Biomaterial Interfaces Division
Room 101B - Session BI+AS+NS-FrM

Characterization of Biological and Biomaterial Surfaces

Moderator: Bill Theilacker, Medtronic

8:20am **BI+AS+NS-FrM1 Novel Insights into Skin Biology and Permeation of Actives using ToF-SIMS and 3D OrbiSIMS.***, **David Scurr**,* The University of Nottingham, UK **INVITED**

This work presents the use of mass spectrometry imaging (specifically ToF-SIMS and 3D OrbiSIMS) as an emerging tool for skin analysis, offering the ability to perform chemical histology and monitor the distribution of xenobiotic compounds, namely antibacterial, cosmetic and pharmaceutical agents. Both 2D and 3D spatial distribution profiles of analytes within skin are achievable for both topically applied compounds following permeation and inherent compounds present in native tissue. Data acquired using the 3D OrbiSIMS can identify a significant number of biological molecules, unavailable using ToF-SIMS, including subtle chemical variations within single skin strata and / or individual cells.

Individual tape stripped layers of human *stratum corneum*, both native and following application of a topical compound can be imaged using ToF-SIMS and 3D OrbiSIMS. The sensitivity of these techniques has also enabled the detection of analytes from native tape stripped samples highlighted differences in the lipid composition of the *stratum corneum* relating to both intrinsic and extrinsic aging effects[1]. In particular, a significant increase in the presence and a localised spatial distribution was observed for cholesterol sulfate, which has been shown to play a key role in desquamation.

In conducting an analysis of native *ex vivo* porcine tissue we were successfully able to detect and spatially map chemical biomarkers of both the *stratum corneum* and underlying epidermis. In addition, using a gas cluster ion beam (GCIB), the 3D distribution of analytes throughout the epidermis could be visualised for both pharmaceutical and cosmetic topical products following Franz cell experiments. These methods can be used to illustrate enhanced topical delivery, for example in the use of supramolecular gels encapsulating ascorbic acid and microneedles applied prior to the application of imiquimod used for cosmetic and pharmaceutical purposes respectively.

[1] Starr, Johnson, Wibawa, Marlow, Bell, Barrett & Scurr, *Anal. Chem*. **2016**, 88 (8), pp 4400–4408

9:00am **BI+AS+NS-FrM3 Multivariate Analysis of ToF-SIMS Data using Mass Segmented Data Matrices: Polymers and Biointerfaces***, R.M.T. Madiona,* La Trobe University, Australia*; N.G. Welch,* CSIRO Manufacturing, Australia*; D.A. Winkler,* La Trobe University, Australia*; J.A. Scoble,* CSIRO, Austalia*; B.W. Muir,* CSIRO, Australia*; **Paul Pigram**,* La Trobe University, Australia

Time-of-flight secondary ion mass spectrometry (ToF-SIMS) is continuously advancing. The data sets now being generated are growing dramatically in complexity and size. More sophisticated data analytical tools are required urgently for the efficient and effective analysis of these large, rich data sets. Standard approaches to multivariate analysis are being customised to decrease the human and computational resources required and provide a user-friendly identification of trends and features in large ToF-SIMS datasets.

We demonstrate the generation of very large ToF-SIMS data matrices using mass segmentation of spectral data in the range 0 – 500 m/z in intervals ranging from 0.01 m/z to 1 m/z. No peaks are selected and no peak overlaps are resolved. Sets of spectra are calibrated and normalized then segmented and assembled into data matrices. Manual processing is greatly reduced and the segmentation process is universal, avoiding the need to tailor or refine peak lists for difficult sample types or variants.

ToF-SIMS data for standard polymers (PET, PTFE, PMMA and LDPE) and for a group of polyamides are used to demonstrate the efficacy of this approach. The polymer types of differing composition are discriminated to a moderate extent using PCA. PCA fails for polymers of similar composition and for data sets incorporating significant random variance.

In contrast, artificial neural networks, in the form of self organising maps (SOMs) deliver an excellent outcome in classifying and clustering different and similar polymer types and for spectra from a single polymer type generated using different primary ions. This method offers great promise for the investigation of more complex bio-oriented systems.

9:20am **BI+AS+NS-FrM4 Can you dig it? ToF-SIMS Tissue Depth Profiling***, **Daniel Graham**, T.B. Angerer, L.J. Gamble,* University of Washington

ToF-SIMS has been shown to provide detailed chemical information about cells and tissues with excellent lateral resolution. This has enabled looking at the 2D chemical distribution of lipids and other biological molecules within tissues and cells. Since cells and tissues are three dimensional constructs, it is of interest to be able to characterize their chemical composition in 3D. With the use of gas cluster ion beams (GCIBs) ToF-SIMS can attain very fine z-resolution (<10 nm) in depth profiles, however the use of ToF-SIMS for 3D imaging of biological samples is limited. This is likely due to the complexity of the materials and artifacts often encountered because of the presence of salts. In this work we use ToF-SIMS 3D depth profiling to optimize accurate reconstruction of depth profiles of planarian worm cross-sections. For this, dual beam depth profiles with a 25 keV $Bi3^+$ liquid metal ion gun (LMIG) for imaging and 10 keV Argon 1000 clusters for sputtering were acquired using an Iontof 5 system. Data reconstruction was carried out using the NBToolbox (https://www.nb.uw.edu/mvsa/nbtoolbox) ZCorrectorGui. It is well known that due to the fixed angle to of the analysis beam, the sequential images taken at each layer of the profile shift as a function of depth. Adjusting the beam steering during data acquisition and image shifting post data acquisition are used to account for this image shifting and more accurately reconstruct a 3D representation of the data. Areas with distinct structural features were chosen for depth profiles in order to aid in ascertaining the accuracy of the 3D data reconstruction. These studies will help establish the viability of 3D data reconstruction of complex biological samples and could be instrumental in being able to localize chemical distributions throughout tissues and cells.

10:00am **BI+AS+NS-FrM6 Novel Insights into Drug Release by a Functionalized Biomaterial and Dispersion into Bone using Surface Analytical Techniques***, **Marcus Rohnke**, C. Kern, B. Mogwitz, S. Ray,* Justus-Liebig University Giessen, Germany*; J. Thomas,* IFW Dresden, Germany

Bone is a complex composite material with similarities to hierarchically structured functional materials. In the case of a fracture or the need for a replacement (e.g. hip prosthesis) filler or replacement materials are necessary. Next generation bone implants are functionalised with drugs to stimulate bone healing locally or to provoke antibiotic effects. Here we focus on the release and dispersion of the anti-osteoporotic agent Sr^{2+} from strontium enriched bone cement. The knowledge of the release and dispersion kinetics of the drug plays an eminent role for the performance optimisation of the biomaterial.

Due to practical and technical reasons it is almost impossible to track the drug release kinetics, drug dispersion and the degradation of the implant material in vivo. Here we apply time of flight secondary ion mass spectrometry (ToF-SIMS) depth profiling to obtain the diffusion coefficient of Sr^{2+} in the mineralised areas of healthy and osteoporotic rat bone in post mortem examinations. For data evaluation of the depth profiles in mineralised bone we applied a simple diffusion model. The obtained diffusion coefficient for trabecular osteoporotic bone is with 1.76×10^{-10} cm^2/s more than two decades higher than that for healthy bone (2.91×10^{-12} cm^2/s). In cortical bone no significant difference in the diffusion coefficient (healthy 1.33×10^{-12} cm^2/s, osteoporotic 4.17×10^{-12} cm^2/s) could be found. The varying diffusion coefficients can be explained by the different bone nanostructure, which was investigated by focused ion beam scanning electron microscopy (FIB-SEM) and high-resolution transmission electron microscopy (HR-TEM).

The data of cement dissolution experiments into water in combination with inductively coupled plasma mass spectrometry (ICP-MS) analysis account for dissolution kinetics following Noyes-Whitney rule. For dissolution in A-MEM cell culture media the process is kinetically hindered and can be described by Korsmeyer-Peppas kinetics. An adsorbed protein layer on top of the cement surface, which was detected by ToF-SIMS, is responsible for the kinetic inhibition. Based on the results of various analytical experiments we developed a two-phase model and performed a finite element calculation for the release and dispersion of Sr^{2+} in bone. The validity of the applied model is proven by animal experiments. We compared the calculated images to mass spectrometric images of bone cross sections and achieved good conformity. It appears that drug removal via the vascular system is negligible. This is a good basis for predictions of drug mobility in bone.

10:20am **BI+AS+NS-FrM7 Spatial Distributions of Epithelial Growth Factors in Hydrogels Studied by ToF-SIMS and TIRF Microscopy for the Development of Biocompatible Multiple-protein Delivery Systems for Wound Healing***, **Shohini Sen-Britain**,* State University of New York, Buffalo*; W. Hicks,* Roswell Park Comprehensive Cancer Center*; J.A. Gardella Jr.,* State University of New York, Buffalo

This work reports the use of ToF-SIMS imaging, TIRF microscopy, and depth profiling to visualize and map the interactions of (hydroxyethyl)methacrylate (HEMA)-based hydrogels with mixtures of growth factors that are often secreted by the epithelium during wound healing. During re-epithelialization, hydrogels can act as both tissue scaffolds at the interface between healing epithelium and surrounding connective tissue, and as delivery vehicles of therapeutic proteins that expedite the wound healing process.

The spatial distribution of multiple growth factors at hydrogel surfaces can influence biocompatibility and release kinetics, orientation and conformation of the individual growth factors. Hydrogels interact with mixtures of growth factors in vivo and also when they are developed into multiple-protein delivery systems. To address these concerns, this work presents 2D and 3D spatial distributions of fluorophore-labeled growth factors varying in size, secondary structure, and hydrophobicity at the hydrogel surfaces to model the interface between porous, phase segregated drug delivery systems and complex macromolecular mixtures.

HEMA hydrogel blends incorporating methyl methacrylate (HEMA/MMA) and methacrylic acid (HEMA/MAA) cause increased hydrophobicity or hydrophilicity at the hydrogel surface, respectively. They also present phase segregation and porous topography at the surface. Depth profiling shows that smaller proteins, such as epidermal growth factor (EGF) permeate deeper into porous regions than larger proteins such as keratinocyte growth factor (KGF) and platelet-derived growth factor (PDGF). SIMS and TIRF imaging shows that proteins with more hydrophobic character such as PDGF and EGF localize at phase segregated regions containing MMA, while those with more hydrophilic character such as KGF localize at phase segregated regions containing MAA or HEMA. Biological ramifications of these results regarding biocompatibility and multiple-protein delivery systems are the focus of future work.

Author Index

Bold page numbers indicate presenter

— A —
Angerer, T.B.: BI+AS+NS-FrM4, 1
— G —
Gamble, L.J.: BI+AS+NS-FrM4, 1
Gardella Jr., J.A.: BI+AS+NS-FrM7, 2
Graham, D.J.: BI+AS+NS-FrM4, **1**
— H —
Hicks, W.: BI+AS+NS-FrM7, 2
— K —
Kern, C.: BI+AS+NS-FrM6, 1
— M —
Madiona, R.M.T.: BI+AS+NS-FrM3, 1
Mogwitz, B.: BI+AS+NS-FrM6, 1
Muir, B.W.: BI+AS+NS-FrM3, 1
— P —
Pigram, P.J.: BI+AS+NS-FrM3, **1**
— R —
Ray, S.: BI+AS+NS-FrM6, 1
Rohnke, M.: BI+AS+NS-FrM6, **1**
— S —
Scoble, J.A.: BI+AS+NS-FrM3, 1
Scurr, D.J.: BI+AS+NS-FrM1, **1**
Sen-Britain, S.: BI+AS+NS-FrM7, **2**
— T —
Thomas, J.: BI+AS+NS-FrM6, 1
— W —
Welch, N.G.: BI+AS+NS-FrM3, 1
Winkler, D.A.: BI+AS+NS-FrM3, 1