A NOVEL INTERFACE COUPLING DROPLET MICROFLUIDICS WITH MALDI-MASS SPECTROMETRY

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ABSTRACT

We present a novel method to interface a continuous-flow droplet-based microfluidic system with matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS). The interface facilitates the automated deposition of over 26,000 individual droplets on a single high-density micro-array plate at high throughput. The micro-array plate is directly compatible with standard MALDI mass spectrometers. Therefore, the method presented here enables label-free content analysis of individual microdroplets.

KEYWORDS

droplet microfluidics, segmented flow, mass spectrometry, MALDI, micro-array plate, interface, spotting, deposition, label-free detection, high-throughput screening.

INTRODUCTION

Over the last ten years, the generation and manipulation of ultrasmall-volume droplets on microfluidic platforms has opened captivating possibilities for chemical and biological research.[1,2] However, the analysis of the droplets is challenging and commonly relies on optical methods such as fluorescence microscopy. This significantly limits the applicability of droplet microfluidics because labeling of target molecules with fluorophores is required. Our newly developed interface enables label-free detection and identification of molecules from individual droplets and overcomes the difficulty of phase separation.

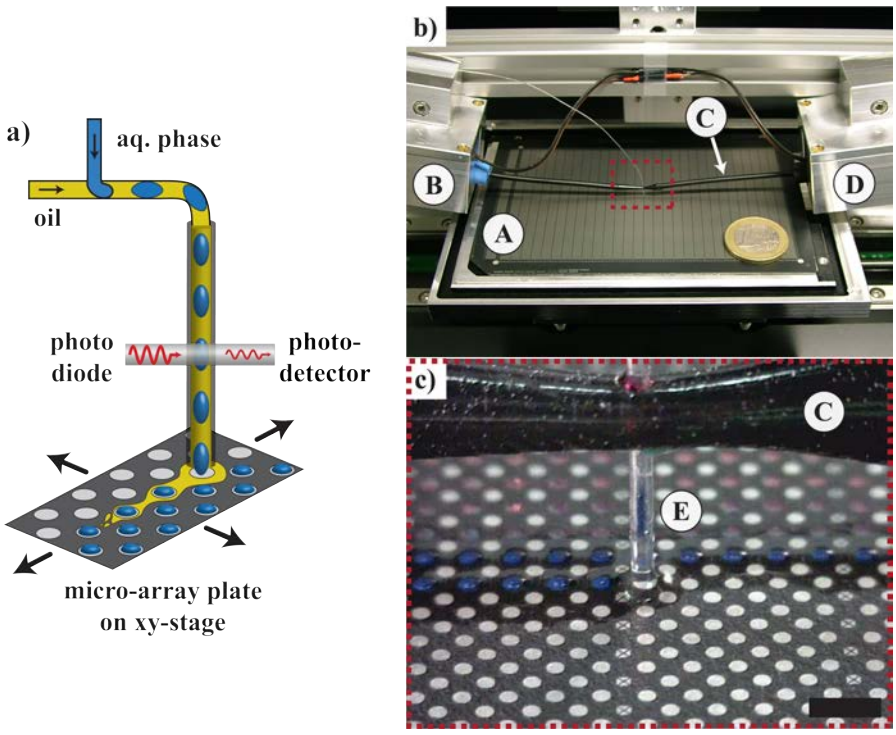

Figure 1. a) Schematic representation of droplet creation, in-line droplet detection and droplet deposition. After deposition, droplet phase and oil phase evaporate quickly which resolves the problem of phase separation. b) Overview of the interface with the micro-array plate (here with a total of 26444 hydrophilic spots of 300 µm diameter) mounted on an xy-stage (A) and the droplet detection setup. The detection setup consists of a photodiode (B), an optical fiber (C) and a photodetector (D). c) Enlargement of b) showing the spotting capillary tip (E) and illustrating the spotting process of 3 nL droplets containing blue food dye (patent blue V) on hydrophilic spots. Scale bar: 1 mm.

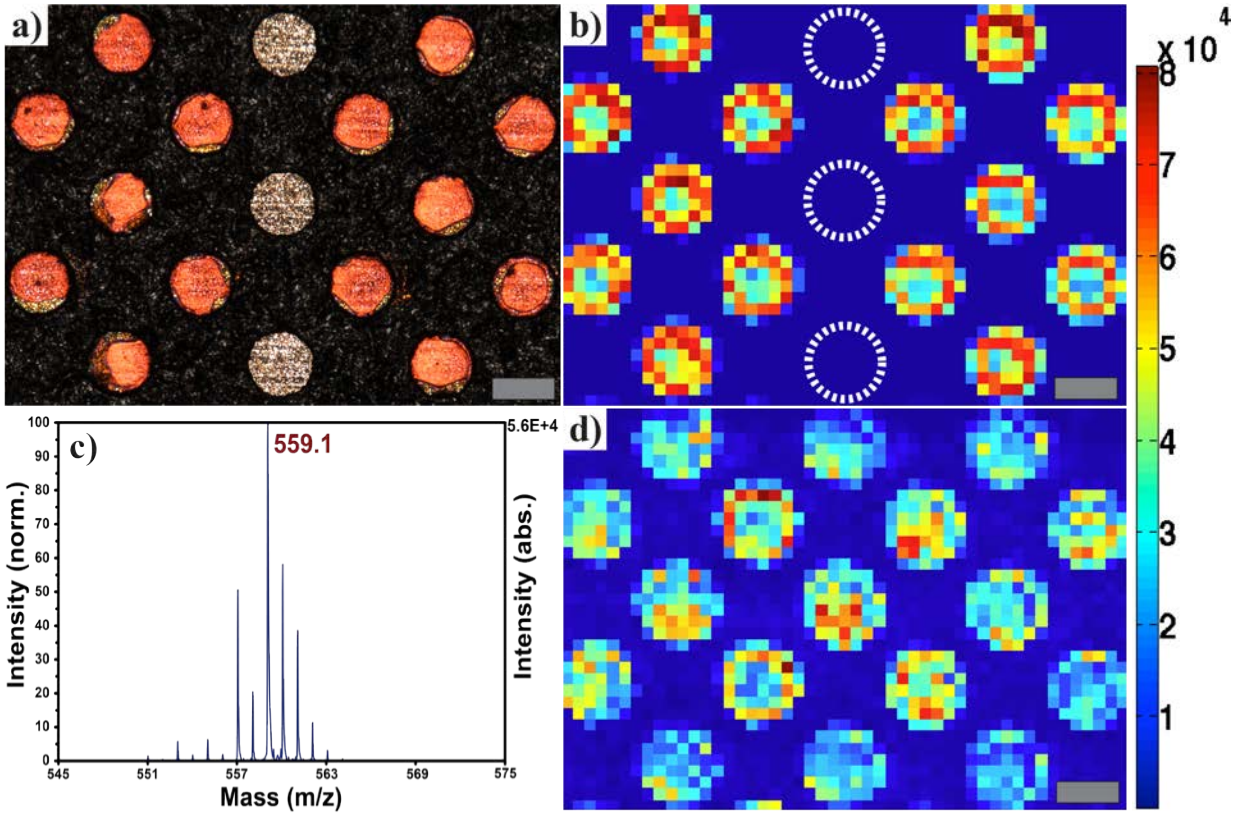

Figure 2. a) Micrograph showing a section of the micro-array with food dye droplets (dried) deposited in vertical lines leaving one column empty. b) and d) MALDI-MS imaging experiment: After spotting DHB (MALDI matrix) in horizontal lines on the array section showed in a), the section has been raster-scanned using 50x50 µm² spots. The color represent the intensity of the patent blue V base peak (559.16 m/z) (b) and of the DHB base peak (153.02 m/z) (d). c) A MALDI-MS spectrum of a hydrophilic spot containing patent blue V. No cross-contamination could be observed. All scale bars: 300 µm.

EXPERIMENT

We created 3 nL microdroplets by injecting an aqueous phase into an immiscible oil phase (perfluorodecaline) using a microfluidic T-junction (Fig. 1a). The microchannel is connected to a capillary, the end of which deposits droplets on a metal plate mounted on an *xy*-stage (Fig. 1b & c). The plate (standard MALDI-MS plate size of 123x81 mm²) is coated with a hydrophobic Teflon layer, which has been structured by laser ablation to form an array of 26,444 hydrophilic spots of 300 µm diameter.[3] A simple optical detection system consisting of a photodiode and a photodetector registers every droplet passing the capillary and automatically triggers the motion of the plate towards the next hydrophilic spot ensuring that only one droplet is deposited per spot.

The hydrophobic coating of the plate prevents cross-contamination between adjacent hydrophilic spots and confines each droplet to a defined position within the array. To demonstrate this, droplets of an aqueous solution of blue food dye were spotted in a specific pattern leaving vertical lines of empty spots (Fig. 2a). After evaporating the oil phase and the analyte-containing aqueous droplets, additional droplets of 2,5-dihydroxybenzoic acid (MALDI matrix) were spotted in a second run across all spots in horizontal lines. As the oil-flow between successive matrix droplets prevents any direct aqueous connection between successive spots, no crossing-over of dye could be observed by eye. The absence of cross-contamination was also verified by MALDI-MS imaging (Fig. 2b & d).

As a first application we monitored the enzymatic conversion of Angiotensin I to Angiotensin II by Angiotensin Converting Enzyme, an important process in the regulation of blood pressure in humans. Substrate and enzyme were mixed, loaded into a syringe, continuously aliquoted into droplets and spotted. For 3 hours 8,000 droplets were deposited and immediately dried using a flow of dry nitrogen gas to stop the reaction. Each spot represents a single time point of the enzymatic conversion. Subsequently, a MALDI-MS spectrum was obtained from each hydrophilic spot and the progress of the conversion for each time point was calculated from the relative peak intensity of Angiotensin II (product) over the total peak intensity of Angiotensin I (substrate) and II. Figure 3a shows the spatial distribution of the calculated peak ratio on the micro-array plate while figure 3b shows the peak ratio as a function of reaction time.

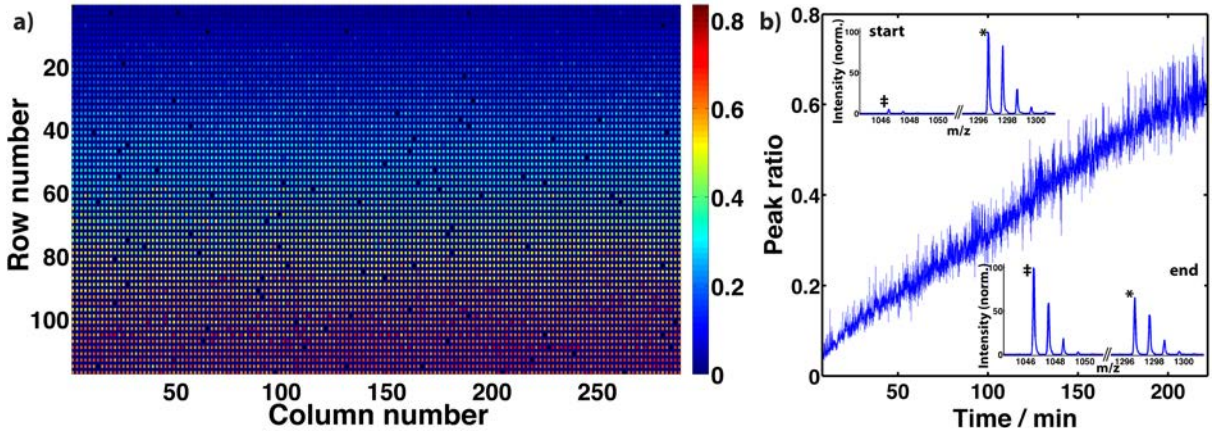

Figure 3. a) Heat map showing the progress of the enzymatic conversion in form of the peak ratio for the 8000 droplets spotted on individual hydrophilic spots and measured by MALDI-MS. b) The peak ratio as a function of time. Insets: MALDI-MS spectra from the beginning and at the end of the enzymatic reaction (Angiotensin I, substrate; ‡ Angiotensin II, product).*

CONCLUSION

Our method combines the advantages of droplet microfluidics such as high throughput and ultra-low sample consumption with the high sensitivity (down to attomoles) and excellent mass resolution of MALDI-MS. The possibility to analyze the content of individual microdroplets without labeling will substantially widen the scope of droplet applications, particularly in proteomics and metabolomics research.

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