Biomaterial Interfaces
Room: 201 B - Session BI-WeM

Cell-Surface Interactions
Moderator: M.R. Alexander, University of Nottingham, UK

8:00am **BI-WeM1 High-Throughput Discovery of Materials for Human Pluripotent Stem Cell Culture**, ***A.D. Celiz***, *J.G.W. Smith, A.K. Patel,* University of Nottingham, UK, *R. Langer, D.G. Anderson,* Massachusetts Institute of Technology, *D.A. Barrett, L.E. Young, C. Denning, M.C. Davies, M.R. Alexander*, University of Nottingham, UK

A key hurdle in translating stem cell therapies from research to industrial scale and clinical application is to produce the necessary numbers of cells. For example, a major heart attack causes loss of 1 billion cardiomyocytes and similar cell numbers are lost during progression of other conditions such as multiple sclerosis and diabetes. To meet the demand for such high cell numbers, a defined growth substrate free of animal-derived components is desirable. To this end, we have employed polymer microarrays to screen for human pluripotent stem cell (hPSC) attachment and phenotype on polymers in a high-throughput manner. Polymer microarrays enable a large combinatorial chemical space to be interrogated on a single glass slide. Furthermore, since monomers can be robotically printed and polymerized on the slide via UV photo polymerization, rapid evolution of large numbers of polymers is facilitated. 'Hit' materials identified from the initial screen can be taken forward to a second generation array and mixed in a combinatorial manner to test hypotheses formed from the first generation array and this iteration continues until scaled up to plastic ware for automated culture protocols to achieve long-term expansion of hPSCs.

We have screened over 140 acrylate and acrylamide homopolymers in an array format for hPSC attachment in mouse embryonic fibroblast (MEF) conditioned medium and defined media including StemPro® and mTeSR™1 after 24 hours in culture. Hit materials were then mixed to produce a second generation array of over 500 unique copolymers to optimise cell attachment. Polymer microarrays were characterized using time of flight secondary-ion mass spectrometry (ToF-SIMS), X-ray photoelectron spectroscopy (XPS), atomic force microscopy (AFM) and water contact angle (WCA) measurements. Multivariate analysis (MVA) was used to successfully predict material wettability and cell performance from the ToF SIMS data. Cell attachment was identified using DAPI (nuclei) staining and maintenance of pluripotency was confirmed by OCT-4 staining and imaged using automated fluorescence microscopy. This approach to materials discovery will provide a defined, synthetic growth substrate for hPSC culture that is amenable to scale up for industrial application and is a step toward xeno-free hPSC culture conditions necessary for clinical application.

8:20am **BI-WeM2 Toxicology of Antimicrobial Conjugated Electrolytes: Interactions with Mammalian Cells**, ***H.E. Canavan***, *K.N. Wilde, D.G. Whitten*, University of New Mexico

Certain cationic phenylene ethynylene (CPE)-based polymers (PPEs) and oligomers (OPEs) exhibit dark- and light-activated antimicrobial activity. Until recently, it was unknown if they would also exhibit similar biocidal activity toward mammalian cells. Based on their biocidal activity and diversity of repeat unit number and functional groups, a variety of CPEs, PPEs, and OPEs were selected for these studies, and were examined for their toxicity toward mammalian cells at three levels: cytoxicity testing of cell monolayers, skin irritation testing of tissues, and intracellular co-localization. As expected, concentration plays the largest role in determining viability. The lack of skin irritation for all substances alleviates initial safety concerns for products based on these CPEs and OPEs. In all cases, the addition of light changed the effects of the compounds on the mammalian cells. The modes of action of these compounds appear to be governed primarily by length.

8:40am **BI-WeM3 *In Situ* ATR-FTIR of Human Mesenchymal Stem Cell Differentiation**, ***D.E. Barlow***, *P.A. Fulmer, T.J. O'Shaughnessy, K.P. Fears, J. Morabito, R. Stine, S.P. Mulvaney, B.R. Ringeisen*, U.S. Naval Research Laboratory

Vibrational spectroscopies are valuable methods for non-destructive characterization of biochemical functionality and physiological changes in stem cells. To date, the primary approaches have almost exclusively used either Raman microscopy or Fourier transform infrared (FTIR) microscopy in transmission or reflection configurations. Another FTIR approach is the interfacially sensitive attenuated total reflectance (ATR) configuration which has often been used for in situ characterization of buried cell-substrate interfaces with live microorganisms such as bacteria. However, so far, the method has rarely been used with mammalian cells. Our approach provides a useful complimentary method for which multiple live specimens can be kept under controlled environmental conditions and analyzed by ATR-FTIR over multi-week periods. As a first step in employing in situ ATR-FTIR, we will present results demonstrating early detection of osteogenic differentiation of live human mesenchymal stem cells (hMSC's). In comparison to control hMSC spectra, hydroxyapetite related bands are clearly observed for cells in osteogenic media within 24 hours. Further hydroxyapetite formation and differentiation characteristics were observed over 2 week periods. Additional results will also be presented comparing interfacial spectra of live hMSC's on surfaces with varying properties, including poly-D-lysine/laminin coated substrates, graphene, and nanocrystaline hydroxyapetite.

9:00am **BI-WeM4 Do Cells Read Braille? High Throughput Screening of Surface Topography-Induced Cellular Responses**, ***J. De Boer***, University of Twente, Netherlands **INVITED**

It is well known that cells can respond to diffusible molecules but it is less well known that they are also able to respond to patterned surface topographies. If we are able to understand how cells respond to these patterns, we can rationally design the surface of medical implants for optimized functionality. To unravel the secret Braille language of cells, we have designed and engineered the TopoChip platform, a library of surface topographies reproduced onto polymeric surfaces. Using high-content imaging, we are able to analyze the response of cells to thousands of different surface patterns simultaneously. Thus, we have found surfaces which induce expression of the early osteoblast marker alkaline phosphatase (ALP) in mesenchymal stromal cells to levels similar to that induced by classical osteoblast inducers such as dexamethasone. In addition to ALP staining, we have also stained the actin cytoskeleton and the nucleus, and using CellProfiler software, we have extracted nearly 300 morphological features for each cell on the TopoChip. Using machine learning algorithms, we are now able to predict ALP expression based on cell morphology alone, and further experiments are in progress to investigate a possible correlation between the actin cytoskeletal organization and ALP expression. In conclusion, using a high-throughput screening approach, we can now start to unravel the secret language of surface topographies and apply the hit surfaces to improve medical implants.

9:40am **BI-WeM6 Less is More: Enhancing the Effect of Fibroblast Growth Factor (FGF2) on Human Dermal Fibroblast Proliferation by Surface Modification**, ***J.D. Whittle***, *D.E. Robinson*, University of South Australia

Often the materials selected for culture-ware, scaffolds or bandages are chosen for their bulk properties and low cost, rather than their suitability for cell culture. Consequently cell culture frequently requires relatively large quantities of expensive growth factors (GFs) and other supplements to be added to the culture medium. Cell response to surfaces is known to be heavily influenced by surface chemistry and topology, which may impact on cell attachment, proliferation or differentiation. These surface properties can affect the growth of cells, they do so by influencing the cellular microenvironment. Often this is by modulating the composition and conformation of adsorbed proteins.

A number of approaches to healing of chronic wounds involve the culture of patient cells on bandages or scaffolds for delivery direct into wounds. In this paper, we describe the approach we have taken in our lab to provide a suitable microenvironment for human dermal fibroblasts.

Inspired by the role of the extra-cellular matrix (ECM) in vivo, we use a plasma polymer surface to bind glycoaminoglycans (GAGs) which are able to capture the growth factor FGF2 from solution. By binding the GAG and growth factor to the culture surface, we achieve significantly higher cell proliferation rates at low serum concentrations than adding these components directly into the culture media. We show that binding GAG and GF to the surface has a cooperative effect, in which the combination of these biomolecules is much more effective than either of them alone. In addition to better performance, the pre-loading of culture surfaces avoids the need to add these reagents to the culture medium and therefore reduces the cost of cell culture. We also show how this approach can be translated from 2d cultures to electrospun scaffolds to provide organised dermal structures of fibroblasts and keratinocytes.

10:40am **BI-WeM9 An Anionic Drug Delivery System using Lecithin Liposomes to Improve Oncolytic Viral Cancer Therapy**, ***N. Mendez***, *V. Herrera, F. Hedjran, S.L. Blair, T. Reid, W.C. Trogler, A.C. Kummel*, University of California San Diego

Several treatment modalities such as surgery, radiation, and chemotherapy are effective for cancer treatment however also face several limitations. Alternatively, oncolytic viruses (OVs) can target multiple mechanisms of action while at the same time exploit validated genetic pathways known to be disregulated in many cancers. In particular, the oncolytic virus TAV-255 has shown viral replication attenuation in normal cells while retaining cytolytic activity in tumor cells by taking advantage of defects in the p53-tumor suppressor pathway. Despite its several advantages, the utility of OVs for cancer therapy is limited by 1) neutralization by antibodies mediated by the immune system, 2) rapid clearance by the reticuloendothelial (RE) system in the liver, and 3) the lack of expression of surface receptors (CAR) in certain cancers necessary for OV transduction. Oncolytic viruses are promising agents to combine with nanoparticle delivery approaches because of the capacity for self-replication of the virus. In systemic delivery, targeting with nanoparticles may focus the viral load to the primary tumor cells as well as metastatic tumors to insure a productive initial infection. A non-toxic liposomal delivery system has been developed for delivery of the virus to tumor cells. Further, with the aim to overcome an immune response and to enhance its potential use to treat primary and metastatic tumors, an encapsulation method involving an anionic non-toxic liposome has been prepared by self-assembly of Lecithin around the viral capsid. The developed method has shown that encapsulated viruses retain their ability to infect cancer cells. Furthermore, an immunoprecipitation (IP) technique has shown to be a fast and effective method to extract non-encapsulated viruses and homogenize the liposomes remaining in solution. Extracting non-encapsulated viruses from solution may prevent an adverse immune response when used in an in vivo model and may enhance treatment for multiple administrations.

11:00am **BI-WeM10 Poly(*N*-isopropyl Acrylamide)-coated Surfaces: Investigation of Biocompatibility with Mammalian Cells**, ***M.A. Cooperstein***, *H.E. Canavan*, University of New Mexico

Poly(*N*-isopropyl acrylamide) (pNIPAM) undergoes a conformation change in a physiologically relevant temperature range. PNIPAM is relatively hydrophobic above its lower critical solution temperature (LCST, ~32ºC), and mammalian cells are easily cultured on pNIPAM-grafted surfaces. When the temperature is lowered below the LCST, the polymer's chains rapidly hydrate, and cells detach as intact sheets capable of being used to engineer tissues ("cell sheet engineering"). Although the NIPAM monomer is toxic, there are conflicting reports as to whether its polymerized form is toxic, as well. Very few (<10) studies exist that investigate the cytotoxicity of pNIPAM, and their results are conflicting. Furthermore, the published studies are not comprehensive. Before the cell sheets detached from pNIPAM can ultimately be used on humans, it is crucial to first assess the cytotoxicity of the surfaces from which they have been obtained. In this work, we present a comprehensive investigation of the cytotoxicity of pNIPAM-grafted surfaces. The relative biocompatibility of substrates prepared using different polymerization (free radical and plasma polymerization) and deposition (spin coating and plasma polymerization) techniques is evaluated using appropriate cytotoxicity tests (MTS, Live/Dead, plating efficiency). Four different mammalian cell types (endothelial, epithelial, smooth muscle, and fibroblasts) were used for the cytotoxicity testing. The pNIPAM-coated surfaces were evaluated for their thermoresponse and surface chemistry using X-ray photoelectron spectroscopy and goniometry. We find that while cell viability on pNIPAM surfaces decreases when compared to controls, the viability also seems to be deposition type dependent, with sol-gel-based pNIPAM surfaces being the least biocompatible. We attribute this difference to surface topography and chemistry. This work will have valuable insights into the cytotoxicity of pNIPAM-coated surfaces, and therefore into the applicability of cells grown on these surfaces for use in human subjects. In addition, the trends observed in the effect of polymer molecular weight, surface modification technique, and cell type may be extrapolated to other bioactive polymers of interest.

11:20am **BI-WeM11 Polymer Microarrays for the High Throughput Discovery of Novel Switchable Materials**, ***A.L. Hook***, *C. Chang*, University of Nottingham, UK, *R. Langer, D.G. Anderson*, Massachusetts Institute of Technology, *P. Williams, M.C. Davies, M.R. Alexander*, University of Nottingham, UK

Polymer microarrays for the high throughput discovery of novel switchable materials

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Polymer microarrays have become a key enabling technology for high throughput materials discovery. This format has been applied to a broad range of biological systems, from stem cell attachment to the resistance of bacterial attachment.[1] Switchable materials have also been a focus of materials research as they provide temporal control of biological systems. Switchable materials are able to alter their surface or physical properties in response to an external specific signal such as a change in temperature, pH, or concentration of a signal molecule. This class of materials has been applied to drug delivery and controlled cell attachment.

We have applied polymer microarrays for the discovery of novel switchable materials that are able to temporally manipulate biological systems.[2,3] Polymer microarrays were formed by printing mixtures of acrylate monomers, followed by UV curing and vacuum extraction. This enabled arrays of hundreds of unique materials to be produced. We screened these materials for switchable properties using AFM, WCA, optical microscopy and ToF-SIMS. The focus of this work was to identify materials with thermally induced changes in chemistry or topography.[2,3] Using these methods we discovered novel materials with unique switchable properties. In particular, ToF-SIMS analysis provided insight into the conformational changes induced in the polymers by the change in temperature.[2] These were applied to investigate the attachment of bacteria to surfaces, where we were able to temporally control the interaction of bacteria with a polymer surface. These materials have potential application for regenerative filtration devices.

[1.] Hook et al., *Biomaterials*, **2010**, 31, (2), 187-198.

[2.] Hook et al., *Surface and Interface Analysis*, **2013**, 45, (1), 181-184.

[3.] Hook et al., *Soft Matter*, **2011**, 7, (16), 7194-7197.

11:40am **BI-WeM12 Antibacterial and Cells Proliferation Studies of Biodegradable Polymeric 3D Scaffolds**, ***A. Pegalajar-Jurado***, *K.A. Wold, M.M. Reynolds, E.R. Fisher*, Colorado State University

Wounds caused by diseases or injuries often present complications, requiring medical intervention to restore biochemical processes needed for proper healing to occur. Several biodegradable polymeric materials have been applied to wounds for protection and to facilitate the healing. These materials, however, often do not inhibit bacterial infection nor support cell growth.

Development of therapeutic materials that release antimicrobial agent from a porous, polymeric fibers scaffold has been previously reported by our group, which was directly related to the release of nitric oxide (NO). These scaffolds showed a release of NO under physiological pH and temperature, and a log2 reduction in *Acinetobacter baumannii* was achieved using 15 mg/mL of NO releasing S-nitrosated poly(lactic-*co*-glycolic-*co*-hydroxymethyl propionic acid (PLGH)-cysteamine. However, fibroblast cells showed low viability when exposed to concentrations higher than 0.1 mg/mL of S-nitrated PLGH-cysteamine polymer. A log5 reduction in bacteria such as *Escherichia coli* and methicillin-resistant *Staphylococcus aureus* is required to be considered medically relevant. To achieve this goal, a multiple therapeutic strategy was implemented. The antimicrobial activity of silver nanoparticles against *E.coli* and *S.*aureus over short period of time is well established. To obtain a long term efficacy as well as local toxicity, silver nanoparticles were incorporated within the NO releasing scaffolds. This combination is expected to result in antibacterial activity against multiple strains included *E.coli* and MRSA.

Plasma processing has been used extensively to modify two-dimensional materials. Recently, however, plasma treatments have been used to successfully modify three-dimensional (3D) structures. Our preliminary data demonstrate that porous (Ɛ-caprolactone) (PCL) scaffolds can be modified by plasma functionalization. Significant increase in the scaffolds' wettability was achieved by H_2O/NH_3 and H_2O/N_2 plasma treatments. XPS analysis corroborated changes in the surface chemistry throughout the 3D structure, with no significant changes in scaffold morphology after plasma treatments (SEM analysis). In addition, enhanced osteoblast proliferation was observed in PCL scaffolds after plasma surface modification. These results support plasma surface modification as a viable technique to improve the surface properties of 3D materials to promote cell growth and ultimately aid in tissue engineering. In addition to these results, cell proliferation and the antimicrobial behavior of S-nitrated PLGH-cysteamine polymer after the incorporation of silver nanoparticles and plasma surface modification will be discussed.

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