ELECTROWETTING-ON-DIELECTRIC FOR ANALYSIS OF PEPTIDES AND PROTEINS BY MATRIX ASSISTED LASER DESORPTION/IONIZATION MASS SPECTROMETRY

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ABSTRACT

A new technique for preparing samples for matrix assisted laser desorption/ionization mass spectrometry (MALDI-MS) is reported. The technique relies on electrowetting-on-dielectric (EWOD) to move droplets containing proteins or peptides and matrix to specific locations on an array of electrodes for analysis. Standard MALDI-MS reagents, analytes, and concentrations are demonstrated to be compatible with the technique. The results suggest that EWOD may be a useful tool for automating sample preparation for high-throughput proteomics and other applications of MALDI-MS.

INTRODUCTION

The field of proteomics is important as a basic science and for clinical applications ranging from drug discovery to assays for gene expression [1]. Standard methods for proteomics rely on mass spectrometry (MS) coupled with matrix assisted laser desorption/ionization (MALDI). Progress in proteomics would be accelerated by the availability of high-throughput analysis techniques.

Typical proteomics analyses with MALDI-MS require many steps, as depicted in Figure 1. A critical step is the mixing of sample and matrix (Figure 1d); this step is time-consuming and can lead to sample loss, dilution, and contamination. High-end commercial instruments accomplish this step with robotically controlled deposition of reagents. This technology has recently been combined with microfabricated picoliter droplet delivery devices, which enables deposition of arrays of thousands of spots for analysis [2, 3].

Figure 1. Schematic depicting the steps required for proteomics analysis with MALDI-MS. The current work focuses on step (d).

We present a novel method for MALDI sample preparation relying on electrowetting-on-dielectric (EWOD) [4-7]. In EWOD, a sequence of potentials is applied to adjacent electrodes buried beneath hydrophobic, dielectric layers. This causes changes in local wettability, which enables transport of aqueous droplets across a surface. EWOD devices are formed in an array geometry, which makes it an attractive technology for use with MALDI-MS.

Unlike droplet dispensing systems [2, 3], EWOD-based devices are reconfigurable, require low power, and require no moving parts. We recently presented the design parameters for EWOD devices to dispense, merge, mix, and cut droplets suspended in air [6]. This is of critical importance, as MALDI signals are severely degraded by the presence of non-volatile liquids. Thus, other array-based droplet manipulation techniques that rely on silicone or hydrocarbon oils as a suspension medium [7, 8] would probably not be suitable for MALDI-MS.

We describe here the fabrication and use of EWOD devices for MALDI-MS analysis of peptides and proteins. This is the first report of using droplet-based microfluidics for proteomics or mass spectrometry applications.

EXPERIMENTAL

EWOD devices were fabricated using procedures similar to those developed previously [4-6]. The devices consisted of a top and bottom plate as shown in Figure 2. Electrodes on the bottom plate were formed from doped polysilicon and were buried under thermally grown oxide (1500 Å) and Teflon-AF (7500 Å). The electrodes were 1 mm² with a 4 µm gap between each electrode. A single electrode on the top plate was formed from indium-tin-oxide (ITO) buried under a thin layer of Teflon-AF (150 Å). The two plates were joined by three layers of double-sided tape (300 µm).

Figure 2. Side view (a) and top view (b) schematics of EWOD device (not to scale).

EWOD devices were used to move droplets of sample and matrix, as shown in Figure 3. Typically, droplets were moved several mm in a few seconds, and then dried under vacuum for ~1-2 min. Droplets were routinely driven on and between each line

of electrodes on each device. Matrix and sample droplets were either moved and dried separately, or two droplets were merged on a device and then dried. Typically, several spots were deposited onto the bottom plate of a device, which was then affixed with double-sided tape to a customized MALDI sample target.

Figure 3. Video sequence (left-to-right) of EWOD-driven movement of 0.5 μ L droplets of insulin and ferulic acid (FA) down the right side of the electrode pattern. In (a), an insulin droplet was moved and dried. In (b), a droplet of FA was driven onto the dried insulin spot.

A Voyager DE-STR spectrometer (Applied Biosystems) was used to collect MALDI mass spectra from deposited spots of sample and matrix. 500 shots were collected per spectrum, with the laser power adjusted for different matrices. Images and mass spectra of insulin co-crystallized with the common matrices 2,5-dihydroxybenzoic acid (DHB, 10 mg/mL, 0.05% TFA, with 5% acetonitrile), ferulic acid (3 mg/mL, 0.0375% TFA, with 15% acetonitrile), or sinapinic acid (SA, 10 mg/mL, 0.1% TFA, with 50% acetonitrile) are shown in Figure 4.

Figure 4. Images and MALDI mass spectra of insulin (5733 Da) co-crystallized with various MALDI matrices. For each trial, a 0.5 μ L droplet of insulin (1.75 μ M, 0.025% TFA) was moved by EWOD to a designated electrode and dried. In (a) and (b), insulin deposition was followed by EWOD-driven deposition and drying of a 0.5 μ L droplet of matrix. In (c), insulin deposition was preceded by manual deposition and drying of a 0.5 μ L droplet of matrix.

In addition to insulin, several other peptides and proteins were analyzed, including insulin chain B (Fig. 5a), cytochrome c (Fig. 5b), and myoglobin (Fig. 5c). The measured molecular weights for cytochrome c and myoglobin were higher than the expected values, which is believed to be caused by the use of a custom sample target that changes the ion flight path length. In future experiments, EWOD-MALDI mass spectra will be calibrated with known standards. These data demonstrate that

EWOD-MALDI can be used to prepare samples with a variety of standard MALDI matrices, and to analyze proteins and peptides having a wide range of molecular weights.

Figure 5. MALDI mass spectra of (a) insulin chain B (3495 Da, 2 μ M, 0.025% TFA), (b) cytochrome c (12.8 kDa 1.85 μ M, 0.025% TFA), and (c) myoglobin (16.9 kDa, 1.45 μ M, 0.0125% TFA). For each spectrum, a 0.5 μ L droplet of peptide or protein was moved by EWOD and dried. The process was then repeated with DHB (10 mg/mL, 0.05% TFA, with 5% acetonitrile).

CONCLUSIONS

This work represents a potentially important breakthrough for proteomics. Future work will concentrate on extending EWOD-MALDI to devices with dense arrays of electrodes for high-throughput analysis, and on integrating additional sample processing steps, such as sample purification, disulfide reduction, and proteolytic digestion (Fig. 1c).

REFERENCES

1. D. Figeys, "Proteomics in 2002: A year of technical development and wide-ranging applications", *Anal. Chem.*, **75**, 2891-2905 (2003).
2. D. P. Little, T. J. Cornish, M. J. O'Donnell, A. Braun, R. J. Cotter, and H. Koster, "MALDI on a Chip: Analysis of Arrays of Low-Femtomole to Subfemtomole Quantities of Synthetic Oligonucleotides and DNA Diagnostic Products Dispensed by a Piezoelectric Pipette", *Anal. Chem.*, **69**, 4540-4546 (1997).
3. P. Onnerfjord, J. Nilsson, L. Wallman, T. Laurell, and G. Marko-Varga, "Picoliter Sample Preparation in MALDI-TOF MS Using a Micromachined Silicon Flow-Through Dispenser", *Anal. Chem.*, **70**, 4755-4760 (1998).
4. J. Lee, H. Moon, J. Fowler, T. Schoellhammer, and C.-J. Kim, "Electrowetting and electrowetting-on-dielectric for microscale liquid handling", *Sens. Actuators, B*, **95**, 259-268 (2002).
5. H. Moon, S. K. Cho, R. L. Garrell, and C.-J. Kim, "Low voltage electrowetting-on-dielectric", *J. Appl. Phys.*, **92**, 4080-4087 (2002).
6. S. K. Cho, H. Moon, and C.-J. Kim, "Creating, Transporting, Cutting, and Merging Liquid Droplets by Electrowetting-Based Actuation for Digital Microfluidic Circuits", *J. Microelectromech. Sys.*, **12**, 70-80 (2003).
7. M. G. Pollack, R. B. Fair, and A. D. Shenderov, "Electrowetting-based actuation of liquid droplets for microfluidic applications", *Appl. Phys. Lett.*, **77**, 1725-1726 (2000).
8. J. A. Schwartz, J. V. Vykoukal, and P. R. C. Gascoyne, "Droplet-based chemistry on a programmable micro-chip", *Lab Chip*, **4**, 11-17 (2004).