ENHANCED MICROFABRICATION CAPABILITIES OF THERMOPLASTICS ELASTOMERS FOR CD LAB SYSTEM INCLUDING: LYSING, PCR AND HYBRIDIZATION MICROFLUIDIC FUNCTIONS

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ABSTRACT

We demonstrate the feasibility to fabricate and assemble at low-cost a complex multi-level microfluidic CD hybrid system made through the assembly of only two polymer layers. The design demonstrated in this work integrate in a single molded thermoplastic elastomer (TPE) layer all the microfluidic features necessary to build a nucleic acid testing device. This design would allow for cell lysis, sample metering and clarification, mixing with PCR buffer, PCR amplification, exonuclease digestion of amplified DNA, self-venting and finally DNA hybridization on a microarray located on the upper layer of the CD.

KEYWORDS: Thermoplastic elastomers, Lab on a CD, Microfluidic Integration, Molecular Diagnostics.

INTRODUCTION

Medical diagnostics is an area of great potential that has recently fuelled an increased interest for the development of disposable devices for point-of-care applications Of particular interest is the use of a centripetal device to integrate the functions needed to perform Nucleic Acid Testing (NAT). Such devices are refered to as Lab on a CD [1]. Beyond the proof of many concepts, the commercialization of lab-on-chip systems has been hindered by the lack of fabrication technologies and materials that allow for rapid and low-cost fabrication. The advent of soft lithography, and the ability of PDMS to achieve intimate contact on a substrate such as glass led to the possibility of quick fabrication of complex microfluidic functions. However, the use of PDMS for commercial applications is limited by its cost and not being suitable for injection molding for mass production. Thermoplastic materials (TP) represent an alternative solution for the low-cost fabrication and a number of thermoplastic materials including poly(methylmethacrylate) seem promising because these materials are relatively inexpensive. However, despite the obvious potential for low cost manufacturing, the use of rigid thermoplastics for the fabrication and assembly was limited by the currently available bonding methods and some major mold making challenges.

EXPERIMENTAL

The design demonstrated in this work integrate in a single molded thermoplastic elastomer (TPE) layer all the microfluidic features necessary to build a nucleic acid testing device. This design would allow for cell lysis, sample metering and clarification, mixing with PCR buffer, PCR amplification, exonuclease digestion of amplified DNA, self-venting and finally DNA hybridization on a microarray located on the upper layer of the CD.Sequential microfluidic steps are controlled by a combination of a series of single or serial siphon valves combined with capillary valves (fig. 1). Hot embossing experiments were carried on an EVG 520 HE equipment using a 5 levels (25, 100, 200, 450 and 1000 µm) low cost epoxy mold. The mold was obtained by replica molding from an initial SU-8 master composed of 5 successive photolithography steps. A 2-mm extruded TPE sheet has been molded by hot embossing (20 kN for 10 minutes at 135 ^{o}C). SEM images (fig. 2B) show quality and fidelity of molded features, de-embossing is performed manually by peeling-off the polymer layer from the mold as easily as can be accomplished with PDMS from a master [2]. Finally the TPE layer has been thermally bonded on a hydrophilic film (3M, hydrophilic polyester) that ensured the proper behavior of the siphon valves, static contact angle of this film after bonding is stable and has been measured to 24 ± 4° (fig. 2A).

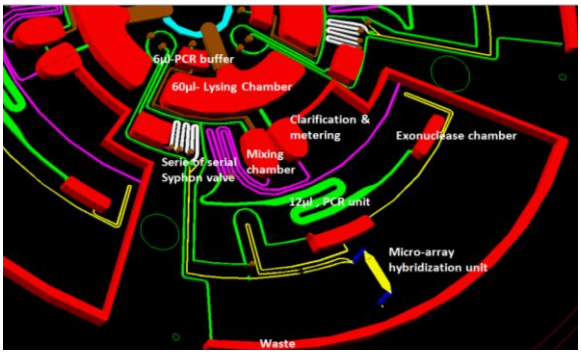

Figure 1. Microfluidic design of integrated CD Lab

RESULTS AND DISCUSSION

Table 1 reports the spin profile required for specific steps completion. The sequential flow are mainly ensure through two mains strategies. i) the implementation of capillary valves allowing the consistent release and liquid flows at desired burst frequencies, while ii) the siphon valves allow to maintain the liquid inside the specific loop of successive channel during the spinning phases, and then rests periods of few tenths seconds allow the liquid through capillary rise passive actuation to pass over the crest of the loop and thus ensure the next step. Clarification/metering chamber communicate via a capillary valve with the mixing chamber form which one 6 µl of PCR buffer is delivered form a reservoir. The hybridization unit under the form of small plastic insert was positioned on the upper layer of the CD. Figure 3, displays snapshots from a video demonstrating the stable and accurate control of fluidic motion though different locations: (a) post lysing/metering sample (b) post mixing of PCR buffer with measured sample and siphon valving in siphon valves structures, (c) PCR chamber, (d) hybridization on micro-array.

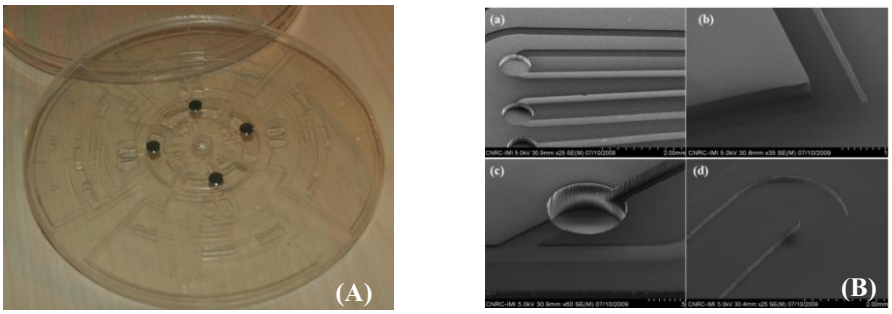

Figure 2. (A) TPE CD bonded on hydrophilic 3M film. (B) SEM images of embossed TPE CD illustrating (a) serial siphon valving structures, (b) over-metering microchannel (c) element of self-venting structures and (d) PCR chamber.

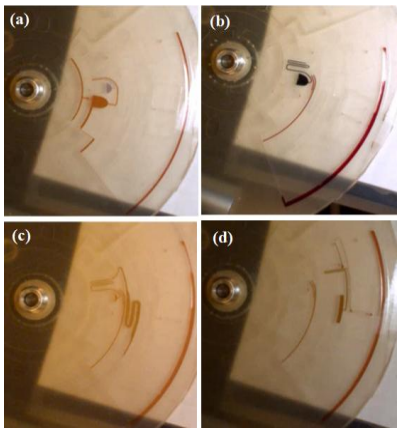

Figure 3. Optical images extracted from the movie.

Steps	RPM@time
Lysing & Burst of lysing chamber	± 650 RPM@4 min, 1650 RPM
Burst PCR reservoir	2100 RPM
Clarification	3500 RPM@ 3 min
Burst Clarification to mixing chamber	4600 RPM
PCR & Lysat Mixing	± 1500 RPM@1 min
Rest (two distinct loop)	Δt = 30 s
Extra sample to waste	2500 RPM@30 secs
Rest	Δt = 30 s
Filling PCR chamber	600 RPM@30 s
Burst and filling Exonuclease chamber	1450 RPM
Rest for siphon valving in exonuclease	Δt = 10 s
Mixing in Re-suspension & burst	± 800 RPM@1 min, 1200 RPM
Hybridization	1400 RPM @2min
Loading 15 µl washing Buffer	rest
Burst washing buffer (15µl) and washing	1210 RPM, 1400 RPM@2min

Table 1 : CD spin profile

CONCLUSION

Through enhanced microfabrication and bonding capabilities of TPE material, we have been able to fabricate a complex multi-level microfluidic CD. We demonstrate all the microfluidic functions necessary to perform : cell lysing, sample metering and clarification, mixing with PCR buffer, PCR amplification, exonuclease digestion, and finally DNA hybridization on microarray.

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