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Advanced mass spectrometry technologies for the study of microbial pathogenesis

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Matrix-assisted laser desorption/ionization mass spectrometry (MALDI MS) has been successfully applied to the field of microbial pathogenesis with promising results, principally in diagnostic microbiology to rapidly identify bacteria based on the molecular profiles of small cell populations. Direct profiling of molecules from serum and tissue samples by MALDI MS provides a means to study the pathogen–host interaction and to discover potential markers of infection. Systematic molecular profiling across tissue sections represents a new imaging modality, enabling regiospecific molecular measurements to be made *in situ*, in both two-dimensional and three-dimensional analyses. Herein, we briefly summarize work that employs MALDI MS to study the pathogenesis of microbial infection.

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Introduction

Hospital and community-acquired infections represent an increasing threat to global public health. Pathogens are rapidly developing resistance to therapeutic intervention, further compounding this threat [1,2]. Therefore, a great need exists for advanced analytical technologies to aid both diagnosis of pathogens in clinical laboratories and the discovery of novel targets for therapeutic intervention in research laboratories. Advanced mass spectrometry technologies can meet this need and aid the ability of clinicians to continue successful treatment of infectious diseases.

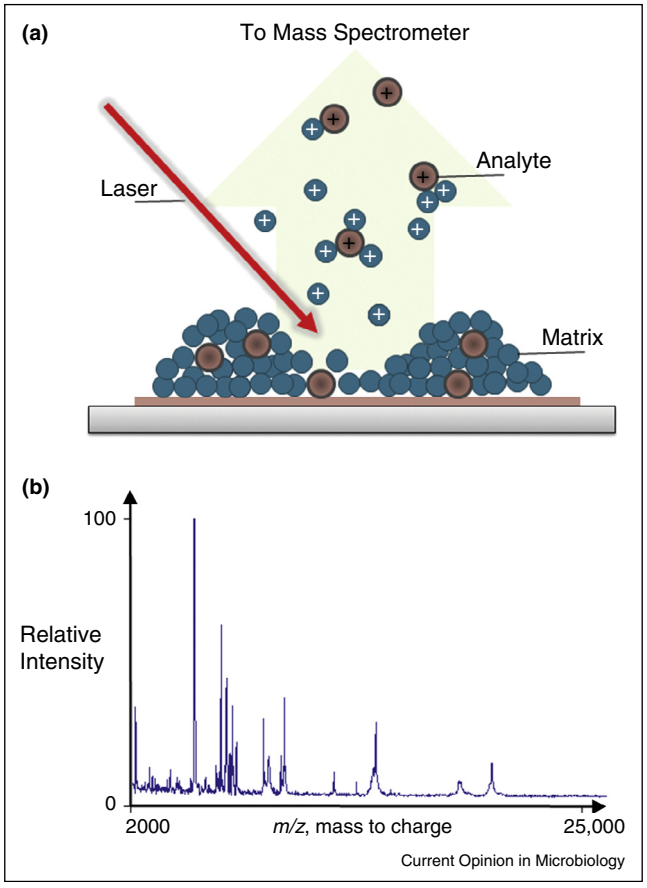
Matrix-assisted laser desorption/ionization mass spectrometry (MALDI MS) is an analytical technology that enables direct analysis of biomolecules. MALDI utilizes a matrix, typically a small organic acid with strong ultraviolet absorbance, mixed with analytes to aid desorption and ionization (Figure 1a) [3•]. The resulting gas phase analyte ions are detected and displayed in a spectrum according to their mass-to-charge ratios (m/z), which yield specific molecular signatures within complex samples (Figure 1b). This label-free technology can be used without *a priori* knowledge of sample composition, allowing for the detection of a variety of analytes, from small molecules to large proteins [4]. Combined with efficient analyte identification strategies and the emergence of online searchable databases [5,6], MALDI MS has been successfully applied to a variety of biological samples, including tissue sections [7], plants [8], insects [9], whole animals [10], and microbial colonies [11]. The ability to analyze such a wide range of systems has led to the use of MALDI MS as a clinical tool, particularly in diagnostic microbiology.

Diagnostic microbiology

MALDI MS can distinguish molecular fingerprints associated with specific microorganisms, allowing for the rapid identification of infectious agents. MALDI MS presents a means to interrogate intact cells from agar plates or liquid media, leading to robust m/z signatures independent of culture conditions [12]. This has been expanded to include identification of fungi and bacteria that are difficult to culture, such as anaerobic or highly infectious bacteria [13–16]. Advances in sample preparation have improved the reproducibility of MALDI MS measurements from intact cells, making it a promising tool for diagnostic microbiology [11,17,18].

Microbial fingerprinting using MALDI MS can provide accurate determination of spectral peaks specific to both species and genus [19,20]. Initial studies have been compiled into a collection of databases that can be used to classify clinical isolates quickly and accurately [11]. Instrumental set-ups, automated analyses, and database searching platforms tailored to these applications are available from commercial instrument manufacturers, including FDA-approved systems like the MALDI Biotyper system (Bruker Daltonics) and the Vitek microbial identification system (Biomérieux) [21]. Highly specific databases can be created by users that facilitate the differentiation of pathogenic and nonpathogenic bacterial serotypes [22] as well as particularly virulent strains [23]. Furthermore, comparative analyses of spectral information can be used to determine microbial lineage, which

Figure 1



(a) Schematic of MALDI ionization process. The sample is first mixed with a matrix. Irradiation by a UV laser initiates the ionization/desorption processes, and newly created ions are guided into a mass spectrometer. Ions produce peaks with unique mass-to-charge ratios, or *m/z* values, which correlate to their protonated molecular weight. **(b)** An example MALDI mass spectrum taken directly from a mouse kidney.

provides a rapid and sensitive tool to diagnose and control outbreaks of pathogenic microbes [24]. Successful implementation of this workflow has been extended from clinical diagnostic laboratories to botany labs for the study of plant pathogens and to the food service industry to study food-spoiling pathogens [25,26].

In addition to rapidly identifying bacteria cultured on media, MALDI MS can be used to identify microbial signatures from complex mixtures, including blood cultures, thus enhancing utility in clinical settings. For example, direct MALDI MS analysis of centrifuged blood culture broths from patients with bacteremia in intensive care units has been utilized to accurately identify microbes from complex, polymicrobial samples. The approach also requires substantially less time than previous diagnostic methods [27•]. Such advancements greatly decrease both time and cost associated with microbial identification, leading to more efficient antimicrobial intervention. Integration of MALDI MS technologies into clinical laboratories therefore has the potential to dramatically affect infectious disease medicine and have a positive impact on human health [28].

MALDI MS profiling of infected tissues to aid diagnostics

The ability to rapidly identify MS signatures specific to microorganisms in culture is a major advancement. However, the challenge remains to detect pathogen-specific signals in the context of infection, as simple cultures do not mimic the conditions encountered by microorganisms within the host and provide little insight into pathogenesis and virulence. Because of limited abundance of microbial signals in the presence of host response markers, the detection of these signals from within the host presents analytical challenges. Moreover, identification of disease-specific host response signatures is needed to provide a more complete picture of the host–pathogen interface during infection.

MALDI MS profiling experiments collect a number of discrete spectra from various cell types within samples for comparison. This approach was used in a recent study that searched for serum host-response biomarkers for sepsis in neonates in intensive care units. Sepsis-associated *m/z* values, including *m/z* 11,528, identified as a variant of the host inflammatory protein serum amyloid A, provided diagnostic markers which could be detected from serum at birth. Scoring of these biomarkers will allow clinicians to determine which patients should be placed on antimicrobial therapies and has prompted the design of a triage strategy for suspected cases of neonatal sepsis [29].

Another study utilized MALDI MS profiling to study the host response to *Staphylococcus aureus* infected skin wounds over time. Skin lesions infected with *S. aureus* were swabbed into sterile water at various time points post-infection. Both swab samples and wound exudate were analyzed by MALDI MS. Comparison of collected spectra from wound beds and cultured *S. aureus* revealed several matching *m/z* values. Additional signals were detected from the wound bed thought to belong to mouse defensins and blood, highlighting the ability of MALDI profiling to monitor bacterial signals, host response to infection, and wound healing over time [30••]. Such analyses could lead to direct microbial analysis from soft tissue infections and aid in diagnosis of chronic wound infections.

In addition to analyzing spotted tissue homogenates or serum, MALDI MS provides the distinct advantage of *in situ* tissue profiling. This method allows molecular signals to be obtained directly from tissue sections in a spatially-targeted approach, allowing pathologists to focus analyses on histological regions of disease [31]. The histology-directed approach has been applied to the study of bacterial sarcoidosis in snap-frozen human tissue. Targeting only