Monday Afternoon, November 6, 2023

Biomaterial Interfaces Division
Room B117-119 - Session BI1-MoA

SIMS and Orbi-SIMS Characterization of Biological and Biomaterials Surfaces

Moderators: Axel Rosenhahn, Ruhr-University Bochum, **Markus Valtiner**, Vienna University of Technology, Austria

1:40pm BI1-MoA-1 Mixing Things Up to Reduce Mix Ups in Lipid and Fatty Acid Analysis, *Daniel Graham, H. Lei, L. Gamble,* University of Washington

Each ToF-SIMS spectrum can contain a combination of molecular species, fragments of these species, rearranged fragments, cluster ions (combinations of molecules and atoms) and atomic species. The complexity of the spectral information increases with the complexity of the surface being analyzed. This is particularly true when analyzing cells and tissues. Each of these systems contain a rich mixture of molecules including proteins, sugars, lipids and fatty acids. The lipids are typically arranged in well-ordered layers that can contain a wide variety of lipid molecules. While ToF-SIMS has been shown to provide detailed chemical information from the lipids and fatty acids from cells and tissues, this information is complex and can be difficult to uniquely interpret. Recently, it has been shown that the fragmentation pattern in ToF-SIMS spectra contains similar information to an MS/MS experiment and that this information can be used to uniquely identify lipids without doing MS/MS.[1] However, additional work needs to be done to better understand which fragments will show up and whether the relative intensity of these fragments might also encode information about the mixture of molecules which are present.

In order to better understand these complex systems we have taken a reductive approach and started by looking at mixtures of fatty acids. Fatty acids make up a large part of lipids and generate unique signals within ToF-SIMS spectra. This presentation will focus on our work looking at binary mixtures of fatty acids with ToF-SIMS. ToF-SIMS spectra were compared with simulated mixture spectra generated using a custom built graphical user interface (GUI) in Matlab. This GUI allows the user to create spectra of mixtures of two molecules based on the peak intensities of pure component spectra from each chosen fatty acid. Peak areas from the simulated spectra were compared with peak areas from experimental data. It was found that the experimental data deviated from the expected intensities of the simulated spectra. These deviations provide insight into mechanisms that enhance or reduce the yield of certain fatty acid peaks in the mixtures. Insights from these studies will be used to look at increasingly complex surfaces simulating mixtures seen in cells and tissues.

1 T.B. Angerer, D. Velickovic, C.D. Nicora, J.E. Kyle, D.J. Graham, C. Anderton, and L.J. Gamble, *Anal Chem*. **91**, no. 23, pp. 15073–15080, (2019).

2:00pm BI1-MoA-2 Native State Physicochemical Characterisation of Drug Delivery Hydrogels using Cryo-OrbiSIMS and SEM, *Julie Watts, D. Scurr,* University of Nottingham, UK

Supramolecular hydrogel formulations have the potential to increase topical delivery of active agents and are well suited being biocompatible, with facile gel formation from cationic surfactant bis-imidazolium salts and combination with anionic, cationic or neutral drugs [Limón et. al., Eur J Pharm Biopharm, 2015]. Although the potential of hydrogels for improved topical skin permeation analysis has been demonstrated using time of flight secondary ion mass spectrometry (ToF-SIMS) [Starr et. al., Int. J. Pharm, 2019] the chemistry of the systems themselves have not been chemically characterised in their native state. This is primarily due to ion beam induced fragmentation and limitations of mass resolving power, as well as the obscuring of the spectra of frozen hydrated samples with water fragment ions.

In this work we investigate the application of cryo-OrbiSIMS in the molecular characterisation of supramolecular hydrogels loaded with two different porphyrins (0.1% w/v). Skin permeation studies were performed to evaluate the delivery of 5,10,15,20-Tetrakis(4-hydroxyphenyl)porphyrin (TPPOH) and 5,10,15,20-Tetrakis(4-carboxylatephenyl)porphyrin (TCPP). It was observed that in *ex vivo* porcine skin permeation studies the TPPOH appeared to have permeated the skin whereas the TCPP had not. Gel monomer skin permeation was below detectable levels in all cases. In order to understand this difference in delivery, cryo-OrbiSIMS and SEM were performed to determine if there were any variations in the physicochemical properties of the gels.

In native state gels as well as those loaded with porphyrin, the cryo-OrbiSIMS spectra show the detection of a range of secondary ions attributable to the gel, [M-H]+at m/z 901, TPPOH[M-4H]+ at m/z 677, and TCPP [M-4Na]- at m/z 788. Ions detected include molecular and fragments ions.The data suggests that the chemistry of the supramolecular gel is confirmed and that the porphyrins have been successfully loaded into the gels and are uniformly distributed. Using a controlled sample sublimation approach to expose the fibrous microstructure of the frozen hydrated gels, cryo-SEM images indicate structural differences between gels with and without porphyrins, with longer, more interconnected fibres present in gels systems without porphyrins. However, the two porphyrin containing systems are comparable, as such the release behaviour is proposed to relate to a difference in their affinity to the gel fibres.

2:20pm BI1-MoA-3 Molecular Characterization of Cells and Bio-interfaces using SIMS: The Foreign Body Reaction, *Morgan Alexander,* The University of Nottingham, UK **INVITED**

New biomaterials are necessary to tackle the challenges of medical device centred infection combined with antimicrobial resistance and the foreign body reaction (FBR). Together, these cause unacceptably high rates of device failure, rejection, mortality and morbidity.

Novel polymers have been discovered which reduce bacterial biofilm formation, infection, and control host immune response.[1,2,3] To understand their mode of action and improve these cell-instructive biomaterials requires detailed characterisation of the biointerface that plays a central role in their achieving homeostasis. Recently lipids have been proposed as critical in controlling FBR using ToF SIMS data;[4] this finding is intriguing since it offers an alternative to the prevalent protein adsorption paradigm.

Whilst ToF SIMS is excellent for imaging metabolites present at sufficient abundance, it struggles in identifying endogenous species in complex biological systems due to its relatively poor mass resolving power when faced with myriad possible peak assignments for each secondary ion peak.[5] The 3D OrbiSIMS approach addresses that by combining an OrbiTrap with a time-of-flight SIMS instrument to undertake direct analysis of solid samples.[6] The 3D OrbiSIMS has been able to undertake single cell metabolomics for primary macrophages[7] which are orchestrate the body's response to implanted medical devices. This has been used to help interpret the complex spectra acquired from the tissue interface with novel implanted novel biomaterials.[8] The outlook for this approach in medical device characterisation, and more widely using unbiased assignment procedures for SIMS will be discussed.[9]

1. Immune-Instructive Polymers Control Macrophage Phenotype and Modulate the Foreign Body Response In Vivo **Matter (Cell Press)** Rostam2020
2. Combinatorial hydrogel library enables identification of materials that mitigate the foreign body response in primates **Nature Biotechnology** Vegas2016
3. Combinatorial discovery of polymers resistant to bacterial attachment **Nature Biotechnology** Hook2012
4. Lipid deposition profiles influence foreign body *responses* **Advanced Materials** Schreib 2023
5. Mass Spectrometry and Informatics **Anal Chem** Green2011
6. The 3D OrbiSIMS: Label-free metabolic imaging with subcellular lateral resolution and high mass-resolving power **Nature Methods** Passarelli2017
7. Single cell metabolomics of macrophages using 3D OrbiSIMS: correlations with phenotype **Anal Chem** Suvannapruk 2022
8. Spatially resolved molecular analysis of host response to subcutaneous medical device implantation achieved using the 3D OrbiSIMS *UnderReview.*
9. Molecular formula prediction for chemical filtering of 3D OrbiSIMS Datasets **Anal Chem** Edney2022

3:00pm BI1-MoA-5 Elucidating of Native Macromolecule Structure in Cryo OrbiSIMS, *Anna Kotowska, M. Alexander, D. Scurr,* University of Nottingham, UK

Analysis of proteins in SIMS has historically been limited due to fragmentation caused by the energetic analysis beam, resulting in only single amino acid secondary ions. In previous work, we successfully demonstrated that the combination of a GCIB with an Orbitrap analyser can return primary structure information from proteins [1]. This was achieved through *de novo* peptide sequencing, with sequence coverage up to 50%.

The presence of water is known to increase ionisation of the sample components, particularly high molecular weight compounds [2]. Analysis of frozen-hydrated samples, spraying water above the sample or using water clusters as primary ion beams have been found to increase the [M+H]+ signals as well as fragments and [M+Na]+ and [M+K]+ adducts [2]. In this work, focusing on macromolecules, this enhancement enabled us to map proteins in human skin and in bacterial biofilm. The additional benefit of analysing large biomolecules in cryogenic conditions is preserving the native state of the molecule, which may enable acquisition of 3D structural information in addition to primary structure.

The extent of chemical information available from cryo-OrbiSIMS analysis is expansive and can be difficult to deconvolute. We have developed a molecular formula prediction (MFP) and level of molecule saturation (double bond equivalents) process to chemically filter multidimensional SIMS data [3]. Chemical filtering is particularly beneficial for the assignment of poorly ionisable molecules (e.g. protein fragments). Here, in addition to filtering protein fragments, we generated a protein fragment database, which facilitates rapid assignment and classification of protein ions and could pave the way for the development of a proteomics-like approach for OrbiSIMS analysis of large biomolecules.

In this work we demonstrate the potential of combining *de novo* sequencing with using cryogenic conditions and advanced data analytics approaches to identify unknown protein samples and obtain structural information from macromolecules.

[1] Anna M. Kotowska *et al.*, *Nat. Comms.*, 2020

[2] Sheraz Née Rabbani *et al.*, *Anal. Chem.,* 2015

[3] Max K. Edney *et al.*, Anal. Chem., 2022

3:20pm **BI1-MoA-6 Comparing Desalination Methods of Bacterial Biofilms for Static ToF-SIMS Analyses**, *Gabriel Parker,* University of Illinois - Chicago*; X. Yu,* Oak Ridge National Laboratory*; A. Plymale, J. Dhas, Z. Zhu,* Pacific Northwest National Laboratory*; L. Hanley,* University of Illinois - Chicago

Time-of-flight secondary ion mass spectrometry (ToF-SIMS) is a central technique for imaging of intact bacterial biofilms. Sample preparation in ToF-SIMS is simpler compared with gas chromatography - mass spectrometry (GC-MS) or liquid chromatography mass spectrometry (LC-MS) for biofilm analysis. Nevertheless, sample desalination is crucial to successful measurements from the native environment of biofilms consisting of complex salt and organic matrices. Without desalination, salt and other undesirable signals could dominate biofilm mass spectra and obscure acquisition of useful signals. Matrix effects in high salt environment can also affect the ion yield by enhancing disproportionate ion signals and nonlinear concentration correlations. This work compares desalination methods for ToF-SIMS of planktonic bacterial cells and biofilms on hard surface substrates. *Paenibacillus* sp. 300A biofilms are grown over the course of one week via static cell incubation at room temperature. Two methods of desalination, water submersion (WS) and centrifugal spinning (CS) of bacterial samples, are compared against each other and against samples with no desalination treatment. Water submersion samples are prepared by plating drops of biomaterial solution (planktonic cells or biofilm) onto a substrate, drying the sample, then submerging it in a water bath, and drying again prior to static SIMS analysis. Centrifugal spinning samples are prepared by centrifuging biomaterial, discarding the supernatant, resuspending biomaterial with deionized water, then plating biomaterial solution on substrate and drying under nitrogen prior to SIMS analysis. Results show that non-desalinated samples have the highest salt signal that arises in part from bacteria growth media with signal suppression of biologically relevant ions. By contrast, both WS and CS desalination display well defined peaks with high signal to noise that correspond to metabolites, amino acids, lipids, fatty acids, and salt adducts up to *m/z* 800. WS displays similar peak intensities compared to CS, but in some cases, the signal is higher for WS samples. Overall, experimental results show that the simple WS method for desalination lowers matrix effects in biofilms for ToF-SIMS analysis while keeping the biofilm structure intact. Centrifugal spinning proves to be a reliable method to reduce matrix effects in ToF-SIMS analyses of biofilms.

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