Side-by-side comparison of disposable microchips with commercial capillary cartridges for application in capillary isoelectric focusing with whole column imaging detection

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Simple-structured, well-functioned disposable poly(dimethylsiloxane) (PDMS) microchips were developed for capillary isoelectric focusing with whole column imaging detection (CIEF-WCID). Side-by-side comparison of the developed microchips with well-established commercial capillary cartridges demonstrated that the disposable microchips have comparable performance as well as advantages such as absence of lens effect and possibility of high-aspect-ratio accompanied with a dramatic reduction in cost.

Introduction

Capillary isoelectric focusing (CIEF) with whole column imaging detection (WCID)[1–4] is a unique CIEF format. A short separation capillary (usually several centimetres in length) is used to separate proteins according to their differential isoelectric points (pI) in a chemically formed pH gradient under the influence of an applied electric field. The focused sample bands in the whole column range are detected with a charge-coupled device (CCD) camera. By using WCID, the mobilization step necessary for conventional CIEF is avoided, offering significant advantages, including maintained high resolution, reduced separation time and ease of implementation. A CIEF instrument with UV absorbance-WCID (iCE280 Analyzer) has been commercially available from Convergent Bioscience (Toronto) since 1998. This instrument has been widely adopted as a powerful tool for research and development as well as quality control by many leading pharmaceutical companies such as Pfizer, Amgen, Bristol-Myers Squibb and Genentech. Recently, a laser-induced fluorescence (LIF) version of WCID was developed.[5] So far, CIEF-WCID has been used for a wide range of applications in the separation and characterization of small molecules,[6] peptides,[7] proteins,[7,8] antibodies,[7,8] viruses[9] and cells.[10] Benefiting from the dynamic detection capability of WCID, CIEF-WCID also has been demonstrated as a powerful tool for the study of reactions of proteins,[11] rapid two-dimensional characterization of proteins[12] and characterization of protein–DNA interactions.[13]

Integration of CIEF-WCID to a microfluidic chip can provide several significant advantages compared with a capillary cartridge, including reduced cost, enhanced speed and efficiency when a smaller channel size is used, integration of multiple unit operations and possibility of multi-dimensional separation. However, only a few attempts[14–18] have been made on chip format WCID. Mao and Pawliszyn[14] first performed CIEF-UV-WCID in microchannels on a quartz chip-based cartridge, which were fabricated using photolithography and chemical etching processes. Ivory and co-workers[15] presented IEF in PDMS microchips with detection on a fluorescence microscope equipped with a CCD camera. The detection was similar to WCID; however, the isoelectric patterns were obtained by splicing a series of images because the imaging area of the microscope was too small to cover the whole separation channel. Dan and Fan[16] investigated the effects of channel length and applied voltages on sample focusing in a plastic microchip with an in-house built LIF-WCID system. Luo and co-workers[17] presented CIEF in glass/PDMS chips with an in-lab built organic light emitting diode (OLED) induced fluorescence-WCID system. Recently, Luo and co-workers[18] performed CIEF with whole column fluorescence imaging detection on a glass/PDMS chip by using a programmed OLED array as a spatial-scanning light source and a single photomultiplier tube as a detector.

In this study, we examined the feasibility of using disposable PDMS microchips for CIEF-WCID applications and comprehensively compared the separation performance of the PDMS microchips with that of the well-established commercial capillary cartridges. The PDMS microchips were designed with a simple structure while maintaining necessary functions for CIEF and UV-WCID. The softlithography fabrication process was much simpler than the manual fabrication process required for the capillary cartridges and thus significantly reduced the cost. Although channels with larger cross-sectional areas were patterned, the microchips exhibited comparable performance compared with the capillary cartridges. Besides, two advantages

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were found for the microchips, including absence of lens effect and possibility of high-aspect-ratio channels, which are favourable to detection sensitivity.

Experimental

Instruments

The CIEF experiments were performed using an iCE 280 Analyzer (Convergent Bioscience, Toronto, ON, Canada) with UV absorption detection at 280 nm. The separation capillary of the cartridges used for comparison was an internally fluorocarbon-coated fused-silica capillary of 5 cm × 100 μm ID × 200 μm OD.

Reagents and materials

The poly(dimethylsiloxane) (PDMS) prepolymer and the curing agent were purchased from Dow Corning (Midland, MI, USA). The negative photoresist SU-8 and propylene glycol monoether acetate (PGMEA) developer were from MicroChem (Newton, MA, USA). Pharmalytes (pH 3–10, 40% in concentration) and polyvinylpyrrolidone (PVP, average molecular weight about 360 000 Da and intrinsic viscosity 80–100 K) were obtained from Sigma (St. Louis, MO, USA). The pI markers (4.65, 5.12, 6.14, 6.4, 7.05, 8.18, 8.79) were from Convergent Bioscience. Human haemoglobin control AFSC was purchased from Helena (Beaumont, TX, USA). Water was purified with an ultra-pure water system (Barnstead/Thermolyne, Dubuque, IA, USA) and was used to prepare all solutions. Quartz slides (3 inch × 1 inch) were from SPI Supplies (West Chester, PA, USA). Nickel slit sheets (3 mm × 6.5 cm; slit size: 65 μm × 5 cm) were tailor-ordered products from StenTech (Mississauga, ON, Canada).

Microchip fabrication

PDMS microfluidic channels were fabricated using standard softlithography techniques.[19] Briefly, photomask designs for straight 5.5 cm-long channels with different widths were created using a computer-aided design (CAD) program and commercially printed at the Photoplot Store (Colorado Springs, CO, USA). To prepare the masters, a silicon wafer was first coated with SU-8 negative photoresist by spin-coating at 1800 rpm for 35 s, giving rise to a 100 μm-thick microchannel. The SU-8-coated wafer was then baked at 65 °C for 5 min and at 95 °C for 15 min, and finally cooled to room temperature. The photomask was placed on the SU-8-coated wafer and the designs were transferred from the photomasks to the coated photoresist through UV exposure for a dose of 800 mJ cm^{-2}. After post-exposure baking at 65 °C for 5 min, at 95 °C for 10 min and then cooling slowly to room temperature, the master was obtained by developing in the PGMEA developer for 10 min. PDMS prepolymer was thoroughly mixed with its curing agent at a weight ratio of 10 : 1 and degassed for 30 min under vacuum. The mixture was then poured onto the master and cured for 1 h at 80 °C. After curing, the PDMS substrate with a thickness of 2 mm was peeled off from the master, and two 3 mm diameter holes were punched at the reservoir locations. The chips were made by bonding the PDMS substrate with the channel features to another PDMS substrate *via* oxygen plasma treatment for 45 s at 29.6 W. After the bonding, the separation channel and the reservoirs were immediately filled with water. For storage, the channel was kept wet and the reservoirs were sealed with plastic cling film. A quartz slide (or a plastic slide with an open slit of 2 mm × 6 cm) was used as a supporting base to prevent chip deformation. A piece of the metal slit was glued to the bottom of the base, with the opening section overlapped with the separation channel. For microchips with different aspect-ratio channels, the layout was kept the same, but the channel aspect ratio was changed. The final microchip consisted of three substrates (20 mm-wide and 75 mm-long): two PDMS substrates with the channel and reservoirs in the upper one and one supporting base (either quartz slide or a plastic slide) glued with a metal slit. The schematic and photograph of a typical PDMS microchip with a 100 μm × 100 μm × 5 cm channel are illustrated in Fig. 1.

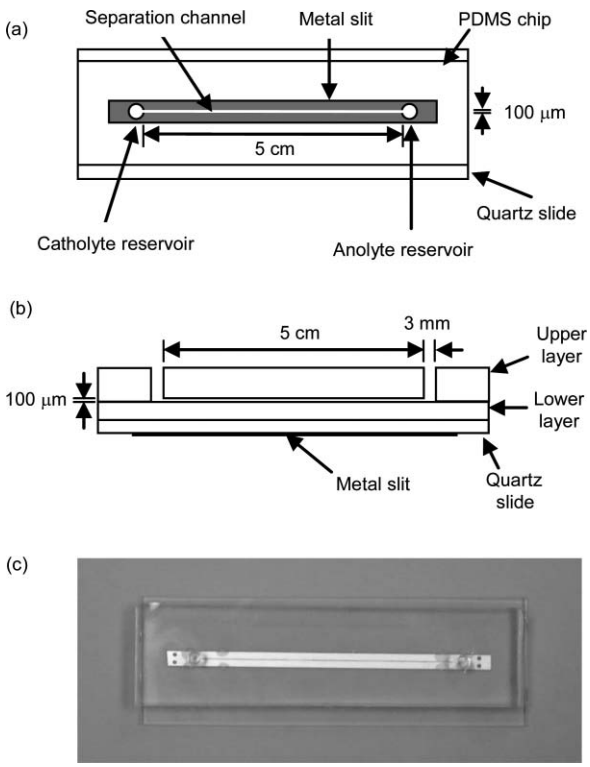

Fig. 1 Top view (a), side view (b) and photograph (c) of a 100 μm × 100 μm × 5 cm-channel PDMS microchip.

CIEF-WCID experiments

Before performing any experiment, the separation channel was conditioned with water and 1% (w/v) PVP aqueous solution for 20 min each. The sample was introduced into the whole channel by vacuum with a simple in-lab built vacuum device. Excess sample solutions in the reservoirs were simultaneously removed with two micropipettes. By using two micropipettes, identical volumes (10–15 μL) of the anolyte (100 mM phosphoric acid containing 1% PVP) and catholyte (100 mM sodium hydroxide containing 1% PVP) were simultaneously added to the left reservoir and right reservoir on the microchip, respectively. The focusing was performed at 1.5 kV for 5 min and then 3 kV until

finished. Images were taken automatically at desired intervals. After each run, the reservoirs and the separation channel were washed with water for 2 min. Before subsequent experiments, the channel was conditioned with 1% PVP solution for 2 min.

Results and discussion

Side-by-side comparison

The performance of the PDMS microchips was comprehensively compared with that of the capillary cartridges in terms of electroosmotic flow (EOF) magnitude, pH gradient linearity, peak capacity, separation resolution, speed, reproducibility and durability. The data for the compared items are summarized in Table 1. The EOF was measured by monitoring the movement of a nearly neutral pI marker (pI 6.4) under the electric field after it has been focused. The electroosmotic mobility was determined by plotting the peak position of the pI marker against the focusing time. The pH gradient linearity, peak capacity and reproducibility were evaluated with a set of six standard pI markers. Due to the focusing mechanism, peak capacity instead of plate number was used to characterize the separation efficiency. The resolving power was evaluated with the resolution of a more complicated sample, human haemoglobin control AFSC, which contains four definite isoforms with much closer pI values (A, 7.0; F, 7.1; S, 7.3; C, 7.5). Representative CIEF patterns are illustrated in Fig. 2 and 3. It can be seen from Table 1 and Fig. 2 and 3 that the overall performance of the microchips is comparable with that of the capillary cartridge. Although the peak capacity in the microchips is slightly lower than that in the capillary cartridge, this item can be considered comparable since larger cross-sectional area was used for the separation channel of the microchips. Although the microchips were designed for disposable use, they could be used for 25 runs within 3 days without apparent lose in performance, compared with 100 runs for the capillary cartridges. The price for the capillary cartridges is currently $250 each. In contrast, the cost was reduced to $2 for one quartz based microchip or $0.5 for one plastic based microchip; at least 100 times lower than that of the cartridge.

Table 1 Side-by-side comparison of the disposable microchip with the commercial capillary cartridge[a]

Items	Microchip	Capillary cartridge
EOF	2.0×10^{-7} cm^2 V^{-1} s^{-1}	4.0×10^{-7} cm^2 V^{-1} s^{-1}
pH gradient linearity	$R^2 = 0.9998$ ($n = 6$)	$R^2 = 0.9996$ ($n = 6$)
Peak capacity[b]	37–62	39–75
Resolution[c]	1.32	1.30
Speed	5–10 min	5–10 min
Run-to-run RSD	$< 1.0\%$ ($n = 4$)	$< 0.5\%$ ($n = 6$)[d]
Durability	25 runs	100 runs
Cost or price	$2 (with quartz base) $0.5 (with plastic base)	$250 (price)

[a] The size of the separation channel was 100 μm × 100 μm × 5 cm or 200 μm × 50 μm × 5 cm (depth × width × length) for the microchip or 100 μm ID × 5 cm for the capillary catridge. [b] The peak capacity was measured with six pI markers. [c] The resolution was measured with the human haemoglobin isoforms S and C. [d] The value was from ref. 3.

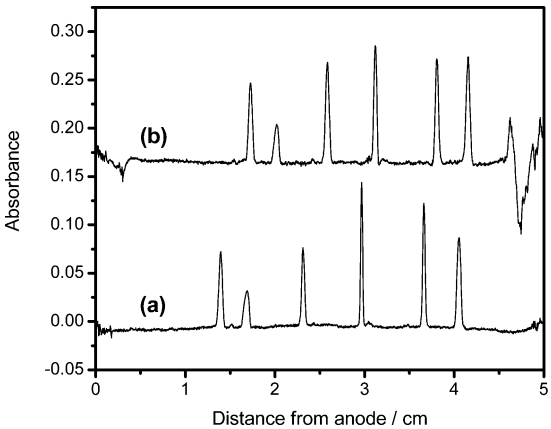

Fig. 2 CIEF profile of pI markers in a capillary cartridge (a) and a microchip (b). Separation channel: (a) 100 μm ID × 5 cm length; (b) 200 μm width × 50 μm depth × 5 cm length. Sample: a mixture of six pI markers (4.65, 5.12, 6.14, 7.05, 8.18, 8.79, 1% of their original concentration) containing 2% Pharmalytes (pH 3–10) and 1% PVP.

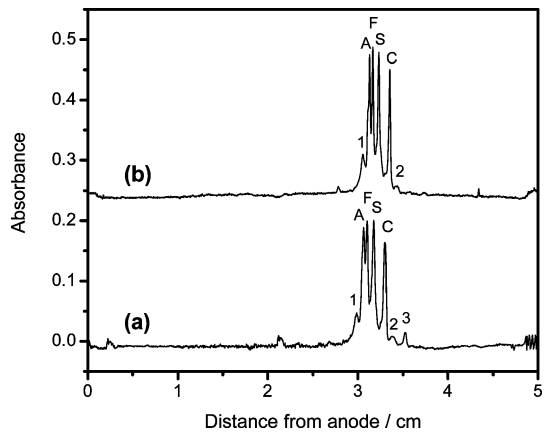

Fig. 3 CIEF profile of human haemoglobin control AFSC on (a) a microchip and (b) a capillary cartridge. Separation channel: (a) 100 μm × 100 μm × 5 cm; (b) 100 μm ID × 5 cm. Sample: 0.1 mg mL^{-1} human haemoglobin control containing 2% Pharmalytes (pH 3–10) and 1% PVP.

Lens effect and high-aspect-ratio channels

Lens effect[20] is an apparent drawback associated with round capillaries for use in path-length-dependent detection schemes such as UV absorbance; the round capillaries act upon the incoming beam like a thick cylindrical lens with a short focal length. The lens effect transforms an incoming collimated light beam into an outgoing strongly uncollimated beam, making light collection inefficient if no extra light focusing means is used. Such a detrimental effect can be eliminated by using microchips with a rectangular channel. A light beam after passing a microchip is expected to have higher intensity than after passing a round capillary. This was confirmed experimentally as shown in Fig. 4. Under otherwise identical conditions, the intensity of an incident light passing through a microchannel of 100 μm × 100 μm was nearly 2 times higher than that passing through a capillary of 100 μm ID. The absence of lens effect is a direct merit of using microchips for CIEF-WCID, which contributes to better detection sensitivity.

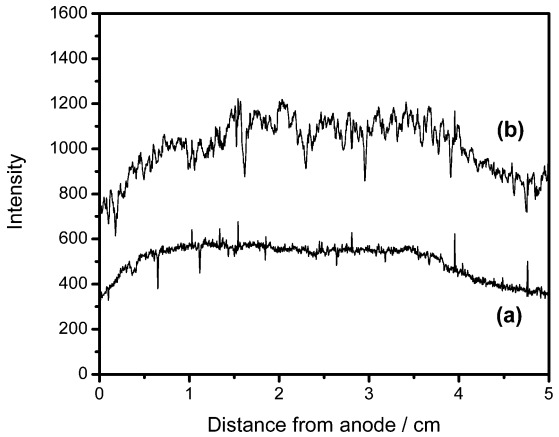

Fig. 4 Comparison of the intensity of the incident light after passing through a capillary cartridge (a) and a microchip (b). Separation channel: (a) 100 μm ID × 5 cm; (b) 100 μm × 100 μm × 5 cm. The separation channels were filled with water. Exposure time, 300 ms.

The use of high-aspect-ratio channels is favourable for detection sensitivity due to the increased light path. Such an advantage is demonstrated with two microchips of different high-aspect-ratio channels, 200 μm depth × 50 μm width and 250 μm depth × 40 μm width. The isoelectric profiles of a mixture of six pI markers are shown in Fig. 5. Except the peak for pI marker 8.79, the peak heights for other five peaks were higher in the higher aspect ratio channel. The average gain in peak height was 24%, which is in good agreement with the increase in the light path (25%).

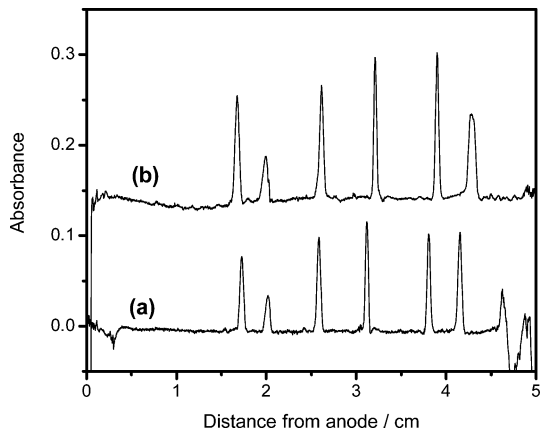

Fig. 5 Comparison of the peak intensity in microchips of different aspect ratio. (a) 200 μm depth × 50 μm width channel; (b) 250 μm depth × 40 μm width channel. Sample: a mixture of six pI markers (4.65, 5.12, 6.14, 7.05, 8.18, 8.79, 1% of their original concentration) containing 2% Pharmalytes (pH 3–10) and 1% PVP.

Conclusions

Simple-structured, well-functioned PDMS microchips have been designed and fabricated, and their performance in CIEF-WCID has been systematically investigated. The comprehensive comparison between the PDMS microchips and the commercial capillary cartridges verified the feasibility of the PDMS microchips for applications in CIEF-WCID and meanwhile revealed two advantages over the capillary cartridges. First, although the channel sizes of the microchips used in this study were larger than those of the capillary cartridges, the overall performance of the microchips was comparable with the capillary cartridges. When the channel size of the microchips is reduced, enhanced efficiency and speed can be expected. Second, due to the elimination of lens effect and the changeable detection light path, microchips can provide better detection sensitivity compared with capillary cartridges. The cost of the microchips was reduced by at least 100-fold compared with the capillary cartridges. The cost can be further greatly lowered by mass production.

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