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From Micro- to Nanofabrication with Soft Materials

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Soft materials are finding applications in areas ranging from microfluidic device technology to nanofabrication. We review recent work in these areas, discuss the motivation for device fabrication with soft materials, and describe applications of soft materials. In particular, we discuss active microfluidic devices for cell sorting and biochemical assays, replicationmolded optics with subdiffraction limit features, and nanometer-scale resonators and wires formed from single-molecule DNA templates as examples of how the special properties of soft materials address outstanding problems in device fabrication.

During the height of the silicon microelectronics revolution. Kurt Peterson wrote a seminal paper entitled "Silicon as a Mechanical Material" (1). Twenty years later, the resounding success of microelectromechanical systems (MEMS) continues to reverberate through the scientific and engineering worlds, and there has been an explosion in new applications for mass-produced, miniaturized devices. Although traditional silicon micromachining techniques have proved to be the methods of choice for electronic and mechanical devices at the micrometer and millimeter scales, other materials and fabrication technologies are coming into play as researchers are exploring fluid applications and trying to push feature sizes below the optical diffraction limit. In this review, we will discuss why soft materials are having an impact in two application areas: micrometerscale fluidic devices and nanometer-scale mechanical and electrical devices.

Why Elastomers?

Although materials such as silicon and glass are excellent choices for electronic and mechanical devices, it is not at all clear that they should be the first choice for applications in biology and chemistry that require fluidic control. Their intrinsic stiffness poses a challenges in making devices with moving parts-the throw to close a valve is much larger than the displacement needed in, for example, a micromachined accelerometer, and the geometry is unfavorable. That is to say, one can make a silicon valve with the requisite displacement but only at the cost of increasing the area of the device by having a proportionally large membrane. Furthermore, all valves need a valve seat to close completely-macroscopic valves use a rubber washer-and it is difficult to integrate a soft material into the rigors of silicon processing. Finally, many biomedical applications require delicate surface chemistry that is difficult to achieve with the high temperatures required to bond silicon and glass.

Soft materials overcome many of the limitations of silicon discussed above and offer further advantages. Fluidic devices by their nature require more surface area than electronic circuits: The components are larger, and the interface with the external world is more complicated. The popular silicone elastomer polydimethylsiloxane (PDMS) is 50 times cheaper than silicon on a per volume basis. Elastomers also have a distinctive mechanical property: The Young's modulus can be tuned over two orders of magnitude by controlling the amount of cross linking between polymer chains (2). Elastomers form a tight seal with silicon and glass, permitting one to design hybrid devices that will contain silicon electronics, light sources, and detectors with silicone fluidics. Finally, elastomers are a forgiving material to work with, requiring less stringent fabrication conditions than silicon and little capital equipment to set up a fabrication facility. Thus, soft material fabrication has two economies of scale-massproduced devices will be inexpensive, but it is also affordable to produce small quantities of devices. Rapid turnaround time for fabrication also allows one to quickly iterate and modify designs (3). Because the cost of entry is low, many people can work in this area, which greatly speeds innovation. An analogous situation is the rapid growth in software due to the development of the personal computer.

The other area of consideration is the inexorable march toward smaller feature size and higher integration density. Although the marked reduction in Young's modulus that accompanies the use of soft materials allows smaller feature sizes for mechanical devices, an even more important consideration is the availability of nonlithographic fabrication techniques for soft materials. The most important of these methods is replica molding, part of a large pool of chemically inspired

fabrication techniques developed by Whitesides and co-workers under the rubric of "soft lithography" (4). They have shown a spectacular array of fabrication technologies to make optical components such as blazed gratings (5), waveguides (6) and lasers (7), and structures with nontrivial geometry and topology, including three-dimensional conducting coils (8), linked rings (9), and basket weave structures (10). An embossing technique called "nano-imprint lithography" developed by Chou and co-workers has also shown the ability to fabricate nanometer features and holds great promise for the future (11, 12). An advantage of replica molding and embossing is that the resolution is determined by the mold feature size, not by the optical diffraction limit. Because the molds are reusable, their cost and fabrication difficulty do not factor substantially into the final cost of a mass-produced device. For example, the molds can be produced with electron beam lithography, a time-consuming and expensive process that has the ability to make nanometer-scale features.

Mechanical Microfluidics with Valves and Pumps

The first functional microfluidic devices made from elastomers performed DNA analvsis (13, 14) and cell sorting (15). These devices used electrokinetic flow control, a popular method used in microfluidic devices made of both hard and soft materials (16). Although electric fields are a powerful tool for molecular separations, they have drawbacks as a general method of fluidic manipulation. For example, electrophoretic demixing occurs when pumping of heterogeneous solutions is attempted. This problem can be partially compensated by alternating plugs of low- and high-conductivity buffer, but other problems then arise (16). Although the devices work well for fixed conditions, the voltages have to be fine tuned whenever buffer composition or salt concentration is changed. Voltage control works well for simple devices with only one switching junction, but it quickly becomes problematic to make more complicated devices with many junctions-it is difficult to set the voltages to compensate for the various pressure and resistive imbalances in the devices.

We have addressed these challenges by fabricating mechanical elastomeric valves that have precise fluid control over a wide range of conditions (17). Choosing an elas-

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tomer allowed us to obtain complete valve sealing, low actuation forces, and a small footprint. These pneumatically actuated valves are surprisingly fast, and we were also able to make peristaltic pumps by arranging three consecutive valves in a row. The valves and pumps have negligible dead volumes and can transport fluid up to a few nanoliters per second, a critical regime for microfluidic assays. They also have very low space requirements; our initial devices were 100 μ m by 100 μ m, and we have since been able to reduce the active area to 20 μ m by 20 μ m.

We used this technology to make a cell sorter with integrated valves and pumps for fluid control (Fig. 1). This cell sorter has more robust performance than our initial device, in that it allows longer running times, higher throughput, and easier integration as a component in complicated fluidic systems. These cell sorters are now being commercially developed and are expected to find applications in a variety of areas, including molecular screening and drug discovery. Besides the cost savings, an important advantage of the microfluidic cell sorters is that they will allow the implementation of sophisticated assays that are impractical to perform with traditional cell sorters or microtiter plates.

We have also used the power of soft lithography to show how microfluidics can be used to increase the sensitivity of diagnostic assays. Most diagnostic assays require reagents to bind to solid supports; traditionally, this has been done with enzyme-linked immunosorbent assays (ELISA), and it is expected that these will be complemented in the future by gene expression arrays. In both cases, when probing for multiple targets, the surface area quickly becomes large, and for sufficiently specific reagents, the sensitivity of the assay becomes limited by its ability to pull down all of the analyte in the solution. Active fluidic pumps can be used to transport analyte material within the binding region of the assay. This allows each of the binding sites to sample all of the analyte, instead of just that portion that can diffuse from a local neighborhood. We designed a chip with a circular channel that could be closed off from the rest of the chip while reagents were pumped within the loop (Fig. 2) (18). This design allows complete recycling of the analyte and optimal sensitivity. Such a configuration is topologically impossible to achieve with electrokinetic flow because of the polarities of the electrodes.

A microfluidic print head was used to pattern the surface of a glass cover slip with biotin (19). The ability of soft lithographic techniques to perform chemical patterning has long been recognized, and early demonstrations showed antibody recognition in microfluidic networks (20) and microcontact printing of self-assembled monolayers (4). We designed an elastomeric print head with a radial pattern of fluidic channels and attached it to a glass cover slip whose surface had been functionalized with carboxyl groups. A biotin-amine conjugate and coupling solution were deposited in a well in the middle, which then filled the microfluidic spokes by capillary action. Although this process only produces a crude pattern with each spoke identically derivatized, more complicated microfluidic networks with active pumping could be designed to distribute reagents in arbitrary patterns. When the biotin coupling reaction was finished, we flushed the channels, removed the print head, and attached the rotary pump. Biotin pixels were thus defined in the regions where the spokes intersected the loop channel of the rotary pump.

Micrometer-sized neutravidin-coated latex beads were used as a simulated analyte; the beads have a diffusion constant comparable to a spore, bacteria, or the genome of the bacteriophage lambda. After a solution containing the beads was pumped into the loop, valves at the entrance and exit to the loop were closed. The beads were then allowed to diffuse within the loop, and after 4 hours, only a small fraction had managed to bind to the pixels. However, when the experiment was repeated with active pumping of the beads around the loop, >80% of the beads in the loop had bound to the biotin pixels within 4 min (Fig. 2). Thus, in this case, active



Fig. 1. A microfabricated cell sorter with integrated valves and pumps. This is a two-layer device; the bottom layer is a T-shaped fluidic channel, and the top layer contains pneumatic control lines for pumps and valves, as well as cavities to smooth out oscillations. One of the advantages of chip-based cell sorters is that they can implement sorting schemes for rare cells that are not limited by the valve switching speed. Scale bar, 1 mm. [Photograph courtesy of Felice Frankel] pumping accelerated analyte binding by at least a factor of 60. Devices incorporating such active pumping principles should have markedly increased sensitivity for a wide variety of biochemical assays and should allow efficient affinity purification in chips (21).

Besides the mechanical approach outlined here, more exotic applications of soft materials are being developed. One such area is chemical control of fluidic devices. Beebe and collaborators have shown how functional hydrogel structures can be fabricated in microfluidic channels (22). The structures swell or shrink depending on the pH of the solution and can be used to regulate flow control with "chemical feedback." Although these valves are substantially slower than the mechanical valves described above, they are interesting in that they





Fig. 2. (A) A rotary pump for sensitive surfacebinding assays. The pump is fabricated in two layers. The bottom layer is a fluidic layer containing a T junction that continues into a loop, with another channel exiting the loop. The top layer contains pneumatic control lines with pumps for each of the inputs, valves to isolate the loop (lower isolation valve not shown), and pumps within the loop. Scale bar, 1 mm. (B) Optical micrograph showing fluorescent neutravidin-coated latex beads bound to biotin pixels in the channels. Binding of the beads was accelerated by a factor of 60 with active pumping. Scale bar, 200 µm.

Fig. 3. (A) An array of diffractive optical lenses fabricated by replica molding of an elastomer. (B) A higher magnification image of one of the lenses shows that the smallest line widths are 80 nm. The feature sizes are 200 nm high.



function without external control or circuitry. There is thus the possibility of constructing autonomous, self-regulating devices.

Molding Nanostructured Diffractive Optics

Because many biochemical assays create optical signals as output, it is desirable to efficiently couple light into and out of microfluidic chips. One way to accomplish this goal is to include optics near the flow channel. Diffractive optical lenses, beam splitters, and other optical elements can be fabricated both within the glass substrate and within the elastomer material that defines the flow channels. For the latter, it is possible to use the inexpensive replication molding step for high-fidelity pattern definition of optical nanostructures.

It is historically well known that very small surface structures can be replicated with very high fidelity into polymer "replicas" (23). In fact, before the advent of modern scanning electron microscopes, the replication-molding process, followed by shadow

evaporation and transmission electron microscopy, formed the basis for microstructural surface analysis. Features as small as a few nanometers could be readily observed in carefully prepared replicas in Formvar, Collidon, Parlodion, and polystyrene, as well as cellulose tape during the 1950s (24, 25). Even higher resolution was available from evaporated carbon replicas. As a natural extension of this work, Whitesides and co-workers (5) and Chou and co-workers (11) demonstrated narrow line gratings and small pillar arrays by using embossing and replication molding steps to fabricate diffraction gratings and other fine structures within elastomeric materials. We have also used such a molding process to define diffractive lenses within the elastomer, which allows us to integrate them with our microfluidic valves and pumps.

Typically, we start by using electron beam lithography (26) rather than photolithography to generate subwavelength features in polymethylmethacrylate (PMMA) resist. Replica dies are then created in quartz or silicon by

Fig. 4. Nanobridges and resonators fabricated from silicon and gallium arsenide. (A) Reflection electron microscopy image of pillars sticking out of a surface after directional etching. The lateral width of the individual pillars is 6 nm. Scale bar, 50 nm. (B) Silicon cantilever ar-



rays with widths of 15 nm. These structures were defined by electron beam lithography, etched with chemically assisted ion beam etch, and undercut by selectively etching the SiO_2 layer below the cantilevers in hydrofluoric acid.



using the PMMA pattern as a mask during an ion etching process. When making silicon molds, we use a chemically assisted ion etch during which the sample is bombarded with argon ions while a jet of reactive XeF₂ is directed at the sample surface. The addition of XeF₂ increases the etch rate and directionality and allows the silicon to be etched at a much higher rate than the PMMA mask. This process results in the easy definition of highfidelity features with sizes as small as 50 nm (27). Of course, the initial silicon die for this process must now be defined with the opposite tone from the real lens, but this requirement is readily accomplished during the electron beam lithography process. Care must also be taken during the replication of highly anisotropic structures in order to minimize the adhesion between the die and the elastomer and to define features with the smallest possible lateral dimensions. The desired delamination between die and replica can be assisted by treating the die with organic vapors such as trimethylchlorosilane before the replication process. Figure 3 shows a replicated diffractive lens and illustrates the resolution possible in such a replication process.

Nanofabrication with Soft Templates

Nanometer-sized structures can be produced with a replication process, but elastomers, like most organic molecules, are poor conductors. To develop electrical nanodevices, one can use soft materials as templates for fabrication with materials with good electronic properties. Similarly, for nanomechanical applications, we sometimes would like to template a rigid material to achieve high resonance frequencies. The ultimate limit of template-assisted fabrication is to use a single molecule as a template, and we and others have recently made steps toward constructing molecular nanostructures. Single-molecule nanobridges, electrical point contacts, ultrasmall mechanical resonators, and wires have

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Fig. 5. Nanowires and nanoresonators formed from a DNA template. The metallized DNA wires form bridges across a lithographically defined 60-nm gap. The individual wires range from 5 to 8 nm in width. The lateral width of the bridge is controlled by the gold metallization thickness. (A) DNA nanowires across a channel. Scale bar, 100 nm. (B) DNA nanowire and diving board resonator. Scale bar, 150 nm.





all been demonstrated in efforts to miniaturize functional devices to molecular dimensions. Here, we will compare the lithographic fabrication of nanobridges with a single-molecule templating method.

Electron beam lithography and highly anisotropic pattern transfer have been used to construct nanostructures with lateral dimensions as small as a few nanometers (28). The ultimate size of the structures is limited in theory by the size of the incident electron beam and forward scattering of the exposing electrons as they interact with the resist and in practice by the resolution of the resist and shot noise in the electron dose. We have fabricated nanopillars only 6 nm in width and lines as small as 10 nm in width by using a combination of high-resolution lithography and anisotropic ion etching (29). Figure 4 shows a reflection electron micrograph of some 6-nm pillars, which were defined in GaAs/InGaAs quantum well material by combining electron beam lithography, mask deposition, lift-off, and very directional chemically assisted ion etching. Although it is possible to construct even narrower pillars, it is very difficult to electrically probe these vertical nanostructures, which are about 60 nm tall and quite fragile. For electrical measurements, it is therefore more desirable to lithographically define the nanowires in plane and use lithographic alignment methods to contact them.

When line patterns are lithographically defined in a single-crystal material grown on top of a sacrificial layer, and the sacrificial material is later removed, it is possible to define a conducting bridge. This surface micromachining method allows the definition of very small suspended structures in almost all of the interesting semiconductor materials systems, such as Si, GaAs, InP, and SiC. We have developed fabrication procedures for constructing nanoscale bridges in silicon, where the development of silicon on insulator (SOI) technology for the fabrication of electronic circuits provides us with an ideal starting wafer with SiO₂ as a sacrificial layer. SOI wafers consist of a thin layer of silicon, which was wafer-bonded onto an already oxidized silicon wafer. Then, we use electron beam lithography and anisotropic etching to transfer narrow lines through the Si layer into the underlying silicon dioxide. To release the bridges from the substrate, samples are then either dipped into hydrofluoric acid or etched in a C_2F_6 plasma. Figure 4 shows a scanning electron micrograph of 15-nm-wide Si bridges that were constructed in this way.

If nanobridges with lateral widths much below 10 nm are to be defined, traditional lithographic techniques are pushed to their limits, and it becomes advantageous to use long molecules such as DNA to construct bridges. DNA has been used as a template to chemically assemble a wire between two electrodes. The DNA molecule was stretched out on a surface and silver was precipitated around it, similar to silver staining of a DNA sequencing gel (30). Although the wires were 100 nm wide, much larger than the 2-nm diameter of a single DNA molecule, they already displayed interesting electrical properties. Others have condensed palladium onto DNA to create structures with 40nm widths (31) and have fabricated comparable structures using microtubules as a template (32). One of the attractions of using DNA as a template is the potential for directed self-assembly through the ability of single strands to recognize complementary sequences and hybridize to each other.

We have used single molecules of DNA as templates for nanowire fabrication with linewidths below 10 nm. The fabrication sequence consists of first defining a very narrow trough in insulating material. In this case, an angleevaporated shadow mask was used to define the trench in quartz. We used molecular combing (33) to stretch single DNA molecules over the trench. These molecules function as templates and vacuum evaporation was used to deposit a thin layer of gold over the molecules. By exploiting the ability to evaporate at an oblique angle, the channels are not connected electrically. The results are shown in Fig. 5-we can make nanoscopic wires with widths of <10 nm and lengths up to 60 nm (34). Thus, the resulting metal-clad DNA structure can be considered a one-dimensional quantum wire. Other structures were found in which the wire was broken, creating a "diving board." The resulting metal wires are some of the smallest resonator structures ever made. Such nanoresonators have an extremely high natural frequency, and calculations show that it may be possible to fabricate mechanical resonators in this fashion with natural frequencies on the order of a gigahertz.

Conclusion

Soft materials have many attributes that make them ideally suited for defining microfluidic, optical, and nanoelectromechanical structures with low-cost replication processes such as replication molding and templating. The ultimate resolution with which such structures can be defined is limited only by the sizes of molecules, and dimensions below 10 nm can be obtained. We believe that soft materials are poised to have a tremendous impact on both laboratory research and the practical commercialization of micro- and nanoscale devices.

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Microfabricating Conjugated Polymer Actuators

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Conjugated polymer actuators can be operated in aqueous media, which makes them attractive for laboratories-on-a-chip and applications under physiological conditions. One of the most stable conjugated polymers under these conditions is polypyrrole, which can be patterned by means of standard photolithography. Polypyrrole-gold bilayer actuators that bend out of the plane of the wafer have been microfabricated in our laboratory. These can be used to move and position other microcomponents. Here we review the current status of these microactuators, outlining the methods used to fabricate them. We describe the devices that have been demonstrated as well as some potential future applications.

The miniaturization of electronic and optical devices has fueled the information technology revolution. During the past decade, a similar miniaturization has been going on for sensors and actuators for mechanical, chemical, and biological applications. The integrated gas chromatograph was an early example (1); today, an integrated analysis system for sample handling for biological characterization has recently been developed (2).

Microstructures promise to be of great importance for the coming biotechnology revolution. There is currently a tremendous increase in both academic and corporate research on micromachined laboratories-on-a-chip, or micrototal analysis systems (3). These devices will find applications in areas such as genomic and proteomic studies, which will require extensive parallelism to allow many small simultaneous experiments. The integration of multiple experiments on a single carrier requires a miniature format. To minimize the chance of cross contamination when handling biological samples, a single-use device is preferred. Therefore, these devices should be disposable and thus be produced with inexpensive materials and patterning techniques. Polymers might be an option.

Polymers can be patterned by inexpensive

methods such as hot embossing and imprinting and therefore make attractive carrier materials. Imprint methods allow submicrometer patterning with dimensions smaller than 100 nm, as has been demonstrated with hard (4)and soft (5) imprint materials.

Polymers can also deliver active functions. Polymer surfaces can be chemically modified in a variety of ways, and this property is important in microstructures, which have a high surface-to-volume ratio. For example, surface-bound processes may be used to alter biomolecular function (6). Some polymers even allow the formation of electronic devices. Field effect transistors with useful carrier mobility have been made (7). Because thin polymer films may be easily prepared by spin coating, they can be integrated into functional systems. This capability may be important in the development of inexpensive, disposable chemical detectors for genomics and proteomics.

Handling, transport, separation, and detection of most biological species are done in liquids. Cells, organelles, and biomolecules are kept in buffer solutions or blood. The rapid handling of small sample volumes will require microfluidic technology. The movement of samples requires some driving force. One approach being pursued for fabricating small laboratories is to emboss compact discs (CDs) with microfluidic systems (8, 9). These devices use the inertial forces on the rotating CD to force liquids through channels. Another approach is to use microactuators to pump or redirect the samples (10). Microactuators that are part of lab-on-a-chip systems may need to operate in biological environments and under liquid flow. The response time of these actuators should preferably be in the range of seconds or faster, depending on the specific application. Such actuators, when included in the chip, should preferably also be simple and inexpensive to produce and should be easily integrated with detector systems. Polymeric microactuators may be able to meet this need.

Conjugated Polymer Actuators

We are currently developing polymer actuators for use in biomedicine and biotechnology. These devices are all based on conjugated polymers, which undergo volume changes during oxidation and reduction. The use of conjugated polymers as volume-changing materials began in the 1980s, primarily in bilayer devices (11-16). One layer of the bilayer was typically a passive substrate onto which the active conjugated polymer layer was applied.

Bilayers were initially used to determine the amount of volume change and to identify the volume change mechanism (16, 17). Bilayers provided a simple way to study classical conjugated polymers, such as polypyrrole, polyaniline (18, 19), and polythiophenes (20, 21). The active polymer layer was deposited onto a substrate, usually by electrochemical synthesis. Under electrochemical oxidation and reduction, volume change in the active layer forced the assembly to bend, and the direction and magnitude of volume change could be deduced from the direction and degree of bending. Such bilayer structures were typically a few centimeters long and a few millimeters wide. These studies showed that the volume change in the conjugated polymer is dominated by ionic movement into and out of the polymer.

Other studies were done on single conducting polymer fibers submerged in a liquid electrolyte and connected to force- and elongation-measuring equipment. Speed of actuation, stress, and strain were measured, veri-

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