FLUOROTHERMOPLASTIC CHIPS FOR DROPLET MICROFLUIDICS AND DNA ANALYSIS

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ABSTRACT

We present a fabrication technique allowing the production of monolithic fluorinated microfluidic chips. We used an new material (Dyneon THV), compatible with fluorinated oils and organic solvents. Microfeatures are produced by hot embossing using flexible molds, and chips are sealed by a new bonding technique based on the use of two different grades of the polymer. We developed a new strategy to obtain round channels, by bonding two blocks containing hemi-circular structures. Bonding was possible by selective transfer of the adhesive layer and alignment was obtained by putting cylindrical spacers in unconnected extra-channels.

KEYWORDS: Fluorinated Polymers, Hot Embossing, Droplet Microfluidics, DNA Amplification

INTRODUCTION

Droplet microfluidic systems have been widely applied for chemical and biological applications [1-2]. The main advantage of this approach is the possibility to manipulate small and discrete quantities of liquids, with volumes ranging from picoliters to microliters and without mutual contamination.

One promising application of these systems is the possibility to perform DNA amplification: our group previously proposed an automated platform for continuous flow PCR amplification in 1µL confined droplets ("plugs") carried by perfluorinated oil inside perfluoro-alkoxy (PFA) capillaries [3]. Plugs were generated by combining in situ reagents and samples from a microtiter-plate, allowing for the production of stable "trains" in which every droplet contains a different DNA sample. The use of fluorinated oil ensured complete suppression of cross-contamination between adjacent plugs [4].

Capillary-based systems are easy to implement, but they have serious limitations regarding integration and miniaturization. The fabrication and manipulation of capillary-based PCR systems is complex, and the minimal dimensions of commercially available capillaries are ~100µm. A microchip approach would thus be suitable for reduced production cost, easier manipulation and full flexibility in droplet volumes. However, fluorinated oils are not compatible with most of the materials used in microfluidics (PMMA, COC or untreated PDMS). Surface treated PDMS chips were successfully used with non-confined droplets [5]. Such droplets, however do not allow for accurate synchronization, and they generally require an internal label to keep track of droplet identity. Confined plugs, in contrast, retain perfect control in spacing and order, but our earlier experience with plugs in fluorinated oil confined in treated PDMS did show occasional droplets pinning and cross contamination, probably due to surface treatment defects.

EXPERIMENTAL

We thus developed novel monolithic chips based on the use of Dyneon THV, a thermoplastic fluoropolymer that retains most of the properties of PFA capillaries (transparency, low surface energy, compatibility with fluorinated oils and organic solvents) and is suitable for hot embossing [6]. We embossed this material with flexible molds (PDMS) to produce microchannels on THV 500 (T_M=160ºC) blocks. We also developed a "monolithic-adhesive" bonding technique, by using a thin sheet of THV 220 (T_M=120ºC) between two THV 500 blocks. The thin sheet is produced by spin-coating on a flat surface of the high T_M grade. Bonding is performed at 120ºC, at which only the low T_M copolymer is deformed, allowing for good sealing without structure collapse. Other properties of these two polymer grades (hydrophobicity, transparency etc.) are comparable, yielding "monolithic" chips. For complex geometries, (e.g. round channels), two structured blocks were sealed using the selective transfer technique (described in Fig.2): a thin sheet of THV 220 is produced by spin coating on a silicon wafer, and then transferred on the structured THV 500 block by putting them in contact at 120ºC. We also propose an original self-aligning method involving cylindrical capillary pieces in opposing hemi-circular extra-channels (Fig.2).

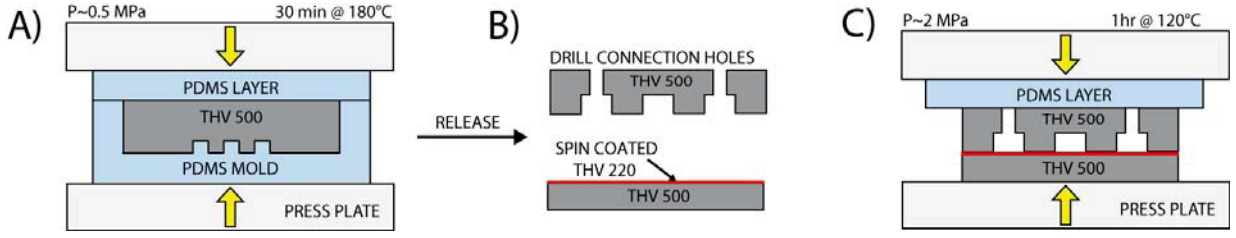

Figure 1: Fabrication technique for the production of one-layer monolithic THV chips: A)Molding of a THV 500 sheet; B)Drilling of connection holes and spin coating of a thin layer of THV220; C) sealing of the channels.

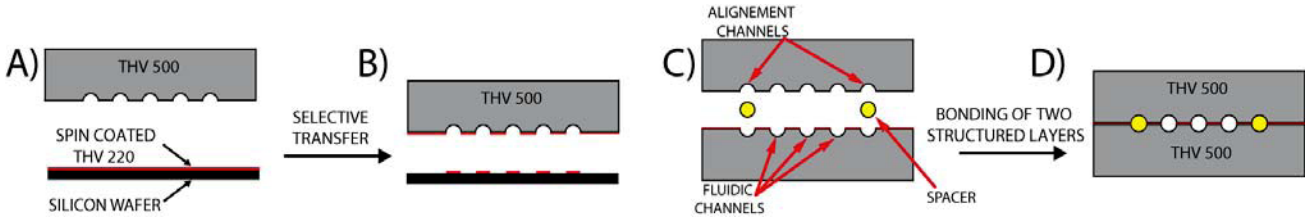

Figure 2: Fabrication process for bonding two structured THV 500 blocks: A) a thin layer of THV220 is spread on a silicon wafer; the structured block is then put in contact with the thin film at 120ºC for 2 minutes, under low pressure (~1MPa); B) the thin layer is selectively transferred only in the contact area; C) both layers are designed to have fluidic channels and extra unconnected channels. They contain cylindrical spacers with the same dimensions, allowing easy and accurate self-alignment on large areas; D) the stack is then bonded as in Fig. 1C to obtain a sealed chip.

RESULTS AND DISCUSSION

We characterized the molding and bonding steps by scanning electron microscopy and optical profilometry. Since THV is an insulator, metallization was required prior to SEM analysis. The visualization of channels sections was possible by using cryo-cut prior to metal coating.

Fig. 3 shows micrographs of open and closed channels, with different geometries (Flow Focusing Device with channels 20µm deep; Y-junction composed of hemi-circular channels with a radius of 150µm and a closed 50x50µm channel). In all cases the mold structures are reproduced with high fidelity, proving the versatility and accuracy of our technique. Optical profilometry showed a difference in dimensions (channel width and height) between PDMS molds and embossed structures always below few percent (data not shown). The minimal dimensions obtained were 20x40µm for rectangular channels, and 200µm ID for round channels. Embossing of smaller structures (arrays of dots 5x5µm) or bigger channels (200µmx2mm) was also easily achieved, increasing the possible applications of this technique.

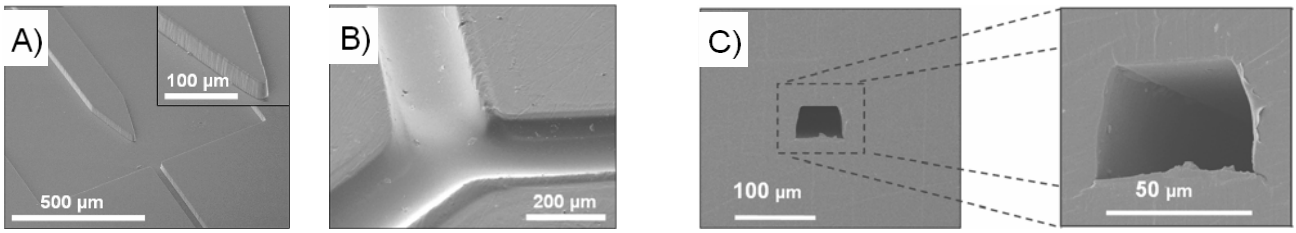

Figure 3: SEM images of channels on THV produced with PDMS molds: A) Flow focusing device with channels 20µm deep; B) hemi-circular channels (radius 150µm) C) sealed 50x50µm channel after cryo-cut.

Chips were also characterized by studying the circulation of plugs trains (aqueous or chloroform solutions) in fluorinated oil. Typical geometries for droplets production (T-junctions and Flow Focusing Devices) were integrated in the chip geometries, and can be used to produce regular trains with constant droplet volumes and spacing. Typical droplet volumes can range from 10pl (for the Flow Focusing devices) to hundreds of nanoliters (in the case of the T-junction with channels size 300x300µm). Plugs trains can be alternatively produced with the automated sampling system described previously [3]. In all cases the oil was Fluorinert (FC-40) with 3% of surfactant (Perfluoro-Decan-ol). Trains were stable over at least 1m and no interaction with the walls was visible. Drops can be continuously produced for days in the same chip, with no detectable sign of deterioration or wetting of the walls. Fig. 4 gives some examples of the different geometries and droplets volumes that can be achieved with our technology.

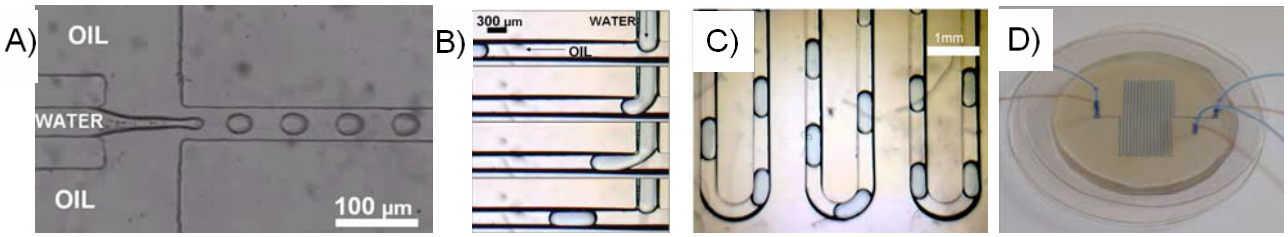

Figure 3: Examples of chips application: A) 10 pL droplets produced with a Flow Focusing device; B) 80nL plug production at a T-junction; C) plug circulation in a serpentine channel; D) image of the whole chip used for plug production and circulation.

We then characterized the optical properties of THV, and especially the possibility of performing on-chip fluorescence detection. Plugs containing different concentrations of DNA and a fluorescent marker (SybrGreenI) were easily produced in the chip. We used a fluorescence microscope and a CCD camera to visualize the plugs trains. Fig.5A shows transmission and fluorescence micrographs of a droplet in a microchannel. A detection zone can be used to study the fluorescence profile over time, thus studying the intensity and spacing of different plugs (Fig. 5B).

Dyneon has a residual fluorescence slightly higher than PDMS, but we were able to detect plugs containing 0,2 ng/µl of DNA. Further reduction in the detection limit can be achieved by reducing the chip thickness and/or by using confocal microscopy for fluorescence detection.

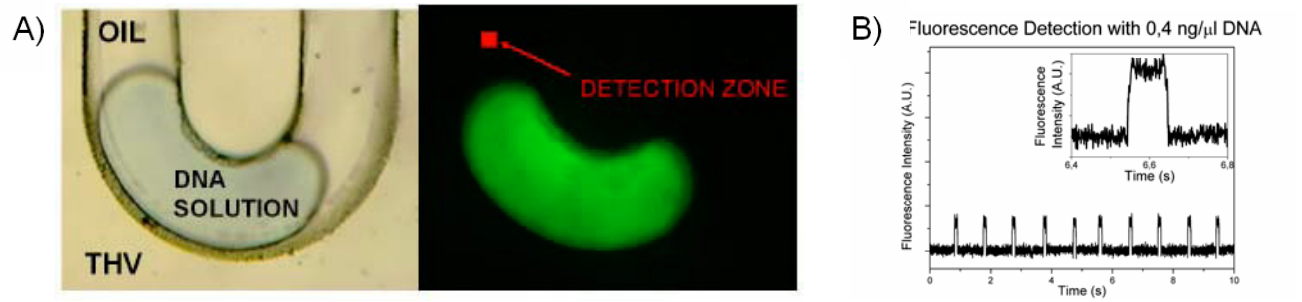

Figure 5: On-chip fluorescence detection: A) transmission and fluorescence images of an 80nL plug circulating in the chip. The red square indicates the area used for fluorescence quantification; B) fluorescence intensity collected over time, each peak corresponding to the passage of one plug containing DNA (0,4 ng/µl) and SybrGreen I. The fluorescence intensity is constant for different plugs, as well as their size and spacing.

CONCLUSION

We presented a simple, versatile, low cost and rapid technique for the production of monolithic microfluidic chips by using Dyneon THV. This material retains most of the properties of previously used fluoropolymers (transparency, hydrophobicity, compatibility with organic solvents and fluorinated oils) but shows a lower melting temperature. This technique is thus well adapted for rapid prototyping at the laboratory scale (the fabrication time for producing one chip is below one day) but it can be extended to mass production. We were able to obtain structures over a large scale of dimensions (from micrometers up to centimeters) and geometries (square, rectangular, hemi-circular or circular channels).

The produced chips are suitable for the production and manipulation of droplets in fluorinated oils, avoiding interactions with the channels walls. Organic solvents can also be manipulated, allowing for possible applications in liquid-liquid extraction or organic chemistry. On-chip fluorescence detection of DNA marked with SybrGreenI is also possible, with a detection limit of 0,2 ng/µl.

We thus believe that this technology will open the road to the extension of our previous work in DNA amplification, by using an integrated microfluidic chip. Future developments will focus on the reduction of the fluorescence detection limit and on the characterization of DNA amplification in sub-microliter plugs.

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