Following Skin Wound Healing using 2D Mass Spectrometry Imaging

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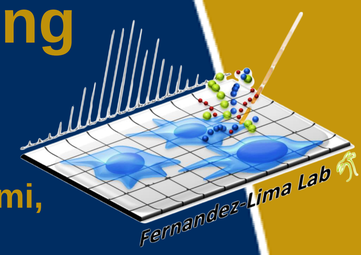

Overview

For the first time, the distribution of lipids from human skin is observed as a function time in an *ex-vivo* acute wound. Using Time-of-Flight Secondary Ion Mass Spectrometry (TOF-SIMS), fatty acids, cholesterol sulfate, and other lipids can be visualized in a label-free manner directly from cryo-sectioned and dehydrated tissue. This work can lead to improved understanding of the lipids role during skin repair, and may lead to better therapeutic tools for treatment of non-healing chronic wounds.

Introduction

Skin repair is a significant aspect of human health. The skin serves as a barrier which protects against pathogens and prevents transepidermal water loss. In order to maintain a functional barrier, it is vital that a precise assembly of lipids be present.[1,2] While the makeup of healthy stratum corneum and epidermis is generally understood, the role of lipids involved in skin repair has not been extensively studied. In the present work, Time-of-Flight Secondary Ion Mass Spectrometry (TOF-SIMS) is applied to characterize the chemical composition of newly formed epidermis following an initial wound.

Experimental Methods

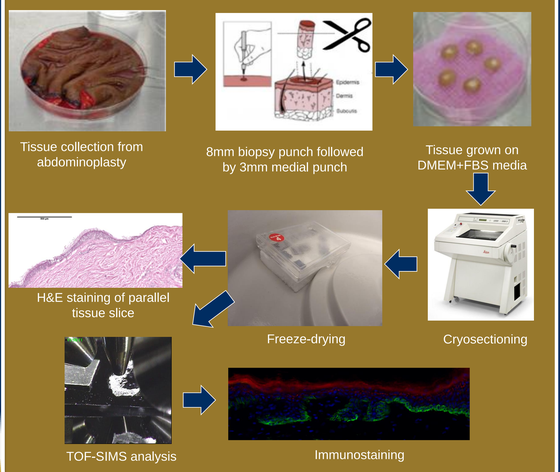

Figure 1. Sample preparation steps during 2D-MSI-TOF-SIMS

References

1. K. R. Feingold, *Journal of Lipid Research*, 2007, **48**, 2531-2546.
2. J. van Smeden, M. Janssens, G. S. Gooris and J. A. Bouwstra, Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids, 2014, 1841, 295-313.
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SIMS Mass Spectra of Skin

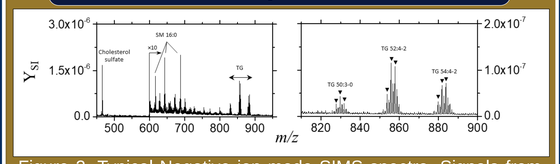


Figure 2. Typical Negative ion mode SIMS spectra. Signals from cholesterol sulfate, sphingomyelin (SM), and Triacylglycerides (TGs) are denoted.

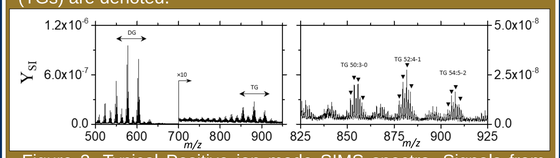


Figure 3. Typical Positive ion mode SIMS spectra. Signals from Diacyglycerides (DGs) and TGs are denoted.

Parallel H&E and TOF-SIMS

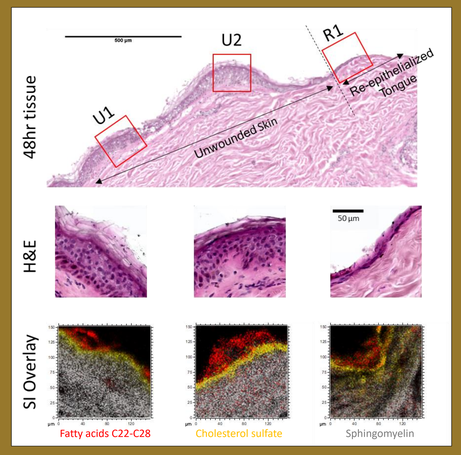
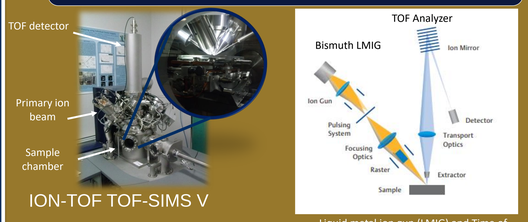


Figure 4. H&E staining of a parallel tissue slice and TOF-SIMS imaging of a similar region of interest.

TOF-SIMS instrumentation



ION-TOF TOF-SIMS V

Liquid metal ion gun (LMIG) and Time of Flight (TOF) schematics

- 25keV Bi_3^+ primary ion beam
- 1.6 µm pixel size, 3×10^{12} ions•cm^{-2} Primary Ion Dose

$$Y_{SI} = \frac{Secondary\ Ion\ count(Area)}{Region\ of\ Interest(cm)^2 \times Primary\ Ion\ fluence\ \left(^{primary\ ions}/_{cm^2}\right)}$$

SIMS and subsequent IHC

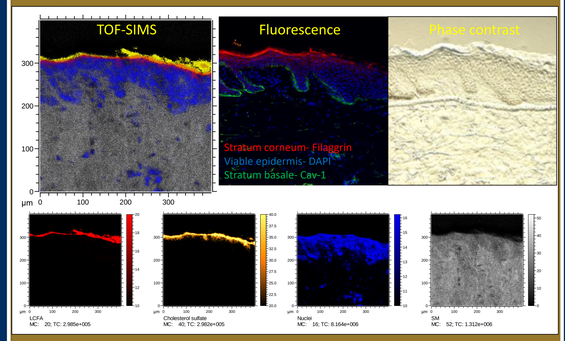


Figure 5. Unwounded tissue region, incubated 48 hrs.

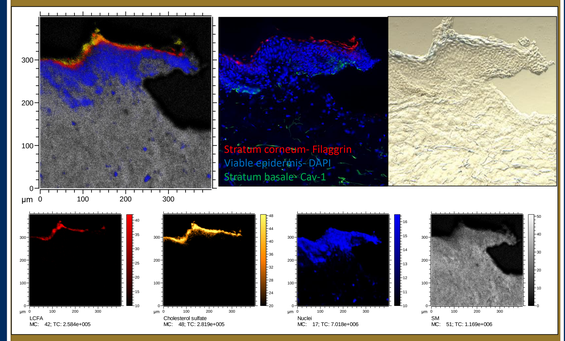


Figure 6. Re-epithelialized tissue region, incubated 48 hrs.

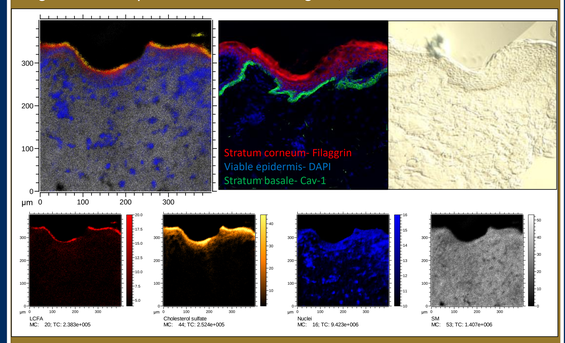


Figure 7. Unwounded tissue region, incubated 0 hrs.

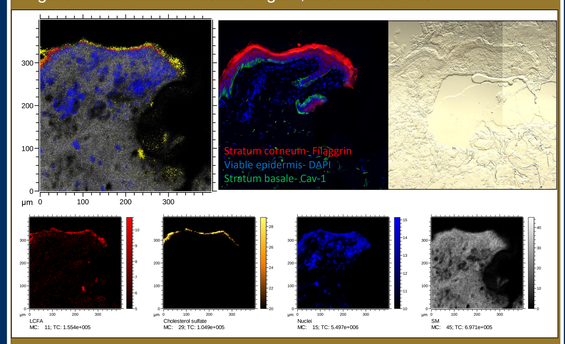


Figure 8. Re-epithelialized tissue region, incubated 0 hrs.

Metabolite semi-quantitation

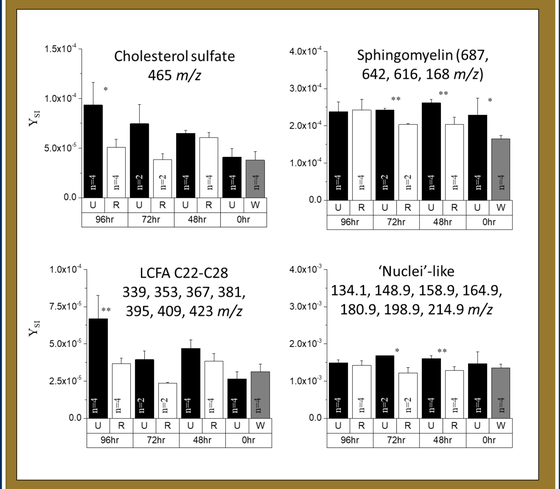


Figure 9. TOF-SIMS based semi-quantitation of cholesterol sulfate, sphingomyelin, 'Nuclei'-like species, and long chain fatty acids. Asterisks denote significate difference (unpaired t-test: **p≤0.01, *p≤0.05).

Conclusions

Mass Spectrometry has been demonstrated to be the tool of choice for identifying and localizing lipid species in human tissue.[3-5] With relatively simple sample preparation steps, human *ex-vivo* skin tissue layers can be described by their composition in a label-free manner. Knowledge of the lipid composition during skin repair may lead to improved treatment of both acute and chronic wounds.

- TOF-SIMS imaging complements H&E and IHC staining, and is a label-free technique.
- Lipid species such as cholesterol sulfate and long chain fatty acids can be observed in the top-most layer of the epidermis, consistent with literature on the stratum corneum.[6]
- Depending incubation time, newly re-epithelialized skin was observed to have relatively deficient levels of cholesterol sulfate, sphingomyelin, and long chain fatty acids (C22-C28).

Acknowledgments

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