HYBRID INTEGRATED PLATFORM OF PDMS MICROFLUIDICS AND SILICA CAPILLARY FOR EFFECTIVE CE AND ESI-MS COUPLING

Ivan K. Dimov[‡,a,b] *Asif Riaz*[‡,a]*, Jens Ducrée* [a] *and Luke P. Lee* [c]*
[a]Biomedical Diagnostics Institute, NCSR, Dublin City University, Glasnevin, Dublin 9, IRELAND
[b]Department of Biomedical Engineering, Universidad de Valparaíso, CHILE
[c]Biomolecular Nanotechnology Center, Berkeley Sensor and Actuator Center
Department of Bioengineering, University of California, Berkeley, USA
‡ These authors contributed equally to this work.

ABSTRACT

We present an effective hybrid integration of PDMS microfluidic devices and fused silica capillaries. These hybrid microfluidic Integrated PDMS and Silica Capillary (iPSC) modules exhibit a novel architecture and method for leakage free CE sample injection requiring only a single high voltage source. Use of the iPSC devices is based on a modular approach which allows the capillary to be reused over 1,000 times whilst replacing the fluidics below it for different experiments. Integrating fused silica capillaries with PDMS microfluidics allows the direct application of a wide variety of well established conventional CE protocols for complex analyte separations and ESI-MS coupling, allowing users to focus on the sample analysis rather than the development of new separation protocols. The iPSC fabrication method is simple (3 steps) and quick (7 min).

KEYWORDS

CE, Microfluidics, ESI-MS, Silica Capillary, Integration.

INTRODUCTION

Microfluidic systems provide a powerful platform for bioanalytical assays. By embedding micro-channels in a chip the sample preparation procedures and the assay formats can be miniaturized. The often mentioned advantages of the miniaturized assays include small reagent and cell volumes, quick reaction times, low cost, as well as the potential for high-level process parallelization, automation and integration. PDMS casting [1] is a convenient and widely accepted way of constructing such microfluidic systems. PDMS casting constitutes a simple and rapid fabrication technique that can replicate with high fidelity micron scale features, optical transparency (down to 280 nm), non toxicity, compatibility to biological samples [2], chemical inertness, reversible and irreversible bonding to itself or other materials such as silicon, glass, SiO_2, glassy carbon and quartz. For bio assays, such as PCR, cell culture, electrophoresis, to be successfully implemented on PDMS microfluidic devices, adequate surface properties are required. This is an especially critical issue when trying to perform capillary electrophoresis (CE) within a PDMS microfluidic device. The highly hydrophobic surface of unmodified PDMS channels usually suffers from analyte adsorption and unstable electroosmotic flow (EOF). These surface coating issues generally reduce the reproducibility and efficiency of separations and thus hinder the use of CE within PDMS devices.

On the other hand electrophoresis using fused silica capillaries has several advantages over PDMS micro-channels. Since its emergence [3] the conventional CE has passed through a rapid developmental phase and a wide body of knowledge and separation protocols are available [4] . Furthermore, various kinds of surface treated fused silica capillaries are commercially available for specialized applications. Monolithically integrating fused silica capillaries with PDMS microfluidics would create a powerful combination that harnesses the advantages of standard fused silica CE with those of highly integrated PDMS microfluidics.

Various strategies have been proposed [5] to connect capillaries with microfluidic devices in substrates such as silicon [6], glass [7] and plastic [8]. Few methods have been shown to work in PDMS. In one approach a specially machined casing and mould with inserted wires and fused silica capillaries is used during the casting process to create capillary connection ports [9] while in another approach holes are punched and capillaries directly inserted into the PDMS device [10]. Although these techniques have been successful in creating fluidic interconnects, the monolithic integration of silica capillaries with PDMS microfluidic devices has not been demonstrated.

In this article, we present a new, simple (3 step) and quick (7 min) method for integrating PDMS microfluidic devices with fused silica capillaries. These integrated PDMS silica capillary (iPSC) modules exhibit a novel architecture and method for leakage free CE sample injection using a single high voltage source. The fabrication of the iPSC devices is based on a modular approach which allows the capillary layer to be reused over 1000 times whilst replacing the fluidics layer for different experiments. Furthermore, due to the silica capillary integration, the device can be easily coupled to ESI-MS with zero dead volume and requiring no non-standard custom made devices or parts.

Authorized licensed use limited to: DUBLIN CITY UNIVERSITY. Downloaded on July 12,2010 at 11:44:44 UTC from IEEE Xplore. Restrictions apply.

FABRICATION OF IPSC

The PDMS module incorporating the silica capillary was fabricated by spin coating a mixture of curing agent and the prepolymer (1:10 w/w) on a Si wafer at 500 rpm for 10 s, to obtain ~400 µm thick layer (Fig. 1A). A fused silica capillary of desired length was obtained and a detection window was made by incinerating the polyimide coating on the capillary. Then water was filled into the capillary through capillary action, just by dipping one end of the capillary in a drop of water. The water filled capillary was placed on the PDMS coated Si wafer and allowed to sink into the PDMS, and the wafer was placed on a hot plate set to 75 °C for 5 minutes. The water inside the capillary prevented the PDMS from entering into the capillary. Due to the expansion of water at elevated temperatures, the contained water formed small bubbles at the openings on either side of the capillary (Fig. 1C) ensuring that the capillary is not blocked by PDMS during the curing process, thus allowing to route fluids directly into the capillary core. Holes were punched with a 1-mm OD flat-tip needle in the PDMS layer to access the capillary inlet and outlet. If required one end of the capillary can be easily cut out of the surrounding PDMS since there is no irreversible bonding between the capillary and the surrounding PDMS. The iPSC module is then used by mounting it on to a fluidics chip and placing the iPSC inlet port over the fluidics chip sample outlet.

CE OPERATION

The process of sample injection, and separation is shown in Fig 2. (steps 1-5). In our operation method, sample leakage is prevented by cleanly removing the remaining part of the sample from the inlet access port with the continuous flow of buffer from the PDMS channel running in the fluidic chip as depicted in Fig. 2

Step 1. The iPSC CE module is mounted onto the fluidic chip by placing the iPSC inlet over the fluidics chip sample outlet. Then the capillary is filled with buffer.

Step 2. Sample is introduced in the inlet port.

Step 3. Voltage is applied across the inlet and the outlet, and sample is introduced into the capillary by electroosmotic flow.

Step 4. Plenty of buffer is flown into the port for washing and discharged to waste directly from the injection port. Fluidic chips were fabricated using standard soft photolithography[1] (other fabrication methods are also compatible with the plug and play module).

Step 5. High voltage is applied for separation and the separated analytes are detected by fluorescence monitoring under a microscope.

Platinum electrodes were used with a high voltage source HVS448 (LabSmith, Livermore, CA, USA). Electropherograms were obtained by using an inverted

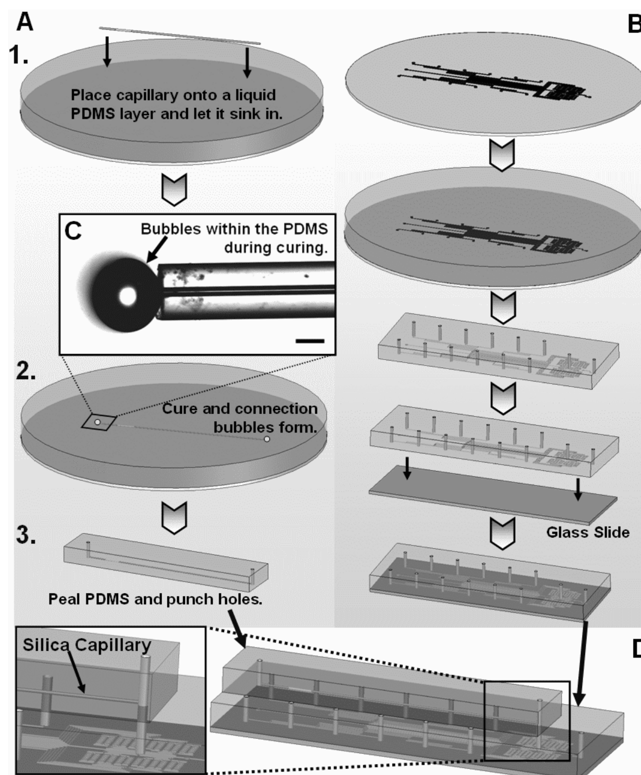

Fig. 1 (A) 3 step fabrication process of an iPMSC module. (B) Standard soft-lithography replication process for manufacturing complex microfluidic devices for sample treatment and preparation (C) Micrograph showing the bubble formed at the capillary edge after PDMS curing, scale bar 150 µm. (D) Mounting the iPMSC module onto the fluidic device.

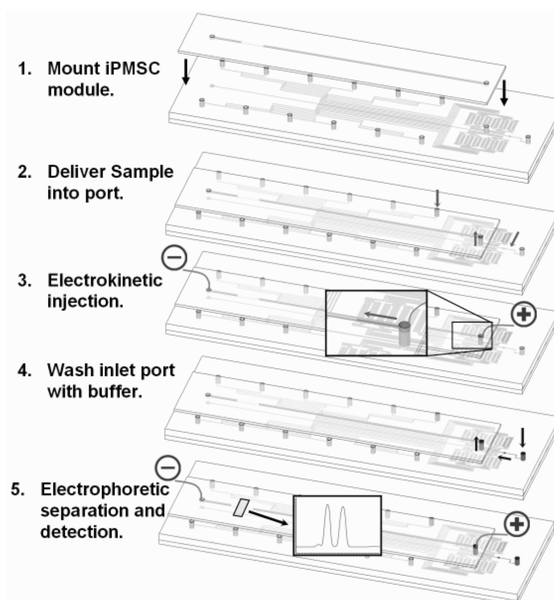

Fig. 2 Device mounting and operation (Step 2-3) Precise electrokinetic injection of the sample is done by applying a voltage on the electrodes (Step 4-5) Using hydrostatic flow the sample port is washed and then the sample plug is CE separated.

Authorized licensed use limited to: DUBLIN CITY UNIVERSITY. Downloaded on July 12,2010 at 11:44:44 UTC from IEEE Xplore. Restrictions apply.

fluorescent microscope (Olympus IX81) focused on the capillary window with a 10X objective. The capillary window was exposed to 492 nm excitation light (excitation filter BP492/18 and a xenon light source CellR MT20, Olympus) for 80 ms and the 530 nm fluorescent light was sampled through a filtercube (U-MF2, Olympus) with a CDD sensor (Hamamatsu C4742-80-12AG). The image sampling rate was 4 Hz. After the CE separation the images were analysed with the CellR software and the fluorescent kinetics plotted. A new capillary was flushed with 1 M NaOH for 10 min by connecting the outlet to a vacuum line and placing a 50 μL drop of 1 M NaOH at the capillary inlet followed by 5 min flushing with water, and then 5 min with 0.1-M NaOH. Finally the separation buffer was loaded.

RESULTS: ON CHIP CE

To demonstrate the reliability of the iPSC CE module we separated a mixture of two analytes FL and DCF using the conditions described previously [11] (Fig. 3). A peak migration time with a standard deviation of 3.9% (n = 3) and a peak area with a standard deviation of 4.0% (n = 3) were obtained. These relative standard deviations can be further lowered by surface regeneration between each run, e.g. by flushing the capillary with 0.1 M NaOH. As seen in Fig. 3 the flat baseline shows that there was no sample leakage or adsorption.

To demonstrate the module's ability to separate complex analytes by using conventional CE protocols we separated green fluorescent protein (GFP) iso-forms (Fig. 4). The separation protocol was adopted from a previously described (conventional CE) method [12]. GFP was purified off chip from *E-Coli* lysate. A single iPSC CE module was used to perform more than 100 separation experiments. To achieve this, the capillary was flushed with 0.1 M NaOH for surface regeneration.

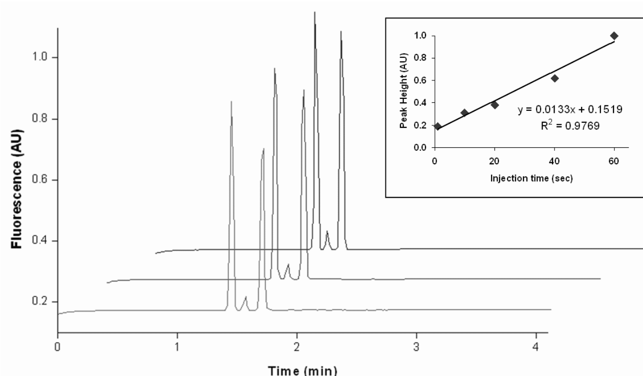

Fig. 3 Reproducible separation of FL and DCF. Inset shows the relation between the peak height and the injection time.

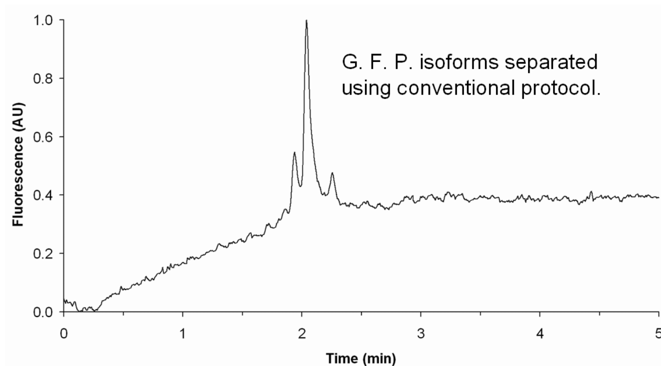

Fig. 4 Separation of green fluorescent protein (GFP) isoforms.

DISCUSSION

The fabrication of the iPSC module can be done within 7 minutes in a conventional lab without micro-fabrication facilities and requires only simple widely available equipment and materials. During the fabrication of the iPSC module uncured PMDS is prevented from entering the capillary by loading it with water which expands and evaporates during the PDMS curing process. This is advantageous since water droplets and vapour bubbles are formed at the edges of the capillary (Fig. 1F) which, after punching the holes, efficiently route fluids from the fluidics layer directly into the capillary core. The bubble size or formation are not critical factors since the punching process after curing eventually defines the connection channel at high precision. For special applications commercially available pre-coated silica capillaries can also be integrated. Finally the plug-and-play iPSC module has to be mounted onto the fluidic chip with minimal alignment complications. Since reversible bonding is sufficient a single capillary may be reused more than a 1000 times which allows for the fluidic chip to be replaced in each new experiment.

Since the capillary can be made to extend out of the main monolithic chip area other detection techniques such as electrical contactless detection[13] or UV detection can be efficiently and easily coupled by using commercially available detector modules and sliding the extended capillary into the sensor module. Furthermore using standard protocols this capillary extension can be easily coupled to ESI-MS with zero dead volume. Finally the integration of fused silica capillaries with PDMS microfluidics allows the direct application of a wide variety of well established conventional CE protocols for complex analyte separations and ESI-MS coupling, allowing users to focus on the sample analysis rather than the development of new separation protocols.

CONCLUSIONS

In this work we have shown a novel method for integrating the advantages of microfluidics with conventional CE into a PDMS microdevice. In addition,

Authorized licensed use limited to: DUBLIN CITY UNIVERSITY. Downloaded on July 12,2010 at 11:44:44 UTC from IEEE Xplore. Restrictions apply.

the on chip CE operation within a monolithic PDMS microdevice method solves important problems such as surface characteristics for low adsorption and stable EOF and leakage free and reproducible injection. The electrode and voltage source requirements were reduced with respect to the popular double-T format CE chips.

The modular approach allows the iPSC module to be reused over 1000 times whilst replacing the fluidic chip for different experiments. Additionally, by extending the capillary out of the chip, these devices can be easily coupled with ESI-MS with zero dead volume and minimal fabrication complexity and equipment requirement. This enables the fabrication of monolithic sample-in-answer-out systems with mass spectrometric accuracy. Finally, since conventional CE capillaries are used, this allows the direct application of the wide variety of well established existing conventional CE protocols for complex analyte separations and electroselectrospray ionization mass spectrometry (ESI-MS) coupling, permitting users to focus on the sample analysis rather than the development of new separation protocols.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Niamh Gilmartin for providing the green fluorescent protein. This work was supported by the Science Foundation Ireland under Grant No. 05/CE3/B754.

CONTACT

*Luke P. Lee Tel:+1 510 642 5855; lplee@berkeley.edu

REFERENCES

[1] Y. Xia and G. M. Whitesides, "Soft Lithography," *Angewandte Chemie International Edition*, vol. 37, pp. 550-575, 1998.

[2] S. K. Sia and G. M. Whitesides, "Microfluidic devices fabricated in Poly(dimethylsiloxane) for biological studies," *ELECTROPHORESIS*, vol. 24, pp. 3563-3576, 2003.

[3] J. W. Jorgenson and K. D. Lukacs, "Zone Electrophoresis in Open-Tubular Glass-Capillaries," *Analytical Chemistry*, vol. 53, pp. 1298-1302, 1981.

[4] D. Vladislav, "Capillary electrophoresis of proteins 2005-2007," *ELECTROPHORESIS*, vol. 29, pp. 143-156, 2008.

[5] C. K. Fredrickson and Z. H. Fan, "Macro-to-micro interfaces for microfluidic devices," *Lab on a Chip*, vol. 4, pp. 526-533, 2004.

[6] I. Fazal, I. Fazal, E. Berenschot, R. deBoer, H. A. J. H. Jansen, and M. A. E. M. Elwenspoek, "Bond strength tests between silicon wafers and Duran tubes (fusion bonded fluidic interconnects) Bond strength tests between silicon wafers and Duran tubes (fusion bonded fluidic interconnects)," presented at Solid-State Sensors, Actuators and Microsystems, 2005. Digest of Technical Papers. TRANSDUCERS '05. The 13th International Conference on, 2005.

[7] N. H. Bings, C. Wang, C. D. Skinner, C. L. Colyer, P. Thibault, and D. J. Harrison, "Microfluidic Devices Connected to Fused-Silica Capillaries with Minimal Dead Volume," *Anal. Chem.*, vol. 71, pp. 3292-3296, 1999.

[8] D. M. Hartmann, J. T. Nevill, K. I. Pettigrew, G. Votaw, P.-J. Kung, and H. C. Crenshaw, "A low-cost, manufacturable method for fabricating capillary and optical fiber interconnects for microfluidic devices," *Lab on a Chip*, vol. 8, pp. 609-616, 2008.

[9] A. P. Dahlin, S. K. Bergstrom, P. E. Andren, K. E. Markides, and J. Bergquist, "Poly(dimethylsiloxane)-Based Microchip for Two-Dimensional Solid-Phase Extraction-Capillary Electrophoresis with an Integrated Electrospray Emitter Tip," *Anal. Chem.*, vol. 77, pp. 5356-5363, 2005.

[10] N. A. Cellar and R. T. Kennedy, "A capillary-PDMS hybrid chip for separations-based sensing of neurotransmitters in vivo," *Lab on a Chip*, vol. 6, pp. 1205-1212, 2006.

[11] Asif Riaz and D. S. Chung, "Transient isotachophoresis of highly saline trace metals under strong electroosmotic flow conditions," *ELECTROPHORESIS*, vol. 26, pp. 668-673, 2005.

[12] E. H. Turner, K. Lauterbach, H. R. Pugsley, V. R. Palmer, and N. J. Dovichi, "Detection of green fluorescent protein in a single bacterium by capillary electrophoresis with laser-induced fluorescence," *Analytical Chemistry*, vol. 79, pp. 778-781, 2007.

[13] J. Z. Andreas, "Capacitively coupled contactless conductivity detection in capillary electrophoresis," *ELECTROPHORESIS*, vol. 24, pp. 2125-2137, 2003.

Authorized licensed use limited to: DUBLIN CITY UNIVERSITY. Downloaded on July 12,2010 at 11:44:44 UTC from IEEE Xplore. Restrictions apply.