Appendix A

Methods

A.1 Fabrication of microfluidic molds

Relief molds for microfluidic device casting were created by photolithographically patterning photoresist on silicon wafers or glass microscope slides.

A.1.1 Photomask preparation

Low resolution (3386 dpi) photomasks were printed on plastic transparencies at Mika Color Corp. (Los Angeles, CA). These masks are acceptable for simple designs, but printed features were found to “overflow” the designed boundaries by as much as 20 μm.

Designs with critical channel widths or closely spaced features were printed at CAD/Art Services, Inc. (Poway, CA) at a resolution of either 8000 or 20000 dpi, depending on accuracy requirements.

Transparency photomasks were adhered to flat borosilicate glass plates (Chemglass Inc., Vineland, NJ) with clear tape.

A.1.2 Mold patterning

Photoresists were processed according to manufacturers’ suggested protocols for the desired channel pattern thickness (channel depth). SJR 5740 (Shipley), SPR 220-7 (Shipley), and AZ 50XT (Clariant) positive resists were typically used for fluid-layer molds. Various negative resists of the SU8 family (MicroChem) were used for control-layer molds.

A.1.3 Mold rounding

It is necessary that fluid channels have a rounded profile to allow complete closing of valves at modest pressures. To cause rounding of photoresist channel features on fluid layer molds, molds were heated above the melting temperature of the resist. Molds patterned with SJR 5740 or SPR 220-7 were heated to 120°C for 20–30 min. Molds patterned with AZ 50XT were heated to 160°C for 1 h.

A.1.4 Fabrication of fluid layer molds with frit valves

Frit valves (for trapping microbeads, for example) were created by including non-rounded (square) channel segments in the fluid layer at the desired valve positions. Typically the square channels were 200 μm wide and were crossed by valves with 200 μm width. The fluid layer design was printed onto two different photomasks—one containing channel segments to be rounded, the other containing the square channel segments. At junctions, the rounded segments were designed to overlap the ends of the square channel segments by 30–50 μm.

Rounded and flat channel segments on the mold were fabricated from two different photoresists—typically SU8 resist for the square segments and SJR 5740, SPR 220-7, or AZ 50XT for the rounded segments. Due to its high chemical stability, SU8 segments were patterned first, according to the manufacturer's protocol using a negative photomask. Processing of the second resist was then carried out directly on top of the SU8-patterned wafer. During the exposure step, the second (positive) photomask was aligned to the SU8 features visible on the wafer. After development, the newly patterned channel features were rounded according to Secton A.1.3. The SU8 photoresist does not undergo rounding under those conditions.

Note that for rounded and square channel features of 10–12 μm in height, a significant jump in height (60–70 μm) can occur at junctions of the two resists. For this reason, frit valves should be positioned at least several hundred microns away from junctions.

A.1.5 Mold treatment with fluorosilane

(Tridecafluoro-1,1,2,2-tetrahydrooctyl)-1-trichlorosilane provides an effective mold release coating for replication molding of many polymers. The coating is applied by placing the mold in a dessicator with a small amount (200μL) of silane in a small dish of paraffin oil. The dessicator is pumped with a vacuum pump for 2 min to vaporize the silane and then is sealed closed. Treatment requires continued exposure for about 1–2 hours in this vapour.

A.2 Glass Protocols

A.2.1 RCA glass cleaning

Standard 1 mm thick 1×3 inch and 2×3 inch glass microscope slides were cleaned as follows. First, slides were mounted in a rack and sonicated for 30 min in a solution of 2% Micro-90 soap (Cole-Parmer Instrument Company, Vernon Hills, IL) in deionized water. Slides were then rinsed 3 times with deionized water. A batch of RCA-1 cleaning solution (6 parts deionized water, 4 parts 27% ammonium hydroxide, 1 part 30% hydrogen peroxide) was prepared and heated on a hot plate until it bubbled vigorously. The slide rack was then immersed in this solution for 60 min with continued heating and stirring. Subsequently, slides were rinsed 3 times with deionized water and then stored underwater in sealed containers to prevent contamination before use.

A variety of alternative glass cleaning methods are reviewed and compared in [51].

A.2.2 Glass microchannel etching

Microchannels were etched in glass using a protocol adapted from Stjernström and Roeraade [254]. Glass microchannel layers were fabricated from RCA-cleaned (Appendix A.2.1) microscope slides. SJR 5740 photoresist (Shipley) was used as an etch mask. Resist was spin-coated at 1500 RPM for 60 s. After soft-baking for 2.5 min at 105°C, the resist was exposed through the desired positive photomask (channels dark) for 1.5 min on a Karl Suss mask aligner (UV lamp intensity 175 W). The resist was developed by immersing for approximately 2 min in Microposit 2401 developer diluted 1:4

in deionized water. A uniform layer of resist was spun and soft-baked on the other side of the glass to prevent etching from the back side. Alternatively, clear tape could be used as an etch barrier. Channels were etched by immersing the slide with the channel surface facing down in a solution of equal parts of 1N HCl, deionized water, and buffered oxide etchant (6:1) at 25°C. Channels were etched until the desired channel depth was reached (etch rate approximately 0.8 μm/min). Slides were then rinsed 3 times with deionized water and then washed in acetone and isopropanol to remove the photoresist. Slides were washed again in water and then dried before use.

A.2.3 Drilling holes in glass slides

Solvents must sometimes be delivered to microfluidic fluid channels through the glass substrate—for example when only the bottom surface of the device is compatible with the solvents.

Holes in glass were drilled using a 0.75 mm diameter diamond-tipped Triple Ripple drill bit (C. R. Laurence Company Inc., Los Angeles, CA). The desired locations of the centers of the holes were first marked on the glass with a water-insoluble marker. With the slide immersed in water, the drill bit (spinning at a high speed setting) was brought down slowly to the glass surface. After drilling to a depth of 100–200 μm, the drill was raised to allow water to enter the hole. This process was repeated until the hole was drilled completely through the glass. To prevent flaking on the back side, it was necessary to firmly push the slide against a second glass slide or other rigid support as the drill bit broke through the back surface.

A.2.4 PDMS/glass oxygen plasma bonding

Oxygen plasma treatment can be used to covalently bond PDMS to itself or to glass. The mechanism is thought to be related to the breaking of bonds on each surface during treatment followed by the formation of Si–O–Si bonds when the two surfaces are brought into contact.

Details of the process are poorly understood, but numerous groups have reported processing parameters that work, and a few have published systematic optimizations of bonding [21]. In general, clean PDMS surfaces (for example, using HCl treatment), low plasma powers, and short treatment

times lead to the highest bond strengths. One set of optimized parameters is 75 W power at 75 mTorr pressure for 10 s duration [137]. Typically, surfaces must be placed into contact within about 1 min of treatment unless oligomers are extracted from the cured PDMS [160]. Drops of non-swelling solvents such as methanol or ethanol can be placed between treated surfaces to act as a lubricant during alignment and to prolong the working time. Assembled devices are heated to evaporate the solvent and complete the bonding process within several minutes.

Typically, I treated surfaces at a pressure of 90–100 mTorr for 15 seconds. The plasma power was not critical: powers from 25 W to 75 W resulted in successful bonding. 1–2 drops of methanol were placed between surfaces during alignment. Capillary action helps to pull the bonding surfaces together as the methanol evaporates during the subsequent bake at 80°C for several hours.

A.2.5 Amino-derivatization of glass slides

RCA-cleaned (Appendix A.2.1) glass slides were derivatized with amino groups by treatment with 3-aminopropyltriethoxysilane (Sigma-Aldrich). Slides were rinsed twice with acetone to remove water and then immersed in a solution of 2 vol% APTES in acetone for 30 min at room temperature with stirring. Slides were then rinsed twice with acetone to remove unreacted silane and twice with deonized water. They were then dried and baked at 120°C for 30–60 min to anneal the silane coating and finally stored dry until use. Toluene or 95% ethanol were also found to be suitable solvents.

A.2.6 Derivatization of glass for DNA synthesis

A silanization solution of 1 vol% silane in 95% ethanol was prepared and stirred for 1 h. The silane, N-(3-(triethoxysilyl)-propyl)-4-hydroxybutyramide, was purchased from Gelest, Inc., Morrisville, PA. Prior to treatment, RCA-cleaned (Appendix A.2.1) slides were immersed in 10% sodium hydroxide for 3 min at 70°C, rinsed with deionized water, immersed in 1% HCl for 1 min, washed twice with water, and dried with nitrogen and transferred to a dry slide rack. Dried slides were wetted by immersion in ethanol and then transferred to the silanization solution for 1 hour with stirring. After treatment, slides were washed three times with ethanol, dried with nitrogen, baked for 2 h

at 115°C, and stored dry until use. Attempts to react this silane from the vapour phase resulted in a poor density of functional groups. Oligonucleotides synthesized on the treated surface should be deprotected by immersion in ethylene diamine (EDA) and ethanol (1:1, v/v) at room temperature rather than by ammonia treatment.

Other silanization procedures suitable for DNA synthesis can be found in the literature. For example, Maskos and Southern described the preparation of a linker that is stable under final ammonia deprotection [184]. Glass is first reacted with a silane (3-glycidoxypropyltrimethoxysilane) to which a hexaethylene glycol linker is attached.

During optimization of derivatization protocols (Section 7.5.3), I prepared several slides by this method. 15 mL silane was added to 50 mL xylene with a trace of diisopropylethylamine and mixed in a staining jar. Slides were placed in this jar, which was heated on a hot plate to a solution temperature of 80°C overnight. Slides were then washed twice in methanol, once in methanol with ether, once again in methanol, and then dried with nitrogen. Next, a PDMS gasket was placed between two silanized slides, resulting in a sealed chamber between them. A solution of 1.5 mL hexaethylene glycol with a trace of sulphuric acid was injected into the enclosed space. The assembly was baked at 80°C overnight.

A.3 Polymer film protocols

A.3.1 Measurement of polymer film thickness

Polymer film thicknesses were measured with an Alpha-step 500 profilometer (KLA-Tencor). This instrument consists of a stylus that is dragged across the surface while maintaining a constant vertical contact force. The stylus thus follows the topography of the sample, and the height profile is recorded.

To measure the film thickness on a silicon wafer mold, a small part of the film was cut or scraped away creating a step edge from the polymer down to the wafer. A profile of length 3–10 mm was taken across the edge from the polymer (high) to the silicon (low). (If profiles were taken in the

reverse direction, the stylus often caught on the polymer edge and peeled it from the surface.) When measuring soft polymers, it was necessary to use a small stylus force (e.g., 1–2 mg). The film thickness was read directly from the profile after leveling. Generally the profile of the polymer was not flat near the step edge due to air gaps between the polymer and wafer created during cutting. It was thus necessary to observe the profile height at a point several millimeters from the edge.

A.3.2 Calibrating spin curve for a new polymer

Fabricating elastomeric microfluidic devices with functional microvalves requires that the thickness of the thin layer be well controlled. For new polymers, it was thus necessary to calibrate the spin-coating procedure.

Several brand new glass slides or silicon wafers were prepared by treating their surfaces with the mold-release agent appropriate for the polymer being investigated. Substrates were then spin-coated with prepolymer at a variety of spin speeds (with duration and ramp speed kept constant). Samples were then cured or polymerized and measured by profilometry according to Appendix A.3.1. The layer thickness was plotted as a function of spin speed, allowing the spin speed for the desired layer thickness to be interpolated from the graph.