EWOD LAB-ON-CHIP FOR MASS SPECTROMETRY AND FLUORESCENCE ANALYSIS

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ABSTRACT
ElectroWetting On Dielectric for Digital MicroFluidic (EWOD-DMF) has already been demonstrated to allow basic microdroplet manipulations on totally hydrophobic surfaces such as creating, cutting, merging and transporting. In this paper, we propose an innovative solution for biological liquid operations by contacting the droplets with superhydrophilic patterned areas on a superhydrophobic substrate as a counter electrode along the EWOD transport path. Liquid/Surface characterization has been performed using both fluorescence measurements and matrix-free Desorption/Ionisation On Silicon Mass Spectrometry (DIOS-MS) analysis. These showed that biomolecules can be transferred on the superhydrophilic pads and that the overall superhydrophobic property of the surface limits the non-specific adsorption of biomolecules.

KEYWORDS: EWOD-DMF, Mass spectrometry, Fluorescence analysis, Lab-On-Chip

INTRODUCTION
Since the emergence of Lab-On-Chip applications for biomedical protocols, EWOD-DMF systems have been implemented in many domains such as enzyme assays, immunoassays, DNA-based applications, cell-based assays, tissue engineering and proteomics [1]. Thus, DMF Lab-On-Chip (LoC) have been coupled with off-line Matrix-Assisted Laser Desorption/Ionisation Mass Spectrometry (MALDI-MS) analysis as an alternative to optical methods. MALDI-MS analysis brings the advantage of time reduction, less complexity of sample preparation and high sensitivity. Srinivasan et al. [2] succeed to coupled an EWOD device with an MALDI-MS analysis by moving protein sample droplets towards a stamping site where the analysis were performed. Next, Wheeler et al. [3-4] used EWOD-DMF to manipulate both the MALDI matrix droplets and proteins. Even if this system is a pioneering one in the LoC applications for MS analysis, several drawbacks can be underlined: proteins or peptides concentrations were not inferior to nmol/µl and the analysis protocol is somewhat time consuming and complicate due to the manipulation of viscous matrix and to the proteins dry operation on the surface. Matrix-free DIOS-MS can therefore increase the sensibility by the use of nanostructured silicon surfaces.

We report an original study by coupling EWOD-DMF using superhydrophobic substrates authorizing a matrix free DIOS-MS analysis for very low concentration (i.e. fmoles/µL) peptides sample analysis through a rapid and simple protocol. The use of superhydrophobic surfaces permit the direct ionization/desorption of molecules on the surface and prevent also from non-specific adsorption on the sample pathway.

EXPERIMENTAL
EWOD-DMF devices were fabricated using conventional clean room facility. The nickel electrodes network were realized on glass substrates by photolithography and Su-8 2002 photoresist were used as an insulating layer. Substrates were finally hydrophobized by a 30nm Cytop® layer deposition. Contact angle and hysteresis contact angle were 112° and 12° respectively (goniometer from Krüss GmbH, Germany).

Counter-electrodes were fabricated on highly doped silicon substrates using the Electroless Metal Deposition (EMD) [5] as previously described by Piret et al. [6]. The nanostructure dimensions were in the range of 10-200nm, with 1µm height, as evidenced by the cross-sectional SEM views Figure 1.

Figure 1: SEM pictures (a, top, and b, cross views) of the electroless nanostructured silicon substrate (1µm height). (c) Picture of a deionized water droplet on the superhydrophobic surface (160° contact angle).

To achieve the superhydrophobicity of the surfaces, an OTS monolayer was deposited using conventional methods [7]. Contact angle and contact angle hysteresis were 164° and 2° respectively leading to a superhydrophobic character.
Superhydrophilic patterns, 100*100µm² and 4000µm spaced, were realized by photolithography step. A 3 minutes plasma O₂ (100W, 100mT, 20sccm) were performed to removed the OTS at the localized patterns and then the photoresist were removed.

EWOD-DMF were carried out as followed: a Labview program controls 24 electrodes through a relay card linked to a signal generator (Centrad GF 265, ELC, FRANCE) and amplifier (Tegam 2340, USA). The actuation voltage was set at 100V_TRMS. A Teflon holder encapsulates a 1µL droplet solution between the electrodes base and the superhydrophobic counter-electrode set by a 300µm gap. A video camera (JVC, 25fps) records the droplet motion. The electrodes network allows to the EWOD-DMF all the microfluidic operations such as separation, transport and merging processes. The simplicity of our system allowed portable, automatic, and high speed manipulations.

The experimental protocol was set by the motion of a 1µL biological droplet at 100mm/s (controlled by the Labview program) and the number of back and forth was modulated between 0.5 and 100. We assume that the time of interaction between liquid and superhydrophobic area is equal to 2ms per passage and that the volume deposited inside these apertures is about 2.6pL (considering a semi spherical droplet of 50µm radius). Two biological solutions were displaced:

- A 50fmol/µL of Lys-Arg-Rhodamin solution diluted in a 10mM ammonium citrate solvent for fluorescence characterizations.
- A 50fmol/µL of sample Mix1 (sequazyme™ peptide mass standard kit from Applied Biosystems (AB, Part number P2-3143-00) containing [Des-Arg¹]-bradykinin (MW = 904.5Da), angiotensin I (MW = 1295.7Da), [Glu¹]-fibrinopeptide B (MW = 1570Da), and neurotensin (MW = 1672.9Da) peptides) diluted in a 10mM ammonium citrate solvent for DIOS-MS characterizations.

Figure 2 shows the schematic view of the biological droplet displacement on the superhydrophobic counter-electrode and the deposition of the sample inside the superhydrophilic patterns. DIOS-MS and fluorescence analysis were then performed inside (Si) and outside (Sout) the superhydrophilic spots.

Figure 2: Schematic views of (a) the droplet displacement by EWOD on the superhydrophobic/superhydrophilic patterned substrate, (b) the DIOS-MS analysis, and (c) the fluorescent measurement inside and outside the plots.

RESULTS AND DISCUSSION

Figure 3 shows the fluorescence pictures of the superhydrophobic/superhydrophilic patterned surfaces after the displacement of the 50mol/µL Rhodamin droplets. We observed that the signal inside the plots (Si) increases until saturation as the number of cycles increases. In the same way, the signal outside the plots (Sout) increases with the number of back and forth. Therefore, we have been able to calculate a Signal to Noise S/N ratio between Si and Sout and optimized the number of cycle to 126 get the best S/N ratio.

For DIOS-MS characterization, a 50fmol/µL Mix1 droplet solution were displaced one time along 5 electrodes followed by a rinsing step with a 1µL deionised water EWOD displaced. Figure 4 shows mass spectra (from Voyager-DE-STR time-of-flight (ToF) mass spectrometer, Applied Biosystem) of the peptides mixture deposited (a) inside a superhydrophilic pattern and (b) outside, on the superhydrophobic surface after the droplet displacement. We can see that the transfer of the peptides in the superhydrophilic regions were successful.

We present here a summary of the best results obtained by fluorescence and DIOS-MS analysis. A more detailed study will be presented in a future paper.
Figure 3: Fluorescence pictures of superhydrophobic/superhydrophilic patterned substrate for (a) 10, (b) 76, (c) 126, (d) 200 cycles of a 50fmol/µL Rhodamined droplet at 100mm/s. The fluorescence is only visible inside the superhydrophilic patterns (100µm sizes, 4000µm spaced). The image has been artificially colored to improve clarity.

Figure 4: Mass spectra of a peptide mixture Mix1 (50fmol/µL) deposited by EWOD displacement (a) inside the superhydrophilic patterns and (b) on the superhydrophobic surface.

CONCLUSION
The main advantages associated with such nanostructured superhydrophobic substrates are: (i) a significant decrease of the droplet’s flow resistance and non-specific adsorption (ii) a simple realization of superhydrophilic pads in the superhydrophobic surfaces allowing analytes trapping and enhancement of the liquid/surface interaction, (iii) and a subsequent analysis by matrix-free DIOS-MS on these pads. Thus, the combination of EWOD-DMF and DIOS-MS by the use of these kinds of surfaces led to fast (1-2min), localized, highly sensitive, and matrix-free analysis.

ACKNOWLEDGEMENT
The research leading to these results has received funding from the European Community’s Seventh Framework Programme (FP7/2007–2013) under grant agreement no. 227243.

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