoparticles, including quantum-dot, have offer significant advantages over conventional antibody. By conjugating Qdots with small antibody fragments targeting cancer markers, such as GRP78, we demonstrated that the Quantum dot-antibody retains its immunospecificity and its distribution can be monitored by visualization of multi-color fluorescence imaging both in vitro and in vivo. Moreover we have shown for the first time that Qdot-GRP78 scFv bioconjugates can be efficiently internalized by breast cancer cells and possess biological anti-tumour activity, showing its potential for cancer diagnosis and treatment.

Selection of isotype for immunomodulatory anti-cancer antibodies

Dr Ann White, Senior Research Fellow, University of Southampton, UK

Recent clinical data have revealed the potential for cancer eradication by immune modulation. Monoclonal antibodies (mAb) designed to block inhibitory or promote stimulatory immune signals and drive anti-cancer immunity are delivering durable responses. However they are effective in only a minority of patients and their mechanisms of action are still not clear. In this talk I will review recent data examining the role of mAb isotype and Fc receptor interaction in dictating immunostimulatory and therapeutic activity and discuss ways to optimise these agents through mAb engineering.

Targeting immune- checkpoints in cancer: Novel mechanistic insights on the role of antibody/Fc receptor interactions

Dr Sergio A. Quezada, Immune Regulation and Tumour Immunotherapy Group, UCL Cancer Institute, UK

The immunological balance in cancer is characterized by the dominant infiltration of regulatory T cells resulting in a low Teff/Treg ratio. Remarkably, antibodies against CTLA-4, a key immune modulatory receptor expressed on T cells, efficiently modify this balance, increasing the ratio of Teff/Treg and promoting tumor rejection.

Here we demonstrate that changes in Teff/Treg ratio and tumor rejection depend on the depletion of tumor-infiltrating Treg cells expressing high levels of CTLA-4. Furthermore, depletion is driven by FcγRIV expression on tumor infiltrating myeloid cells illustrating the relevance of antibody/Fc Receptor interactions within the tumor environment on the final outcome of antibody-based immune-modulatory therapies.

Protein based nano and micro particles for biological applications

Deepak Kalaskar, Centre for Nanotechnology and Tissue Engineering, UCL, London

Various organic or inorganic nanoparticles are been investigated in nanomedicine for range of applications in drug delivery, in vivo/vitro cell tracking , gene therapy , MRI contract agent, cancer targeting , biosensor applications etc. However, majority of materials currently in use or being investigated are made from materials which are alien to human body such as carbon nanotubes, quantum dots and other similar nanoparticles. There are still concern regarding use of these particles in biological applications owing to their toxicity, adverse immunological response and retention in tissues or organs.

Measurements' protocol for the investigation of physicochemical and conformational stability and aggregation kinetics of therapeutic IgGs

Dr Ali A. Dahab BSc, MSc, PhD, MBPS, MRSC, FRSM Microseparation Group Pharmaceutical Sciences Research Division King's College, UK

Characterisation of therapeutic monoclonal antibodies (mAbs) represents an ongoing challenge due to their diverse 3-dimensional structures that can affect their stability, immunogenicity and/or toxicity. Although Circular dichroism (CD) spectroscopy provides rapid determinations of protein secondary structure in solutions, there is a pressing need for an improvement in current practices in applying the technique for batch QC. There is a lack of experimental evidence in the literature which is concerned with improving the current practices. This work is based on an effective protocol for the study of IgG2a stability in solution using the simultaneous measurements of Absorbance, Turbidity and CD.

Poster Presentation Abstracts

VEGF-SPECIFIC APTIDES INHIBIT CHOROIDAL AND RETINAL NEOVASCULARIZATION

Jin Hyoung Kim[1*], Dong Hyun Jo[1], Sunghyun Kim[2], Sangyong Jon[2], Jeong Hun Kim[1, 3**]

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Age-related macular degeneration and diabetic retinopathy are leading causes of blindness. Vascular endothelial growth factor (VEGF) is known to be the main factor that induces pathological angiogenesis in these diseases. In this study, we investigate the therapeutic potential and safety profiles of high-affinity peptides targeting VEGF which are identified using an 'aptide' technology. We show that two VEGF-binding aptides, APT_{VEGF1} and APT_{VEGF2}, demonstrate high binding affinity and specificity to VEGF. Furthermore, they suppress VEGF-induced activation of VEGF receptor-2, *in vitro* angiogenesis, and *in vivo* pathological choroidal and retinal neovascularization. Despite potent anti-angiogenic effects, both VEGF-binding aptides do not induce any definite toxicity at the level of cellular viability, histological integrity, and gene expression. Our data show the therapeutic potential of VEGF-binding peptides for the treatment of choroidal and retinal neovascularization.

DECREASE OF BAFFR EXPRESSION DOES NOT COMPENSATE FOR HIGH LEVEL OF BAFF IN SJÖGREN'S SYNDROME PATIENTS

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B cell activating factor (BAFF), a member of tumor necrosis factor ligand superfamily, controls B cell proliferation, maturation, and survival. High level of BAFF is detected in blood cells and serum of patients with autoimmune diseases, such as Systemic lupus erythematosus. As a result, BAFF antagonist (an inhibiting fusion protein) and blocking monoclonal antibody against BAFF are under investigation in clinical trials for treatment of several autoimmune diseases, including Systemic lupus erythematosus and Sjögren's syndrome.

BAFF is mainly produced by monocytes and exerts its function through binding to BAFF receptor (BAFFR), BCMA, and TACI. While the two later receptors also bind APRIL, BAFFR specifically interacts with BAFF. BAFFR signaling involves canonical and non-canonical NF-κB pathway, but there is evidence that BAFFR induces other pathways, as well. BAFF via BAFFR activates ERK and PI3K which suports cell survival. Survival induced by BAFFR leads to phosphorylation of Syk and BCR-associated Igα signaling subunit.

However, a signal from BCR is required for complete phosphorylation of Syk. p38MAPK/CREB and JNK/AP-1 pathway is started after engagement of BAFFR and TACI with BAFF. Such an activation results in isotype switching in B cells.
Crosstalk between BAFFR and other receptors suggests that cooperation of BAFFR-signaling pathway with other pathways is necessary to transduce the signal of BAFF-binding. Therefore, blocking BAFF may decrease clinical symptoms of previously mentioned autoimmune diseases by supressing autoreactive B cell clones, but other important mechanisms that take part in pathophysiological response of immune system remain to be unraveled. We should also be aware that $BAFF^{-/-}$ mice are seriously deficient in B cells, and analogously, patients treated with BAFF antagonist or BAFF-blocking antibody are likely to have their immunoglobulin-based immunity, such as ADCC and mucosal immunity, impaired, too.
We detected BAFF and BAFFR in peripheral blood cells of 19 Sjögren syndrome (Sjs) patients and 20 control samples. We combined cell-surface and cytoplasmatic staining to display membrane expression and total expression of both proteins. We also detected soluble BAFF in serum.
BAFF was localized on monocyte cell-surface, as well as intracellularly. BAFF was also found in both comparments of B cells, but at much lower level than in monocytes. While BAFFR was highly expressed on the surface and in cytoplasma of B cells, we measured only low expression of BAFFR in monocytes. High concentration of sBAFF was found in serum.
Unsurprisingly, expression of monocyte BAFF in Sjs patients was much higher than in controls. Similar finding was observed in B cells of Sjs patients. Also concentration of sBAFF was higher in Sjs patients. On the other hand, BAFFR expression in Sjs patients' B cells was significantly decreased when compared to controls.
These results indicate that activated monocytes interact with B cells via BAFF and BAFFR so that B cells are stimulated, but BAFF is also produced to stimulate cells in autocrine way. However, the technique we used did not enable us to distinguish between autocrine BAFF and BAFF produced outside the cell.
The decrease of BAFFR expression in Sjs patients suggests that there is a mechanism that attempts to take over in order to balance the high level of BAFF. Since the level of BAFF is still high in Sjs patients' B cells, therapy targeting BAFF is likely to bring benefits to the patients.

LABEL-FREE DETECTION OF HUMAN PROSTATE-SPECIFIC ANTIGEN (HPSA) USING FILM BULK ACOUSTIC RESONATORS (FBARS)

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Label-free detection of cancer biomarkers using low cost biosensors has promising applications in clinical diagnostics. In this work, ZnO-based thin film bulk acoustic wave resonators (FBARs) with resonant frequency of ~1.5 GHz and mass sensitivity of ~1.5 ng/cm^2 have been fabricated for their deployment as biosensors. Mouse monoclonal antibody, anti-human prostate-specific antigen (Anti-hPSA) has been used to bind human prostate-specific antigen (hPSA), a model cancer biomarker used in this study. Ellipsometry was used to characterize and optimise the antibody adsorption and biomarker antigen binding on gold surface. It was found that the best amount of antibody at the gold surface for effective antigen binding is around 1 mg/m^2, above or below which resulted in the reduced antigen binding due to either the limited binding sites (below 1 mg/m^2) or increased steric effect (above 1 mg/m^2). The FBAR data were in good agreement with the data obtained from ellipsometry. Antigen binding experiments using FBAR sensors demonstrated that FBARs have the capability to precisely detect antigen binding, thereby making FBARs an attractive low cost alternative to existing cancer diagnostic sensors.

DETERMINING THE ORIENTATION AND POSITION OF THE BLOCKING AGENT IN MODEL PREGNANCY TESTS USING DEUTERATED HSA MEASURED WITH NEUTRON REFLECTIVITY

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Immunoassays utilize the specific binding of an antibody to its antigen to measure the concentration of the antigens in a test solution. The antibodies need to be immobilized onto a solid substrate for most of these applications. However, the antigen binding efficiency of a surface-immobilized antibody is far less than for an antibody in free solution. In order to produce more sensitive immunoassays, it is essential to understand the interfacial behavior of immobilized antibodies. In this work, experimental studies of antibody adsorption and antigen binding that mimicked pregnancy test immunoassays have been performed using neutron reflectivity studies of a model antibody/antigen system immobilized on the silica/water interface. The study revealed the nature of the antibody/antigen interaction and also the importance of a blocking protein, in this case human serum albumin (HSA) which enhances the immunoassay's specificity and efficiency. Of central importance to this study has been the use of a perdeuterated human serum albumin (d-HSA), providing contrast that highlights the orientation and position of the blocking agent within the adsorbed layer. It was found that the adsorbed HSA filled the gaps between the preadsorbed antibodies on the substrate, with decreased adsorption occurring as a function of increased antibody surface coverage. In addition, hCG antigen was also found inserted into both the inner layer and the outer layer of the adsorbed antibody layers. These results are of importance for a full understanding of immunoassay systems that are widely used in clinical tests and in the detection of environmental contaminants.

BRIDGING DISULFIDES FOR THE FORMATION OF BETTER DEFINED ANTIBODY DRUG CONJUGATES

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Antibody drug conjugates (ADC) are a growing class of chemotherapeutic agents for targeted delivery of highly-potent anti-cancer drugs by linking them to an antibody or an antibody fragment.[1] Significant advances in the selection and optimisation of the target, antibody, payload and antibody-drug linker used have led to the recent clinical success of two antibody-drug conjugates (ADC).

ThioBridge™ is a technology platform developed by PolyTherics that conjugates a cytotoxic payload to reduced interchain disulfide bonds of antibodies via covalent re-bridging. ThioBridge™ conjugation is site-specific, highly stable and does not disrupt the tertiary structure of the protein.[2] The resulting antibody drug conjugate has the benefits of improved homogeneity and stability compared to traditional lysine or cysteine conjugated ADCs, with no requirement for the antibody to be recombinantly re-engineered for site-specific conjugation.

Disulfide re-bridging reagents comprising two different payloads: monomethyl auristatin E and F (MMAE or MMAF) were prepared and conjugated to trastuzumab (TRA). The achieved % conversion of antibody to ADC was in a range of 80-90% with a drug to antibody ratio (DAR) of 4 with no unconjugated antibody remaining. The resulting homogeneous conjugates retained antigen-binding, were stable in serum and demonstrated potent and antigen-selective cell killing *in vitro.* In addition, the trastuzumab-MMAE ADC was further tested and showed efficacy in an *in vivo* cancer model.

Reference:
1. Sassoon I, Blanc V. (2013) Antibody-drug conjugate (ADC) clinical pipeline: a review. *Methods Mol Biol. 1045*:1-27.
2. Brocchini *et al 2008*. Disulfide bridge based PEGylation of proteins. *Adv. Drug Delivery Reviews. 60, 3-12.*

SITE-SPECIFIC IMMOBILIZATION OF BMP-2 ON SOLID SCAFFOLD FOR BONE REGENERATION

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Maintenance of skeletal homeostasis and remodeling of bone and joint structures are the key factors in preserving a proper skeleton functionality and hence life quality. Bone tissue has a high natural regenerative potential which is determined by an interplay between hormonal and cytokine signaling. This is often impaired in certain pathological conditions, such as diabetes, senescence, osteoporosis, cancer and many others.
To date, an autograft replacement is a gold standard in treatment of non-healing critical-size bone defects. Nevertheless, low availability, high failure rate, and morbidity of the donor site are the limiting factors paving a way towards application of biocompatible polymeric scaffolds. In order to improve the properties of these scaffolds, certain osteogenic growth factors, e.g. BMP-2 have been incorporated using a variety of coupling techniques, such as surface adsorption or encapsulation within polymeric matrices. The drawback of such systems is the need of tremendous amounts of BMP-2 being immobilized (6-12 mg for long bones non-unions), which in combination with a weak release-dose control often leads to so called initial burst release phenomenon. Additionally, the high local concentration of BMP-2 might cause serious side effects, such as bone overgrowth, osteolysis or inflammation. Some encapsulation technologies may also influence protein conformation and in turn lead to a partial loss of its biological activity. BMPs have also been immobilized onto scaffolds via non site-specific covalent interactions. However, this manner of immobilization may lead to random positioning of growth factors within a scaffold, resulting in suboptimal exposure of receptor binding sites.
Considering the above challenges we developed a system for a site-specific covalent BMP-2 immobilization onto a solid scaffold material, potentially eliminating the necessity of high-dose scaffold loading. We created 3 different BMP-2 variants comprising only one unique amino acid substitution at N-terminal site of the mature polypeptide, which allows site-specific interaction with target structures. Since molecules are then protected from a sequestration, and thus the removal by an extracellular fluid, not only the half-life of such scaffolds may be substantially extended, but also much lower loading capacities might be required. Additionally, this system enables a correct orientation of the immobilized ligand within the scaffold, thus allowing the best possible ligand-receptor interaction. The same system may also facilitate immobilization of series of different growth factors acting in concert for complete bone regeneration.

PRE-LIGAND RECEPTOR ASSEMBLY (PLAD) AS A THERAPEUTIC TARGET

S. Albogami, L. Fairclough, I. Todd and P. Tighe
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University of Nottingham, UK:

The TNF and TNF receptor (TNFR) superfamilies are valuable models for understanding aspects of immunological signaling. TNFR1 contains an extracellular pre-ligand binding assembly domain (PLAD), which is distinct from the ligand-binding domain. This region is necessary for assembly of TNFR complexes in the absence of a ligand and to facilitate trimerization upon activation by TNF. The discovery of PLAD has revealed unexplained aspects of TNFR biology, providing a powerful strategy for designing novel therapeutics against TNFR-mediated inflammation. It is therefore necessary to understand how PLADs interact with each other on the cell surface before we can fully explore their therapeutic potential. This understanding goes hand in hand with possible improvements in the present knowledge of the nature of TNF/TNFR1 interaction. Currently, very little is known about how PLADs contribute to receptor function. Previously, substantial work advocated various models to understand the nature of TNF/TNFR1 interaction mechanisms, but these models have some weak points. In order to fully understand the molecular mechanisms involved in PLAD interactions, the MBTI group produced both in-silico and physical models of the TNFβ-TNFR1 crystal structures generated by Banner et al. at a scale of 10^7:1. In particular, this study aims to examine a new model of TNF/TNFR1 interaction, by investigating the effect of mutations on the PLAD (CRD1) interaction surfaces. This can help us infer how CRD1 interacts with each other and their effect on the whole nature of TNF/TNFR1 interaction and subsequent signaling is orchestrated at the molecular level.

Day 3:

Invited Speakers Abstracts

Micro-cariers: its role in reducing the cost, time, and efforts towards industrial cells proliferation and protein productions

Dr Ali Hilal-Alnaqbi, Assistant Professor UAEU, Visiting Assistant Professor, Harvard, USA

High-Throughput and Miniaturisation in Process Development

Dr Adrian Haines, Senior Principal Scientist, Lonza Biologics plc, UK

The use of well-designed screens early in the selection and development of cell lines, bioreactor processes and DSP conditions to ensure fit to the manufacturing plant's normal-operating ranges increase the probability that the first clinical batch is delivered on-time. Using automation platforms and miniaturisation in cell culture, DSP and process analytics and bringing them together, makes possible a paradigm shift in cell line process development and selection.

Xanthine Oxidase might serve as a target therapeutic molecule for the treatment of stroke

Dr. Kristine Danielyan, National Academy of Science, Armenia

Delineation of Xanthine Oxidase and its organ specific forms features as the key regulating final enzymes of purine catabolism, which is formed in the conditions of hypoxia due to the limited proteolysis, is vitally important for cells proliferation processes. From the other hand, generation of the hydroxyl peroxide, as the final product of this enzyme activity, might have an exacerbating impact in the early stages of tissue damaging after stroke.

We are proposing and proving experimentally, that regulation of the organ specific forms of above-mentioned enzyme might be the regulating keys in above mentioned physiological processes.

Integration of high-throughput data and mathematical modelling in bioprocess engineering

Dr Ajoy Velayudhan, Principal Research Fellow & EPSRC Manufacturing Fellow, Department of Biochemical Engineering, University College London, United Kingdom

High-throughput data is generated in many different formats, with varying degress of scalability to processes used in biologics manufacturing. Here, HT data for downstream processing in various adsorptive formats—microtiter plates, batch systems, differential reactors--as well as conventional batch chromatography are evaluated for accuracy and scalability. A related issue is developing appropriate mathematical models and fitting them to the HT experimental data. In order to improve model fits, additional terms may be included. However, the experimental data may not be rich enough to identify uniquely all the parameters within a particular model. Model identifiability is evaluated, and global optimization methods are used to ensure appropriate parameter estimation.

New integrated approach to online process monitoring in Cell Culture applications

Berno Lüpkes, Hamilton Robotics

A new approach to online process monitoring and control for all Cell Culture applications. Viable biomass measurement with capacitance measurement technology. Optical DO measurement with digital data transfer. pH measurement with digital data transfer

Integrated Single-Use Technologies for Continuous MAb Processing

Dr. Stephen Cross, EU Process Development Scientist Manager, BioManufacturing Science Network, Merck Millipore.

The properties of monoclonal antibodies have allowed development of platform manufacturing processes following a similar template of affinity capture, ion exchange chromatography and virus removal filtration. There are now new challenges to meet to evolve the current platforms towards incorporating single use technologies and continuous processing. Merck Millipore are developing a single-use, fully-connected mAb flow-through process to simplify purification from a Protein A elution pool. High yield and robust impurity clearance are achieved while maintaining critical product quality attributes. Process data and economic modeling will be presented to demonstrate the advantages of the continuous process.

Micro-Matrix, the next generation in microbioreactors for high throughout processing

Mark Peacock, Applikon Biotechnology UK.

The adoption of high throughput technology for upstream bioprocessing is becoming increasing common in the industrial setting. Reduced time lines associated with rapid cell line screening, process development and optimisation, together with targeted validation campaigns is seeking to drive down costs, improve process efficiency and ultimately improve time to market. Applikon has developed the unique micro-Matrix bioreactor system offering total control over 24 independent bioreactors. This presentation will aim to give an overview of the bioreactor market in general with a focus on the adoption of high throughput bioprocessing in industry.

Poster Presentation Abstracts

INTEGRATED SOLUTIONS FOR CONTINUOUS PROCESSING IN MOBIUS® CELLREADY BIOREACTOR SYSTEMS

Mr Andrew Clutterbuck, Merck Millipore, France

Single-Use Bioreactors are now commonly used for Process Development and production activities, as seeding bioreactors or to produce Drug Substances. The advantages of such equipment have been well demonstrated over the last years in time and cost saving. The combination of a single use bioreactor and a continuous production process strategy leverages powerful and synergistic technologies to maximise productivity. The aim of this presentation is to determine media preparation logistics at 50L and 1000L scale in the Mobius® Mix vessels and assess the performance and scalability of the 3L and 50L Mobius® Single-Use CellReady bioreactors used in combination with the Refine ATF® perfusion system for continuous processing.