Adam Pruška[1,2], *Karl Christian Schäfer*[3], *Jarmila Navrátilová*[4], *Jan Šmarda*[4], *Bernhard Spengler*[3], *Jan Preisler*[1,2]

[1]Department of Chemistry, Faculty of Science, Masaryk University,
Kamenice 5, 625 00 Brno, Czech Republic
[2]Central European Institute of Technology, Masaryk University,
Kamenice 5, 625 00 Brno, Czech Republic
[3]Institute of Inorganic and Analytical Chemistry, Justus-Liebig University Gießen,
Heinrich-Buff-Ring 17, 35392 Gießen, Germany
[4]Department of Experimental Biology, Faculty of Science, Masaryk University,
Kamenice 5, 625 00 Brno, Czech Republic

Quasi-homogeneous biological tissues as a universal tool for study of matrix layer homogeneity in MALDI MSI

Key words: MALDI MSI, homogeneity, quasi-homogeneous tissue, matrix characterization

Introduction:

At the present, the matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI MSI) places considerable emphasis on increasing the speed of analysis, reproducibility of sample preparation, achieving higher spatial resolution and efficient statistical data processing. One of the most critical steps in the MALDI MSI workflow is the application of a homogeneous matrix layer with efficient analyte extraction and reduced planar diffusion. Unfortunately, natural untreated tissue cannot be used to study behavior and characterization of the matrix layer because the tissue itself usually have diverse structures with varying analyte distributions. Therefore, two biological materials and several treatment processes for the matrix layer characterization independent of the used deposition system, place, and time of preparation have been introduced.

Methods:

Chemicals: 2,5-dihydroxybenzoic acid, α-cyano-4-hydroxybenzoic acid, trifluoroacetic acid, acetone and redistilled water. Chicken liver and lab-cultivated 3D colorectal cancer cell cultures were chosen as a suitable biological material for the preparation of quasi-homogeneous tissues (QHT). The tissue was frozen in liquid nitrogen, ground using a tissue grinder (in the case of the liver), ultrasonicated and finally stored at −78 °C. Two pneumatic spraying systems were employed for matrix deposition. Namely, SMALDIPrep (TransMIT GmbH, Germany)

iMatrixSpray (Tardo GmbH, Switzerland). Q Exactive MS analyzer (Thermo Fisher Scientific, Germany) with atmospheric ion source "AP-SMALDI^{10}" (TransMIT GmbH, Germany) and MALDI TOF "Autoflex Speed" (Bruker, Germany) were employed for the chemical maps acquisition.

Results:

The main aim of this work was the design of a universal, homogeneous sample with composition and behavior corresponding to real biological tissues. At the same time, it would be suitable for the complex characterization of the deposited matrix layer. For this purpose, the procedure of the QHT preparation was developed. A procedure including crushing, proper grinding, and using of ultrasonication bath was found out to provide the most homogeneous QHTs. Subsequently, these QHTs were used to optimize the matrix deposition parameters employing both SMALDIPrep and iMatrixSpray. The detailed optical characterization of the deposited layer followed by MSI analyses of small areas of the QHT and glass surface was performed for all suggested deposition conditions. The matrix layer homogeneity was assessed according to calculated relative standard deviation (RSD) of the particular intensities of both the QHT and the matrix on the glass surface. Evident correlation between changes of deposition parameters and intensity RSDs was observed. In the case of the liver QHT, the best deposition conditions yielded RSD of the tissue signal intensities below 25 % without any normalization. Based on the microscopic observation, the matrix crystal size measurements, and the comparison of the signal intensity RSDs, optimal deposition conditions were carefully selected. The possibility of utilizing optimized deposition conditions is demonstrated on slices of different types of mouse tissue and cancer cell cultures reaching pixel size in range 8 □ 25 μm for AP-SMALDI^{10} and 30 □ 50 μm for vacuum MALDI TOF MS.

Conclusions:

This contribution outlines an approach for the matrix layer characterization suitable for MALDI MSI using two different QHTs as universal samples. The major part is focused on comprehensive matrix layer characterization and development of the optimal deposition conditions. Final methods are demonstrated on the real tissues and cancer cell cultures.