Integrated Interface Technology for Microfluidic Systems

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ABSTRACT

This paper introduces a generic packaging approach for microfluidic systems. The 'integrated microfluidic interface' (IMI) provides a simple method for realizing complex arrangements of on-chip to off-chip fluid interconnects, optical windows, and general fluid control components such as pumps / valves into a single package. The fluid interconnects were designed for "one step" fabrication using stereolithography and post assembly of o-rings to ensure a tight seal. The normally open latex microvalves had zero dead volume and were pneumatically actuated by a gauge pressure of ~400kPa. The IMI was demonstrated in a glass-based genetic sample preparation application in which the total analysis system contained microchannels and sub-compartments with different analytical functions. Optical windows in the IMI were utilized for an infrared mediated polymerase chain reaction compartment and for the fluorescence detection in the capillary electrophoresis compartment. The performance of the sample preparation system with the IMI was experimentally characterized, showing that this approach can provide a simple and reliable macro-micro interfacing solution for complex microfluidic systems.

INTRODUCTION

For more than two decades, analytical microfluidic devices [1-3] have been developed for manipulating biological substances. Some of the advantages, when compared to macroscale devices, include smaller size, shorter analysis times, less sample/reagent consumption, and disposability. Many researchers have successfully demonstrated single-functional microfluidic devices [4,5] and transducers [6,7] for fluid manipulation. However, the development of integrated complex microfluidic systems has shown more modest growth, because integration of analytical functions and multiple chips into a total analysis system has technical difficulties such the need for more complex packaging, the possibility of cross biochemical contamination, and the possible need for different substrate materials for the compartment functionalities.

In this work, the IMI fabricated by stereolithography (SLA) is demonstrated to be a flexible method for packaging microfluidic systems with complex arrangements of fluid interconnects and the need for on-chip fluid control. One of the benefits of the IMI is the ability to incorporate microfluidic interconnects, which enables interconnection between chips and interconnections between macroscale and microscale system components. Since the approach results in monolithic fluid interconnects as opposed to the conventional method of post assembling individual connectors, it enables much closer spaced, complex arrangements of fluid interconnects. The IMI can be aligned and bonded to glass, silicon, or plastic microfluidic systems by using common registration techniques such as tooling holes or edge / feature alignment. Furthermore, using o-rings to achieve tight fluid seals avoids the possibility of clogging the fluid microchannels with adhesive when the IMI is bonded to the underlying microfluidic system. In addition, it allows for the integration of other component functionalities such as the incorporation of electrical, mechanical, and optical components into the interface as part of the build process. We demonstrate integration of multiple latex microvalves into the build process for on-chip fluid control. Additionally, we demonstrate optical windows for infrared heating and fluorescence detection.

DESIGN

The miniaturized sample preparation system consists of a cell separation system, cell lysing operation, DNA extraction from the cell lysate, PCR amplification of the DNA, and a DNA separation system, as shown in Fig.1. The cell separation chip contains functionality for introduction of whole blood, purification and separation of red/white blood cells, and cell lysing. The DNA chip contains the functionality for solid phase extraction (SPE), polymerase chain reaction (PCR), and capillary electrophoresis (CE).

First, the cell separation system separates out white blood cells from whole blood. The separated white blood cells are chemically broken down in the cell lysis compartment. In the DNA separation system, the SPE compartment extracts genomic DNA from the white blood cells, and the infrared mediated PCR compartment allows amplification of the target DNA fragments from the extracted DNA. Next, the amplified target DNA fragments are separated using on-chip CE, and subsequently detected with laser-induced florescence.

We have previously shown SPE of DNA from biological samples on independent microdevices [8-10], with a discussion of the technical difficulties associated with integrating these analytical functions with additional functions [10]. These issues include prevention of the solid phase matrix from being established in other regions of the integrated device, contamination of the PCR region with inhibitory reagents used in the SPE process, specifically guanidine and isopropyl alcohol, and prevention of the PCR chamber coating from coating the SPE compartment. Elimination of these problems in simple glass chips without the use of the IMI is not possible.

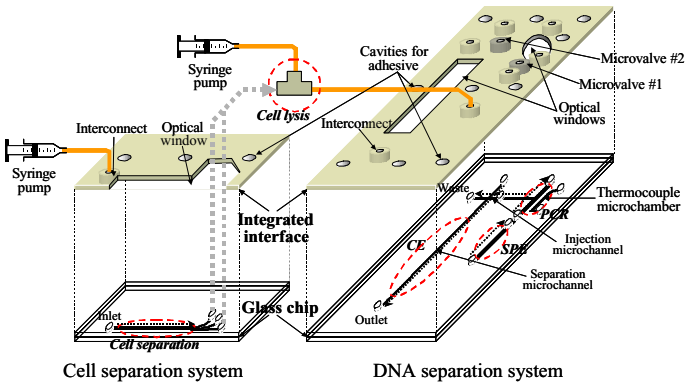

***Figure 1**. Schematic view of the IMI for the miniaturized sample preparation system.*

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Integration of the PCR and the CE compartments has been shown by a number of groups [10-14], but again difficulties remain [10]. Inhibitor effects of CE sieving buffers on the PCR amplification are possible, and the coating used in the PCR chamber can adversely affect the injection process if it gets into the separation domain. Direct sizing of the PCR fragments during the CE separation is also an important issue to ensure correct amplification or to allow multiplex amplification with sizing. This would require addition of internal standards after PCR amplification or co-injection of a DNA marker. One other problem encountered in the integration of PCR with a separation domain was the heat denaturing of the CE sieving matrix during the PCR thermocycling. This led to broad peaks in the subsequent CE separation.

To solve these problems, the IMI for the DNA separation system consists of seven microfluidic interconnects, two microvalves and two optical windows, as shown in Fig.1. The IMI fluid interconnects are designed as a "one step" plug-in interconnect (Fig.2) to facilitate simple connection of capillary tubing. The o-rings are used to avoid clogging of the microchannels by the UV-adhesive during bonding of the glass chip with the IMI and to provide a tight seal between the external capillary tubing and the IMI fluid interconnects. Figure 3 shows the formation of the monolithic microvalve when the IMI is aligned and bonded to the surface of the glass based sample preparation system. As can be seen from the schematic, the microvalve is designed to have zero dead volume.

FABRICATION PROCESS

Stereolithography is a technique where a laser is used to cure a photosensitive polymer structure layer-by-layer. The laser beam

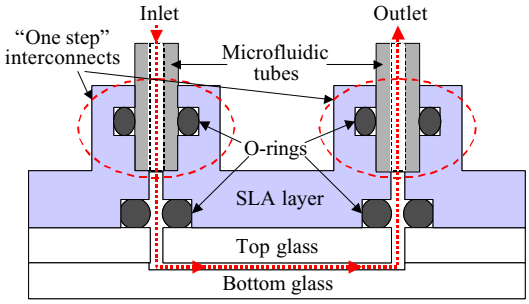

***Figure 2**. Schematic cross-section view of the microfluidic interconnects.*

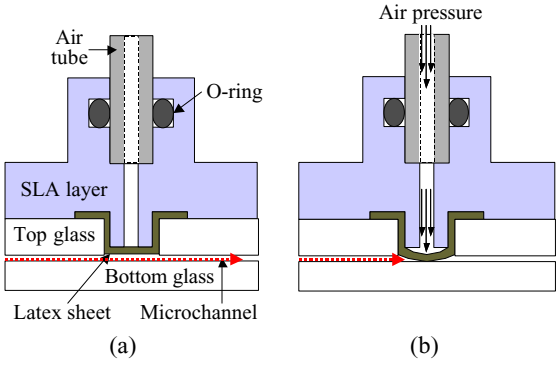

***Figure 3**. Schematic cross-section view of the microvalve: (a) normally open state; (b) pneumatically closed state.*

diameter of the SLA system (Viper SI2, 3D Systems Corp., USA) is 75 µm ± 15 µm, and the vertical resolution of the platform motion is 25 µm. First, the integrated interface is drawn using 3-D design software (Pro/Engineer®, Parametric Technology Co., USA). The drawing is imported into the SLA system, and then multiple copies of the single drawing are built in an automated fashion with the photosensitive polymer (SL5510™, 3D Systems Corp., USA). Once the part is formed, it is removed from the platform and cleaned to remove the excess uncured photopolymer resin. A five-minute immersion in 100% isopropyl alcohol in an ultrasonic bath is used as the cleaning process, followed by curing using an ultraviolet source.

Figure 4 shows the fabrication process of the sample preparation system with the integrated interface fabricated by SLA. Bottom glass wafers (Borofloat glass of 1.1 mm thick, Howard Glass Co., Worchester, MA) of the cell and DNA separation systems are etched 50 µm and 60 µm in 25% HF solution, respectively. The bottom glass wafers contain microchannels for each of the sample preparation compartments (i.e., cell separation, SPE, PCR, and CE). The cell separation compartment consists of an etched microchannel (3 cm long, 400 µm wide) with an electroplated nickel wire (50 µm high and 100 µm wide) centered in and running the length of the microchannel. The SPE compartment consists of an etched microchannel 2.2 cm long with a width of 400 µm. For the DNA separation system, the top glass wafer, containing only the PCR and thermocouple microchambers, is etched 150 µm deep to allow access for the thermocouple. For proper alignment between the microchannels of the bottom glass and the fluidic ports of the top glass, a drilling guide fabricated by SLA is used, as shown in Fig.4(a). By using glass-to-glass thermal bonding (Fig.4(b)) at 685°C for 3.5 hours, the glass chips of the cell and DNA separation systems are completed.

The IMI is used to realize the microfluidic interconnects, microvalves and optical windows on the glass chip, as shown in Fig.4(c). Nitrile rubber o-rings (Size 001-1/2, McMASTER-CARR, Atlanta, GA) are used for sealing the microfluidic interconnects, and a latex sheet (120 µm thick, McMASTER-CARR, Atlanta, GA) is used for the microvalves. The integrated interface and the glass chip are aligned and clamped in a clamping jig fabricated by SLA, as shown in Fig.4(d). A UV adhesive (Product 1187-M, DYMAX Co., Torrington, CT) is dropped into the openings on the integrated interface, and capillary forces pull the adhesive into the gaps between the integrated interface and the glass chip. The UV adhesive is then cured by placing it under a UV light for 30 minutes, completing the fabrication of the microfluidic device, as shown in Fig.4(e).

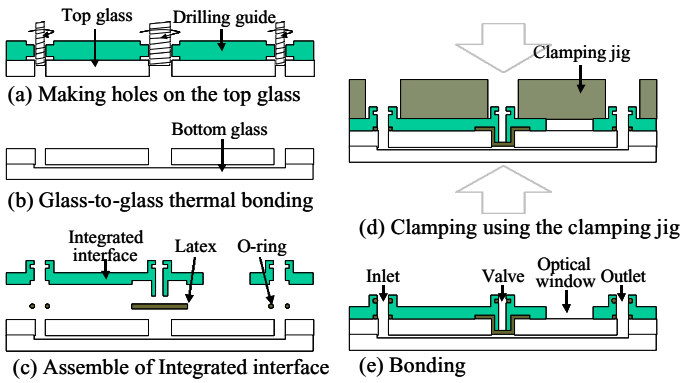

***Figure 4**. Fabrication process.*

RESULTS AND DISCUSSION

Figures 5(a) and (b) show the fabricated cell separation system and DNA separation system with the integrated interfaces. In the cell separation system, the microfluidic interconnect and the optical window are used for readily and effectively introducing the whole blood, and for monitoring the cell separation, respectively. Figure 6 shows that white blood cells are separated from whole blood in microchannel of the cell separation system. In the DNA separation system, microfluidic interconnects are used for introducing the biological/chemical samples into their respective compartments. Microvalve #1 is used to isolate the SPE channel during filling and curing of the sol-gel. Microvalve #2 is used to isolate the CE microchannel while filling the microchannel with polymer, and for preventing the PCR reagent from leaking into the CE compartment during the PCR amplification. Figure 7 shows the maximum fluid pressure before valve leakage for a range of valve pressures from 0 to 400 kPa. Figure 8 shows red dye flowing through each single-function microfluidic compartment in the DNA separation system controlled by the pneumatically actuated microvalves (gauge pressure of ~400 kPa). A DNA extraction profile from the integrated SPE compartment is shown in Fig.9. The optical window for the PCR compartment enables rapid thermocycling between 60 °C and 94 °C using IR-mediated, non-contact heating instrumentation, as shown in Fig.10. These experimental results demonstrate the usefulness of integrated interfaces for complex microfluidic systems.

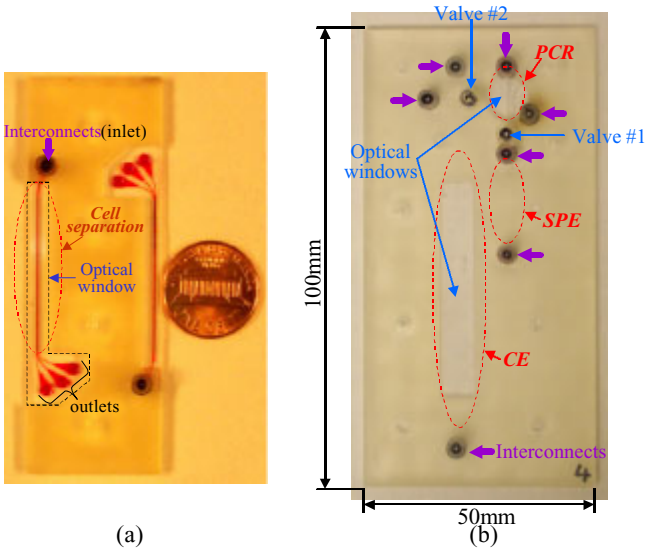

***Figure 5**. Fabricated sample preparation system: (a) cell separation system; (b) DNA separation system.*

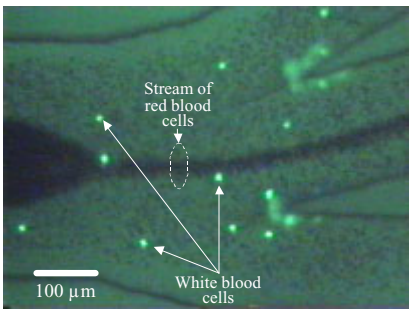

***Figure 6**. Blood cells being separated in the cell separation system.*

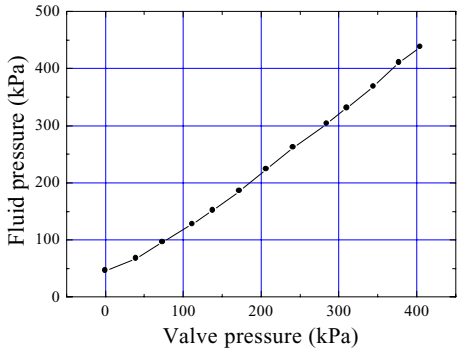

***Figure 7**. Fluid pressure before valve leakage for varying valve pressures.*

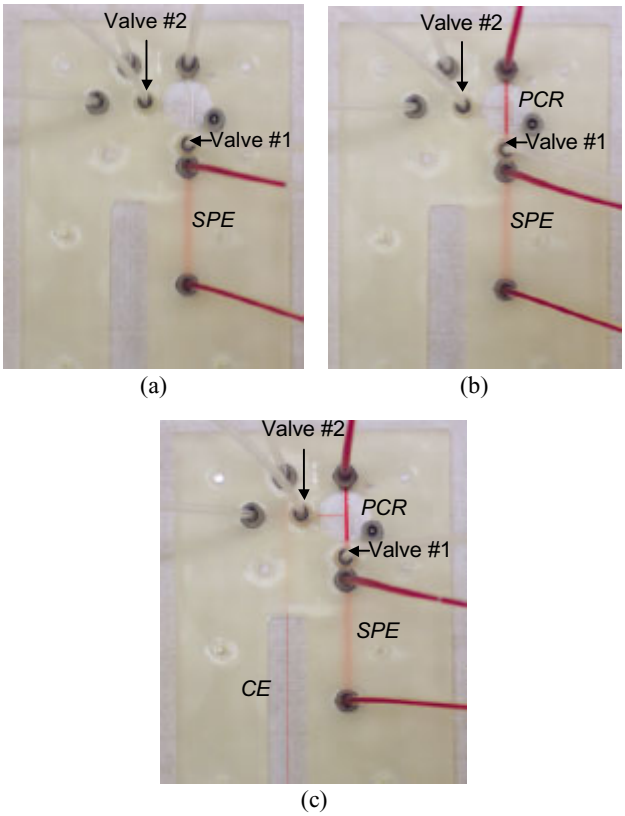

***Figure 8**. Red dye flowing through the microfluidic compartments in the DNA separation system with microvalve #1 and microvalve #2: (a) closed, closed; (b) open, closed; (c) open, open, respectively.*

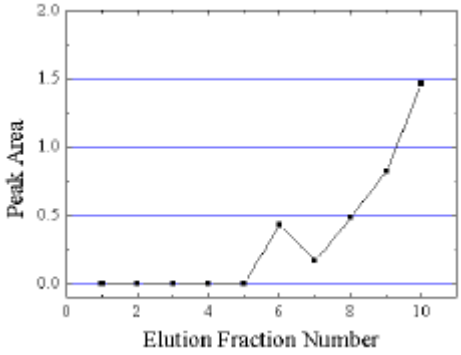

***Figure 9**. Extracted DNA profile from the SPE compartment.*

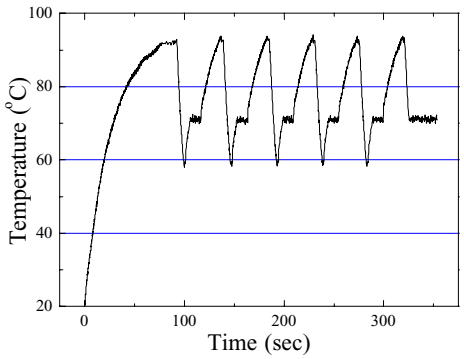

Figure 10. *PCR thermocycling using IR-mediated, non-contact heating.*

CONCLUSION

In this paper, we presented an integrated interface based on stereolithography for a sample preparation system, which consists of a cell separation system and a DNA separation system. The integrated interface contains microfluidic interconnect, microvalve, and optical window components for realizing the integrated sample preparation system.

In the cell separation system, the integrated interface has a "one step" microfluidic interconnect for readily introducing whole blood to the inlet, and one optical window for monitoring the cell separation. In the DNA separation system, the integrated interface consists of seven "one step" microfluidic interconnects, two microvalves for controlling fluids, and two optical windows for optically interfacing with the PCR and CE compartments. The microfluidic interconnects were easy to use and effective for creating fluidic interconnections for each compartment. The microfluidic interconnects were used for curing of the SPE sol-gel, and enabled stopping the flow through the PCR microchamber during DNA amplification. The normally open microvalves, designed to have zero dead volume, were pneumatically actuated by a gauge pressure of ~400 kPa. Microvalve #1 was used to isolate the SPE chamber. Microvalve #2 was used to prevent contamination of the PCR microchamber while filling the CE separation microchannel with polymer, and for preventing the PCR reagent from leaking to the CE compartment during DNA amplification. The optical window over the PCR chamber enabled rapid thermocycling between 60 °C and 94 °C using IR-mediated, non-contact heating instrumentation.

By demonstrating the performance of the single-function compartments of the sample preparation system, this paper has proven that the IMI is a facile and reliable solution for interface technology of complex microfluidic systems.

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REFERENCES

1. S. C. Terry, J. H. Jerman, and J. B. Angell, "A Gas Chromatographic Air Analyzer Fabricated on Silicon Wafer", *IEEE Transactions on Electron Devices, ED-26*, 1880 (1979).
2. O. Bakajin, T. A. J. Duke, J. Tegenfeldt, C.-F. Chou, S. S. Chan, R. H. Austin, and E. C. Cox " Separation of 100-Kilobase DNA Molecules in 10 Seconds", *Analytical Chemistry, 73*, 6053 (2001).
3. Y. Huang, S. Joo, M. Duhon, M. Heller, B. Wallace, and X. Xu, "Dielectrophoretic Cell Separation and Gene Expression Profiling on Microelectronic Chip Arrays", *Analytical Chemistry, 74*, 3362 (2002).
4. A. Y. Fu, C. Spence, A. Scherer, F. H. Arnold, and S. R. Quake, "A Microfabricated Fluorescence-Activated Cell Sorter", *Nature Biotechnology, 17*, 1109 (1999).
5. K. S. Chang, S. Tanaka, C. L. Chang, and M. Esashi, "Combustor-Integrated Micro-fuel Processor with Suspended Membrane Structure", *in Tech. Dig. 12th Int. Conf. Solid-State Sensors and Actuators Workshop (Transducers'03)*, Boston (2003), pp.635-638.
6. W. H. Grover, A. M. Skelley, C. N. Lui, E. T. Lagally, and R. A. Mathies, "Monolithic Membrane Valve and Diaphragm pumps for Practical Large-Scale Integrated into Glass Microfluidic Devices", *Sensors and Actuators B, 89*, 315 (2003).
7. A. Han, O. Wang, M. Graff, S. K. Mohanty, T. L. Edwards, K.-H. Han, and A. B. Frazier, "A Multi-Layer Plastic/Glass Microfluidic Systems Containing Electrical and Mechanical Functionality ", *Lab-on-a-Chip, 3*, 150 (2003).
8. K. A. Wolfe, M. C. Breadmore, J.P. Ferrance, M.E. Power, J.F. Conroy, P.M. Norris, and J.P. Landers, "Toward a Microchip-Based Solid-Phase Extraction method for Isolation of Nucleic Acids", *Electrophoresis, 23*, 727 (1997).
9. M. C. Breadmore, K. A. Wolfe, I. G. Arcibal, W. K. Leung, D. Dickson, B. C. Giordano, M. E. Powers, J. P. Ferrance, S. Feldman, P. M. Norris, and J. P. Landers, "Microchip-Based Purification of DNA from Biological Samples", *Analytical Chemistry, 75*, 1880 (2003).
10. J. P. Ferrance, Q. Wu, B. C. Giordano, C. Hernandez, Y. Kwok, K. Snow, S. Thibodeau, and J. P. Landers, "Developments Toward a Complete Micro-Total Analysis System for Duchenne Muscular Dystrophy Diagnosis", *Analytical Chimica acta, 500*, 233 (2003).
11. E. T. Lagally, P. C. Simpson, and R. A. Mathies, "Monolithic Integrated Microfluidic DNA Amplification and Capillary Electrophoresis Analysis System", *Sensors and Actuators B, 63*, 138 (2000).
12. A. T. Woolley, D. Hadley, P. Landre, A. J. deMello, R. A. Mathies, and M. A. Northrup, "Functional Integration of PCR Amplification and Capillary Electrophoresis in a Microfabricated DNA Analysis Device", *Analytical Chemistry, 68*, 4081 (1996).
13. J. Khandurina, T. E. McKnight, S. C. Jacobson, L. C. Waters, R.S. Foote, and J.M. Ramsey, "Integrated System for Rapid PCR-Based DNA Analysis in Microfluidic Devices", *Analytical Chemistry, 72*, 2995 (2000).
14. E. T. Lagally, I. Medintz, and R. A. Mathies, "Single-Molecule DNA Amplification and Analysis in an Integrated Microfluidic Device", *Analytical Chemistry, 73*, 565 (2001).