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MICROFABRICATION IN BIOLOGY AND MEDICINE

Joel Voldman[1,2], Martha L. Gray[1,2,3], and Martin A. Schmidt[1,2]

[1]*Department of Electrical Engineering and Computer Science,* [2]*Microsystems Technology Laboratories, and* [3]*Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139; e-mail: voldman@mtl.mit.edu, mgray@mit.edu, schmidt@mtl.mit.edu*

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■ **Abstract** Microfabrication uses integrated-circuit manufacturing technology supplemented by its own processes to create objects with dimensions in the range of micrometers to millimeters. These objects can have miniature moving parts, stationary structures, or both. Microfabrication has been used for many applications in biology and medicine. These applications fall into four domains: tools for molecular biology and biochemistry, tools for cell biology, medical devices, and biosensors. Microfabricated device structures may provide significantly enhanced function with respect to a conventional device. Sometimes microfabrication can enable devices with novel capabilities. These enhancing and enabling qualities are conferred when microfabrication is used appropriately to address the right types of problems.

Herein, we describe microfabrication technology and its application to biology and medicine. We detail several classes of advantages conferred by microfabrication and how these advantages have been used to date.

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INTRODUCTION

Microfabrication is a process used to construct physical objects with dimensions in the micrometer to millimeter range. It takes advantage of established semiconductor fabrication processes, used to make integrated circuits, and augments these with processes specially developed for microfabrication.

Microfabricated objects or devices can be comprised of a range of miniature structures, including moving parts such as cantilevers and diaphragms, static structures such as flow channels and wells, chemically sensitive surfaces such as proteins and cells, and electrical devices such as resistors and transistors.

Microfabricated devices, also known as microelectromechanical systems (MEMS), micromachining, lab-on-a-chip, microsystems, and micro-total analysis systems (micro TAS), have existed for >30 years, with several applications attaining commercial and/or scientific success. Although there have been a few applications to biology or medicine during that time, only in the past decade has a closer union emerged. Several factors have driven this recent fusion. Commercially, high-throughput, low-volume-consumption technologies such as whole-genome sequencing projects and drug discovery have created a need for these devices. Scientifically, the ability to design and control experiments at the micrometer scale has attracted the interest of biologists, who have started devising fundamental studies using this technology.

When applied in the right instances, microfabrication can either significantly enhance a device in relation to its conventional counterpart or enable entirely new devices. An example of an enhancement is the more uniform, accurate, and reproducible flow chamber geometries that can be constructed by microfabrication as opposed to conventional machining (Figure 1). Enabling means that some aspect of microfabrication allows for the design of a novel device, such as precise geometrical control enabling one to pattern the specific spatial relationships of two cell types in cocultures (Figure 2).

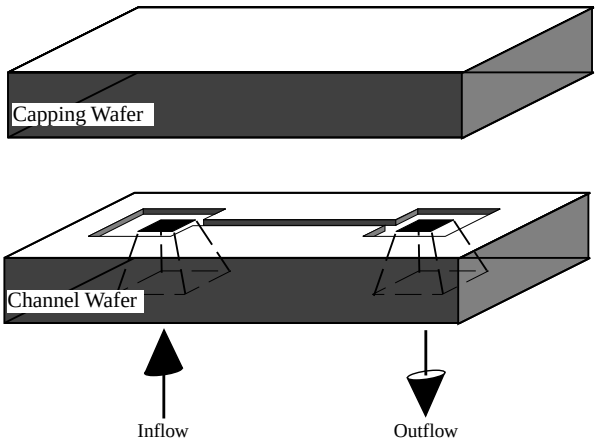

FIGURE 1 Schematic of flow chamber microfabricated in silicon (9). Many microfabricated devices have flow structures of this type.

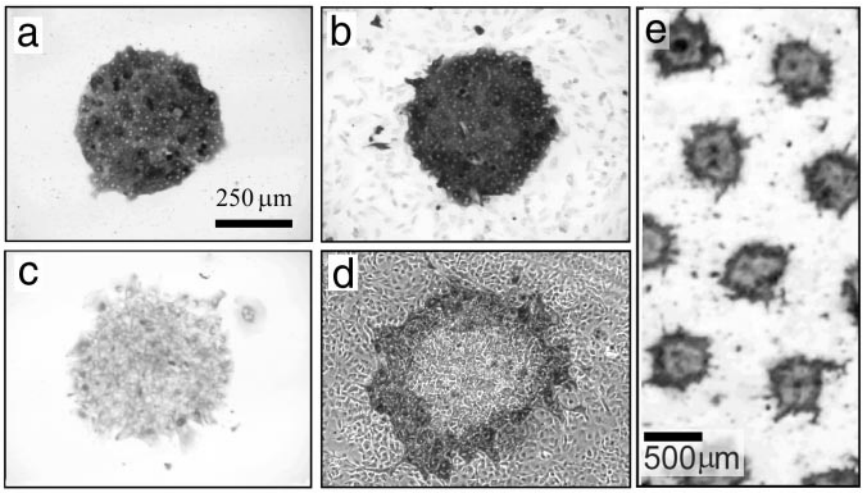

FIGURE 2 Circular micropatterns of hepatocytes (*b, d*) with and (*a, c*) without fibroblasts cocultured on the perimeter, fixed and stained for albumin directly (*a, b*) after culture or (*c, d*) on day 6. Dark staining on day 1 indicates viable, functioning hepatocytes. (*d*) By day 6, only hepatocytes in proximity to fibroblasts continue to synthesize albumin. Panel (*e*) is a large-area view of (*d*), showing pattern reproducibility. Bhatia et al used microfabricated methods to ''pattern'' cell arrangements to explore how heterotypic cell interactions influenced cell function (16).

In this review, we describe the ways in which microfabrication has been applied to medicine and biology. First, we discuss microfabrication technology. This gives a background of the processes and materials that are used to create these structures. This knowledge is crucial because devices cannot be separated

from their fabrication processes; knowing how all the processes work together determines what can be made.

Next, we describe the areas in which microfabrication has made an impact on biology and medicine. Our approach is to emphasize the different advantages that microfabrication can confer and describe a few devices in terms of these advantages. This is more useful than simply reciting a set of applications because it will help the readers discern the proper role of microfabrication in medicine and biology and determine how and when to use it themselves.

Besides this article, the reader is referred to other general reviews (49, 75, 87, 137). In addition, a repository of micromachining information resides on the World Wide Web (96a).

MICROFABRICATION TECHNOLOGY

Overview

Microfabrication uses a sequence of process steps (a process flow)—some taken from semiconductor fabrication technology and some developed specifically for micromachining—assembled together in a given order to produce a physical structure. The variety of process steps and materials leads to a large range of possible devices.

In a microfabrication process, one takes a substrate and builds a device either out of its bulk material or on its surface. The former is referred to as bulk micromachining (74), whereas the latter is known as surface micromachining (29). Figure 3 is a schematic example of each type of process. Often, devices are built with a combination of both types of machining. In either case, four basic processes are used. The first is photolithography, which transfers a pattern into a material. The second is thin-film growth/deposition, in which thin films (usually on the order of micrometers in thickness) are grown or deposited onto a substrate. Etching, the third kind of process, creates features by selectively removing materials (either thin films or substrate) in defined patterns. The final kind of process is bonding, where two substrates (often structured and with thin films) are bonded together. In this section, we will give a brief overview of the technology. More detailed discussions can be found in books on microfabrication (90, 110) or semiconductor processing (125, 139).

Substrate Materials

Silicon Silicon is the most common material in microfabrication, owing to its role in the fabrication of integrated circuits. It comes in a single-crystal wafer form, with typical diameters of 75–200 mm and thicknesses of 0.25–1.0 mm. In addition to its excellent electrical properties, silicon also possesses outstanding mechanical properties, enabling the design of micromechanical structures (106). There exists a wide range of ways to micromachine silicon, and the ability to do

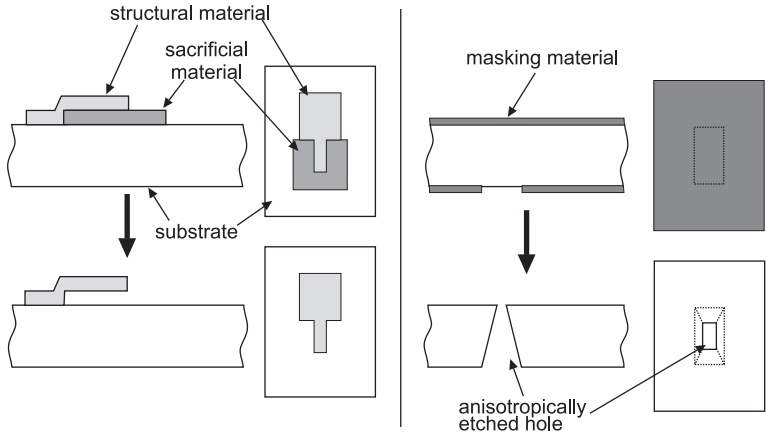

FIGURE 3 Side and top views of examples of (*left*) surface and (*right*) bulk micromachined structures. On the left, a sacrificial material and a structural material are deposited and patterned. The sacrificial material is removed to create a released cantilever. On the right, a hole is anisotropically etched in silicon (see text).

this in combination with integrated-circuit fabrication leads to the potential to form monolithically integrated microsystems.

For biological or medical microsystems, silicon may not be the material of choice. It is not optically transparent, preventing the use of transmission microscopy, and its cost can potentially be too large for disposable devices.

Glass Although the range of micromachining processes for glass is less extensive than for silicon, glass provides some unique features, most notably optical transparency. Glass wafers are available in many different compositions and sizes. Two important examples are fused silica wafers and borosilicate wafers. Fused silica wafers are pure amorphous silicon dioxide (SiO_2). They can withstand high temperatures ($T_{\text{softening}} = 1580°C$), are optically transparent down to short wavelengths, and have very low autofluorescence. Borosilicate wafers, of which the most common is Pyrex© (Corning 7740), are much less expensive than fused silica (and can be less expensive than silicon). They can be easily bonded to silicon but cannot be exposed to the high temperatures needed for some thin-film depositions and have higher autofluorescence than fused silica.

Plastics Plastic is often the least expensive substrate material. The availability of mass production processes (e.g. injection molding, embossing) that can be extended to the microscale means that plastic devices can be extremely inexpensive to produce in volume. This allows for disposable devices, which minimizes issues of sterilization, clogging, and drift. For these reasons, a majority of commercial enterprises are using plastic microdevices, especially for disposable clini-

cal applications. Most devices to date have been separation channels for capillary electrophoresis. Methods of fabrication include injection molding (92, 96), hot embossing (12, 93), and casting [especially of poly(dimethylsiloxane)—PDMS] (43, 45), all of which are batch processes capable of replicating a whole wafer at a time. While these methods are not conventional microfabrication processes, they all require standard silicon or glass microfabrication in the beginning to make the master/mold. In addition, the softness of plastics can mean poor dimensional tolerance and stability, and autofluorescence is often a problem.

Photolithography

Photolithography is used to transfer a pattern envisioned by the designer into a material. The process is depicted in Figure 4. A pattern, drawn with a computer-assisted design (CAD) program (Figure 4*a*), is transferred onto a mask (Figure 4*b*). The mask is a glass plate that has on its surface a photodefinable opaque material (usually chrome) in the desired pattern and is typically prepared by a mask vendor. If the features and tolerances in the pattern are relatively large (>20 μm), then one can use a simpler mask-making process (43). After mask making, the pattern transfer begins when the substrate (Figure 4*c*) is spin-coated with photoresist (Figure 4*d*), a photosensitive organic polymer. The substrate and mask are brought into contact, and UV light is shown through the mask and onto the photoresist (Figure 4*e*). Photoresist under the transparent portions of the mask will be exposed, causing it to become soluble in a developing solution. This is known as a positive photoresist (negative photoresist gives the inverse pattern). The wafer and mask are separated, and the exposed photoresist is removed in the developing solution (Figure 4*f*). The photoresist can now be used as a protective mask to transfer the pattern into the underlying material via etching. When finished, the photoresist is removed.

A different method of pattern transfer called microcontact printing has recently been introduced (140). Microcontact printing uses a soft polymeric stamp, usually

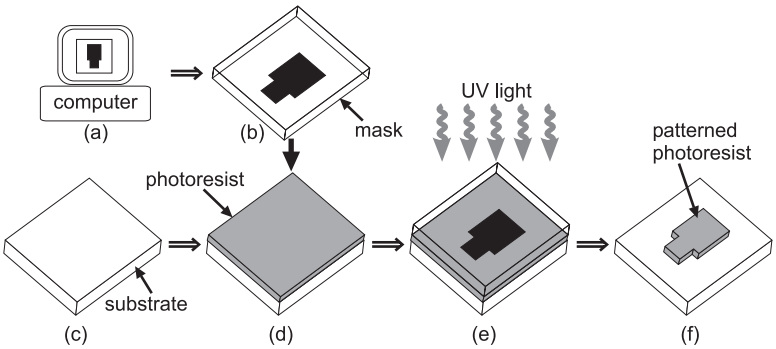

FIGURE 4 Pattern transfer with photolithography.

made of PDMS, which has been formed by molding to a master made by conventional microfabrication. The stamp is ''inked'' with alkanethiols or alkylsilanes and placed on a gold- or silicon dioxide–coated surface, respectively. This transfers the molecules from the stamp to the substrate, where they form a self-assembled monolayer in the same pattern as the stamp. These patterned self-assembled monolayers can then be used as resists for etching or as passivation layers to prevent deposition. This method of pattern transfer is advantageous when working with non–cleanroom-compatible materials or chemicals, or nonplanar substrates, although unresolved issues exist with multilevel pattern registration.

Thin-Film Growth/Deposition

Thin films are used for a variety of different purposes in microstructures—masking materials, structural materials, sacrificial materials, and electrical devices, to name a few. They are formed by either chemical-reaction–driven processes or physical processes.

Dielectrics Two of the most common films are silicon dioxide and silicon nitride, often used for electrical isolation or as etch masks. Thermal silicon dioxide is grown by placing a silicon substrate in a high-temperature (900–1200°C) oxidizing ambient. Growth is limited to $\sim$1 μm because thicker layers take prohibitively long to grow. Thicker films (10–20 μm) can be obtained with chemically deposited oxides, although they are not as robust as thermally grown oxide films. Silicon nitride is always deposited.

Silicon Polycrystalline and amorphous silicon thin films, deposited by chemical-reaction–driven processes, are frequently used as structural materials in microsystems (29). In addition, dopant atoms can be introduced into the surfaces of silicon wafers to make thin doped films of single-crystal silicon that can be used as etch stops for wet silicon etching (116).

Metals Metals (Al, Au, Pt, etc), physically deposited (90) or electroplated (58), are usually used for electrical interconnects and electrodes or as replication-process masters, although they can also be used as surfaces for self-assembled monolayers (140).

Plastics Plastics can be used as compliant mechanical structures, as thick structural layers for molding, or as chemically sensitive films. Polyimides have been utilized for many years in microfabrication (84), as have spin-on silicone-rubber films (20, 133) and photoresist (118). SU-8, an epoxy-type photoresist that can be spun on into thick layers ($>$100 μm) and can make anisotropic structures (81, 88), has been used as a mold for PDMS casting (43) and as a structural layer for microchannels (65). Plastic films can also be deposited by a range of methods building off of microcontact printing, including micromolding in capillaries (73,

140), microtransfer molding (140), and solvent-assisted micromolding (140). Finally, transparent plastic films (parylene C) can be vapor deposited (91).

Biomolecules Deposition and patterning of biomolecules, most commonly proteins, are quite important in biological applications of microfabrication. Three predominant methods to accomplish this have been reported (18). The first method, protein adsorption, relies on physical adsorption of proteins in solution onto a substrate. The patterning is achieved either by dissolution of protein-covered photoresist patterns (18) or by constraining where the protein solution flows via microchannels (18, 39, 51). The cocultures shown in Figure 2 were made by patterning extracellular-matrix proteins in the former manner. The second method of biomolecular patterning is using photochemistry, where UV light shown through a patterned mask is used to activate or deactivate chemical species (18). The final method uses patterned self-assembled monolayers to selectively inhibit or allow protein attachment (18, 99, 140).

Etching

Etching can be divided into wet (via liquid chemicals) or dry (via gas-phase chemistry) etching. Either method can lead to isotropic or anisotropic etching. Isotropic etching etches in all directions equally, leading to mask undercutting and a rounded etch profile (Figure 5, *left*). Anisotropic etching is directional (Figure 5, *middle*, *right*) and is either chemically or physically (sputter etching) induced. In general, wet etching is more selective than dry etching, whereas anisotropic etches are more common with dry etching. Chemical etches are more selective than physical etches but amenable to fewer materials. The aim is to find a complementary set of materials and etchants, thus allowing selective pattern transfer.

Thin Films Thin films—to remove or structure them—can be wet etched with a variety of different chemistries, and certain ones are amenable to dry etching. An extensive list of wet chemical etchants for different thin films can be found in the book chapter by Kern & Deckert (70).

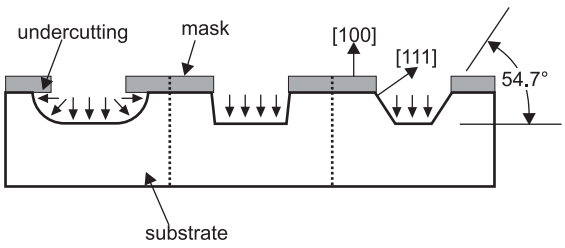

FIGURE 5 Overview of (*left*) isotropic and (*middle*, *right*) anisotropic etching. Anistropic etching by (*middle*) dry etching or by (*right*) wet anisotropic etching.

Silicon Wet etching of silicon, commonly used in bulk micromachining (Figure 3, *right*), can be either anisotropic or isotropic. Wet isotropic etching can be performed with solutions of hydrofluoric acid and nitric acid (111). Wet anisotropic etchants, such as would be used to make the hole in Figure 3 (*right*), exploit the crystallinity of silicon by etching the {111} crystal planes slower than the {100} planes, leaving a characteristic 54.7° sidewall on a [100]-oriented wafer (Figure 5, *right*). The primary wet anisotropic etchants are potassium hydroxide (KOH), tetramethylammonium hydroxide (TMAH), and ethylenediamine-pyrocatechol (EDP) (115, 116, 126). These wet-etching processes require minimal equipment and are easy to set up.

Standard dry etching can be used to etch silicon to depths ranging from submicrometer to ~10 μm, giving either isotropic (Figure 5, *left*) or anisotropic (Figure 5, *middle*) profiles. This process is commonly employed to make shallow trenches in silicon or to pattern thin silicon films. A new variant of dry etching is deep-reactive-ion etching (DRIE) (10). This technique, introduced in the past 5 years, is revolutionizing microfabrication. Although the technology is quite expensive, it has the capability to make very deep and narrow structures in silicon. A final dry silicon etchant is xenon difluoride (XeF_2), a gas-phase etchant that etches silicon isotropically at room temperature (32).

Glass Glass wet-etching, such as is used to make fluidic channels, is performed using a hydrofluoric acid–based chemistry, with the amorphous nature of the glass leading to an isotropic etch. Pure SiO_2 (fused silica and thin-film silicon dioxide) can be etched in hydrofluoric acid or buffered oxide etch ($HF:NH_4F$). Wet chemistries have been developed, using metal/photoresist (48) or silicon masks (34, 35, 57, 121), to etch non-pure glass substrates, which pose the additional problem of removing the impurities present within. Pure SiO_2 can be dry etched, whereas non-pure glass substrates cannot and so must be sputter etched (79). Thru-holes can be machined in glass via ultrasonic drilling (90), electrochemical-discharge machining (47, 121), or conventional drilling.

Plastics Plastics are usually sputter etched, although some (e.g. photoresist, parylene C) can be etched in oxygen plasmas.

Bonding

In many processes, there will be a desire to bond two substrates (possibly with thin films) together to form a hermetic seal. A common example is the bonding of a glass capping wafer to a structured silicon wafer to form an optically accessible sealed system. Many technologies have been developed to bond different materials together, either with or without intermediary layers (113).

Anodic bonding is a widespread form of bonding in biological microstructures. This bond occurs between an impurity-laced glass wafer (most often Pyrex©) and a silicon wafer. By applying heat (~400°C), a high electric field, and pressure to

the two materials, a hermetic, irreversible, high-strength bond can be obtained. Owing to the high temperatures involved, the glass and silicon must have similar thermal expansion coefficients.

Fusion bonding, which occurs via chemical reactions between the bonding surfaces, can be used to bond a larger variety of materials together. A bond is formed by contacting two ultraclean surfaces and annealing them at high temperature (~1000°C). Although it is most often used with silicon or thermally grown silicon dioxide surfaces, fusion bonding can also be obtained with deposited oxides and nitrides, although they must be smoothed first with a chemical-mechanical polish (5, 59).

Other bonding techniques abound. To join two metal layers together, one can use eutectic or thermocompression bonding (113). Substrates can be bonded with adhesives (92), whereas plastics can be bonded by heating them to above their glass transition temperature and then compressing them (93). PDMS can be reversibly hermetically bonded to glass or to itself by simple contact (45) and can be irreversibly bonded to itself by oxidizing two pieces and placing them together (43).

Sample Process Flow

With all the information on substrates, layers, and the ways to shape them, we can now step through the process flow for a simple device—the flow channels shown in Figure 1. Starting with a silicon substrate (Figure 6*a*), we grow a thin

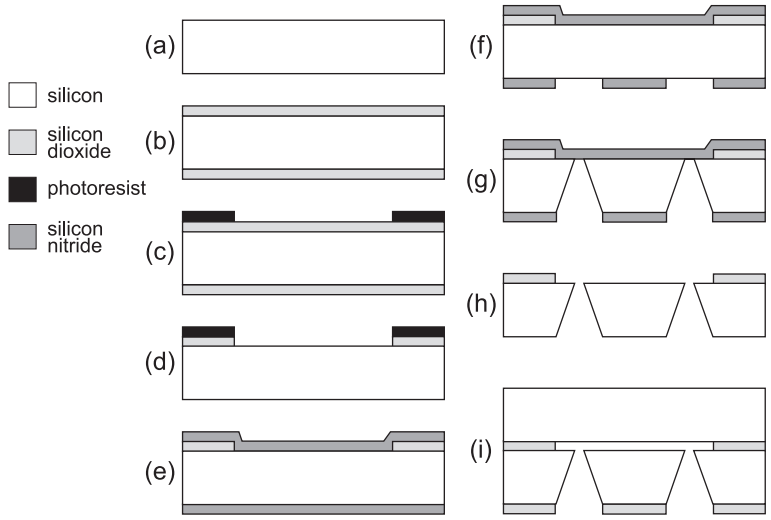

FIGURE 6 Cross-sectional view of the process flow for the flow channels shown in Figure 1. Note that the drawing is not to scale; the silicon substrate is ~500 μm thick, whereas the other layers are on the order of micrometers in thickness.

layer of silicon dioxide on it (Figure 6*b*). This layer, owing to the accuracy and uniformity of its thickness, will be used to define the channel height. We then perform a photolithography step on the front side of the wafer to pattern the channel in photoresist (Figure 6*c*). The patterned photoresist is used as a mask during a wet etch of the oxide, which selectively stops at the silicon surface (Figure 6*d*). After stripping the photoresist, we deposit a thin film of silicon nitride (Figure 6*e*). This layer, because it is not etched by KOH, will be used as a mask for the subsequent silicon etch. We perform another photolithography step on the backside of the wafer to define the inflow and outflow holes, transfer this pattern into the nitride with a dry etch, and then remove the photoresist (Figure 6*f*). Next, we anisotropically etch the silicon in KOH to form the inflow and outflow holes (Figure 6*g*), and then remove the silicon nitride (Figure 6*h*). Finally, we fusion bond a bare silicon wafer to our structured wafer to form the flow channels (Figure 6*i*).

APPLICATIONS OVERVIEW

With an understanding of the technology, we can now examine the many applications of microfabrication to biology and medicine. For organizational purposes, we have distributed them into four domains: tools for molecular biology and biochemistry, tools for cell biology, tools for medicine, and biosensors. Specific examples of devices in the first three domains and how each takes advantage of microfabrication are listed in Tables 1, 2, and 3. In this section, we will give an overview of each application domain and the regions of interest within them. This will be followed in the next section by a discussion of a few devices that cleverly exploit microfabrication's advantages.

TABLE 1 Microfabricated devices with applications in molecular biology and biochemistry

Description	Advantages of μfab.[a]	Reference(s)
Channels for molecular separations	High SA/V[a] ratio High throughput Small volumes	(11, 12, 27, 28, 43, 45, 48, 93, 94, 96)
Nucleic acid arrays	Batch processing High throughput Small volumes	(27, 66, 94, 103)
On-chip PCR chambers	High SA/V[a] High throughput Small volumes Integration	(27, 94)
Piercing structures for DNA injection	Batch processing Geometrical control	(63)

[a]Abbreviations: SA/V, surface area to volume; μfab., microfabrication; PCR, polymerase chain reaction.

TABLE 2 Microfabricated devices with applications involving cells or cell biology

Description	Advantages of μfab.[a]	Reference(s)
Chambers for studying MT dynamics	Constrained geometry	(42, 67)
Sperm/embryo tools		
Sperm motility, in vitro fertilization	Constrained geometry	(77, 78)
Embryo branding	Geometrical control	(134)
Force measurements with bending cantilevers	Small size Single-cell analysis Geometrical control	(54, 83, 101)
Cell mechanics	Single-cell analysis Geometrical control	(24, 30, 71, 104, 124, 135)
Cell dynamics	Small volumes Constrained geometry	(2, 22, 33, 36, 95, 102, 138)
Flow cytometry/sorting	Small size Small volumes	(3, 6, 7, 17, 98, 121)
Dielectrophoresis and electrorotation	High SA/V[a] ratio Small size Single-cell analysis	(52, 53, 107)
Electrodes for recording/ stimulating electrogenic cells	Small size Integration Batch processing	(4, 19, 23, 76, 100, 122, 123, 136)
Impedance monitoring for cell motility and micromotion	Single-cell analysis	(55, 61, 69, 85)
Chemical/physical substrate patterning	Geometrical control Batch processing	(16, 18, 31, 37, 99, 119, 122, 140)

[a]See footnote a in Table 1.

Before describing the application domains, though, we first discuss microfluidics, which provides the foundation for the applications. Liquids on small scales behave quite unfamiliarly. They flow without turbulence and mix due to diffusion only. Purging of bubbles becomes a difficult problem. Microfluidics is the study of fluid flow and the design of devices that operate at these scales (26, 46, 56, 108).

Because the vast majority of biological and medical applications of microfabrication utilize liquids, building devices to handle liquids is crucial. Many conventional fluidic elements have been scaled down, including pumps (105, 131, 141), valves (20, 84, 130, 133), filters (25, 40), and mixers (21, 97, 114). It is much more difficult and rare to integrate (i.e. fabricate on a single or hybrid chip, rather than assemble) diverse components together and with analysis components (8, 28, 132). The designer must decide which components should be integrated into a microsystem; total integration is usually not the best solution.

TABLE 3 Microfabricated devices with applications in medicine.

Description	Advantages of μfab.[a]	Reference(s)
Blood pressure sensors	Mechanical properties Integration	(44)
Micro-velcro for tissue fastening	Geometrical control Batch processing	(62)
Minimally invasive surgery	Small size	(38, 60, 68, 72, 80, 82, 128)
Medical therapeutics		
Functional electrical stimulation	Small size Integration	(13, 14, 127)
Cell transplantation	Geometrical control	(40)
Drug delivery		
Internal	Small size Integration	(112)
Transdermal	Geometrical control Batch processing	(64)

[a]See footnote a in Table 1.

Tools for Molecular Biology and Biochemistry

The ability of microfabricated devices to interrogate and manipulate biomolecules is rapidly emerging (Table 1). Applications relating to genome sequencing and genomics have received widespread attention because of their use in clinical diagnostics and human disease. These applications are among the most mature (>10 years) and are starting to enter the commercial sector. Coupled with the bioinformatics revolution, they are poised to have a significant impact on people's everyday lives.

Tools for Cell Biology

Moving up in length scale from biomolecules, microfabricated devices can also be used to interrogate and manipulate cells themselves. In some ways, it is more challenging to manipulate cells than biomolecules because of the need to maintain viability. Although less publicized, this application area is very exciting because of its potential to enable experiments that can answer fundamental scientific questions. Published work in this area spans from devices for examining subcellular components, such as the cytoskeleton, to devices involved in cell biology on single cells, such as single-cell force sensors, to devices for multicellular analysis, such as flow cytometers (Table 2).

Tools for Medicine

Microfabricated blood pressure sensors are routinely used in clinical practice. They represent one of the few areas where microfabrication has already made a

large impact on clinical medicine. Other applications in this domain (Table 3), which includes devices or instruments with primarily medical purposes, are less well established in clinical medicine, and their acceptance will continue to remain a challenge.

Biosensors

Many biologically relevant substances either need to be sensed or can act as sensors themselves. In fact, many of the applications cited in this review can be strictly defined as biosensors. A biosensor is a system (not necessarily microfabricated) that converts a biological signal into a (usually) electrical one. Examples of biosensors include sensors for small molecules (oxygen, pH, glucose) and large molecules (immunosensors). The field of biosensors is quite developed, having been around for $>$30 years. Thus, we refer the reader to the appropriate literature. Many review articles and monographs deal at least in part with microfabricated biosensors (15, 41, 86, 89, 90, 109, 120, 129). In addition, several journals exist that cover biosensors and microfabrication, such as *Sensors and Actuators B, Biosensors and Bioelectronics*, and *Biosensors*.

ADVANTAGES CONFERRED BY MICROFABRICATION

Microfabrication should be used only when it will significantly enhance an existing device or enable a new device. The most successful applications cleverly exploit the advantages bestowed by microfabrication. As stated previously, we have chosen to describe applications in terms of the advantages that they utilize, as opposed to simply reciting the applications themselves. In Tables 1–3, we have listed the two or three most significant advantages for each application area. Taking this list of advantages, we will now describe each one in turn and selected devices that make use of them.

Small Device Size

Miniaturization itself can be a reason to microfabricate. Size effects can have either enhancing or enabling characteristics.

Dielectrophoresis and electrorotation devices (Table 2) fall into the first class. In these devices, nonuniform electric fields, applied by electrodes, are used to generate forces that can manipulate cells or large molecules. The forces generated scale with the gradient of the square of the electric field intensity. Miniaturizing the spaces between driving electrodes increases these gradients, and thus the forces, while simultaneously reducing the driving voltages necessary to generate them. This enhances the devices, allowing them to operate with a few volts instead of a few hundred volts.

The second class of size advantages is in enabling something that could not be done before. Small devices are portable and can be placed in constrained spaces. One trend is in systems for point-of-care use, such as in the medical

practitioner's office or the field (instead of in a centralized lab). Such systems need to either be hand-held or fit onto a small tabletop. Microfabricating key elements, which reduces system size, enables the application. One example is microfabricated flow cytometers (Table 2), which use microfabrication to make miniature flow chambers. Although it will be difficult for these devices to compete with the power and versatility of conventional flow cytometers, their portability may be useful for point-of-care hematological tests.

High Surface-Area-to-Volume Ratio

As devices are miniaturized, their surface area relative to their volume increases. At small enough scales, this leads to a situation where surface effects dominate volume effects. Remarkable physical enhancements result. One benefit exploited by electrophoretic channels (Table 1), polymerase chain reaction chambers (Table 1), and dielectrophoresis and electrorotation devices (Table 2) is that heat removal is enhanced as the device is miniaturized. For both dielectrophoresis and electrorotation devices and electrophoretic channels, this means that higher electric fields than in conventional systems can be used without adverse heating effects. This gives faster and better separations for electrophoretic channels and larger forces for the dielectrophoresis and electrorotation devices. For polymerase chain reaction chambers, the high heat removal decreases thermal response times, allowing for more rapid temperature cycling.

The most notorious disadvantage of increased surface-area-to-volume ratio is that surface adsorption of biomolecules increases, lowering yields. There is ongoing research into this problem (117).

Integration with Electronics

In principle, the close relationship between microfabrication and conventional semiconductor fabrication allows one to integrate electronics or electrical components with microfabricated systems. The challenge lies in establishing mutually acceptable process steps to achieve this integration.

One simple level of integration is fabricating piezoresistors in silicon. Piezoresistors transduce mechanical stress into electrical resistance changes. Some blood pressure sensors (Table 3) integrate these onto pressure-sensitive diaphragms. Upon deflection by an applied pressure, the piezoresistors change resistance. Thus, the electronic devices enable the integration of the transduction element (the piezoresistor) with the mechanical element (the diaphragm).

Higher levels of integration include fabricating an integrated circuit with the microfabricated device. This is used by some probe-style electrode arrays that record signals from neurons in intact cortical tissue (100, 136) (Table 2). These devices consist of sharp silicon needles with integrated electrodes. They are inserted into cortical tissue and record extracellular signals from neurons. The integrated circuits generate stimulus currents, amplify recorded signals, and process the data to reduce the number of electrical leads needed.

High Throughput

Sometimes miniaturization leads to high-throughput devices by either parallelization or enhanced serial transfer. This is common in systems for genomic research and drug discovery. A rate-limiting step for researchers in these fields is throughput. Miniaturization of channels for capillary electrophoresis (Table 1) not only means faster analysis time because of the gain in surface area to volume but also the ability to array many capillaries in a small space and operate them in parallel. Both of these effects increase throughput.

Microfabricated nucleic acid arrays (Table 1) are another example. Nucleic acid arrays essentially perform a Southern or Northern blot at each active site, and so constructing arrays with tens of thousands of sites allows for that many simultaneous assays. Thus, these arrays can be used to probe the expression of many genes simultaneously or to look for mutations at many places in a genome.

Small Sample Volumes

Decreasing the volume of sample consumed in an assay can be beneficial for several reasons. From a financial standpoint, reducing reagent volumes and waste disposal by a large factor can reduce assay costs. In addition, for drug discovery applications or some medical diagnostics, the sample materials are scarce. Reducing the needed volume can thus extend the use of each sample.

Systems for monitoring cell dynamics (Table 2) often make use of small sample volumes. The Cytosensor Microphysiometer (Molecular Devices Corp., Sunnyvale, Calif.) uses a microfabricated planar pH sensor to sense the extracellular acidification rate as a means of monitoring cell physiology (95). The pH sensor can be used in a small volume, allowing it to be placed at the bottom of a small cell-culture chamber. Operating in such a small volume effectively increases the volume cell density. This increases the rate of acidification, easing detection. In this case, the small volume decreases detection requirements, enabling the system.

One disadvantage of small volumes is that the detection of molecules in dilute solutions becomes more difficult. This is because, for a given solute concentration, the number of molecules scales as the cube of the volume. At small enough volumes, the number of molecules may approach detection limits.

Batch Processing

Many microfabrication processes can be performed as easily on one device as on a thousand. Such batch processing can make thousands of identical devices not subject to the variations present in individually constructed objects. This is exploited by planar electrode arrays that record from and stimulate neurons in culture (23, 122, 123) (Table 2). After one electrode is made, it is only incrementally more difficult to make a large array of them. One can then record from and stimulate many neurons. Contrast this with conventional intracellular electrodes, where the use of multiple electrodes becomes logistically difficult.

Another application that well illustrates the advantages of batch processing is nucleic acid arrays fabricated by photolithography (Table 1). For an $n \times n$ nucleic acid array, there are n^2 different oligonucleotides of length l. Synthesizing each oligonucleotide individually would require n^2l chemical steps. Fodor et al's method uses selectively masked photochemistry to synthesize the oligonucleotides (50). It requires four chemical steps (one for each base) per unit length, or $4l$ steps irrespective of the number of different oligonucleotides. Thus, one can make a 4×4 array of octamers as easily as a 200×200 array. This dramatically decreases the difficulty of making large arrays.

Geometrical Control

Geometrical control can be very important for microstructures. Photolithography allows one to pattern largely varying geometries (1 μm to >1 cm) in the same space with micrometer dimensional accuracy. In addition, one can vary dimensions of the same feature on a mask, instantly making tens of different but similar structures.

One creative use of this concept is by Bhatia et al to investigate cell–cell interactions in cocultures (16) (Table 2). Using microfabrication, they could precisely control the spatial organization of hepatocytes and fibroblasts. This made it possible to eliminate variations present in random cocultures, such as amount of heterotypic interface between the two cells types, amount of homotypic interface, and hepatocyte:fibroblast ratio. They found that liver-specific function (as measured by albumin and urea synthesis) is dependent on the amount of heterotypic interface in the coculture and that, as shown in Figure 2, albumin production is localized to hepatocytes at this interface. Such a study would be impossible to perform without microfabrication.

Constrained Geometries

Often all one needs is a small constrained geometry, such as a small well. Constrained geometries can be used to confine either molecules or mechanical forces.

Confining molecules prevents diffusion out of a volume, increasing a molecule's local concentration. This is cleverly exploited by applications involving electrochemical or optical probing of cells in small wells (22, 33, 36) (Table 2). Here the microfabricated wells allow the analyte being probed to remain concentrated, instead of being diluted into a large solution volume.

The advantages of confining forces are well illustrated by the work of several investigators examining the assembly of microtubules (MTs) in microfabricated structures (42, 67) (Table 2). Using cell-sized chambers microfabricated in glass coverslips, Holy et al examined the assembly of MTs from artificial MT-organizing centers consisting of tubulin-covered beads (67). In these constrained geometries, results showed that MT polymerization alone could position the artificial MT-organizing centers in the middle of the well, suggesting that these forces are important when considering MT dynamics. Another study used shallow chan-

nels with MTs attached to the bottom surface (42). By looking at MT bending as it polymerized and hit the wall of the channel, they could determine its force-velocity relationship. Both of these experiments would not work in free solution; microfabricated constrained geometries enable the experiments.

It is worth noting that sample evaporation can be a problem when using small wells; picoliters of fluid can evaporate in seconds. Special precautions are needed to avoid this (33).

Single-Cell Analysis

Shrinking devices can enable single-cell analysis for any of the aforementioned reasons (e.g. constrained geometries). The power of this lies in the heterogeneity of cell populations, which bulk measurements cannot discern. Analyzing multiple single cells can reveal the variations within populations.

This is illustrated by a device for measuring erythrocyte mechanics (124) (Table 2). These researchers have used microfabrication technology to create a device with optically accessible uniform grooves. This device, coupled with an image acquisition system, allows them to measure the volume and velocity profile of every cell (in a population) as it passes through the grooves. Thus, they can obtain individual cell data from a statistically significant population. This compares favorably to conventional methods that can measure these properties either on single cells or bulk populations but cannot measure them on many individual cells. In this case, microfabrication's strengths (geometrical control) have been used to create a device that can perform single-cell analyses.

Disadvantages of Microfabrication

There are reasons why a particular device would not be microfabricated. If only a few devices are needed and the dimensions are reasonable (>100 μm), it is often possible to machine them conventionally. Microfabrication also usually has long development times, although this depends on the complexity of the system. Finally, the range of structures or materials available might not be compatible with the application.

CONCLUSIONS

As can be seen from the preceding discussion, microfabrication has already made an impact in many areas of biology and medicine. The range of impact is quite large. It varies from commercial applications of molecular biology, such as nucleic acid arrays for hybridization analyses, to basic studies of MT dynamics.

The success of various applications resides in whether the utilization of microfabrication technology has enabled or significantly enhanced the device. Microfabrication can confer different types of advantages, such as higher surface-area-to-volume ratio, small size, small sample volumes, geometrical con-

trol, constrained geometries, single-cell analysis, batch processing, high throughput, or integration. When used appropriately, these can significantly impact current biological or medical problems. Often, very simple microfabricated solutions exist.

A danger exists, though, in proclaiming this technology a panacea for a large portion of biological or medical problems. Such assertions are doomed to failure; entrenched, mature conventional technologies will continue to dominate much of biology and medicine. Microfabrication technology is meant to supplement, not replace, these established technologies.

In the future, expect to see even more cooperation between microfabrication and biology and medicine. Commercial technologies based on microfabricated devices will start to become part of the biological and medical-diagnostic tool kit. In addition, basic biology should see an increase in the numbers of microfabricated devices custom-built to answer individual questions. These devices will likely contain a higher level of sophistication, taking advantage of the new microfabrication technologies and/or more complicated structures.

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