

Biomaterial Interfaces Division

Room A120-121 - Session BI+AS-TuM

Characterization of Biological and Biomaterial Surfaces

Moderators: Karyn Jarvis, Swinburne University of Technology, Sally McArthur, Swinburne University of Technology

8:00am BI+AS-TuM1 Characterizing Protein Fiber Structures in Solution with Vibrational Sum-Frequency Scattering Spectroscopy, *David G. Castner, P.K. Johansson*, University of Washington

Sum frequency generation vibrational spectroscopy has been developed as a powerful technique for investigating the structure of proteins at flat liquid-solution interfaces. However, many biological processes are regulated by interactions at the interface of 3D structures. Collagen forms large fibers that are responsible for the structural integrity of tissues. The structure, organization and interactions of these fibers are important for the survival, communication, migration, and proliferation of cells. Investigating protein fiber interactions is challenging, particularly under biological conditions. However, vibrational sum-frequency scattering (SFS) spectroscopy, with inherent contrast for local molecular ordering, can be utilized towards these important goals. We have applied SFS to collagen type I fiber networks self-assembled in aqueous environments. Signals were detected from the amide I band stretching vibrations (associated with the protein backbone structure) and signals from the C-H stretching and bending vibrations (associated with the protein side-chains). The angular scattering patterns for the backbone (amide I) and side-chain (C-H stretches and bends) signals are different, making the spectra dependent on the angle of detection. For randomly oriented fiber networks the amide I chiral signals are dominant in the phase-matched direction. In contrast, amide I achiral and chiral signal strengths are comparable at scattering angles above 6°. The backbone signal intensity decreases significantly as the scattering angle increases. In contrast, the side-chain signals remain high at large scattering angles. Distinctions in the organizational symmetry and the relative fiber surface contribution to the overall signal are probable reasons for these observations. The amide I band the spectra acquired at a scattering angle of 22 degrees provided good specificity to the surface region of the collagen fibers. This surface sensitivity was used to investigate how a dilute sodium dodecyl sulfate surfactant solution affects the spectra and scattering patterns of the SFS signals. The amide I SFS polarization ratios at a scattering angle of 22° provided insights to early changes to the collagen fiber structure. This shows the promise of SFS as an important technique for providing detailed information about the surface structure and chemistry of protein fibers, complementary to what can be obtained from other techniques such as SHG imaging or IR spectroscopy. Thus, SFS can provide a molecular level understanding of the changes to collagenous tissues during decellularization and help optimize the protocols for tissue engineered organs.

8:20am BI+AS-TuM2 Near-Ambient Pressure XPS Surface Characterisation of Bacteria and Biofilms - Model Systems and Sample Preparation, *Marit Kjaervik*, Bundesanstalt für Materialforschung und -prüfung, Germany; *P. Dietrich, A. Thissen*, SPECS Surface Nano Analysis GmbH, Germany; *K. Schwibbert, W.E.S. Unger*, Bundesanstalt für Materialforschung und -prüfung, Germany

Bacterial samples are typically freeze dried or cryo-prepared prior to XPS analysis to allow for measurements in ultra-high vacuum (UHV). The sample environment in the near-ambient pressure (NAP) XPS instrument EnviroESCA allows for measurements in up to 15 mbar water vapor, thus, sample preparation is no longer restricted to UHV-compatible techniques.[1] For instance, biofilms grown in medium can be transferred directly from the medium to the measurements chamber, maintaining a humid environment throughout the measurements.[2] Considering the complexity of bacterial samples, sample preparation must be carefully considered in order to obtain meaningful and reproducible results.

In this talk, various strategies for sample preparation of bacteria and biofilms for NAP-XPS measurements will be discussed. Model systems of planktonic bacteria, artificial biofilms resembling the exopolysaccharide matrix and biofilms have been characterised in various conditions. The stability and homogeneity of the samples were assessed by monitoring the C1s core-level peak at different sample locations. The quality of the XP spectra is also influenced by the gas environment, which will be exemplified by core level spectra of *P. Fluorescens* acquired in air, water vapor and ultra-high vacuum.

Furthermore initial results from iodine doped model biofilms will be presented. The in-depth chemical composition profile of these model films was obtained using an argon gas cluster ion gun.

Acknowledgements

This project has received funding from the EMPIR programme co-financed by the Participating States and from the European Union's Horizon 2020 research and innovation programme.

[1] P. M. Dietrich, S. Bahr, T. Yamamoto, M. Meyer, and A. Thissen, "Chemical surface analysis on materials and devices under functional conditions – Environmental photoelectron spectroscopy as non-destructive tool for routine characterization," *J. Electron Spectros. Relat. Phenomena*, vol. 231, pp. 118–126, Feb. 2019.

[2] M. Kjaervik, K. Schwibbert, P. Dietrich, A. Thissen, and W. E. S. Unger, "Surface characterisation of *Escherichia coli* under various conditions by near-ambient pressure XPS," *Surf. Interface Anal.*, vol. 50, no. 11, pp. 996–1000, Nov. 2018.

8:40am BI+AS-TuM3 ToF-SIMS Imaging of Plant seed Interactions with Plant-growth Promoting Bacteria, *Yuchen Zhang, X.-Y. Yu*, Pacific Northwest National Laboratory

Presentation Summary:

This presentation aims to show that we have successfully used delayed image extraction in time-of-flight secondary ion mass spectrometry (ToF-SIMS) to study the interaction between Brachypodium seed and plant growth-promoting bacteria (PGPB) for the first time.

Abstract

The use of time-of-flight secondary ion mass spectrometry (ToF-SIMS) is a relatively unexplored in plant biology that is undergoing fast development [1]. The majority of existing research in plant biology that has utilized ToF-SIMS mostly involves the study of plant stems and leaves, and only a handful of studies apply it on the analysis of plant roots and/or seeds. Additionally, the use of Brachypodium distachyon (Brachypodium) as a model system for research has become more prominent in plant biology. Brachypodium, a C3 model, can provide more insight into the biological studies of other species including small grain crops such as wheat and barley [2], therefore it has a significant scientific impact in plant biology. To the best of our knowledge, this will be the first systematic ToF-SIMS imaging of Brachypodium. In this work, we obtained chemical mapping of the interaction of grains of Brachypodium with plant growth-promoting bacteria (PGPB) [3], namely, *Pseudomonas* and *Arthrobacter*, using ToF-SIMS. Specifically, the use of the delayed image extraction mode in ToF-SIMS provides chemical speciation of the Brachypodium seed surface and simultaneously captures the morphological features of the plant-bacteria interface. Our findings provide high resolution spatial distributions of fatty acids (e.g., palmitic acid, stearic acid, and arachidic acid) and phospholipid (e.g., cardiolipin) present on the Brachypodium seed surface. Spectral PCA results indicate that the biofilm and planktonic cells both have effects on the seed surfaces. In terms of seedling potentials, the seed brush is the most active after PGPB attachment on the biointerface.

Key words: ToF-SIMS, delayed image extraction, PGPB, Brachypodium, *Pseudomonas*, *Arthrobacter*

References:

1. Boughton, B.A., et al., Mass spectrometry imaging for plant biology: a review. *Phytochem Rev*, 2016. 15: p. 445-488.
2. Delaplace, P., et al., Influence of rhizobacterial volatiles on the root system architecture and the production and allocation of biomass in the model grass *Brachypodium distachyon* (L.) P. Beauv. *BMC Plant Biol*, 2015. 15: p. 195.
3. Scholthof, K.B.G., et al., Brachypodium: A Monocot Grass Model Genus for Plant Biology. *Plant Cell*, 2018. 30(8): p. 1673-1694.

9:00am BI+AS-TuM4 Visualization of Signaling Molecules in Brain Tissue by Multimodal Imaging with Matrix Assisted Laser Desorption/Ionization Mass Spectrometry and Time-of-Flight Secondary Ion Mass Spectrometry, *Matthias Lorenz, S.T. King, N. Borodinov, C.A. Steed, J. Chae, A.V. Ievlev, O.S. Ovchinnikova*, Oak Ridge National Laboratory

Matrix Assisted Laser Desorption/Ionization (MALDI) is commonly used for the chemical imaging of biological tissue samples with mass spectrometry due to its capability to desorb and ionize large organic molecules with limited fragmentation, thus preserving a high degree of molecular information. MALDI is suitable to analyze species such as peptides and proteins, and the intact molecular ion is observable in many cases.[1] The

achievable spatial resolution using MALDI mass spectrometry imaging (MSI) is limited to about 30 μm using standard matrix compounds, primarily due to the dimensions of matrix crystals and the stability of the matrix coating.[2] Time-of-Flight (ToF) Secondary Ion Mass Spectrometry (SIMS) is another mass spectrometry based chemical imaging technique that can achieve a spatial resolution below 100 nm.[3] The chemical information obtained from ToF-SIMS analyses is, however, limited to smaller organic molecules and elemental species due to a more significant fragmentation of intramolecular bonds and decreasing ion yields with increasing molecular weight. We present here a workflow comprising the consecutive application of ToF-SIMS and MALDI-ToF-MS MSI to combine the strength of both chemical imaging techniques. Even though mass spectrometry based surface analysis techniques are inherently destructive in nature, the volumes of sample material that the two imaging techniques extract at each sampling location differs significantly ($\sim 30 \mu\text{m}$ vs. $\sim 100 \text{ nm}$ craters). This difference enables the assumption of a non-destructive nature for the ToF-SIMS imaging cycle relative to the MALDI sampling volume and spatial resolution, leaving a virtually pristine sample surface for a subsequent MALDI imaging cycle of the same sample area. We showcase the application of our workflow for the multimodal imaging of a coronal mouse brain tissue section, with automated co-registration of the two imaging data sets. We demonstrate how the MALDI mass spectral data enable to complement the high spatial resolution ToF-SIMS MSI data set with an additional degree of molecular structural information and discuss our workflow based on the visualization of signaling molecules in the mouse brain tissue.

[1] Todd, P.J.; Schaaff, T.G.; Chaurand, P.; Caprioli, R.M. *J. Mass Spectrom.* **2001**, *36*, 355–369.

[2] Yang, J.; Norris, J.L.; Caprioli, R. *J. Mass Spectrom.* **2018**, *53*, 1005–1012.

[3] Kollmer, F.; Paul, W.; Krehl, M.; Niehuis, E. *Surf. Interface Anal.* **2013**, *45*, 312–314.

9:20am BI+AS-TuM5 *In situ* Observation of Triacylglycerol (C39:0) and Acylceramide (C17) Colocalization in Lipid Droplets of Apoptotic Cells using ToF-SIMS, Shohini Sen-Britain, N. Li, G.E. Atilla-Gokcumen, J.A. Gardella Jr., State University of New York, Buffalo

The formation of phase segregated lipid droplets storing triacylglycerol containing polyunsaturated fatty acyl chains (PUFA-TAGs) during apoptosis, or programmed cell death, has been previously observed [1,2]. Polyunsaturated fatty acids are incorporated into PUFA-TAGs by diacylglycerol acyltransferases (DGATs) [1]. The acylation of ceramide by DGATs also produces acylceramide which has been found in lipid droplets as well [3]. The accumulation of ceramide and PUFA phospholipids sensitizes cells to cell death. Therefore, acylation of these molecules into phase segregated droplets is thought to have a protective effect [1,2].

Previous studies observing these acylated molecules in lipid droplets have utilized LC-MS of fractionated lipid droplets [1-3]. However, colocalization of these lipids has not been observed *in situ*. Previous imaging studies have been limited by the use of nonspecific lipid dyes, such as Nile red, in observing lipid droplets.

In this study, we have utilized metal-assisted time-of-flight secondary ion mass spectrometry (ToF-SIMS) to image the colocalization of TAG (C39:0) and acylceramide (C17) in apoptotic HCT-116 human colorectal carcinoma cells. We maintained sample preparation conditions used in previous microscopy studies by cutting out squares of cell culture plates containing lyophilized apoptotic HCT-116 cells. Imaging of lipids within the cells was accomplished by milling off the top half of the cells using a focused ion beam-scanning electron microscope (FIB-SEM). Imaging of gold sputter coated samples in negative ion mode allowed for the observation of high molecular weight secondary ions ($>1000 \text{ m/z}$) and of unique spectra of both TAG (C39:0) and AC (C17). Colocalization of endogenous TAG and AC were observed in apoptotic cells. TAG and AC fragmentations were determined by analyzing (1) gold sputter coated TAG and AC standards on cell culture plates and (2) standard additions of TAG and AC onto milled lyophilized apoptotic cells that were also gold sputter coated.

The work accomplished in this study illustrates the potential of identifying the spatial localization of large biomolecules in cells on insulating, high topography containing samples through the use of standard additions and high mass resolution, metal-assisted ToF-SIMS. The results are also the first reported *in situ* observation of TAG and AC colocalization in apoptotic cells.

[1] *Biochemistry* **2018**, *57*, 72–80

[2] *ACS Chemical Biology* **2016**, *11*, 2583–2587

[3] *Cell Metabolism* **2017**, *25*, 686–697

Tuesday Morning, October 22, 2019

9:40am BI+AS-TuM6 Customizing Decellularized Biopolymer Matrices to Serve as Cell-instructive Microenvironments: A ToF-SIMS Study, Valentina Magno, M. Nitschke, R. Zimmermann, N.R. Dennison, Leibniz Institute of Polymer Research Dresden, Germany; C. Werner, Leibniz Institute of Polymer Research Dresden, Germany, Deutschland, Germany

Decellularized extracellular matrix (ECM) preparations provide highly valuable options for the *in vitro* reconstitution of tissue-specific niches. In this approach, control over the ECM composition and structural assembly can be achieved through the modulation of cell culture conditions. We have previously demonstrated that adding ascorbic acid and using macromolecular crowding (MMC) allows for tuning the ECM deposition by human mesenchymal stem cells by boosting procollagen synthesis and enhanced complexation/deposition of soluble matrix components [Prewitz *et al. Biomaterials* **73** (2015) 60]. Combining both options, we have now explored the fabrication of a large set of cell-derived ECM variants which were analyzed by time-of-flight secondary ion mass spectrometry (ToF-SIMS) and immunostaining. Principle component analysis (PCA) of the ToF-SIMS spectra and quantitative immunofluorescence data revealed distinct differences and trends in the complex ECM composition. The introduced methodology is validated by cell culture experiments using the decellularized matrix variants and concluded to provide a new level of control in tailoring matrix properties for tissue and organoid models (authors MN and VM contributed equally).

11:00am BI+AS-TuM10 Hierarchical Changes in Protein Structure: from Surface Influence to Cell Control, Sapun Parekh, University of Texas at Austin

INVITED

Protein structure, not just identity, is now appreciated as a critical variable that determines downstream biochemical reactivity. In biomaterials research, proteins are often coated onto materials to make them biocompatible; however, the structure of particular proteins on the material surface is often unknown or not taken into account, leading to inconsistent biological responses. The same protein on different biomaterial surfaces can take on distinct structures that can, for example, lead to differential receptor activation or stem cell differentiation into specific lineages. In this work, we demonstrate how both chemical and physical stimuli modulate protein structure and ultimately direct cell response. In the first part of this talk, I will show how graphene materials, with their unique physico-chemical properties and potential applications in tissue engineering, can strongly modulate fibronectin structure, cellular integrin binding, and stem cell differentiation. In the second part, I will show how physical forces on protein-based fibrin hydrogels can modulate protein structure, modifying enzymatic and integrin binding sites and drastically reducing platelet adhesion. The work presented here shows that physical and chemical properties of materials strongly influence protein structure and downstream biological responses, showing that biomaterial design should include considerations to control protein structure in addition to protein capture.

11:40am BI+AS-TuM12 The Role of Cr-N phases Prepared by Plasma Processes on 316L Stainless Steel and the Potential Use in Biocompatible Systems, Diana Galeano-Osorio, S. Vargas-Giraldo, C. Castano, Virginia Commonwealth University

The corrosion performance of chromium nitride (CrN) phases obtained by two different plasma-based techniques on 316L stainless steel was investigated by electrochemical testing in simulated body fluid, SBF. One method consisted of the surface treatment of 316L stainless steel by plasma nitriding. The other approach comprised the deposition of Cr-N thin film layers on 316L stainless steel substrates by reactive sputtering technique. The structural analysis of the Cr-N phases on the plasma nitrided samples by X-ray diffraction (XRD) showed an expanded austenite phase (S-Phase), while X-ray photoelectron spectroscopy (XPS) analysis revealed the presence of both the S-Phase and CrN. In the case of thin films, the XRD and XPS characterization predominantly showed a CrN phase. The different topographical characteristics of both approaches coupled with the surface energy characteristics and the electrochemical behavior in SBF provided valuable information for the potential use of these materials in biocompatible applications.

12:00pm BI+AS-TuM13 Direct Interspecies Electron Transfer (DIET) in Syntrophic Microbes, Cuiyun Yang, X.-Y. Yu, Pacific Northwest National Laboratory

Presentation Summary:

Tuesday Morning, October 22, 2019

This presentation will show our recent results of metabolic performance of direct interspecies electron transfer between syntrophic *Geobacter* species by using *in situ* liquid time-of-flight secondary ion mass spectrometry (ToF-SIMS).

Abstract

Direct interspecies electron transfer (DIET) is deemed important and effective for electron exchange among syntrophic *Geobacter* species. DIET facilitates coupling of carbon, nitrogen, phosphorus biogeochemical cycles in the natural anaerobic environment [1]. In this presentation, *Geobacter sulfurreducens* and *Geobacter metallireducens* were employed to investigate the metabolic behavior of a syntrophic community cultured in a SALVI microfluidic reactor and analyzed by *in situ* liquid time-of-flight secondary ion mass spectrometry (ToF-SIMS). More types of molecules that facilitate metallic-like electron conductivity pili or cellular outer-membrane cytochrome (e.g., OmcS) formation in the *Geobacter* co-culture community were observed than the planktonic cells. Characteristic peaks observed include aromatic acids m/z^+ 82 ($C_4H_6N_2^+$, histidine), 120 ($C_8H_{10}N^+$, phenylalanine), and 166 ($C_9H_{12}NO_2^+$, phenylalanine), benzene polymers m/z 93 ($C_6H_5O^+$), 94 ($C_6H_6O^+$), and 133 ($C_9H_9O^+$) in the co-cultured aggregate. The compositions of specific fatty acid also changed according to the culture condition when comparing the single population vs. co-cultured community. Abundance of water clusters were observed in this work and the water cluster differences observed among the cultured community, single population biofilms, or planktonic cells also suggest that other living activities of cells is possible, for instance, moderation of the solvation spheres when forming the aggregates due to IET and/or DIET. Alternatively, we hypothesize that proton-coupled electron transfer (PCET) may play a role in the syntrophic community besides DIET based on *in vivo* spectral comparisons. Our *in situ* molecular imaging results lead to the following conclusions: 1) interspecies electron transfer in co-cultured planktonic states may be mainly mediated by reduced molecular hydrogen; and 2) DIET in co-cultured aggregates functions via direct contact or microbial nanowire. Our findings improve the understanding of the electron transfer in syntrophic communities based on *in vivo* molecular imaging.

Key words: direct interspecies electron transfer (DIET), interspecies electron transfer, *Geobacter sulfurreducens*, *Geobacter metallireducens*, *in situ* liquid TOF-SIMS

References:

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