Tuesday Afternoon, October 20, 2015

Biomaterial Interfaces
Room: 211D - Session BI-TuA

Cells and Microorganisms at Surfaces
Moderator: Axel Rosenhahn, Ruhr-University Bochum, Markus Valtiner, Max-Planck Institut für Eisenforschung GmbH

2:20pm **BI-TuA1 Control of Surface Physical Properties for Effectively Promoting and Maintaining Cell Clusters such as Stem Cell Colonies at Interfaces**, ***YingChih Chang***, *P.Y. Yeh*, Academia Sinica, Taiwan, Republic of China

A series of biomimetic polypeptide layer-by-layer conjugated supported lipid bilayers as lubricated thin films were constructed and characterized for their physical properties under cell-surface contact. The construct was used to promote the selection and maintenance of stem/progenitor cell colonies from the primary culture in one example, and to isolate circulating epithelial cells from human peripheral blood in another example. The adsorption of serum proteins, and nonspecifically bound cells are clearly reduced when a lipid coating was employed as the underneath layer, as studied by quartz-crystal microbalance with dissipation, and immunohistochemistry.

2:40pm **BI-TuA2 Immobilized Liquid Layers for Controlled Bacterial, Fungal, and Mammalian Cell Attachment**, ***Caitlin Howell***, *N. Juthani, N. MacCallum, Y. Kovalenko, S. Kelso, J. Lin, C. Nemr, P. Kim, J. Aizenberg*, Harvard University

Immobilized liquid layers, inspired by the *Nepenthes* pitcher plant, are emerging as a powerful new approach to the control of cellular attachment to surfaces. These layers present a "moving target" for the adhesion of fouling organisms and have shown promise as biofilm-resistant coatings. Tests on clinically-relevant bacteria and fungi such as *E. coli*, *S. aureus*, *S. epidermidis*, *P. aeruginosa*, and *C. albicans*, have shown significantly decreased adhesion without toxic effects. Immobilized liquid layers also show promise as tunable platforms for the attachment and detachment of mammalian cells, opening new directions in the area of tissue engineering. Finally, these surfaces can be made to be continuously self-replenishing through the incorporation of a bio-inspired vascular system, extending their longevity. We anticipate that these layers will prove a unique and adaptable platform for controlling the attachment of cells on surfaces.

3:00pm **BI-TuA3 Quantitative Characterization of Bacterial Cells Mixed with Nanoparticles**, *P.M. Martins, A.R. Silva,* University of Minho, Portugal, *I.M. Pinto, C. Sousa,* International Iberian Nanotechnology Laboratory, Portugal, *S. Lanceros-Méndez,* University of Minho, Portugal, ***Dmitri Petrovykh***, International Iberian Nanotechnology Laboratory, Portugal

The unique physicochemical properties of nanoparticles (NPs) are the basis for their potential applications in nanomedicine and biomedical research, whereby NPs interacting with cells provide a means for detecting, monitoring, or controlling cell functions via non-biological (magnetic, electronic, optical, mechanical) properties of NPs. When considering interactions of NPs with bacterial cells, the single-digit micrometer or even submicron sizes of typical bacteria have to be taken into account, in addition to their biological properties. For mixed suspensions of bacteria and NPs, therefore, both biological and physicochemical properties are involved in creating the corresponding micrometer- and nanometer-scale biointerfaces. While some methods are available for characterizing the biological properties of bacteria in suspension, reliable characterization of their physicochemical properties remains challenging, even for basic parameters, such as size distribution and concentration. *Staphyloccus aureus* bacteria are a convenient model system for developing and validating such physicochemical analysis of bacterial cells, due to their robust viability and nearly-spherical shapes with diameters of approximately 1 micrometer. We will describe the use of multiple complementary techniques, including flow cytometry, high-resolution microscopy, and optical spectroscopy, scattering, and absorption, for quantitative characterization of *S. aureus* suspensions and for extending these methods to investigations of NP-bacteria interactions.

3:20pm **BI-TuA4 Tethered Antimicrobial Peptide WLBU2 for Capture of Circulating Bacteria and Endotoxin in Sepsis**, ***Ramya Raman***, *K.F. Schilke*, Oregon State University

Severe sepsis is a blood infection that affects over 750,000 people each year in the US alone, killing 28-50% (more than prostate cancer, breast cancer and AIDS combined). Symptoms result from a highly dysregulated immune response, which, if untreated, can lead to multiple organ failure and death. Currently, treatment uses wide-spectrum antibiotics, but this is hindered by the rise of antibiotic-resistant 'superbugs'. One potential novel treatment is a high-throughput microfluidic hemoperfusion device, which specifically removes circulating bacteria and cell wall fragments ("endotoxin") from blood. A device with a biocompatible and bioactive surface coating could selectively bind circulating bacteria and endotoxins from blood, enabling rapid, safe treatment of bacterial sepsis. WLBU2 is an α-helical, cationic amphiphilic peptide (CAP) with 13 positively-charged arginine and 11 hydrophobic tryptophan/valine residues oriented on opposite faces of the helix. WLBU2 has high anti-microbial potency against a variety of pathogens, and integrates into bacterial cell membranes (Deslouches, et al. J. Antimicrob. Chemother. 2007; 60: 669-672). Biocompatible, non-fouling surfaces can be made by covalently tethering a dense brush of polyethylene oxide (PEO) polymer chains at the surface. Longer PEO tethers terminated with WLBU2 should enable increased mobility and solvent accessibility to tethered WLBU2, allowing it to bind bacterial cells, without compromising the biocompatibility of the coated surface. Poly-L-arginine and poly-L-Lysine served as controls for charge effects, and Cys-WLBU2 served as a tether-free control. The surface chemistry is consistent with peptide immobilization at the surface using X-ray Photoelectron Spectroscopy (XPS). Atomic Force Microscopy (AFM) images demonstrated the uniform coverage of gold surfaces with PEO and peptides. Scanning Electron Microscopy (SEM) and Quartz Crystal Microbalance with Dissipation (QCMD) were used to demonstrate capture of bacteria at the coated surfaces. Tethered WLBU2 may more effectively bind P. aeruginosa than surface-bound WLBU2. Future work will focus on optimization of the coating to enable high loading of tethered bioactive molecules, without compromising surface biocompatibility. We are also developing a novel surface coating platform, using self-assembly and immobilization of PEO-based surfactants. This method shows promise in providing biocompatibility and biological function to a variety of polymers used in medical devices, without requiring expensive and toxic crosslinking reagents.

4:20pm **BI-TuA7 Concentration Dependent Acceleration of hMSC Differentiation on Orthogonal Concentration Gradients of RGD and BMP-2 Peptides**, ***Matthew Becker***, The University of Akron **INVITED**

Self-assembled monolayer substrates containing tethered orthogonal concentration profiles of GRGDS and BMP-2 peptides are shown to synergistically accelerate the proliferation and osteoblastic differentiation of human mesenchymal stem cell (hMSC) populations *in vitro* without the use of osteogenic additives. Concurrently, the single peptide gradient controls (RGD or BMP-2 only) were found to induce significantly different proliferation and differentiation behavior from the orthogonal substrates. hMSC cells were individually isolated for qPCR at specified points along the gradients using laser capture microdissection. Bone sialoprotein (BSP) and Runt-related transcription factor 2 (Runx2) qPCR data corresponded spatially and temporally to protein marker data obtained from immunofluorescent imaging tracking the differentiation process. Genomic and protein data at high concentrations of both BMP-2 (25 pmol/cm^2) and GRGDS (71~83 pmol/cm^2) were shown to have a cooperative acceleration on the hMSC differentiation timeline relative to the individual peptide concentrations. These data highlight the utility of the orthogonal gradient approach to help identify the synergistic concentrations of peptides and growth factors that can be advanced in translationally relevant systems.

5:00pm **BI-TuA9 How Does Plasma Surface Modification Affect Biological Responses?**, ***Adoracion Pegalajar-Jurado***, *M.J. Hawker, M.N. Mann, E.R. Fisher*, Colorado State University

Biofouling causes severe and costly problems in industries including, but not limited to water filtration, food packaging and preservation, marine operations and biomedical devices. Depending on the industrial context, the term biofouling assumes different meanings including bacterial attachment and biofilm formation, undesired protein adsorption, or prevention of cell growth and tissue regeneration. Nevertheless, the process commences with undesired interactions of biological agents with the material surface.Consequently, the ability to tune surface properties to tailor biological response highlights an exceptional route towards preventing issues associated with biofouling. Although surface micro- and nanotopography, surface free energy, and surface chemistry are known to affect biological agent-surface interactions, this presentation will focus specifically on the effects of surface chemical modifications of 3D constructs (i.e. drug delivery systems and polymeric membranes and scaffolds) on biological responses. Among others, plasma surface modification offers a tunable and versatile parameter space for tailored and

reproducible surface modification while retaining the morphology of the material, to produce *bio-nonreactive materials* (limit bacterial and cell attachment and low cytotoxicity). On 2D substrates, plasma polymerized cineole films have demonstrated limited *Escherichia coli* (*E. coli*) attachment over 18 hours and non-cytotoxic to mammalian fibroblast .[1] Herein, such films were used to conformally encapsulate 3D constructs. Results from both *E. coli* attachment studies as well as cytotoxicity studies will be presented. Alternatively, allylamine/allyl alcohol plasma copolymerized films applied to 3D materials and water plasma treated nitric-oxide releasing materials will be included as *bio-reactive* materials. In this case, human dermal fibroblast attachment and growth was enhanced in comparison to unmodified materials. Through these model systems, we will explore the use of plasma surface modification to minimise fouling and/or enhance biocompatibility of a 3D material resulting in the extension of device lifetime, and enhancement of performance.

Keywords: plasma surface modification, bio-reactive, bio-nonreactive

[1] Pegalajar-Jurado, A., Easton, C. D., Styan, K. E., McArthur, S. L., Journal of Materials Chemistry B, 2, no. 31 (2014): 4993-5002.

5:20pm **BI-TuA10 Stereoscopic Tracking Reveals Responses of Barnacle Larvae to Surface Cues**, *S. Maleschlijski, G.H. Sendra, S. Bauer,* Karlsruhe Institute of Technology (KIT), Germany, *A. Di Fino,* Newcastle University, UK, *L. Leal-Taixe,* Leibnitz University Hannover, Germany, *T. Ederth,* Linköping University, Sweden, *N. Aldred,* Newcastle University, UK, *B. Liedberg,* NTU Singapore, *A.S. Clare,* Newcastle University, UK, *B. Rosenhahn,* Leibnitz University Hannover, Germany, ***Axel Rosenhahn***, Ruhr-University Bochum, Germany

The critical step in surface colonization by marine biofouling organisms is surface exploration and settlement of the sessile stages (larvae, spores). Barnacles are one of the most important biofouling organism and surface selection of their larvae is a highly selective process [1]. 3D stereoscopic tracking enables quantitative analysis of the pre-settlement behavior and thus to understand how larvae respond to chemical and physical surface cues. We developed a transportable, submersible stereoscopic system which can be applied to record three dimensional video data and to extract swimming trajectories of multiple, label-free objects. The pre-settlement ritual can be classified in different motion patterns which vary in characteristic parameters, such as distance to the surface, velocity, or the curvature of the motion [2]. In general the larvae favor both, liquid-solid and liquid-air interfaces. The distribution within the water column and the fraction of larvae exploring the solid surface is determined by its chemistry. Using different self assembled monolayers we found a positive correlation of the settlement probability with both, the fraction of larvae exploring the interface and their mean swimming velocity [3]. Thus, 3D tracking provides a predictor for settlement probability. A combination of stereoscopic tracking with imaging surface plasmon resonance reveals that a temporary adhesive is an important ingredient in the mechanosensing process. Surfaces with high settlement probability and low swimming speeds tend to bind the adhesive stronger than the sensory setae while inert surfaces with low settlement probability and high swimming speeds interact only very weakly.

[1] N. Aldred, A.S. Clare, Biofouling 2008, 24, 351–363.

[2] S. Maleschlijski, S. Bauer, A. DiFino, H. Sendra, A.S. Clare, A. Rosenhahn, J. Roy. Soc. Interf. 2014, 12(102), 20141104

[3] S. Maleschlijski, S. Bauer, A. Di Fino, G.H. Sendra, A.S. Clare, A. Rosenhahn, Biofouling 2014, 30(9), 1055

5:40pm **BI-TuA11 New Materials Toolboxes for Tissue Engineering and Regenerative Medicine Applications**, ***Adam Celiz****, Harvard University, *J. Smith,* University of Nottingham, UK, *A. Patel, R. Langer, D. Anderson,* Massachusetts Institute of Technology, *D. Mooney,* Harvard University, *L. Young, M. Davies, C. Denning, 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 in xeno-free, defined culture systems. For example, a heart attack can cause a 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, materials scientists have been challenged to discover new synthetic biomaterials as xeno-free growth substrates.[1] We apply a high throughput materials discovery approach to identify a novel polymer for hPSC culture using microarray screening of an unprecedented chemical space (141 monomers, polymerized alone and mixed to form 909 unique polymers, tested in 4356 individual assays). This identified the first synthetic polymeric substrate that achieves both pluripotent hPSC expansion (in the commercially available defined culture media, StemPro and mTeSR1) and subsequent multi-lineage differentiation into representatives of the three germ layers, namely cardiomyocytes, hepatocyte-like cells and neural progenitors.[2] Surface analysis techniques such as ToF-SIMS and XPS were used to identify chemical moieties at the biomaterial interface that contributed to maintaining hPSC pluripotency. The identification of these controlling surface moieties was essential in the development of a facile scale up procedure from arrayed spots to coated cultureware that can be used *off-the-shelf*.

An alternative strategy for cell and tissue regeneration is to harness the natural regenerative capacity of the human body through activation of quiescent cell populations. Biomaterials such as hydrogels that mimic native extracellular matrix can be synthesized and implanted *in vivo* to present biophysical and biochemical cues to their surroundings and activate/traffic these cell populations towards a desired therapeutic effect.[3] Novel bioactive hydrogels, synthesized through bioorthogonal click chemistry methods, will be presented that can activate and regulate quiescent cell populations to aid regeneration of lost tissue after trauma or injury. The utility of these new materials will be demonstrated through muscle regeneration in a hind limb ischemia mouse model.

1. A. D. Celiz *et al.* Nat. Mater. (2014) *13*, 570.

2. A. D. Celiz *et al.* Adv. Mater. ***in press*** adma.201501351R1.

3. C. A. Cezar *et al.* Adv. Drug Deliv. Rev. (2014) http://dx.doi.org/10.1016/j.addr.2014.09.008.

*** BID Early Career Researchers Award**

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