TOF-SIMS IMAGING ALLOWS LIPID MAPPING OF HUMAN DYSTROPHIC AND CONTROL MUSCLE SECTIONS

Nora TAHALLAH[1], Alain BRUNELLE[1], Sabine DE LA PORTE[2] and Olivier LAPRÉVOTE[1]

[1] Institut de Chimie des Substances Naturelles (ICSN-CNRS); [2] Institut de Neurobiologie Alfred Fressard (INAF-CNRS) - Gif-sur-Yvette - FRANCE

1. OVERVIEW

• Analysis of human paravertebral striated muscle samples, from male Duchenne Muscular Dystrophy (DMD) affected and control 14 years old patients, by cluster-time-of-flight secondary ion mass spectrometry (cluster-ToF-SIMS) imaging.

• Micrometer scale molecular distribution mapping.

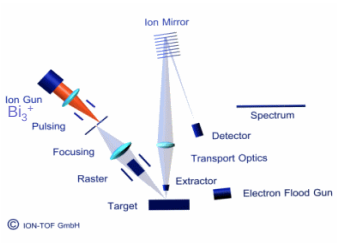
• Characteristic distributions of various lipids: Phosphocholines (PCs) in necrotic areas, together with sphingomyelin species (SMs); Phosphatidylinositols (PIs), Triglycerides (TGs) varied depending on the region.

2. INTRODUCTION

The input of cluster time-of-flight secondary ion mass spectrometry (ToF-SIMS) is continuously expanding in the field of lipidomics and drugs/biomarkers mapping in biological tissues, at a micrometer scale (1). The use of Bismuth (2) primary ion clusters has particularly enhanced the secondary ion emission and allowed the investigation and specification of the degeneration extent of the muscle of the mouse model of DMD (3). DMD is a severe neuromuscular X-linked recessive disease affecting in average one boy out of 3500 (4). Mass spectrometry imaging has been used for the analysis of human control and DMD-affected muscle.

3. METHODS

The *Banque de Tissus pour la Recherche* (BTR, AFM) provided human muscle surgery residues from DMD-affected and control children.
20 µm thick sections were cut at -20°C using a cryostat (Leica Mycrosystemes SA, Rueil-Malmaison, Fr) and deposited on glass plates. Primary ion dose density: 2-5 x 10^{11} ions/cm^2. Secondary ion images obtained with IonImage software (Ion-Tof GmbH, Muenster, Germany). Images field of view: 500 x 500 µm^2 (256 x 256 pixels). Final lateral resolution of 4 µm due to a compression to 128 x 128 pixels and an averaging to increase contrast.



TOF-SIMS IV mass spectrometer (Ion-Tof GmbH, Muenster, Germany): fitted with a bismuth (Bi_3^+, 25 keV) cluster ion source, a TOF analyzer, an electrostatic reflector. The flight path is 2 m, the ion kinetic energy is 2 keV, the post-acceleration is at 10 keV before the detector, an electron flood gun was used for insulating samples. The mass resolution was M/ΔM > 10^4 (FWHM).

5. CONCLUSIONS

• Complete preservation of sample molecular and structural integrity: no prior sample treatment,
• Direct analysis and chemical imaging of intact biological tissues and organ sections,
• Possibility of simultaneous but separate analysis of different muscle cells at µm scale,
• High molecular specificity and sensitivity, and no molecular delocalization at µm scale,
• Specific localization of different compounds in positive ion mode images and confirmation in negative mode,
• Association of spectra with images provides molecular information even with too low image signal, on one hand, and, on the other hand, spatial information even with insufficient spectrum signal-to-noise ratio,
• Confirmation in human of similar trends observed previously in the *mdx* mouse model.

This work was supported by the European Union. (contract LSHG-CT-2005-518194 COMPUTIS)

Alain.Brunelle@icsn.cnrs-gif.fr
Nora.Tahallah@icsn.cnrs-gif.fr

REFERENCES

1. Brunelle A, Touboul D, Laprévote O. *J Mass Spectrom.* **2005**;40 (8):985-99.
2. Touboul D, Kollmer F, Niehuis E, Brunelle A, Laprévote O. *J Am Soc Mass Spectrom.* **2005**;16 (10):1608-18.
3. Touboul D, Brunelle A, Halgand F, De La Porte S, Laprévote O. *J Lipid Res.* **2005**;46 (7):1388-95.
4. Blake DJ, Weir A, Newey SE, Davies KE. *Physiol Rev.* **2002**;82 (2):291-329.

4. RESULTS

Negative Ion Mode:
PIs localization was maximal in the remaining dystrophic cells, while triglycerides accumulated in the adipocytes.

Positive Ion Mode:
SMs are present in DMD necrosed (green ROI) or intercellular regions, but not in the control
⇒ disorder in regulation of membrane sphingolipid composition (*i.e.* structure) and/or dysfunctionning of ceramide-mediated apoptosis pathway.
Besides, PCs distribution and composition undoubtedly shows a modification in fatty acyl chains composition, revealing a perturbation of the fatty acid incorporation in membrane PCs, in order to lower membrane rigidity.