Microfiber Assisted Fabrication of Microfluidic Channels Using Poly(dimethylsiloxane)

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A microfluidic device is typically formed using bulk silicon etching techniques on a silicon substrate (Kovacs et al., 1998). A photolithography step defines the desired pattern on the silicon substrate with photoresist. Etching using acids or gases, followed by a solvent and acid cleaning process to remove residual photoresist, leaves micron-scale features. Such devices are capable of providing rapid identification of nucleic acids, proteins, drugs, or other important biological compounds with enhanced sensitivity and time-to-result, while reducing consumption of expensive reagents compared to microtiter plates or test tube scale analyses (Stone and Kim, 2001; Khandurina and Guttman, 2002; Meldrum and Holl, 2002).

Polydimethylsiloxane (PDMS), created by mixing a silicone elastomer base and a curing agent in a 10:1 ratio, gives an alternate material for fabricating microfluidic devices (McDonald and Whitesides, 2002). The liquid pre-polymer is poured over a master generated either from photolithography using a high resolution transparency as a photomask, or by laser ablation or Solid-Object Printing to form the device (Grzybowski et al., 1998; McDonald et al., 2002; McDonald and Whitesides, 2002). We report formation of a master by directed placement of glass microfibers on silicon or glass substrates, followed by pressing a preformed PDMS sheet onto the substrate to from microfluidic channels. Wells are formed by criss-cross fiber patterns, while functionalized microbeads coated onto fibers result in microscale channels that separate proteins. We believe this approach is an attractive research tool, because it places rapid prototyping capability within the reach of laboratories that have access to glass slides, an optical microscope, digital camera, tweezers, and PDMS.

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Materials and Methods

Microscopy

Most images were acquired using a Nikon Eclipses L150 microscope with a Spot Insight digital camera, and Spot advanced imaging software. Fluorescent images were acquired using a Nikon Eclipses E600FN microscope with a triple filter block (DAPI/FITC/TRITC) and a Kodak DC290 digital camera. Scanning electron microscope images were obtained using a JEOL JSM-840 type scanning EM via sputter coating with Au/Pd.

Formation of microfluidic channels and wells

The Dow Corning Sylgard 184 silicone elastomer kit was used to form a flat PDMS cover with a 1 mm thichness using a ratio of a 10:1 elastomer base: curing agent. Glass wool fibers were removed from a glass wool fiber bundle using sharp tweezers. Individual fibers were carefully laid onto a clean 76 x 50 mm laboratory glass slide. One end of the glass wool fiber was secured by pressing a small piece of PDMS against it, while the free end remained suspended. Then, holding the PDMS with a tweezer, the free end of the glass fiber was dipped into absolute EtOH and ultrasonically cleaned for 3 min. The fiber was then placed onto a glass slide that had also been cleaned in ethanol. Alternately, the fiber was placed on a SiO_2 substrate obtained from a 76 mm dia. silicon wafer $<100>$ with thermally oxidized SiO_2. Using the tweezers, the glass wool fiber(s) were carefully cleaved

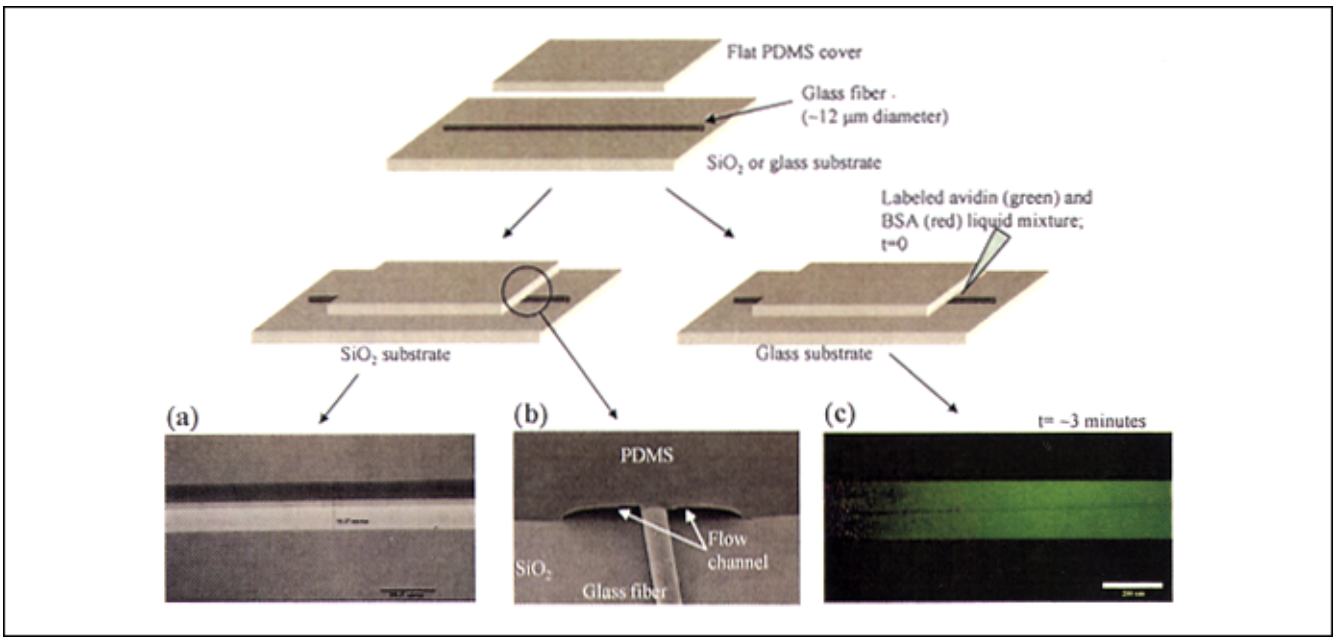

Figure 1. Microfiber-assisted fabrication of microchannels.

(a) 91 μm wide channel formed with 12 μm wide glass fiber. (b) End-view of 5 nL channel formed using a 5 mm long PDMS cover. (c) Mixture of fluorescein red labeled BSA (10 μg/mL) and fluorescein green labeled avidin (10 μg/mL), in fluid-filled microchannel.

and placed on the slice or oxide substrate. Microchannels were formed when a flat PDMS cover was carefully laid and pressed onto the top of a straight fiber (Figure 1a and 1b), while a 1 nanoliter (nL) well was formed when PDMS is pressed over two fibers laid one on top of the other (Figure 2a). Capillary action fills the microchannel that consists of the pocket between the fiber and PDMS (Figure 1b) within 10 s, as observed for an aqueous mixture of labeled avidin and biotin (Figure 1c).

Functionalized glass fiber for microscale separation

The free end of the glass wool fiber, secured in the PDMS, was dipped into an aqueous slurry of dimethylamino (DMA)

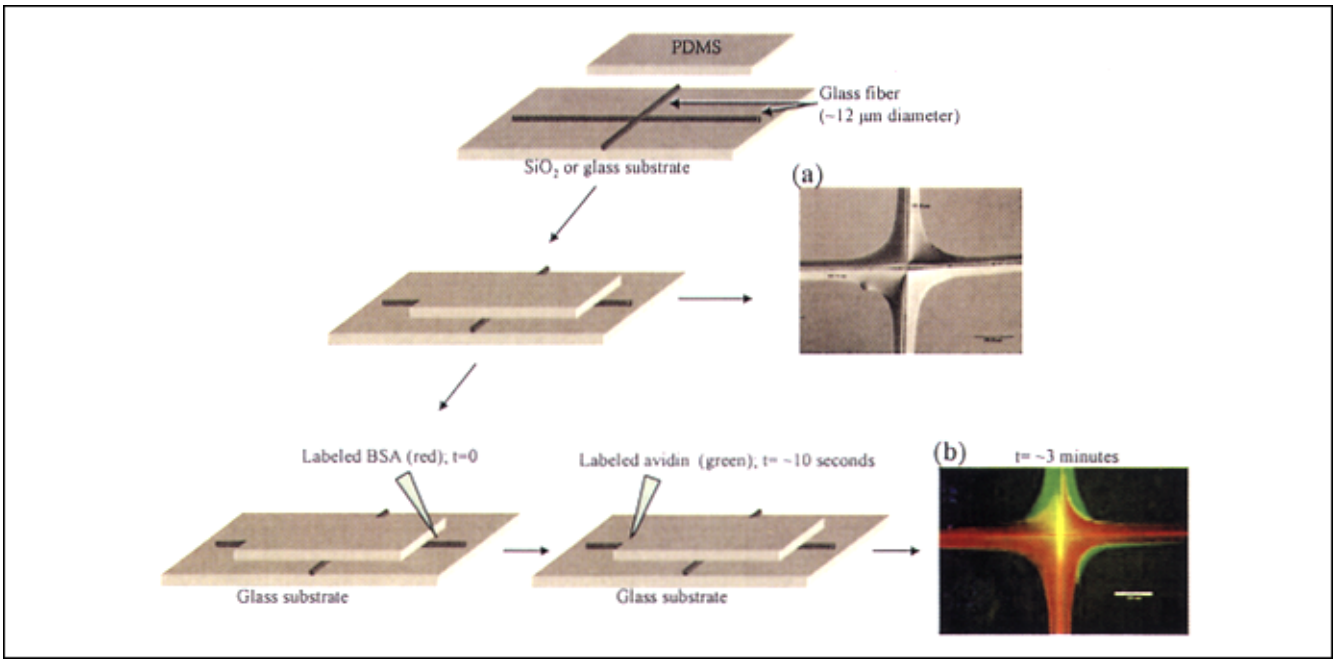

Figure 2. Assembly and formation of (a) 1 nL well at the intersection of two fibers; (b) labeled BSA (red; 200 μg/mL) and avidin (green; 200 μg/mL) transported to liquid well mixed to give a yellow color.

Area of the image in Figure 2b is approxiamtely 1 mm^2.

beads, 0.8 μm in diameter (Catalog No. DP-08-10, Spherotech Inc., Libertyville, IL), 1.78 x 10^{11} beads/mL, in a 1.5 mL Eppendorf tube. After 2 min. the glass wool fiber was slowly drawn out from the microbead solution and air dried. The DMA beads, held in place by hydrophobic and ionic forces, coated the fiber (Figure 3 a).

Streptavidin beads, 0.8 μm dia. (Spherotech Catalog No. SVP-08-10), adsorbed onto a fiber coated with biotinylated BSA (8 moles of biotin/mole of BSA (Catalog No. 29130) Pierce Inc., Rockford, IL). The glass fiber was biotinylated by dipping it into a 2 mg/mL concentration of biotinylated BSA in PBS (pH 7.2) for 5 min, and then washing it by dipping it into a PBS buffer (pH 7.2) for 3 min. The biotinylated fiber was then immersed in streptavidin beads (3.6 x 10^{10} beads/mL) for 2 min and air dried to give the fiber of Figure 3b. Rationale and experimental protocols for micropatterning beads on oxide surfaces is described elsewhere (Huang et al., 2003). The DMA or biotin fibers, thus provided, were laid onto a clean glass slide. A square PDMS cover (5 by 5 mm) was then pressed onto a 6 mm long derivitized microfiber. The resulting microchannel was open with both ends of the glass fiber protruding from the PDMS cover (Figure 3).

Results

Fluid flow in a microchannel is at a Reynolds' number of less than 100 (Stroock et al., 2002), so mixing is diffusively controlled, but occurs relatively rapidly given the small dimensions of the well. Placement of 2 μL of labeled BSA (red; 200 μg/mL) at one entrance, followed 10 s later by 2 μL of avidin (green; 200 μg/mL) at the opposite entrance, results in fluid transport into the well via capillary action, and mixing of the two fluids to form a yellow color at the intersection (Figure 2b).

Separation of a protein mixture containing BSA (60 KDa and pI = 4.8) labeled with a red fluorescent dye, TRITC, (from Sigma-Aldrich Catalog No. A-2289), and avidin (67 KDa and pI = 10.2) labeled with a green fluorescent dye, FITC (from Pierce Catalog No. 21221), is shown in Figure 3. A pipette was used to place 2 μL of protein mixture onto the open end of a 6 nL microchannel. The protein mixture filled the channel through capillary action within 10 s. Labeled BSA (red) was separated from avidin (green) in 3 min due to diffusive de-mixing where the BSA adsorbed on the DMA fiber (red color), while the avidin remained distributed across the width of the channel (Figure 3c). An axial and radial concentration gradient of labeled BSA (red) is evident near the entrance of the microchannel (Figure 3c). Electrostatic interaction causes the positively charged dimethylamino microbeads to capture the negatively charged BSA (pI = 4.8) when the BSA is in the PBS buffer (pH 7.2). Avidin (pI = 10.2) is positively charged at pH 7.2, so this protein is not adsorbed on the DMA fiber. However, avidin adsorbs on biotinylated BSA coated glass fiber (Figure 3d), since biotin has a strong affin-

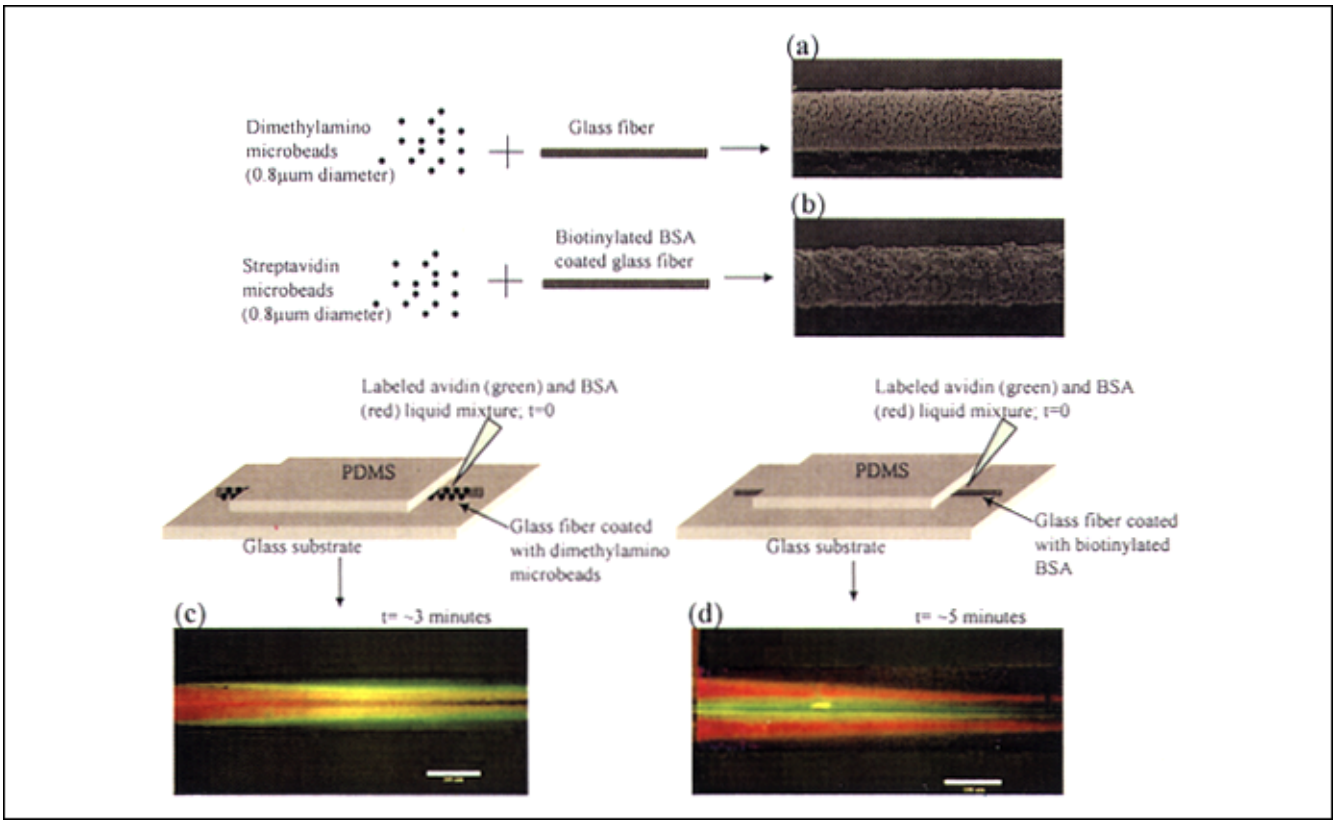

Figure 3. Microscale separation device.

(a) DMA derivatized microbeads (0.8 μm dia.) on glass fiber. (b) Streptavidin derivatized microbeads (0.8 μm dia.) on glass fiber previously coated with biotinylated BSA. A spiral pattern is indicated, although we have no explanation for it at this time. (c) Separation of labeled avidin and BSA (10 μg/mL) mixture, with BSA (red) preferentially binding DMA coated fiber in 3 min. (d) Fractionation of avidin and BSA, with avidin (green) preferentially binding biotin on fiber in 5 min.

ity for avidin with a dissociation constant of 10^{-15} M (Creighton, 1993). The capture of avidin labeled microbeads by biotinylated glass fiber is demonstrated by 0.8 μm particles fixed onto the biotin coated microfiber (Figure 3b), while the adsorption of avidin on the biotin coated microfiber itself is shown in Figure 3d.

Glass wool fiber coupled with silane chemistry represents an additional scaffold upon which bio-specific or ion-exchange ligands may be grafted. Other fibers could be used such as hydrophilic cellulose and glass, or hydrophobic polypropylene, aramid, and acrylic fibers. Biotinylated BSA on the surface of the fiber blocks nonspecific adsorption and also anchors biotinylated antibodies through avidin. The antibody enables the selective capture of a target species from a background of other components. Derivatized forms of cellulose (DEAE or sulfated cellulose) may also provide scaffolds for ion-exchange microscale chromatography of proteins, while hollow fibers would enable gas and/or liquid exchange (Yang et al., 1992; 1993; Hamaker et al., 1998; Ladisch, 2001).

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