## Chapter 5

# Solvent-Resistant Perfluoropolyether (PFPE) Microfluidic Devices

## 5.1 Introduction

Poly(dimethylsiloxane) (PDMS) has rapidly become the material of choice for many microfluidic device applications due to its numerous attractive properties [218, 272, 188, 268, 170], as discussed in Chapter 2. Upon crosslinking, it becomes an elastomeric material with a low Young's modulus of about 750 kPa [272]. This enables it to conform to surfaces and form both reversible and irreversible seals. These properties have enabled the fabrication of very simple but effective microvalves that can be densely integrated to produce extremely sophisticated devices [272, 268]. PDMS has a low surface energy, usually around 20 erg/cm<sup>2</sup>, which facilitates easy release from molds after patterning [218, 188]. Another important feature of PDMS is its high gas permeability. This allows trapped air within channels to permeate out of the device (e.g., for blind filling) and is also useful for sustaining cells and microorganisms inside channels, or for evaporating solvents.

Many current PDMS devices are based on Sylgard 184 (Dow Corning, Midland, MI), which cures thermally through a platinum-catalyzed hydrosilation reaction. With this material, complete curing

Text of Sections 5.1 and 5.2 in this chapter copyright the American Chemical Society, 2004. Originally published in [230]. Used with permission.

can take up to 5 hours. To expedite this process, Rogers *et al.* have recently reported the synthesis of a photocurable PDMS material with mechanical properties similar to that of Sylgard 184 for use in soft lithography [42]. This material cures through the use of free radical photoinitiators in just a few minutes, yet still has a long pot life.

Despite the advantages of PDMS for microfluidics technology, this material suffers from a serious drawback in that it swells in most organic solvents (Chapter 3). Swelling can lead to plugging of microchannels or device delamination, and other incompatibilities with solvents can lead to contamination of reactions and assays or to device damage. Instead of reverting back to devices made from inert but rigid materials such as silicon and glass [160] with their numerous drawbacks, it would be preferable to fabricate devices from a solvent-resistant elastomer with PDMS-like properties.

In a collaboration with DeSimone's research group at the University of North Carolina (UNC), we have taken this approach and replaced PDMS with perfluoropolyethers (PFPEs). PFPEs are a unique class of fluoropolymers that are viscous liquids at room temperature prior to curing, and that exhibit low surface energy, low modulus, high durability and toughness, high gas permeability, and low toxicity, with the added feature of being extremely chemically resistant [237]. They are highly fluorinated polymers with the chemical unit  $-(CF_2CF_2O)_m-(CF_2O)_n-$ . DeSimone *et al.* have reported extensively on the synthesis and solubility of PFPEs in supercritical carbon dioxide [29]. These materials have the potential to greatly extend the use of microfluidic devices to a wide variety of new chemical applications. With the added advantage of photocuring capability, the production time of microfluidic devices can be reduced from several hours to a matter of minutes.

With materials designed at UNC specifically for microfluidic device applications, we demonstrated the first fabrication of devices based on photocurable PFPEs. The next section describes the preparation and characterization of first-generation PFPEs and our success in fabricating solventresistant microfluidic devices with functional valves. This served as a proof-of-principle, from which further generations of material were evolved to improve the robustness of fabrication and the reliability of device operation. These improvements are described in subsequent sections. Based partly on the successes described herein, DeSimone and others recently founded a company, Liquidia Technologies (Morrisville, NC), to manufacture these materials and develop, among other things, microfluidic applications.

## 5.2 **Proof of principle**

#### 5.2.1 Materials synthesis and characterization

The synthesis and photocuring of these materials (Figure 5.1) is based on earlier work done by Bongiovanni *et al.* [217]. The reaction involves the methacrylate-functionalization of a commercially available PFPE diol ( $M_n = 3800 \text{ g/mol}$ ) with isocyanato-ethyl methacrylate. Subsequent photocuring of the material is accomplished by blending it with 1 wt% of 2,2-dimethoxy-2-phenylacetophenone (DMPA) and exposing it to UV radiation ( $\lambda = 365 \text{ nm}$ ).



Figure 5.1: Synthesis and crosslinking of photocurable PFPEs. PFPE diol is reacted with isocyanato-ethyl methacrylate to form a PFPE dimethacrylate (PFPE DMA) that can subsequently be cured into an elastomeric material. Polymerization occurs by free-radical polymerization when PFPE DMA is mixed with 1 wt% 2,2-dimethoxy-2-phenylacetophenone and exposed to UV light. (Reproduced from [230] with permission. Copyright the American Chemical Society, 2004.)

To evaluate solvent resistance, tests using classical swelling measurements [234] were performed on both the crosslinked PFPE DMA and Sylgard 184. Sample weight was compared before and after immersion in dichloromethane for several hours. The data show that after 94 h the PDMS network had swelled to 109% by weight, while the PFPE network showed negligible swelling (< 3%). Using the same technique, we quantitated PFPE swelling in a wide variety of additional solvents (Table 5.1).

Solvent	Swelling $(wt\%)$ after 7 days	Comment
acetone	4.1	
acetonitrile	2.0	
ammonium hydroxide $(27\%)$	0.4	
chlorobenzene	2.6	
chloroform	8.3	spotting on surface
cyclohexane	1.6	
dichloromethane	< 3	data from [230]
diisopropylamine	2.3	darkened in colour
dimethylformamide	4.2	
dimethyl sulfoxide	2.3	data from 3-day measurement
ether	3.6	
Fluorinert FC-75	66.3	
formaldehyde	0.7	
hexane	0.8	
isopropanol	2.3	
nitric acid $(70\%)$	4.5	
pyridine	5.2	slight discolouration
sulphuric acid $(96\%)$	10.3	surface turned black
tetrahydrofuran	5.3	
toluene	2.0	
trichloroethylene	6.7	spotting on surface
triethylamine	2.6	darkened in colour
xylene	1.7	

Table 5.1: **Swelling of PFPE in various solvents**. Swelling was quantified by performing classical immersion tests. Most solvents do not swell PFPE significantly, except for perfluorinated solvents such as Fluorinert FC-75. A few cause discolouration, suggesting that more in-depth studies of the interaction must be conducted if those solvents are to be used in devices.

The PDMS and PFPE precursor materials and the fully cured networks have similar processing and mechanical properties. Rheology experiments showed the viscosity of the uncured PFPE DMA at 25°C to be 0.36 Pa·s, which is significantly lower than that of 3.74 Pa·s for the uncured Sylgard 184. However, because both materials are sufficiently viscous oils at room temperature, standard PDMS device fabrication methods can be employed.

Dynamic mechanical thermal analysis (DMTA) was performed on the fully cured materials. Both the PFPE and PDMS networks exhibited low temperature transitions ( $-112^{\circ}$ C and  $-128^{\circ}$ C respectively) as evidenced by maxima in the loss modulus E'' (Figure 5.2). This transition accounts for the similar elastic behavior of the two crosslinked materials at room temperature.



Figure 5.2: Dynamic mechanical thermal analysis of PDMS and PFPE. DMTA traces of crosslinked PDMS and PFPE materials showing maximum in the storage modulus as a function of temperature. (Reproduced from [230] with permission. Copyright the American Chemical Society, 2004.)

Static contact angle measurements were made on both elastomers (Table 5.2). The PFPE DMA elastomer showed a higher contact angle than Sylgard 184 for water and methanol. Toluene and dichloromethane instantly swelled Sylgard 184 on contact, which prevented measurements to be taken. However, values for these solvents were obtained for the PFPE DMA material, as no swelling occurred.

Material	water	methanol	toluene	dichloromethane
PFPE-DMA	107	35	40	43
Sylgard 184	101	22	-	-

Table 5.2: Static contact angles for PFPE and PDMS. All values are in degrees. Note that measurements were not possible for droplets of toluene and dichloromethane on PDMS due to swelling.

#### 5.2.2 Device fabrication

Device fabrication was accomplished according to the procedure illustrated in Figure 5.3. This method was first reported by Quake *et al.* for PDMS devices and utilizes partial curing techniques to adhere the two layers without compromising feature sizes [272]. The PFPE DMA material was easily spin-coated and molded in a similar manner to Sylgard 184. As a result of viscosity differences, spin-coating rates used in PFPE device fabrication were lower than those employed for

PDMS materials (800 RPM vs. 2000 RPM). Channels in the thick layer were 50  $\mu$ m × 100  $\mu$ m while channels in the thin layer were 12  $\mu$ m × 100  $\mu$ m. Devices exhibited superior adhesion between layers than between the device and the substrate—thus, we fabricated devices with push-down valves due to the lower pressure requirements at the device-substrate interface in this architecture.



Figure 5.3: **PFPE device fabrication procedure**. (a) A small drop of PFPE DMA containing 1 wt% DMPA is spin coated onto a patterned silicon wafer to a height of 20  $\mu$ m. Separately, a thicker layer (roughly 5 mm) is formed by pouring PFPE DMA containing 1 wt% DMPA into a temporary PDMS barrier mold surrounding a patterned wafer. Both wafers are then exposed to UV light until barely solidified. (b) The thick layer is peeled from its wafer and aligned on top of the thin layer. The entire device is then exposed to UV light to adhere the two layers together. Later generations of PFPE material involved complete curing of the two layers followed by a distinct adhesion step. (c) The device is peeled from the wafer and adhered to a substrate for microfluidic operation. (Reproduced from [230] with permission. Copyright the American Chemical Society, 2004.)

To compare the solvent compatibility of devices made from the two materials, a solution containing dichloromethane, acetonitrile, methanol, and the blue dye xylene cyanol FF was introduced into both a PFPE and a PDMS channel by capillary action (Figure 5.4). The PFPE channels showed no evidence of swelling as the solution traveled easily through the channel. A pronounced reverse meniscus was observed indicating good wetting behavior. In contrast, no solution entered the PDMS device because the channel was plugged shut when it made contact with the droplet. As a control, a dyed methanol solution was easily introduced into the PDMS channel in the same manner.

Actuation of valves was accomplished by introducing pressurized air (about 25 psi) to small holes that were punched through the thick layer at the beginning of the channels. When dyed solvent was present in the channel, valve actuation was easily observed (Figure 5.5). While fluid easily flows into channels due to capillary action, it was necessary to pressurize fluid channels to at least 4–5 psi



Figure 5.4: **PFPE microchannels are not swelled shut by solvents**. (a) Dyed solution of methylene chloride, acetonitrile, and methanol entering the end of a microchannel that is open to the side of a PFPE device. (b) This solution did not enter a PDMS channel of the same size due to swelling. (c) As a control, dyed methanol (which does not swell PDMS) does enter the same PDMS channel. (Adapted from [230] with permission. Parts *a* and *b* copyright the American Chemical Society, 2004.)

to eliminate air bubbles that otherwise form due to the very high wettability of the PFPE channel surfaces (Figure 5.6). The CAD design and photographs of the test devices are shown in Figure 5.7.

Solvent resistance enables a wide variety of chemistry to be performed inside microfluidic devices. Figure 5.8 shows the design and fabrication of a prototype chip that could be used for solid phase combinatorial array synthesis on a glass surface. For example, a combinatorial DNA array could be synthesized. Unlike PDMS, PFPE is compatible with all solvents involved in DNA synthesis reactions. The channel features in this device design were widely spaced to simplify layer alignment.

#### 5.2.3 Methods

#### 5.2.3.1 Materials

Poly(tetrafluoroethylene oxide-*co*-difluoromethylene oxide)  $\alpha, \omega$ -diol (ZDOL, average M<sub>n</sub>  $\approx$  3800 g/mol, 95% Aldrich), 2-isocyanatoethyl methacrylate (EIM, 99% Aldrich), 2,2-dimethoxy-2-phenyl acetophenone (DMPA, 99% Aldrich), dibutyltin diacetate (DBTDA, 99% Aldrich), and 1,1,2-trichlorotrifluoroethane (Freon 113, 99% Aldrich) were used as received.

104



Figure 5.5: Microvalve actuation in PFPE microchannels containing solvents. (a) Top-down view of channels containing no solvent. The channels in the thin layer (to carry fluid) run top to bottom, while those on the thick layer (to carry pressurized air) run left to right. (b) Thin layer channel filled with dyed solution of acetonitrile, methylene chloride, and methanol. The thick layer (control) channel is unpressurized, and the valve is open. (c) Valve actuated by introducing 25 psi of air into the control channel. Beneath each photograph, a drawing of the valve cross-section is shown. (e,f) Photographs of additional valves of different dimensions with solvents present in the fluid channels. Valves closed at 12–14 psi could generally block fluids pressurized to 4 psi. Increasing both pressures results in faster response times during closing and re-opening. (Adapted from [230] with permission. Parts a, b, and c copyright the American Chemical Society, 2004.)



Figure 5.6: Extreme wetting leads to air bubbles in fluid channels. In this sequence of five video frames, fluid is being pushed from left to right under low pressure (< 5 psi). The control channel (oriented top to bottom) is unpressurized. Due to the extreme wetting of PFPE by dichloromethane (and many other solvents), fluid prefers to flow in narrower parts of the channel. In frame 2, as the leading edge of the meniscus reaches the valve, it pulls the valve membrane downward (towards the fluid channel), causing a slight constriction of the fluid channel. Fluid prefers to collect here, bypassing an air bubble that forms upstream of the valve. In frames 3–5, the air bubble expands to the left, even though the net flow of fluid is still to the right. Only by flowing fluid at sufficient pressures to deflect the valve membrane *away* from the fluid channel can this be prevented. Similarly, flushing out channels with air is difficult as the air tends to flow down the middle (tallest) part of the channel, leaving stagnant fluid at the sides.



Figure 5.7: Valve test pattern used to evaluate PFPE valves. (a) CAD design for valve size test pattern. A series of parallel fluid channels (blue) ranging from 124–250  $\mu$ m in width is controlled by a series of control channels (red) in the perpendicular direction with the same size range. (A previous design used a wider range of channel widths, 30–500  $\mu$ m, but the largest channels tended to collapse, and poor wetting of such wide photoresist lines by the liquid PFPE precursor led to fabrication defects in the thin layer.) At the right, images of actuated valves are shown (12 psi control pressure; no fluid) for each valve in the column where the control channel is 200  $\mu$ m wide. (b) Top-view photograph of the PFPE device fabricated from this pattern with red and blue food colouring filling the control and fluid channels respectively. Note that the control channels are dead-end channels while the fluid channels have ports at both ends. In this particular device, one control channel had a defect and could not be filled. (c) The same device viewed from a different angle to illustrate how thin (2 mm) these devices were (to conserve material during testing). It is mounted on a 2×3 inch glass slide. (d) Photograph of the device during valve testing on a stereoscope.



Figure 5.8: Design of a primitive combinatorial array synthesizer. (a) CAD design of a  $4 \times 4$  array synthesizer based on the solid-phase combinatorial synthesis principle introduced by Southern et al. [248]. The device is designed to synthesize all 2-mers of DNA on a glass surface, for example, though it can also be used for other combinatorial chemistry applications. Combinatorial synthesis proceeds by first flowing each of the four nucleotides in a dedicated row to couple single nucleotides to the surface in a stripe pattern. Next, each of the four nucleotides is flowed in a dedicated column, forming 2-mers where the columns intersect the originally synthesized row stripes. All 16 possible DNA 2-mers are synthesized at the 16 intersections. The device is designed to implement this technique as follows. Pressurizing the row flow selector closes a set of valves that allows flow in fluid channels (blue) only in the horizontal direction. Each of the four nucleotides is fed into the row fluid inlet in succession. The row/column valves are configured such that the nucleotide flows along the single desired row in each case. Similarly a bank of valves switches the flow path to the column direction for introducing reagents in the second step. (The design and details of operation are described in significantly more detail in Chapter 7.) The device has a push-down valve architecture so that the contents of fluid channels are in direct contact with the glass substrate, where the coupling reactions occur. In the inset is a micrograph of a portion of a PFPE device corresponding to the region inside the green square. (b) Photograph of a PFPE combinatorial synthesis chip. (c) Photograph of PFPE device mounted on a glass slide with off-chip connections in place. (d) Micrograph of corner of PFPE chip. The fluid channels have been filled with a blue dyed solution of dichloromethane, acetonitrile, and methanol.

#### 5.2.3.2 Preparation of PFPE DMA

In a typical synthesis, ZDOL (5.7227 g, 1.5 mmol) was added to a dry 50 mL round bottom flask and purged with argon for 15 min. EIM (0.43 mL, 3.0 mmol) was then added via syringe along with Freon 113 (2 mL) and DBTDA (50  $\mu$ L). The solution was immersed in an oil bath and allowed to stir at 50°C for 24 h. The solution was then passed through a chromatographic column (alumina, Freon 113, 2×5 cm). Evaporation of the solvent yielded a clear, colourless, viscous oil that was further purified by passage through a 0.22  $\mu$ m polyethersulfone filter. 1H-NMR (ppm): 2.1, s (3H); 3.7, q (2H); 4.4, t (2H); 4.7, t (2H); 5.3, m (1H); 5.8, s (1H); 6.3, s (1H).

#### 5.2.3.3 Photocuring of PFPE DMA

In a typical cure, 1 wt% of DMPA (0.05 g, 2.0 mmol) was added to PFPE DMA (5 g, 1.2 mmol) along with 2 mL Freon 113 until a clear solution was formed. After removal of the solvent by vacuum degassing, the cloudy viscous oil was passed through a 0.22  $\mu$ m polyethersulfone filter to remove any DMPA that did not disperse into the PFPE DMA. The filtered PFPE DMA was then irradiated with a UV source (Electro-Lite ELC-500 UV curing chamber,  $\lambda = 365$  nm, intensity: 24–28 mW/cm<sup>2</sup>) while under a nitrogen purge for 10 min. This resulted in a clear, slightly yellow, rubbery material. Prior to exposure, samples were left in the UV curing oven for at least 5 min under nitrogen purge. All generations of PFPE material were photocured in this manner.

#### 5.2.3.4 Device fabrication with PFPE DMA

In a typical fabrication, PFPE DMA containing photoinitiator (as described in Section 5.2.3.3) was spin coated to a thickness of 20  $\mu$ m (800 RPM) onto an untreated silicon wafer containing the desired photoresist pattern. This wafer was then placed into the UV curing chamber and irradiated for 6 s. Separately, a thick layer (roughly 5 mm) of the material was produced by pouring the PFPE DMA containing photoinitiator into a PDMS barrier mold surrounding the silicon wafer containing the desired photoresist pattern. This wafer was irradiated with UV light for 1 min. Following this, the thick layer was removed, and inlet holes were carefully punched in specific areas of the device. Punching was performed with a hole-punching press (Technical Innovations, Inc., Brazoria, TX), as holes punched manually with Luer stubs tended to have rough edges that did not completely seal around stainless steel tubing. The thick layer was then carefully aligned with and placed on top of the thin layer and the entire device was irradiated for 10 min. Once complete, the entire device was peeled from the wafer with both layers adhered together. Additional inlet holes were punched through the device for channels in the thin layer, and the device was sealed to a clean glass slide or slide coated with fully cured PFPE. The above curing times were determined to be the optimal exposure times to achieve a good balance between structure failure and proper adhesion of the two layers. It should be noted that the later generations of PFPE material employed a distinct bonding mechanism, such that curing and bonding processes were not intertwined in this way.

#### 5.2.3.5 Swelling experiments

Swelling experiments were performed by soaking fully cured PFPE DMA and fully cured Sylgard 184 (Dow Corning) in dichloromethane. Percent swelling was determined using the following equation:

% swelling = 
$$100\% \cdot \frac{(W_t - W_0)}{W_0}$$
 (5.1)

where  $W_t$  is the weight of the material after soaking in dichloromethane for time t (measured immediately after removing the sample from the solvent and patting the surface dry with a Kimwipe), and  $W_0$  is the original weight of the material. The same procedure was used to determine swelling of crosslinked PFPE DMA in other solvents.

#### 5.2.3.6 Rheometry

Viscosities of the two elastomer precursors (PFPE DMA and Sylgard 184) were measured on a TA Instruments AR2000 Rheometer. Measurements were made with approximately 3–5 mL of material. Measurements on the Sylgard 184 precursors were taken immediately after mixing the two components. The shear rate for Sylgard 184 was varied from  $0.03 \text{ s}^{-1}$  to  $0.70 \text{ s}^{-1}$ , and a constant viscosity was observed at all rates. The shear rate for PFPE DMA was varied from  $0.28 \text{ s}^{-1}$ 

to  $34.74 \text{ s}^{-1}$ , also resulting in a constant viscosity. Viscosities were obtained by taking an average of the viscosity values over all measured shear rates. The raw data for these experiments is shown in Figure 5.9.



Figure 5.9: Viscosity vs. shear rate for PFPE DMA and Sylgard 184 precursors. In both materials, viscosity is constant over the range of shear rates. A single viscosity value was computed for each by taking an average of the raw data here. (Reproduced from [230] supplementary information with permission. Copyright the American Chemical Society, 2004.)

#### 5.2.3.7 Dynamic mechanical analysis

Modulus measurements were taken on a Perkin Elmer DMA 7E Dynamic Mechanical Analyzer. Samples were cut into  $4 \times 8 \times 0.5$  mm (width×length×thickness) rectangles. The initial static force on each of the two samples was 5 mN, and the load was increased at a rate of 500 mN/min until the sample ruptured or the load reached 6400 mN. The tensile moduli were obtained from the initial slope (up to about 20% strain) of the stress/strain curves shown in Figure 5.2.

#### 5.2.3.8 Dynamic mechanical thermal analysis

Thermal transitions of the two elastomers were obtained on a Seiko DMS 210 Dynamic Mechanical Thermal Analyzer. Samples were cut into  $4 \times 20 \times 0.5$  mm (width×length×thickness) rectangles. The following settings were used: L<sub>amp</sub>: 10; minimum tension/compression force: -10.000 g; tension/compression correction: 1.2; and force amplitude: 100. The temperature sweep ranged from – 140°C to 50°C. T<sub>g</sub> values were obtained from the corresponding temperature at the maxima in a plot of E'' (loss modulus) vs. temperature.

#### 5.2.3.9 Contact angle measurements

Static contact angles were measured using a KSV Instruments, Ltd. CAM 200 Optical Contact Angle Meter. Droplets were placed on each of the fully cured elastomers using a 250  $\mu$ L screw-top syringe.

## 5.3 Improvements in mechanical properties

The first-generation PFPE polymer exhibited many of the desirable properties of PDMS including its elasticity, modulus, and precursor viscosity. However, it was somewhat brittle. Cracks were created during the hole punching step, preventing complete sealing of inserted stainless steel tubing connectors. In addition, devices were fragile and difficult to handle as bending the PFPE too far resulted in cracking or breaking. Furthermore, the material exhibited poor elongation (< 20%), making it unsuitable for push-up valves that, depending on channel dimensions, can require the membrane to be stretched much further.

We initially devised engineering solutions to these issues. For example, droplets of PFPE cured around inserted inlet pins acted as a sealant to create air-tight connections (see Figure 5.10). However, modification of the polymer material itself was a more flexible and effective long term solution. Rolland *et al.* synthesized a PFPE mono-methacrylate (PFPE MMA) to be mixed with PFPE DMA to reduce the crosslink density in the final polymer. A 1:1 mixture of these two monomers yielded polymers with significantly improved flexibility and toughness, yet sufficient rigidity to prevent collapse of channel features. Punching holes in the new material seldom resulted in crack formation.

## 5.4 Improvements in device bonding

In the construction of 2-layer microfluidic devices there are two bonding interfaces—between layers and between the device and the substrate. Each generation of material synthesized by Rolland *et al.* 



Figure 5.10: **PFPE sealing of off-chip connections**. First-generation PFPE devices suffered from cracking near the inlet holes during punching that resulted in fluid or air pressure leaks. These cracks were sealed by depositing and curing droplets of PFPE after inserting stainless steel tubing. Due to the thinness of early devices (typically 2 mm, to conserve PFPE), the tubing could easily be dislodged by normal handling thus breaking the seals. One solution to this problem was to encapsulate the entire device in a thick layer of PDMS. Although PDMS does not adhere to PFPE, the layer holds tubing firmly in place, extending the life of the seals and thus the devices.

was developed to evaluate a new chemical bonding strategy. I discuss the evolution of bonding at both interfaces.

#### 5.4.1 First-generation PFPE

#### 5.4.1.1 Layer bonding

Bonding of devices fabricated from first-generation PFPE was achieved by a delicately balanced partial curing technique. The thin layer was undercured by using a very short UV exposure such that a very thin liquid layer remained on the upper surface to act as a glue. The thick layer was cured the minimum time necessary until it solidified. If either layer was cured for too long, we found the bonding to be very weak, and devices quickly delaminated at relatively low pressures (2–10 psi). Undercuring too severely resulted in an excessive amount of liquid between layers that clogged channels. To a certain extent, the clogging could be reduced by baking at 80°C for several minutes prior to the UV bonding step. Heating reduced the viscosity, allowing the excess material to spread out along channels, therefore reducing the accumulation at any one point.



Figure 5.11: Adjusting crosslinking density of PFPE. (a) Simplified structure of PFPE DMA, a long-chain molecule containing a methacrylate group (represented by a double line) at each end, and PFPE MMA, the same molecule with only a single methacrylate. At the far right is a further simplified view of each molecule: imagine each circle to be a coiled-up polymer chain. (b) Schematic of the formation of a PFPE MMA network. During the polymerization process, methacrylate groups are joined together to form a linear polymer (with PFPE as a side group). The final elastomer material is a tangle of these individual chains. When using di-functional PFPE DMA, one can imagine a similar picture but with the *other* ends of each PFPE group interconnected in complex ways to form a highly crosslinked network. (Adapted from the web, http://emu0.emu.uct.ac.za/EMforBiologists/lecture2/Lecture-2.htm.) (c) Mixing both PFPE MMA and PFPE DMA results in an intermediate polymer with greater average distance between crosslinks when compared with pure PFPE DMA. The material is more flexible as a result. (Adapted from Isaure *et al., Journal of Materials Chemistry* 13(11), 2701–2710, 2003. Copyright the Royal Society of Chemistry, 2003.)

The optimal curing time for the thin layer was determined to be 6 s. However, it was difficult to precisely reproduce this exposure time in our curing oven due to a relatively long bulb ignition delay and a timer that could be adjusted only in 0.1 min increments. The large uncertainties in timing led to poor reproducibility of layer-layer adhesion. To reduce the *relative* error, we increased the time needed for curing by reducing the UV exposure intensity. Two of the four UV lamps were removed, and UV absorbers such as platic sheets were inserted into the light path. However, even with improved exposure time accuracy, optimal bonding was not consistently achieved. Batch-tobatch and even day-to-day variation in quality was observed. The lack of repeatability resulted in a low yield of functional microfluidic devices.

Furthermore, we realized that even the optimal bond achieved by this technique is a purely mechanical one. Invariably, 2-layer devices could be peeled apart without damage to either surface, suggesting that devices would be likely to eventually fail under normal operating conditions. We suspected that adhesion was due primarily to van der Waals interactions between the two smooth surfaces and also to mechanical interactions between the thick layer features and the thin liquid layer that cures closely around it. The lack of chemical bonding can also be demonstrated simply by curing some prepolymer on a fully-cured layer: it seals, but the layers can be peeled cleanly apart.

In an initial attempt to achieve chemical bonding, we treated samples with FluoroEtch (Acton Technologies, Inc., Pittston, PA), a chemical that strips fluorine atoms from Teflon and other uncrosslinked fluorocarbon polymers [265]. Once stripped, surface groups reorganize into a variety of functional groups that are capable of bonding to conventional adhesives such as epoxy (or perhaps to one another). Though PFPE is crosslinked and did not visibly respond to treatment as Teflon does, a change in surface-wetting properties was observed after immersion for several hours at 55°C. We chose not to use conventional adhesives for layer bonding due to their susceptibility to chemical attack, but did observe that treatment of the thick layer with FluoroEtch led to qualitatively improved bonding to the partially cured thin layer.

#### 5.4.1.2 Substrate bonding

Initial PFPE devices were simply sealed to a clean glass slide or to a slide coated with fully cured PFPE. The amount of adhesion provided in this manner was sufficient to pressurize fluid channels up to about 5 psi before delamination of the device from the substrate.

Eventually, a superior substrate adhesion method based on partial curing was developed. A two-layer device was fabricated as described in Section 5.2.3.4 with the exception that the final UV exposure to bond the layers together lasted only 5 min instead of 10 min. Next, PFPE was spin coated (800 RPM) onto a cleaned glass slide and cured for approximately 3 s under a nitrogen purge. After punching holes in the 2-layer device, it was placed on the coated slide and allowed to sit for 5 min. Bonding was completed by a 10 min UV exposure of the whole device. Typically, the bond strength was improved compared to simply sealing the device to the substrate; however, it was typically less than the strength of the first layer-layer bond.

Due to the relative weakness of the device-substrate bond, we generally fabricated push-down devices. This valve architecture exposes that bonding interface to less pressure than the layer-layer interface during device operation (see Section 2.3.1).

#### 5.4.2 Second-generation PFPE

#### 5.4.2.1 Layer bonding

Later generations of materials developed by Jason Rolland *et al.* at UNC incorporated functional moities specifically for chemical bonding between layers. In second-generation PFPE, the prepolymer mixture in each layer was "doped" with 5–10% of PFPE derivatives with different end groups: isocyanate and hydroxyl. These dopants, poly(tetrafluoroethylene oxide-*co*-diffuoromethylene oxide)  $\alpha, \omega$ -diisocyanate (PFPE-A) (average M<sub>n</sub>  $\approx$  3000) and poly(tetrafluoroethylene oxide-*co*-diffuoromethylene oxide)  $\alpha, \omega$ -diol (PFPE-B) (average M<sub>n</sub>  $\approx$  3800), were originally synthesized at UNC but in later experiments were purchased from Aldrich. The hypothetical bonding mechanism is illustrated in Figure 5.12. PFPE-A and PFPE-B do not participate in the UV curing process, instead becoming entangled in the elastomer matrix as it cures. A certain fraction will display functional groups near the PFPE surface. When two cured polymers (one containing PFPE-A, the other containing PFPE-B) are brought into contact under heated conditions (120 °C), hydroxyl and isocyanate groups react, forming covalent bonds between the two layers, joining the layers by "polymer stitching". In fact, two layers containing PFPE-A should be bondable due to the reaction between isocyanates.



Figure 5.12: Chemical layer-bonding mechanism in second-generation PFPE. (a) Schematic of layer-bonding procedure between two PFPE polymers. One contains a small fraction of PFPE-diisocyanate (PFPE-A) (top, red), while the other contains a small fraction of PFPE-diol (PFPE-B) (bottom, blue). Groups at the surface react with one another to covalently bond chains in one layer with those in the other to stitch the two polymers together. One might expect superior bonding if the dopants were bifunctionalized such that one end would be covalently linked to the PFPE elastic network while the other participated in interlayer bonding. (b) Reaction of hydroxyl group with isocyanate group.

The revised device fabrication procedure was as follows. Both the thin and the thick layers were prepared as usual, except that each was *fully* cured by a 10 min UV exposure with a nitrogen purge. After removal of the thick layer from its mold, holes were punched, and the layer was aligned to the thin layer still affixed to its mold. Baking for 2 h at 110–120°C provided sufficient adhesion that the 2-layer device could be peeled from the mold for hole punching.

#### 5.4.2.2 Substrate bonding

Substrate bonding was hindered by two effects. First, the 2-layer device developed a substantial curvature (upward at the edges) after the 2 h baking step. We attempted to use weights to flatten

the device during subsequent processing. Second, the 2 h bake seemed to remove all bonding capability from the bottom layer. Even when sealed onto a freshly cured coated glass slide and baked up to 48 h at 120°C, no bonding occurred. Typically we attempted to fabricate devices as a B-A-B/A sandwich, such that the thick layer was doped with PFPE-B, the thin layer with PFPE-A, and the glass coating with PFPE-B or PFPE-A.

In another approach we sought to bond devices to uncoated glass. PFPE-A provides isocyanate groups that should in theory allow a doped PFPE sample to bond to a variety of substrates including glass derivatized with hydroxyl, epoxide, or amine groups. Some of these substrates are ideal for microfluidic applications involving *in situ* solid-phase synthesis of DNA or peptides (using a push-down valve architecture). However, numerous experiments did not result in successful bonding to these surfaces, even with PFPE-A samples that had not previously been baked. In a typical experiment, a sample of UV cured PFPE-A was placed on a clean, dry, derivatized substrate and baked at 120°C overnight or longer with one or two glass slides placed on top as weights. We observed that the sample could easily be peeled from the substrate after cooling. Three types of commercial derivatized slides were tested: SuperAmine and SuperEpoxy (TeleChem International, Inc., Sunnyvale, CA) and Xenoslide A (Xenopore Corp., Hawthorne, NJ). Glass slides that I treated with aminopropyltriethoxysilane (APTES) or N-(3-(triethoxysilyl)-propyl)-4-hydroxybutyramide were also evaluated (see Appendices A.2.5 and A.2.6, respectively).

Although channels in the upper layer (between the thick and thin layers) could routinely be pressurized up to 25 psi without delamination due to a strong initial bond, the lack of any adhesion of the device to the substrate allowed the push-down valves to overdeflect. As membranes deflected downwards, they weren't stopped by the substrate surface; rather, they continued deflecting, lifting the device from the surface in the process, until they ruptured.

#### 5.4.3 Third-generation PFPE

Third- and fourth-generation PFPE were developed after the formation of Liquidia Technologies Inc. and structures of these materials are proprietary. However, both continue to use a heat-activated bonding mechanism that is orthogonal to the UV curing (crosslinking) process. Unlike the secondgeneration material, the third and fourth use an identical material composition in all device layers.

The device fabrication protocol was identical to that for second-generation PFPE. Layer-layer bonding reliably withstood 20–30 psi actuation pressure under static conditions. When valves were actuated repeatedly, however, layers were observed to delaminate rapidly (see Figure 5.13) and would not be suitable for operation of actual microfluidic devices. We observed very significant batch-tobatch and day-to-day variation in bonding that was eventually attributed to phase separation that occurs over time and sometimes occurred during transport of material samples from UNC to Caltech. Usually filtering and blending with fresh photoinitiator restored the bonding capability.

It was found that bonding could be significantly improved by curing third-generation PFPE in *liquid* form onto an already cured layer and baking at  $120^{\circ}$ C. Because our usual device fabrication process involves the bonding of two solidified layers, it was necessary to consider alternatives. One strategy is to cure a thin layer of PFPE on the fluid layer mold, then pattern a sacrificial material on top of that layer to define the pattern of channels in the second layer. Liquid PFPE is then poured over this sacrificial material and cured, and the sacrificial material is removed. To test this idea, I patterned SPR 220-7 photoresist (Shipley) on top of a cured PFPE layer. Spin coating, exposing, and developing were carried out according to the manufacturer's protocol and were observed to have no adverse effect on the existing PFPE layer. A PDMS mold barrier was placed around this pattern and filled with PFPE. The sample was exposed to UV light for 10 min to cure the top liquid layer, followed by baking at 120–130°C overnight. Unfortunately, the prolonged baking step hard-bakes the photoresist making it nearly impossible to remove. Immersion in a variety of organic solvents with sonication and heating for several days had almost no effect on the resist. Eventually, in some samples, valve intersections exhibited local delamination as if solvents had gradually accumulated underneath the PFPE. Fabrication in this manner might be successful if an alternative sacrificial material (e.g., wax, see Chapter 6) can be used.



Figure 5.13: **Delamination in third-generation PFPE devices**. (a,b) In an optimally bonded device, layers do not delaminate under static conditions. Shown here is a valve held in the closed state by a fixed pressure of 7 psi (top) and 30 psi (bottom). At high pressures, the first signs of delamination are evident as roughness at the edge of the pressurized control channel (clear channel). (Photographs courtesy Jason Rolland, Liquidia Technologies, Inc.) (c,d) In a non-optimally bonded device, the same signs of delamination are visble at lower pressures. Top: 0 psi; bottom: 8 psi. (e,f,g) Micrographs showing progressive delamination (peeling apart) when a valve pressure of 8 psi is oscillated on and off at about 1 Hz. The horizontal channel is an empty fluid channel. The vertical channel is a control channel in this push-down device. Note that the jagged-edged, rounded region in the middle of the valve intersection is simply a fabrication defect where the control channel partially collapsed. As the pressure is oscillated, the rough channel edges progressively expand in an intricate pattern as the layers gradually peel apart. In this case, complete separation of layers occurred in about 1 min.

#### 5.4.4 Fourth-generation PFPE

A fourth generation of material was synthesized recently by Liquidia and evaluated in the context of fabricating microfluidic chips for the synthesis of [<sup>18</sup>F]-fluoro-2-deoxyglucose (FDG) [159].

Materials were mixed in proportions as specified by Liquidia and degassed in a vacuum chamber. The thin layer mold and plain or aminated glass substrate are spin coated with PFPE and additional PFPE is poured into a PDMS gasket around the thick layer mold pattern. All three components are photocured by exposure to UV light for 20 min under a nitrogen purge. The cured thick layer is removed from the mold and holes are punched for inlets. The thick layer is then aligned to the thin layer (still affixed to its mold), taking care to ensure none of the channels are collapsed. (If not re-opened prior to the adhesion step, collapsed channels become permanently bonded shut.) The layers are bonded by baking for 20 min at 105°C. The two-layer device is then peeled from the wafer, additional holes are punched, and the device is placed on the coated glass. The entire device is heated at 105°C for 1 h to perform substrate bonding and to complete the first layer-bonding reaction. After this step, the material yellows slightly and the initial cloudiness disappears.

Athough theoretically possible to bond directly to aminated glass [229], attempts to do so by baking were unsuccessful. For now, this will preclude the use of PFPE devices for the class of applications involving *in situ* synthesis on glass.

Due to the high reactivity of fourth-generation PFPE with various photoresists, the mold for the thin layer (on which the material is baked for 20 min) must be passivated by sputtering a metal layer such as palladium-gold several nanometers thick. CYTOP or possibly parylene coatings could be used instead.

Mechanical properties have been dramatically improved in this material generation compared with previous ones. Increased flexibility and elongation permit hole punching without cracking and permit the fabrication of devices with push-up valves.

We fabricated partially functional microfluidic devices designed for FDG synthesis. Control channels withstood in excess of 60 psi of pressure exhibiting no signs of delamination either between layers or between the device and the substrate. Permeability was sufficient to perform dead-end channel filling and solvent evaporation. The material surface is quite tacky and valves are slow to reopen if fluid channels are empty. However, the presence of fluids allows valves to be actuated quickly (tens of Hz).

### 5.5 Summary

With novel PFPE elastomer materials designed and synthesized by Rolland *et al.*, we have demonstrated successful fabrication of microfluidic devices with integrated microvalves. Device fabrication and valve actuation were accomplished using established procedures for PDMS devices. Due to the properties of PFPE, these devices offer most of the same advantages as PDMS devices (see Chapter 2) with the added benefits of photocurability (which reduces fabrication time from hours to minutes) and remarkably high solvent resistance. By solving the solvent-resistance problem in elastomeric microfluidics, these new devices have the potential to expand the field to many novel applications and should be of great interest to anyone wishing to perform chemistry in microfluidic devices.

## Acknowledgment

I thank Jason Rolland and his colleagues at Liquidia and in Joe DeSimone's group at UNC for their tremendous contributions to this work. In particular, they were responsible for material design, synthesis, and characterization and were involved with the optimization of the device fabrication protocol. The supply of PFPE precursors (various generations) for performing bonding optimization experiments and device fabrication is greatly appreciated. I also thank Saurabh Vyawahare at Caltech for help with several experiments, in particular the solvent-resistance testing. Jason Rolland and Arkadij Elizarov fabricated and tested the fourth-generation chips discussed here.