Vacuum Advances in BIOTECHNOLOGY

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Mass Spectrometry Imaging of Clinically and Pharmaceutically Relevant Biological Samples

Visualization of Spatial Distributions: The Role of Mass Spectrometry Imaging in Biotechnology

In the clinical and pharmaceutical sectors, it is often required to estimate the efficacy and safety of a drug compound. To accomplish this task, it is very important to have knowledge about the specific location of the compound as well as the concentration that is present at these points within the target tissue. This knowledge gives the ability to detect not only the level of exposure, but also the spatial distribution within the target tissue. This eventually helps understand the complex biochemical interactions that occur between a drug and its target tissue [1]. To this end, researchers have employed liquid chromatography tandem mass spectrometry (LC-MS/MS) for homogenized tissues for quantitative assessments to determine drug exposure in the target tissues. However, LC-MS/MS does not give the information on the spatial distribution for the compound in the target tissue [1].

Mass spectrometry imaging (MSI) can overcome this challenge by providing information on the spatial distribution and chemical composition of analyte molecules on complex surfaces [2]. MSI has emerged as an established tool in the field of biotechnology research including clinical practice and the pharmaceutical industry, where spatial information in tissue samples is required. Recent studies have shown that the combination of information gained from MSI along with visualization of spatial distributions in biological samples can be a valuable chemical analysis tool for a range of biological specimen characterization [2]. MSI can be employed to image thousands of molecules, such as metabolites, lipids, peptides, proteins, DNA and glycans, in a single experiment without labeling. This also includes multivariable analysis that can be used for simultaneous analysis of various compounds [2]. Based on the high spatial resolution, sample surface sensitivity, and superior statistical analysis, the application of MSI has opened a vast array of new avenues in analysis of pharmaceuticals and complex metabolites of biological systems that include biomarker discovery for degenerative brain disease and cancers, and drug tracking in the body, just to name a few examples [2, 3].

Components and Workflow of Mass Spectrometry Imaging Analysis

Researchers have shown that improvement in ionization efficiency of the conversion of target molecules into gas phase ions is extremely important in order to have the widespread adoption of MSI as an analytical tool for surface analysis. To this end, high vacuum and ultra-high vacuum technology have been shown to play a vital role in enhancing the ionization efficiency for high-resolution mass spectrometry imaging [2]. **Figure 1** (top) shows the various components of mass spectrometer [4]. Mass spectrometers usually comprise of an ion source that creates ions, a mass analyzer, a detector, and a data system to control the instrument and to acquire the data (**Figure 1**) [4]. The role of the ion source is to create a focused ion beam that is injected into the mass analyzer.

The ion source housing is designed in such a way that it provides various means of transferring the biological sample into the vacuum environment of the ion source where ionization is effected. In this way, the inlet system transfers the gaseous form of sample into the vacuum of the ion generation chamber of mass spectrometer [4]. In the ion generation chamber, neutral sample molecules are ionized and then accelerated into the mass analyzer tube, which is the most important part on which a range of the mass spectrometer depends [4]. This step is followed by the separation of generated ions that are either in space or in time, according to their mass-to-charge ratio (m/e). Subsequently, the

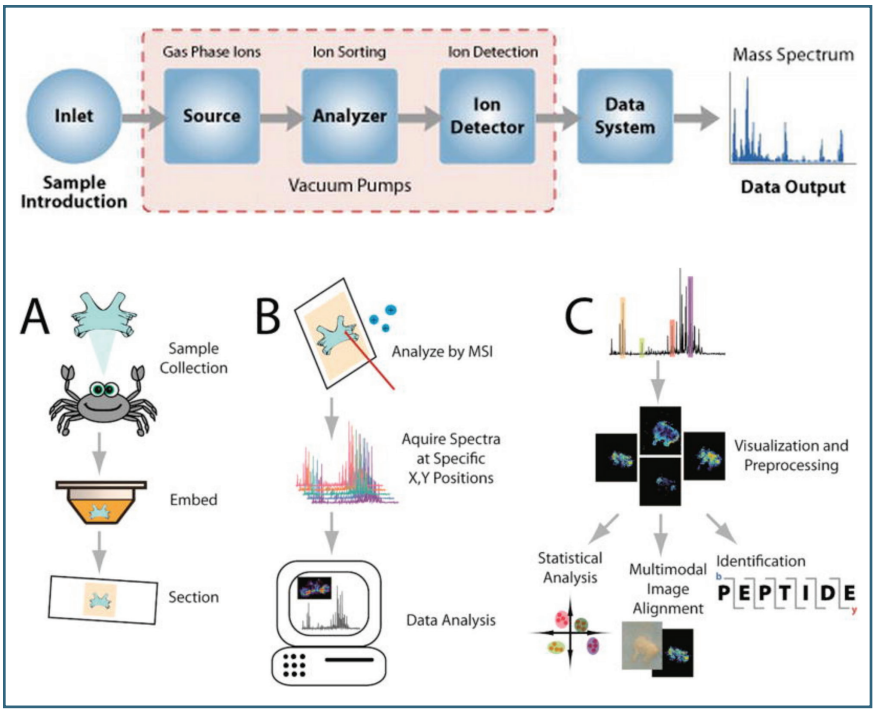

Figure 1. (*Top*) Components of a mass spectrometer are shown [*Source:* IntechOpen, (2017)]. (*Bottom*) [A-C] Visual workflow for the mass spectrometry imaging analysis is schematically illustrated that involves a crustacean's brain as an example tissue for sample preparation. The sample is then embedded in a supporting medium for sectioning onto slides, which is followed by sample analysis and acquiring of a spectrum at each (x,y) grid point on the tissue. Software tools are used to process and visualize the data. After preprocessing the data (e.g., baseline correction), the distribution of selected molecules can be visualized. This is followed by identification (e.g. Peptide) of the m/z values and statistical analysis between different images or image co-registration with other image modalities [*Source:* Anal Chem (2018)].

separated ions are collected and detected in ion collector chamber. The signal is ten transferred to a data collection system for data investigation [4]. The high vacuum or ultra-high vacuum, which is applied between the ion generation chamber, analyzer tube and ion collector maintains the required low pressure in the system that helps minimize the chances of ion-molecule reaction as well as scattering and neutralization of the ions (**Figure 1**) [4]. The detector, which is often a secondary electron multiplier can convert the event of ion impact into an electrical signal. This can be then used for data acquisition.

Figure 1 (bottom) shows a summary workflow of mass spectrometry imaging of biological samples. This workflow involves a careful sample preparation as the first step. Subsequently, the general setup of an MSI experiment deals with defining an (*x, y*) grid over the surface of the sample, where the grid area is chosen by the user [3]. The next step is ionizing of the molecules by the mass spectrometer on the surface of the sample, which results in the collection of a mass spectrum at each pixel on the section. The resulting spatial resolution defined by the pixel size [3]. The spectra is subsequently collected, which is followed by the application of a suitable computational software to select an individual mass-to-charge *(m/z)* value. The intensity of the *m/z* is extracted from each pixel's spectrum. These intensities are finally then combined into a heat map image that depicts the relative distribution of that *m/z* value throughout the sample's surface [3].

In the following section, we will describe some of the notable applications of mass spectrometry imaging.

Murine Brain Atlas of Serotonin, Dopamine, and Norepinephrine Levels Using Mass Spectrometry Imaging

Monoamine neurotransmitters are a family of small molecules that include serotonin (5-hydroxytryptamine, 5-HT), dopamine (DA), and norepinephrine (NE). These neurotransmitters are secreted by specialized neurons that regulate behavioral, motor, and cognitive functions [5]. Researchers have shown that the monoamine neurotransmitters in the brain cannot cross the blood-brain barrier, and their total amount is determined by the ratio between local synthesis and degradation. Further, it is known that the changes in behavior, such as impulsiveness, or the sleep-wake cycle pharmacological or even cognitive functions can be caused by genetic alterations in monoamine neurotransmitter levels can cause. It is therefore, believed that the abundance and localization of monoamine neurotransmitters in the brain must be tightly controlled in view of their essential roles in controlling goal-directed and adaptive behavior. While the localization of monoaminergic neurons in the brain is known, it is challenging to gain insights into the distribution and kinetics of monoamines [5].

To address this challenge, researchers generated a murine brain atlas of serotonin (5-HT), dopamine (DA), and norepinephrine (NE) levels using mass spectrometry imaging (MSI) [5]. They used MSI to generate an atlas of 5-HT, DA, and NE in the whole brain of the C57BL/6J mouse (**Figure 2**). Their MSI-based study revealed an unexpected accumulation of multiple monoamines, particularly 5-HT and a catecholamine (DA or NE), in several brain nuclei [5]. They showed that quantitation of 5-HT revealed the paraventricular nucleus of the thalamus (PVT), which received a raphe-derived dense serotonergic innervation (**Figure 2**) [5].

These results paved the way to advance our understanding about monoamine distribution in the brain and the regional differences in 5-HT metabolism. Further, this study suggested the serotonergic nervous system targeting different brain regions with different priorities. This shows that MSI can a powerful tool, which can be employed to detect monoamine fluctuations in the brain of mice subjected to a behavioral experiment. This could eventually facilitate a better understanding of the complexity of the serotonergic network in the brain [5].

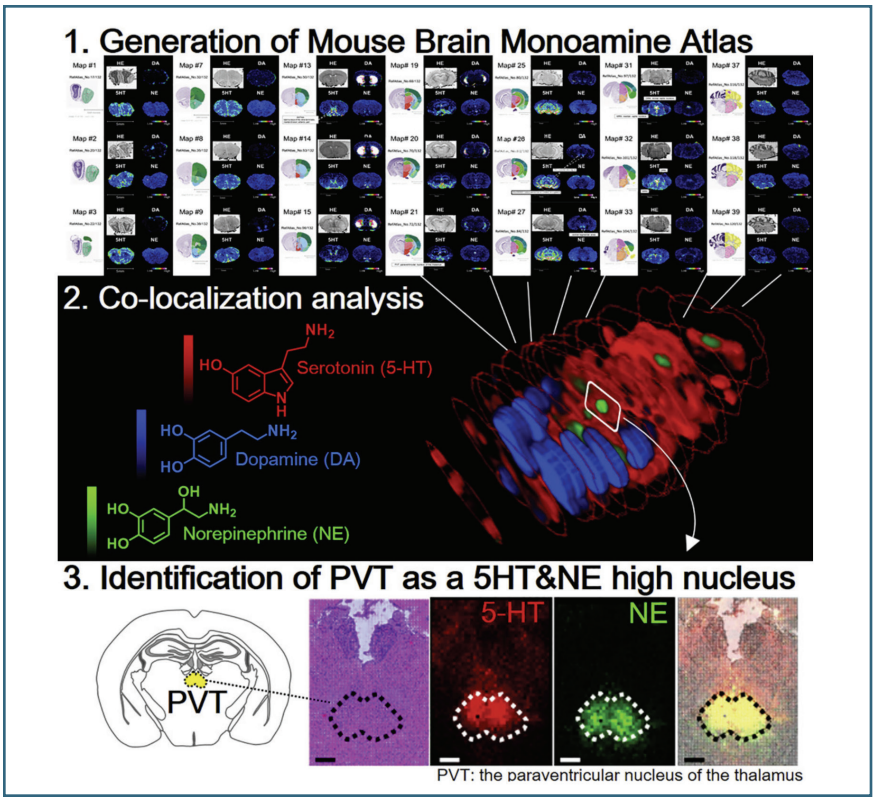

Figure 2. [1-3] A murine brain atlas of monoamine (5-HT, DA, NE) levels that was generated via mass spectrometry imaging and subsequent data of paraventricular nucleus of the thalamus (PVT) that had high levels of 5-HT and NE [*Source:* iScience (2019)].

MALDI and SIMS Based Mass Spectrometry Imaging for Clinical Analysis of Hard Tissue in Abnormal Bone Fracture Healing

Bone fractures and injury-related articular cartilage damage are considered common traumas to the skeletal system. The healing process can be impaired in most of the cases that can result in non-unions of bone in about 5–10% of the bone fractures and up to 75% of the cases of cartilage damage in posttraumatic osteoarthritis (PTOA) [6]. While there have been studies in the areas of fracture healing and cartilage repair as well as non-unions and PTOA and the knowledge of some key molecules in the healing processes has been gained, it is still challenging to predict the outcome of a bone fracture or articular cartilage damage [6]. In addition, there is a lack of understanding on the exact molecular contribution and interactions with these processes as well as how these molecules are involved in non-union and PTOA development are not fully known [6]. This poses huge clinical challenges related to abnormal fracture healing. It is believed that advancing the knowledge of molecules involved in abnormal fracture healing can potentially lead to the development of new prediction, diagnosis, and treatment options [6].

To this end, researchers have employed MSI of hard tissue. Especially, matrix-assisted laser desorption/ionization (MALDI) and secondary ion mass spectrometry (SIMS) imaging (**Figure 3**) have been used to imaging hard Tissue for Clinical Analysis in abnormal bone fracture healing [6]. While MALDI uses a matrix to be deposited on the tissue section during to support the desorption and ionization of the molecules from the tissue, SIMS works on the basis of the generation of secondary ions created and ejected from the tissue by sputtering its surface with a focused primary ion beam [6]. It has been shown that the analytical abilities of MALDI are broader compared to SIMS. A wide range from the analysis of small molecules such as metabolites to larger (intact) proteins, including also lipids, peptides, and glycans is possible by MALDI. On the other hand, a higher spatial resolution (down to 50 nm) can be achieved by SIMS than the current achievable spatial resolution with MALDI (commonly down to 10 µm) [6].

Using MSI, researchers investigated risk factors and key molecules involved in the repair process, together with the main challenges associated with the prediction of outcome of these injuries [6]. They employed MSI for detecting a wide variety of molecules in tissues. This contributed to extending molecular understanding of impaired healing and the discovery of predictive biomarkers [6].

Recent studies have suggested that MSI analysis of bone, cartilage, and surrounding tissue can potentially pave the way to new breakthroughs in advancing our knowledge and fundamental understanding of the key molecular processes involved in bone fracture healing and cartilage repair. Studies of a broad range of molecules can result in defining key molecules including lipids and proteins and the pathways well as their distribution. This could be used for the development of new therapeutics in traumas to the skeletal systems [6].

Mass Spectroscopy Imaging in the Cardiovascular Diseases and Clinical Diagnosis

In the cardiovascular research field, MSI is an emerging technology for structural analysis and identification of lipids, proteins/peptides, and metabolites. MSI has shown the potential for implementation in (pre)clinical research and complementing the diagnostic tests in cardiovascular diseases particularly in ischemic heart disease and stroke. The application of MSI is believed to provide new insights into disease progression and thereby contribute to the understanding of underlying mechanisms related to cardiovascular diseases [7]. The characterization by MSI can provide vital information of biochemical changes that can help understand the pathophysiology, which may eventually be used in clinical applications [7].

In many cellular processes, lipids play a vital role. Lipids includes among others fatty acids (FA), steroids and many classes of phospholipids (PL). High-resolution mass spectrometry imaging analysis has been employed in lipid identification that has enabled assignment of lipid classes. Tandem MIS is used for the identification

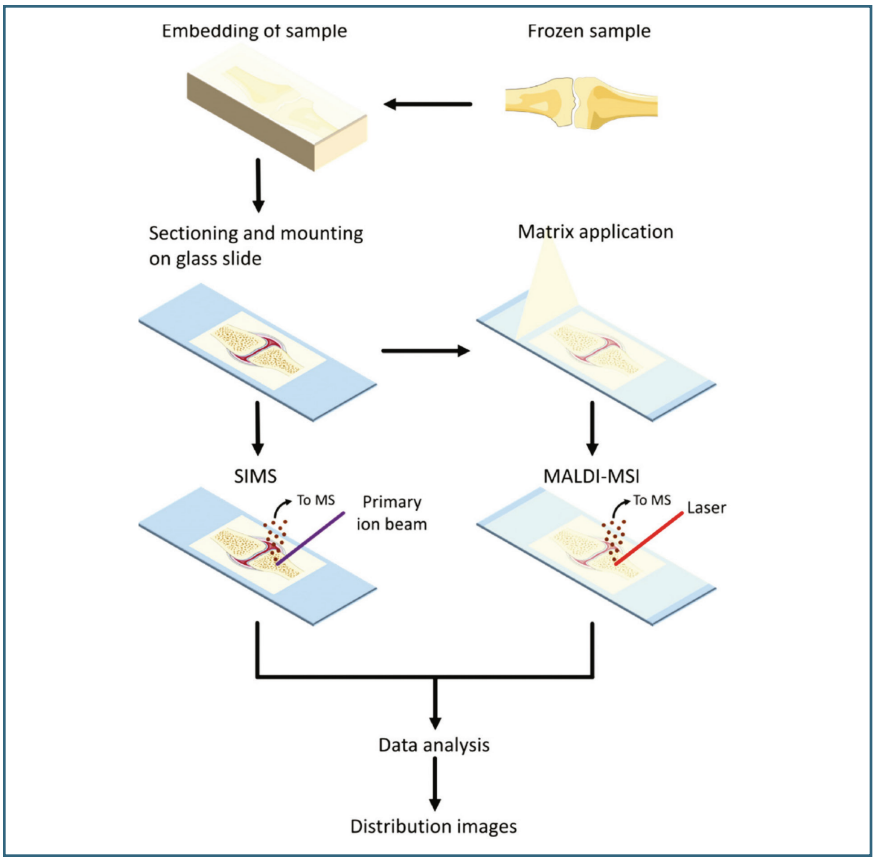

Figure 3. A schematic depiction shows the collection of frozen bone hard tissue samples for secondary ion mass spectrometry (SIMS) and for matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI). Per pixel in the image, a mass spectrum is created of the generated ions, which is followed by distribution images of mass-to-charge values (m/z) [*Source:* Clin Chem Lab Med (2020)].

of the individual FA chains. The human heart uses FA as its major energy substrate. In addition to FA, its lipidome consists mainly of (lyso)phospholipid, sphingolipid species, and neutral lipids. Thus, structural lipid identification can help understand lipid biochemistry and their roles in (patho)physiological processes [7]. To this end, cardiovascular lipid imaging was done with a time-of-flight spectrometry combined with MALDI or SIMS [7].

In addition to Lipids identification and imaging, researchers have employed peptide and protein MSI to cardiovascular diseases. They investigated the role of the enzyme aminopeptidase A (APA) in the metabolism of cardiac angiotensin (ANG), focussing on ANG II and ANG-(1-7) [7]. A MALDI-MSI enzyme assay was used to visualize the metabolism pathway. To achieve this visualization, the heart sections were incubated with ANG II. Subsequently, higher APA protein levels were detected in MI compared with sham mouse hearts, which suggested the degradation of ANG-(1-7) in cardiac repair and reduction of the ventricular function after MI [7]. Human MI cardiac tissue was used to identify proteins reflecting cardiomyocyte viability, where the damaged lesions showed an enhanced signal for hemoglobin subunit α, adenosine triphosphate synthase subunit alpha (ATPA), and the sarcomeric proteins myosin-6, myosin-7, myosin light chain 3 (MYL3), and alpha actin 2 (**Figure 4**) [7].

The application of MSI to image cardiac proteins to investigate their role in cardiac (dys)function could open up new therapeutic pathways to treatment and diagnostics for personalized medicine. The advantage of MSI in this case is that it allows to obtain the combination of spatial information and the relative abundance of cardiac proteins in different phases of disease progression. It is believed that in the future, together with MSI information, the clinical analysis can be performed that can eventually contribute to the understanding of involved pathways in cardiovascular diseases [7].

Concluding Remarks

Mass spectrometry imaging (MSI) has emerged a very powerful tool in modern

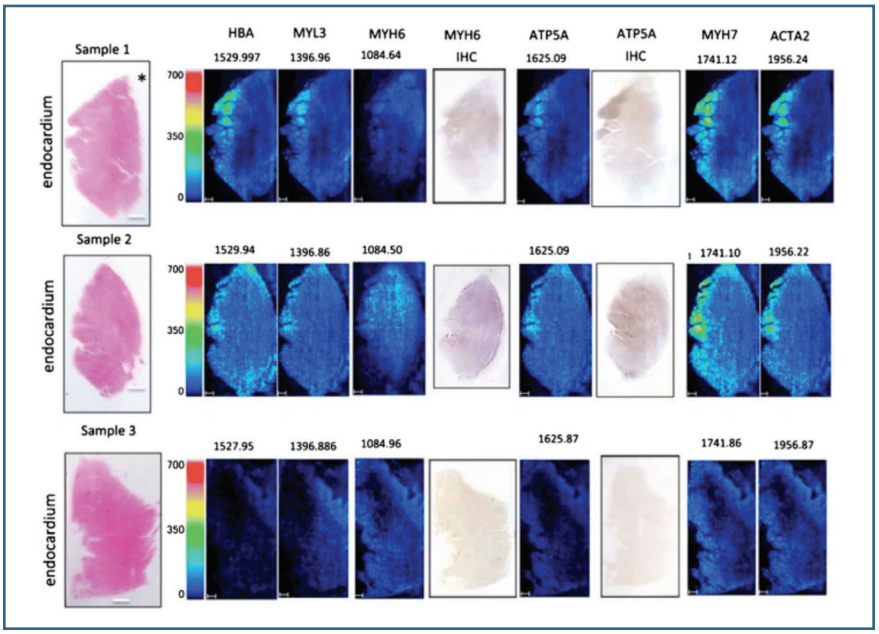

Figure 4. Peptide MALDI-MSI analysis of cardiac tissue shows the left column containing H&E staining and orientation (endocardium and pericardium) of the samples. Other columns show ion images of the identified proteins including m/z values or the corresponding immunohistochemistry [*Source:* Anal Bioanal Chem (2019)].

biotechnology to analyze and image biological samples that are of clinical and pharmaceutical significance. This field is a truly a multidisciplinary area of research where experts in vacuum technology, biotechnology, spectroscopy and medicine have come together to find solutions to some of the most complex medical challenges in modern healthcare. We anticipate that MSI based analysis and diagnostics will further evolve and expand into other areas of biomedical science and engineering in the future.

References for Further Reading

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