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Desorption electrospray ionization mass spectrometry for lipid characterization and biological tissue imaging ☆

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ABSTRACT

Desorption electrospray ionization mass spectrometry (DESI-MS) imaging of biological samples allows untargeted analysis and structural characterization of lipids ionized from the near-surface region of a sample under ambient conditions. DESI is a powerful and sensitive MS ionization method for 2D and 3D imaging of lipids from direct and unmodified complex biological samples. This review describes the strengths and limitations of DESI-MS for lipid characterization and imaging together with the technical workflow and a survey of applications. Included are discussions of lipid mapping and biomarker discovery as well as a perspective on the future of DESI imaging.

1. Introduction

DESI is one of a recently developed group of ambient ionization techniques in mass spectrometry in which samples are examined in the ambient environment with minimal pretreatment [1–3]. DESI has been increasingly used for direct lipid analysis from biological samples, especially in the medical field [4–6]. These characteristics combined with its ease of execution make DESI a technique of some significance in the field of imaging mass spectrometry [7]. The DESI-MS imaging experiment is carried out by directly scanning the unmodified sample in the *x* and *y* directions through an impinging spray of charged droplets; the chemical information obtained can then be plotted as two-dimensional images recording the abundance of specific ions [8]. Many of the promising results for the analysis of biological samples are based on differences in lipid composition [9,10]. Thus far, DESI-MS imaging has been used for the analysis of multiple types of human tissue encompassing diseased tissue, adjacent normal tissue and non-diseased tissue. The measurements are highly reproducible although not strictly quantitative. In this review we cover the principles of DESI-MS imaging, its use for lipid analysis and characterization, describe the lipid classes detected and discuss the technique.

High performance liquid chromatography (HPLC), gas chromatography (GC) and thin-layer chromatography (TLC) are traditionally used for lipid separation and can be followed by MS analysis [11,12]. However, these are lengthy methods and do not provide information on spatial distribution as is achieved by MS imaging techniques. Imaging mass spectrometry allows direct analysis of the spatial distribution of a variety of compounds without the need for fluorescent or radioactive labeling normally used in histochemical protocols [13,14]. Most imaging MS is currently done using the desorption ionization methods of secondary ion mass spectrometry (SIMS) [15,16] and matrix assisted laser desorption ionization (MALDI) [17]. Both ionization methods are vacuum techniques which require sophisticated instrumentation. As an electrospray based ionization method, DESI-MS imaging brings different and complementary capabilities, although MALDI remains the main imaging MS technique for biological sample analysis. MALDI has been traditionally used to image larger biomolecules such as proteins and peptides in biological tissues [18], even though it is also effective for the analysis of smaller molecules such as lipids [19]. One limitation

Abbreviations: DESI-MS, desorption electrospray ionization mass spectrometry; HPLC, high performance liquid chromatography; GC, gas chromatography; TLC, thin-layer chromatography; SIMS, secondary ion mass spectrometry; MALDI, matrix assisted laser desorption ionization; FA, fatty acids; GP, glycerophospholipids; GL, glycerolipids; SP, sphingolipids; ST, sterol lipids; SM, sphingomyelin; PS, glycerophosphoserine; GalCer, galactoceramide; H&E, hematoxylin and eosin; PE, glycerophosphoethanolamines; plasm-PE, plasmenyl glycerophosphoetanolamines; PI, glycerophosphoinositols; PG, glycerophosphoglycerols; ST, sulfatides; PC, glycerophosphocholine; CE, cholesteryl esters; LIT, Linear ion traps; hST, hydroxylated sulfatide; DHSM, dihydrosphingomyelin; TAGs, triacylglycerides; DAG, diacylglycerols; PCA, principal component analysis; PLS-DA, partial least squares discriminant analysis; RCC, renal cell carcinoma; GBM, glioblastoma; LPC, lysoglycerophosphocholines; LAESI, laser ablation electrospray ionization; LA-FAPA, laser ablation coupled to flowing atmospheric-pressure afterglow; AP fs-LDI, atmospheric pressure femtosecond laser desorption ionization; REIMS, rapid evaporative ionization mass spectrometry

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for small molecule analysis by MALDI is the interference of matrix ions in the low *m/z* region of the mass spectrum [20]. In contrast, lipids are easily ionized and measured by DESI-MS. DESI-MS has been used for the direct analysis, characterization and imaging of many classes of lipids including fatty acyls (FA), glycerophospholipids (GP), glycerolipids (GL), sphingolipids (SP) and sterol lipids (ST). More recently, DESI-MS has been mainly applied for tissue section analysis in an attempt to enable disease diagnosis based on lipid profiles and the presence of specific lipid species as characterized by tandem MS experiments, complemented by the use of multivariate statistical analysis of the data (Fig. 1) [21].

Lipids comprise a group of diverse molecules with different structures and functionalities that play important roles in cellular processes [22]. The study of the lipid composition as means of characterizing biological samples is potentially important since understanding the role that lipids play in normal cells can lead to an understanding of how lipids function in disease states [9]. Extensive investigations have reported that the lipid composition of tissues can vary with disease state [23]. In the area of cancer research, a number of studies focus on the molecular changes that occur in cells, signaling the beginning of malignancy and providing a means for early detection and treatment intervention [24,25]. GPs for example reflect cellular growth, maturation and differentiation as well as histological cell type. Cell membranes have non-uniform distributions of GPs with particular species appearing specifically in the inner or outer membrane [26]. Alterations in this distribution are indicative of cell transformations, including malignancy [27]. In colon cancer, it has been shown that both primary and colon cancer liver metastasis exhibit abnormal GP distributions compared to normal tissue, indicating structural and functional modifications of the cell membrane [28,29]. Changes in lipid tissue content also indicate tumor progression in human brain cancer, with significant alterations in sphingomyelin (SM), glycerophosphoserine (PS) and galactoceramides (GalCer) content during glioma development [30]. As building blocks for GPs, FAs are important to cell structure and function and therefore it is expected that the FA composition of cancerous cells would differ from that of normal cells. Many studies have sought to examine the changing profiles of FAs in order to understand the metabolic pathways in malignancy. For example, changes in FA composition occur between intraepithelial cervical lesions, cervical cancer and normal cells showcasing the path toward carcinogenesis [31,32]. FA patterns have also been found to differ between stomach cancer tissue and normal gastric mucosa, with overall FA content being increased in cancerous tissue [33]. Besides cancer, lipid composition alterations have been reported for many other diseases, such as in the neurodegenerative Alzheimer's disease [34] and cardiovascular diseases including atherosclerosis [35]. These findings reported by an increasing number of research studies in lipidomics emphasize the importance of determining the composition of lipids in biological tissues for its prognostic value in determining and diagnosing disease.

2. DESI-MS methodology for lipid analysis

In the DESI-MS experiment, a spray of charged droplets is directed towards the sample. When the spray impacts the sample, a thin layer of solvent is formed into which the analytes may dissolve. As other primary droplets arrive at the sample surface, they splash secondary microdroplets containing the dissolved analytes from the solvent film. This mechanism, "droplet pickup" [36], causes analyte containing droplets to be generated in the open air, and then delivered to the mass spectrometer through a heated extended capillary. Fundamental studies have shown that under typical DESI experimental conditions the average velocity of the primary droplets is about 120 m/s, with an average diameter of about 3 μm [37]. Simulations of the DESI process show the release of dozens of microdroplets in the range of 0.8 to 3.3 μm, from a single droplet-thin film collision event [36]. After the desorption process, ionization occurs via mechanisms that are similar to those of electrospray ionization. Tandem MS or high mass resolution experiments are normally used for detailed characterization of individual lipids.

2.1. Biological sample preparation

Biological samples analyzed by DESI-MS include tissue extracts [38], bacterial colonies [39], plant tissue [40,41] and animal tissue sections [7]. Samples can be directly spotted or deposited on a solid surface for analysis while in the case of tissue sections, imaging analysis is done from a single piece of tissue normally 5–25 μm thick, mounted onto a glass slide and stored at −80 °C until analysis. While tissue analysis by DESI has so far been limited to fresh frozen tissue, expansion to formalin fixed paraffin embedded samples is expected. Surfaces of choice for DESI-MS include polytetrafluoroethylene printed glass which gives the most intense, stable and long-lasting signals for standard phospholipid samples applied directly to the surface [38]. Nevertheless, glass slides are the most commonly used substrates for lipid analysis [42] when done for tissue imaging. This is largely due to the fact that traditional

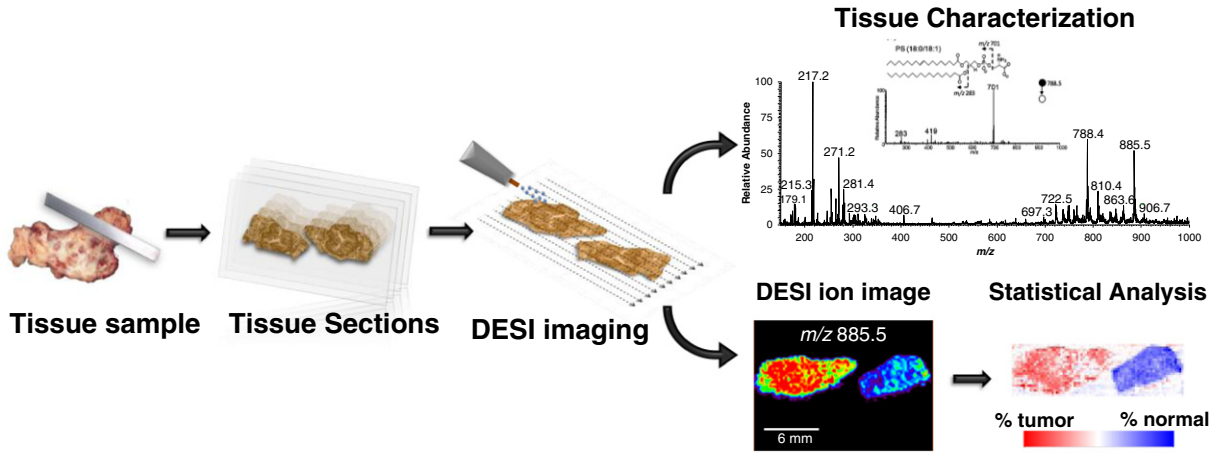

Fig. 1. Schematic of DESI-MS imaging of biological tissue. Tissue samples are cryosectioned and tissue sections thaw mounted onto glass slides, then directly analyzed by conventional or reactive DESI imaging. Tissue characterization is performed based on the lipid profiles detected and identification of particular lipid species by tandem MS analysis. For cancer diagnostics, statistical analysis of the entire image data is performed with the goal of generating classification rules. Figure adapted in part from
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