

Video Article

Whole-cell MALDI-TOF Mass Spectrometry is an Accurate and Rapid Method to Analyze Different Modes of Macrophage Activation

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Abstract

MALDI-TOF is an extensively used mass spectrometry technique in chemistry and biochemistry. It has been also applied in medicine to identify molecules and biomarkers. Recently, it has been used in microbiology for the routine identification of bacteria grown from clinical samples, without preparation or fractionation steps. We and others have applied this whole-cell MALDI-TOF mass spectrometry technique successfully to eukaryotic cells. Current applications range from cell type identification to quality control assessment of cell culture and diagnostic applications. Here, we describe its use to explore the various polarization phenotypes of macrophages in response to cytokines or heat-killed bacteria. It allowed the identification of macrophage-specific fingerprints that are representative of the diversity of proteomic responses of macrophages. This application illustrates the accuracy and simplicity of the method. The protocol we described here may be useful for studying the immune host response in pathological conditions or may be extended to wider diagnostic applications.

Video Link

The video component of this article can be found at <http://www.jove.com/video/50926/>

Introduction

Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS) is a popular mass spectrometry technique to study biological samples. Using a laser beam and an energy-absorbing matrix allows a soft ionization process: the evaporation and genesis of large mostly single-charged biomolecules. This process is called desorption/ionization, justifying the acronym MALDI. These ions are then accelerated by application of voltage and enter a TOF analyzer that allows the separation of these ions and the quantification of their respective masses¹.

MALDI-TOF MS has been extensively used in biology, chemistry, and medicine to identify molecules and biomarkers²⁻⁴ or to monitor post-translational modifications on proteins^{5,6}. Recently, several groups applied MALDI-TOF MS to the identification of microorganisms from clinical samples^{7,8}. This microbiological application is now used routinely in the clinical settings. Whole cell MALDI-TOF has many advantages compared to classical applications of MALDI-TOF MS. Samples containing whole cells are directly processed, avoiding time consuming steps to fractionate or separate large amounts of material. Moreover, no characterization of the various peaks is needed: the whole spectrum is considered as a fingerprint of the sample, and matching algorithms compare the tested spectrum with a database of reference spectra.

We and others have applied this whole-cell analysis technique to eukaryotic cells. Many applications may be derived from this technique: (1) identify the main cell types from a mixed sample⁹⁻¹¹; (2) assess the viability of cell cultures over time (including quality control industrial applications)¹²; (3) monitor activation states of a single cell type¹³; (4) assess the malignant transformation of a clinical sample^{14,15}.

Here, we describe the use of whole-cell MALDI-TOF MS to explore the various polarization phenotypes of macrophages in response to cytokines or heat-killed bacteria. Macrophages play a pivotal role in the immune response to microbial pathogens. They detect infectious agents in the tissues through pattern recognition receptors able to detect conserved microbial patterns, such as lipopolysaccharide (LPS)¹⁶. Macrophages are professional antigen-presenting cells that interact with T cells to mount the adaptive immune response. T cells influence macrophages by releasing cytokines that either reinforce or regulate the microbicidal activity of macrophages. By analogy to the Th1/Th2 lymphocyte polarization, inflammatory, microbicidal, and tumoricidal macrophages have been classified into M1 macrophages and immunoregulator macrophages as M2 macrophages¹⁷⁻¹⁹. The term M1 refers to the classical activation of macrophages by type I cytokines, such as interferon (IFN)- γ and tumor necrosis factor (TNF), or bacterial products, such as LPS^{18,20-23}, whereas macrophages activated by alternative pathways (interleukin (IL)-4, IL-10, Transforming Growth Factor- β 1) are considered M2 macrophages^{19,24,25}. The high phenotypic and functional plasticity of macrophages in response to their microenvironment renders these macrophages useful to analyze subtle changes by a MALDI-TOF MS approach.

Protocol

In the present protocol, the whole-cell MALDI-TOF technique is used to obtain a mass spectrum considered as a fingerprint of the sample. A bioinformatic analysis allowed the comparison and the classification of these fingerprints. There were three main parts in this protocol:

1. The preparation of the biological samples: control macrophages and macrophages stimulated with different agonists.
2. The analysis of each type of sample with technical replicates by whole-cell MALDI-TOF MS.
3. The bioinformatics analysis of raw data.

Prepare sterile solutions for cell isolation and culture. Prepare and store all reagents at 4 °C

1. Preparation of Human Monocytes

1. Prepare cell culture medium. Add 55 ml of human serum AB+ or fetal bovine serum (FBS) and 5 ml of the antibiotic solution (penicillin at 10,000 UI/ml and streptomycin at 10,000 µg/ml; final concentration of 100 UI/ml for penicillin and 100 µg/ml for streptomycin) to RPMI 1640 medium (500 ml).
 1. Isolate peripheral blood mononuclear cells (PBMCs) from leukopacks (leukocyte concentrates).
 1. Prepare 50 ml tubes containing 15 ml Ficoll. Dilute the blood in saline (vol/vol, 1/10). Deposit 30 ml diluted PBMCs on Ficoll as previously described²⁶.
 2. Centrifuge at 700 x g for 20 min. Recover PBMCs at the interface between Ficoll (density of 1.077 g/ml) and diluted plasma. Dilute PBMCs in culture medium and centrifuge at 300 x g for 5 min.
 2. Prepare CD14+ monocytes from PBMCs using magnetic beads coated with anti-CD14 antibodies.

Note: Keep products and cells at 4 °C until monocyte obtention.

1. Prepare running buffer consisting of phosphate-buffered saline (PBS) pH 7.2, 0.5% bovine serum albumin (BSA) and 2 mM ethylenediaminetetraacetic acid (EDTA).
2. Gently dissociate pelleted PBMCs (10^7 cells per assay) into 80 µl of running buffer.
3. Add 20 µl CD14 MicroBeads to PBMCs. Mix and incubate PBMCs for 15 min. Wash PBMCs with 5 ml of running buffer and centrifuge at 300 x g for 5 min. Gently dissociate pelleted PBMCs into 500 µl of running buffer for 10^8 PBMCs.

Note: use 500 µl of running buffer for PBMC concentrations lower than 10^8 PBMCs.

4. Proceed to magnetic separation.

Note: for less than 2×10^8 PBMCs, use MS column; for more than 2×10^8 PBMCs and less than 2×10^9 PBMCs, use LS column.

5. Rinse precolumn and column with running buffer (500 µl for MS column or 3 ml for LS column). Add PBMCs into the precolumn (wait for unlabeled cells to pass through the column). Rinse 3 times with running buffer (500 µl for MS column or 3 ml for LS column). Remove the precolumn.
6. Place the column on 15 ml tubes. Add running buffer (1 ml for MS column or 5 ml for LS column) on the column and elute CD14+ cells by applying a pressure on the column.
7. Centrifuge the eluate at 300 x g for 5 min. Discard the supernatant. Wash monocyte pellet with 10 ml of culture medium.

Note: Analyze the purity of monocyte preparation using anti-CD14 antibodies and flow cytometry (classically higher than 95%).

2. Differentiation of monocytes into macrophages.
 1. Incubate monocytes (10^6 monocytes per well in 6 well plates) in 3 ml of culture medium containing 10% human serum AB+ at 37 °C. After 4 days, replace the culture medium containing human serum by 3 ml culture medium containing 10% FBS for 3 additional days.
 2. Identify the obtained cell population as monocyte-derived macrophages (MDMs) by flow cytometry (see **Figure 1**).
 1. Replace the culture medium by 3 ml PBS. Scrape MDMs with a rubber policeman and collect MDM suspensions. Centrifuge at 400 x g for 5 min. Add PBS containing 2% BSA to pelleted MDMs and gently agitate cell suspension. Adjust the cell concentration (10^6 MDMs in 200 ml PBS) and incubate at 4 °C.
 2. Add 10 ml of anti-CD14 antibodies and 10 ml of anti-CD68 antibodies to MDM suspension and incubate at 4 °C for 20 min in the dark.

Note: these antibodies must be labeled with two different fluorochromes consistent with flow cytometry analysis. For example, anti-CD14 antibodies may be conjugated with phycoerythrin coupled (for monocytes staining) and anti-CD68 antibodies with Alexa Fluor 647 coupled (for macrophages staining).
 3. Centrifuge MDMs at 400 x g for 5 min. Remove supernatants. Gently dissociate the cell pellet and incubate MDMs in 250 ml 3% paraformaldehyde (PFA) for 15 min at room temperature. Centrifuge PFA-fixed MDMs at 400 x g for 5 min. Wash MDMs with 3 ml PBS and centrifuge MDMs at 400 x g for 5 min. Gently dissociate MDM pellet in 400 ml PBS.
 4. Analyze the differentiation of monocytes (that express CD14 but not CD68) into monocytes (that express CD68 but not CD14).

Note: classically more than 95% of cells are MDM.
3. 18 hr stimulation of MDMs.
 1. Replace the culture medium of adherent MDMs by fresh 3 ml culture medium (containing 10% FBS).

2. To induce a M1 or M2 polarization, use the following human recombinant cytokines: IFN- γ and TNF for M1 polarization, and IL-4 for M2 polarization. M1 macrophages are also obtained by stimulation with LPS. Note that stock-solutions of cytokines are conserved at -80 °C and that the stock-solution of LPS is conserved at -20 °C.
 1. Dilute the stock-solution of IFN- γ (1 mg/ml) at 1/100 in culture medium to obtain a dilution of 10 ng/ml. Add 6 μ l of this IFN- γ dilution to MDMs to obtain a final IFN- γ concentration of 20 ng/ml.
 2. Dilute the stock-solution of TNF (1 mg/ml) at 1/100 in culture medium to obtain a dilution of 10 ng/ml. Add 6 μ l of this TNF dilution to MDMs to obtain a final TNF concentration of 20 ng/ml.
 3. Dilute the stock-solution of IL-4 (1 mg/ml) at 1/100 in culture medium to obtain a dilution of 10 ng/ml. Add 6 μ l of this IL-4 dilution to MDMs to obtain a final IL-4 concentration of 20 ng/ml.
 4. Add 3 μ l of the stock-solution of LPS (1 mg/ml) to MDMs to obtain a final LPS concentration of 1 μ g/ml.
3. To stimulate MDMs with bacteria, use heat-killed bacteria. Wash living bacteria with PBS and heat them at 95 °C for 1 hr. Use *Orientia tsutsugamushi* (strain Kato (CSUR R163), *Mycobacterium bovis* (Bacillus Calmette-Guérin, BCG CIP strain 671203) and *Coxiella burnetii* (Nine Mile in phase I) were because these pathogens are known to infect macrophages. Thus, prepare a *O. tsutsugamushi* suspension at 10^9 bacteria/ml. Add 50 μ l to MDMs. Repeat the same step for *M. bovis* and *C. burnetii*.
4. Preparation of biological samples for MALDI-TOF MS.
 1. Wash stimulated MDMs with PBS without Ca^{2+} or Mg^{2+} (cells may agglutinate in the presence of Ca^{2+} or Mg^{2+}). Scrape MDMs with a rubber policeman and collect MDM suspensions. Centrifuge MDMs. Wash again MDMs in PBS without Ca^{2+} or Mg^{2+} at 400 x g for 5 min to discard FBS contamination.
 2. Adjust the cell concentration (2×10^6 MDMs per assay). Centrifuge MDMs at 400 x g for 5 min and discard supernatants. Collect cell pellets in 20 μ l of PBS without Ca^{2+} or Mg^{2+} .
 3. Analyze samples immediately or store them in PBS at -80 °C before analysis.

2. Analysis of Macrophages by Whole-Cell Maldi-TOF MS

1. Preparation of CHCA matrix.
 1. Add 500 μ l of acetonitrile, 250 μ l 10% trifluoroacetic acid, and 250 μ l of high-performance liquid chromatography (HPLC) water to a vial. Dilute 10 mg of CHCA in this solution to a final concentration of 10 mg/ml. Mix and sonicate for at least 20 min.

Note: sonication will improve the saturation of the matrix but this step is not mandatory.

 2. Centrifuge at 13,000 x g for 5 min. Discard the pellet and keep the supernatant.

Note: prepare the matrix solution just before use. A matrix solution that contains crystals does not allow a good ionization of sample molecules, and this may affect the quality of the spectra.
2. Preparation of MALDI steel target.
 1. Moisten the polished steel target with hot tap water. Rub with precision wipe paper. Add 70% ethanol and rub. Rinse with water by rubbing. Add 70% ethanol and rub with precision paper.
 2. Immerse the target in 70% ethanol and sonicate for at least 15 min. Cover the target with 500 μ l to 1 ml of trifluoroacetic acid at 80%. Rub and wipe with precision paper. Rinse with HPLC water without rubbing. Dry the target at room temperature.

Note: an improperly cleaned target may affect the quality of the spectra.
3. Preparation of deposits.
 1. Place the clean target on a horizontal and level support to obtain uniform deposits.
 2. Gently thaw MDM samples on ice. Note: rapid and vigorous thawing may alter samples, thus affecting the quality of the spectra. Homogenize MDMs (by pipetting back and forth the cell suspension) before deposition of 1 μ l (containing approximately 1×10^5 cells) on the MALDI target. Add 1 μ l of the MALDI matrix solution on the sample. Avoid mixing spot with the pipette. Mixing spots with pipettes alters the quality of the spectra. Depose 12-16 samples/assay (technical replicates).
 3. Evaporate spontaneously at room temperature. Note: evaporation takes place gradually and leads to the formation of matrix/sample crystals. The deposits may be immediately analyzed or stored in the dark for several days before analysis (up to 2 weeks).
 4. User should control that the cytokines or heat-killed bacteria used in the experiment do not provide peaks within the range of stimulated macrophages' spectra. Here, we checked that the cytokines and heat-killed bacteria alone did not provide any signal within the studied range (0-20 kDa).
4. Mass spectrometer tuning and data acquisition.
 1. Insert the steel target containing samples in the mass spectrometer.
 2. Configure the mass spectrometer and run data acquisition. Use the default configuration for automated acquisition of the data.
 3. Note: a detailed view of the configuration of flexControl software is given in the appendix/Table 1. This may vary according to mass spectrometer and software used.

3. Bioinformatic Analysis

Note: the bioinformatic analysis was performed using the free and open source statistical analysis software R, along with specific analysis libraries (*MALDIquant*). R can be downloaded freely from its website <http://cran.r-project.org/>. A detailed description of the script is provided as supplementary material.

1. Loading and pretreatment of raw data.
 1. Store raw data on the computer associated with the mass spectrometer in multiple files and folders. Retrieve the root folder of the experiment with all subfolders. Copy root folder to personal computer for analysis.
 2. Use *readBrukerFlexData* and *MALDIquant* libraries to load and analyze raw data.

Note: *readBrukerFlexData* allows the loading of raw data from the mass spectrometer into a specific object in R for further analysis. See *MALDIquant* description for further information²⁷.

3. Analyze generated spectra.

Note: each spectrum consists of a list of peaks with their respective masses and intensities (relative abundance). Use the square root of the intensities to enhance the graphical visualization of the spectra. Correct the background using a statistic-sensitive nonlinear peak-clipping algorithm for baseline estimation²⁷. Use a signal-to-noise ratio of 6 to detect peaks. Consider that the detected peaks are similar across spectra when the mass/charge (m/z) values are within a 2,000 ppm window.

2. Score definition and computation. Classify the spectra using a hierarchical clustering with a ward algorithm for agglomeration and a dissimilarity matrix based on the Jaccard distance.

Note: the Jaccard index measures the similarity between Boolean sample sets (*i.e.* the presence/absence of a list of peaks).

3. Comparison of spectra and viewing.
 1. Analyze raw spectra plots. Note: x-axis represents the m/z ratio (in Daltons) and the y-axis represents the intensity (relative abundance).
 2. Assess the similarity between spectra by hierarchical clustering. Represent similarity (or divergence) as dendrogram.
 3. Assess the reproducibility by the mean of virtual gel-view representation. Note: virtual gel-view representation is a modified heatmap plot where relative abundance is color-coded with increasing intensities of blue.

Representative Results

The aim of the present protocol is to demonstrate the accuracy of whole-cell MALDI-TOF MS to assess the responsiveness of macrophages to their microenvironment.

Figure 1 describe preparation of stimulated macrophages from blood samples. **Figure 2** represents the analysis of monocytes and MDMs by flow cytometry. Note that monocytes expressed CD14 but not CD68 (**Figure 1A**). Conversely, MDMs expressed CD68 but not CD14 (**Figure 1B**).

Figure 3 describes the principle of whole-cell MALDI-TOF MS. Cells are deposited with matrix on the target plate. Within the mass spectrometer, a laser beam induces the desorption and ionization of molecules by shooting multiple times on the sample (240 shots). The produced ions are accelerated by a magnetic field and separated according to their m/z ratio in the tube. The TOF analyzer records the impact of the various ions at the end of the tube. According to the time of flight, each impact is converted into a m/z ratio, and impacts corresponding to the same m/z ratio are summed up to generate the full raw spectrums.

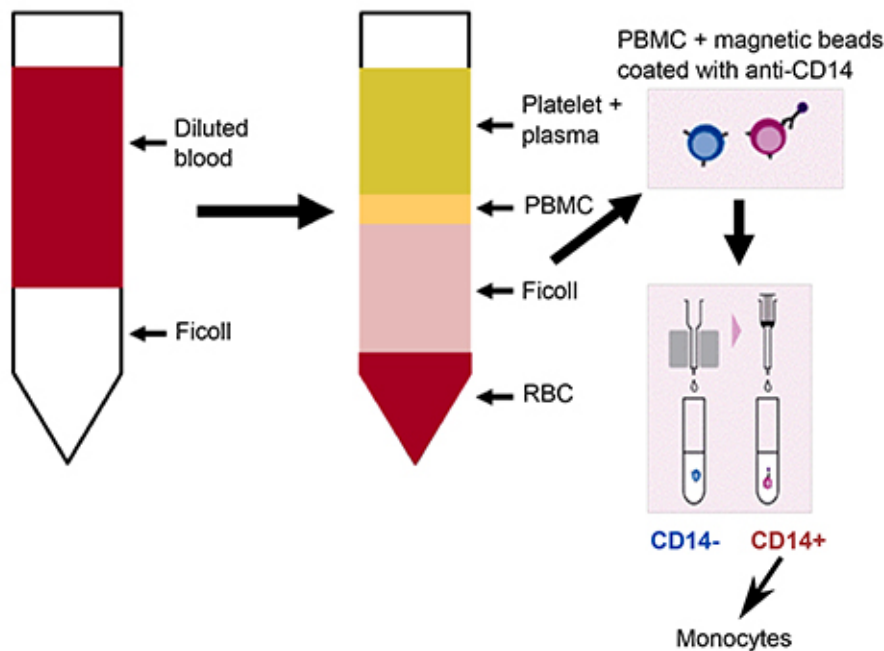
Figure 4 illustrates the role of sample preparations in the interpretation of MALDI-TOF MS results. A good quality spectrum is represented in **Figure 4A**. It usually contains a major peak around 5 kD ($m/z = 4,965$). A minimum cell concentration is required to obtain good samples: **Figure 4B** shows a poor quality spectrum obtained with a low cell concentration. However, raising MDM concentration above $1 \times 10^5/\mu\text{l}$ does not improve the quality of spectra. Similar poor results are obtained when the sample is mixed with matrix before deposition on the target plate. If mixing is done on the target plate, it may also result in heterogeneous crystallization, as shown in **Figure 4C**. Hence, deposition of the samples on the target is a tricky and critical step in this protocol.

The reproducibility of the spectra is shown in **Figure 5**. Here, spectra from various samples are represented as a heatmap. Relative abundance (intensity) is color-coded by intensities of blue. This virtual gel-view representation illustrates the reproducibility of the samples within each class. The normalization and alignment of the spectra is a critical step to obtain such results. An unsupervised analysis by hierarchical clustering is summarized as a dendrogram on the right hand side of the figure. It illustrates that all samples clustered within three different groups: unstimulated MDMs (NS), IFN- γ -stimulated or IL-4-stimulated MDMs.

Figure 6 illustrates the discrimination of M1 macrophages (MDMs stimulated with IFN- γ) from M2 macrophages (MDMs stimulated with IL-4) and unstimulated MDMs. Indeed, the peak representation of a reference spectrum for IFN- γ , IL-4 or unstimulated MDMs shows specific peaks for each class. This representation is obtained using the R *MALDIquant* library²⁷.

Figure 7 illustrates the specific fingerprints induced by several agonists. It is commonly accepted that IFN- γ , TNF and LPS induce an inflammatory (M1-type) response in macrophages. We used MDM samples stimulated with these cytokines alone or in combination to illustrate the accuracy of whole-cell MALDI-TOF MS. Indeed, spectra from all stimulated samples were clearly separated from those of unstimulated macrophages (**Figure 7A**). However, we obtained a specific fingerprint from each type of stimulation, as illustrated by the clustering of the samples according to the stimuli. Interestingly, MDMs also exhibited specific fingerprints induced by heat-killed bacteria (**Figure 7B**). These results support the hypothesis that MALDI-TOF MS may be used to analyze circulating cells to assess the host-response to infection or inflammatory diseases in the clinical setting.

1. Monocytes isolation



2. Macrophages: Monocyte derived macrophages (MDM) obtained by differentiation in culture media with human and calf fetal serum in 7 days.

3. Macrophages stimulation

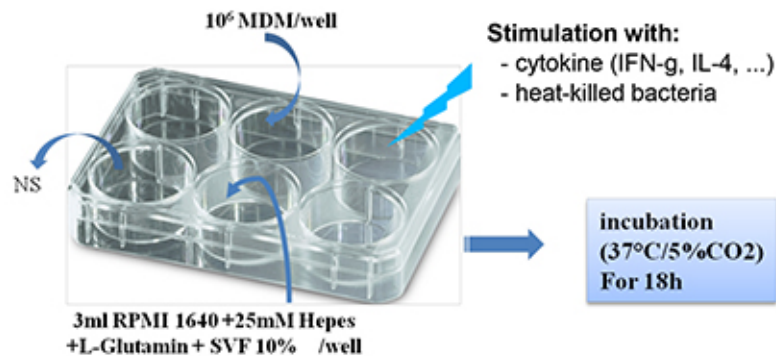


Figure 1. Preparation of biological samples. 1. Monocytes were selected from peripheral blood mononuclear cells (PBMC) by positive selection with magnetic beads coated with anti-CD14⁺ antibodies. 2. Macrophages were obtained in 7 days by culture of monocytes in RPMI. 3. Stimulated samples were obtained by adding either cytokines or heat-killed bacteria for 18h on differentiated macrophages. RBC: Red blood cells. [Click here to view larger image.](#)

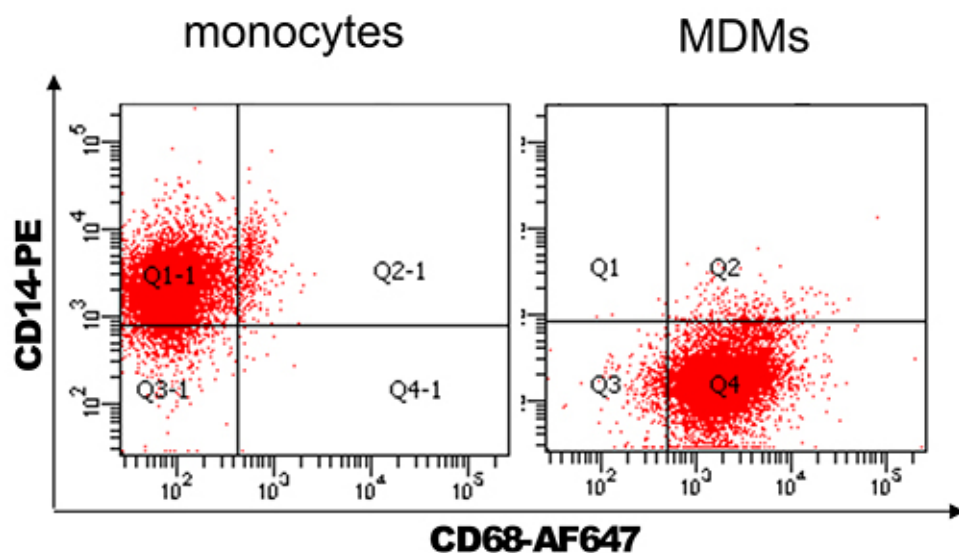


Figure 2. Assessment of CD14 and CD68 expression by flow cytometry. Monocytes (left panel) or MDMs (right panel) were labeled with anti-CD14-PE and anti-CD68-AF647 antibodies to assess membrane expression of these molecules. The differentiation of monocytes into MDMs is accompanied by the down-modulation of CD14 expression and the up-modulation of CD68 expression. [Click here to view larger image.](#)

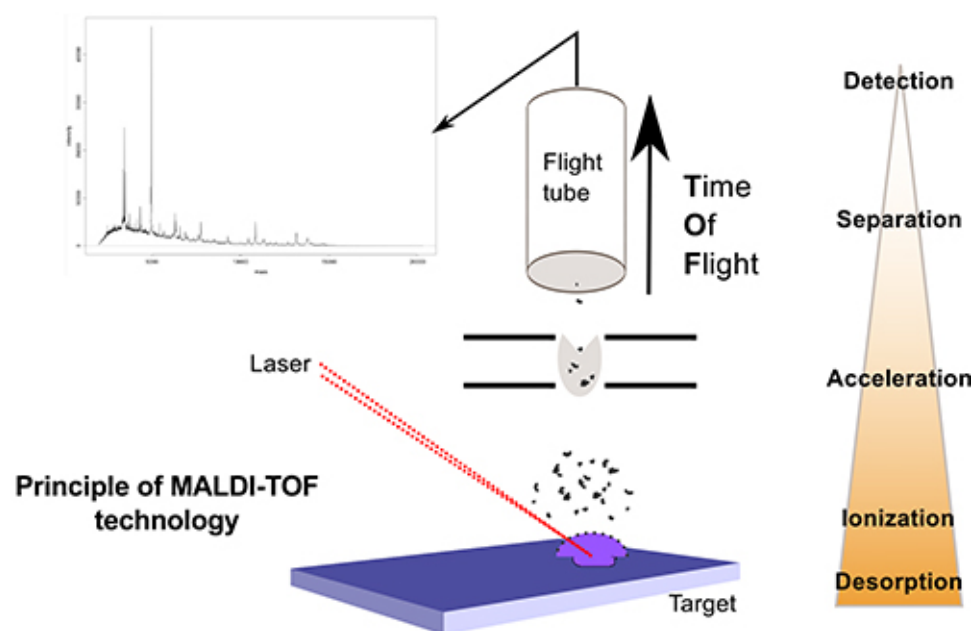


Figure 3. Principle of MALDI-TOF MS technology. This drawing describes the principle of the MALDI-TOF mass spectrometry. [Click here to view larger image.](#)

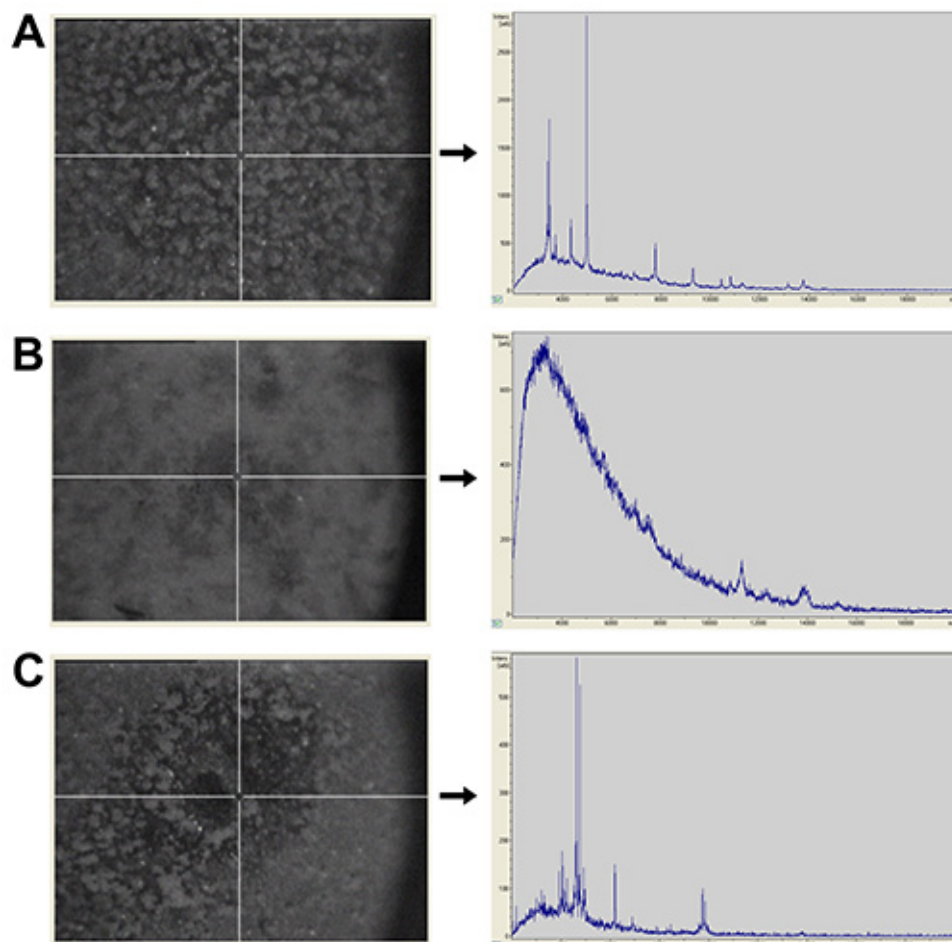


Figure 4. Whole-cell MALDI-TOF MS spectra. A zoomed view of spots deposited on the MALDI target (left panels) with corresponding spectra (right panels). Note that a good quality spot leads to an accurate spectra (A) whereas bad quality spots lead to spectra with a very poor signal to noise ratio (B, C). [Click here to view larger image.](#)

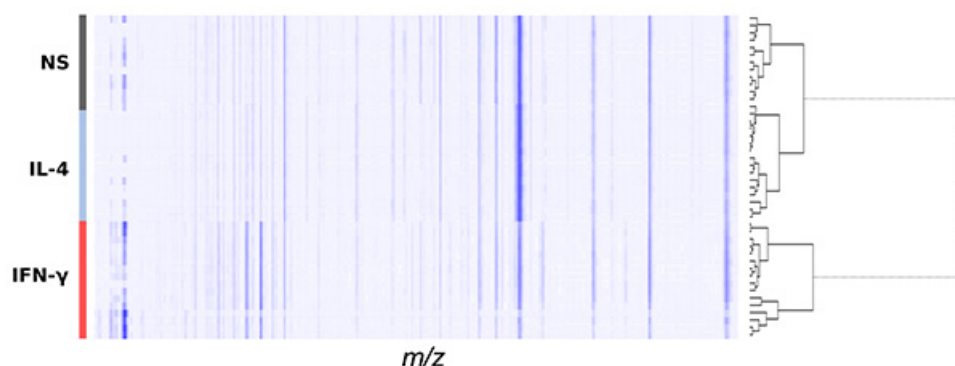


Figure 5. Reproducibility of MALDI-TOF MS spectra. Virtual gel-view of the whole spectra obtained from control and IL-4- or IFN- γ -stimulated MDMs are presented as a heatmap. Horizontal axis refers to the m/z ratio. Spectra are classified according to the presence/absence of peaks. NS: non stimulated; IFN- γ : interferon-gamma; IL-4: interleukin 4. This figure was reproduced from Ouedraogo *et al.*¹³ with permission. [Click here to view larger image.](#)

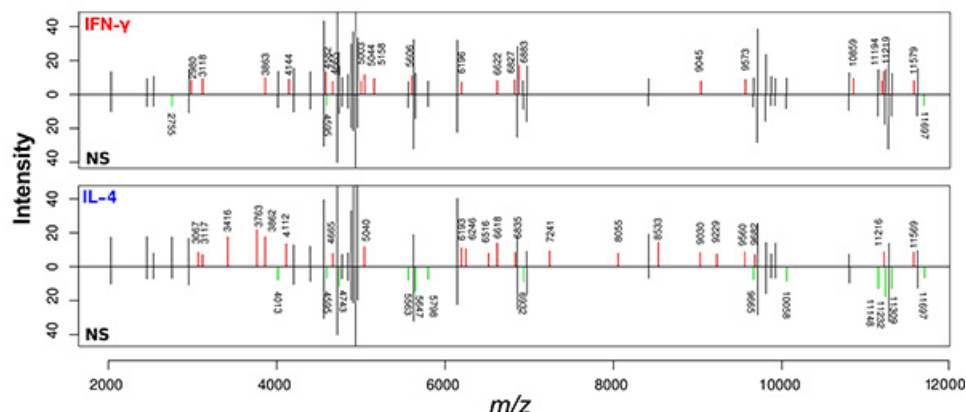


Figure 6. Reference spectra for M1 and M2 macrophages. The reference spectra for IFN- γ - and IL-4-stimulated MDMs are compared to the reference spectrum of nonstimulated (NS) MDMs. The peaks that are shared by stimulated and NS MDMs are in black. The peaks that are induced by stimulation are in red, whereas peaks that are detected only in NS MDMs are in green. m/z : mass/charge ratio; IFN- γ : interferon-gamma; IL-4: interleukin 4. This figure was reproduced from Ouedraogo *et al.*¹³ with permission. [Click here to view larger image.](#)

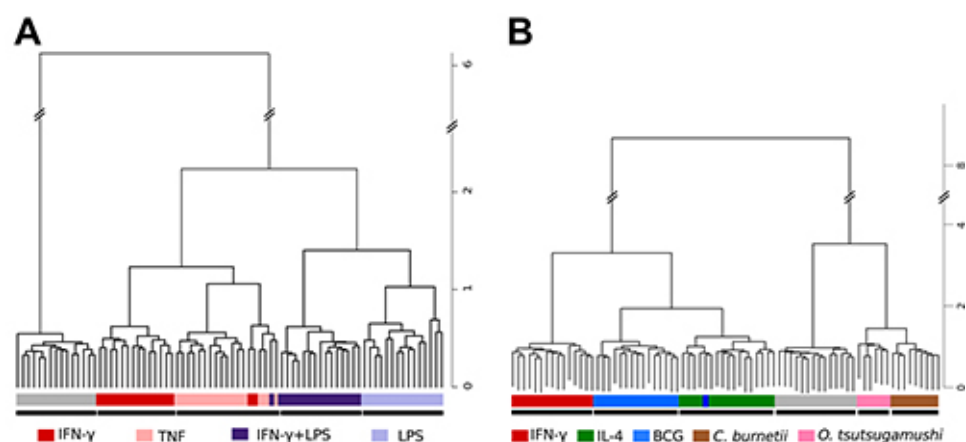


Figure 7. Hierarchical clustering of activated MDMs. MDMs were stimulated with different agonists for 18 hr. The results are shown as hierarchical clustering of the data. MDMs were activated with M1-related agonists (A) and intracellular bacteria or IL-4 (B). Unstimulated MDMs are presented in grey. IFN- γ : interferon-gamma; LPS: lipopolysaccharide from *Escherichia coli*; TNF: tumor necrosis factor; IL-4: interleukin 4; BCG: bacillus Calmette-Guérin; *C. burnetii*: *Coxiella burnetii*; *O. tsutsugamushi*: *Orientia tsutsugamushi*. This figure was reproduced from Ouedraogo *et al.*¹³ with permission. [Click here to view larger image.](#)

Discussion

This protocol describes the use of MALDI-TOF-MS on eukaryotic whole cells. Here, we illustrate the accuracy of the method by analyzing the multiple activation states of macrophages in response to their microenvironment.

The success of the protocol relies on few critical steps. First, any solution contaminant may alter the spectra. For example, it is important to wash cells in PBS to remove culture medium and serum proteins before deposition on the target. A cell concentration of 1×10^5 cells/ μ l is also needed to ensure reproducible results. Second, the crystallization is an important step in the protocol. To ensure good quality results, the target plate must be carefully washed and the matrix should be prepared before the deposition of samples on the target. The best results are obtained when the samples are deposited on the target just before the matrix solution (avoid mixing the samples with the matrix before the deposition on the target). Correct spontaneous mixing between samples and the matrix solution needs homogeneous deposits. Third, whole-cell MALDI-TOF-MS is a high-throughput technique, which can rapidly result in high amounts of raw data. Bioinformatics analysis is thus a major tool to systematically analyze the data in a reasonable amount of time. Quality assessment, background correction and normalization can be automated. The selection of relevant peaks (e.g. above a given signal-to-noise ratio) and the comparison of spectra based on the presence/absence of these peaks require important computational steps. These methods are described in details in the supplementary material of this article.

Although the acquisition of a mass spectrometer may represent a significant investment, daily running costs are low, and a high number of biological and technical replicates may be easily obtained in one run. For example, our university hospital is able to routinely identify bacteria in 200 clinical samples each day with a similar whole-cell technique. The cell concentration may be a limit for specific clinical applications such as the analysis of needle biopsies or cells harvested from broncho-alveolar lavages. A recent article described an automated approach of whole-cell MALDI-TOF analysis that allowed the robust classification of samples with as few as 250 cells on each spot¹¹. A proof of concept of the clinical application of this technique to the diagnosis of oral cancer has also been recently published¹⁵. The matrix choice may limit the type of analyzed molecules. Some matrices will favor the ionization of specific type of molecules (proteins, lipids, sugars...) and of a given mass

range. In our conditions, we were not able to retrieve good quality spectra with ions above 20 kDa. In our protocol, we focused on the analysis of whole spectra as a fingerprint of a given activation state of cells. Therefore, we did not try to identify the proteins that form the main peaks of the spectrum. The identification of specific biomarkers requires an alternative use of mass spectrometry.

In conclusion, we describe here the application of a whole-cell MALDI-TOF MS approach for the accurate and rapid analysis of macrophage activation. This method allowed the identification of macrophage-specific fingerprints that are representative of the diversity of proteomic responses to cytokines and bacterial pathogens. The protocol we described here may be useful for studying the immune host response in pathological conditions or may be extended to wider diagnostic applications.

Disclosures

The authors have no conflict of interest to declare. RO, JLM and CC are inventors of an international patent WO 2011/154650, named "Procédé d'identification de cellules de mammifères par spectrométrie de masse MALDI-TOF".

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