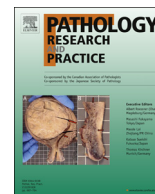




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Review

Mass spectrometry in pathology – Vision for a future workflow

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ABSTRACT

Mass spectrometric (MS) techniques are applied in various areas of medical diagnostics. For the detection of microbiological germs and genetic mutations, MS is a method used in routine. Since MS also allows the analysis of proteins and peptides, it seems an ideal candidate to supplement histopathological diagnostics. Matrix-assisted laser desorption/ionization time-of-flight Imaging MS links molecular analysis of numerous analytes with morphological information about their spatial distribution in cells or tissues. Herein, we review principle MS techniques as well as potential applications in pathology and discuss our vision for a future workflow.

1. Introduction

Mass spectrometric (MS) techniques are applied in various areas of medical diagnostics.

In microbiological germ detection, MS is a routine diagnostic method since the last years [17,58]. Similarly, this technique has been introduced into toxicology [51], as well as in forensic medicine [6,28]. For the analysis of DNA, this technique allows the detection of several hundred different mutations and is therefore applied in molecular pathology already today [41,45]. However, as the identification of mutations by MS is based on prior PCR amplification, the method is most suitable for the detection of hotspot mutations and there are limitations when it comes to the identification of complex mutations or genetic aberrations.

As MS also allows the analysis of proteins [68] and peptides [40,48], it seems an ideal candidate for histopathological diagnostics which strongly relies on the detection of proteins and peptides by immunohistochemical (IHC) methods [71]. An advantage of MS as compared to IHC, is the detection of numerous proteins or peptides without the need for target-specific antibodies [56]. On the other hand, the identification of the detected ion peaks might be a challenge. MS can be applied in the profiling mode where a region of a tissue section has been chosen for application of the laser which ionizes the proteins or peptides or in the imaging mode, where numerous proteins or peptides can

be localized in a given tissue section [2].

Furthermore, other chemical classes can be detected in tissue such as lipids or phospholipids [14,31,39,70], carbohydrates and glycoconjugates [37], exogenous or endogenous small molecules, especially molecules playing a role in drug metabolism [34,50,65] and nucleic acids [38,57].

Matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF) Imaging MS (MALDI-IMS), first described by Caprioli et al. in 1997 [9], links molecular analysis of numerous analytes with morphological information about their spatial distribution in cells or tissues [7,12,22,63] and provides unbiased visualization of the arrangement of biomolecules [29].

The information contained in tissues cannot be replaced by investigation of serum or blood [67]. Therefore, pathology is not only a large field of medical research but also a basis for diagnostics of various diseases and treatment decisions.

2. General principle of mass spectrometry

MS is a wide field with various different specialized methods applied. However, all are based on the fact that molecules from a target e.g. a tissue section are ionized and subsequently measured. MALDI is the most common method applied for ionization. In MALDI experiments, the time-of-flight of the respective analyte is measured by a

Abbreviations: DNA, deoxyribonucleic acid; EGFR, epidermal growth factor receptor; H&E, hematoxylin and eosin; KRAS, V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; LC, liquid chromatography; MALDI, matrix-assisted laser desorption/ionization; MALDI-IMS, matrix-assisted laser desorption/ionization imaging mass spectrometry; MS, mass spectrometry; NRAS, neuroblastoma RAS viral oncogene homolog; PCR, polymerase chain reaction; TOF, time-of-flight

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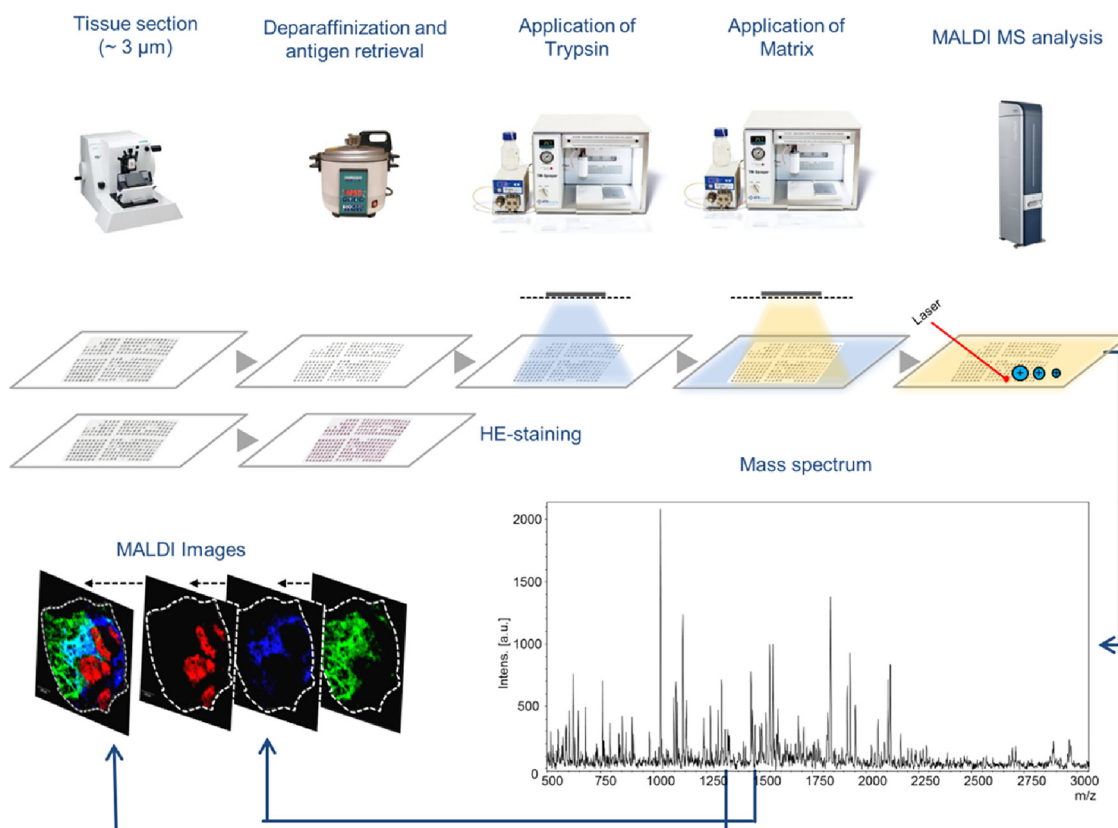


Fig. 1. Workflow of a MALDI-IMS experiment.

A typical workflow of a MALDI-IMS experiment is illustrated. Tissue sections are mounted on an indium-tin-oxide covered glass slide, the sample is prepared for the MALDI analysis and mass spectra are acquired in a rastered fashion at a resolution of 10–50 µm. For each peak, the distribution throughout the tissue can be visualized.

detector and the ions are categorized based on their mass-to-charge ratio (m/z value). Matrix is applied to a sample in order to prepare the sample for analysis and a laser subsequently irradiates the solid preparation. This leads to the ionization and desorption of the molecules (Fig. 1). Various substances such as proteins, peptides, carbohydrates or lipids can be detected by MALDI. IMS allows to localize specific molecules throughout a tissue section. Other common ionization methods applied in IMS are summarized in Table 1. An example of a pancreatic cancer specimen analyzed by MALDI-IMS is shown in Fig. 2. The detection of genetic mutations is based on DNA isolation followed by PCR-amplification of the respective DNA sequence. If a genetic mutation is present, a mass peak of the wild-type allele and a second mass of the mutated allele are detected at specific locations in the mass spectrum.

3. Detection of different molecules

The following paragraph illustrates examples for the detection of various molecules. With regard to proteins, Meding et al. analyzed 171 fresh frozen samples from adenocarcinomas of esophagus, breast, colon, liver stomach and thyroid. A primary set was used for training and a secondary set was utilized for confirmation. In the confirmation set, an accuracy of > 80% for the classification of the six different tumor origins was achieved [53]. Concerning peptides, most studies have analyzed formalin-fixed paraffin-embedded tissue samples from routine diagnostic archives [44,72,74]. In a recent study, we show that adenocarcinoma and squamous cell carcinoma of the lung could be reliably differentiated by MALDI-IMS. Out of 118 samples, 117 could be correctly classified, which surpasses the accuracy of a single IHC marker [42]. Among the 339 m/z values that were used for classification in this study, some were subsequently identified by tandem MS and validated by IHC. Interestingly, we found known immunohistochemical

discriminative markers such as cytokeratin 5, but also potential new markers such as cytokeratin 15 or Heat-shock-protein-beta 1. Although these results should be validated in a larger dataset, they highlight the potential of MALDI-IMS on routine paraffin material.

Also carbohydrates and glycoconjugates may be detected for classification purposes [20] and it is well documented that alterations and changes in cell surface glycosylation occur during tumorigenesis [16]. Thus, it is not surprising that tumor and stroma show distinct N-glycan distributions, which has recently been demonstrated in high-grade serous ovarian cancer and hepatocellular carcinoma [25,62].

Lipids are another molecule class that can be detected by MS. In a prove of concept study including 36 gliomas, a correct classification (subtype and grade) on an independent test set could be made in 79%, based purely on lipids [24]. Naturally, pathologists do have a sound understanding of proteins and peptides, as the application of histochemistry and IHC targets these molecules. However, other molecule classes might very well contribute to a more detailed understanding and ultimately to a better classification of diseases.

Besides proteins, carbohydrates, glycoconjugates and lipids, nucleic acids can be detected. This application of MS is already well established in many laboratories; either for the detection of e.g. viruses such as human papilloma virus, bacteria including their resistances to antibiotics and parasites [13,45,55,76,78], or for the detection of genetic mutations in various genes including *KRAS*, *NRAS*, *EGFR* and others [30,41,49,75].

4. Perspective workflow in pathology including mass spectrometry

The regular workflow in pathology includes as a first step diagnosis on hematoxylin and eosin (H&E)-stained tissue sections [33]. Adjunct

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