DROPLET-BASED COMPARTMENTALIZATION AFTER ISOELECTRIC FOCUSING IN A SLIPCHIP

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ABSTRACT

Isoelectric focusing (IEF) is a widely used technique for protein separation. We describe a novel Slipchip device that allows '*in situ*' compartmentalization of separated analytes into droplets after IEF in microfluidic channel. The device prevents remixing of separated components and provides facile hyphenations for subsequent analysis. The droplets can be analyzed either on chip or collected for off line analysis, such as electrophoresis or mass spectrometry. We demonstrate IEF separation using standard markers and proteins, with on chip pH calibration. Compared with other approaches of sample collections, this method is diffusion free, scalable and can be easily hyphenated with the other analytical methods.

KEYWORDS: Droplet, Isoelectric focusing, Slipchip

INTRODUCTION

IEF is a widely used technique that separates and focuses amphoteric molecules, e.g. proteins and peptides, according to their isoelectric point (pI). There are many embodiments of miniaturized IEF separation process such as Capillary IEF (cIEF) [1, 2] and Microchip IEF (μIEF) [3, 4], they consume very small amounts of sample compared to other methods, and have the potential for high throughput and automatic hyphenation to analytical methods like mass spectrometry.

However, hyphenation of miniaturized IEF platforms is not without challenges; a significant hurdle remains in how to collect the discrete bands for subsequent transfer to a downstream analytical system. The focused analyte must be removed from the capillary or channel by pressure-driven, electroosmotic flow, or chemical mobilization; this introduces severe dispersion and remixing of the focused bands. There have been efforts to compartmentalize chemically separated components using microdroplets. However, these separation methods do not work for IEF. In this paper we describe a Slipchip [5] based method that achieves in situ compartmentalization of an IEF separated sample into droplets, as shown schematically in Figure 1. The working principle is as follows: IEF separation is first performed in the microfluidic zig-zag channel that is composed of a sequence of wells formed in the two halves of the Slipchip as shown in Figure 1A. A pH gradient is then established in this channel and an analyte focused along the channel by application of an electric field (Figure 1B). Slipping the chip in a single operation disconnects these wells leaving the analyte in separated compartments or single droplets in each of the wells as shown in Figure 1C.

EXPERIMENTAL

The Slipchip device was formed from two acrylic plates, with wells, ducts and holes patterned on each plate. Two reservoirs for electrolytes were placed on the top plate and connected to the separation channel via inlet/outlet holes. The dimension of each well was 250μm × 1.7mm and 250μm deep (Figure 1D), giving a volume of 106nL for each well. The separation channel consists 29 wells and the total length is 5cm giving a total sample volume of 5μL.

The acrylic plates were patterned by micro machining, then the chips were exposed to chloroform vapor for 3min to reflow the surface. A 0.5μm thick Parylene C layer was deposited over the acrylic and the chip was further treated with Duxcoat solution, and dried in an oven at 60°C for 10min. The devices were held in a homemade chip holder with two pairs of magnets to clamp the chips. A micrometer head was used to precisely control the slipping distance (Figure 1D).

IEF buffer was prepared from 3 M Urea cIEF gel, 3% Pharmalyte pH 3-10 broadband carrier ampholyte, 0.5 mM Iminodiacetic acid, 6 mM L-Arginine, and 4% (w/v) PVA. 26 mM H_3PO_4 and 4 mM NaOH in cIEF gel with 3% (w/v) HPMC were prepared for anolyte and catholyte separately.

Prior to use, FC-40 oil was applied between the two plates to wet the surfaces. The oil created a seal preventing leakage; it also acted as a lubricant. The 5μL IEF sample was slowly pipetted into the separation channel to avoid leakage. Then the reservoirs were loaded with electrolyte, and the electrodes connected to the high voltage power supply to give an electric field of 100V/cm.

RESULTS AND DISCUSSIONS

To perform IEF separation, the chip was slipped to connect the wells to form a "zig-zag" continuous channel. Platinum electrodes were inserted into the reservoirs to establish an electric field (Figure 1A). During IEF separation, a pH gradient is established within the carrier ampholyte. Any proteins in the sample are driven by the electric field along the pH gradient and focused to their respective isoelectric points (Figure 1B). After separation, the focused bands are compartmentalized into droplets in wells by a simple slipping of the two plates (Figure 1C). The electric field was kept on during the slipping until the wells were fully separated and droplets were created.

We performed IEF with 6 pI makers (pI 4.0, 5.5, 7.2, 7.6, 8.1 and 9.0) in the zig-zag channels as shown in Figure 2. The peak capacity was calculated to be $n = L/w \approx 140$, where L is the total channel length and w is a measure of the

average analyte bandwidth. Accordingly, the theoretical minimum difference in isoelectric point, $\Delta(pI)$ = 0.05. Such separation results are comparable with the other µIEF results [3, 4], where peak capacities were reported from 36 to 133.

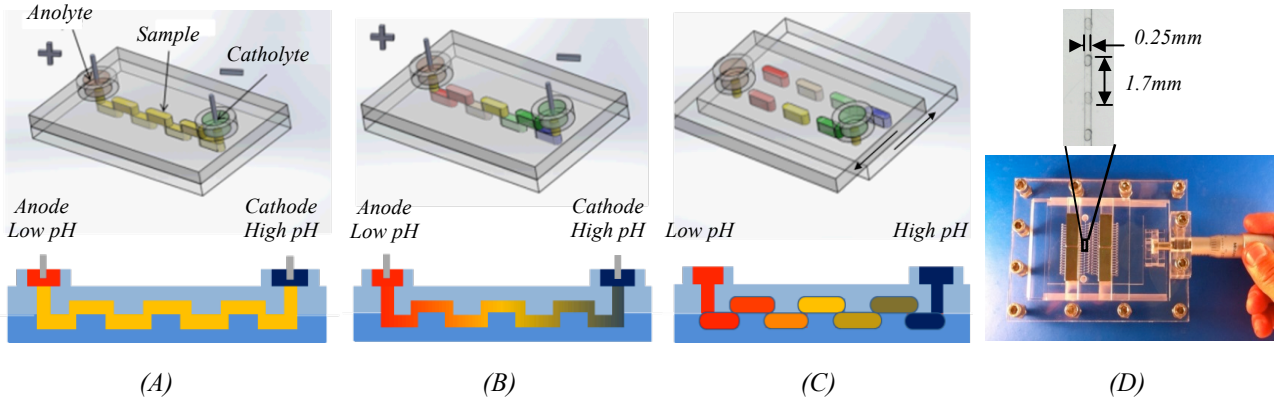

Figure 1: The schematics and prototype device for IEF separation and in situ compartmentalization in a Slipchip. (A) Sample loading to a continuous "zig-zag" channel. (B) pH gradient is established and IEF performed under an electric field. (C) in situ compartmentalization after IEF separation.(D)The prototype chip and platform made of PMMA plastic material, contains a "zig-zag" channel with dimensions of 250µm × 250µm × 5cm (1.7mm long for each well).

pH gradient formation is a critical step in an IEF process. Ideally we want to know the pH gradient distribution in the whole channel, therefore a focused unknown protein can be reliably allocated to a pI point/range, or known proteins collected from their pI points. However, whole channel/capillary pH gradient calibration is difficult in µIEF or cIEF [6] [7].

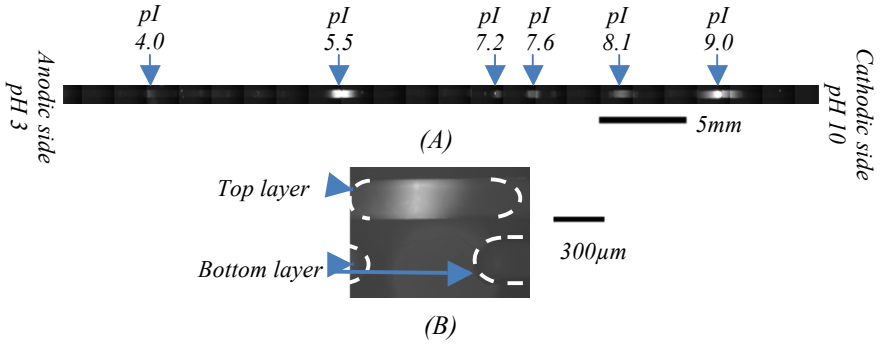

Figure 2: Fluorescence image of 6 focused pI makers (pI 4.0, 5.5, 7.2, 7.6, 8.1 and 9.0) in zig-zag channel (A) In the channel before compartmentalization; (B) after compartmentalization.

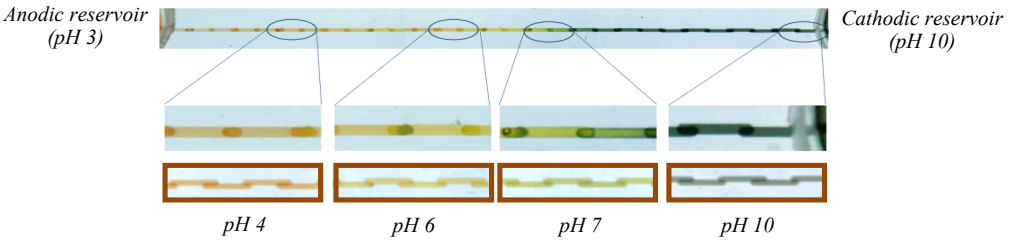

Figure 3: Snapshot of colorimetric image for on-chip pH gradient calibration. Bottom panels show controls for each representative pH values.

In our Slipchip based IEF platform, the pH gradient can be easily calibrated by mixing the compartmentalized droplets with pH indicator droplets, as shown in Figure 3. Channels for pH indicator solution were fabricated on each side of the IEF separation channel. After IEF separation, the top plate was slipped, and both the sample and the pH indicator were compartmentalized into droplets in wells. By further moving the plated, the sample and pH indicator droplets in different layers were merged and mixed by diffusion. After 10 minutes, the plate was moved back to the original IEF separation position, and an image of the entire channel was taken immediately as shown in Figure 3. The color distribution in the channel was compared with images from the other channels containing mixtures of pH indicator with solutions of known pH, as shown in the inset of Figure 3 for a few representative pH values. All the images show colorimetric similarity to their corresponding pH points, suggesting a continuous and near linear pH gradient can be reliably established in the Slipchip IEF channels. Note that this method of visual comparison is crude and further efforts are needed to calibrate the pH gradient quantitatively.

As a proof of principle for the whole system, we performed IEF separation of a mixture containing 5 standard proteins, Trypsin Inhibitor, β-Lactoglobulin A, Carbonic Anhydrase Isozyme II, Myoglobin, and Lectin. After 30minutes of IEF separation with the same conditions used in the previous sections, the sample was compartmentalized into droplets, and collected then analyzed with an Agilent 2100 Bioanalyzer.

A total of 21 single droplets were collected (Figure 4A and B) covering the pH range from 3.7 to 8.6. To simplify verification, these droplets were mixed with 3 consecutive droplets, to give a sample volume of 4μL; each sample droplet covers a 0.7 pI range. The samples droplets were analyzed directly with a Bioanalyzer without desalting.

The carrier ampholyte and urea can affect the resolution in CE, but all of the proteins can be clearly seen in the electropherogram as shown in Figure 4. The lane 8 is the control (mixtures containing all of the 5 proteins). The rest of the lanes show the electropherograms from each combined sample droplets. Lane 1 covers the pH range from 3.7 to 4.4. There is no protein in this range. Lane 2 covers the pH range from 4.4 to 5.1, where the Trypsin Inhibitor can be found. β-Lactoglobullin A has a pI of 5.1, and appears in the lane 3. Carbonic Anhydrase Isozyme II, Trypsin Inhibitor and Myoglobin appear in lane 4, 6, 7 respectively.

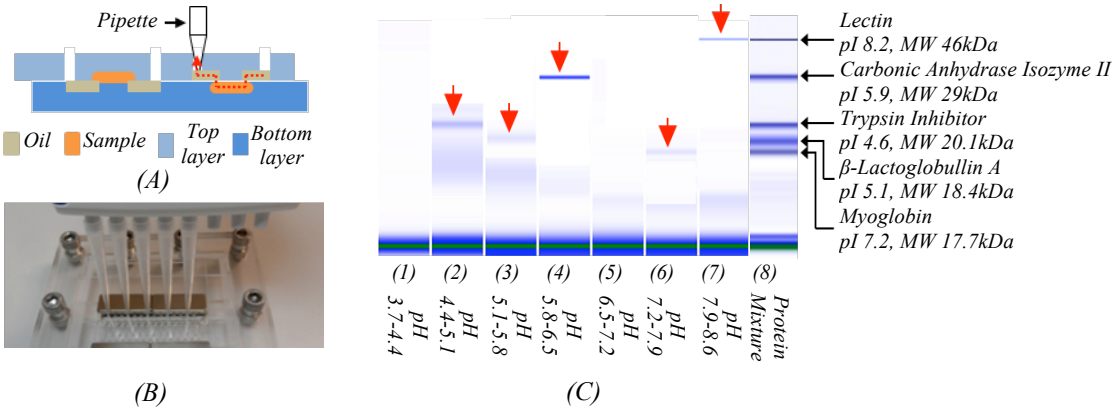

Figure 4: (A) and (B) Droplet collection and (C) Gel electropherograms of the collected droplets (with Agilent 2100 Bioanalyzer), the sample contains 5 proteins with different pIs and molecular weights, as marked in the right.

CONCLUSION

We describe a novel IEF system, which can compartmentalize separated proteins *in situ* into droplets, without remixing of the focused species. We demonstrated the function of the device using standard IEF markers. The droplets can be analyzed on chip, for pH calibration or with other labeling and detection techniques, or collected either in parallel or serial and transferred off chip for further analysis. Five standard proteins were separated and the separation was quantitatively demonstrated with CE. The device has the potential to reduce the volume of each partition to analyze complicated samples, or be hyphenated with the other analytical devices for multiple dimensional separations.

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REFERENCES

[1] Silvertand, L.H.H., et al., *Recent developments in capillary isoelectric focusing.* Journal of Chromatography A, 2008. 1204(2): p. 157-170.

[2] Righetti, P.G., R. Sebastiano, and A. Citterio, *Capillary electrophoresis and isoelectric focusing in peptide and protein analysis.* Proteomics, 2013. 13(2): p. 325-340.

[3] Ou, J.J., et al., *Fabrication of a hybrid PDMS/SU-8/quartz microfluidic chip for enhancing UV absorption whole-channel imaging detection sensitivity and application for isoelectric focusing of proteins.* Lab on a Chip, 2009. 9(13): p. 1926-1932.

[4] Cui, H.C., et al., *Isoelectric focusing in a poly(dimethylsiloxane) microfluidic chip.* Analytical Chemistry, 2005. 77(5): p. 1303-1309.

[5] Du, W.B., et al., *Slipchip.* Lab on a chip, 2009. 9(16): p. 2286-2292.

[6] Shimura, K., et al., *Fluorescence-labeled peptide p1 markers for capillary isoelectric focusing.* Analytical Chemistry, 2002. 74(5): p. 1046-1053.

[7] Weiss, N.G., et al., *Isoelectric focusing in a drop.* Langmuir : the ACS journal of surfaces and colloids, 2011. 27(1): p. 494-8.

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