SIMS Solutions in Materials and Life Sciences
Room Great Lakes A2-A3 - Session SS-TuA4

Cells and Tissue IV

Moderators: Gregory Fisher, Physical Electronics USA, **Sebastiaan Van Nuffel**, Maastricht University

2:00pm **SS-TuA4-1 Probing the Human Epidermis from a Materials Science Point of View**, ***Xavier Delvaux***, University of Namur, LISE Research unit, Namur Institute of Structured Matter, Belgium; *Y. Poumay,* University of Namur, Namur Research Institute for Life Sciences, Belgium; *L. Houssiau,* University of Namur, LISE Research unit, Namur Institute of Structured Matter, Belgium

The mammalian epidermis, the most topical cellular layers of the skin, may be considered as a continuously renewing and highly complex structure composed of multiple biomolecular layers. The most fundamental functions of the epidermis are to provide a barrier shielding the organism from its environment and to mitigate dehydration. This is achieved through a specific cellular death pathway known as cornification. Keratinocytes undergoing cornification produce a protein-rich cellular envelope as well as an intercellular matrix composed mainly of lipids and hydrophilic molecules, resulting in a specific histological layer referred to as the *Stratum Corneum* (SC). However, a wide range of pathologies can affect the formation of the epidermis and impair its function. In the context of dermatological research, understanding the molecular changes induced by these pathologies is paramount for their efficient treatment and prevention.

In the recent years, analytical techniques derived from the materials science field have been of increasing interest for the investigation of complex biological systems. Among those techniques, ToF-SIMS has proven to be a particularly useful tool in the field of lipidomics, as it combines a very high sensitivity with a high mass and spatial resolution. In this work, we aimed at developing a rigorous and reproducible investigation methodology of the human epidermis by applying ToF-SIMS to an in vitro epidermal model known as Reconstructed Human Epidermis (RHE). This model is composed of keratinocytes layers cultured in order to reproduce the main histological features of a real human epidermis. The ToF-SIMS characterization of these RHEs was performed under static SIMS conditions on freeze-dried cryosections and combined both high mass and lateral resolution acquisitions. Data processing was assisted by Principal Components Analysis (PCA). This approach allowed the successful decorrelation of the highly complex data sets into a few principal components (PC) carrying the essential biological information about RHE cross sections. Most notably, PCA yielded one specific PC highlighting relevant spectral features needed to distinguish the viable cells from the cornified region. Furthermore, we obtained high lateral resolution molecular maps of the major species identified by PCA. Finally, we demonstrated that this methodology was reproducible, therefore allowing the production of experimental replicates. Ultimately, these results suggest that this methodology could be of significant interest for the field of dermatology by allowing the effective characterization of molecular modifications induced by various skin pathologies.

2:20pm **SS-TuA4-3 Ambient Mass Spectrometry Imaging of Lipid Molecules from Live Cells and Tissues Using Nanomaterials**, *J. Kim,* Kyungpook National University, Republic of Korea; *H. Lim,* ***DaeWon Moon,*** Daegu Gyeongbuk Institute of Science and Technology (DGIST), Republic of Korea **INVITED**

We have been developing new methods to analyze cells and tissues in ambient condition without any harsh chemical fixation or physical freezing and drying for last several years. The first approach, an atmospheric pressure mass spectrometry imaging method, is based on laser ablation in atmospheric pressure assisted by atmospheric plasma and nanomaterials such as nanoparticles and graphene to enhance laser ablation. The second one is based on secondary ion mass spectrometry (SIMS) imaging of live cells in solution capped with single layer graphene to preserve intact and hydrated biological samples even under ultrahigh vacuum for SIMS bio-imaging in solution.

Recent activities such as the extension of the molecular analysis range from lipids to proteins, applications to neuronal and cancer cell using confocal, SIMS, and SEM/HIM will be discussed.

References

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[2] Heejin Lim, Sun Young Lee, Yereum Park, Hyeonggyu Jin, Daeha Seo, Yun Hee Jang, Dae Won Moon, "Mass Spectrometry Imaging of Untreated Wet Cell Membranes in Solution Using Single-Layer Graphene", Nature Methods 18, 316-320 (2021)

3:00pm **SS-TuA4-7 SiLC-MS (Single-Live-Cell Mass Spectrometry) Analysis in the Context of Drug Discovery**, ***Carla Newman,*** GSK, UK

In the last few decades, the pharmaceutical industry has transformed people's lives. However, the development of new drugs possesses challenges and a paradigm shift in the drug discovery workflow would be desired to reduce attrition and transform conventional drug screening assays into translatable analytical techniques for the analysis of drugs in complex environments, both in-vitro and ex-vivo.

The ability to visualise unlabelled compounds inside the cell at physiological dosages can offer valuable insight into the compound behaviour both on and off-target.

SiLC-MS is a semi-automated methodology that allows the collection of intracellular contents using a modified CQ1 imaging system developed by Yokowaga. The instrument is equipped with a confocal microscope that allows bright field imaging as well as fluorescence imaging with 4 lasers (405, 488, 561 and 640 nm). Sampling is performed using the tips developed by Professor Masujima (1-4). The tip, holding the cellular contents, is then used for static nanospray of the contents into an Orbitrap Fusion Lumos (Thermo Scientific) and the resulting data processed using Compound Discoverer (Thermo Scientific).

In this study, we show the applicability of the SiLC-MS technology to drug discovery, as it is crucial to identify compound and its metabolites when incubated in a mammalian cell at a therapeutic dose. We report on the validation studies performed using the SiLC-MS platform, in these validation studies we assess the ability to distinguish different cell types based on their metabolomic fingerprint, furthermore we have also evaluated if this assay was sensitive enough to detect drugs intracellularly.

We are currently establishing a multi-omics platform on the modified CQ1 that allows both metabolomics and transcriptomics at the single cell level. For that we have sampled the cells first for metabolomics and then for transcriptomics.

We demonstrate that dosed compound can be identified in a single cell after samplingusing the modified CQ1, endogenous metabolites can also be identified that can further the understanding of the drug's mechanism. This technique has direct relevance for assessing compound effects on disease relevant cells and its low sample requirement makes it applicable to studying rare cell types. The use of high content imaging system enables the effect of compounds on live cells to be studied and suitable time points selected for sampling cell contents.

3:20pm **SS-TuA4-9 TOF-SIMS Study of Pharmacological Active Components in Cordyceps Sinensis**, *Q. Zhan,* School of Chemical and Environment Engineering, China University of Mining and Technology , China; *M. Xia,* Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, China; *S. Sun, L. Cai,* Department of Chemistry, Tsinghua University, China; *H. Liang,* School of Chemical and Environment Engineering, China University of Mining and Technology, China; ***Zhanping Li,*** Key Laboratory of Organic Optoelectronics and Molecular Engineering of Ministry of Education, Department of Chemistry, Tsinghua University, China

Cordyceps sinensis is a well-known traditional Chinese medicine. This study showed TOF-SIMS was used to identify the pharmacological active substances, reveal the pharmacological active substances at different developmental stages and visualize spatial differentiation of the pharmacological active substances in Cordyceps sinensis. Base on the high mass resolution (M/ΔM) of TOF-SIMS, the positive fragment ion detected at m/z 251 might not be the molecular ion M^+ of cordycepin $C_{10}H_{13}N_5O_3$ (m/z 251). There are some "splicing" ions, which are formed between pharmacological active compounds and weakly polar compounds and/or themselves, appeared in the TOF-SIMS mass spectrum of Cordyceps sinensis. The changes of the pharmacological activesubstances of Cordyceps sinensis with time (different stages of development during growth cycle) at different parts (stroma, worm body and the base of stroma) were studied. The amino acid class showed different changes in the different parts due to the metabolic regulation in development. The

changes of nucleosides are similar in the same part of Cordyceps sinensis but there are great differences betweenstroma and worm body. The content of ergosterol first rises and subsequently falls in the stroma, rises at the base of the stroma, and at the worm body first falls and subsequently rises. The visualization of spatial differentiation of ergosterol and other active components in whole Cordyceps sinensis was first realized by developing a feasible and simple “segmentation-imaging-splicing” strategy based on TOF-SIMS. Whole-body chemical mapping of Cordyceps sinensis was then accomplished by splicing these ion images with normalized size and signal intensity. Ergosterol was found more enriched in host caterpillar, especially enriched in the top of host caterpillar, than in fruiting body. Moreover, ergosterol and lipids showed obviously complementary distribution pattern in some special structures of Cordyceps sinensis.

References

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