Fabrication of reversibly adhesive fluidic devices using magnetism

Marjan Rafat,[a] Danielle R. Raad,[b] Amy C. Rowat[a] and Debra T. Auguste*[a]

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Fluidic devices are often made by irreversibly bonding a polydimethylsiloxane (PDMS) mold to itself or a glass substrate by plasma treatment. This method limits the range of materials for fluidic device fabrication and utility for subsequent processing. Here, we present a simple and inexpensive method to fabricate fluidic devices using magnets to reversibly adhere PDMS and other polymer matrices to glass or gel substrates. This approach enables fluidic devices to be fabricated from a variety of materials other than PDMS and glass. Moreover, this method can be used to fabricate composite devices, three-dimensional scaffolds and hydrogel-based fluidic devices.

Introduction

Capable of manipulating picoliter to nanoliter volumes of fluid, microfluidic devices are used in fields ranging from biotechnology to analytical chemistry.[1] The most common method for bonding materials to each other to form such devices is plasma oxidation. This treatment is applied to glass and silicon-based materials, such as PDMS, and generates reactive surfaces which subsequently form an irreversible bond when placed in contact with one another. In addition, this method requires expensive machinery and cannot be used to fabricate devices from a wide range of polymers.[2] For example, soft lithography is used to mold polymers, including epoxies, polyurethanes, polyethylene glycol, agar, and agarose, but these materials cannot easily be attached to glass, limiting their use in flow applications.[3] Several biological assays, which manipulate cells and reagents, would benefit from reversible assembly by allowing step-wise analyses at various stages. A technique that can reversibly adhere PDMS and other polymers to substrates would be useful for applications requiring reversible adhesion and fabrication using diverse materials.

Devices can be fabricated by exploiting the inherent adhesion properties of the materials or by design. PDMS can form reversible bonds with metal or photoresist because of its high surface energy.[4] Reversibly-adhered devices have been produced with this method for microelectrode fabrication,[5] spatial control of cell deposition,[6] capillary electrophoresis,[7] and chemical patterning of substrates.[8] This technique can be used for applications with pressures that do not exceed approximately 35 kPa.[9] For applications that require stronger adhesion of PDMS to glass, an aspiration technique has been developed that allows for pressures of up to 100 kPa within microfluidic channels.[10] However, this requires fabrication of a specialized network of microchannels, which adds additional stages to the manufacturing process. These approaches cannot be easily implemented for a wide array of materials and devices.

In this study, we present the use of bar magnets or iron powder for adhering PDMS to glass. The use of magnets is inexpensive, reversible and allows for straightforward reconstruction of microfluidic devices when cleaning and replacing parts as necessary. As surface treatment is not required, this method is easily extended for securing many types of gels to glass or other substrates. In addition, this method is simple and does not require additional fabrication steps. We show that the adhesion of PDMS to glass via magnets is stable over a range of gel elasticities. We also demonstrate the use of these channels for staining cells with small volumes of reagents. The potential to use a wide variety of materials for fluidic device fabrication could support applications in drug delivery, biosensors and tissue engineering, which use novel materials or combinations of materials.

Materials and methods

Channel design and fabrication

Channels were machined out of a polyoxomethylene slab to be 70 mm long × 7.5 mm wide × 1.6 mm high (Harvard University Engineering Sciences Laboratory Machine Shop; Fig. 1a–c). The master was used for casting fluidic constructs using soft lithography as described previously.[11] Briefly, PDMS base and curing agent (Sylgard® 184 Silicone Elastomer Kit) were mixed in ratios varying from 5:1 to 20:1 (w/w), degassed, poured onto the master, and cured for 1 hour at 65 °C.

PDMS channels were adhered to soda lime glass (Moliterno, Inc., Lowell, MA) using neodymium bar or slab magnets (SuperMagnetMan, Birmingham, AL) or fine iron powder with a magnetic susceptibility χ of 1.7×10^{-3} (Sigma, St. Louis, MO).[12] The magnets were reported by the manufacturer to be extremely resistant to demagnetization. Indeed, measurements of the surface gauss level showed no degradation over 15 years. A rectangle with the exact dimensions of the channel was machined out of the slab magnets (Harvard University Engineering Sciences Laboratory Machine Shop). Fig. 1 illustrates the magnet adhesion techniques.

Channels were also fabricated using agarose. Gels were made by dissolving agarose powder (Invitrogen, Carlsbad, CA) in 1X tris acetate EDTA (TAE) buffer solution at 100 °C, pouring over the master, and cooling to room temperature. Concentrations of gels explored ranged from 1 to 8% agarose.

[a]*School of Engineering and Applied Sciences, Harvard University, Cambridge, MA, 02138, USA. E-mail: auguste@seas.harvard.edu; Fax: +1 (617) 495-9837; Tel: +1 (617) 384-7980*
[b]*Department of Chemistry, Brown University, Providence, RI, USA*

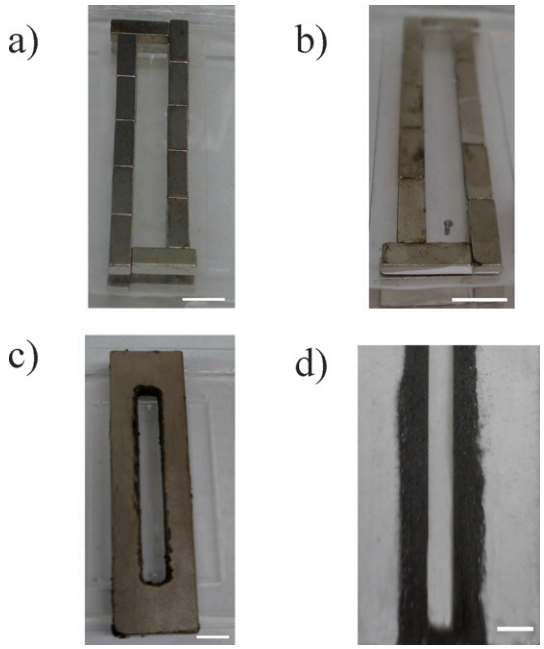

Fig. 1 (a) Adhesion of PDMS to glass using bar magnets (15 mm × 5 mm × 3.2 mm); (b) Channel fixed by magnets (15 mm × 5 mm × 3.2 mm) embedded in the PDMS gel; (c) Magnet slab (102 mm × 25.4 mm × 6.4 mm) used to adhere the PDMS to glass; (d) Iron filings embedded in PDMS around the channel and using bar magnets underneath the glass. Scale bar is 1 cm.

Leakage tests

To assess the maximum pressures and flow rates the magnet-bonded devices can withstand, we attached a pressure gauge to the flow system (Fig. 2). Magnets of 6.4 mm thickness were placed around the perimeter of the channel to adhere the PDMS to glass, and water was flowed through the channels for approximately 5 minutes. A pressure regulator was used to measure the air pressure applied to the water column; the water reservoir was connected to the fluidic device with polyethylene tubing. Flow rates were determined by measuring the accumulated volume for 30 seconds (n = 4). The maximum pressures and flow rates were determined at the point where leakage occurred.

Mechanical testing

To evaluate the range of elastic properties of materials required for magnet adhesion, the Young's modulus of each gel concentration was determined. Tensile tests were conducted with each gel using an Instron BioPuls machine (Instron, Norwood, MA). Gels (10 mm × 1 mm × 30 mm) were extended at a rate of 1 mm/min at room temperature (n = 9).

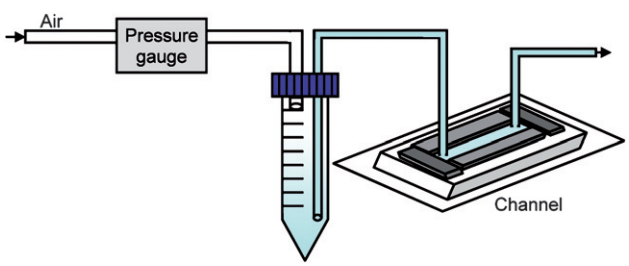

Fig. 2 Schematic of leakage experiment.

Cell culture and staining

HeLa cells (CCL-2, ATCC, Rockville, MD) were cultured in Dulbecco's Modified Essential Medium/F12 (Invitrogen, Carlsbad, CA) supplemented with 5% fetal bovine serum and 1% penicillin and streptomycin at 37 °C and 5% CO_2. Cells were seeded into channels adhered to glass by embedding magnets into PDMS (Fig. 1b) at a cell density of 2×10^5 cells/ml and grown to confluency.

Nuclear and actin stains were performed on HeLa cells in the channels using the Actin Cytoskeleton and Focal Adhesion Staining Kit (Millipore, Billerica, MA). Cells were fixed in acetone for 5 min at −20 °C. To stain actin, cells were incubated with 0.6 μg/ml tetramethylrhodamine isothiocyanate (TRITC)-conjugated phalloidin in phosphate buffered saline for 60 min at room temperature. The cells were incubated with the nuclear stain 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI) concurrently at 0.1 μg/ml. Fluorescent images were acquired using a Zeiss Axiovert 200 M microscope (Carl Zeiss MicroImaging, Thornwood, NY).

Results and discussion

We tested four different methods to bond fluidic channels using magnets: (1) placing several bar magnets around the channel perimeter on top of the PDMS gel and below the glass (Fig. 1a); (2) embedding bar magnets into the PDMS gel and placing them below the glass to bring the magnets closer together (Fig. 1b); (3) positioning a slab magnet above the gel and below the glass (Fig. 1c); and (4) embedding iron filings in the PDMS around channel edges and using bar magnets underneath the glass (Fig. 1d). Tightness of the seal was determined by visual inspection. Out of the four methods tested, the bar and slab magnet methods (Fig. 1a–c) provided tight sealing of the PDMS to glass. Using iron filings for adhesion (Fig. 1d) resulted in the least tightly sealed channel when compared to the other methods. Although the iron filings did not form the tightest seal, this was the most useful method for bonding devices with irregular geometries. Because the bar magnet method (Fig. 1a) is the most amenable to changes in channel geometry and disassembly, we utilized this set-up for our measurements.

We evaluated the maximum pressures and flow rates our magnet-adhered devices could withstand by assessing when leakage occurred (Table 1). The stability of the bond between the bar magnets and their substrate was maintained over long

Table 1 Maximum pressure and flow rates of magnetically-adhered fluidic devices (see setup in Fig. 1a).[a]

PDMS ratio	P (kPa)[b]	Q_{max} (ml/min)
5:1	50	100
10:1	60	115
15:1	75	145
20:1	145	215

[a] Error is estimated to be within 2 kPa. [b] Plasma bonded PDMS devices can withstand 210–345 kPa[13]

periods of time. Magnetic strength increases with magnet thickness; we used magnets with 6.4 mm thickness. Failure (or leakage) of the devices occurred when pressure through the channels was increased. The maximum pressure in the magnetically-adhered device was approximately 145 kPa. In comparison, irreversibly plasma bonded PDMS devices can typically withstand pressures in the range of 210–345 kPa.[13]

Since gels with different mechanical properties may be useful for a variety of applications, we probed a range of elasticities by varying the ratio of PDMS elastomer base to curing agent. We tested a range of base to curing agent ratios ranging from 5:1 to 20:1, including the commonly used 10:1 composition. We found that the maximum flow rates vary from approximately 100 to 215 ml/min. Within this range, we achieve higher flow rates using more deformable gels with a lower base to curing agent ratio and Young's modulus. Flow rates used with cells to simulate physiological shear stresses can vary between 0.1 to 10 ml/min,[14] whereas flowrates for microchannels are generally in the range of 1 to 600 μl/min.[15]

While PDMS is a widely used material in fluidic devices, a variety of hydrogels are also desirable for many biological applications. However, these materials can be much softer than the often used 10:1 PDMS (9% curing agent). To test the compatibility of materials ranging in stiffness with our magnetic-adhesion method, we fabricated channels with a range of agarose concentrations. The agarose gels had Young's moduli ranging from 0.2 to 3.1 MPa. This was comparable to the Young's moduli of PDMS that ranged from 0.2 to 2.7 MPa (Fig. 3).[16] Magnets of 3.2 mm were able to secure devices for all gel concentrations investigated without breaking the gel. Agarose is used for gel electrophoresis,[17] the repair of spinal cord defects,[18] and for testing drug delivery.[19]

Other hydrogels including polymethacrylic acid (PMAA), poly-2-hydroxyethyl methacrylate (PHEMA), and alginate can be prepared to have Young's moduli from 0.07 to 13.4 MPa (data not shown). These polymeric systems were successfully used to create fluidic devices using magnetic adhesion. These materials are broadly used in oral drug delivery,[20] micro-electrode arrays

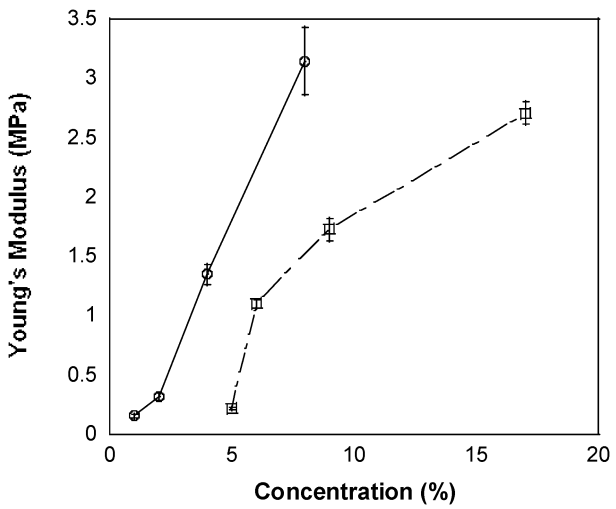

Fig. 3 Average Young's moduli for varying concentrations of PDMS curing agent (squares) and agarose (circles). A 10:1 PDMS base to curing agent ratio is equivalent to 9% curing agent. Error bars represent the standard error with n = 9.

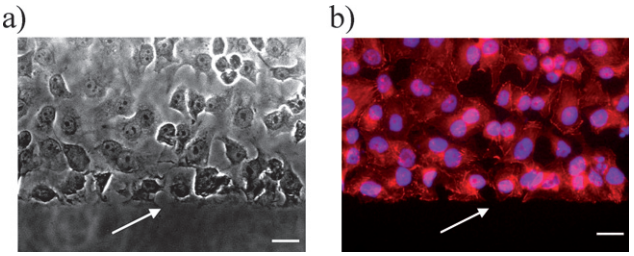

Fig. 4 Staining HeLa cells in magnet-adhered channels: (a) Phase contrast image; (b) F-actin and nuclear stain. Scale bar is 25 μm. The arrows indicate the channel edge.

to construct drug delivery devices for *in vivo* neural networks,[21] and scaffolding for tissue engineering.[22]

Magnetically-adhered devices were used to fabricate chambers for cell culture. As shown in Fig. 4, cells can be grown in magnetically-adhered channels and intracellular structures in HeLa cells can be stained. The reversibility of the magnet adhesion method allowed the saving of expensive reagents by enabling us to (1) disassemble the device and (2) drop small volumes of staining solution onto attached cells as opposed to the conventional method of filling the entire channel. Filling the channel required a minimum of 840 μl of reagent; however, by opening the device, we were able to apply 250 μl directly to the cells. This also demonstrated the feasibility of micropatterning cells on glass substrates which could then be placed underneath a fluidic device. The channels or wells can be removed and replaced easily to survey an array of different conditions. This method thus facilitates advancements in medical diagnostics where the flow of reagents can be controlled via sophisticated microfluidics but uniform analyses may be conducted quickly and easily by disassembling the device.

Reversible adhesion for device fabrication is an important step towards 3D hydrogel devices for cell culture, a major goal in tissue engineering.[23] Such devices would enable cells to be cultured with improved exchange of nutrients and waste products. In addition, magnet adhesion is not limited to hydrogels and glass: magnets can be used to affix any two gels of the same or different materials together to make composite devices, such as 3D or asymmetric constructs. More generally, this technique can be extended to any process where materials other than PDMS and glass need to be incorporated into fluidic devices, including porous membranes and filters.

Conclusions

We demonstrate the use of magnets as a practical and feasible method for constructing fluidic devices by binding PDMS and other hydrogels to a variety of surfaces. This simple, cost-effective method provides an alternative to the routinely used plasma oxidation and allows for reversible adhesion of glass to PDMS, as well as the use of a wide range of hydrogel materials that cannot be plasma treated.

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