

## Review

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# Matrix-assisted laser desorption ionisation (MALDI) mass spectrometry (MS): basics and clinical applications

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## Abstract

**Background:** Matrix-assisted laser desorption ionisation (MALDI) mass spectrometry (MS) has been used for more than 30 years. Compared with other analytical techniques, it offers ease of use, high throughput, robustness, cost-effectiveness, rapid analysis and sensitivity. As advantages, current clinical techniques (e.g. immunoassays) are unable to directly measure the biomarker; rather, they measure secondary signals. MALDI-MS has been extensively researched for clinical applications, and it is set for a breakthrough as a routine tool for clinical diagnostics.

**Content:** This review reports on the principles of MALDI-MS and discusses current clinical applications and the future clinical prospects for MALDI-MS. Furthermore, the review assesses the limitations currently experienced in clinical assays, the advantages and the impact of MALDI-MS to transform clinical laboratories.

**Summary:** MALDI-MS is widely used in clinical microbiology for the screening of microbial isolates; however, there is scope to apply MALDI-MS in the diagnosis, prognosis, therapeutic drug monitoring and biopsy imaging in many diseases.

**Outlook:** There is considerable potential for MALDI-MS in clinic as a tool for screening, profiling and imaging

because of its high sensitivity and specificity over alternative techniques.

**Keywords:** biomarker; clinical; disease; MALDI; mass spectrometry.

## Introduction

Matrix-assisted laser desorption ionisation (MALDI) is an ionisation technique that uses a laser energy-absorbing matrix to create ions with little fragmentation. As a clinical application tool, it is commonly used in microbiology for the identification of bacterial and fungal isolates; however, its application in other areas of laboratory medicine is minimal. This review explores current and future clinical applications of MALDI-mass spectrometry (MS) and its benefits and features.

## Matrix-assisted laser desorption ionisation (MALDI) mass spectrometry (MS): basics

### Origins

The term and concept of MALDI was introduced by Karas et al. [1] in 1985, who discovered that the ionisation of alanine was enhanced at a laser wavelength of 266 nm, in the presence of tryptophan. This was followed by a breakthrough for large molecule laser desorption/ionisation in 1987, when Tanaka et al. [2] managed to ionise proteins with a molecular mass of up to 34,000 Da using fine metal powder matrix dispersed in glycerol and a pulsed N<sub>2</sub> laser at 337 nm. There were further reports from the Hillenkamp group demonstrating MALDI on larger peptides and the use of a matrix for pulsed laser desorption MS [3–5].

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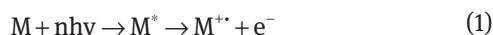
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## Mechanism

MALDI is a process that involves photovolatilisation of a sample (e.g. plasma) cocrystallised in an excessive amount of appropriate energy-absorbing matrix. Cocrystallisation of the analyte-matrix complex is achieved by spotting the mixed analyte and matrix on to a target plate and allowing to dry. The mechanism involves desorption and ionisation of the analyte-matrix crystal from the solid phase.

The initial photochemical reactions occur from pulsed laser beam irradiation resulting in the ionisation of the matrix followed by fast desorption [6]. This is sometimes referred to as primary ionisation and can be explained by Eq. 1 (photoionisation) and Eq. 2 (proton disproportionation) [7]. Photoionisation occurs via pooling of electrons in photoexcited matrix molecules, resulting in the formation of matrix-derived primary ions generated by energy pooling/annihilation processes [8]. Proton disproportionation postulates that absorbed photon energy is converted to thermal energy via ultrafast nonradiative relaxation to create ionised matrix species [9]. Both models can be shown by Eqs. 1 and 2, where M and M\* represent the matrix in the electronic ground and excited states, respectively:



Desorption begins with photoabsorption by the matrix from a laser beam, bringing the crystallised sample from the solid phase into the gas phase. The efficiency of desorption depends on the properties of the analyte and excitation conditions such as molecular weight, laser conditions and crystal morphology [10]. Typically, a decrease in the MALDI crystal size and an increase in system temperature result in increased desorption efficiency [11].

Ion molecule reactions are open to debate; however, two main models have been proposed: (i) the gas phase protonation and (ii) the lucky survivor model [12, 13]. The gas phase protonation model occurs as secondary ionisation [7]. This model assumes that the analyte is released in a neutral form and gas phase collisions with matrix ions lead to ion molecule reactions, resulting in the ionisation of the analyte. It can be represented by the following equation, where X is the analyte and MH<sup>+</sup> is the charged matrix, which transfers the charge over to the analyte (XH<sup>+</sup>), becoming uncharged (M) [14, 15]:

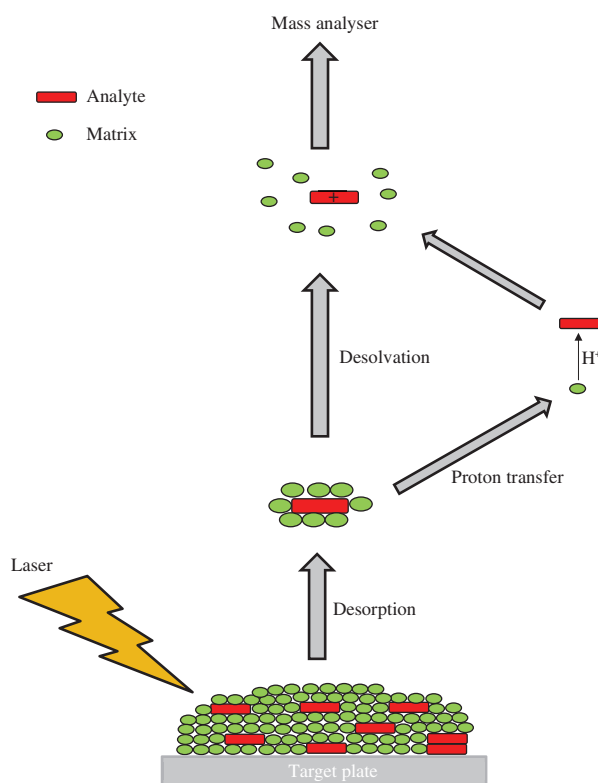


In contrast to the gas phase protonation model, the assumption of the lucky survivor model (also known as the cluster model) is that the analyte occurs as a pre-formed ion inside the matrix crystal and is released after laser irradiation as a cluster of analyte ions, matrix molecules and corresponding amounts of counterions. In this model, quantitative charge neutralisation occurs where positively charged clusters (analyte ions, matrix molecules and counterions) undergo desolvation of neutral matrix molecules, releasing singly charged analyte ions. These are known as the “lucky survivors” [16].

The ratio of occurrences of the described theories depends on the experimental parameters, analyte and matrix, with lower matrix proton affinities and increased laser fluency favouring gas phase protonation, whereas increased quantitative analyte protonation in solution and intramolecular ion stabilisation favours the lucky survivor model [17]. Both proposed models are depicted in Figure 1.

## Laser

Originally, MALDI-MS development used the quadrupled Nd:YAG lasers at 266 nm wavelength [4]. Thereafter, most analytical work used ultraviolet lasers such as nitrogen



**Figure 1:** Illustration of the MALDI process.

lasers at 337 nm [2], which were less expensive. However, low-throughput and life span limitations led the field onto solid-state lasers for high-throughput applications. Nitrogen lasers achieve a maximum repetition rate of about 50 Hz, with an average life span of only  $2 \times 10^7$ – $6 \times 10^7$  shots, whereas solid-state lasers are able to achieve considerably higher repetition rates (>1000 Hz) and have a life span typically of  $10^9$  shots [18]. Solid-state lasers such as the diode-pumped frequency-tripled Nd:YAG lasers (355 nm) offer improved robustness, higher acquisition rates, longer laser life spans and reproducibility for high-throughput applications [18]. Although not as common, infrared MALDI lasers such as Er:YAG or the tuneable optical parametric oscillator lasers [19] are also useful and offer some advantages over nitrogen lasers such as low mass interferences [20] and its compatibility with matrix free approaches [21].

## Matrix

The choice of matrix is one of the most critical components in ensuring that the ionisation of the analyte occurs. Three major functions have been suggested for the matrix, incorporating the analyte in a cocrystallised complex, absorption and ablation [22]. The role of the matrix in soft ionisation is central to minimise internal energy transfer to the analytes [23]. Analytes are cooled through collisions with neutral matrix molecules that further reduce their internal energy and prevent the ions from fragmenting. Studies showed that the temperature in the early plume was 300–400 K higher than the late plume, attributed to expansion cooling [24].

Matrix choice depends on the type of analysis. The most common matrices used are alpha-cyano-4-hydroxycinnamic acid ( $\alpha$ -CHCA), sinapinic acid (SA), 2,5-dihydroxybenzoic acid (DHB) and 3-hydroxypicolinic acid (HPA) [12]. DHB is a useful matrix for proteins, peptides, DNA and synthetic polymers, whereas  $\alpha$ -CHCA is a useful matrix for proteins and peptides, but not DNA because the ionic signal of DNA cannot be detected. SA is reportedly used for proteins and polymers, whereas HPA was shown to be the best matrix for DNA [25]. For low molecular weight compounds such as metabolites and lipids, 9-aminoacridine proved to be a suitable matrix in the negative ionisation mode [26], whereas trihydroxyacetophenone is useful for the detection of oligonucleotides and phosphorylated peptides [27].

## Sample and type of analytes

The development of MALDI-MS focussed on the detection of peptides and proteins. However, with the identification

of further matrix formulations, sample enrichment, improved sensitivity and mass range capabilities, a range of biological analytes can be accurately detected and analysed. The type of analytes routinely studied includes lipids [28], carbohydrates [29], nucleotides [30], metabolites [31] and hormones [32].

Samples can be analysed directly (i.e. without pretreatment), which results in a faster analysis, or indirectly, with pretreatment steps, that are used to increase the concentration of the analyte and decrease the degree of interference present in biological samples. Pretreatment depends on the nature of the sample, the analyte of interest and the type of matrix.

Among clinical samples, the use of biofluids has significantly increased because of routine analysis in clinical laboratories. Plasma and/or serum represent the most commonly used specimen for analysis and contain an abundance of analytes, which display the genotype and phenotype of the individual [33]. Other biofluids include cerebrospinal fluid [34], saliva [35], tears [36], seminal fluid [37], bronchoalveolar lavage fluid [38] and synovial fluid [39].

Nonbiofluid sample types include exhaled breath [40], hair [41], nails [42], skin [43] and tissue [44]. MALDI-MS imaging is becoming increasingly useful to study solid sample matrices such as tissue for diagnosis, especially from cancerous biopsies.

## Mass range

MALDI-MS is able to accurately measure compounds across a very wide mass range. To achieve the extreme mass ranges, modifications to sample preparation, matrix and operating procedures are required, such as using a mixture of two matrices (i.e. DHB and  $\alpha$ -CHCA).

Tanaka et al. [2] reported the detection of proteins and polymers up to 100,000  $m/z$  by laser desorption ionisation time of flight (ToF), highlighting its purpose in the detection of high-molecular mass organic compounds. For analyses of compounds in the small mass range, MALDI has shown its capability of accurate measurement of masses  $\leq 1500$  Da [45].

## Reproducibility and quantitation

The reproducibility of MALDI-MS spectra depends on a number of factors, including the homogeneity of the matrix, analyte crystals, ion suppression and amount of matrix. A variety of results have been reported for

reproducibility and a range of reported %CV from 4% to 26% [46–48]. For example, in complex biofluids, the average mass accuracy reported a relative standard deviation of 0.0003% for 36 measurements, and a %CV for the intensity of 60 monoisotopic peaks was 37% (4%–134%). The challenge with reproducibility suggests that MALDI-ToF analyses are suitable for a semiquantitative application [49].

There have been attempts to expand the utility of using MALDI-MS for quantitative measurements; however, the heterogeneity with the matrix crystal poses difficulties [50]. Using complex mixtures such as biological samples has shown poor reproducibility (sample-to-sample and shot-to-shot) for quantitation because of the so-called sweet spots. A common problem with the dried droplet method, sweet spots are laser-irradiated zones that give much higher sensitivities than other positions. Sweet spots can give saturating intensities, and without the use of internal standards, the intensities from sweet spots may need to be classified as outliers. Although the sweet spot issue is not fully understood, it could be due to the variation in the crystallisation of the matrix [51]. To avoid the sweet spots and increase reproducibility, numerous manufacturers use a random walk movement whereby the laser irradiation spot moves automatically (i.e. after every 10 pulses) and randomly in different locations on the sample surface and a spectrum is averaged over the numerous laser pulses [52, 53].

Furthermore, suppression effects also hinder quantitative measurements, which can distort complex biological samples in a wide concentration range. Because of ionisation efficiencies, compounds of a similar nature and dynamic range can affect quantitation due to detector saturation [54]. Efforts have been made in quantitation by using isotopically labelled versions of the molecule(s) of interest to reduce the complex interferences and to improve precision and accuracy [54].

To improve reproducibility, an accurate optimisation is recommended through approaches including sample automation, use of internal standard and algorithms for normalisation and peak detection [46].

## Sensitivity and specificity

Several studies have reported very high sensitivity and specificity for MALDI-MS of >90% [55–57].

Sensitivity in the attomolar ( $10^{-18}$ ) range can be achieved through sample enhancement, for example, with affinity capture of peptides [58] or *in situ* signal amplification using micro- or nanoparticles [59]. Compared

with liquid chromatography-mass spectrometry (LC-MS), sample volumes used for analysis are generally lower, with 0.5–1  $\mu$ L analyte-matrix solution deposited onto the target plate [60]. This allows for easier sample storage and reanalysis, with stability of spotted analytes showing no adverse effects to peak intensities after 1 month storage on a target plate in a sealed container [61].

In terms of specificity, MS directly measures the mass of the analyte itself rather than a secondary product (e.g. light or fluorescent signals), offering a much higher specificity compared with conventional clinical biochemistry techniques such as immunoassays. Pretreatment of a sample using immunoassays that isolate the analyte of interest offers a further improvement in specificity [62].

## Throughput

MALDI-ToF MS is recognised for high-throughput detection. Because pretreatment is offline, large sample sets can be analysed and prepared in parallel. Automation allows for even higher throughput by using 96- or 384-well plates, which require less sample volume and a sampling rate of ~5 s. Similarly, coupling MALDI-ToF MS with nanoliter liquid handling allows for over 6000 samples to be analysed on one plate [63].

Manufacturers continue to improve the speed of analyses. More recently, the MALDI PharmaPulse™ has achieved speeds of 0.5–1.0 s/sample with throughput of more than 100,000 samples/day [64].

## Matrix-assisted laser desorption ionisation (MALDI) mass spectrometry (MS): applications

The versatility of MALDI-MS makes it a platform with great potential for clinical applications. It has routinely been used in microbiology as a successful diagnostic tool offering high-throughput, cost-effectiveness and efficient output of data [62, 65]. Further advantages are its fast and accurate determinations of molecular mass covering a wide mass range and requiring little or no sample preparation. However, because biological samples are made up of many thousand analytes that vary in molecular weight, composition and abundance, sample preparation offers a pretreatment that aims to reduce sample complexity, to improve analytical performance and to improve sensitivity to target the desired analyte. Chromatography, extraction,

electrophoresis and isolation are commonly used pretreatment techniques for targeted analysis. Applications that use MALDI-MS include quantitative, semiquantitative or qualitative and depend on the type of analysis (i.e. screening or quantitation).

The subsequent section reports on the clinical and research applications of MALDI-MS, variations of MALDI and potential future applications. Table 1 summarises the discussed applications.

## Current clinical applications

### Microbiology

In recent years, the acquisition of data for bacterial libraries has shown greater accuracy in genus identification ( $\geq 94.9\%$ ) than conventional methods (86.4%) [92]. MALDI-MS profiling or identification of bacterial species is particularly applicable in a clinical setting; a single sample can be identified in a few minutes [93], it is cost-effective and reliable and the libraries are updated regularly by the manufacturer.

Currently, only two MALDI-ToF MS developments, Bruker Biotyper (Bruker Daltonics) and VITEK MS (bioMérieux), have been commercialised and gained regulatory approval from the US Food and Drug Administration for the identification of microbial species. Both instruments consist of a MALDI-ToF MS, software and a database containing a list of microorganisms with validated spectral classifiers cleared for identification, unique to each system [66]. MALDI-ToF MS applications for these systems have been extensively studied and shown suitability for the identification of bacteria, viruses and fungi for testing in urine and blood culture broth [66, 67].

One of the first studies using the Bruker Biotyper (Bruker Daltonics) reported the development of a database of mass spectra showing the identification of non-fermenting gram-negative bacteria with corresponding 16S rRNA gene sequencing [94]. This was followed with the commercial database for the identification of 1660 bacterial isolates by Bruker, reporting rapid analysis and accurate identification of 95% by MALDI-ToF MS: 84% at the species level and 11% at the genus level [95]. The rapid identification was reported at 23–83 h faster for gram-positive and 34–52 h faster for gram-negative bacteria compared with routine methods with 89% and 97.8% accuracy, respectively [96]. The high reproducibility and accuracy coupled with improved curated databases containing mass spectral libraries has gained reputation for

the Biotyper as a routine clinical instrument into laboratories [97].

Studies performed with the VITEK MS (bioMérieux) system, with the curated database, provided 96% correct identification of bacterial isolates with 87% to the species level and 8% to the genus level [98]. The coupling of MALDI-ToF MS with a curated database as with the Biotyper and the VITEK MS systems are advantageous for rapid turnaround and useful for the identification of common aerobic gram-negative bacilli and gram-positive cocci. However, they are less suitable compared with other methods, for anaerobic bacteria [99]. The performance of both instruments and the databases are similar for accuracy [100].

Although not yet approved for clinical applications, the MALDI-ToF MS platform, ASTA MicroIDSys (ASTA, Korea), was developed for microbial species identification. It includes a simple extraction method for the rapid identification of microbial isolates with a database containing reference spectra for 2604 species. For the identification of frequently isolated bacterial species, results were comparable with the Bruker Biotyper (98.9%–100%) [101].

### Prenatal and newborn screening

Prenatal screening allows for a praecox diagnosis and aims to inform the parents about the risk of having a baby affected by genetic disease. Although invasive techniques are accurate, they are associated with the risk of miscarriage to the foetus. Noninvasive prenatal diagnostic approaches have been developed; however, minority foetal traits may be hindered by majority DNA background. MALDI-ToF MS allows for the detection of single gene disorders [102], foetal blood group genotyping [103] and foetal aneuploidies in maternal plasma [104]. For prenatal and newborn screening, MALDI-ToF MS presents clinically approved and commercially available assays for conditions, including sickle cell disease (SCD), haemoglobinopathies and thalassaemia carried out by MAP Sciences Ltd using home testing kits to obtain a sample of blood or urine on a sampling card. Further research using MALDI-ToF MS is in fertility testing [68].

For the diagnosis of neonatal SCD and  $\beta$ -thalassaemia major, dried blood spots are analysed for screening using automated preparation coupled to a MALDI-MS to monitor the mass and relative abundance of the primary haemoglobin (Hb)  $\alpha$  and  $\beta$  subunits and HbS subunit [69, 84]. Sensitivity and specificity were reported to be  $>99\%$  and 100%, respectively, for the HbS homozygous phenotype



**Table 1:** Applications of MALDI-MS.

Application	Method/systems	Analyser	Comments	References
Clinical				
Microbiology	Bruker Biotyper (bacterial identification) VITEK MS (yeast identification)	Linear ToF	Clinical and FDA approval Routine application of MALDI-MS in clinical microbiology	[66, 67]
Prenatal and newborn screening	Commercial assays by MAP Sciences Ltd with Shimadzu MALDI-8020	Linear ToF	Clinically approved and commercially available  Carried out by MAP Sciences Ltd for SCD, haemoglobinopathies and thalassaemia	[68, 69]
Plasma cells disorders	Bruker Microflex LT for M-proteins. Assay is termed MASS-FIX	Linear ToF	Clinically approved for M-proteins detection	[70]
MALDI profiling	Bruker Biotyper (bacterial identification) VITEK MS (yeast identification) VeriStrat® developed by Biodesix Inc.	Linear ToF	Clinically approved for microbiology with curated databases (see Microbiology) VeriStrat® is an approved test in the USA for patient prognosis from cancer treatment by EGFR inhibitors	[71–74]
Research				
Metabolic diseases	MALDI-TOF/TOF 4800 Analyzer (Applied Biosystems) and sample pre-clean up using Sep-Pak C <sub>18</sub> and carbograph columns	Reflectron ToF/ ToF	Promising clinical application for multiplex assays with high throughput	[75]
Drug testing	Bruker Ultraflex II reflectron for cocaine in hair analysis Bruker UltrafleXtreme MALDI-TOF for therapeutic drug monitoring	Reflectron ToF	Promising clinical application for therapeutic drug monitoring in real time	[76–79]
Cardiometabolic diseases	Shimadzu Axima Performance MALDI-ToF for BNP molecular forms captured using MSIA Bruker Reflex IV MALDI-TOF for glycated haemoglobin	Linear ToF	Promising clinical application that investigates molecular forms of current biomarkers	[80–83]
MALDI profiling	Bruker Ultraflex II Linear ToF/ToF for SCD MALDI-TOF/TOF 4800 Analyzer (Applied Biosystems) for proteomic profiling coupled with nano-LC system	ToF ToF/ToF LC-MALDI-ToF/ToF	Promising clinical applications such as that observed in microbiology	[84–86]
MALDI imaging	Bruker rapifleX MALDI TissueTyper for cancer and Alzheimer's disease with initial tissue preparation	Linear ToF Reflectron ToF	Promising clinical applications in cancer biopsies and neurodegenerative diseases	[87, 88]
Variations of MALDI	Bruker UltraFlex II MALDI-TOF/TOF for imaging following lipid fixation and histological staining for dye-assisted laser desorption ionisation AB SCIEX 4800 MALDI TOF-TOF for iMALDI application using stable isotope labelling and antibody conjugated beads Ciphergen SELDI ProteinChip® Array Technology	Linear ToF/ToF Reflectron ToF/ ToF Linear ToF	Currently, research grade with potential for clinical approval with further research	[89–91]

EGFR, epidermal growth factor receptor; FDA, Food and Drug Administration; LC, liquid chromatography; MALDI, matrix-assisted laser desorption ionisation; MS, mass spectrometry; MSIA; mass spectrometric immunoassay; SELDI, surface-enhanced laser desorption ionisation; ToF, time of flight.

in two independent MS facilities. Compared with conventional screening methods, MALDI-MS allowed for automation, cost-effectiveness, higher throughput and rapid acquisition of 600–1000 samples per day [57].

## Plasma cell disorders

Diagnosis and monitoring of plasma cell disorders (e.g. multiple myeloma) is currently achieved by a combination of three serum-based diagnostic tests to maximise sensitivity: protein electrophoresis, immunofixation electrophoresis and measurement of free light chains [105]. A single, highly sensitive method would improve diagnostic confirmation. A clinical assay for detecting M-proteins in plasma cell disorders coupling immunoenrichment with MALDI-ToF MS was developed and validated to show analytical sensitivity beyond current approaches. The method, MASS-FIX, has the potential to succeed current clinical applications for M-protein detection, isotype and quantitative information [70].

## MALDI-MS profiling

Profiling is a method that focuses on identifying features (e.g.  $m/z$  and peak intensity) that show statistical differences in populations (e.g. control vs disease) rather than the accurate detection of analytes. It is associated with minimal sample processing to retain the complexity of the sample; however, the more abundant analytes may still be present in the sample and mask analytes of lower abundance, which may be of interest. For this reason, pretreatment steps that are often used to ensure the removal of these abundant analytes may confound attempts to quantitate naturally occurring biomarkers and increase difficulties with identification [106].

Certainly in clinical microbiology, MALDI-MS profiling has been demonstrated to be advantageous and versatile. Promising applications of MALDI-MS profiling include cancer [56], rheumatoid arthritis [85] and as a first-tier screen for SCD in neonates [84], with early efforts for proteomic profiling in heart failure [86].

The VeriStrat® (Biodesix Inc, USA) is a commercially available serum-based protein test, developed to identify patient response to cancer treatment (epidermal growth factor receptor inhibitors) by profiling acute phase reactant proteins in the blood [71]. The test uses MALDI-ToF MS to assign binary categories of VeriStrat Good or VeriStrat Poor, indicating the outcomes of treatment. The test was developed by comparing mass spectra from

pretreated samples from patients with either long-term stable disease or early progression on gefitinib therapy (reference set) and identifying eight features for differentiating between the groups [72]. The algorithm used for prognosis compares the eight features in a patient sample with the reference set and has been successfully demonstrated in a number of studies, including two randomised studies and a phase III trial in non-small cell lung cancer (NSCLC) patients who have failed at least one line of prior chemotherapy [73]. Current data suggest clinical application for NSCLC patients in the second-line setting [74].

For successful MALDI-MS profiling of large clinical samples, it is necessary for a curated database of analytes. Although there are many individual mass spectral libraries available, three commercial systems exist to integrate equipment, software and database for identification: BioTyper, Bruker Daltonics; SARAMIS, Shimadzu and Anagnostec; and MicrobeLynx™, Waters Corporation [107].

## Research applications

### Metabolic disease

MALDI-ToF MS has been investigated for rapid screening in genetic metabolic diseases. In particular, lysosomal storage diseases (i.e. oligosaccharidoses) are a group of inherited metabolic disorders resulting from the loss of function of one or more enzymes involved in the lysosomal degradation of macromolecules. Because these disorders are not specific for a single enzyme deficiency, the use of enzyme assays or thin layer chromatography, which are usually performed, is limited by high costs. MALDI-ToF/ToF MS has been used to screen for oligosaccharidosis in urine without pretreatment. In total, 43 characteristic-free oligosaccharide combinations were identified, providing diagnostic features for 10 lysosomal storage conditions [75]. This method had a short run time of 30 min and was semiautomated, sensitive and specific, and is therefore easily adaptable in a clinical chemistry laboratory [108]. Although not yet clinically approved, MALDI-ToF MS has shown successful application and the potential for multiplex assays in metabolic disease.

### Drug testing

Drug testing can usually be conducted with urine, hair or plasma. However, hair rather than urine or plasma represents a routinely powerful tool for drug testing, with the

advantage that it is a noninvasive method and provides a larger surveillance window; the growth rate and length of hair (e.g. 1 cm length approximates to 1 month of growth) covers a retrospective record of drug adherence over time [109]. Specifically, hair samples require a drying process followed by pulverisation, centrifugation and direct deposition of powdered hair samples treated with graphite. This method has been applied for the rapid detection of cocaine, benzoylecgonine and cocaethylene with high sensitivity and specificity (100%) for cocaine [41, 76]. Beard hair, due to daily shaving habit, can reveal incorporation mechanisms as was demonstrated in volunteers for the detection of ethyl glucuronide, associated with excessive alcohol consumption [77].

MALDI-MS offers a high-throughput method, the ability to monitor samples over time, and accurately. Although not currently clinically approved, these demonstrations imply that MALDI-MS can be applied to therapeutic drug monitoring and quantifying drugs in real time [78, 79].

## Cardiometabolic disorders

MALDI-MS applications in cardiometabolic disorders investigate molecular forms of current recommended clinical biomarkers (such as BNP and HbA<sub>1c</sub>). There is extensive research in place into these molecular forms to understand their niche in clinical practice.

In cardiovascular cohorts, MALDI-ToF MS was used to detect three common degradation peptides of B-type natriuretic peptide (BNP), the current gold standard biomarker in heart failure diagnosis. The analysis of the molecular forms was achieved by MS immunoassay (MSIA) using magnetic beads coupled to anti-BNP antibody for semiquantitative analysis. MALDI-ToF MS showed that clinical BNP is a combination of a number of degradation products, rather than BNP alone [110]. BNP 5-32, BNP 4-32 and BNP 3-32 were all able to show prognostic capabilities in a comparable manner with current standards in acute heart failure and acute myocardial infarction patients [80, 81]. Although these peptides did not show superiority over current markers, they were useful for the early screening of heart failure.

The development of a method for quantitative analysis of the protein glycation level of globulins in diabetes demonstrated high levels of reproducibility and specificity, making it a potential diagnostic test not only for diabetes but also for oxidative stress [82]. MALDI-ToF MS allowed for the differentiation of glycosylated and glyco-oxidised products of both  $\alpha$ - and  $\beta$ -globulins, such that further studies showed that clinical HbA<sub>1c</sub> measurements are the combination of glycosylated and glyco-oxidised products of both  $\alpha$ - and  $\beta$ -globulins rather than glycosylated

$\beta$ -globulin as initially thought. This was followed up by a semiautomatic robotic procedure to increase throughput and allows for rapid screening as a sensitive and specific biomarker in diabetes [83].

## MALDI imaging

Current applications of MALDI-MS imaging (MALDI-MSI) include tissue-based research for analysing tissue specimens or biopsies. This enables the analysis of a broad spectrum of analytes ranging from proteins and peptides to small molecules, drugs and their metabolites, endogenous metabolites and lipids [111].

There are several applications of MALDI-MSI, particularly in proteomics for biomarker discovery [44], diagnostic screening and molecular pathology to correlate molecular detail with histopathological changes in tissue. This augments the possibility to identify candidates for disease biomarkers. The most common condition where the method has been applied is cancer such as breast cancer [112], ovarian cancer [113], brain cancer [114] and prostate cancer [44]. Recently, in epithelial ovarian cancer (EOC), Klein et al. [113] showed the advantages of this technique for the classification of histotypes from a tissue microarray to enable the development of new prognostic parameters in the tissue assessment of EOC, to enhance personalised treatment.

MALDI-MSI has also been applied in the field of molecular pathology, where high spatial resolution of membranous nephropathy was studied. This enabled the generation of specific tryptic peptide profiles of the different cellular regions within the glomerulus, thus assisting in the routine prognostic assessment of membranous nephropathy patients [115]. In particular, the technique enables the unbiased analysis and spatial mapping of a variety of molecules directly from clinical tissue sections. This allows for the expression of distinct biomarkers in a biopsy, essential for clinical decision making and forming a key goal of translational research.

Currently, applications for MALDI-MSI have been used in research for diagnosis, prognosis and survival markers; however, many studies have demonstrated the scope for future clinical application, e.g. in cancer biopsies and neurodegenerative diseases [87, 88].

## Variations of MALDI

Variations of MALDI present novel approaches that show promising future clinical applications where MALDI-MS may experience difficulties.



Immuno-MALDI (iMALDI) uses antipeptide antibodies for sample enrichment and on-target detection of the captured analytes of interest. Because MALDI may present with difficulties with complex samples and LC-MS lacks the robustness and the ability to automate, iMALDI has been developed to overcome these potential problems. It is able to quantitate peptides and proteins from clinical samples while having the potential for an automated workflow [89]. This technique has been used to develop an assay for the quantitation of angiotensin I for the diagnosis of secondary hypertension in cardiology [116] and the quantitation of cell-signalling proteins in cancer [90]. A relatively newer technique, iMALDI, will be increasingly used for clinical proteomic biomarker analysis certainly because of its high throughput, sensitivity, specificity and the ability to integrate with other -omics fields to aid precision medicine.

Other variations of MALDI used for clinical research include surface-enhanced laser desorption ionisation (SELDI) and dye-assisted laser desorption ionisation (DALDI). The main difference between these spectrometry techniques is the way the analyte is captured [114]. Both SELDI and DALDI present as promising ionisation technique for clinical research in bone disease [117] and the diagnosis of lipid diseases, respectively [91].

## Analytical and practical limitations

Although MALDI-MS has revolutionised the identification of microbial species, it currently has analytical limitations in clinical research in other fields.

Quantitative measurements are difficult because of ion suppression, complex sample nature and wide concentration range [54]. When a complex sample is analysed directly, ionisation suppression can occur where analytes with the same abundance but lower ionisation efficiencies are suppressed by higher ionisation efficiencies, resulting in a partial representation of the sample. Sample complexity is reduced by pretreatment to separate the different types of compounds [118, 119].

The MALDI-ToF MS has undergone enormous transformation over the last three decades in terms of size, robustness, affordability and performance. Manufacturers are continually developing the MALDI-MS to make it portable and user-friendly for clinical applications. The cost of a MALDI-MS system varies, depending on the application. For example, for bacterial identification, MALDI-MS neither requires a reflectron ToF nor a negative mode operation, thus a reduction in cost, size and complexity. On the other hand, there is a requirement for enhanced electronics, complex software and higher laser

repetition rate in MALDI-MS imaging. In terms of cost, the MALDI-ToF MS system may cost ~200,000 euros, and for maintenance ~20,000 euros per year [120]. Despite the substantial up-front investment associated with purchasing a MALDI-MS system, an annual saving of 51.7% was estimated in one clinical laboratory, including technologist and maintenance [121]. Labour-intensive protocols are both in favour and out of favour with MALDI-MS, depending on the type of analyte. For example, where bacterial species are considered, 5311 beta-haemolytic *Streptococci* require 210 h less labour time for MALDI-MS compared with standard microbiology protocols; however, 1675 isolates of *Candida albicans* require an estimated 96 h more labour time [122]. Compared with most analytical techniques such as LC-MS, the operating and maintenance costs are modest, and the cost per sample analysis is also low [123].

## Comparisons with current clinical techniques

Currently, most clinical biomarker analysis is conducted using large automated immunoassays such as ELISA or radioimmunoassays that use colorimetric, fluorescent or radioactive detection methods [124]. Both MS and immunoassay have advantages and disadvantages for clinical use. MS provides accurate mass information and, therefore, specificity. Compared with immunoassay, difficulties are exhibited when translating MS methods onto automated systems and require high-cost specialised training. Immunoassays can be easily automated and require less training; however, assays are susceptible to interference [125]. These assays pose difficulties with cross-reactivity, and changes, mutations and posttranslational modifications are usually undetectable. The analytical detection limit for MALDI-ToF MS is usually in the low femtomolar to high attomolar range and less sensitive to immunoassays; however, MALDI-ToF MS provides high mass accuracy in the lower molecular weight range [126]. MALDI-ToF MS also allows multiplexed assays to be developed, facilitating hundreds of analytes in a single run, reducing time and cost [127]. A comparison with current clinical techniques is summarised in Table 2.

## Future clinical prospects

Aside from the current clinical and near-clinical applications of MALDI-MS, biomarker discovery studies using

**Table 2:** Comparison of MALDI-MS with current clinical techniques.

Clinical detection method	Advantages	Disadvantages
MALDI-MS	Fast Accurate Ability to multiplex assays High specificity Allows for automation	High start-up cost Data acquisition is time consuming Highly trained personnel
Immunoassay	Fully automated High throughput Easy to operate	Issues with cross-reactivity Susceptible to interference
Culture in microbiological media and biochemical test for microbial identification	High sensitivity Cost-effective	Time consuming (24–48 h)

MALDI-MS have identified novel markers in a range of diseases that include single and multimarker platforms [44, 128]. The validation and method development of sensitive and specific assays in this study could be critical in the future.

MALDI-MS has great potential to become a powerful technique in future clinical applications. The method development and optimisation of existing protocols and the discovery of suitable matrices extend its applications into newer disciplines. The development of MALDI imaging is highly regarded, and its future potential in drug research and analysis of cancer biopsies underpins the future direction of MALDI-MS. The launch of bench-top MALDI-MS systems offering automation, robustness, sensitivity and a smaller footprint is designed for clinical diagnostic research and development [129].

## Conclusions

Since the inception of MALDI-MS over 30 years ago, the analytical capabilities have improved substantially and the potential clinical applications have broadened. MALDI-MS systems are affordable, easy to operate and robust, making MALDI-MS a useful tool in clinical laboratories for a wide range of applications. Currently, the prevalent use of MALDI-MS as a clinical application is in microbiology; however, with increasing research, the potential for widespread clinical applications is imminent.

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