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Invited critical review

Matrix-assisted laser desorption ionization/time-of-flight mass spectrometry for clinical diagnosis

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ABSTRACT

Background: Matrix-assisted laser desorption ionization/time-of-flight (MALDI-TOF) mass spectrometry is known as an extremely sensitive analytical tool for characterizing different types of biological compounds including proteins, peptides and lipids. Since MALDI-TOF analysis requires very simple sample pretreatment, the technique can be used for rapidly detecting biochemical compounds serving as disease biomarkers.
Results: This mini-review focuses on the applications of MALDI-TOF in the detection of potential disease biomarkers in various biological samples.
Conclusions: The potential disease biomarkers are mostly abundant proteins, peptides, or lipids including: albumin; hemoglobin; α-defensins; trimethylamine; phospholipids; and glycatedα- and β-globin, which are indicators of albuminuria; fecal occult blood and ischemic stroke; dry eye disease and/or aging; trimethylaniuria; breast cancer; and diabetes, respectively.

Contents

1. Introduction

Matrix-assisted laser desorption ionization/time-of-flight (MALDI-TOF) mass spectrometry is an extremely sensitive technique that permits the detection of chemical and biological compounds at abundances below sub-femtomole ($<10^{-15}$ mol) [1–3]. The technique offers soft ionization potential, a relatively low degree of fragmentation, and uncomplicated spectra comprised of mostly singly charged ions. It is therefore a powerful analytical tool for the analysis of fragile biomolecules such as peptides and proteins. MALDI-TOF has also been renowned for its extreme ease of operation [4,5] and requiring inexpensive matrix for sample preparation; more importantly, the instrumentation is capable of automation, thus making the screening large sample numbers possible. Compared to liquid chromatography/mass spectrometry (LC/MS),

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MALDI-TOF can tolerate much higher salt concentration, this means proteins or peptides can be rapidly detected in biological sample without undergoing tedious desalting processes.

Conventionally, MALDI-TOF is one of the main analytical tools used in proteomics to identify proteins, peptides, and other molecules of biological interest [6–9], and is often combined with 2-dimensional polyacrylamide gel electrophoresis (2D-PAGE) for protein separation and identification. In clinical proteomics, this approach has been used to evaluate the variations of specific protein or peptide expressions in biofluids from controls and patients [10–12]. Unfortunately, the applicability of the approach for clinical screening and diagnosis is limited by problems with method reproducibility and the time- and labor-intensive factually [13].

Actually, the experimental procedure required to complete a typical MALDI-TOF analysis without 2D-PAGE is extremely simple: the sample solution is mixed with a matrix solution on a sample plate, air-dry to form a crystallized sample spot, irradiates with a focused pulsed laser, and then run through a TOF mass analyzer. Different TOF modes are available, where linear-TOF mode allows for the detection of molecules with high molecular weights, while reflective-TOF mode provides high resolution and mass accuracy for small molecule detection for bottom-up proteomics, metabolomics, and lipidomics. These features make MALDI-TOF a useful standalone tool for rapidly detecting specific biochemical compounds.

For the aforementioned reasons, several diagnostic applications have been evolved in recent years using MALDI-TOF as the analytical tool [14,15], including: (i) studying the distributions of particular biochemical compounds on tissues; (ii) identifying microorganisms or pathogens by their protein biomarkers; (iii) analyzing nucleic acids for genotyping single nucleotide polymorphisms (SNPs); and (iv) diagnosing biomarkers in biological samples for clinical purposes (Fig. 1).

Because of the high spatial resolution of its laser beam, MALDI-TOF has been applied to study the distribution of particular biochemical compounds on tissues. This MALDI-based imaging mass spectrometry (IMS) has emerged as a powerful tool for biomarker discovery and allows protein or lipid expression to be correlated with histology. Several excellent reviews about MALDI-IMS have been published in the last few years [16–20]. Conventional approaches for diagnosing microorganisms are tedious and time-consuming, while in contrast, MALDI-TOF has been employed to characterize bacteria and yeast based on differences between their peptide and protein biomarkers [21–24]. Many studies have demonstrated the reliability and accuracy of this approach [21–24]. MALDI-TOF has also been proven useful for the analysis of nucleic acids for genotyping single nucleotide polymorphisms (SNPs) [25–28].

In addition, selectively capture of disease biomarkers prior to MALDI-TOF analysis has being researched to increase the technique's effectiveness in clinical diagnostics; such a method circumvents problem with molecular interferences from other molecules in the sample. Various affinity methods, such as on beads, membranes, columns, nanomaterials, and biochips, have been employed to capture biomarkers for MALDI-TOF analysis [29–33]. Many reviews have described the diagnostic applications of affinity MALDI-TOF [34–39]. Furthermore, various sample pretreatment approaches and devices have been developed to remove abundant proteins from samples so that differences in low abundant proteins and peptides biomarkers between those of patients and controls can be revealed by MALDI-TOF analysis [40].

In this mini-review, we will focus on the fourth application listed in Fig. 1—diagnosing biomarkers in biological samples for clinical purposes. Screening for disease biomarkers in biological samples is an important aspect of clinical diagnosis [41–76]. Unlike existing biochemical diagnostic detection methods that use chemical reactions and UV, VIS or fluorescence spectroscopy, MALDI-TOF detects molecules of interest by their mass-to-charge (m/z) ratios. This approach allows unambiguous and objective diagnosis of biomarkers, avoiding interferences from reactive chemical compounds in the sample. If proper analytical strategies are developed, the detection of biomarkers for clinical diagnostics using MALDI-TOF will become efficient and practical, given the extremely simple sample pretreatment involved.

2. Directly characterizing disease biomarkers in biological samples with MALDI-TOF

Many biochemical tests that involve the detection of specific disease biomarkers via reaction with chemical reagents have been developed for clinical diagnosis. Unfortunately, this indirectly diagnostic approach suffers from false positives or false negatives due to the presence of some reactive compounds in biological samples. Directly detecting

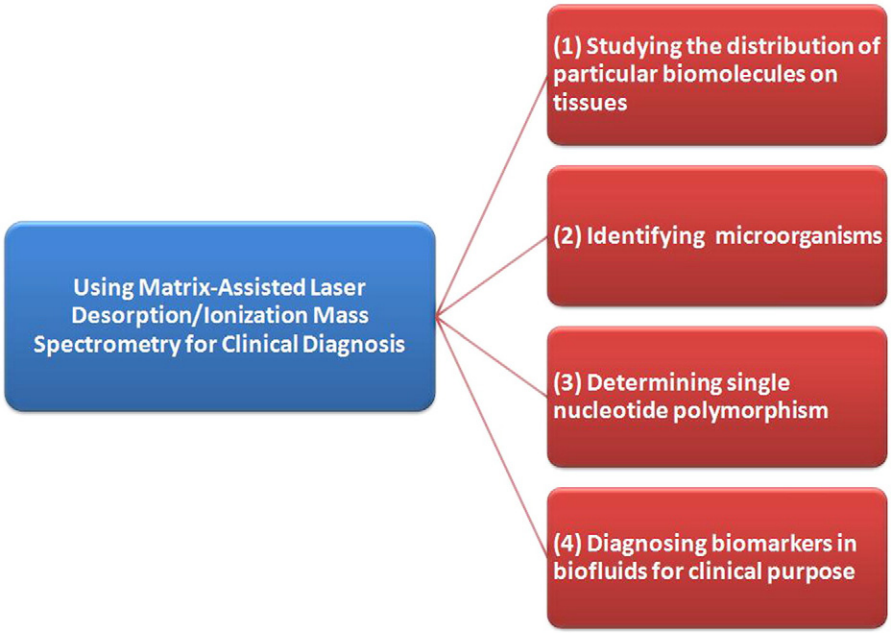

Fig. 1. Applications of MALDI-TOF/MS in clinical diagnosis.