Annu. Rev. Biomed. Eng. 2001. 3:335–73

SOFT LITHOGRAPHY IN BIOLOGY AND BIOCHEMISTRY

George M. Whitesides[1], Emanuele Ostuni[1,2], Shuichi Takayama[1,3], Xingyu Jiang[1], and Donald E. Ingber[4]

[1]Department of Chemistry and Chemical Biology, Harvard University, Cambridge, Massachusetts 02138, and [4]Departments of Surgery and Pathology, Children's Hospital and Harvard Medical School, Enders 1007, Boston, Massachusetts 02115; e-mail: gwhitesides@gmwgroup.harvard.edu, eostuni@gmwgroup.harvard.edu, takayama@eecs.mich.edu, xjiang@gmwgroup.harvard.edu, and ingber@a1.tch.harvard.edu

Key Words surface engineering, self-assembled monolayers (SAMs), patterning, μTAS, microfabrication

■ **Abstract** Soft lithography, a set of techniques for microfabrication, is based on printing and molding using elastomeric stamps with the patterns of interest in bas-relief. As a technique for fabricating microstructures for biological applications, soft lithography overcomes many of the shortcomings of photolithography. In particular, soft lithography offers the ability to control the molecular structure of surfaces and to pattern the complex molecules relevant to biology, to fabricate channel structures appropriate for microfluidics, and to pattern and manipulate cells. For the relatively large feature sizes used in biology ($\geq$50 μm), production of prototype patterns and structures is convenient, inexpensive, and rapid. Self-assembled monolayers of alkanethiolates on gold are particularly easy to pattern by soft lithography, and they provide exquisite control over surface biochemistry.

CONTENTS

[2]Current Address: Surface Logix Inc., 50 Soldiers Field Place, Brighton, MA 02135.
[3]Current Address: Department of Biomedical Engineering, University of Michigan, 2350 Hayward, 3304 GG Brown, Ann Arbor, Michigan 48109-2125.

INTRODUCTION

Microfabrication has become important to biology. The decoding of the genome (1–3) and the development of combinatorial methods of organic synthesis (4, 5) have generated both therapeutic targets and drug candidates; both require microfabricated or microstructured components. Pharmaceutical and biotechnology laboratories perform thousands of assays daily, with an increasing number based on sophisticated uses of complex biochemical pathways. These assays are often carried out using small volumes of analytes and reagents and in small reaction vessels. Combinations of disease-specific genetic information and miniaturized assays are making it possible to develop new classes of diagnostic tools (6–8).

Many of these technological achievements have been made possible by the convergence of microfabrication, automation sciences, and biology. The microelectronics industry developed photolithography and associated techniques to fabricate integrated circuits. Silicon micromachining had been developed for the fabrication of microelectromechanical systems, and these techniques were the first to be adapted to the fabrication of microstructures for biological research. The use of photolithography in the fabrication of DNA arrays was the first example that attracted wide attention (9–12). Although the most highly developed technology for micropatterning, photolithography is limited in its application to biotechnology and biology. (*a*) It is intrinsically expensive, (*b*) it gives limited control over surface properties, (*c*) it often is not directly applicable to proteins and cells, (*d*) the time to go from a design to prototype can be long, and (*e*) the techniques are unfamiliar and inaccessible to most biologists.

We have developed a set of techniques called soft lithography that offers tools for micropatterning that complement and extend conventional fabrication methods (13). Two of the key features of soft lithography are the use of elastomeric (that is, mechanically soft) materials to fabricate the pattern transfer elements by molding, and the development of techniques that pattern complex biochemicals (14–18). Both features involve organic materials and polymers—"soft matter," in the language of physics (19, 20).

This review describes the central techniques of soft lithography and their applications to biology. The first section introduces the major techniques of soft lithography and discusses their advantages. Understanding the principles behind soft lithography helps rationalize its applications to problems in biology. We focus on the application of soft lithography in the fabrication of microsystems useful in biology. The second section describes the components and systems for microfluidics that can be fabricated using soft lithography. The third section discusses the extensive application of soft lithography to problems in cell biology and uses these applications to illustrate the characteristics of each of the variants of soft lithography.

KEY FEATURES OF SOFT LITHOGRAPHY AND THEIR RELEVANCE TO APPLICATIONS IN BIOLOGY

Surface Engineering

As the size of devices decreases, their surface-to-volume ratios increase, and their surface properties become increasingly important in determining their performance. In microscopic systems, and especially those used in biology, it is often necessary to engineer the properties of the surfaces with molecular-level detail. Self-assembled monolayers (SAMs) of alkanethiolates on gold have become indispensable systems for modeling and, in some cases, controlling biologically relevant surfaces (Figure 1) (21, 22). Soft lithography is well suited to pattern the composition, topography, and properties of surfaces (13). The combination of soft lithography and SAMs has proved invaluable in the formation of microsystems for use in biology.

SELF-ASSEMBLED MONOLAYERS The surfaces of noble metals such as gold and silver react with organic thiol groups and form SAMs. The most ordered structures are formed from compounds with the structure $HS(CH_2)_nX$, where $n \sim 16$–18 and X = a small, nonpolar, organic functional group (22–27). Selective reaction of the metal atoms on the surface with the thiol groups generates a dense array of thiolate groups; the organic groups then order and reach densities approaching those of a crystalline solid (Figure 1) (23, 24, 28). SAMs of alkanethiolates with more than 10 methylene (CH_2) units are essentially impermeable to water, and the properties of their surfaces are largely determined by the functional group X (22, 29, 30). SAMs also function as etch resists in the fabrication of patterned structures (31, 32).

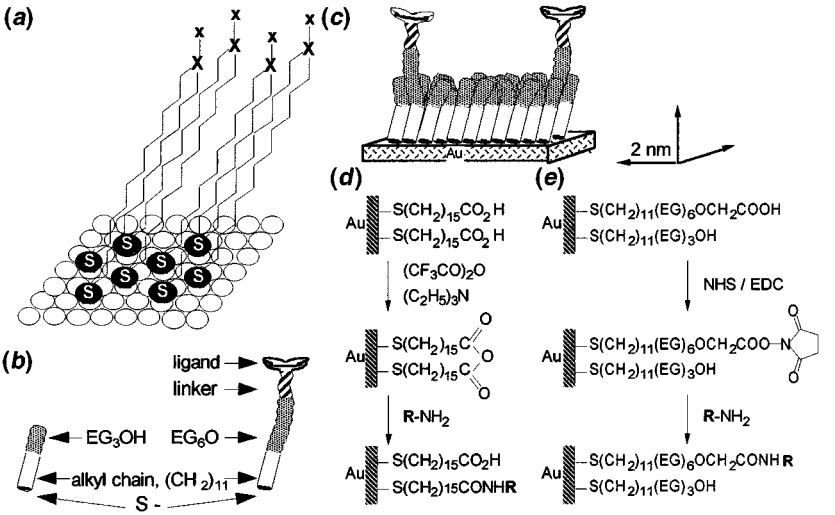

Figure 1 (*a*) Schematic illustration of the structure and packing of a self-assembled monolayer (SAM) on gold. The sulfur atoms coordinate the gold surface to expose the terminal groups X at the interface. The alkyl chains pack at an angle of 30° with the surface, where X is small; this orientation maximizes the van der Waals' contacts between adjacent alkyl chains. (*b*) The structure of typical alkanethiols used in biological studies. (*c*) A diagram of a mixed SAM drawn using the structures of alkanethiols illustrated in panel *b*. (*d*) The "common intermediate method" described in the text as a procedure for attaching ligands to preformed SAMs. (*e*) The "anhydride method" described in the text.

Because the end group X of the alkanethiols can be determined or modified through organic synthesis, SAMs offer the ability to generate well-defined surfaces with a broad range of characteristics.

SAMs of alkanethiols on gold have been used extensively in fundamental studies of wetting, adhesion, tribology, multilayer formation, protein adsorption, and cell adhesion (21, 22, 27, 28 , 30, 33, 34). Biological processes—for example, adsorption of protein—that occur at the surfaces of SAMs can be studied with surface plasmon resonance (35–39), quartz crystal microbalance (40–42), ellipsometry (43), contact angle (44, 45), infrared spectroscopy (46, 47), atomic force microscopy (48, 49), sum frequency spectroscopy (50), X-ray photoelectron spectroscopy (51), surface acoustic wave and acoustic plate mode devices (52, 53), confocal and optical microscopies (15), low-angle X-ray reflectometry (54, 55), electrochemical methods (56, 57), and scanning elecron microscopy (58, 59). SAMs on gold are also used in commercial products that require stringent control of surface properties: DNA arrays (12) and biosensors (35, 37) are examples. SAMs can also be formed with molecules other than alkanethiols, on surfaces

other than coinage metals. The most important alternative system is that formed with alkyltrichlorosilanes ($RSiCl_3$) or alkyltrialkoxysilanes [$RSiO(CH_3)_3$] on SiO_2-terminated surfaces (glass or silica). Alkylsiloxane SAMs are useful, but they are less ordered than are SAMs of alkanethiolates on gold, and neither the synthesis of the $RSiCl_3$ nor the preparation of the SAMs is simple. They have, however, been used extensively in industry for modification of surfaces (for example, to improve adhesion or wettability). Alkylsiloxane SAMs have been reviewed extensively elsewhere (60–62).

INERT SURFACES One important application of soft lithography in biology is to generate patterns of proteins or cells. The formation of these patterns, in turn, is based on patterning the surfaces into regions that either promote or resist the adhesion of proteins and cells. Many surfaces allow proteins and cells to adhere. Most proteins, for example, adhere to hydrophobic surfaces; far fewer surfaces resist the adsorption and the adhesion of biological species. We have explored such surfaces (which we call inert surfaces for brevity) extensively. The most straightforward method to generate inert surfaces is to cover the surface with an appropriate density of derivatives of oligo(ethylene glycol) [$(EG)_n$] or poly(ethylene glycol) (63). This density depends on the length of the $(EG)_n$ group: Longer groups require lower densities (64, 65, 66).

These $(EG)_n$– or poly(ethylene glycol)-terminated surfaces are useful but have some deficiencies: They may autoxidize in the presence of dioxygen (O_2) and transition metals [especially Cu(II) and Fe(III), both common in biological systems] (67–69), and in vivo, they are modified by alcohol dehydrogenase (70, 71). We have used SAMs to define the molecular properties that make surfaces inert. A survey of approximately 60 surfaces indicated that most inert surfaces have four characteristics: (*a*) They are polar, (*b*) they have hydrogen bond acceptor groups but (*c*) no hydrogen bond donor groups, and (*d*) they are overall electrically neutral (72, 73; E Ostuni, RG Chapman, RE Holmlin, S Takayama & GM Whitesides, submitted for publication). Luk et al (75) have reported that surfaces terminating in mannitol apparently are also inert, although they do not meet the third criterion; the origin of the properties of these interesting surfaces remains to be established.

Thin polymer films with these same four characteristics grafted onto surfaces also resist protein adsorption and bacterial adhesion (76). For many applications in biotechnology, these surface-grafted films may provide the best methods available to generate usefully inert surfaces.

VERSATILE METHOD OF ATTACHING LIGANDS By synthesizing alkanethiols that are terminated with a molecule, such as a ligand, for an enzyme (77) or a tripeptide that is specific for the integrin receptors of mammalian cells (78), it is possible to engineer the surfaces of SAMs to bind proteins or to promote the adhesion of mammalian cells biospecifically. Complex problems such as protein-ligand binding or cell-surface interactions are best studied using a surface that presents a low density of the ligand of interest in a background that is otherwise inert.

A useful strategy for preparing these kinds of surfaces is to form mixed SAMs with two alkanethiols (45, 79–81): one (the majority species) terminated with tri(ethylene glycol) [$(EG)_3OH$], and one (a minority) terminated with $(EG)_6OR$, where R is the ligand, or a group such as CH_2CO_2H that can be easily activated for attachment of the ligand (Figure 1*c*) (77, 78, 82–84). A mixed SAM of this type makes it possible for binding events to occur biospecifically between the ligand and the protein or the cell in the absence of steric interference from neighboring ligands, and without nonspecific interactions with the surrounding substrates (84). This capability is particularly useful when studying cell adhesion because the cells themselves secrete adhesive molecules that change the properties of the surface to which they are attached (as well as the surrounding surface) if it is not inert ("surface remodeling") (78).

In these kinds of studies, the synthesis of the alkanethiol $HS(CH_2)_{11}(EG)_6OR$ that is required can be a technical challenge, even for synthetic organic chemists (78). The design of experiments requiring a surface that presents a specific ligand is simplified greatly by procedures that use a common intermediate that can be activated to react with a variety of ligands. We have developed a system that allows attachment of ligands and is relatively straightforward (and operationally familiar to biochemists) by forming mixed SAMs using alkanethiols terminated with $(EG)_3OH$ and $(EG)_6OCH_2CO_2H$ groups. The acid groups in these mixed SAMs can be activated toward coupling with amines using standard methods (Figure 1*d*) (82–84). The method is useful for the immobilization both of small ligands and of proteins. Incorporation of as much as 2% (mol/mol) of acid-terminated groups into a SAM otherwise terminated in $(EG)_3OH$ groups leaves the surface inert but allows it to be activated for attachment of ligands or protein. At surface densities of the CH_2CONHR groups greater than 2%, the surfaces cause increasingly larger amounts of nonbiospecific adsorption of proteins.

Mrksich and coworkers have developed a method for derivatizing SAMs that uses electrochemically switchable surfaces (56, 57, 85 , 86). Mixed SAMs are formed with a small percentage of hydroquinone-terminated alkanethiols in an inert background of $(EG)_5$. A brief oxidizing electrochemical pulse converts the hydroquinone to a benzoquinone derivative that can react with a functional group that carries a reactive diene moiety. The method is simple to use if the proper molecules are available, and it has been adapted to the immobilization of proteins (57).

A method for functionalizing a surface that is operationally simpler than the ones described above begins with a homogeneous SAM terminated with carboxylic acid groups. This surface can be functionalized by dehydration with trifluoroacetic anhydride to generate interchain carboxylic anhydride groups. The subsequent reaction of the activated surface with amine groups ($HNR'R$, $R' = H$, R = organic group) generates amide bonds (Figure 1*e*) (87). SAMs formed using the "anhydride method" present an approximately 1:1 distribution of CONR'R groups and CO_2^-/CO_2H groups. The properties of a SAM formed by the reaction of

undecylamine with an anhydride-terminated surface are, none the less, similar to those of a single-component SAM formed with hexadecanethiol; the longer terminal group [in this instance, the $-(CH_2)_{10}CH_3$ group] seems to shield the CO_2H/CO_2^- group (87, 88). The anhydride method is particularly useful in screening the properties of surfaces quickly to evaluate the need to undertake the synthesis of the alkanethiol that presents the functional group of interest (73, 89). In some experiments, the presence of a high surface density of CO_2^-/CO_2H groups may introduce complexities that would be absent in experiments performed with mixed SAMs, but the operational simplicity of the method makes it attractive for screening and exploratory experiments (73).

Poly(dimethylsiloxane) Stamps: Fabrication Using Replica Molding

The ability to control surface properties using SAMs is most useful when combined with spatially defined patterns. Soft lithography allows the creation of patterned surfaces using a combination of SAMs and microcontact printing (μCP). In μCP, a microstructure (a stamp) fabricated of an elastomer transfers materials to a surface; the use of elastomers allows the micropatterned surface to come into conformal contact with the surfaces over large areas (Figure 2*b*).

The key strategy for fabricating stamps and molds used in patterning is to replicate, in an elastomer, the three-dimensional topography of a patterned, solid surface by replica molding (Figure 2*a*) (90–92). Because masters are typically rigid, the use of an elastomer facilitates separation of master and replica (Figure 2*a*). Polymeric stamps can also be used as masters for fabrication with rigid materials that cannot be molded and separated on conventional, brittle masters. Replica molding is successful even with features that are only tens of nanometers wide and tall (13, 92).

Poly(dimethylsiloxane) (PDMS) and other siloxane-based polymers are widely used in making stamps and molds for soft lithography, although other elastomers can also be used (93). PDMS has a useful combination of properties. It has a Young's modulus that makes it a moderately stiff elastomer (1 MPa) (94). It is nontoxic and readily available commercially. It is optically transparent to $\sim$300 nm. It is intrinsically very hydrophobic (advancing contact angle of water, $\theta_a^{H2O} \sim 110^\circ$), but its surface can be converted to a hydrophilic form ($\theta_a^{H2O} \sim 10^\circ$) by brief treatment with a plasma (95, 96). Contact of two freshly oxidized PDMS surfaces results in irreversible contact adhesion, presumably by a spontaneous dehydration of SiOH groups ($\equiv SiOH + HOSi\equiv \rightarrow \equiv SiOSi\equiv$) (96–98). Treatment of the plasma-oxidized PDMS with an alkyltrichlorosilane ($RSiCl_3$) introduces R groups onto the PDMS surface; this process is commonly used to introduce perfluoroalkyl groups that reduce interfacial free energies and thus reduce adhesion. The conversion of PDMS to a form with high surface free energy by plasma oxidation (95, 96) generates a negatively charged surface that can support electroosmotic flow for applications in biochemical separations based on capillary

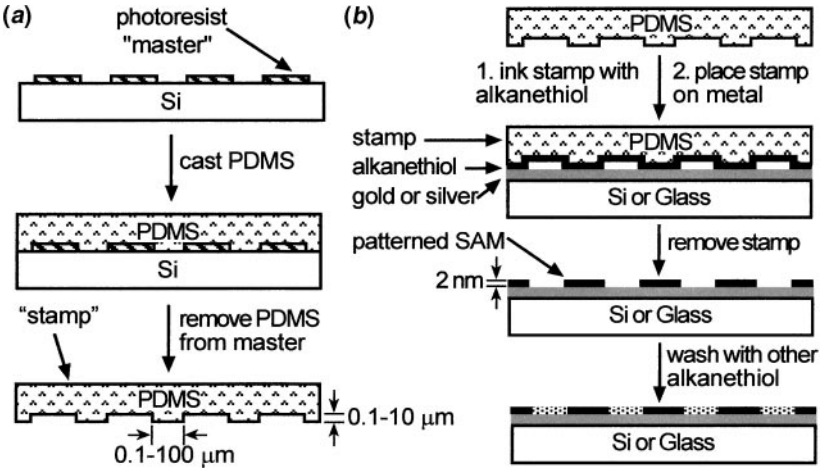

Figure 2 A schematic outline of patterning by preparation of a poly(dimethylsiloxane) (PDMS) stamp using replica molding, and pattern transfer by microcontact printing (μCP). (*a*) The processes begin by exposing photoresist to ultraviolet light on a silicon support through a mask (which, for $\geq$50-μm features, can be prepared by commercial or desktop printing). On dissolving the unexposed photoresist, the cured photoresist remains on the silicon support in a bas-relief pattern defined by the mask (a structure we call a master). The master is exposed to vapors of $CF_3(CF_2)_6(CH_2)_2SiCl_3$ overnight to reduce its tendency to adhere to the stamp. An elastomer is poured over the master; typically PDMS (Sylgard 184) is used as the elastomer (curing is for 2 h at 60°C). After curing, the PDMS stamp can be peeled off the master. (*b*) In μCP, stamps are inked with an ethanolic solution of an alkanethiol, and the ethanol is removed by evaporation in a stream of nitrogen and then brought into contact with a thin, supported film of gold for 30 s to 5 min. (See text for details.)

electrophoresis (97). The ability to change the surface properties of PDMS is useful when designing a stamp for pattern transfer of a molecular species; the amount of material that is loaded on the stamp can be maximized by using a stamp with properties similar to those of the compound—for example, a hydrophilic stamp may be required to transfer a pattern of hydrophilic molecules (83). PDMS is relatively permeable to nonpolar gases, including O_2, N_2, and CO_2: This property is essential to its use in channel systems for mammalian cell culture.

Formation of Masters: Rapid Prototyping Methods

Typically, soft lithographic techniques rely on photolithography for the formation of masters. One of the most time consuming and expensive (ca \$500 per in^2 for features between 1 and 5 μm) parts of conventional photolithography is the

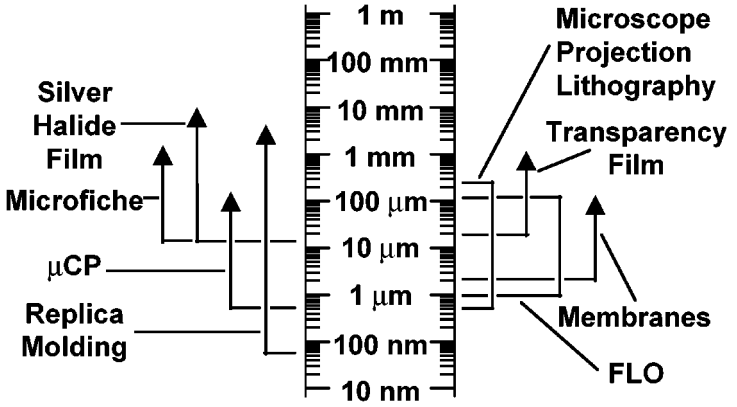

Figure 3 Schematic illustration of the size ranges that are accessible by the techniques of soft lithography that are being applied to biological problems. The upper limits of some of the techniques are not indicated because they have not yet been tested.

production of the photomask; the mask also limits the area that can be patterned in a single step. We have developed several methods to make the masks required in soft lithography rapidly and inexpensively (Figure 3).

TRANSPARENCY SHEETS The patterns to be created in photoresist can be designed using commercial computer drawing packages. The graphic designs are printed onto transparencies using a commercial printer operating at 5080 dpi. These transparencies are used instead of chrome masks in contact photolithography and can routinely generate features of photoresist with dimensions of 50 μm as well as (with some degradation in quality) features as small as 20 μm (99, 100). It is possible to use this method to produce continuous patterns with dimensions of at least 20 cm^2, and larger printed areas should allow larger areas of reproduction.

MICROFICHE OF PHOTOGRAPHIC NEGATIVE An alternative method for producing masks involves the use of an office printer to generate low-resolution (600 dpi) prints of the mask designs, followed by reduction of the features using photographic methods onto 35-mm film or microfiche. The film and the microfiche can be used for contact photolithography to produce 15-μm features with a 1.5-μm edge roughness (101, 102). This method can produce patterns with areas of approximately 4 cm^2.

MICROSCOPE PROJECTION LITHOGRAPHY Transparency masks can also be used with a conventional microscope to produce features that are 5–25 times smaller than the features printed on the masks. The microscope lenses are used as the reducing elements, and the transparency is placed in the path of the light before it reaches the lenses. Microscope projection lithography can produce features with widths of

1 μm and an edge roughness of 0.2 μm (JC Love, DB Wolfe, HO Jacobs, & GM Whitesides, unpublished results). It is limited to areas of approximately 10000 μm^2.

Microcontact Printing

The ability to engineer the properties of surfaces with SAMs has made it possible to study biological processes, such as cell adhesion, that occur at interfaces as well as to conduct miniaturized and high-throughput assays. Most current biological uses of micropatterning require features with sizes between 1 and 100 μm.

μCP is a method for patterning by printing material using a PDMS stamp (Figure 2*b*) (31, 32, 104 , 105). It can routinely transfer patterns with features having 1-μm dimensions and with an edge roughness of $\leq$100 nm, provided the necessary masters are available (13); the fabrication and patterning of simple 40-nm features, such as lines and circles, is possible with some additional steps (106–109). This procedure has been most widely used in printing alkanethiolates on films of gold and silver. The general procedure for μCP involves inking the stamp with a solution of the substance to be printed. As the solvent (typically ethanol) evaporates, the alkanethiolate ink is deposited on the relief structure (104); it may also dissolve in the PDMS (110, 111). The stamp is then brought into conformal contact with the surface for a period ranging from 30 s to several minutes, depending on the application (Figure 2*b*). On removing the stamp from the surface, a pattern is left that is defined by the raised bas-relief structure of the stamp. If required by the intended application, the remainder of the surface can be filled in with a second SAM simply by dipping it into a solution of the alkanethiol. μCP is convenient and suitable for patterning large areas (ca 100 cm^2) in a single impression. This technique has also been used to print alkylsiloxanes [the quality of the patterned features (112) can be improved by printing at high temperatures (113)] and would probably work with most systems that form SAMs.

SAMS ON GOLD AND SILVER Alkanethiols can be printed onto both gold and silver films with equally good results, although all applications of printed SAMs in cell biology use films of gold, because silver is cytotoxic. Alkanethiols with a broad range of structures can be patterned with μCP (33). In a typical experiment, the hydrophobic hexadecanethiol is printed onto a gold-coated surface to generate patterns that promote the adsorption of proteins; the remainder of the surface is covered with an alkanethiol that is terminated with $(EG)_3OH$ groups; this surface resists the adsorption of proteins and the adhesion of cells (15, 17).

The quality of the SAMs formed by microcontact printing, and the resolution of the printed features, depends on the concentrations of the alkanethiols and the conditions used to perform the printing. Delamarche and coworkers found that using ethanolic solutions of dodecanethiol $>$10 mM for printing caused the resulting SAM to be structurally comparable to a high-quality SAM formed from

a solution (111, 114). The resolution of the printed features decreases as the vapor pressure of the alkanethiol increases. Short alkanethiols can transfer from the stamp to the substrate through the vapor and give blurred edges or broadened lines (110). This blurring is important only for $<$500-nm features and is irrelevant for most biological applications (115). Maracas and coworkers have shown that μCP can be used to print 550-nm-wide lines with $\geq$40-nm accuracy over a circular area with a 7.5-cm diameter by using a rigid backing and a precision-controlled jig to place the stamp on the surface (116).

TRANSFER OF OTHER MATERIALS μCP is also useful for generating patterns of materials such as proteins on surfaces other than gold (polystyrene, glass); the only requirement is that the material being transferred can be deposited on the surface of the stamp. PDMS stamps have been incubated with solutions of proteins and the adsorbed proteins transferred to glass, polystyrene, or silicon (14, 16, 18). The activities of the proteins are generally 50%–100% of those measured with protein layers adsorbed from solution.

We have also used μCP to transfer poly(ethyleneimine) (PEI) onto a SAM terminated with anhydride groups; the PEI was grafted covalently (117, 118). The free, residual amino groups on the PEI were then used to attach other groups to the pattern (76).

METHODS BASED ON WRITING WITH A "PEN" Our initial work in the area of printing alkanethiols used simple pens to write lines of alkanethiols on a gold surface with widths of $\geq$0.1 μm (31, 104). Mirkin and coworkers have used an operationally related, but mechanistically different, method [dip-pen nanolithography (DPN)] to write patterns of alkanethiols on gold surfaces with line widths as small as 15 nm using AFM tips (119). In DPN, Piner et al have suggested that as the AFM tip coated with alkanethiol is carried across the surface, water that condenses from vapor into liquid between the tip and the surface carries the alkanethiol molecules from the tip to the substrate; the reaction of the alkanethiol with the gold surface forms a SAM only in the regions traced by the AFM tip. The size of the features depends on the duration of contact between the AFM tip and the surface (119). DPN has also been extended to the writing of adjacent features with different terminal groups and to the parallel writing of features with eight different AFM tips (120, 121).

Elastomeric Membranes for Dry Lift-off

Surfaces can be patterned by the direct transfer of material with a stamp—as in μCP—or by selectively blocking the access of material using physical barriers. "Lift-off" is a technique commonly used in microelectronics fabrication. A surface patterned with photoresist is exposed to a metal vapor. The photoresist is then dissolved; as it dissolves, it "lifts off" the metal deposited on it but leaves the metal deposited on the unprotected surface. The result of this procedure is a pattern

of metal that corresponds to the pattern of holes in the exposed and developed photoresist.

Conventional lift-off involves several steps—especially treatment with an alkaline or organic solution to dissolve the photoresist—that are incompatible with patterning proteins or cells. We have developed a method for fabricating elastomeric membranes containing through-holes (122). The elastomeric membranes can be used to provide patterned access to the surface. After patterning, the membranes can be physically lifted off the substrate. The fabrication of such membranes is straightforward; it only involves spinning a layer of PDMS on a master of photoresist posts to a thickness lower than the height of the posts (Figure 4). On curing the PDMS, the membrane can be removed from the master as a free-standing film with features in the shape of the posts on the master (122). The thinnest membranes we have made are 5 μm thick with 3-μm diameter holes. Elastomeric membranes have been useful in patterning metals, sol-gels, electroluminescent materials, and proteins in arrays of dots (122–124). Multiple materials can be patterned using multiple, stacked membranes (122).

Folch et al (125) fabricated similar membranes using a procedure that is not as straightforward as the one we have described, but it does not require a spin coater. After pouring PDMS on a pattern of photoresist, the substrate is covered with an adhesive film made to adhere to the posts by pressure or by removing the excess PDMS with a syringe. The adhesive film seals against the photoresist and allows the PDMS to cure around the posts to generate a film that has through-holes (125).

Fabrication Using Laminar Flow in Microfluidic Systems

In microfluidic systems—with typical channel sizes of 50 μm—fluids flow laminarly. A buffer that flows inside a microscopic channel (50 μm wide) with a velocity of 0.6 cm/s has a Reynolds number ($Re = vl\rho/\mu$) $\sim$1 (126). *Re* is a dimensionless parameter that describes a fluid's tendency to become turbulent; laminar conditions in a fluid are obtained at low values of the velocity (v), the diameter of the capillary [l, (m)], and the density of the liquid [ρ, (kg/m^3)], and high values of the viscosity [μ, (kg m^{-1} s^{-1})]. Fluids with $Re \ll 2000$ flow laminarly. Because l is small for microfluidic systems, *Re* is typically 0.1–1 for flow rates commonly used in biology. Two streams of fluid that flow into a common channel of a microfluidic structure from independent inlets do not mix turbulently but flow in adjacent, separate laminar streams that mix only at their interface by diffusion (Figure 5*a*).

We have used the laminar flow of fluids in microfluidic structures to pattern surfaces and to fabricate structures at the interface between different fluid streams (127, 128). Figure 5 shows a silver wire that was created inside a microfluidic structure by precipitation at the interface between two liquid streams of an electroless plating solution; these structures can be used to generate electrodes inside a channel and should be useful in electrochemical assays and in probing the behavior of cells potentiometrically (127). The ability to fabricate electrodes that

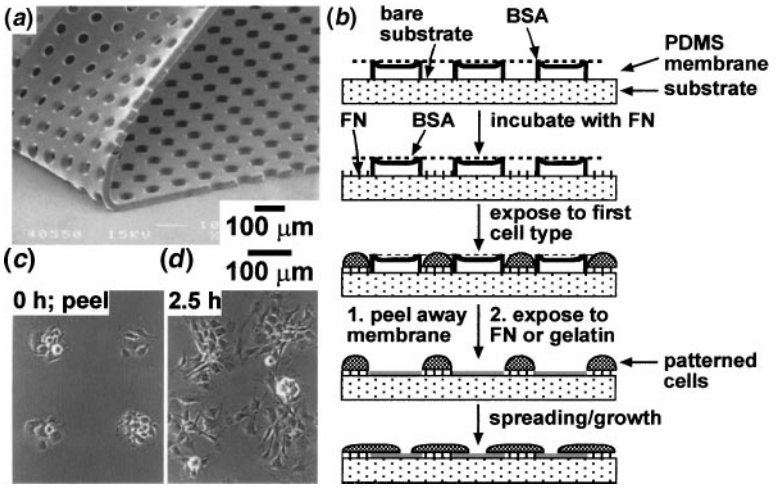

Figure 4 Use of elastomeric membranes in studies of cell spreading. (*a*) Scanning electron micrograph of a membrane ca 50 μm thick with holes 50 μm in diameter. Poly(dimethylsiloxane) (PDMS) was spun on a master of photoresist to a thickness slightly smaller than the height of the features of photoresist. (*b*) Schematic illustration of the procedure for the use of membranes in studies of cell spreading. A PDMS membrane coated with bovine serum albumin (BSA) is placed on a Petri dish and exposed to a solution of fibronectin (FN); the system is exposed to vacuum for three 30-s intervals to allow the solution to fill the wells. The FN adsorbs to the surface of the dish that is exposed by the holes of the membrane and directs the selective adhesion of the cells to those parts of the surface. Removal of the membrane leaves cells on the surface of the Petri dish in a pattern as in *panel c*; brief exposure of the Petri dish to a solution of gelatin renders the rest of the surface adhesive. (*d*) Micrograph taken 2.5 h after that in *panel c* showing the cells spread onto the remainder of the surface. After ca 4 h, the surface becomes covered with cells and no longer exhibits a pattern.

are smaller than, and aligned to, the channels of a microfluidic system is unique to soft lithographic methods and will find application in the design of assay systems (129).

Patterning Using Two-Dimensional Microfluidic Structures

Channel systems, in combination with laminar flow, can be used to pattern material onto a surface from solution. Delamarche et al used multiple, adjacent channels to pattern proteins onto a surface and to direct chemical reactions on surfaces (130, 131). The system uses small volumes of reagents and may be useful in immunoassays. Laminar flow of liquids can be used to generate simple patterns of proteins and cells inside a channel (127, 132).

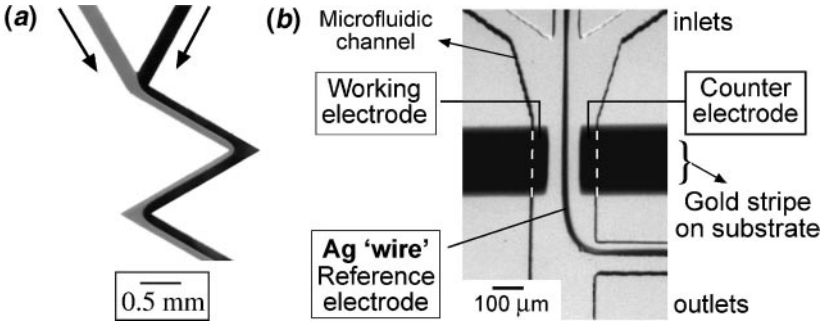

Figure 5 (*a*) Laminar flow in microfluidic systems. Aqueous solutions colored with a black and a red dye were combined in a channel. The two solutions only mix at their interface because they are flowing laminarly. (*b*) A three-electrode system that was fabricated using the laminar flow of fluids inside the 200-μm-wide channel of a microfluidic structure. The microfluidic channel was placed on top of a stripe of evaporated gold. Etchant solution was allowed to flow in the central channel of a three-channel inlet to remove a central strip of gold and thus to create the working and counter electrodes. The reference electrode was fabricated as a silver wire by flowing solutions of the components of electroless silver plating mixture from the two outer inlets of the system. The silver wire extended to a silver contact pad.

Patterning Using Three-Dimensional Microfluidic Structures

By stacking membranes, it is possible to fabricate three-dimensional microfluidic structures (Figure 6) (133, 134). These structures can generate complex patterns of different materials in one step on a surface by deposition from solution. Simple μCP is restricted to printing crossing lines (135).

Generation of Gradients Using Microfluidic Systems

Appropriately designed microfluidic channels can be used to create gradients. Figure 7 shows a "Christmas tree structure" fabricated to generate gradients and illustrates its use with fluorescein using three inlet reservoirs (136, 137). Using a small number of inlet streams allowed to mix diffusively within the serpentine channels that separate the crossing points of the channels, it is possible to create smooth gradients of two substances over a dimension of a few hundred micrometers in the common channel (136, 137). A range of other methods for producing gradients has also been demonstrated. These methods include diffusion of alkanethiols through porous matrices (138, 139), photochemical activation of SAMs for peptide coupling (140, 141), electrochemical desorption of SAMs (142), and depletion of protein inside microfluidic channels by adsorption (143). Methods that use pipettes for forming gradients in solution have also been described (144–147).

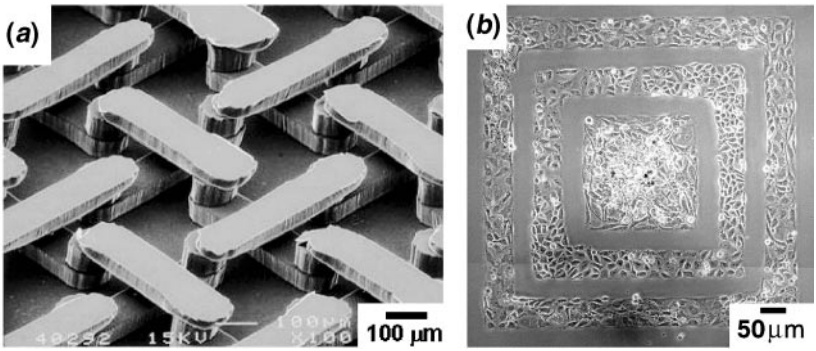

Figure 6 Fabrication of three-dimensional (3D) microfluidic systems and their application in cell biology. (*a*) Stacked membranes were used to fabricate a 3D network of channels. The channels were filled with epoxy and cured before dissolving away the poly(dimethylsiloxane). (*b*) A microfluidic system similar to that in *panel a* was used to deliver cells to an adhesive tissue culture surface in concentric squares. (*Outer* and *inner squares*) Covered by bovine capillary endothelial cells; (*middle square*) covered by human bladder cancer cells. The system also makes it possible to deposit the same type of cell on all surfaces defined by the channels and to treat each area with different substances.

APPLICATIONS OF SOFT LITHOGRAPHY IN MICROSCOPIC BIOCHEMICAL ASSAYS

Overview

The important advantages of miniaturizing biochemical assays are the minimization of the amounts of analytes required for analysis and the maximization of the number of assays that can be carried out in parallel, using systems that require only a small amount of laboratory space and labor (148). Miniaturized devices that perform complete laboratory tasks, such as polymerase chain reaction or sequencing, are called micro total analysis systems (μTAS). A fully complete μTAS would be a device capable of performing all phases of an analysis. For example, the device might (*a*) sample and concentrate organisms from a fluid, (*b*) lyse them to release nucleic acids, (*c*) separate the DNA from the rest of the cell debris, (*d*) amplify the DNA using polymerase chain reaction, and (*e*) sequence the DNA. Currently, no μTAS of this sophistication has been fabricated, but products that perform simpler tasks—such as separations of proteins and DNA, sequencing, or binding assays in parallel arrays—are beginning to emerge.

One of the challenges to the use of microfluidic structures is the ability to create systems that manipulate laminar flow of fluids moving in 50- to 200-μm channels. These microfluidic systems require the same types of functional components—pumps, connectors, valves, mixers—that are used for manipulating fluids at higher

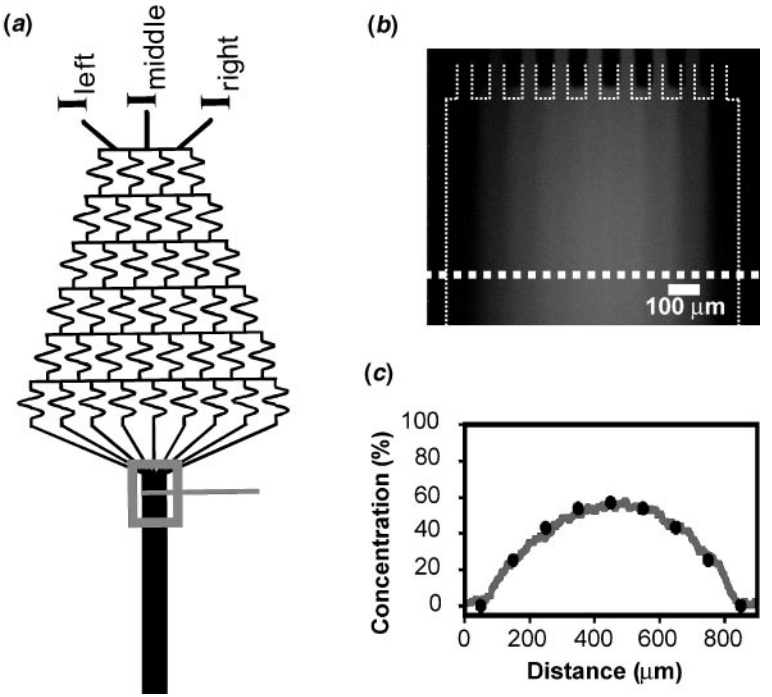

Figure 7 Generation of gradients using microfluidic networks. (*a*) Schematic of the microfluidic structure used to generate gradients. Solutions from the three inlets were combined repeatedly and allowed to mix diffusively before being recombined to generate a smooth gradient in the main channel. (*b*) Fluorescence micrograph of a gradient of fluorescein isothiocyanate. (*c*) The calculated fluorescence intensities in each channel. The shape of the gradient is influenced by the flow speed of the liquids.

Reynolds' number (*Re*), but they require fundamentally new designs that take into account the behavior of the fluids when there is no turbulence. Some of these components have been fabricated using silicon micromachining, but the methods are complex, and some devices (especially valves) malfunction when particles are present. The miniaturization of fluidic components can also be achieved with soft lithographic methods and elastomeric materials (13). These procedures, when combined with advances in flow imaging, make it possible to test and characterize the design of components quickly (149, 150).

The initial work in fabrication of μTAS was carried out using silicon (151, 152); current versions of μTAS are being fabricated using glass (153, 154). The use of plastics (including elastomers such as PDMS) in the fabrication of μTAS is rapidly becoming more common because of the advantages—especially in cost—offered by these materials (13, 97).

Components

PUMPS Microfluidic pumps can be classified as mechanical [piezoelectric (155–158), electrostatic, electromagnetic (159), pneumatic (160), thermopneumatic (161), ultrasonic (162), or bubble formation (163)] or nonmechanical [surface tension (131, 164–166), electrohydrodynamic (167–169), electroosmotic (150, 170, 171), or gravitational (132)]. Microfluidic pumping inside channels created by soft lithographic methods can operate by mechanisms in either category. A successful example of mechanical pumping is the pneumatically driven peristaltic pump fabricated by Quake's group (160). Examples of nonmechanical pumping schemes in PDMS channels include electroosmosis (148, 150, 172), gravitational pumping (132), electrokinetic generation (173, 174), surface tension (96, 175), and wetting by capillary force (131). External macroscopic pumps can also be used to drive fluid flow inside the microfluidic channels (127). An important consideration with the different pumping schemes is the flow profiles (parabolic, plug-like, complex) they generate. Inside uniformly charged channels, electroosmotic pumping gives plug-like flow; pressure-driven pumping gives parabolic flow. Complicated flow profiles can be produced in electroosmotically pumped systems by creating surfaces patterned with different charges using microcontact printing (176).

VALVES The direction of fluid flow at junctions where three or more capillaries meet can often be controlled without mechanical valves (150, 171). For example, hydrophobic patches can be used to regulate flows of aqueous solutions (177). Most sophisticated microfluidic devices will, however, include valves, and elastomeric materials have the advantage that they allow motion of fluids with simple designs. Valves can be built into microfluidic systems made from elastomers by taking advantage of the softness of the material. We have built a passive check valve using PDMS flaps that open only when liquid flows in one direction (NL Jeon, DT Chiu, CJ Wargo, H Wu, IS Choi, et al, submitted for publication). By using a crossed-channel architecture, where channels in one layer run perpendicular to the direction of the channels in an adjacent layer, Quake and coworkers fabricated a valve in which a pneumatically expanded channel in one layer pinched off a perpendicular channel in an adjacent layer (160). Beebe and coworkers have used pH-responsive hydrogels as the actuating component to prepare a variety of microvalves (179).

MIXERS Mixing of fluids in microfluidic channels is challenging because the flow is usually laminar (i.e. nonturbulent). In a channel with a diameter smaller than 10 μm with the fluids introduced side by side, the mixing by diffusion would be relatively fast ($\sim$0.1 s for molecules with diffusion coefficients of 5×10^{-6} cm^2/s). Channels for most microfluidic devices are, however, 50–200 μm wide. In these channels, mixing by diffusion is slow, especially for particles (e.g. cells or beads) and for macromolecules (proteins, DNA). A variety of mixers have been developed for microfluidic mixing: laminating mixers (180), plume mixers (181), and chaotic advection mixers (163, 182). Laminating mixers work by generating multiple thin

layers of two or more liquids to increase the contact area and enhance diffusive mixing. Plume mixers enhance diffusive mixing by injecting small plumes of one liquid into another liquid through arrays of micronozzles. In chaotic advection, volume elements of fluid that are initially close to each other become widely separated because of chaotic flow (the type of flow where initial conditions profoundly influence the path and final position of individual particles).

DETECTORS The majority of miniaturized assays have been developed to use changes in fluorescence emission as a means of detection; fluorescence is sensitive and the technology for fluorescent labeling of reagents is highly developed. A common strategy in microarray or microfluidic devices is to utilize external, separate, macroscopic detectors, such as slide readers, epi-fluorescence optical microscopes, and confocal microscopes (150, 183). The strategy of separating device and detector is satisfactory in many applications, but not when the entire system must be portable; developing small, low-cost detectors would also allow each device to operate simultaneously and would eliminate detector bottleneck.

The fabrication of detectors integrated in the microscopic devices poses several challenges. Major problems include their size, cost, and requirements for power. There is also a serious issue of the incompatibility of materials used to fabricate the device (typically plastic and glass) with those required by the detector (which normally requires metals and often incorporates microprocessors). The integration of optical detectors into the microfluidic device for DNA analysis developed by Burns et al (184) was simplified by the use of silicon to fabricate the entire device. Integrated systems fabricated in polymers, or using soft lithography, are still being developed.

An alternative to detecting analytes by fluorescence is to analyze changes in the index of refraction (185); examples of detectors include a liquid-core waveguide (186) and a microfluidic diffraction grating (187) made from PDMS. Chemiluminescence and electrochemical detection are also possible (127). Cell-based sensors that monitor the electric response of cells to toxins have been fabricated from PDMS and silicon (188). Miniature nuclear magnetic resonance systems based on surface coils have been described (189). Capillary electrophoresis (CE) systems and other microfluidic systems are also often interfaced with mass spectrometers (190, 191). We have recently demonstrated that a micro avalanche photodiode can be embedded in microfluidic structures made of PDMS and can function as a detector for biochemical separations; the system is sufficiently sensitive and potentially inexpensive enough to make it suitable for manufacturing low-cost, disposable μTAS devices (DT Chiu, ML Chabinyc, J Christian, JC McDonald, AD Stroock, et al, submitted for publication).

Separation Systems

Capillary electrophoresis (CE) was the first separation technique used in miniaturized analytical systems. The commercially available CE instruments already in

use, with small (50-μm inner diameter) capillaries to separate proteins and DNA, were prime candidates for miniaturization into more compact systems (193–195). The first miniaturized CE system fabricated in PDMS, developed by Effenhauser et al (196–198), was used to separate DNA fragments. This system was based on PDMS that was sealed reversibly against a flat glass surface by external pressure; although the ability to disassemble such systems is useful for cleaning and reusing the channels, the electrical weakness of the seal between the PDMS and the glass limits the voltage that can be applied.

We and others have made microfluidic systems to carry out separations with CE by using plasma oxidation to seal PDMS capillaries irreversibly to glass surfaces (97, 199). The oxidation of the PDMS is important to CE because it generates surface charges that support electroosmotic flow.

Quake and coworkers developed an elegant microfabricated fluorescence-activated cell sorter and a microfabricated, single-molecule DNA sizing/sorting device (200) in PDMS. Austin and coworkers demonstrated that a lattice of small PDMS channels sorts white blood cells (201, 202). Cells adhered to different regions of the lattice depending on their size, nuclear morphology, and surface properties.

Systems for Polymerase Chain Reaction and DNA Detection/Analysis

A variety of companies and academic research groups have developed systems for nucleic acid amplification and/or detection (8, 184, 203–208). Because of the low cost of replica molding, a number of commercial nucleic acid analytical chips incorporate polymeric components.

Biochemical Assays

Yager and coworkers have developed two devices that take advantage of laminar flows, using the differences in the rates of diffusion of molecules and particles of different size, to achieve separations in the direction perpendicular to that of the flow (126, 209, 210). The T-sensor has been used to analyze blood (211). When blood is allowed to flow laminarly adjacent to a stream that contains an acid-sensitive indicator, the protons diffuse rapidly ($D \approx 10^{-5}$ cm^2/s) from the blood into the adjacent indicator stream (209, 212). The resulting change in color allows the determination of their concentration. There is little colorimetric background from the erythrocytes because they diffuse slowly ($D < 10^{-9}$ cm^2/s) (209–211). The constant flow in the microfluidic systems ensures that reagents are replenished and avoids photobleaching when using fluorescent markers. Similar principles were used to fabricate the H-filter—a device that filters complex mixtures such as blood (209). Filtration and sorting of complex biological media are essential steps in sample preparation for analysis by μTAS.

APPLICATIONS OF SOFT LITHOGRAPHY IN CELL BIOLOGY

Overview

The ability to position anchorage-dependent cells on a surface with control over their size and spatial arrangement—a capability that soft lithography has made routine—is being developed for fundamental biological research; we believe it will ultimately be used commercially in high-throughput screening, and perhaps in cell-based sensors and human diagnostic systems. Approaches to patterning cells generally involve the patterning of proteins in the geometry desired for the cells; proteins and cells are delivered to the patterned surfaces from solutions or suspensions (15, 17). The isolation of cells on a surface makes it possible to study events occurring in each individual cell instead of relying on statistical distributions based on ensembles of cells; it also prevents cells from migrating across the surface and thus makes it straightforward to address and observe single cells repeatedly. These same techniques also make it practical to isolate pairs and triplets of cells, with the potential (so far relatively unexplored) of studying interactions between them (213).

The combination of patterned cells and reagents delivered to the cells in laminar flows in microchannels makes it possible to study the influence of these reagents on cells with high accuracy. The single-cell systems are not complicated by cell-cell interactions but have the disadvantage that the behavior of isolated cells may be very different from that of the same classes of cells in vivo surrounded by other cells. They also use substrates with unnatural mechanical properties. Collagen and polymer gels are used routinely in patterned cell culture to study the effect of substrate elasticity, but they do not provide well-defined surfaces (214, 215). Recent advances in cell patterning and coculture are making it possible to create and study well-defined multiple cell systems that resemble the physiological environment of the cell in vivo (133, 216, 217).

Patterned Cell Culture

STUDY OF GROWTH AND CELL CYCLE ON MICROPATTERNED SAMS We have used μCP to pattern the size and shape of bovine capillary endothelial (BCE) cells by patterning the sizes and shapes of the adhesive islands of hydrophobic alkanethiolate SAMs; the area separating these islands was covered with inert (nonadsorbing) SAMs. The hydrophobic islands adsorbed extracellular matrix (ECM) proteins such as fibronectin and laminin from solution and promoted cell adhesion (15, 218, 219). BCE cells also attached to patterns consisting of multiple adjacent islands with diameters smaller (3–5 μm) than that of the cell (Figure 8) (15).

Examination of the adhesion of cells to micropatterned SAMs revealed that the surface area over which a cell spreads determined the metabolic program it followed (15, 220). The number of BCE cells that entered the growth phase was

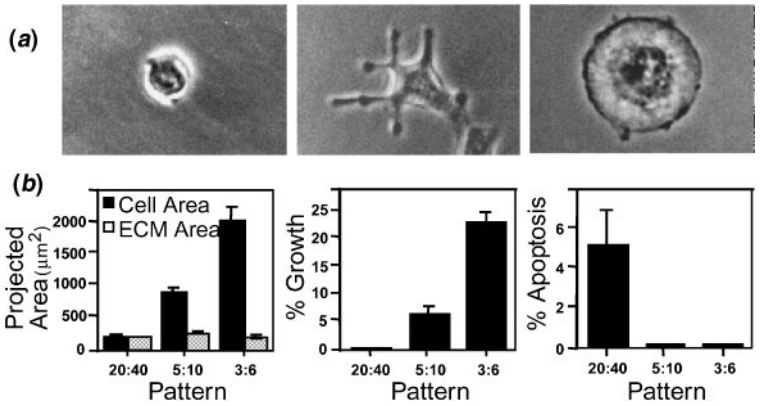

Figure 8 The extent of cell spreading determines the genetic programs engaged by a bovine capillary endothelial (BCE) cell. (*a*) Micrographs of BCE cells attached to individual 20- or 50-μm-diameter islands, or to multiple 5-μm-diameter islands patterned with microcontact printing. (*b*) Plots of the values of the extracellular matrix contact area and projected cell area, the percentage of cells in the growth phase, and the percentage of cells entering apoptosis for cells patterned on circular areas with diameters of 20, 5, and 3 μm, separated by 40, 10, and 6 μm, respectively.

largest when the cells were confined to the largest areas (15). This result raised the question of whether the adhesive area or the projected footprint of the cell determined its behavior (Figure 8). BCE cells attached to surfaces composed of multiple adhesive islands in an inert sea—where each island was too small to allow growth—spread over an area that was larger than the aggregate area of the adhesive spots and entered the growth phase (15). This observation suggests that it is the projected footprint of the cell that is crucial in determining the progression of the cell through the cell cycle; the total adhesive area is less important.

PATTERNING CELLS WITHOUT SAMS μCP is not always the best method of patterning cells: It may be difficult to release the cells from the pattern after they have attached. Not all biological laboratories have access to gold-coated substrates and alkanethiols terminated with ethylene glycol groups and/or ligands required for biospecific attachment. The basic techniques of microfabrication are often unfamiliar. The lift-off (see comments above) membranes described earlier are useful alternatives to μCP for patterning ECM proteins on surfaces to direct cell adhesion (for brevity, we call this method of membrane patterning MEMPAT) (Figure 4*c*) (124). We have used MEMPAT to pattern BCE cells on the surfaces of glass, PDMS, polyurethane, and silicon (Figure 4*c*). Toner et al have also described the patterning of cells on surfaces with membranes (125).

STUDIES OF CELL SPREADING One approach to studying cell spreading is to prepare cells constrained in a pattern and then to release them from the constraint of that pattern and allow them to spread. Soft lithographic techniques have provided two methods for studying cell spreading by this strategy.

We used membranes coated with bovine serum albumin (BSA) to pattern a Petri dish with fibronectin when BCE cells were allowed to attach to the composite systems (dish plus membrane). The cells adhered only to the regions of the dish coated with adsorbed fibronectin; they did not attach to the BSA-coated membranes even at the edge directly exposed to the cell. After removing the membranes, the remainder of the surface was coated with gelatin to render it adhesive. The attached cells spread out of the original pattern in a time-dependent fashion to cover most of the surface (Figure 4*c*, *d*) (124).

Mrksich developed an elegant method to study the spreading of fibroblasts on substrates patterned with alkanethiols based on μCP and SAMs that can be activated electrochemically (56, 85). The initial pattern is created by μCP with hexadecanethiol on gold, and the remainder of the surface is covered with a SAM terminated with penta(ethylene glycol) groups and a low density ($\sim$1%) of a hydroquinone-terminated alkanethiol (86, 221, 222). This surface is inert and cells do not attach to it, except in the pattern defined by the regions of hexadecanethiolate SAMs. A diene terminated with an RGD tripeptide reacts with the surface through a Diels Alder reaction, under conditions sufficiently mild that they do not damage the cells (56, 85). The presence of the RGD groups introduced into the area between the islands patterned with cells makes it possible for the cells to spread over that surface from the pattern (86, 221, 222).

CELLS ATTACHED IN ARRAYS OF MICROWELLS The ability to generate arrays of cells on a surface at densities higher than even 1536 well-assay formats is potentially useful in cell-based screens. In these screens, the cells must be separated spatially from their neighbors to allow testing different compounds. We have developed a method for patterning the adhesion of cells in high-density arrays of microscopic wells (ca 10,000 wells/cm^2, 50-μm well diameter) that are fabricated by replica molding of PDMS against a master of photoresist (223). We were able to coat the interior surfaces of the wells with an ECM protein, and the space between the wells with BSA (to which BCE cells do not adhere) (Figure 9). A drop of aqueous solution of BSA placed on top of wells fabricated in hydrophobic PDMS does not fill the wells, traps air in them, and delivers the BSA only to the surface between wells. The inner surfaces of the wells are then coated with ECM proteins; the appropriate solution can be delivered to the wells by exposing them briefly to a vacuum to release the trapped air bubbles.

Cocultures

PHOTOLITHOGRAPHIC METHODS Bhatia et al developed a photolithographic method for patterning the attachment of hepatocytes and fibroblasts to adjacent regions

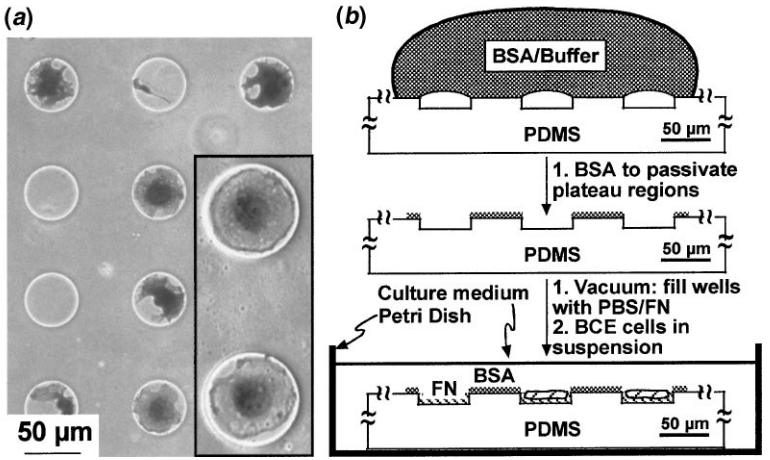

Figure 9 Mammalian cells arrayed in microwells using the behavior of liquids on contoured surfaces. (*a*) Micrographs of bovine capillary endothelial (BCE) cells that were deposited in an array of wells 50 μm in diameter (1.3 μm tall). (*Inset*) Two BCE cells that spread to cover the entire area of their wells. (*b*) Schematic illustration of the procedures used to produce the array of BCE cells shown in *panel a*. A solution of bovine serum albumin (BSA) was deposited on the array of microwells and trapped air bubbles in the wells; BSA adsorbed selectively in the space separating the wells. (*Drop*) The shape is spherical but here it is flattened to minimize the size of the image. After rinsing with buffer, the system is exposed to vacuum for three 30-s intervals to release the air bubbles from the wells. The buffer is then exchanged for a fibronectin-containing (FN) buffer that enters the wells and coats them with a layer of protein. Cells from suspension adhere selectively to the microwells that are coated with FN.

of a surface (224). A pattern of photoresist on glass was exposed to a solution of collagen to allow the protein to adsorb to the glass and the photoresist; upon lift-off (see above) of the photoresist, the collagen remained patterned on the glass and, as important, remained active in promoting cell attachment (225). Hepatocytes adhered selectively to the pattern of collagen; fibroblasts were subsequently deposited in the areas separating the islands of hepatocytes to create a patterned coculture (216). Cocultures of hepatocytes and fibroblasts maintained the phenotype of the hepatocytes in vitro for several weeks (217); this result is remarkable in light of the known instability of individual hepatocyte cultures in vitro. Cocultures can also be created using MEMPAT (124).

3D CHANNEL SYSTEMS Three-dimensional channel systems have allowed us to pattern suspensions of two or more types of cells onto tissue culture surfaces (133). We have used the system described in Figure 6*b* to deposit human bladder cancer cells and BCE cells in concentric squares separated by 50 μm. On removing the

channel structures used to deliver the cells, both types of cells began to spread on the adhesive tissue-culture surface. The BCE cells spread faster than the cancer cells; the two spreading cultures came into contact over a 20 h period after spreading over ca 200 μm.

These studies demonstrate the ability both to generate systems in which cells migrate in defined environments and geometries and to study rates of spreading by direct comparison in cocultures.

Influence of Surface Topography on Cell Behavior

Epithelial and neuronal cells that adhere to topographical features that are long and narrow with respect to the typical length scale of a cell ($\sim$50 μm) align their bodies and cytoskeletons in the direction parallel to the length of the feature (226). This alignment is influenced by the height of the microfabricated features used to direct the attachment of the cells; the extent of alignment increases with increasing height of the features (227). Wilkinson and coworkers found that cells that adhered across several lines develop more focal adhesions and actin fibers in the areas of contact with the lines than in the areas between the lines. We have used soft lithography to produce contoured substrates that cause cells to align their bodies and stress fibers; the subtrates were made using patterned SAMs (228) or etched microfluidic structures (128). There is some indication that surfaces roughened with features of $<$0.5 μm prevent the adhesion of some cell types (229, 230).>

Patterning Over a Single Cell

The laminar flow of fluids in microfluidic channels can be used to deliver reagents from solutions to a part of the surface of a single cell by positioning the interface between two liquid streams containing different solutes, flowing laminarly, over one cell (132; S Takayama, E Ostuni, P LeDuc, K Naruse, DE Ingber, & GM Whitesides, submitted for publication). If the material being delivered is able to diffuse across the cell membrane, this technique can also generate patterns inside the cell. The nature of these patterns depends on the relative rate of diffusion across the cell membrane and of diffusion within the cell. We call the collection of techniques that depend on delivering reagents to part of a cell using laminar flows partial treatment of cells using laminar flow (PARTCELL) (Figure 10) (S Takayama, E Ostuni, P LeDuc, K Naruse, DE Ingber, & GM Whitesides, *Nature*, In press).

Bradke & Dotti have recently described a method for patterning fluid flow over single neurites that involves the use of two pipettes with tips positioned close to the cell: one to deliver fluid to the neurite outgrowth and one to remove the fluid (144). Each technique has useful characteristics, but PARTCELL can deliver fluids to parts of cells more accurately and with sharper boundaries between different liquid streams. The technique based on pipettes enables programmed motion of the pipette tips in the plane of the substrate.

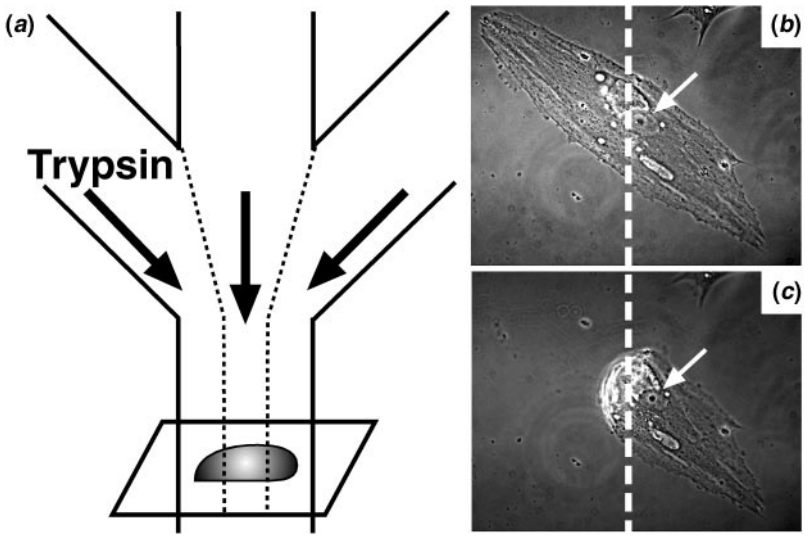

Figure 10 PARTCELL (partial treatment of cells using laminar flow) makes it possible to treat parts of cells selectively with small molecules as well as with proteins. (*a*) Schematic illustration of a typical PARTCELL experiment. Flows of different fluids (trypsin-EDTA and culture media) are positioned over different parts of a single, live bovine capillary endothelial (BCE) cell. (*b*) The BCE cell before trypsin treatment. (*c*) The same BCE cell after 5 min of trypsin treatment over the left side of the cell. The matrix proteins that maintain cell attachment were digested in the trypsin-treated side, causing cell detachment. The untreated side of the cell remained practically unchanged. Note that the position of the nucleus (*arrows*) does change after treatment of the cell with trypsin.

PATTERNING THE MEDIUM OVER A SINGLE CELL PARTCELL made it possible to flow a solution containing an acetylated low-density lipoprotein (LDL) derivative over a part of a BCE cell. The LDL binds its receptors on the cell surface and is endocytosed selectively in the area of the cell that was contacted by the flow. Over time, the endocytosed LDL is distributed throughout the cell.

PATTERNING THE INSIDE OF A CELL PARTCELL was used to deliver latrunculin A—a membrane-permeable, actin-disrupting molecule—to part of a cell with fluorescently labeled mitochondria. The disassembly of the actin filaments in one area of the cell caused a shift in the position of mitochondria—typically associated with cytoskeletal structures—in the cytoplasm, while the morphology of the cell remained largely unchanged.

PATTERNING THE CELL SUBSTRATE PARTCELL is also useful in targeting the extracellular proteins that determine interaction between a cell and the substrate to

which it adheres. We have accomplished the selective detachment of a part of a cell from a surface using PARTCELL to deliver a stream of trypsin to only that part of the cell. Trypsin digests the ECM and the cell surface proteins only in the region of the cell that it contacts (Figure 10).

Cell Behavior in and on a Gradient

Techniques we have developed for generating gradients make it possible to study the biological problems in the areas of migration, proliferation, development, and differentiation that, in vivo, occur along a gradient. These techniques enable us to generate gradients flexible in shape or spatial extent conveniently (140, 141, 144). The transfer of a gradient of protein in solution to a surface occurs on contact and should make possible the study of a range of cellular problems (136, 137).

OUTLOOK AND CONCLUSIONS

Soft lithography has features that are unique in microfabrication and that complement conventional techniques: (*a*) It can be carried out conveniently, rapidly, and inexpensively especially for the relatively large features ($\geq$50 μm) commonly used in biology, (*b*) it can pattern delicate biological matter and is applicable to a wide range of materials, and (*c*) it provides the ability to control the properties of surfaces at the molecular level. The characteristics of soft lithography are the basis for new techniques for micropatterning and for new microdevices for biology.

The formation of surfaces with well-defined molecular characteristics and complex topographies using the techniques of soft lithography has made it possible to miniaturize existing biochemical and cell-based assays and to create new ones. Applications of soft lithography to the patterning of proteins and cells have relied on the availability of inert and biospecifically adsorbing surfaces based on SAMs as well as on the ease of fabrication of elastomeric stamps for printing. The combination of surface engineering and μCP has yielded methods for patterning single and multiple cells and cocultures. The convenience of the fabrication of polymeric components, combined with the unique ability of elastomers to seal conformally to surfaces, underlies microcontact printing, microfluidics, and membrane-based patterning methods—MEMPAT.

Complex structures fabricated by soft lithography provide the basis for microanalysis systems that use microfluidic channels. Soft lithography makes the fabrication of microfluidic systems (both two and three dimensional) straightforward. The flow of fluids inside microfluidic systems is characterized largely by laminar flow; this kind of fluid flow is the basis for useful fluidic devices, but it also poses challenges in fluid manipulation. (*a*) New and sensitive methods of separation and detection of biochemical species have been developed using laminar flow. (*b*) The ability to carry out reactions at the interface of two fluids flowing laminarly in adjacent streams has made it possible to fabricate structures such as electrodes inside microfluidic systems. (*c*) Generation of gradients in solution and on surfaces with microfluidic systems has the spatial resolution that makes it

applicable to biological problems. And (*d*) the combination of laminar flow in microfluidic systems with surface patterning was key in developing new capabilities in the analysis of single cells using PARTCELL.

The microanalysis systems that can be fabricated using soft lithographic techniques are broadly applicable to (*a*) rapid and low-cost manufacturing of patterned arrays of the structure needed for high-throughput screening, (*b*) proteomics (when combined with surface engineering), (*c*) studies of cell biology (e.g. the cell cycle and stem cells), and (*d*) studies of cocultures for tissue engineering.

Rapid prototyping using soft lithography is particularly useful in the area of μTAS. Components such as valves and mixers are more easily fabricated in elastomers than in glass and silicon, and some designs can only be fabricated in elastomers. Some components—for example valves and interconnects—are remarkably tolerant of size variation when fabricated using soft materials. One of the outstanding problems in the field of μTAS is the integration of miniaturized detectors with microanalysis systems; this problem exists regardless of the material with which the systems are fabricated. It is, however, possible in principle to integrate systems fabricated in elastomers using soft lithography with all-organic electronic devices; these systems may be processed and fabricated more easily than those based on silicon or glass. Implantable devices made with soft polymers may have the advantage of being more biocompatible and more comfortable for the recipient than devices made with rigid materials.

The tools provided by soft lithography for the study of cell biology are making it possible to address new problems. PARTCELL is useful for studying phenomena at the subcellular level; organelle trafficking and endocytosis are two relevant examples. The application of a stimulus to a cell in a well-defined, localized region with PARTCELL allows the observation of the "global" response of the cell to such localized stimuli. The simplicity with which gradients of proteins can be generated in solution or on a surface using soft lithography will be useful in the study of areas of biology in which gradients are important: Embryonic development, neuronal polarity, and stem cell differentiation are three areas in which the ability to generate gradients over the length of one cell will provide new capability.

The use of polymeric materials in soft lithography will allow the application of the broad range of properties available in these materials in microfabricated systems: Electrical conduction, actuation, tunable optical reflection, shear-induced deformation, liquid crystalline behavior, and gelation are examples of such properties. The new capabilities made possible by the biological applications of soft lithography, combined with the ease and low cost with which devices and arrays can be fabricated, promise to make these techniques important components of both basic and applied biological research.

ACKNOWLEDGMENTS

Financial support for the majority of this work was provided by grants from NIH (GM30367, CA 45548), and DARPA. We also used MRSEC shared facilities supported by NSF. EO thanks Glaxo-Wellcome Inc. for a pre-doctoral fellowship.

ST acknowledges a post-doctoral fellowship from the Leukemia and Lymphoma Society. We are grateful to many coworkers who have made significant contributions to this field throughout the last 10 years: Nicholas Abbott, Jose-Luis Alonso, Janelle Anderson, Colin Bain, Hans Biebuyck, Scott Brittain, Robert Chapman, Christopher Chen, Daniel Chiu, Tao Deng, Stephan Dertinger, Laura Dike, David Duffy, Sui Huang, Lyle Isaacs, Rustem Ismagilov, Rebecca Jackman, NooLi Jeon, Paul Kenis, Enoch Kim, Amit Kumar, Paul Laibinis, Joydeep Lahiri, Philip LeDuc, Gabriel Lopez, Milan Mrksich, Kateri Paul, Kevin Parker, Kevin Prime, Dong Qin, Carmichael Roberts, John Rogers, Olivier Schueller, George Sigal, Rahul Singhvi, Howard Stone, Abraham Stroock, Younan Xia, Lin Yan.

LITERATURE CITED

1. Hudson TJ, Stein LD, Gerety SS, Ma J, Castle AB, et al. 1995. An STS-based map of the human genome. *Science* 270:1945–54
2. Chervitz SA, Aravind L, Sherlock G, Ball CA, Koonin EV, et al. 1998. Comparison of the complete protein sets of worm and yeast: orthology and divergence. *Science* 282:2022–28
3. Adams MD, Celniker SE, Holt RA, Evans CA, Gocayne JD, et al. 2000. The genome sequence of *Drosophila melanogaster*. *Science* 287:2185–95
4. Fodor SP, Read JL, Pirrung MC, Stryer L, Lu AT, Solas D. 1991. Light-directed, spatially addressable parallel chemical synthesis. *Science* 251:767–73
5. Lam KS, Salmon SE, Hersh EM, Hruby VJ, Kazmierski WM, Knapp RJ. 1991. A new type of synthetic peptide library for identifying ligand-binding activity. *Nature* 354:82–84
6. Hacia JC, Fan JB, Ryder O, Jin L, Edgemon K, et al. 1999. Determination of ancestral alleles for human single-nucleotide polymorphisms using high-density oligonucleotide arrays. *Nat. Genet.* 22: 164–67
7. Gilles PN, Foster CB, Dillon PJ, Chanock SJ, Wu DJ. 1999. Single nucleotide polymorphic discrimination by an electronic dot blot assay on semiconductor microchips. *Nat. Biotechnol.* 17: 365–70
8. Xu X, Miller C, Wang L, Edman CF, Nerenberg M, Westin L. 2000. Anchored multiplex amplification on a microelectronic chip array. *Nat. Biotechnol.* 18: 199–204
9. Schena M, Shalon D, Davis RW, Brown PO. 1995. Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science* 270: 467–70
10. Lockhart DJ, Dong H, Byrne MC, Follettie MT, Gallo MV, et al. 1996. Expression monitoring by hybridization to high-density oligonucleotide arrays. *Nat. Biotechnol.* 14:1675–80
11. Fodor SP, Rava RP, Huang XC, Pease AC, Holmes CP, Adams CL. 1993. Multiplexed biochemical assays with biological chips. *Nature* 364:555–56
12. Chee M, Yang R, Hubbell E, Berno A, Huang XC, et al. 1996. Accessing genetic information with high-density DNA arrays. *Science* 274:610–14
13. Xia Y, Whitesides GM. 1998. Soft lithography. *Angew. Chem. Int. Ed. Engl.* 37: 550–75
14. Bernard A, Delamarche E, Schmid H, Michel B, Bosshard HR, Biebuyck H.

1998. Printing patterns of proteins. *Langmuir* 14:2225–29
15. Chen CS, Mrksich M, Huang S, Whitesides GM, Ingber DE. 1997. Geometric control of cell life and death. *Science* 276: 1425–28
16. James CD, Davis RC, Kam L, Craighead HG, Isaacson M, et al. 1998. Patterned protein layers on solid substrates by thin stamp microcontact printing. *Langmuir* 14:741–44
17. Singhvi R, Kumar A, Lopez GP, Stephanopolous GN, Wang DIC, et al. 1994. Engineering cell shape and function. *Science* 264:696–98
18. St John PM, Davis R, Cady N, Czajka J, Batt CA, Craighead HG. 1998. Diffraction-based cell detection using a microcontact printed antibody grating. *Anal. Chem.* 70:1108–11
19. deGennes PG. 1996. Introductory lecture—mechanics of soft interfaces. *Faraday Discuss*., pp. 1–8
20. deGennes PG. 1996. Soft adhesives. *Langmuir* 12:4497–500
21. Mrksich M, Whitesides GM. 1996. Using self-assembled monolayers to understand the interactions of man-made surfaces with proteins and cells. *Annu. Rev. Biophys. Biomol. Struct.* 25:55–78
22. Whitesides GM, Gorman CB, eds. 1995. *Self-Assembled Monolayers: Models for Organic Surface Chemistry*. Boca Raton, FL: CRC
23. Whitesides GM, Laibinis PE. 1990. Wet chemical approaches to the characterization of organic surfaces: self-assembled monolayers, wetting and the physical-organic chemistry of the solid-liquid interface. *Langmuir* 6:87–96
24. Troughton EB, Bain CD, Whitesides GM, Nuzzo RG, Allara DL, Porter MD. 1988. Monolayer films prepared by the spontaneous self-assembly of symmetrical and unsymmetrical dialkyl sulfides from solution onto gold substrates: structure, properties, and reactivity of constituent functional groups. *Langmuir* 4: 365–85
25. Nuzzo RG, Zegarski BR, Dubois LH. 1987. Fundamental studies of the chemisorption of organosulfur compounds on au(111). Implications for molecular self-assembly on gold surfaces. *J. Am. Chem. Soc.* 109:733–40
26. Nuzzo RG, Allara DL. 1983. Adsorption of bifunctional organic disulfides on gold surfaces. *J. Am. Chem. Soc.* 105:4481–83
27. Dubois LH, Nuzzo RG. 1992. Synthesis, structure, and properties of model organic surfaces. *Annu. Rev. Phys. Chem.* 43:437–63
28. Bain CD, Whitesides GM. 1989. Modelling organic surfaces with self-assembled monolayers. *Angew. Chem. Int. Ed. Engl.* 28:506–16
29. Dubois LH, Zegarski BR, Nuzzo RG. 1990. Fundamental studies of microscopic wetting on organic surfaces. 2. Interaction of secondary adsorbates with chemically textured organic monolayers. *J. Am. Chem. Soc.* 112:570–79
30. Nuzzo RG, Dubois LH, Allara DL. 1990. Fundamental studies of microscopic wetting on organic surfaces. 1. Formation and structural characterization of a self-consistent series of polyfunctional organic monolayers. *J. Am. Chem. Soc.* 112: 558–69
31. Abbott NL, Folkers JP, Whitesides GM. 1992. Manipulation of the wettability of surfaces on the 0. 1-to 1-micrometer scale through micromachining and molecular self-assembly. *Science* 257:1380–82
32. Abbott N, Kumar A, Whitesides GM. 1994. Using micromachining, molecular self-assembly and wet etching to fabricate 0. 1 to 1 micrometer–scale structures of gold and silicon. *Chem. Mater.* 6:596–602
33. Ostuni E, Yan L, Whitesides GM. 1999. The interaction of proteins and cells with self-assembled monolayers of alkanethiolates on gold and silver. *Colloids Surf. B* 15:3–30
34. Mrksich M. 2000. A surface chemistry

approach to studying cell adhesion. *Chem. Soc. Rev.* 29:267–73
35. Karlsson R, Roos H, Fagerstam L, Persson B. 1994. Kinetic and concentration analysis using BIA technology. *Methods Compan. Methods Enzymol.* 6:99–110
36. Raether H. 1977. Surface plasma oscillations and their applications. In *Physics of Thin Films*, ed. G Hass, M Francombe, R Hoffman, pp. 145–261. New York: Academic
37. Szabo A, Stolz L, Granow R. 1995. Surface plasmon resonance and its use in biomolecular interaction analysis. *Curr. Opin. Struct. Biol.* 5:699–705
38. Malmqvist M. 1993. Biospecific interaction analysis using biosensor technology. *Nature* 361:186–87
39. Mrksich M, Sigal GB, Whitesides GM. 1995. Surface plasmon resonance permits in situ measurement of protein adsorption on self-assembled monolayers of alkanethiolates on gold. *Langmuir* 11:4383–85
40. Keller CA, Kasemo B. 1998. Surface specific kinetics of lipid vesicle adsorption measured with a quartz crystal microbalance. *Biophys. J.* 75:1397–402
41. Hook F, Rodahl M, Kasemo B, Brzezinski P. 1998. Structural changes in hemoglobin during adsorption to solid surfaces: effects of pH, ionic strength, and ligand binding. *Proc. Natl. Acad. Sci. USA* 95:12271–76
42. Ward MD, Buttry DA. 1990. In situ interfacial mass detection with piezoelectric transducers. *Science* 249:1000–7
43. Azzam RMA, Bashara NM. 1977. *Ellipsometry and Polarized Light*. Amsterdam: N. Holland
44. Sigal GB, Mrksich M, Whitesides GM. 1998. Effect of surface wettability on the adsorption of proteins and detergents. *J. Am. Chem. Soc.* 120:3464–73
45. Bain CD, Whitesides GM. 1988. Correlation between wettability and structure in monolayers in alkanethiols adsorbed on gold. *J. Am. Chem. Soc.* 110:3665–66
46. Cheng S-S, Chittur KK, Sukenik CN, Culp LA, Lewandowska K. 1994. The conformation of fibronectin on self-assembled monolayers with different surface composition: an FTIR/ATR study. *J. Colloid Interface Sci.* 162:135–43
47. Liley M, Keller TA, Duschl C, Vogel H. 1997. Direct observation of self-assembled monolayers, ion complexation, and protein conformation at the gold/water interface: an ftir spectroscopic approach. *Langmuir* 13:4190–92
48. O'Brien JC, Stickney JT, Porter MD. 2000. Preparation and characterization of self-assembled double-stranded DNA (dsDNA) microarrays for protein: dsDNA screening using atomic force microscopy. *Langmuir* 16:9559–67
49. Sheller NB, Petrash S, Foster MD. 1998. Atomic force microscopy and X-ray reflectivity studies of albumin adsorbed onto self-assembled monolayers of hexadecyltrichlorosilane. *Langmuir* 14:4535–44
50. Petralli-Mallow TP, Plant AL Lewis ML, Hicks JM. 2000. Cytochrome c at model membrane surfaces: exploration via second harmonic generation-circular dichroism and surface-enhanced resonance raman spectroscopy. *Langmuir* 16:5960–66
51. Feldman LC, Mayer JW. 1986. *Fundamentals of Surface and Thin Film Analysis* . Englewood Cliffs, NJ: Prentice Hall
52. Dahint R, Grunze M, Josse F, Renken J. 1994. Acoustic plate mode sensor for immunochemical reactions. *Anal. Chem.* 66:2888–92
53. Calabrese GS, Wohltjen H, Roy MK. 1987. Surface acoustic wave devices as chemical sensors in liquids. Evidence disputing the importance of rayleigh wave propagation. *Anal. Chem.* 59:833–37
54. Wasserman SR, Whitesides GM, Tidswell IM, Ocko BM, Pershan PS, Axe JD. 1989. The structure of self-assembled monolayers of alkylsiloxanes on silicon: a comparison of results from ellipsometry

and low-angle X-ray reflectivity. *J. Am. Chem. Soc.* 111:5852–61
55. Tidswell IM, Ocko BM, Pershan PS, Wasserman SR, Whitesides GM, Axe JD. 1990. X-ray specular reflection studies of silicon coated by organic monolayers (alkylsiloxanes). *Phys. Rev. B* 41:1111–28
56. Hodneland CD, Mrksich M. 1997. Design of self-assembled monolayers that release attached groups using applied electrical potentials. *Langmuir* 13:6001–3
57. Yousaf MN, Mrksich M. 1999. Diels-alder reaction for the selective immobilization of protein to electroactive self-assembled monolayers. *J. Am. Chem. Soc.* 121:4286–87
58. López GP, Biebuyck HA, Whitesides GM. 1993. Scanning electron microscopy can form images of patterns in self-assembled monolayers. *Langmuir* 9:1513–16
59. López GP, Biebuyck HA, Härter R, Kumar A, Whitesides GM. 1993. Fabrication and imaging of two-dimensional patterns of proteins adsorbed on self-assembled monolayers by scanning electron microscopy. *J. Am. Chem. Soc.* 115:10774–81
60. Brzoska JB, Benazouz I, Rondelez F. 1994. Silanization of solid substrates—a step toward reproducibility. *Langmuir* 10: 4367–73
61. Wirth MJ, Fairbank RWP, Fatunmbi HO. 1997. Mixed self-assembled monolayers in chemical separations. *Science* 275:44–47
62. Allara DL, Parikh AN, Rondelez F. 1995. Evidence for a unique chain organization in long-chain silane monolayers deposited on 2 widely different solid substrates. *Langmuir* 11:2357–60
63. Harris JM, Zalipsky S. 1997. *Poly*(*ethylene Glycol*)*: Chemistry and Biological Applications*. Washington, DC: Am. Chem. Soc.
64. Andrade JD, Hlady V. 1986. Protein adsorption and materials biocompatibility: a tutorial review and suggested hypotheses. *Adv. Polym. Sci.* 79:1–63
65. Jeon SI, Lee LH, Andrade JD, de Gennes PG. 1991. Protein-surface interactions in the presence of polyethylene oxide. I. Simplified theory. *J. Colloid Interface Sci.* 142:149–58
66. Jeon SI, Andrade JD. 1991. Protein-surface interactions in the presence of polyethylene oxide. II. Effect of protein size. *J. Colloid Interface Sci.* 142:159–65
67. Hamburger R, Azaz E, Donbrow M. 1975. Autoxidation of polyoxyethylenic nonionic surfactants and of polyethylene glycols. *Pharm. Acta Helv.* 50:10–17
68. Gerhardt W, Martens C. 1985. Oxidation of polyethylene oxides and polyethylene oxide ethers: formation of acetaldehyde during the oxidation of diethylene glycol with oxygen. *Z. Chem.* 25:143
69. Crouzet C, Decker C, Marchal J. 1976. Characterization of primary oxidative degradation reactions in the course of the autooxidation of poly(oxyethylene)s at 25°C: study in aqueous solution with initiation by solvent irradiation. 8. Kinetic studies at pH between 1 and 13. *Makromol. Chem.* 177:145–57
70. Herold DA, Keil K, Bruns DE. 1989. Oxidation of polyethylene glycols by alcohol dehydrogenase. *Biochem. Pharmacol.* 38:73–76
71. Talarico T, Swank A, Privalle C. 1998. Autoxidation of pyridoxalated hemoglobin polyoxyethylene conjugate. *Biochem. Biophys. Res. Commun.* 250:354–58
72. Holmlin RE, Chen X, Chapman RG, Takayama ST, Whitesides GM. 2000. Zwitterionic SAMs that resist the nonspecific adsorption of protein from aqueous buffer. *Langmuir.* In press
73. Chapman RG, Ostuni E, Takayama S, Holmlin RE, Yan L, Whitesides GM. 2000. Surveying for surfaces that resist the adsorption of proteins. *J. Am. Chem. Soc.* 122:8303–4
74. Deleted in proof
75. Luk Y-Y, Kato M, Mrksich M. 2000.

Self-assembled monolayers of alkanethiolates presenting mannitol groups are inert to protein adsorption and cell attachment. *Langmuir* 16:9604–08
76. Chapman RG, Ostuni E, Liang MN, Meluleni G, Kim E, et al. 2001. Polymeric thin films that resist adsorption of proteins and the adhesion of bacteria. *Langmuir* 17:1225–33
77. Mrksich M, Grunwell JR, Whitesides GM. 1995. Bio-specific adsorption of carbonic anhydrase to self-assembled monolayers of alkanethiolates that present benzenesulfonamide groups on gold. *J. Am. Chem. Soc.* 117:12009–10
78. Roberts C, Chen CS, Mrksich M, Martichonok V, Ingber DE, Whitesides GM. 1998. Using mixed self-assembled monolayers presenting RGD and $(EG)_3$oh groups to characterize long-term attachment of bovine capillary endothelial cells to surfaces. *J. Am. Chem. Soc.* 120:6548–55
79. Bain CD, Whitesides GM. 1988. Formation of two-component surfaces by the spontaneous assembly of monolayers on gold from solutions containing mixtures of organic thiols. *J. Am. Chem. Soc.* 110:6560–61
80. Bain CD, Evall J, Whitesides GM. 1989. Formation of monolayers by the coadsorption of thiols on gold: variation in the head group, tail group, and solvent. *J. Am. Chem. Soc.* 111:7155–64
81. Laibinis PE, Nuzzo RG, Whitesides GM. 1992. Structure of monolayers formed by coadsorption of two *n*-alkanethiols of different chain lengths on gold and its relation to wetting. *J. Phys. Chem.* 96:5097–105
82. Lahiri J, Isaacs L, Tien J, Whitesides GM. 1999. A strategy for the generation of surfaces presenting ligands for studies of binding based on an active ester as a common reactive intermediate: a surface plasmon resonance study. *Anal. Chem.* 71:777–90
83. Lahiri J, Ostuni E, Whitesides GM. 1999. Patterning ligands on reactive sams by microcontact printing. *Langmuir* 15:2055–60
84. Lahiri J, Isaacs L, Grzybowski B, Carbeck JD, Whitesides GM. 1999. Biospecific binding of carbonic anhydrase to mixed sams presenting benzenesulfonamide ligands: a model system for studying lateral steric effects. *Langmuir* 15: 7186–98
85. Hodneland CD, Mrksich M. 2000. Biomolecular surfaces that release ligands under electrochemical control. *J. Am. Chem. Soc.* 122:4235–36
86. Yousaf MN, Mrksich M. 2000. Electroactive substrates that modulate cell growth. *Biochemistry* 39:170–75
87. Yan L, Marzolin C, Terfort A, Whitesides GM. 1997. Formation and reaction of interchain carboxylic anhydride groups on self-assembled monolayers on gold. *Langmuir* 13:6704–12
88. Yan L, Zhao X-M, Whitesides GM. 1998. Patterning a preformed, reactive sam using microcontact printing. *J. Am. Chem. Soc.* 120:6179–80
89. Chapman RG, Ostuni E, Yan L, Whitesides GM. 2000. Preparation of mixed sams that resist adsorption of proteins using the reaction of amines with a SAM that presents interchain carboxylic anhydride groups. *Langmuir* 16:6927–36
90. Zhao X-M, Xia Y, Whitesides GM. 1996. Fabrication of three-dimensional microstructures: microtransfer molding. *Adv. Mater.* 8:837–40
91. Xia Y, Kim E, Zhao X-M, Rogers JA, Prentiss M, Whitesides GM. 1996. Complex optical surfaces by replica molding against elastomeric masters. *Science* 273: 347–49
92. Xia Y, McClelland JJ, Gupta R, Qin D, Zhao X-M, et al. 1997. Replica molding using polymeric materials: a practical step toward nanomanufacturing. *Adv. Mater.* 9:147–49
93. Schmid H, Michel B. 2000. Siloxane polymers for high-resolution, high-accuracy

soft lithography. *Macromolecules* 33: 3042–49
94. Spiering VL, Bouwstra S, Spiering R. 1993. On chip decoupling zone for package-stress reduction. *Sens. Actuators A* 39:149–56
95. Chaudhury MK, Whitesides GM. 1991. Direct measurement of interfacial interactions between semispherical lenses and flat sheets of polydimethylsiloxane and their chemical derivatives. *Langmuir* 7:1013–25
96. Chaudhury MK, Whitesides GM. 1992. Correlation between surface free energy and surface constitution. *Science* 255: 1230–32
97. Duffy DC, McDonald JC, Schueller OJA, Whitesides GM. 1998. Rapid prototyping of microfluidic systems in poly(dimethylsiloxane). *Anal. Chem.* 70: 4974–84
98. Morra M, Occhiello E, Marola R, Garbassi F, Humphrey P, Johnson D. 1990. On the aging of oxygen plasma-treated polydimethylsiloxane surfaces. *J. Colloid Interface Sci.* 137:11–24
99. Qin D, Xia Y, Black AJ, Whitesides GM. 1998. Photolithography with transparent reflective photomasks. *J. Vac. Sci. Technol. B* 16:98–103
100. Qin D, Xia Y, Whitesides GM. 1996. Rapid prototyping of complex structures with feature sizes larger than 20 μm. *Adv. Mater.* 8:917–19
101. Deng T, Wu H, Brittain ST, Whitesides GM. 2000. Prototyping of masks, masters, and stamps/molds for soft lithography using an office printer and photographic reduction. *Anal. Chem.* 72:3176–80
102. Deng T, Arias F, Ismagilov RF, Kenis PJA, Whitesides GM. 2000. Fabrication of metallic microstructures using exposed, developed silver halide-based photographic film. *Anal. Chem.* 72:645–51
103. Deleted in proof
104. Kumar A, Whitesides GM. 1993. Features of gold having micrometer to centimeter dimensions can be formed through a combination of stamping with an elastomeric stamp and an alkanethiol ink followed by chemical etching. *Appl. Phys. Lett.* 63:2002–4
105. Kumar A, Biebuyck HA, Abbott NL, Whitesides GM. 1992. The use of self-assembled monolayers and a selective etch to generate patterned gold features. *J. Am. Chem. Soc.* 114:9188–99
106. Rogers JA, Paul KE, Jackman RJ, Whitesides GM. 1998. Generating ~90 nanometer features using near-field contact-mode photolithography with an elastomeric phase mask. *J. Vac. Sci. Technol. B* 26:59–68
107. Aizenberg J, Rogers JA, Paul KE, Whitesides GM. 1998. Imaging profiles of light intensity in the near field: applications to phase-shift photolithography. *Appl. Optics* 37:2145–52
108. Rogers JA, Paul KE, Jackman RJ, Whitesides GM. 1997. Using an elastomeric phase mask for sub-100 nm photolithography in the optical near field. *Appl. Phys. Lett.* 70:2658–60
109. Rogers JA, Paul KE, Whitesides GM. 1998. Quantifying distortions in soft lithography. *J. Vac. Sci. Technol. B* 16:88–97
110. Delamarche E, Schmid H, Bietsch A, Larsen NB, Rothuizen H, et al. 1998. Transport mechanisms of alkanethiols during microcontact printing on gold. *J. Phys. Chem. B* 102:3324–34
111. Larsen NB, Biebuyck H, Delamarche E, Michel B. 1997. Order in microcontact printed self-assembled monolayers. *J. Am. Chem. Soc.* 119:3017–26
112. Jeon NL, Nuzzo RG, Xia Y, Mrksich M, Whitesides GM. 1995. Patterned self-assembled monolayers formed by microcontact printing direct selective metallization by chemical vapor deposition on planar and nonplanar substrates. *Langmuir* 11:3024–26
113. Koide Y, Wang Q, Cui J, Benson DB, Marks TJ. 2000. Patterned luminescence

of organic light-emitting diodes by hot microcontact printing (hmcp) of self-assembled monolayers. *J. Am. Chem. Soc.* 122:11266–67
114. Delamarche E, Michel B. 1996. Structure and stability of self-assembled monolayers. *Thin Solid Films* 273:54–60
115. Xia Y, Whitesides GM. 1995. Use of controlled reactive spreading of liquid alkanethiol on the surface of gold to modify the size of features produced by microcontact printing. *J. Am. Chem. Soc.* 117: 3274–75
116. Burgin T, Choong V-E, Maracas G. 2000. Large area submicrometer contact printing using a contact aligner. *Langmuir* 16: 5371–75
117. Huck WTS, Yan L, Stroock A, Haag R, Whitesides GM. 1999. Patterned polymer multilayers as etch resists. *Langmuir* 15:6862–67
118. Yan L, Huck WTS, Zhao X-M, Whitesides GM. 1999. Patterning thin films of poly(ethylene imine) on a reactive sam using microcontact printing. *Langmuir* 15: 1208–14
119. Piner RD, Zhu J, Feng X, Hong S, Mirkin CA. 1999. "Dip-pen" nanolithography. *Science* 286:523–25
120. Hong SH, Zhu J, Mirkin CA. 1999. Multiple ink nanolithography: toward a multiple-pen nano-plotter. *Science* 286: 523–25
121. Hang SH, Mirkin CA. 2000. A nanoplotter with both parallel and serial writing capabilities. *Science* 288:1808–11
122. Jackman RJ, Duffy DC, Cherniavskaya O, Whitesides GM. 1999. Using elastomeric membranes as dry resists and for dry liftoff. *Langmuir* 15:2973–84
123. Duffy DC, Jackman RJ, Vaeth KM, Jensen KF, Whitesides GM. 1999. Patterning electroluminescent materials at feature sizes as small as 5 μm using elastomeric membranes as masks for dry liftoff. *Adv. Mater.* 11:546–52
124. Ostuni E, Kane R, Chen CS, Ingber DE, Whitesides GM. 2000. Patterning mammalian cells using elastomeric membranes. *Langmuir* 16:7811–19
125. Folch A, Jo BH, Hurtado O, Beebe DJ, Toner M. 2000. Microfabricated elastomeric stencils for micropatterning cell cultures. *J. Biomed. Mater. Res.* 52:346–53
126. Brody JP, Yager P, Goldstein RE, Austin RH. 1996. Biotechnology at low reynolds numbers. *Biophys. J.* 71:3430–41
127. Kenis PJA, Ismagilov RF, Whitesides GM. 1999. Microfabrication inside capillaries using multiphase laminar flow patterning. *Science* 285:83–85
128. Takayama S, Ostuni E, Qian X, McDonald JC, Jiang X, et al. 2001. Topographical micropatterning of poly(dimethylsiloxane) using laminar flows of liquids in capillaries. *Adv. Mat.* In press
129. Kenis PJA, Ismagilov RF, Takayama S, Whitesides GM, Li S, White HS. 2000. Fabrication inside microchannels using fluid flow. *Acc. Chem. Res.* 33:841–47
130. Delamarche E, Bernard A, Schmid H, Bietsch A, Michel B, Biebuyck H. 1998. Microfluidic networks for chemical patterning of substrate: design and application to bioassays. *J. Am. Chem. Soc.* 120: 500–8
131. Delamarche E, Bernard A, Schmid H, Michel B, Biebuyck H. 1997. Patterned delivery of immunoglobulins to surfaces using microfluidic networks. *Science* 276:779–81
132. Takayama S, McDonald JC, Ostuni E, Liang MN, Kenis JA, et al. 1999. Patterning cells and their environments using multiple laminar fluid flows in capillary networks. *Proc. Natl. Acad. Sci. USA* 96:5545–48
133. Chiu DT, Jeon NL, Huang S, Kane RS, Wargo CJ, et al. 2000. Patterned deposition of cells and proteins onto surfaces by using three-dimensional microfluidic systems. *Proc. Natl. Acad. Sci. USA* 97: 2408–13
134. Anderson JR, Chiu DT, Jackman RJ, Cherniavskaya O, McDonald JC, et al. 2000. Fabrication of topologically

complex three-dimensional microfluidic systems in PDMS by rapid prototyping. *Anal. Chem.* 72:3158–64
135. Bernard A, Renault JP, Michel B, Bosshard HR, Delamarche E. 2000. Microcontact printing of proteins. *Adv. Mater.* 12:1067–70
136. Dertinger SKW, Chiu DT, Jeon NL, Whitesides GM. 2001. Generation of gradients having complex shapes using microfluidic networks. *Anal. Chem.* 73:1240–46
137. Jeon NL, Dertinger SKW, Chiu DT, Choi IS, Stroock AD, Whitesides GM. 2000. Generation of solution and surface gradients using microfluidic systems. *Langmuir* 16:8311–16
138. Liedberg B, Tengvall P. 1995. Molecular gradients of omega-substituted alkanethiols on gold—preparation and characterization. *Langmuir* 11:3821–27
139. Liedberg B, Wirde M, Tao YT, Tengvall P, Gelius U. 1997. Molecular gradients of omega-substituted alkanethiols on gold studied by X-ray photoelectron spectroscopy. *Langmuir* 13:5329–34
140. Herbert CB, McLernon TL, Hypolite CL, Adams DN, Pikus L, et al. 1997. Micropatterning gradients and controlling surface densities of photoactivatable biomolecules on self-assembled monolayers of oligo(ethylene glycol) alkanethiolates. *Chem. Biol.* 4:731–37
141. Hypolite CL, McLernon TL, Adams DN, Chapman KE, Herbert CB, et al. 1997. Formation of microscale gradients of protein using heterobifunctional photolinkers. *Bioconjugate Chem.* 8:658–63
142. Terrill RH, Balss KM, Zhang Y, Bohn PW. 2000. Dynamic monolayer gradients: active spatiotemporal control of alkanethiol coatings on thin gold films. *J. Am. Chem. Soc.* 122:988–89
143. Caelen I, Bernard A, Juncker D, Michel B, Heinzelmann H, Delamarche E. 2000. Formation of gradients of proteins on surfaces with microfluidic networks. *Langmuir* 16:9125–30
144. Bradke F, Dotti CG. 1999. The role of local actin instability in axon formation. *Science* 283:1931–34
145. Gallardo BS, Gupta VK, Eagerton FD, Long LI, Craig VS, et al. 1999. Electrochemical principles for active control of liquids on submillimiter scales. *Science* 283:57–60
146. Weiner OD, Servant G, Welch MD, Mitchison TJ, Sedat JW, Bourne HR. 1999. Spatial control of actin polymerization during neutrophil chemotaxis. *Nat. Cell Biol.* 1:75–81
147. Wilkinson PC. 1998. Assays of leukocyte locomotion and chemotaxis. *J. Immunol. Methods* 216:139–53
148. Manz A, Becker H. 1998. *Microsystem Technology in Chemistry and Life Sciences*. Berlin: Springer-Verlag
149. Ismagilov RF, Stroock AD, Kenis PJA, Stone HA, Whitesides GM. 2000. Experimental and theoretical scaling laws for transverse diffusive broadening in two-phase laminar flows in microchannels. *Appl. Phys. Lett.* 76:2376–78
150. Duffy DC, Schueller OJA, Brittain ST, Whitesides GM. 1999. Rapid prototyping of microfluidic switches in poly(dimethyl siloxane) and their actuation by electro-osmotic flow. *J. Micromech. Microeng.* 9: 211–17
151. Harrison DJ, Glavina PG, Manz A. 1993. Towards miniaturized electrophoresis and chemical-analysis systems on silicon—an alternative to chemical sensors. *Sens. Actuators B* 10:107–16
152. Manz A, Harrison DJ, Verpoorte EMJ, Fettinger JC, Paulus A, et al. 1992. Planar chips technology for miniaturization and integration of separation techniques into monitoring systems—capillary electrophoresis on a chip. *J. Chromatogr.* 593: 253–58
153. Chiem NH, Harrison DJ. 1998. Microchip systems for immunoassay: an integrated immunoreactor with electrophoretic separation for serum theophylline determination. *Clin. Chem.* 44:591–98

154. Harrison DJ, Fluri K, Chiem N, Tang T, Fan ZH. 1996. Micromachining chemical and biochemical analysis and reaction systems on glass substrates. *Sens. Actuators B* 33:105–9
155. Smits JG. 1990. Piezoelectric micropump with 3 valves working peristaltically. *Sens. Actuators A* 21:203–6
156. Shoji S, Esashi M. 1994. Microflow devices and systems. *J. Micromech. Microeng.* 4:157–71
157. Vanlintel HTG, Vandepol FCM, Bouwstra S. 1988. A piezoelectric micropump based on micromachining of silicon. *Sens. Actuators* 15:153–67
158. Koch M, Harris N, Evans AGR, White NM, Brunnschweiler A. 1998. A novel micromachined pump based on thick-film piezoelectric actuation. *Sens. Actuators A* 70:98–103
159. Gong QL, Zhou ZY, Yang YH, Wang XH. 2000. Design, optimization and simulation on microelectromagnetic pump. *Sens. Actuators A* 83:200–7
160. Unger MA, Chou HP, Thorsen T, Scherer A, Quake SR. 2000. Monolithic microfabricated valves and pumps by multilayer soft lithography. *Science* 288:113–16
161. Vandepol FCM, Vanlintel HTG, Elwenspoek M, Fluitman JHJ. 1990. A thermopneumatic micropump based on micro-engineering techniques. *Sens. Actuators A* 21:198–202
162. Moroney RM, White RM, Howe RT. 1991. Microtransport induced by ultrasonic lamb waves. *Appl. Phys. Lett.* 59: 774–76
163. Evans J, Liepmann D, Pisano AP. 1997. *Planar laminar mixer*. Presented at 10th Annu. Workshop Micro Electro Mechanical Systems (MEMS '97), January 26–29, Nagoya, Jpn.
164. Beni G, Tenan MA. 1981. Dynamics of electrowetting displays. *J. Appl. Phys.* 52: 6011–15
165. Beni G, Hackwood S. 1981. Electrowetting displays. *Appl. Phys. Lett.* 38: 207–9
166. Burns MA, Mastrangelo CH, Sammarco TS, Man FP, Webster JR, et al. 1996. Microfabricated structures for integrated DNA analysis. *Proc. Natl. Acad. Sci. USA* 93:5556–61
167. Bart SF, Tavrow LS, Mehregany M, Lang JH. 1990. Microfabricated electrohydrodynamic pumps. *Sens. Actuators A* 21:193–97
168. Fuhr G, Schnelle T, Wagner B. 1994. Traveling wave-driven microfabricated electrohydrodynamic pumps for liquids. *J. Micromech. Microeng.* 4:217–26
169. Jang JS, Lee SS. 2000. Theoretical and experimental study of mhd (magnetohydrodynamic) micropump. *Sens. Actuators A* 80:84–89
170. Manz A, Effenhauser CS, Burggraf N, Harrison DJ, Seiler K, Fluri K. 1994. Electroosmotic pumping and electrophoretic separations for miniaturized chemical-analysis systems. *J. Micromech. Microeng.* 4:257–65
171. Seiler K, Fan ZHH, Fluri K, Harrison DJ. 1994. Electroosmotic pumping and valveless control of fluid-flow within a manifold of capillaries on a glass chip. *Anal. Chem.* 66:3485–91
172. Harrison DJ, Manz A, Fan ZH, Ludi H, Widmer HM. 1992. Capillary electrophoresis and sample injection systems integrated on a planar glass chip. *Anal. Chem.* 64:1926–32
173. Arnold DW, Neyer DW, Paul PH. 2000. *On-chip electrokinetic high-pressure generation*. Presented at Meet. Am. Chem. Soc., March 25–29, San Francisco, CA
174. Paul PH, Garguilo MG, Rakestraw DJ. 1998. Imaging of pressure- and electrokinetically driven flows through open capillaries. *Anal. Chem.* 70:2459–67
175. Chaudhury MK, Whitesides GM. 1992. How to make water run uphill. *Science* 256:1539–41
176. Stroock AD, Weck M, Chiu DT, Huck WTS, Kenis PJA, et al. 2000. Patterning electro-osmotic flow with patterned surface charge. *Phys. Rev. Lett.* 84:3314–17

177. Handique K, Burke DT, Mastrangelo CH, Burns MA. 2000. Nanoliter liquid metering in microchannels using hydrophobic patterns. *Anal. Chem.* 72:4100–9
178. Deleted in proof
179. Beebe DJ, Moore JS, Bauer JM, Yu Q, Liu RH, et al. 2000. Functional hydrogel structures for autonomous flow control inside microfluidic channels. *Nature* 404: 588–92
180. Bessoth FG, de Mello AJ, Manz A. 1999. Microstructure for efficient continuous flow mixing. *Anal. Comm.* 36:213–15
181. Miyake R, Tsuzuki K, Takagi T, Imai K. 1997. *A highly sensitive and small flow-type chemical analysis system with integrated absorptiometric micro-flow cell.* Presented at 10th Ann. Workshop Micro Electromech. Syst. (MEMS '97), January 26–29 Nagoya, Jpn.
182. Liu RH, Stremler MA, Sharp KV, Olsen MG, Santiago JG, et al. 2000. Passive mixing in a three-dimensional serpentine microchannel. *J. MEMS* 9:190–97
183. Taton TA, Mirkin CA, Letsinger RL. 2000. Scanometric DNA array detection with nanoparticle probes. *Science* 289: 1757–60
184. Burns MA, Johnson BN, Brahmasandra SN, Handique K, Webster JR, et al. 1998. An integrated nanoliter DNA analysis device. *Science* 282:484–87
185. Burggraf N, Krattiger B, de Mello AJ, de Rooij NF, Manz A. 1998. Holographic refractive index detector for application in microchip-based separation systems. *Analyst* 123:1443–47
186. Schueller OJA, Zhao XM, Whitesides GM, Smith SP, Prentiss M. 1999. Fabrication of liquid-core waveguides by soft lithography. *Adv. Mater.* 11:37–41
187. Schueller OJA, Duffy DC, Rogers JA, Brittain ST, Whitesides GM. 1999. Reconfigurable diffraction gratings based on elastomeric microfluidic devices. *Sens. Actuators A* 78:149–59
188. Pancrazio JJ, Bey PP, Cuttino DS, Kusel JK, Borkholder DA, et al. 1998. Portable cell-based biosensor system for toxin detection. *Sens. Actuators B* 53:179–85
189. Trumbull JD, Glasgow IK, Beebe DJ, Magin RL. 2000. Integrating microfabricated fluidic systems and NMR spectroscopy. *Ieee Trans. Biomed. Eng.* 47:3–7
190. Oleschuk RD, Harrison DJ. 2000. Analytical microdevices for mass spectrometry. *Trac Trends Anal. Chem.* 19:379–88
191. Lazar IM, Ramsey RS, Sundberg S, Ramsey JM. 1999. Subattomole-sensitivity microchip nanoelectrospray source with time-of-flight mass spectrometry detection. *Anal. Chem.* 71:3627–31
192. Deleted in proof
193. Dolnik V, Liu SR, Jovanovich S. 2000. Capillary electrophoresis on microchip. *Electrophoresis* 21:41–54
194. McDonald JC, Duffy DC, Anderson JR, Chiu DT, Wu HK, et al. 2000. Fabrication of microfluidic systems in poly-(dimethylsiloxane). *Electrophoresis* 21: 27–40
195. Roche ME, Oda RP, Landers JP. 1997. Capillary electrophoresis in biotechnology. *Biotechnol. Progr.* 13:659–68
196. Effenhauser CS, Bruin GJM, Paulus A. 1997. Integrated chip-based capillary electrophoresis. *Electrophoresis* 18: 2203–13
197. Effenhauser CS, Manz A, Widmer HM. 1993. Glass chips for high-speed capillary electrophoresis separations with submicrometer plate heights. *Anal. Chem.* 65:2637–42
198. Effenhauser CS, Manz A, Widmer HM. 1995. Manipulation of sample fractions on a capillary electrophoresis chip. *Anal. Chem.* 67:2284–87
199. Effenhauser CS, Bruin GJM, Paulus A, Ehrat M. 1997. Integrated capillary electrophoresis on flexible silicone microdevices: analysis of DNA restriction fragments and detection of single DNA molecules on microchips. *Anal. Chem.* 69: 3451–57
200. Chou HP, Spence C, Scherer A, Quake S.

1999. A microfabricated device for sizing and sorting DNA molecules. *Proc. Nat. Acad. Sci. USA* 96:11–13
201. Carlson RH, Gabel CV, Chan SS, Austin RH, Brody JP, Winkelman JW. 1997. Self-sorting of white blood cells in a lattice. *Phys. Rev. Lett.* 79:2149–52
202. Bakajin O, Carlson R, Chou CF, Chan SS, Gabel C, et al. 1998. Sizing, fractionation and mixing of biological objects via microfabricated devices. *Micro Total Anal. Syst. Proc.* Banff, Can., pp. 193–98
203. Belgrader P, Benett W, Hadley D, Richards J, Stratton P, et al. 1999. PCR detection of bacteria in 7 minutes. *Science* 284:449–50
204. Cheng J, Sheldon EL, Wu L, Uribe A, Gerrue LO, et al. 1998. Preparation and hybridization analysis of DNA/RNA from *E-coli* on microfabricated bioelectronic chips. *Nat. Biotechnol.* 16:541–46
205. Kopp MU, de Mello AJ, Manz A. 1998. Chemical amplification: continuous-flow PCR on a chip. *Science* 280:1046–48
206. Lagally ET, Simpson PC, Mathies RA. 2000. Monolithic integrated microfluidic DNA amplification and capillary electrophoresis analysis system. *Sens. Actuators B* 63:138–46
207. Sanders GHW, Manz A. 2000. Chip-based microsystems for genomic and proteomic analysis. *Trac Trends Anal. Chem.* 19:364–78
208. Woolley AT, Hadley D, Landre P, de Mello AJ, Mathies RA, Northrup MA. 1996. Functional integration of PCR amplification and capillary electrophoresis in a microfabricated DNA analysis device. *Anal. Chem.* 68:4081–86
209. Brody JP, Yager P. 1997. Diffusion-based extraction in a microfabricated device. *Sens. Actuators A* 58:13–18
210. Weigl BH, Yager P. 1999. Microfluidic diffusion-based separation and detection. *Science* 283:346–47
211. Weigl BH, Kriebel J, Mayes KJ, Bui T, Yager P. 1999. Whole blood diagnostics in standard gravity and microgravity by use of microfluidic structures. *Mikrochim. Acta* 131:75–83
212. Kamholz AE, Weigl BH, Finlayson BA, Yager P. 1999. Quantitative analysis of molecular interactions in a microfluidic channel: the t-sensor. *Anal. Chem.* 71:5340–47
213. Brangwynne C, Huang S, Parker KK, Ostuni E, Ingber DE. 2000. Symmetry-breaking in mammalian cell populations migrating in vitro. *In Vitro Cell Dev. Biol. Animal* 36:563–65
214. Brown RA, Prajapati R, McGrouther DA, Yannas IV, Eastwood M. 1997. Tensional homeostasis in dermal fibroblasts: mechanical responses to mechanical loading in three-dimensional substrates. *J. Cell. Physiol.* 175:323–32
215. Harris AK. 1984. Tissue culture cells on deformable substrata: biomechanical implications. *J. Biomech. Eng.* 106:19–24
216. Bhatia SN, Balis UJ, Yarmush ML, Toner M. 1998. Microfabrication of hepatocyte/fibroblast co-cultures: role of homotypic cell interactions. *Biotechnol. Prog.* 14:378–87
217. Bhatia SN, Balis UJ, Yarmush ML, Toner M. 1999. Effect of cell-cell interactions in preservation of cellular phenotype: cocultivation of hepatocytes and nonparenchymal cells. *FASEB J.* 13:1883–900
218. Chen CS, Mrksich M, Huang S, Whitesides GM, Ingber DE. 1999. Micropatterned surfaces for control of cell shape, position, and function. *Biotech. Prog.* 14:356–63
219. Mrksich M, Dike LE, Tien J, Ingber DE, Whitesides GM. 1997. Using microcontact printing to pattern the attachment of mammalian cells to self-assembled monolayers of alkanethiolates on transparent films of gold and silver. *Exp. Cell Res.* 235:305–13
220. Huang S, Chen CS, Ingber DE. 1998. Control of cyclin d1, p27(kip1), and cell cycle progression in human capillary endothelial cells by cell shape and cytoskeletal tension. *Mol. Biol. Cell* 9:3179–93

221. Youssaf MN, Mrksich M. 2000. Dynamic substrates: modulating the behaviors of attached cells. In *New Technologies for Life Sciences: A Trends Guide*, ed. E Wilson et al. New York: Elsevier. pp. 28–35
222. Yousaf MN, Houseman BT, Mrksich M. 2000. Turning on cell growth with electroactive substrates. *Angew Chem. Int. Ed. Engl.* In press
223. Ostuni E, Chen CS, Ingber DE, Whitesides GM. 2001. Selective deposition of proteins and cells in arrays of microwells. *Langmuir.* In press
224. Bhatia SN, Yarmush ML, Toner M. 1997. Controlling cell interactions by micropatterning in co-cultures: hepatocytes and 3T3 fibroblasts. *J. Biomed. Mater. Res.* 34: 189–99
225. Bhatia SN, Balis UJ, Yarmush ML, Toner M. 1998. Probing heterotypic cell interactions: hepatocyte function in microfabricated co-cultures. *J. Biomater. Sci. Polymer Ed.* 9:1137–60
226. Clark P, Connolly P, Curtis ASG, Dow JAT, Wilkinson CDW. 1990. Topographical control of cell behavior: II. Multiple grooved substrata. *Development* 108: 635–44
227. Wojciak Stothard B, Curtis A, Monaghan W, Macdonald K, Wilkinson C. 1996. Guidance and activation of murine macrophages by nanometric scale topography. *Exp. Cell Res.* 223:426–35
228. Mrksich M, Chen CS, Xia Y, Dike LE, Ingber DE, Whitesides GM. 1996. Controlling cell attachment on contoured surfaces with self-assembled monolayers of alkanethiolates on gold. *Proc. Natl. Acad. Sci. USA* 93:10775–78
229. Craighead HG, Turner SW, Davis RC, James C, Perez AM, et al. 1998. Chemical and topographical surface modification for control of central nervous system cell adhesion. *J. Biomed. Microdevices* 1: 49–64
230. Wilkinson CDW, Curtis ASG, Crossan J. 1998. Nanofabrication in cellular engineering. *J. Vac. Sci. Technol. B* 16:3132–36