A Rapid and Low Cost Manufacturing for Polymeric Microfluidic Devices

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Abstract-A rapid manufacturing process was demonstrated to fabricate a microfluidic device to amplify specific DNA fragments in less than 8 hours. Microfluidics was derived from microelectromechanical system (MEMS) with lithography technique on the substrates of silicon and glass, which made the microfluidic product have a higher fabrication cost and laborious fabrication steps. This rapid approach only requires three steps for a PDMS microfluidic device: metal mold insert manufacturing, PDMS casting, and glass bonding. Each step did not require complicated equipments or procedures, and make this approach very attractive in rapid prototyping and experimental optimization with microfluidic devices. In this work, a brass mold insert was manufactured by a micromilling machine, followed by the standard PDMS casting and glass bonding to fabricate a microfluidic device. Polymerase chain reaction (PCR) to amplify specific DNA fragments, a typical microfluidic example, was successfully realized on this PDMS microfluidic device. This rapid and low cost (compared to conventional lithography) fabrication approach can provide researchers a lower entry to polymeric lab-on-a-chip either on PDMS or thermoplastic substrate for various applications.

Keywords – Micromilling, Microfluidics, Polymeric Microfluidics, Polymerase Chain Reaction.

I. INTRODUCTION

Microfluidic technology has been developing for three decades since the first micro-gas chromatograph was reported in 1979 [1]. This elucidated that a bio/chemical reaction can be realized on a small footprint with the benefits of lower reagent demand, faster reaction rate, minimized labor to reduce the contamination, and the potential to integrate with other functional components as a micro total analysis systems (µTAS). The original microfabrication techniques were derived from the semiconductor industry and the major substrate materials in the late 90's were silicon and glass. To obtain the goal of developing a microfluidic device that is low-cost, disposable, less hazardous fabrication processes, and mass produced, new substrate materials with associated fabrication techniques were explored such as polycarbonate, poly(methylmethacrylate) (PMMA), polydimethyl-siloxane (PDMS), and even paper [2].

To achieve the mass production of microfluidic devices, polymer has been a mainstream candidate from 2000. Polymeric microfluidic devices can be generated by various methods such as hot embossing and injection molding, and both techniques can promise rapid and accurate replication of microstructures from the metal mold insert. While the mold insert is the very fundamental component to this manufacturing process, using lithography (LIGA or UV-LIGA) for manufacturing is very time-consuming, resource-consuming, expensive, and highly professional required, which makes the overall price of a microfluidic device less acceptable in the market and inaccessible for most of the researchers. Since microfluidics has the potential to miniaturize and integrate most of the bio/chemical process equipments on a tiny platform with delivering very competitive performance, researchers have been looking for alternative manufacturing methods for metal mold insert to lower the overall cost. Some methods were reported with simpler fabrication process and lower cost, such as electron discharge machining and high-precision micromilling [3,4].

Micromilling is a very convenient tool in manufacturing a metal mold insert for replication of polymeric devices because it only requires three steps including CAD design, CNC machining, and polishing, which is much faster than the lithography process which usually requires 10 steps with UV exposure source or 15 steps with X-ray source [5,6]. In addition, micromilling can also be used to fabricate microfluidic prototyping on polymer substrates for the purposes of rapid testing and optimization. Micromilling can fabricate multi-level microfluidic structures (Figure 1) during the same milling procedure simply by typing the parameters into the control system, which allows combining more functions and applications into the microfluidic device like the passive alignment structures shown in Figure 2 [7] for integrating microfluidic system. Since micromilling is following the design pattern to remove materials from the substrate, so it can be used on different substrate materials from polymers to metals and create more possibilities in microstructure patterns and applications.
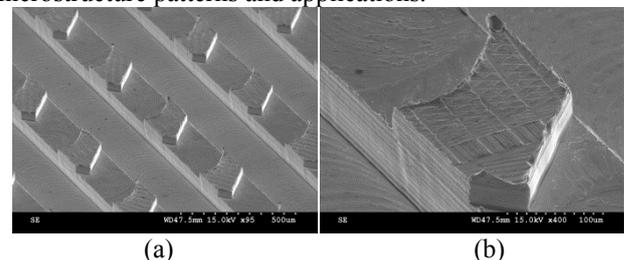

(a)   (b)

Fig 1 SEM images of a multi-level microstructures on the brass substrate.
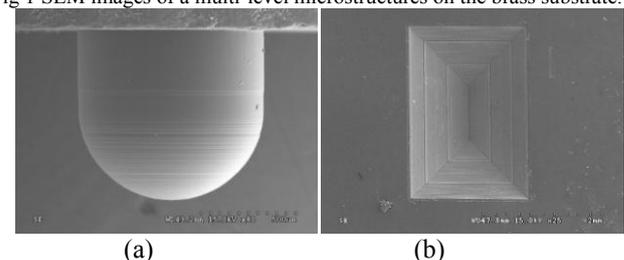

(a)   (b)

Fig 2 SEM images of a typical embossed alignment structures: (a) hemispherical post and (b) v-groove [7].

A criticism to use the micromilling machine for microfluidic device is the roughness generated from the mechanical milling on the wall of the microchannel. Since the surface-to-volume ratio in the microchannel is relatively large and some of the phenomenon like electrokinetic flow depends on the surface property, so milling was thought as an inappropriated method for microfluidic device. Another criticism is the round corner at the microchannel intersection due to the radius of the milling bit, which is a limitation of the milling machine. Some of the application in the microfluidics requires sharp corner at the intersection to improve the performance, but a competitive performance can be achieved with minimum radius and reported [9,11].

Soper's group [8,9] used a micromilling machine to fabricate brass mold insert to replicate the microchannels on the PMMA substrates by hot embossing. They measured the roughness of the polished mold insert at different locations including the top surface, sidewall, and the bottom floor of the microchannel. The results showed the average roughness (R_a) were 20 nm for the top surface, 85 nm for the bottom floor, and 65-110nm for the sidewall depending on the measurement direction. To determine the effect of the roughness to the microfluidic device, they compared the electrophoresis plug generation in two polymer device, one was replicated from micromilled mold insert and the second one was replicated from the LIGA mold insert which has a roughness of 20 nm [8]. Although the experimental results showed a larger plug generated from the device replicated from the micromilled mold insert because of the round corner, but there had no significant difference in the DNA separation efficiency between the two polymeric devices.

Limbach's group [11,12] used conventional milling machine to manufacture a aluminum mold insert for hot-embossing polymer microfluidic device. To study the performance of a PMMA microfluidic device replicated from a CNC machine, other microfluidic devices were used for comparison, including a PMMA microfluidic device replicated from a LIGA mold insert, a commercially Borofloat glass microfluidic device, and a PMMA microfluidic device fabricated by wire-imprinting. The fluorescence plug was injected into the four microfluidic device and the fluorescence microscope was used to monitor the fluorescence separation in the microchannel. The experiments reveal that the separation performance from the microfluidic device replicated from the CNC machine had a very competitive performance to the devices replicated from the LIGA mold insert and from the commercial companies.

Brazill's group [10] used the micromilling machine as the mask production for PDMS casting. An aluminum block was micromilled as a template to hot-emboss PMMA substrates, then those embossed PMMA substrates were used as templates for PDMS casting. With this fabrication approach, a PDMS/glass microfluidic device was fabricated followed by injection of agarose gel for DNA separation.

Polymerase chain reaction (PCR) is a typical multi-discipline example in microfluidics; a successful PCR requires design and manufacturing of microfluidic platform associated with correct chemical composition and temperature. The PCR is a powerful technique used to exponentially amplify specific DNA sequences of interest through repetitive temperature cycling [13]. The temperatures that are typically used in PCR include 90°C–94°C for denaturation of the double-stranded (ds) DNA molecule, 50°C–70°C for renaturation of the primers to the single-stranded (ss) DNA template, and 70°C–75°C for extension of the primers. Many types of micro PCR have been reported in the literature, which can be separated into two major groups: micro chamber PCRs [14-16] and continuous flow PCRs (CFPCR) [17-19]. Micro chamber PCR is a direct miniaturization of commercial bench top PCR machines. Its primary attraction is the potentially reduced demand for biochemical reagent volume; devices as small as the picoliter scale have been reported [16]. CFPCRs rely on a continuous flow of reagents through three thermostatic zones. The advantages are rapid amplification and the potential for approaching the theoretical limit of the reaction, which is determined by the kinetics of polymerase extension [17].

In this research, we demonstrated a rapid and simple method to manufacture a Polydimethylsiloxane (PDMS) microfluidic device for PCR experiment. This manufacturing process includes three steps, shown in Figure 3, metal mold insert fabrication, PDMS casting, and bonding with glass substrate. A mold insert was fabricated by micromilling machine on a brass substrate followed by two different cleanup methods, polishing with different-level sand papers and embossing with polymer substrates. Then the mold insert was used for PDMS casting to fabricate microfluidic PCR devices.
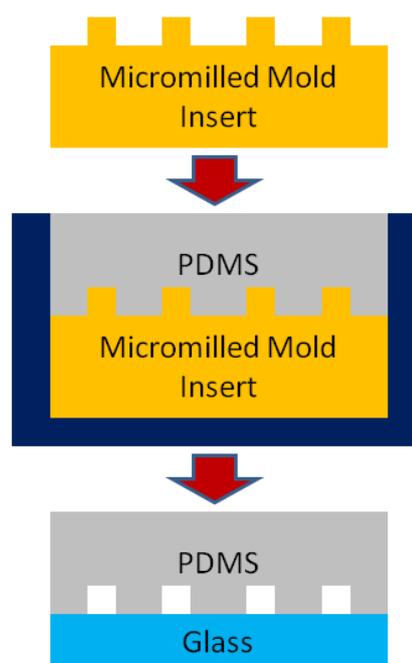

Fig 3 The manufacturing process for a PDMS CFPCR with micromilling machine.

II. EXPERIMENT

A. Design and Fabrication of the Mold Insert

A serpentine continuous flow polymerase chain reaction (CFPCR) was designed and it was composed of 6 major parts: preheating, isothermal zones of denaturation, renaturation and extension, post-heating, and lastly transition (Figure 4a). The chemical reagent was injected from the inlet, went through the preheating zone at 95ºC, cycled through the three isothermal zones for 40 times, and collected from an outlet in the 72ºC zone. To keep the chemical reagent exposed to the correct temperature sequence in the serpentine CFPCR device, the transition micro-channel connecting the denaturation and the renaturation temperature zones was designed with a smallest cross sectional area, 50 μm wide × 50 μm deep, to minimize the dwell time of the chemical reagent in the extension temperature zone (Figure 4b). To achieve the dwell time ratio of 1:1:4 for the chemical reagent in the denaturation, renaturation, and the extension zones, two different width of micro-channel with an universal depth of 50 μm were designed: (1) 100 μm wide in the preheating, denaturation, and renaturation zones; and (2) 250 μm wide in the extension and postheating zones. The overall dimension of this hybrid device was 75 mm × 50 mm and all the micro-channels were built within a central area of 50 mm × 55 mm with concerns of (1) avoiding side-wall cooling to affect the temperature distribution of the micro-channels, and (2) to standardize the location of inlet/outlet for further development in integrating with other functional components.
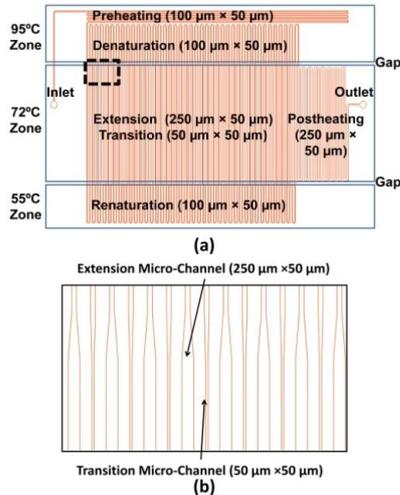

(a)

(b)

Fig 4 The design of a continuous flow polymerase chain reaction (CFPCR) device.

Brass is selected as the substrate material for micromilling since it is easy to machine and its strength is enough for hot embossing [20-21]. According to manufacturer specifications, the micromilling machine (Mini-Mill/4, Minitech, Machinery, USA) is capable of achieving positional accuracy of ±2.5 μm. A software, VisualMill, was used to convert the dxf. file, automatically setup the milling path, and save into a txt. file. The txt file is loaded into the micromilling machine controlling system. The brass substrate was prepared in a rectangle dimension of 50 mm × 75 mm with a thickness of 10 mm. A pre-cut of the entire surface with a 1 mm milling bit (DIXI 7240-1 mm diameter, Precise Tooling System, Singapore) was carried out to ensure the parallelism between both faces of the brass substrate. A 200 μm milling bit (DIXI 7240-0.2 mm diameter, Precise Tooling System, Singapore) was used for the overall milling process. Two milling steps were realized to achieve 50 μm deep microstructures on the brass mold insert: first cut was 30 μm deep at 30,000 rpm with a feed rate of 100 mm/min, and the second cut was 20 μm deep at 30,000 rpm with a feed rate of 80 mm/min. The total time required for fabrication of each mold insert was about 4-5 hours.

B. Cleanup Process

To cleanup the burrs generated from the micromilling manufacturing process, two procedures were applied, polishing and hot embossing with PMMA substrte. Polishing was used to remove the burrs left on the top of the microstructures, and different grain size polishing papers were used. Figure 5(a) shows the brass mold insert after the micromilling process and lots of burrs were on the surface. Figure 5(b) shows the brass mold insert after polishing with different grain size sand papers and dried with compressed air. Most of the burrs were removed after polishing but there still had some burrs left at the bottom corner of the microstructures. To remove these burrs, hot embossing with PMMA substrates was used. During the demolding process, the friction force between the PMMA substrate and the mold insert was used to cleanup the burrs left on the mold insert. Figure 5 (c) shows the brass mold insert after embossing with several pieces of PMMA substrates. Figure 6(a) shows the brass mold insert for CFPCR. Profile meter was used to investigate the flatness and the height of the microstructures. The designed height of the microchannels was 50 μm and the measured height was between 55μm to 60 μm due to the manual zeroing in Z-direction before milling and the resolution limitation of the milling machine. Roughness was measured at several locations of the mold insert and the results were between 3-4μm which was much larger than the roughness reported from Soper's group, less than 1μm [8,9]. Several methods might improve the surface finished quality by optimizing the cutting parameters and setting up the appropriated burss removal mechanism during the cutting process.
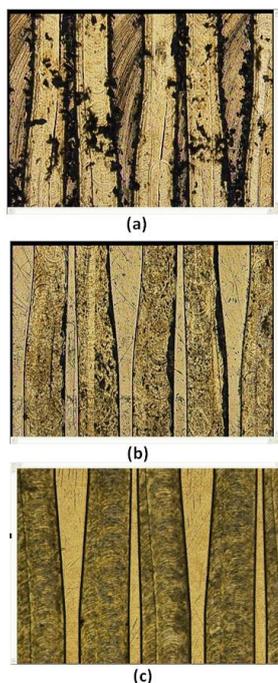
Fig 5 (a) The mold insert before polishing; (b) The mold insert after polishing; and (c) the mold insert after hot embossing with PMMA substrate.
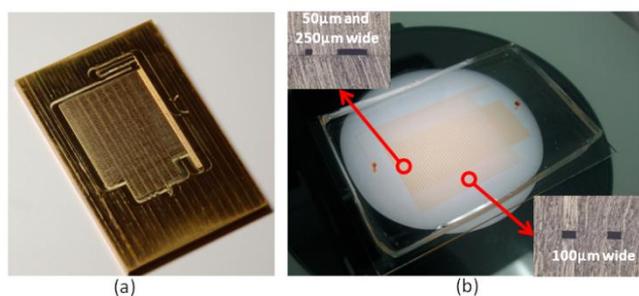

Fig 6 (a) The brass mold insert for CFPCR; (b) The PDMS CFPCR device bonded with a glass substrate for leakage test and DNA amplification experiments.

C. PDMS Microfluidic Device

PDMS can reproduce sub-micron features when casted with a mold insert. The PDMS pre-polymer and curing agent were well mixed at a weight ratio of 10:1 and was degassed to remove bubbles generated during the mixing step. The degassed PDMS mixture was poured onto the brass mold insert to completely replicate the patterns and degassed again to remove any unwanted bubbles which might affect the replication fidelity. Then, the PDMS mixture on the brass mold insert was cured in the oven at 80ºC for 20 minutes and peeled from the mold insert. After punching the inlet/outlet on the cured PDMS substrate, a pyrex glass and the PDMS substrate were exposed to the plasma and bonded permanently for experiments.

D. PCR Experiment

Due to the high surface-to-volume ratio in a microfluidic device and the hydrophobic surface property of the PDMS substrate, protein adsorption is a concern for enzymatic reaction in a microfluidic platform like PCR. There are two methods reported to minimize the enzyme adsorption in a microfluidic device including static and dynamic coating

methods. The basic idea of these two methods was to use a dummy molecule to block the adsorption site inside the micro-channel leading to a smaller chance for the enzyme to be adsorbed during the reaction. For the static coating method, a dummy molecule was pre-coated on the micro-channel walls before injecting the PCR cocktail. And for the dynamic coating method, the dummy molecule was mixed with the PCR cocktail and injected into the micro-channel together. Both methods were applied to this CFPCR device and the dummy molecule used was Bovine Serum Albumin (BSA). The BSA solution with a concentration of 1 µg/µl was injected into the micro-channel at a flow velocity of 1 mm/s for an hour followed by deionized (DI) water washing at 1 mm/s for 30 minutes for static coating procedure. Then the BSA with a final concentration of 0.5 µg/µl was added into the PCR cocktail for dynamic coating.

A customized thermal system (Figure 7) was built to support three steady-state temperature zones of a hybrid CFPCR device. Three heaters were made from a single printed-circuit-board (PCB) and were separated from each other by making through-board grooves. To attach the pyrex glass of the hybrid CFPCR on the PCB heaters, a double-side tape with high thermal conductivity was used to ensure a good contact between the pyrex glass and the PCB heater. This leads to a reliable heating environment and minimize the generation of hot spots to fail the experiment.

A tubing was inserted into the inlet reservoir and connected to a syringe pump. The PCR cocktail in the glass syringe was steadily and smoothly pumped into the CFPCR device and the injection flow velocity was controlled by the syringe pump. Another tubing was inserted into the outlet reservoir and connected to the centrifuge tube. The amplicon was pumped into the centrifuge tube after 40 thermal cycles on the hybrid CFPCR device for subsequent analysis process.
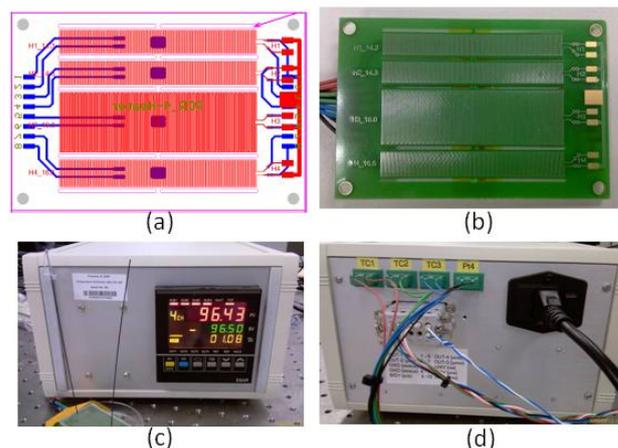

Fig 7 (a) layout of the 4-zone heater on a printed-circuit-board; (b) the printed-circuit-board heater; (c) front panel of the temperature controller; (d) backside of the temperature controller.

The DNA sample was PUC 19 and the PCR cocktail includes the template, primers to generate a 225 bp DNA fragment, and master mix (PCR Master Mix (2X), Fermentas, Thermo Scientific, USA). This DNA cocktail was amplified in a bench top thermal cycler to validate the composition and as a reference for the CFPCR results. The cycling conditions were 30 s at 95°C for preheating, 5 min

at 72°C for a final extension, and 40 cycles consisting of denaturation for 30 s at 95°C, renaturation for 30 s at 60°C, and extension for 30 s at 72°C. The total time needed for this process on the commercial system was about 2 h. In the CFPCR experiment, the DNA cocktail was injected into the micro-channel using a syringe pump (Harvard Apparatus, MA, USA) at a flow velocity of 4 mm/s. The amplicon was collected from the outlet (Figure 4), mixed with 1X Blue/Orange Dye (Promega, Madison, WI), and loaded into an agarose gel (Bio-Rad, Herculus, CA) for electrophoresis. The product was imaged and quantified using a molecular image system (Bio-rad, Gel Doc XR, Herculus, CA).

E. Results and Discussion

Figure 8 represents an agarose gel image of the PUC 19 amplicons generated from a commercial thermal cycler (c) and the PDMS CFPCR at different flow velocities.
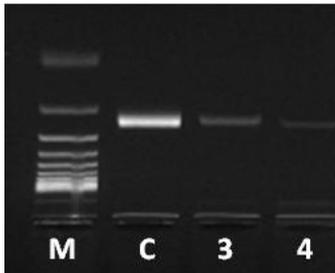

Fig 8 The amplification of PUC 19 DNA fragments from a benchtop thermal cycler and PDMS microfluidic devices.

III. CONCLUSIONS

A micromilling machine was used to manufacture a brass mold insert for PDMS microfluidic devices. The entire fabrication process except the design was less than 8 hours, which made this approach very useful in prototyping and experimental optimization. A typical microfluidic example, polymerase chain reaction (PCR) to amplify specific DNA fragments, was used to demonstrate this approach and the experiment results endorsed this rapid manufacturing of a microfluidic device. A metal mold insert not only can be used for PDMS microfluidic device, it can also be used in thermoplastics like polycarbonate, poly(methyl methacrylate) (PMMA), and cyclic olefin copolymer (COC) via hot embossing or injection molding. Although using micromilling machine to manufacture a metal mold insert caused a rougher finished surface than a mold insert from lithography process, which can be improved by optimizing the cutting parameters and make this fabrication approach suitable for wider applications with lower surface quality demand. With this rapid and low-cost manufacturing technique, researchers can have lower entry barrier for polymeric lab-on-a-chip for various applications.

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