# Thursday Morning, December 15, 2022

## Biomaterial Surfaces & Interfaces

## Room Naupaka Salon 4 - Session BI-ThM1

## Bacteria Biomaterial Interactions

**Moderator: Sally M. McArthur**, Swinburne University of Technology, Australia

8:00am **BI-ThM1-1 Why Mechanism Matters for Antimicrobial Biomaterials**, ***Bryan Coad,*** The University of Adelaide, Australia **INVITED**

Many new antimicrobial biomaterials and coatings are being developed to address the need for implantable medical devices that prevent infections. Researchers, medical device manufacturers, and regulatory bodies are all interested in seeing "promising research results" translated to clinical use, but how are these claims evaluated? What experimental evidence is needed to improve translation of *in vitro* antimicrobial materials to the clinic?

It is illustrative to explore one of the most interesting classes of antimicrobial biomaterials: those which purport to have a contact-inhibition mechanism of action. These materials present covalently attached antimicrobial molecules from surfaces which inhibit or kill adhering microbes on contact. On the one hand, this surface design could open up new paradigms for antimicrobial therapy by virtue of making implants with potentially long-lasting antimicrobial activity. On the other hand, questions about whether these materials release antimicrobials and whether their surface activity would be nullified by protein fouling deserve serious investigation.

This presentation will delve into these issues by explaining why mechanism matters for antimicrobial biomaterials. It can also be viewed generally by biomaterials researchers from other fields who design bioactive surfaces using covalent surface immobilization and face questions about how to interpret the results of cellular assays. The presentation aims to raise awareness of the potential role of confirmation bias in antimicrobial susceptibility experiments which could lead researchers to unwittingly misinterpret antimicrobial mechanisms of action. It discusses ways to avoid this pitfall by proposing a methodological approach that emphasizes the importance of surface analysis. It is hoped that greater awareness of these issues will help "promising" *in vitro* antimicrobial surface technologies to have greater uptake in animal studies and clinical trials.

8:40am **BI-ThM1-3 High Throughput Screening for Antibiotics Using Droplet Microarrays**, *W. Lei, A. Popova,* Karlsruhe Institute of Technology (KIT), Germany; ***Michael Grunze,*** Max Planck Institute for Medical Research, Germany; *P. Levkin,* Karlsruhe Institute of Technology (KIT), Germany

Multidrug-resistant (MDR) bacteria are a severe threat to public health and it is urgent to identify novel antibacterial compounds or pathogen-specific mixtures of antibiotics. In a first paper we reported the application of Droplet Microarrays developed by *Aquarray* as a cost effective high-throughput screening method for the evaluation of drug resistance of *Pseudomonas aeruginosa*, an opportunistic human pathogen /1/. The DMA consists of an array of hydrophilic spots divided by superhydrophobic borders to generate arrays of hundreds of nanoliter-sized droplets containing bacteria and different drugs for screening applications. A novel simple colorimetric readout method compatible with the nanoliter size of the droplets was established. Furthermore, the drug-resistance of *Pseudomonas aeruginosa* 49, a multi-resistant strain from an environmental isolate was investigated by screening of a small library containing 18 antibiotics. We were able to show that our methology reproduces the data obtained with a 96 well microplate.

In the study reported here the search for antibiotic compounds was extended to screen over 2000 compounds for their antimicrobial properties against carbapenem-resistant *Klebsiella pneumoniae* and and methicillin resistant *Staphylococcus aureus* (MRSA). A fast single-step detection method measured the inhibitory effect of the compounds on bacterial growth on the whole array. Six hit compounds, including coumarins and structurally simplified estrogen analogs are identified in the primary screening and validated with minimum inhibition concentration assay for their antibacterial effect. This study demonstrates that the DMA-based high-throughput screening system identifies potential antibiotics from novel synthetic compound libraries, and thus offering opportunities for development of new treatments against multidrug-resistant bacteria.Due to its simplicity, the method is suitable for rapid screens in personalized medicine. If time allows, further examples of rapid screens using DMA´s which habe been designed for personalized medicine will be presented.

/1/ W. Lei, K. Demir, J. Overhage, M. Grunze, T. Schwartz, P. A. Levkin, Droplet-Microarray: Miniaturized Platform for High-Throughput Screening of Antimicrobial Compounds, Adv. Biosys., 2000073 (1-9), 2020

9:00am **BI-ThM1-4 NAP-XPS Studies of a *Pseudomonas fluorescens* Bacterial Cell-Envelope and Other Biomaterial Surfaces**, ***Paul Dietrich,*** SPECS Surface Nano Analysis GmbH, Germany; *N. Wasio, J. Hilton,* SPECS-TII, Inc.; *A. Thissen,* SPECS Surface Nano Analysis GmbH, Germany

Bacterial interactions with the environment are based on processes involving their cell-envelope. Thus, techniques that can analyze their surface chemistry are attractive tools for providing an improved understanding of bacterial interactions. One of these tools is x-ray photoelectron spectroscopy (XPS) with an estimated information depth of <10 nm for Al Kα-excitation. XPS-analyses of bacteria have been performed for several decades on freeze-dried specimens to be compatible with the classical ultra-high vacuum conditions needed. A limitation of these studies has been that the freeze-drying method may collapse the cell structure. However, recent developments in XPS enable the analysis of biological samples at near ambient pressure (NAP-XPS) or as frozen hydrated specimens (cryo-XPS) in vacuum. In this talk, we present the analysis of bacterial samples from a reference strain of the Gram-negative bacterium *Pseudomonas fluorescens* using both techniques. XPS results and reference data from the bacterial strain are provided, and we propose to use planktonic cells of this strain (DSM 50090) as a reference material for surface chemical analysis of such bacterial systems. Further selected examples of NAP-XPS on other biomaterial surfaces will be presented.

9:20am **BI-ThM1-5 Tungsten Disulfide Bio-Nanofabrication Using Dissimilatory Metal-Reducing Bacteria *Shewanella oneidensis* MR-1**, ***Lauren Brady,*** *J. Rees, S. Sawyer,* Rensselaer Polytechnic Institute

A type of bacteria known as dissimilatory metal-reducing bacteria (DMRB) can "breathe metals" to reduce heavy metal ions as part of their metabolic process. *Shewanella oneidensis* MR-1 is a type of DMRB that was first discovered in Lake Oneida, New York for its ability to reduce manganese, but has since been shown to reduce a variety of different electron acceptors including Fe(III), As(V), Cr(VI), and thiosulfate. In previous literature, *S. oneidensis* MR-1 has been grown anaerobically in media enhanced with sulfur and metal ions in order to produce several types of nanoparticles, including molybdenum disulfide, zinc sulfide, and cadmium sulfide.

This work presents the bacterial nanofabrication of tungsten disulfide particles using *S. oneidensis* MR-1. Bacterial nanofabrication synthesis, where the bacteria is the main catalyst for the production of nanomaterials, has numerous advantages compared to traditional chemical synthesis methods in that it can be conducted at room temperature and requires less chemical reagents in the reaction. One of the primary reasons to produce nanomaterials using bacteria is for a low resource input alternative to material fabrication for use in electronic devices.

Tungsten disulfide ($WS_2$) is a two-dimensional transition metal dichalcogenide which has a bandgap transition from an indirect bandgap in the bulk material to a direct bandgap in its monolayer form. Tungsten disulfide has a range of potential applications including photodetection, biosensing, and chemical catalysis. These applications are further enhanced when control can be exerted over crystal growth and thickness.

An anaerobic batch culture of *S. oneidensis* MR-1 was incubated at room temperature in the presence of tungsten trioxide, resulting in the production of tungsten disulfide crystalline nanostructures of varied shapes and size. Several characterization techniques were employed to identify the material, including scanning electron microscopy, transmission electron microscopy, Raman spectroscopy, absorbance spectroscopy and X-ray diffraction. In addition to confirming that tungsten disulfide can be produced by *Shewanella* bacteria, the data collected using these methods provide insight on the size, morphology, and photoresponse of nanoparticles generated this way.

9:40am **BI-ThM1-6 Nanoengineered Implant Surfaces with Enhanced Osteogenic and Antimicrobial Properties**, *R. Shahbazian ,* University of Illinois Chicago; ***Tolou Shokuhfar,*** University of Illinois at Chicago

The lack of osseointegration and implant-related infections are two major complications leading to failure of dental and orthopedic implants. Therefore, the development of effective implant surfaces able to display enhanced osteogenic activity and antimicrobial properties is required. This work aims to study the ability of bio-functionalized TiO2 Nanotube engineered surfaces to induce osseointegration, and concomitantly, to

avoid infection. TiO2 NTs were bio-functionalized with calcium (Ca), phosphorous (P) and zinc (Zn), by reversepolarization anodization. Morphological and topographical features of NTs were observed through scanning electron microscopy (SEM), while surface chemistry was investigated by X-ray photoelectron spectroscopy (XPS). Biocompatibility studies were conducted with MG-63 and human mesenchymal stem cells (hMSCs) through MTT assay. Furthermore, cell morphology and cytoskeleton organization were observed by SEM and laser scanning confocal microscopy (LSCM). The osteoblastic differentiation capacity of hMSCs was studied by real-time PCR, as well as their angiogenesis ability by measuring the total release of vascular endothelial growth factor (VEGF). Finally, viability of Staphylococcus aureus (S. aureus) was assessed by live/dead bacterial viability assay. Results show that bio-functionalized TiO2 nanotubular surfaces are biocompatible and modulated cell morphology. In particular, NTs enriched with Ca, P, and Zn, induced to significantly up-regulated levels of bone morphogenetic protein 2 (BMP-2) and osteopontin (OPN) genes of hMSCs, when compared to conventional NTs. TiO2 nanotubular surfaces induced hMSCs to release a higher amount of VEGF, and significantly reduced the bacterial viability, both when compared to adequate Ti controls. Osseointegration and antibacterial properties have been shown in vitro and in vivo to improve when implants have modified surfaces that have biomimetic nanostructures designed to mimic and interact with biological structures on the nano-scale. Pre-clinical evaluations show that TiO2 nanotubes (TNT), produced by anodization, on Ti6Al4V surfaces positively enhance the rate at which osseointegration occurs and TNT nano-texturization enhances the antibacterial properties of the implant surface. In conclusion, the superimposition of TiO2 nanotubular- textured surfaces and their enrichment with Ca, P, and Zn, is a highly promising approach for the development of novel bio-selective and multifunctional implant surfaces able to improve osseointegration and avoid infection.

# Author Index

## Bold page numbers indicate presenter