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PRODUCTION OF FLUOROPOLYMER MICROCHIPS FOR DROPLET MICROFLUIDICS AND DNA AMPLIFICATION

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ABSTRACT

In this paper we present a novel fabrication technique for production of monolithic microfluidic chips made from a fluoropolymer (Dyneon THV). This material retains numerous properties of commonly used fluoropolymers (low surface energy and compatibility with chemicals such as organic solvents or fluorinated oil), and is easily processable at relatively low temperatures (lower than 180°C). We used hot embossing to mold microstructures on flat sheets of this polymer. The microchips are sealed through a combination of thermal and solvent bonding by applying uniform pressure with a flexible membrane. These closed channels can be used for the production and circulation of aqueous droplets in fluorinated oil. This droplet microfluidic configuration is suitable for DNA amplification since it avoids cross contamination between adjacent droplets.

INTRODUCTION

Droplet Microfluidics has recently raised a strong interest in the microsystems, chemistry and biological communities, as demonstrated by the publication of various review articles [1-2]. Basically it consists in the production and manipulation of discrete volumes of a liquid (from picoliters to microliters), carried by an immiscible fluid. This approach retains the typical advantages of microfluidic systems (e.g. low reagent consumption, fast heat transfer, integration of different reaction steps in the same lab-on-a-chip platform), while avoiding the contact with the walls of microchannels. Moreover, it allows for compartmentalization of reagents and high throughput analyses.

Aqueous droplets in fluorinated oil are an interesting example, as this configuration was successfully used for the amplification of DNA by Polymerase Chain Reaction (PCR) with no cross-contamination between adjacent droplets [3]. Our group also proposed an automated platform [4] for performing DNA amplification in 1μL confined droplets ("slugs") in fluorinated oil. Droplets were generated inside perfluoro-alcoxy (PFA) capillaries, using a pipetting robot coupled to a microtiterplate platform. The confinement provides a perfect control on slug position and order, allowing for multiple experiments in the same slug train. The transparency of the material provides good reproducibility and ease of detection. Moreover, the surface properties of PFA (which is highly hydrophobic and is preferentially wetted by fluorinated oils) promotes the formation of a thin oil film between the plugs and the capillary wall, thus avoiding cross contaminations. However, the use of capillaries limits the integration and the minimal droplet volume, which depends on the internal diameter. A microchip approach would thus give full flexibility in terms of geometry and droplet volume.

Since PFA has a very high melting point (300°C), it is not well adapted for the fabrication of microfluidic devices in the laboratory scale that requires rapid prototyping with limited equipment. A different material should thus be chosen for this purpose, that should keep the same properties of PFA: transparency, compatibility with fluorocarbon oils (and especially low surface energy for the polymer-oil interface), hydrophobicity and if possible a limited cost.

Compatibility with fluorocarbon oils cannot be achieved with traditional polymers used for microfluidic applications, such as polydimethylsiloxane (PDMS), polymethylmethacrylate (PMMA), polycarbonate (PC) or cyclic olefin copolymer (COC). Fluorinated materials are needed for these applications, and they can be used for coating the microchannels or for producing the whole chip. The first approach was used for example by the groups of Griffiths and

Weitz [5], and consists in the use of PDMS microchips treated with a commercial coating for glass (Aquapel® Glass treatment). These experiments were devoted to spherical droplets which are not confined in the microchannels. Such droplets, compared to slugs, have fewer interactions with the walls and their speed is affected by small fluctuations in their position in the channel (e.g. a droplet in the channel center moves faster than one in proximity of the walls). As a result, it is more difficult to keep their reciprocal order and spacing over long distances. Our own experience with slugs confined in surface treated PDMS chips (using Aquapel®, fluorosilanes or Teflon AF by Dupont) showed occasional droplet pinning and cross contamination that can be potentially attributed to surface treatment defects.

The use of "monolithic" chips seems to be a proper way to overcome these problems. Unfortunately, most of the fluorinated polymers commercially available are not transparent (e.g. Viton®, Dupont) or too expensive (e.g. photo-reticulable PFPE [6], which cost is in the order of 10K$/Kg). We have chosen a fluorinated and thermoplastic polymer: Dyneon THV. This material is a ter-polymer of tetrafluoro ethylene ($F_2C=CF_2$), hexafluoro propylene ($F_2C=CF\text{-}CF_3$) and vinylidene ($H_2C=CF_2$), supplied in different grades depending on the relative monomer ratio. It offers an excellent combination of properties:

(i)It retains all the properties of commercial capillaries in PFA, in terms of wetting (static water contact angle around 100°), transparency (the transmission of a 100µm film is 87% @ 200nm and 93% @ 600nm [7]) and compatibility with fluorocarbon oils;

(ii) It is cheap (less than 50$/Kg) and does not require any curing step, which allows for possible industrial application;

(iii) Its grades THV220 and THV500 have melting temperatures (respectively 120°C and 160°C) which are significantly lower than that of PFA (300°C), allowing easier processability by hot embossing;

(iv) It is soft and elastic allowing easy direct connection with tubing as achieved e.g. with PDMS chips, as opposed to other thermoplastics conventionally used in microfluidics (PMMA, PC, COC);

(v) It is moderately resistant to organic solvents (e.g. chloroform, toluene, alkanes), and thus can be used for chemical applications [7].

To our knowledge this polymer has never been used before for microfluidic applications. We developed a complete set of fabrication techniques for the production of microfluidic chips completely made by THV.

FABRICATION TECHNIQUE

THV was supplied by Dyneon Corp in pellets with typical dimensions of few millimeters. The material was first cleaned by sonication in water and ethanol for 1 hour, and then dried at 100°C for 30 minutes in an oven. The pellets were then treated overnight at 180°C and under low vacuum, in order to obtain flat, bubble-free sheets with a thickness of approximately 5mm.

We used commercial silicone molds (Mastrad) or flat PDMS surfaces (Sylgard 164, Dow Corning) in a vacuum oven (Fisher Bioblocks), as shown in Fig 1. The step can be substituted, for routinely applications, with an industrial or semi-industrial extrusion or injection molding system (as is commonly done for PMMA, PC or COC).

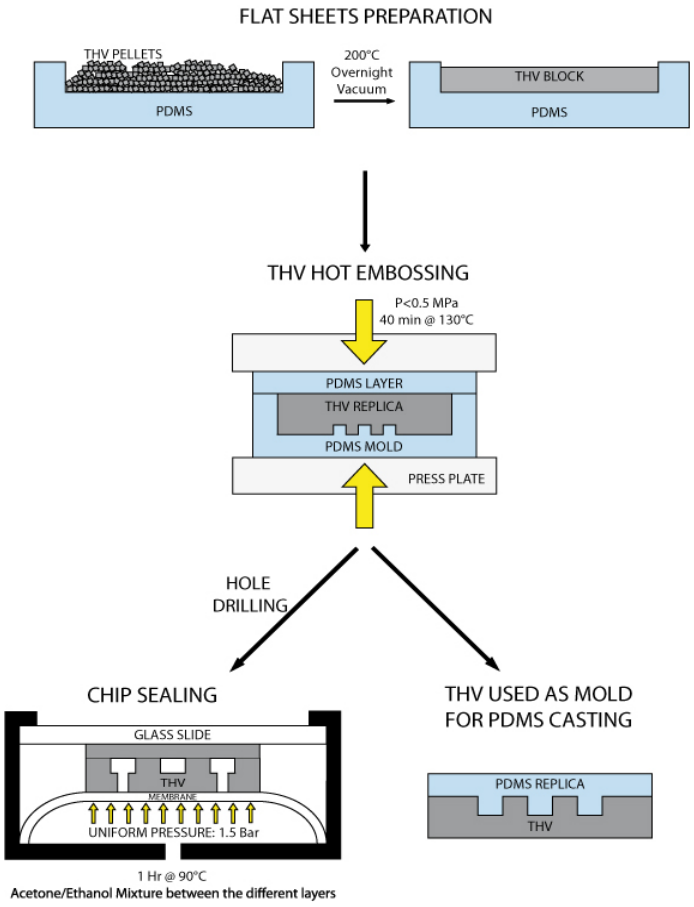

Figure 1: Fabrication technique for the production of THV 220 microchips.

The fabrication of microchannels was performed by hot embossing (Fig. 1) in a heated hydraulic press (Specac). We prepared PDMS molds by soft lithography, using single or double casting of traditional masters made by silicon wafers and SU-8 (Microchem) or SIPR positive resist (Shin Etsu MicroSi). The temperature chosen was slightly above the polymer melting temperature Tm (130°C for THV 220 and 180°C for THV 500), allowing us to apply very low pressures (less than 0.5 MPa) and thus reducing mold deformations during the embossing step. Since PDMS is permeable to air, no bubble can be trapped between the structures and the polymer. After 30 minutes the press was cooled at room temperature, and finally the pressure was released. 1.4mm via holes for connections were then produced using a driller (Proxxon).

The sealing step is performed using a combination of pressure, solvents and heat. A mix of poor (ethanol) and good

(acetone) solvent is poured between the two layers to be sealed. Uniform pressure is then applied through a flexible membrane, as shown in Fig. 1, in a configuration similar to that of nano-imprint lithography. The stack was then put inside a oven at 90°C for 1 hour, and pressure was released only after reaching room temperature. Chips were then left overnight before use to allow complete sealing.

MICROCHIP CHARACTERIZATION

We characterized the embossed microstructures by Scanning Electron Microscopy (SEM) and optical profilometry. Metallization was required prior to SEM analysis, in order to obtain a thin conductive layer (few nm) on the polymer structures. In Fig. 2 some structures are shown. In particular, four geometries were tested: the first one is a flow focusing device used for cell encapsulation in droplets [8], and consists of a chip with rectangular microchannels (height 20µm, variable widths ranging from 60µm to 1mm); a second geometry consists of an array of cylinders (height 2µm, width 7µm) that was used to test the resolution of our technique; a third one is a design usually applied for microchip electrophoresis, in which 50x50µm square microchannels form a cross; the last one consists of hemicircular channels with a radius of 150µm. The versatility of our technique was confirmed by the fact that we were able to mold structures with typical dimensions ranging from few millimeters down to 5 micron, with different geometries and channel sections (square, rectangular, semicircular).

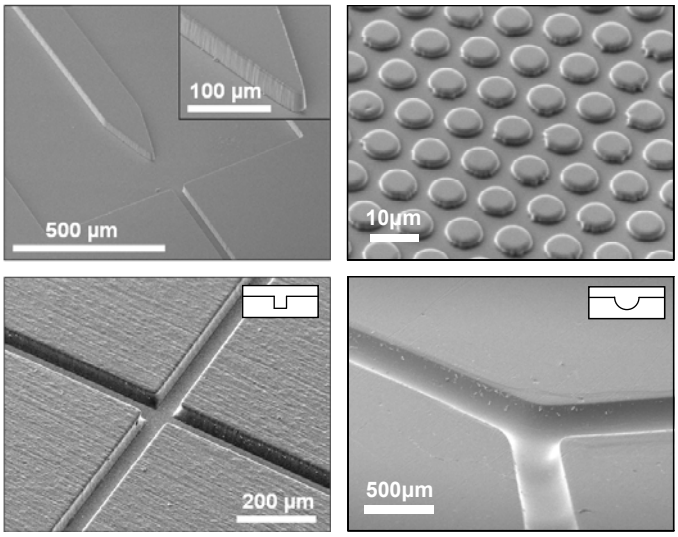

Figure 2: SEM micrograph of structures produced by hot THV hot embossing: flow focusing device with channels 20µm deep, array of dots (height 2µm, width 7µm), square channels (50x50µm) and hemicircular channels (R=150µm).

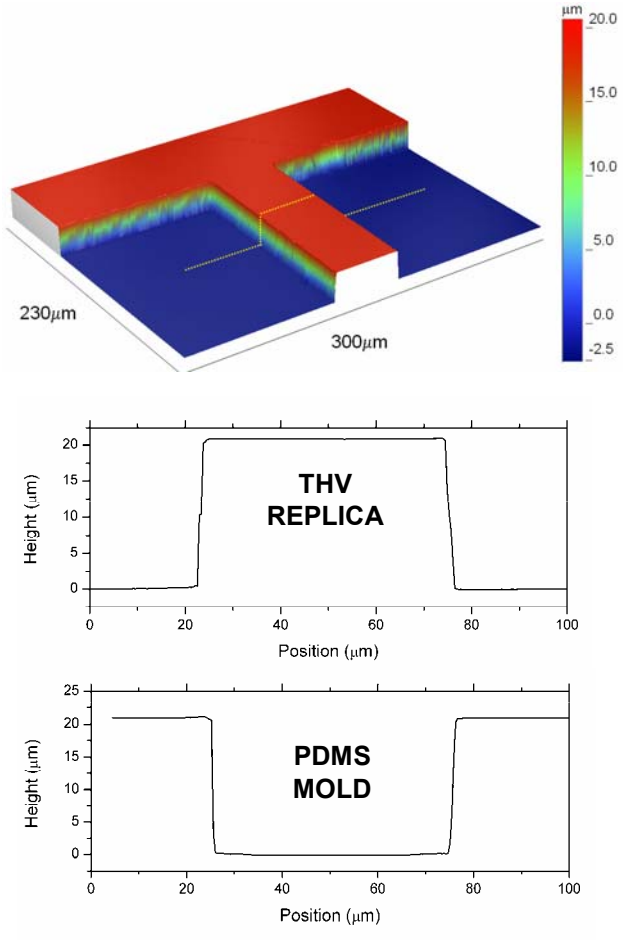

Figure 3: 3D Height profile of a structure mold on THV (height 21µm) and linear profiles taken on the yellow line. The second profile is taken from the PDMS mold used for the hot embossing.

Furthermore, these structures can be used to mold new PDMS sheets, allowing for the production of flexible molds that in routinely use can replace those made with fragile silicon wafers.

Optical profilometry was used to confirm that the structures were well transferred. In Fig. 3 a comparison between the PDMS mold and the THV replica of a microchannel is shown. The profiles show that shape is preserved. Analysis of several samples showed that the patterns are always well transferred. Difference in dimensions (width and height) between mold and replica was always below 4%.

The bonding step was first characterized by imaging of the channel cross section, which was accessible by cutting a sealed chip (Fig. 4). For bigger channels (at least one hundred micrometers) the cut was made with a razor blade, and the image was obtained using an optical microscope. In the case of smaller channels a cryo-cut step was necessary (working temperature -20°C) in order to reduce the deformations due to the cutting step. In this case images were taken with a SEM,

after metallization of the samples. The images show that a good bonding was achieved, with low deformation and no collapsing even for larger channels.

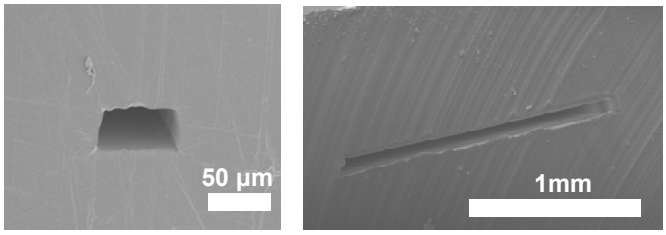

Figure 4: Micrographs of sealed THV chips with different dimensions (50x50µm and 200µmx1.5mm).

DROPLET CIRCULATION AND GENERATION

The wetting properties of the produced microchannels were tested by studying the circulation of distilled water or TAE slugs carried by fluorinated oil (FC-40, 3M) with 3% of surfactant (Perfluoro decan-1-ol, Fluorochem). The fluorinated polymer is also compatible with organic solvents (e.g. chloroform slugs in FC-40 oil can be easily produced in our chips). Fig. 5 shows a micrograph of a 100nL droplet circulating in a 300µm channel (speed=2mm/s, Ca~10^{-5}). The good wetting properties are confirmed by the symmetrical shape of the slug [3]. Slug trains remained stable over long distances (up to 1meter) and for few hours, showing no detectable change in volume or spacing over time.

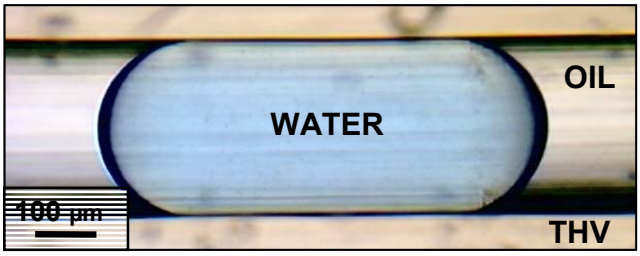

Figure 5: Micrograph of a 100nL droplet circulating in a 300 µm channel

Droplets were produced using the typical geometries used in microfluidic: flow focusing devices and T-junctions. Both the geometries were easily achievable using our fabrication technique. We used syringe pumps (Nemesys, Cetoni) to drive the liquids in the chips. Fig. 6 shows the high-throughput generation of droplets, in a flow focusing device. This configuration was previously applied for the encapsulation and sorting of single cells inside picoliter droplets [8] using mineral oil in a PDMS device. Our technique could be applied for extending this application to fluorinated oils. As previously mentioned, such oils show low affinity for biological molecules (reducing contamination between different droplets), but are permeable to oxygen, allowing for cells survival inside the drops [5].

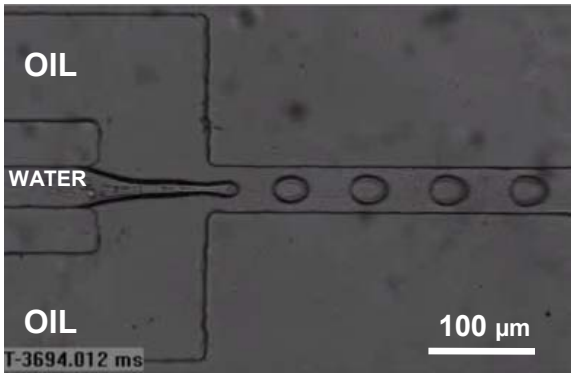

Figure 6: Generation of droplets in a Flow Focusing Device.

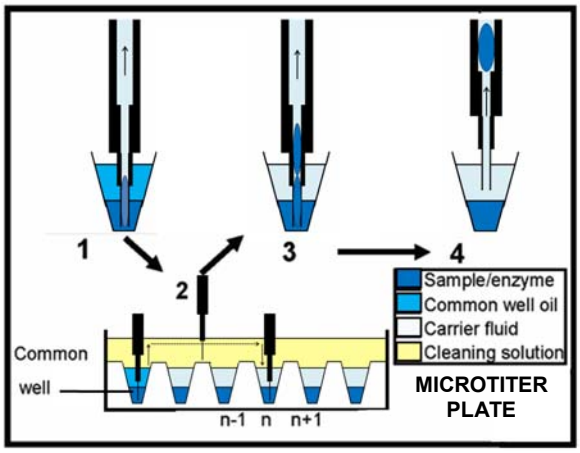

Figure 7: Schematic representation of slug formation method. Each slug is produced by combining a droplet of 2X PCR mix (stored in the common well) and a droplet of DNA solution (stored in well "n"). The following slugs can be produced using different DNA solutions (stored in wells "n+1", "n+2"…), allowing for the production of a train of independent reactors.

Our bonding step proved to be stable, as droplets can be continuously produced in our chips for several days at pressures in the order of 500 millibars, without detaching or leakage.

Alternatively, droplets or slugs can be generated elsewhere and brought inside the chip by drilling holes (1,6 mm diameter) and inserting rigid PFA tubing (OD=1,6mm, ID=0,25mm, Upchurch) to provide good connection. One possible application is the connection to the automated sampling system shown in Fig. 7 [4]. This setup integrates two syringe pumps (Nemesys, Cetoni) and a displacing system (Max) to produce droplets using the samples stored in a micro-titer plate. The main advantage of this approach is that it provides the possibility to control the composition of each slug separately.

This allowed the production of slug "trains" in which a different experiment can be performed in each slug, with no cross-contamination [3]. For capillary experiments thermal treatment is performed by using a heated cylinder, divided in three parts (each with a different temperature). A 10m long capillary is wrapped around the cylinder: plugs are pushed in the capillary, and every tour around the cylinder corresponds to a PCR cycle.

The produced microchips are comparable to capillary systems in terms of wetting properties, but they also assure a complete control in geometry and channel dimension, thus allowing for a possible reduction of slug volumes and in the miniaturization of the heating system.

FLUORESCENCE EXPERIMENTS

Fluorescence detection is one of the most common techniques for biological applications. The possibility to perform on-chip fluorescence detection is desirable, but for this the material constituting the chip should exhibit low autofluorescence.

As a first step we used a fluorescence microscope to observe slugs constituted of Fluoresceine (FITC) solution and circulating in our chips. Both excitation and emission wavelength of this dye are comparable to SybrGreen, a marker for DNA commonly used in PCR experiments. Droplets with a Fluoresceine concentration as low as 1µM were easily detectable. Fig. 8 shows a fluorescence image taken using a slug with 2,5µM of Fluoresceine. In our case the flow is segmented and the carrier oil has negligible autofluorescence. We attributed the low fluorescence signal visible in the intensity profile of the oil region to autofluorescence of the polymer in the upper and lower layers of the chip. The detection limit could be thus lowered by reducing the height of the detection zone (e.g. by confocal microscopy) and/or using the oil as a reference for the fluorescence signal.

CONCLUSIONS

We presented a simple, versatile, low cost and rapid technique for the production of monolithic microfluidic chips made of Dyneon THV. To our knowledge this is the first time that a thermoplastic fluoropolymer has been used for microfluidic applications. This technique is well adapted for rapid prototyping as the fabrication time of one chip is below one day and could be easily extended to mass production.

The produced chips showed good stability and reproducibility, allowing for the production and circulation of aqueous droplets in fluorinated oil with no wetting of the microchannel walls. The material autofluorescence is low enough to allow for fluorescence imaging of droplets circulating in the microchannels. These properties open the road to the integration of our previous DNA amplification platform in a microfluidic chip. Future experiments will be focused on the study of the lower detection limit for DNA in slugs and then on the performance of on-chip PCR amplification in sub-microliter slugs.

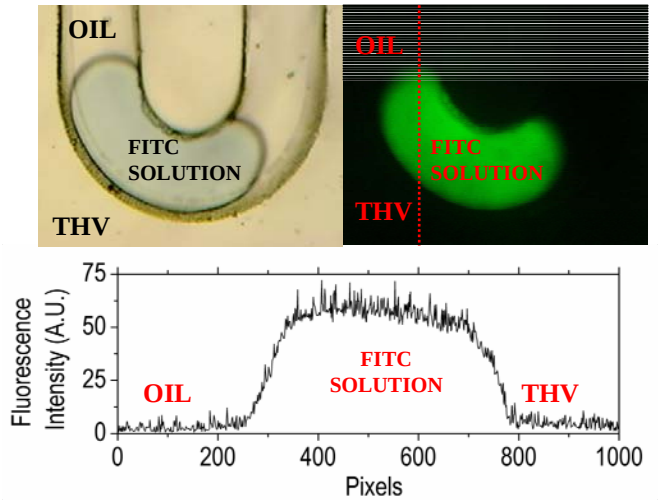

Figure 8: Transmission and fluorescence image of a 2.5µM Fluoresceine (FITC) solution in water. The intensity profile shows the signal along the red dashed line.

Moreover, our technique allows for other applications: the production of flexible molds for PDMS casting and the encapsulation of single cells in droplets using fluorinated oil.

We thus believe that this technology will find numerous applications in microfluidic systems for biology and chemistry.

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