Integration of Dialysis Membranes into a Poly(dimethylsiloxane) Microfluidic Chip for Isoelectric Focusing of Proteins Using Whole-Channel Imaging Detection

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A poly(dimethylsiloxane) microfluidic chip-based cartridge is developed and reported here for protein analysis using isoelectic focusing (IEF)−whole-channel imaging detection (WCID) technology. In this design, commercial dialysis membranes are integrated to separate electrolytes and samples and to reduce undesired pressure-driven flow. Fused-silica capillaries are also incorporated in this design for sample injection and channel surface preconditioning. This structure is equivalent to that of a commercial fused-silica capillary-based cartridge for adapting to an IEF analyzer (iCE280 analyzer) to perform IEF-WCID. The successful integration of dialysis membranes into a microfluidic chip significantly improves IEF repeatability by eliminating undesired pressure-driven hydrodynamics and also makes sample injection much easier than that using the first-generation chip as reported recently. In this study, two microfluidic chips with a 100-μm-high, 100-μm-wide and a 200-μm-high, 50-μm-wide microchannel, respectively, were applied for qualitative and quantitative analysis of proteins. The mixture containing six pI markers with a pH range of 3−10 was successfully separated using IEF-WCID. The pH gradient exhibited a good linearity by plotting the pI value versus peak position, and the correlation coefficient reached 0.9994 and 0.9995 separately for the two chips. The separation of more complicated human hemoglobin control sample containing HbA, HbF, HbS, and HbC was also achieved. Additionally, for the quantitative analysis, a good linearity of IEF peak value versus myoglobin concentration in the range of 20−100 μg/mL was obtained.

Capillary electrophoresis (CE) has been the workhorse for DNA and protein separation due to its high efficiency, ease of automation, low sample and reagent consumption, and multiple separation modes.[1–3] Isoelectric focusing (IEF) as one of the high-resolution CE techniques has been widely applied for the separation of zwitterionic biomolecules, such as proteins and peptides. Basically zwitterionic biomolecules can be focused at their isoelectric points (pI) in an environment with a linear pH gradient. Most IEF is conducted in a single capillary where a pH gradient is set up axially along the capillary. The use of a capillary limits the integration of IEF with other processes such as precolumn sample treatment, other separation schemes, and postcolumn sample labeling. To resolve these problems, microfluidic chips are receiving much attention because of their inexpensive mass production, low reagent consumption, increase of separation efficiency, and portability. The potential of parallel processing on a single microfluidic chip is also attractive as it leads to higher analytical throughput.[4–8]

In most IEF applications, after the samples are focused at their corresponding pIs, the focused zones must be pumped through the detection point by chemical, hydrodynamic, or electroosmotic mobilization methods to obtain an electropherogram.[9–14] This single-point detection method imposes extensive restrictions for IEF applications because a mobilization process requires additional time to process and lowers the resolution and repeatability of the

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separation.[15] An excellent alternative is the whole-column imaging detection technology (WCID) developed by Pawliszyn et al.,[16−21] which does not require mobilization and thereby avoids the above-mentioned disadvantages.

A combination of WCID and chip-format IEF has been investigated by Mao and Pawliszyn[22] on an etched quartz chip and by Ren et al.[23] and Das et al.[24] on a glass chip and a plastic chip, respectively. However, the chips used in these studies require conventional photolithography technology to fabricate, which is very expensive. An attractive alternative for fabricating microfluidic chips is softlithography technology,[25,26] which utilizes poly(dimethylsiloxane) (PDMS) as chip material. PDMS has unique properties such as nontoxic, optically transparent down to 280 nm, elastomeric, and hydrophobic surface chemistry, which are suitable for most chemical analyses. Yao et al.[27] designed a glass/PDMS microfluidic chip integrated with WCID for IEF of R-phycoerythrin, which cannot be directly adapted to the iCE280 analyzer because of lower detection sensitivity of UV absorption obtained on the glass chips.

Previous studies[28−32] have demonstrated that commercial membranes can be integrated into microchips for microdialysis, protein digestion, solid-phase extraction, desalting, and pumping. In addition, the integrated membranes can minimize undesired hydrodynamic flow, serving as filters. In this study, dialysis membranes were integrated with a PDMS microfluidic chip to separate the electrolyte solutions (anolyte and catholyte) in the reservoirs and the sample mixture in the microchannel. Two fused-silica capillaries were inserted into the two ends of the channel for sample injection and discharge and channel surface preconditioning if necessary. The analysis repeatability obtained on this design was dramatically increased, which will be demonstrated below.

EXPERIMENTAL SECTION

Materials. Sylgard 184 PDMS prepolymer base and curing agent were purchased from Dow Corning (Midland, MI). SU-8 photoresist was obtained from Microchem (Newton, MA). Low molecular mass pI markers of 4.65, 5.12, 6.14, 7.05, 8.18, and 8.79 were purchased from Convergent Bioscience (Toronto, ON, Canada). Myoglobin, carrier ampholytes (Pharmalytes 3−10), and methyl cellulose (MC) were purchased from Sigma (St. Louis, MO). Poly(vinylpyrrolidone) (PVP; MW, 360 000) and poly(vinyl alcohol) (PVA; MW, 13 000−23 000) were from Aldrich (Milwaukee, MI). Regenerated cellulose dialysis membrane (MWCO, 12 000−15 000) was from Spectrum Laboratories, Inc. (Rancho Dominguez, CA). Human hemoglobin control containing HbA, HbF, HbS, and HbC was purchased from Helena (Beaumont, TX). Other chemicals were of analytical-reagent grade. Water was purified with an ultrapure system (Barnstead, IA). Fused-silica capillaries with 50 μm i.d. × 100 o.d. and 100 μm i.d. × 168 o.d. were purchased from Polymicro Technologies (Tucson, AZ). Nickel slit sheet (3 mm × 6.5 cm; slit size, 65 μm × 5 cm) was a custom-ordered product from StenTech (Mississauga, ON, Canada). Capillary cartridges with internally fluorocarbon-coated fused-silica capillary of 5 cm × 100 μm i.d. were purchased from Convergent Bioscience.

Microchip Cartridge Design. Although a simple PDMS chip-based cartridge has been successfully fabricated and validated previously,[33] there are several drawbacks associated with that design. First, the samples need to be injected into the channel by applying vacuum at one end of the channel, which can cause deformation of the metal slit, leading to poor detection. In addition, the conditioning solution (PVP) must be fully drained out from the reservoirs before the sample mixture can be injected. Second, anolyte and catholyte solutions must be filled into the two reservoirs, respectively, right after the sample is filled into the channel and simultaneously a voltage difference should be applied to the liquid through the electrodes in the reservoirs to minimize the diffusion between the electrolytes and the samples. It is difficult to maintain the same liquid heights in the two reservoirs; as a result, undesired pressure-driven flow occurs easily as discussed previously. Third, in that design, membranes are not used to separate the electrolytes and samples. IEF separation tends to be deteriorated by the infusion of electrolyte solutions, leading to poor repeatability of IEF-WCID analysis.

In this study, the proposed design addresses the above-mentioned problems. First, sample injection and discharge are realized by incorporating capillaries with the PDMS microchannel, which allows for pressure-driven injection and eliminates the problems of chip deformation. Second, dialysis membranes are integrated into the PDMS chips similar to the hollow fibers used in the commercial capillary-based cartridge.[34] The integrated membranes separate the electrolytes and samples, which can resolve the cross-contamination problem, simplify the operation, and also suppress the induced pressure-driven flow.

Up to date, many approaches have been described to integrate membranes into glass/quartz or polymeric microfluidic chips. A simple method is the direct incorporation by gluing or clamping

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commercial flat membranes[35,36] with chip substrates. A major problem of this method is sealing. Very likely leakage will happen around the membranes due to capillary force and the gaps generated along the edge of the membrane. More recently, a technique for leakage-free integration of porous membranes into PDMS chips was reported by Chueh et al.[37] In their study, the PDMS prepolymer was diluted by toluene and used as mortar to fill the gap and bond the membranes with the PDMS layers.[38] This approach was initially attempted to sandwich dialysis membranes into a PDMS chip for IEF-WCID. Unfortunately, it received little success because the thickness of the membrane resulted in leakage of the electrolytes or blockage of pores in the dialysis membrane. Thus, a new approach of sandwiching dialysis membrane was developed as schematically indicated in Figure 1 and described later in the section of fabrication.

Due to the flexibility of PDMS chips, a commercial chip holder (Convergent Bioscience) is used to support PDMS chips. This holder has a 1-mm-wide, 6-cm-long opening slit for passing through detection light and can be well mounted into the chamber of the IEF analyzer.

Microchip Cartridge Fabrication. The procedures used in this study for fabricating PDMS chip-based cartridges are illustrated in Figure 1. First, a 100-μm-wide, 100-μm-high, 56-mm-long microchannel was fabricated in the bottom PDMS substrate and two 6-mm-wide, 6-mm-long, 40-μm-high recesses were fabricated in the upper PDMS substrate with a distance of 57 mm using standard softlithography technology. In brief, a clean and dehydrated silicon wafer was spin-coated with SU-8 2075 Photoresist. The thickness of the SU-8 layer varied in this study between 50 and 200 μm, which depends on the spin-coating speed and the viscosity of the SU-8 recommended by the manufacturer of SU-8. The designs printed on a transparency mask were transferred to the spin-coated wafer through UV exposure. After developing in the developer solution for several minutes, the silicon wafer containing positive relief of the designs (channel and recess) was dried by nitrogen gas. The mixture of PDMS prepolymer base and curing agent with a weight ratio of 10 to 1 was degassed under vacuum, poured onto the wafer, and then cured at 80 °C for 2 h, resulting in a PDMS substrate with the desired features. The thickness of the bottom and upper PDMS substrates was kept at 2 mm. Once the two substrates were made, two reservoirs with a 2-mm diameter were punctured through the center of the recesses, respectively.

Second, dialysis membranes were incorporated into the chip. To do so, the PDMS prepolymer diluted with toluene (1/2 or 1/1, v/v) was used as mortar, which was made by spin-coating the mixture onto a clean glass microscope slide. The thickness of the mortar layer was controlled by the spin-coating speed and duration time. The dialysis membranes with a desired size (6 mm × 6 mm) were stamped onto the glass slide spin-coated with the PDMS mortar for 1 min and then placed onto the recesses of the upper PDMS substrate for 1 min. Two fresh dialysis membranes were placed into the recesses to replace those membranes with stamped
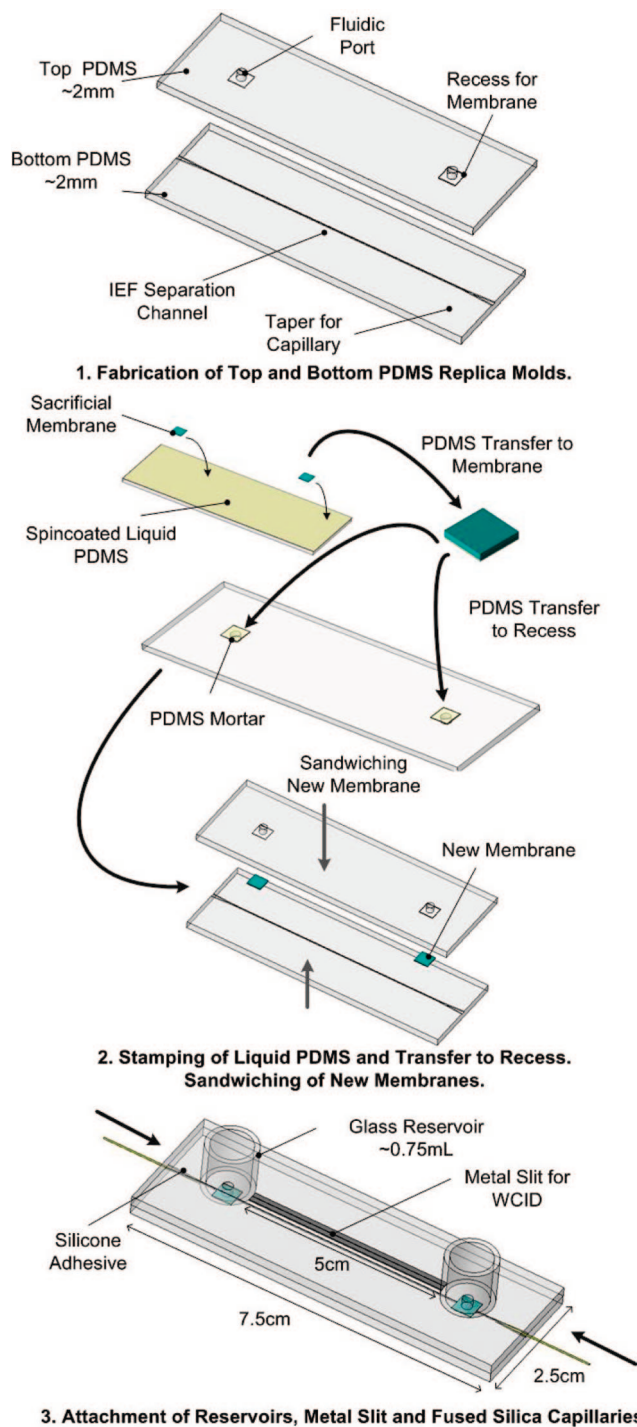
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Figure 1. Schematic procedure of sandwiching dialysis membrane into PDMS chips for fabrication of chip-based cartridge in IEF-WCID.

PDMS mortar, respectively. The PDMS substrate with dialysis membranes was irreversibly bonded with another PDMS substrate with the channel after being pretreated by oxygen plasma. The combined PDMS chip was cured at 65 °C to harden the PDMS mortar.

Third, the capillaries for channel surface preconditioning and sample injection and discharge were incorporated into the chip. A layer of glue was applied to the outer wall of the capillaries. Then, the capillaries with 50 μm i.d. × 100 μm o.d. were manually inserted into the two ends of the microchannel until the capillaries

reached the edge of dialysis membrane. This step was done under a microscope.

Finally, the chip with sandwiched dialysis membranes and embedded capillaries was cut to the desired dimension and placed on a cartridge holder. It is very important to keep the channel at the center of the cartridge so that the detection light can be well aligned with the channel. A metal slit with a 65-μm-wide, 50-mm-long opening was carefully aligned and glued to the top of the PDMS chip under an optical microscope so that the slit was exactly positioned on the top of the channel. All the PDMS microfluidic chips were fabricated using this method.

Measurement of Chip-Based Cartridge for Isoelectric Focusing. IEF experiments were performed on an iCE280 analyzer (Convergent Bioscience), which consists of a deuterium (D2) lamp as a light source and a whole-column optical absorption imaging detector operated at 280 nm. During the process of sample focusing, the light beam from the lamp was focused onto the separation channel by a bundle of optical fibers and a cylindrical lens. The UV adsorption image of the whole-channel was captured by a CCD camera.

Sample solutions were prepared in deionized water containing 1.0% PVP, 2% Pharmates (3−10), and pI markers or proteins. The anolyte and catholyte were 0.1 mol·L^{-1} phosphoric acid and 0.1 mol·L^{-1} sodium hydroxide, respectively. The IEF was performed at room temperature. All of the solutions were filtered using a 0.2-μm pore size cellulose acetate filter (Sartorius, Gottingen, Germany) prior to use.

The channel in the PDMS chip was preconditioned with a PVP solution (1%, w/v) for 30 min. The optimization of the selection of the conditioning solution and operation conditions was conducted and will be explained later. Afterward, the sample mixture was injected into the channel with a syringe. Focusing was performed by first applying a voltage difference of 1500 V for 4 min and then maintaining it by applying a voltage difference of 3 kV. Between runs, the channel was washed with the PVP solution for 5 min. The IEF was repeated three times for each sample.

RESULTS AND DISCUSSION

Optimization of Fabrication Conditions for the Integration of Dialysis Membranes. As mentioned above, two recesses were introduced for integrating dialysis membranes into a microfluidic chip. To fabricate a reliable and repeatable membrane-integrated microfluidic chip, optimization of several fabrication procedures was conducted in this study.

The thickness of the spin-coated PDMS mortar layer on a glass slide for stamping membranes plays an important role for bonding membranes with PDMS substrates and sealing the gap around the membranes. The mortar thickness is affected by several factors including the weight ratio of PDMS prepolymer to toluene, the spin-coating speed, and the duration time. A too thin PDMS mortar layer on a glass slide normally results in a thin layer of PDMS mortar on the PDMS substrate after stamping, which usually does not provide sufficient sealing between membranes and PDMS substrates leading to leakage. On the other hand, a too thick PDMS mortar layer on a glass slide results in too much PDMS around the membranes, leading to blockage of dialysis pores and potentially entering the channel. The optimal conditions were developed as follows: to make a 5-μm-high mortar layer on a microscope glass slide, the PDMS mortar with a 1:1 weight ratio
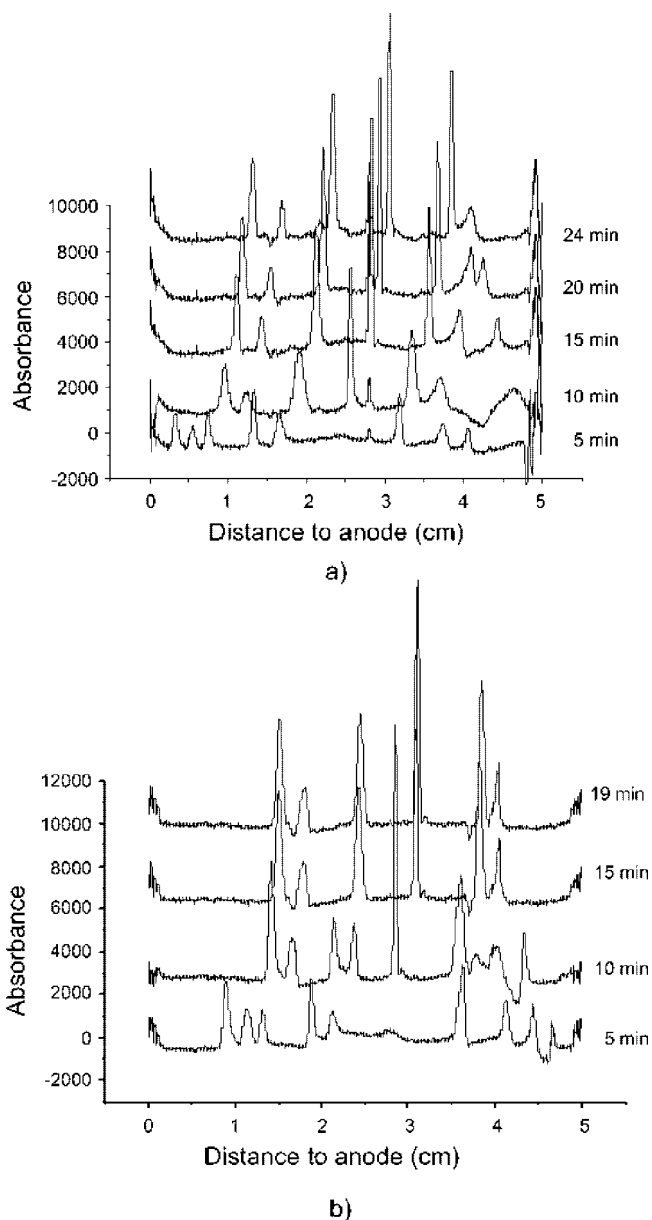
Figure 2. Effect of the mode of sandwiching membranes on the separation of pI markers obtained on PDMS chips using IEF-WCID. (A) Two-sided mortar application and (B) one-sided mortar application. The channel is 100 μm high and 100 μm wide. The sample contains 6 pI markers (4.65, 5.12, 6.14, 7.05, 8.18, 8.79), 2% Pharmalytes (pH 3−10), and PVP (1%, w/v). The applied voltage was set at 1500 V for 5 min and then at 3000 V for the rest of the time.

of PDMS to toluene should be spin-coated at 6000 rpm for 40 s. By using this 5-μm-thick mortar layer, the gap formed between the dialysis membrane and the substrate is very small. In addition, the introduction of the recess design further ensures excellent sealing between two PDMS substrates.

Additionally, stamping one side or two sides of a dialysis membrane from the PDMS mortar has an effect on the bonding strength and IEF separation performance. If only one side of the membrane is stamped, the bonding strength is so weak that leakage occurs after only a few runs. If two sides of the membrane are stamped, the bonding is strong and the chip-based cartridge could be used for several weeks. However, it might lead to blockage of pores in the membrane. As indicated in Figure 2, the

IEF process on the chip with the two-sided stamping mortar application is slower than that with the one-sided mortar application. It is likely due to the blockage of dialysis pores resulting from the two-sided mortar stamping. As a result, it is difficult for hydrogen ions and hydroxyl ions to penetrate into the channel forming the required pH gradient. In most of the presented experiments here, the double-sided stamping application was used for strong bonding and long-time use.

The repeatability of the obtained microfluidic chip was investigated as well. Both run-to-run and chip-to-chip repeatability, expressed as RSD ($n = 5$), were lower than 1%. This result is better than that obtained on the first-generation chip, which did not use dialysis membranes. This is because the integrated dialysis membranes allow the sample injection and channel surface preconditioning to be separated from the anolytes and catholytes and eliminates the induced hydrodynamic pressure between the two reservoirs. The lifetime of this chip was also evaluated. It was found that the column efficiency and separation ability was not remarkably reduced even if the chip was used consecutively for two weeks.

Optimization of IEF Conditions. Based on the surface properties of PDMS, it can be deduced that the IEF performance on PDMS microfluidic chips should be similar to that on a fused-silica capillary. However, PDMS microfluidic chip-based cartridges do offer advantages over commercial capillary-based cartridges. For example, in general, the inner wall of the capillary used in the commercial cartridge needs to be immobilized with a layer of linear polyacrylamide to suppress electroosmotic flow (EOF), which is very time-consuming. This surface immobilization process was eliminated in this study without sacrificing IEF performance because the EOF generated in a PDMS channel is several orders of magnitude lower than that in a fused-silica capillary.

Addition of water-soluble nonionic polymers to sample solutions is required in the capillary-based cartridge, which was also performed in this study to suppress EOF and prevent protein adsorption to channel walls. Native PDMS is hydrophobic, causing protein adsorption and interfering IEF processes. Even after the surface is oxidized by oxygen plasma pretreatment to become hydrophilic, it goes back to hydrophobic gradually over several hours,[39] MC, hydroxylpropylmethyl cellulose, etc., have been used for prevention of protein adsorption in chip-based separation.[27,40,41] In this study, the influences of MC and two other commonly used additive polymers, PVP and PVA, were evaluated and PVP was used for the rest of the tests.

It was found that the current through the channel decreased slower when MC was used as an additive than that when PVA or PVP was used (results not shown). Additionally, it required a higher pressure and took a longer time to introduce samples even if the concentration of the MC decreased to 0.5% (w/v). This may be attributed to the high viscosity of the MC solutions, resulting in a long focusing process.[27] Figure 3 indicates the IEF separation of four pI markers that contained PVA of a concentration of 1.0% (w/v). It can be seen that the four pI markers were well separated within 14 min; however, the focused zones drifted from anode to
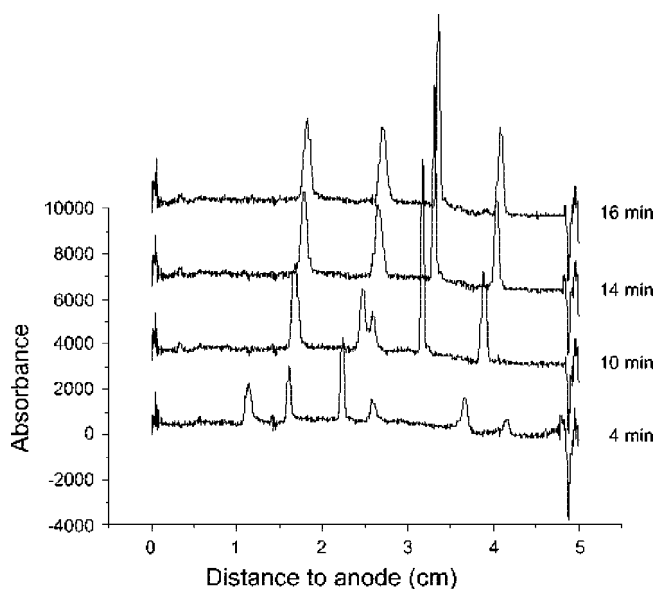
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Figure 3. IEF profile of a mixture of four pI markers in a microchip. Sample is a mixture of four pI markers (4.65, 6.14, 7.05, 8.18, 1% of their original concentration) containing 2% Pharmalytes (pH 3–10) and 1% PVA. Other conditions are the same as in Figure 2.

cathode after focusing. This could be attributed to the remnant EOF although the PVA was used to suppress EOF. As a result, PVP was selected as the additive for the IEF experiments; as exhibited in Figure 2b, the peaks did not move after IEF was completed. The effect of PVP concentration on the IEF process and separation was also investigated, and the optimum concentration was determined to be 1% w/v (data not shown).

Under the optimized conditions, the separation of pI markers was performed on the obtained PDMS microfluidic chip. As exhibited in Figure 4a and b, six pI markers could be well separated on two PDMS chips with a 100-μm-high, 10-μm-wide channel and a 200-μm-high, 50-μm-wide channel, respectively, using the IEF-WCID technology. All the peaks were sharp and symmetric, indicating that both EOF and protein adsorption were suppressed to a large extent by the dynamic coating of PVP. The plots of peak position versus pI point suggested a good linearity of the pH gradient formed in the channels. The linear correlation coefficients for these two chips were 0.9995 and 0.9994 ($n = 6$), respectively.

Comparison between Commercial Capillary-Based Cartridges and PDMS Chip-Based Cartridge. Under the same experimental conditions, the IEF of the above-mentioned pI markers was performed on a commercial capillary-based cartridge, and the result is shown in Figure 4c. Comparing these results, one can see that the column efficiencies in microfluidic chips were slightly lower than that in the capillary. There are a few possible reasons causing this. The first possible reason is the slow heat dissipation occurring in microfludic chips due to the low thermal conductivity of PDMS. It was observed that both the currents passing through the commercial capillary cartridge and the microfluidic channels dropped exponentially. However, the current reached a constant residual value at 160 s in a commercial cartridge and 300 s in microfluidic channels under the same focusing conditions (data not shown). This implied that heat dissipation in a fused-silica capillary is faster than that in a microfluidic channel under the same IEF conditions. Fast heat
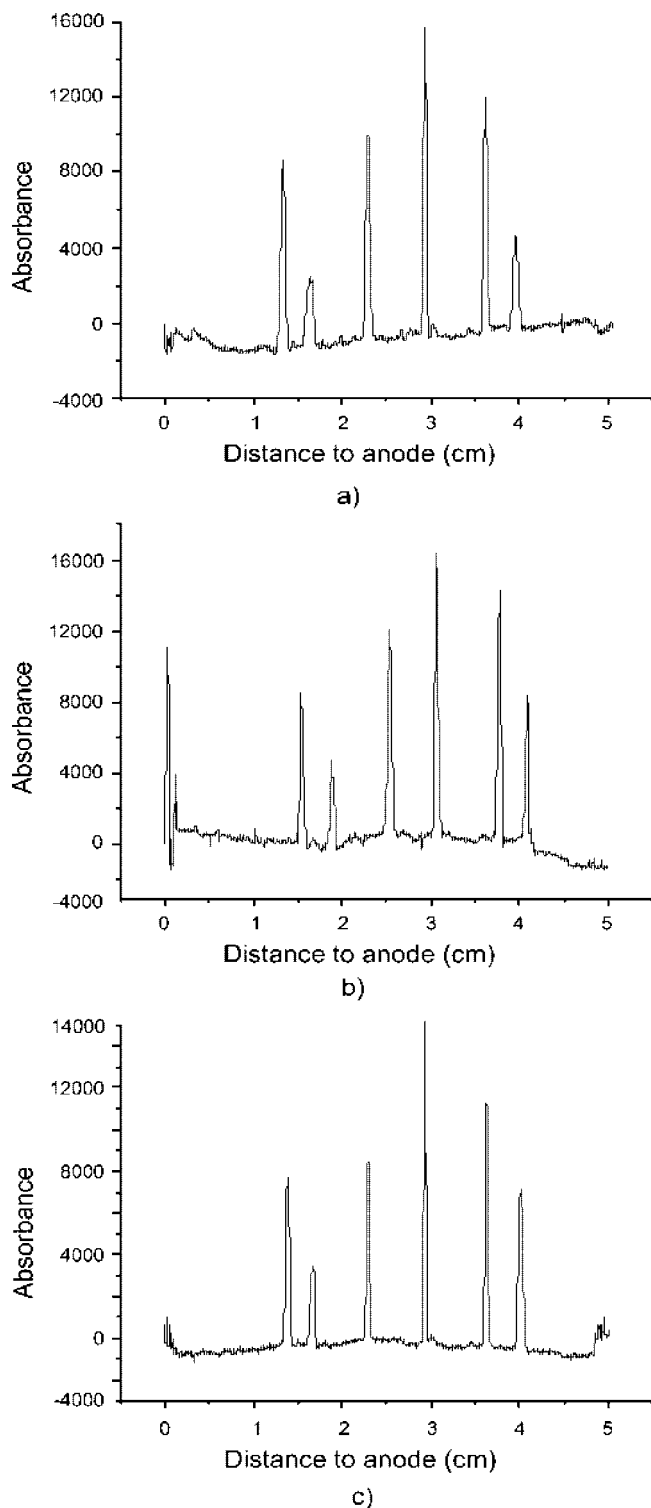
Figure 4. Separation of pI markers on (a) a PDMS chip with a 100-μm-high, 100-μm-wide channel, (b) a PDMS chip with a 200-μm-high, 50-μm-wide channel, and (c) a commercial capillary-based cartridge using IEF-WCID. The sample contains 6 pI markers (4.65, 5.12, 6.14, 7.05, 8.18, 8.79), 2% Pharmalytes (pH 3–10), and PVP (1%, w/v). Other conditions are the same as in Figure 2.

dissipation results in a higher separation efficiency. The second possible reason is the slow permeation of hydrogen and hydroxyl ions through the dialysis membranes, resulting in a longer focusing time. The longer the focusing time, the wider the focusing bands, which leads to a lower separation efficiency. This

can be evidenced by the fact that the average peak width at half-height in the microfluidic chips was about 21 and 28% broader than that in the capillary cartridge, respectively. As a result, the peak capacity values calculated for the microfludic chips were 32–59, which were less than that of capillary cartridges (37–72) as well.

The peak heights for most pI markers in the 100-μm-high, 100-μm-wide channel were almost similar to that in the capillary except the peaks for the pI markers of 4.16 and 8.79, which may be due to their low column efficiencies. However, for the high-aspect ratio microfluidic channel (height-to-width ratio: 200 μm/50 μm), the column efficiency reduced because the effective area of the integrated dialysis membrane decreased. As expected, the peak heights in the high-aspect ratio channels were increased compared to low-aspect ratio channels due to their longer light paths. It can be seen that the peak heights only slightly increased. This is because the opening of the metal slit glued on the top of the microchannel for passing through the detection light is 65 μm wide, which is larger than the channel width (i.e., 50 μm) resulting in high detection noise. It is anticipated that the peak heights in the high-aspect ratio channel can be increased further if a metal slit with a narrower opening is used.

Application of PDMS Chip-Based Cartridge for Qualitative and Quantitative Analysis of Proteins. Figure 5a shows that the mixture of human hemoglobin control AFSC containing four known isoforms (HbA, HbF, HbS, HbC) and two pI markers of 6.14 and 8.18 were separated on the PDMS chip with a 100-μm-high, 100-μm-wide channel using IEF-WCID. The successful separation demonstrated that the developed PDMS chip has strong separation ability. According to the linearity of the pH gradient, these four isoforms with pI values of 7.0, 7.1, 7.3, and 7.5, respectively, could be detected. An unknown isoform in the human hemoglobin control marked asterisk in Figure 5a could be detected besides the definite four isoforms A, F, S, and C. The myoglobin from horse hearts contains two isoforms, whose pI values are 6.8 and 7.2, respectively. It can be seen from Figure 5b that these two isoforms were well separated on the PDMS chip using IEF-WCID. The peaks 1 and 2 could be assigned to the two isoforms according to their pIs. The pI of the unknown peak marked asterisk was measured to be 6.25. The sample containing the above pI markers and carrier ampholyte was stored at 4 °C more than one month and then analyzed by IEF-WCID. The result was shown in Figure 5c. Some peaks became broad and some unknown peaks emerged, which indicated that the sample property has changed, and some pI markers may have been decomposed. These results proved that the IEF-WCID on this PDMS microfluidic chip is an excellent approach for qualitative analysis of proteins.

Furthermore, quantitative analysis of proteins was also obtained on the PDMS chips. Figure 6 exhibits the IEF profiles of myoglobin at a concentration (c) of 20–100 μg/mL obtained on the chip with a 100-μm-high, 100-μm-wide channel. As the peak height (h) was used for quantitative analysis ($h = 0.058c + 0.001$), a good correlation coefficient was achieved and reached 0.996 ($n = 5$) for three repetitions. As a result, the limit of detection and limit of quantitation were calculated to be 0.52 (S/N, 3) and 1.7 μg/mL (S/N, 10).
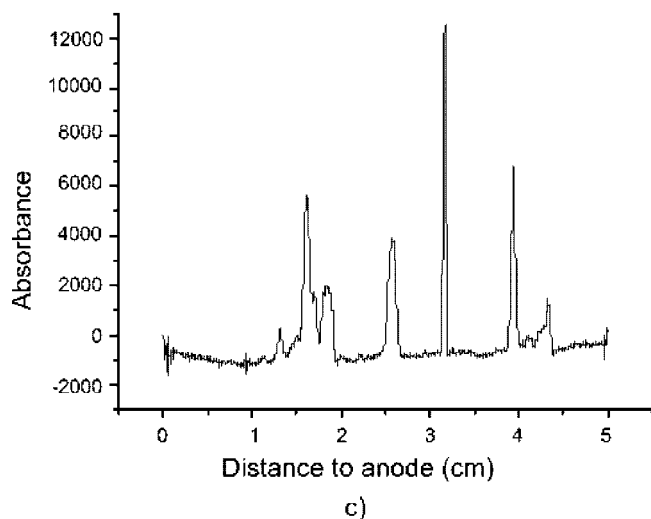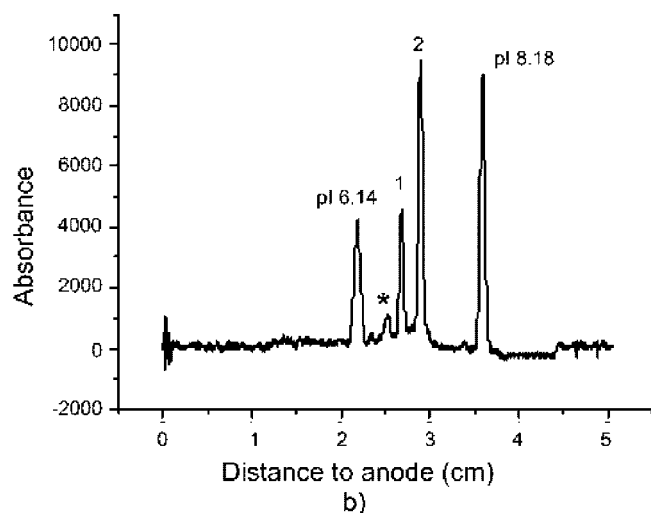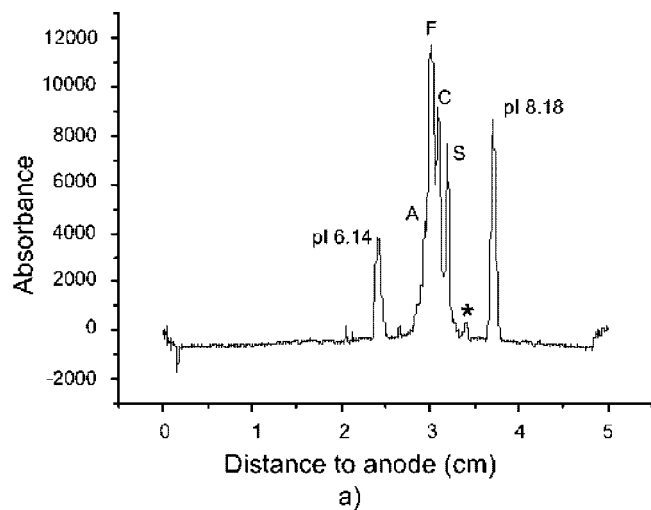
Figure 5. IEF separation of (a) hemoglobin control AFSC, (b) myoglobin, and (c) the degenerative pI markers mixture on a PDMS chip with a 100-μm-high, 100-μm-wide microchannel using IEF-WCID. Other conditions are the same as in Figure 2.

CONCLUSIONS

A new method for integrating commercial dialysis membranes into poly(dimethylsiloxane) microfluidic chips was developed and
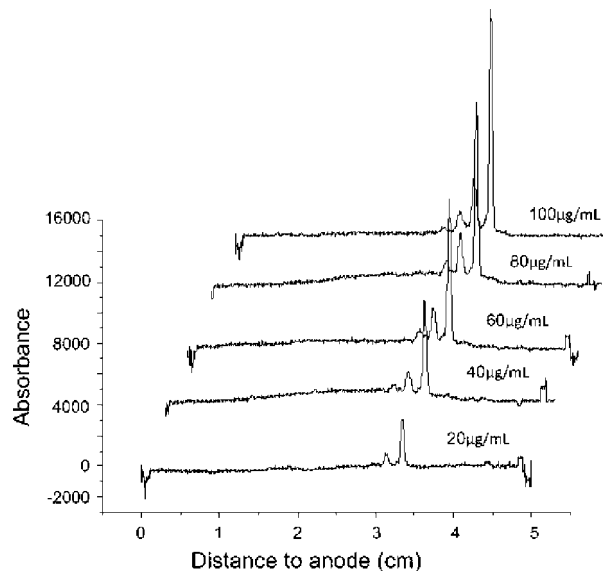

Figure 6. Electropherograms of myoglobin at different concentrations obtained on a PDMS microfluidic chip using IEF-WCID. Other conditions are the same as in Figure 2.

validated. A few appealing features include the following: (i) the introduction of the recesses design for membrane integration decreases the gap generated between membranes and chip substrates; (ii) the use of stamping toluene-diluted PDMS mortar eliminates the crevice around the membrane providing better sealing between membranes and PDMS substrates; and (iii) the integration of commercial membranes into microfluidic chips significantly reduces undesired pressure-driven flow and allows the sample to be injected in an easier manner, which improves the chip-based IEF repeatability.

By comparing the column efficiency and detection sensitivity, good separation ability can be obtained using the developed chip-based cartridge and qualitative and quantitative analysis of proteins is enabled by using the cartridge as well. Although only one channel was utilized in this PDMS microfluidic chip-based cartridge, it is possible to fabricate multiple channels in one PDMS microfluidic chip using soft lithography technology without additional procedures.[42] Therefore high-throughput analysis of IEF-WCID can be realized though the detection system needs to be modified for to accommodate multiple channels. It is a direction of development and will be devoted into the future work.

(42) Duffy, D. C.; McDonald, J. C.; Schueller, O. J. A.; Whitesides, G. M. *Anal. Chem.* **1998**, *70*, 4974–4984.