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Short Communication

Brief Note on the Crucial Steps Involved in the Mass Spectrometry Imaging in Tissue Analysis

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ABOUT THE STUDY

The first and most important part of MSI is sample management following tissue collection. It is critical to keep the tissue properly in order to maintain structural information and cease biological activity. Ischemia of tissue can result in molecular alterations that impact MSI findings extremely fast. In pathology, Formalin-Fixed Paraffin Embedding (FFPE) is widely used to maintain proper spatial information, reduce deterioration, and delocalize. However, because of the cross-linking generated by formalin, FFPE tissue is less compatible with MSI, making ionisation and identification challenging [1]. MSI capabilities for the utilisation of FFPE samples for metabolite and protein/peptide imaging have increased due to advancements in sample preparation. Cryo-preservation with immediate flash freezing following tissue resection/collection remains the recommended tissue preservation approach for MSI that allows access to a wide range of molecular classes.

The next stage is tissue sectioning, which is done with a cryo-microtome for frozen tissue. The operating temperature varies from 5 to 25°C depending on the tissue [2]. Temperatures ranging from 20 to 25°C have been utilised to freeze arterial and cardiac tissue. To minimise cracking or excessive drying durations, typical section thickness range between 10 and 20 m. An embedding media, such as gelatin or carboxymethyl cellulose, can be used to support fragile cardiovascular tissue during sectioning.

The use of an optimum cutting temperature compound is not advised due to the possibility of polymeric contamination of the MS spectra, which obscures some of the crucial chemical information. Nonetheless, Optical Coherence Tomography (OCT) has been employed in several cardiovascular disease research [3]. Tissue slices are then thawed and put on glass slides or electrically conductive Indium Tin Oxide (ITO)-coated glass slides, depending on whether the instrument is orthogonal or non-orthogonal. For FFPE material, a microtome is employed, and the section thickness ranges from 3 to 15 m. Before proceeding with additional sample preparation, the FFPE sample must be deparaffinized and, if peptide/protein analysis is required, antigen retrieval.

It has been demonstrated that adding an adhesive component to the glass slide before mounting the tissue is advantageous; in cardiovascular research, poly L-lysine is employed. This avoids tissue loss during lengthy wash procedures. The process may involve washing and/or digesting stages depending on the analyte of interest. The removal of salts, small compounds, and/or lipids during the washing process reduces ion suppression. As a result, these processes are employed for protein/peptide imaging rather than lipid or metabolite imaging. Digestion stages are incorporated to shrink bigger biomacromolecules like proteins and glycans and render their fragments (proteolytic peptides and oligosaccharides) accessible to MSI.

MALDI-next MSI's step is to apply a matrix layer on top of the tissue. This matrix solution is made up of an organic acid (the matrix) and an organic solvent, with trifluoroacetic acid added in certain circumstances to help with protonation of the analytes. The organic acid crystallises as analyte-matrix crystals, which absorb laser light, allowing ionisation and functioning as a proton source [4]. Spraying, vibrational vaporisation, nebulization, and sublimation are some of the ways available to assure the creation of a homogenous matrix layer on top of the tissue surface. These processes produce layers of matrix crystals of varying sizes and thicknesses. To boost molecular ion yield in metal-assisted SIMS, a thin film of gold can be put on the sample. The spatial resolution achievable in matrix-based MSI techniques is governed by the size of the matrix crystal and the laser point. SIMS's spatial resolution beats MALDI by one to two orders of magnitude due to the small width of the ion beam and the decreased sample preparation. The size, form, stability, and solvent employed for the spray all have an impact on the spatial resolution in DESI-based MSI. For some endogenous chemicals with poor ionisation efficiencies, reactive DESI can be useful. The analyte is derivatized with a reagent in the spray solvent in this method.

MSI mass accuracy, mass resolution, and resolving power are determined by the mass analyzer employed. The Time of Flight

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(TOF) mass analyzer is the most often utilised mass analyzer for MALDI-MSI because it offers a potential limitless mass-to-charge (m/z) range and single ion detection capabilities [5]. A TOF or even a TOF-TOF has frequently been employed in cardiovascular research to analyse distinct molecular groups. MALDI can also be used in conjunction with a Fourier transform ion cyclotron mass spectrometer (FT-ICR), which gives more mass accuracy and resolution than a TOF-MS but is less appropriate for high m/z analysis. A quadrupole TOF, an LTQ XL linear ion trap, and an orbitrap Fourier Transform MS (FTMS) are optional mass analyzers [6]. A TOF-based mass spectrometer is most commonly used for SIMS MSI. An ion source, such as a gold or bismuth liquid metal ion cannon, produces a pulse of primary ions that are propelled to the surface.

CONCLUSION

The source for DESI-MSI may be connected with several mass analyzers that can deal with continuous beams, such as a triple quadrupole, q-TOF, FT-ICR, and orbitrap. Tandem MS, in addition to the procedures stated above, is used to identify specific chemicals. Tandem MS fragments are compared with database libraries and utilised to identify the molecule; tandem MS is necessary for good structural identification. The entire mass is utilised to determine the composition, database matching, and identification when utilising accurate mass analysis. In lipid identification, for example, high-resolution mass analysis allows for the reliable assignment of lipid classes; Tandem MS is necessary for the identification of individual Fatty Acid (FA) chains. The discovery of structural lipids will improve knowledge of lipid biochemistry and their functions in (patho) physiological processes.

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