WATER-IN-OIL DROPLET-BASED MICROFLUIDIC SYSTEM FOR ENZYMATIC STUDIES, COUPLED TO OFF-CHIP ELECTROSPRAY IONIZATION MASS SPECTROMETRY

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ABSTRACT

Droplet-based microfluidic systems coupled to electrospray ionization (ESI) mass spectrometry (MS) has a number of advantages for the study of enzyme kinetics: minimal reagent consumption, a well-defined reaction time control, and label-free and multiplex detection analysis. However, most studies have been dependent on ESI sources integrated on chips, which can be a challenge for practical application. Here, we report a simple trick for coupling of a standard ESI source to a droplet-based microfluidic chip that can also be made with standard techniques. With the new coupling, we demonstrated a label-free kinetic analysis of an enzymatic reaction in 50 nL droplets.

KEYWORDS

Droplets, ESI-MS, Reaction Kinetics, Coupler

INTRODUCTION

Droplets in microfluidic channels have many advantages for enzyme kinetic studies. For example, droplets offer minimal reagent consumption due to a small reaction volume, and well-defined reaction time control due to fast mixing and no dispersion [1-2]. Most of the kinetic analyses in droplets have successfully employed fluorescence as detection methods [1,3]. However, fluorescent labels incorporated in the reaction system can cause challenges like increased material costs, possible effects on the nature of the reaction, and limited ability of multiplex detection. ESI-MS is an attractive option because it provides label-free and multiplex detection. Coupling of droplet-based microfluidic system to ESI-MS has been reported [4-5], where the droplet was separated into an aqueous carrier and flowed into ESI sources integrated with the microfluidic chips. Although such a fully integrated system is an ideal solution, it in many cases offers practical solutions to couple a partly integrated system via well-established conventional routes to ESI-MS. A recent work [6] has shown that droplets in a capillary can be sprayed as they are, with the oil phase in between. However, there remained a challenge that the droplets in the chip should be transferred into the capillary through a connection between them. Droplets can suffer unfavorable coalescence or split at a normal “Chip to Capillary” connection made with a via-hole and a ferrule. In this study, we introduced novel droplet-compatible connections in order to avoid coalescence or split of droplets. The connections enable us to couple a droplet-based microfluidic system to an off-chip ESI source without separation, with the important advantage that the droplet is not diluted into an aqueous carrier.

EXPERIMENTAL

Figure 1 represents the schematic diagrams of the system used for MS analysis of enzyme kinetics. The system consists of a microfluidic chip, an ESI source, a quadrupole time of flight (QTOF)-MS system, syringe pumps, and additional connecting capillaries. The main functionalities of the system to execute enzyme kinetics analysis are (1) preparing droplets of solution for enzyme reaction, (2) controlling time for reaction, (3) droplets delivery to ESI-MS via “Chip to Capillary” connection, and (4) ESI-MS analysis of the droplets. The microfluidic chip was fabricated by standard microfabrication processes. Channels and via-holes for inlets and outlets were formed by deep reactive ion etching in a silicon wafer, which was bonded to a glass wafer.

In order to realize a coalescence-free delivery of droplets, dead volume at “Chip to Capillary” connection should be eliminated. Dead volume can trap droplets and cause coalescence and split. Two types of novel connections were designed for coupling of the microfluidic chip to the capillary bound for the ESI source (Figure 2). The first one, the connection with a channel on glass, is shown in Figure 2(a). The via-hole and the channel on the silicon wafer (channel on silicon) were connected with a channel formed on the glass wafer (channel on glass). The width of the channel on glass is the same as the inner diameter of the capillary. The capillary was inserted into the via-hole guided by the ‘Nanoport’ ferrule to reach the bottom of the via-hole so that the opening of the capillary is placed at the end of the channel on glass. Figure 2(c) shows the

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other design, the connection with a PDMS ferrule. A ferrule with a channel on the surface of it was made with PDMS and placed in the via-hole together with the capillary. The ferrule seals the space between the capillary and the via-hole, while the fluid path from the chip to the capillary is kept open due to the channel on the ferrule.

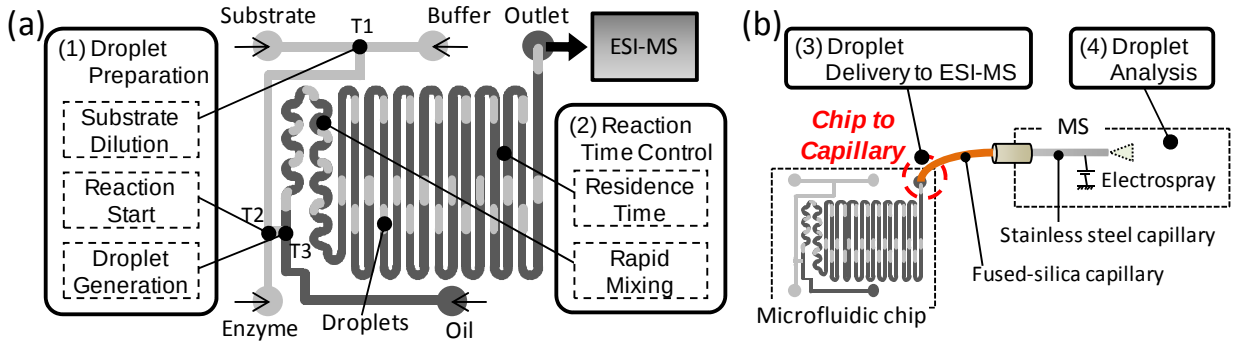

Figure 1. Overview of the microfluidic channel (a) and the whole system (b). Four main functionalities of the system are indicated (1)-(4).

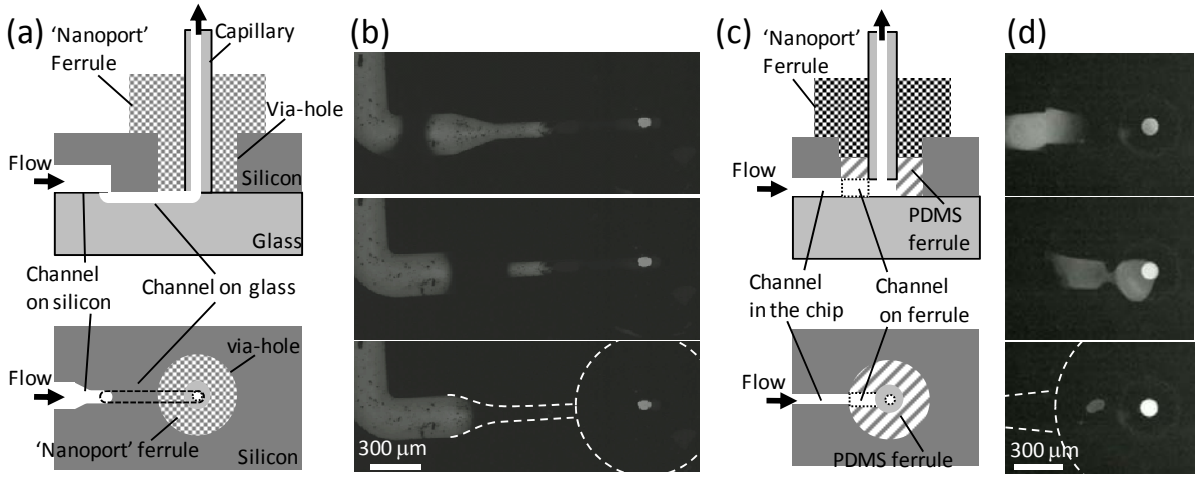

Figure 2. "Chip to Capillary" connection to avoid coalescence of droplets. The design schematics of the cross-section and the bottom view of (a) the connection with a channel on glass, and (c) the connection with a dedicated ferrule fabricated of polydimethylsiloxane (PDMS). (b and d) Time-lapse microphotographs of the fluorescein droplet flowing out of the chip into the capillary via two types of connections.

To evaluate the system, the kinetic profile of peptide digestion by trypsin was analyzed with the system. We employed two peptides, ACTH18-39 as substrate and leucine enkephalin as internal standard (LeuEnk) for MS analysis. Solutions of enzyme, substrate and buffer were mixed and compartmentalized into 50 nL droplets within the channel. Concentrations of the components are tuned by the flow rate ratio of the solutions, while reaction time is defined by the total flow rates. The concentrations of the substrate, the product and the internal standard were simultaneously measured with ESI-MS.

RESULTS AND DISCUSSION

Two types of "Chip to Capillary" connections were tested. Figure 2 (b) and (d) show fluorescein droplets at either of connections flowing out of the chip into the out-going capillary without coalescence. Because the connection with a channel on glass was more robust and easier to setup, we used this one to build the system.

To evaluate the performance of the present system for ESI-MS analysis of droplets, we analyzed two peptides, ACTH18-39 and LeuEnk, which can work as a substrate and an internal standard for the following trypsin analysis, respectively. Figure 3 represents peptide traces pulse-like showing pulse-like pattern as was previously reported [6]. Each pulse corresponds with a droplet. Although both signals showed rather large errors, a clear correlation between them is seen (R=0.969). This suggests the possibility of effective correction by the internal standard. The relative intensity was derived by dividing the ACTH18-39 trace by that of LeuEnk. The relative intensity was averaged across scans within each pulse to give a single value for

each droplet. The coefficients of variance (CV, standard deviation normalized by average value) of the averaged relative intensity was as low as 4.7%, which proves the system has performance of an acceptable level.

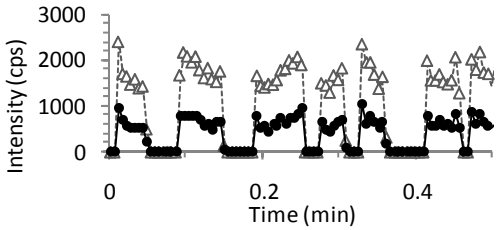

Figure 3. Traces of ion currents corresponding to the substrate (circle, ACTH18-39) and the internal control (triangle, Leucine Enkephalin). Both show pulse-like signals. The gaps between pulses correspond oil phase flowing between droplets. The vertical axis is intensity in counts per second (cps). Droplets of about 50 nL were analyzed with MS at scan cycle of 0.32 s.

A time profile of tryptic digestion of the peptide was obtained with the present system, which is required for the analysis of enzyme kinetics. To obtain a time profile of reaction product, the reaction time was varied from 2.6 to 8.6 min by changing the total flow rate, keeping the ratio between the flow rates constant. The concentration of the reaction product at each time point are plotted in Figure 4. As a result, the initial rate of the reaction was successfully obtained.

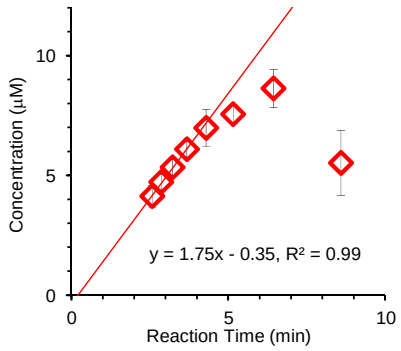

Figure 4. Time profile of enzyme reaction obtained with the present system. The trace of the product was plotted. The solid line is a linear regression derived from earlier four points.

CONCLUSION

We have developed a droplet-based microfluidic system for reaction kinetic analysis. It is shown that the system can control reaction time and concentrations of materials in 50 nL droplets as well as detecting reaction products in label-free manner. The profile of the reaction product showed a linear increase with time, which is a typical characteristic of an enzymatic reaction in an early stage. We believe that the present results demonstrate the feasibility of droplet-based microfluidics coupled with ESI-MS for a variety of chemical analyses and assays. Fluidic connections suitable for droplet delivery with capillaries were also developed, which minimized droplet coalescence by reduction of dead volume in the interconnection. This expands possibilities for further application of droplet-based microfluidics.

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