CBS One Day Symposium - 3rd July 2012

Coates Road Auditorium

Morning Session – Chaired by David Foley

Time	Speaker	Title
10.00-10.05		Opening Remarks
10.05-10.30	Jane Grove	Functional characterisation of a novel type IV secretion system implicated in the pathogenesis of *Helicobacter pylori* infection
10.30-10.55	Andrea Vernall	Fluorescent ligands as drug discovery tools for the human adenosine A3 receptor
10.55-11.20	James Dixon	Rapid Micropatterning of Cell lines and Human Pluripotent Stem Cells on Elastomeric Membranes
11.20-11.50		***Break***
11.50-12.15	Matthew Twigg	Talking to distant relatives; signal molecule based interactions between the green seaweed *Ulva* and marine bacteria.
12.15-12.40	Joel Fulton	Molecular Basis of Selective Interactions of Nuclear Receptors and Cofactors: Novel Targets for Drug Discovery
12.40-14.00		***Lunch and Poster Session***

Afternoon Session – Chaired by Jeroen Stoof

Time	Speaker	Title
14.00-14.25	Lisa Coneyworth	Automation of scalable pluripotent stem cell culture for high through-put screening using a novel Freedom Evo 200 platform
14.25-14.50	Sophie Darch	Understanding the Social Lives of Microbes
14.50-15.15	Alex Cousins	Tandem G-Quadruplexes: Ligand-binding and topological variations
15.15-15.45		***Break***
15.45-16.10	Hassan Rashidi	Growth factor-loaded microparticles to promote the natural healing process of bone
16.10-16.35	Vinoj George	*In vitro* disease modelling using human embryonic stem cell-derived cardiomyocytes
16.35-17.00	Shanika Crusz	Clinical Microbiology at Nottingham University Hospitals NHS Trust
17.00-17.05		Closing Remarks
17.30		***BBQ at the Johnson Arms***

Speaker Abstracts

Jane Grove

Functional characterisation of a novel type IV secretion system implicated in the pathogenesis of *Helicobacter pylori* infection.

Although over half of the world's population is infected with the pathogen *Helicobacter pylori*, only about 20% develop gastric diseases/cancer depending on multiple genetic and environmental factors via largely undefined mechanisms. Recently, a fourth type IV secretion system has been identified in some *H. pylori* genomes, which contains a gene associated with disease development. The prevalence of this novel secretory system has been determined in strains from patients and variant forms identified. Putative secreted effector proteins have been identified and characterised. The potential role of the secretory system in delivering bacterial toxins to host cells has been investigated to elucidate its function and provide insights into the molecular mechanism of pathogenesis.

Andrea Vernall

Fluorescent ligands as drug discovery tools for the human adenosine A3 receptor

G protein-coupred receptors (GPCRs) are the largest family of transmembrane signalling proteins, and are the target of 30-40% of currently marketed drugs. The adenosine A3 receptor (A3AR) is a GPCR that shows promise as a therapeutic target for cancer, glaucoma, ischaemic heart disease, and various autoimmune inflammatory disorders. Our team uses fluorescence to study A3ARs in living cells, via the synthesis of fluorescent conjugates and use of these tools for drug discovery. We have developed a fluorescent probe for the A3AR with high affinity and selectivity, and shown that this can selectively label the A3AR in cells containing a mixed receptor population. In another project we used a fluorescent antagonist to develop a high content screening assay in living cells, to determine binding affinity constants of competing ligands at human A1AR and A3ARs. Here we screened a commercial fragment library and optimised the lead fragments as new A3AR antagonists. This technology represents a powerful platform to measure ligand affinity at receptors in their physiological membrane environment.

Speaker Abstracts

James Dixon

Rapid Micropatterning of Cell lines and Human Pluripotent Stem Cells on Elastomeric Membranes

Tissue function during development and in regenerative medicine completely relies on correct cell organization and patterning at micro and macro scales. We describe a rapid method for patterning mammalian cells including human embryonic stemcells (HESCs) and induced pluripotent stem cells (iPSCs) on elastomeric membranes such that micron-scale control of cell position can be achieved over centimeter-length scales. Our method employs surface engineering of hydrophobic polydimethylsiloxane (PDMS) membranes by plasma polymerization of allylamine. Deposition of plasma polymerized allylamine (ppAAm) using our methods may be spatially restricted using a micro-stencil leaving faithful hydrophilic ppAAm patterns. We employed airbrushing to create aerosols which deposit extracellular matrix (ECM) proteins (such as fibronectin and MatrigelTM) onto the same patterned ppAAm rich regions. Cell patterns were created with a variety of well characterized cell lines (e.g., NIH-3T3, C2C12, HL1, BJ6, HESC line HUES7, and HiPSC line IPS2). Individual and multiple cell line patterning were also achieved. Patterning remains faithful for several days and cells are viable and proliferate. To demonstrate the utility of our technique we have patterned cells in a variety of configurations.

The ability to rapidly pattern cells at high resolution over macro scales should aid future tissue engineering efforts for regenerative medicine applications and in creating in vitro stem cell niches.

Matthew Twigg

Talking to distant relatives; signal molecule based interactions between the green seaweed *Ulva* and marine bacteria

Quorum sensing is defined as a population dependent bacterial signalling mechanism which regulates gene expression through the production and transduction of chemical signal molecules referred to as autoinducers. In Gram negative bacteria the predominantly utilised autoinducers are *N*-Acyl homoserine lactones (AHLs). It has previously been reported that motile zoospores produced by the green seaweed *Ulva spp.,* commonly found in the intertidal zone of the UK coastline, are attracted to AHLs produced by several biofilm dwelling species of marine bacteria. This project has focused on the relationship between *Ulva* and its cognate bacterial community. The species represented in the bacterial community associated with *Ulva* was identified by generating a 16S phylogenetic clone library from bacterial DNA isolated from the surface of the seaweed. These data revealed that the majority of the population belonged to the Proteobacteria or Bacteroidetes phyla. In order to investigate whether QS signalling affected the rate of zoospore germination in addition to zoospore attraction, *Ulva* zoospores were settled and allowed to grow on synthetic AHLs, biofilms derived from AHL producing model organisms and strains relevant to the *Ulva* cognate population which were shown to produce AHLs. These experiments revealed that AHLs effect zoospore germination and the early growth of the *Ulva* germling as zoospores germinated and grown in the absence of AHLs were significantly longer than those germinated in the presence of AHLs. We therefore theorise that reduced germling growth in the presence of AHLs allows Ulva to obtain a health epiphytic bacterial community which is vital for the seaweeds later development. Further understanding of Ulva growth biology could have potential applications in preventing marine biofouling by this genus of seaweed.

Speaker Abstracts

Joel Fulton

Molecular Basis of Selective Interactions of Nuclear Receptors and Cofactors: Novel Targets for Drug Discovery

Nuclear Receptors (NRs) are biomedically important transcription factors that regulate gene expression networks in response to developmental, endocrine or metabolic signals. Humans express 48 different NRs and their isoforms, which can interact with more than 300 known cofactor proteins, many of which are chromatin modifying enzymes. These interactions are mediated by signature motifs (LXXLL in coactivators; or LXXXILXXLL in corepressors) that are essential for NR/cofactor function (Heery *et al., Nature,* 1997; Heery *et al.,* JBC 2001; Coulthard *et al.,* JBC 2003). Cofactor recruitment can be regulated by binding of specific ligands such as steroids, retinoids, fatty acids, haem, vitamin D, thyroid hormones and xenobiotics to the NRs, making these functional complexes amenable to drug targeting. However, the orphan NRs, which lack known ligands, are not as easily amenable to this approach.

We are investigating selective interactions of NRs with their cofactors. This approach yields insight into the structural determinants for selective NR/ cofactor cofactor recruitment and provides new targets for drug discovery. We will describe novel LXXLL-related motifs that permit highly selective interactions of the developmental regulator BCL11A with the NR2E and NR2F subfamily (COUP-TFs, Tailless, PNR). Differential sensitivities of these NRs to substitution mutations in these motifs provide an example of how related NRs can form selective contacts with cofactors.

Lisa Coneyworth

Automation of scalable pluripotent stem cell culture for high through-put screening using a novel Freedom Evo 200 platform

Current protocols for pluripotent stem cell (pSC) culture are labour intensive and provide numerous sources of variation. Automation of pluripotent stem cell culture enables a robust and reproducible culture method scalable to levels unobtainable using manual laboratory methods.

We have developed and optimised the operation of a novel Tecan Freedom Evo 200 platform for human embryonic stem cell (hESC) culture and demonstrated continuous growth and expansion of the hESC line HUES7 over multiple passages. Pluripotency was maintained throughout growth and expansion, demonstrated by the expression of pluripotency stem cell markers including Oct4, Nanog, Sox2, Tra-1-81, SSEA3 and SSEA4.

The cell maintenance processes were developed further into production processes for plating of hESCs into a range of multi-well plates (including 6, 12, 24 and 96 well plate formats). Cells were seeded at different cell seeding densities and subsequent treatment with complex combinations and concentrations of specific additives allows for automated high throughput screening.

These cell production processes have been implemented in a robust and reproducible three stage hepatocyte differentiation protocol optimised on the Freedom Evo 200 platform. Hepatocyte markers specific for each differentiation stage were expressed including FOXA2, AFP and ASGRP1 for differentiation stages 1, 2 and 3 respectively. Final optimisation of automated cardiomyocyte and neural cell differentiation will allow differentiation of hESCs into all three germ layers, methods extremely valuable in pluripotent stem cell research.

To summarise, we have optimised a novel automated cell culture platform proficient in large scale hESC culture, high-throughput screening, complex medium formulation and cell differentiation.

Speaker Abstracts

Sophie Darch

Understanding the Social Lives of Microbes

It has been argued that bacteria communicate using small diffusible signal molecules to coordinate, among other things, the production of factors that are secreted outside of the cells in a process known as quorum sensing (QS). The underlying assumption made to explain QS is that the secretion of these extracellular factors is more beneficial at higher cell densities. However, this fundamental assumption has never been tested experimentally. We directly test this by independently manipulating population density and the induction and response to the QS signal, using the opportunistic pathogen *Pseudomonas aeruginosa* as a model organism. We found that the benefit of QS was relatively greater at higher population densities, and that this was because of more efficient use of QS-dependent extracellular "public goods." In contrast, the benefit of producing "private goods," which are retained within the cell, does not vary with cell density. Overall, these results support the idea that QS is used to coordinate the switching on of social behaviours at high densities when such behaviours are more efficient and will provide the greatest benefit. These experiments have been repeated using three dimensional picolitre-scale microcavities referred to as 'bacterial lobster traps. Here, individual and small aggregates of cells at a high density can be isolated and manipulated, which we hope will help to further define the relationship of cell density and diffusion within QS.

Alex Cousins

Tandem G-Quadruplexes: Ligand-binding and topological variations

G-quadruplexes are a four-stranded secondary structure adopted by guanine-rich nucleic acids. Potential for biological roles and utility as a therapeutic target has prompted interest in how these structures may interact when in close proximity. Here we investigate sequences capable of forming multiple quadruplexes in tandem and how the interactions between their constituent subunits features in their biophysical behaviour. Further we discuss the relationship between ligand-binding and topological variations in these sequences.

Hassan Rashidi

Growth factor-loaded microparticles to promote the natural healing process of bone

Critical sized defects in bone resulting from trauma and primary tumour resections have presented obstacles to the current treatment for bone repair. The identification of growth factors which play key roles in tissue regeneration have attracted great deal of attention in the past decade, however, the outcome from several clinical trials has been largely disappointing. The results from previous studies have suggested that spatio-temporal control is crucial to achieve optimal therapeutic effects of growth factors. Therefore, scaffolds that can regulate such a release represent an attractive therapeutic path for bone tissue engineering. In the current study, the effect of different growth factors on osteogenesis was initially studied in organotypic culture of the chick femur. Different protocols were developed to produce various formulations and sizes of microparticles containing selected growth factors using single and double emulsion techniques. Assessment of *in vitro* release kinetics showed that tailored release of growth factors was achieved by the inclusion of a triblock co-polymer in the microparticle formulations. Finally, the microparticles were transplanted into the chick femur and cultured for 10 days. The results from release of growth factors from microparticles by *in situ* hybridisation and immunostaining demonstrate the potential for controlled spatio-temporal release of bioactive growth factors for chondrogenesis and/or osteogenesis in the chick femur.

Speaker Abstracts

Vinoj George

In vitro disease modelling using human embryonic stem cell-derived cardiomyocytes

While cardiovascular diseases dominate the cause of death in the developed world, understanding these diseases at the cellular, molecular and functional level has been facilitated by the advent of technologies using human pluripotent stem cells (hPSCs). A lot of these technologies depend on differentiation of hPSCs and hiPSCs (human induced pluripotent stem cells) into cardiomyocytes – in their efficiencies, functional outputs and reproducibility to realise *in vitro* disease modelling and drug development. As many animal models (such as mouse genetic knockouts) fail to recapitulate human genetic diseases, work on hiPSCs has gained substantial importance in recent years. Research work in our lab has focused on using hiPSCs derived from Long QT patients to evaluate pharmacological responses and potential gene therapy. Long QT syndrome type 2 (LQTS2), which is caused by a mutation in the I_{kr} potassium channel encoding *KCNH2* gene (also known as HERG), is characterised by cardiac arrhythmias (extended action potential duration) and sudden cardiac death. When LQT2–hiPSC cardiomyocytes were exposed to E4031 (an I_{Kr} blocker), arrhythmias developed as evident from their electrophysiology, representative of patient phenotype and these presented as early after depolarizations (EADs) in their action potentials. LQT2–hiPSC cardiomyocytes also developed EADs when challenged with the clinically used stressor, isoprenaline, an effect reversed by the β-blockers, propranolol and nadolol, the latter being used for the patient's therapy. Treatment of cardiomyocytes with potassium channel enhancers, nicorandil and PD118057, caused action potential shortening and in some cases could abolish EADs. Allele-specific knockdown of *KCNH2* provided insights into the trafficking of the HERG channel protein and also reduced the action potential duration when compared to the unmodified LQT2-hiPSCs. These findings illustrate the ability of hiPSCs to model the abnormal functional phenotype of an inherited cardiac disorder and represent a promising paradigm in the development of patient therapy.

Shanika Crusz

Clinical Microbiology at Nottingham University Hospitals NHS Trust

As members of the Clinical Microbiology Department of Nottingham University Hospitals NHS Trust, we are involved in the provision of a comprehensive medical microbiology service (including virology) for the whole Nottingham Health Community, which involves the processing of a range of specimens from patients (the department handles in excess of 870,000 samples per year), clinical liaison, infection prevention, control and surveillance activities and service development. As a multidisciplinary department (biomedical scientists, laboratory assistants, administrative staff, clinical scientists and medics), we also engage actively in teaching, training, audit and research and development activities. We would like to use this presentation as an opportunity to introduce key members of the team and outline their roles, with the aim of promoting future interaction and collaboration with our academic colleagues.

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