Optical Biosensors: Present and Future
F.S. Ligler and C.A. Rowe Taitt (editors)

799

CHAPTER 18

SOFT LITHOGRAPHY AND MICROFLUIDICS

RAVI S. KANE[1], PH.D., ABRAHAM D. STROOCK[1], NOO LI JEON[1], PH.D., DONALD E. INGBER[2], PH.D. AND GEORGE M. WHITESIDES[1], PH.D.

[1]Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA 02138

[2]Departments of Pathology and Surgery, Children's Hospital and Harvard Medical School Boston, MA 02115

Optical biosensors necessarily involve an interface between synthetic materials and biological systems. This chapter describes the application of soft lithography to create and control this interface. Soft lithography is a set of techniques that includes 1) methods of fabricating microstructures in polymers, especially elastomers, 2) uses of these methods in combination with organic surface chemistry to generate micron-scale patterns on synthetic surfaces, and 3) uses of microfluidic systems to pattern the composition of the fluid medium adjacent to a surface. These techniques allow the immobilization of biomolecules and cells at surfaces with micron-scale resolution, and for the control of the subsequent interaction of these species with liquid media. These techniques are compatible both with optical and electronic materials and with biological systems. This review focuses on the use of soft lithography to fabricate microfluidic systems and to position and manipulate living cells on surfaces.

1. Technical Concept

1.1. Introduction

This chapter describes the use of a set of non-conventional (i.e., not based on photolithography) microfabrication techniques known as soft lithography (Xia

and Whitesides, 1998; Whitesides et al., 2001) to create and control the interfaces between synthetic materials and biological systems. The term, "soft lithography," describes an integrated set of techniques for fabricating microstructures in an elastomeric material, for modifying the chemical properties of surfaces, and for controlling flows of fluid adjacent to surfaces. In the context of the interface between synthetic materials and biological systems, soft lithography makes use of elastomeric stamps, membranes, and microfluidic channels to deposit small molecules, biological molecules, and living cells on synthetic substrates with micron-scale spatial resolution. Microfluidic channels made with soft lithography provide an environment for cell culture in which reagents and analytes can be delivered non-destructively to cells with sub-cellular precision (Duffy et al., 1998; Takayama et al., 1999). These soft lithographic techniques are inexpensive, procedurally simple, and do not require stringent control of the laboratory environment (i.e., a cleanroom is not required). They provide greater flexibility and convenience than photolithography for patterning organic and biological materials.

Soft lithographic techniques offer a means for tailoring the interface between the "optical" and the "bio" components of optical biosensors. Figure 1 illustrates this interface schematically. The characteristics of the interface that must be controlled are 1) the position (on the scale of microns) of biological elements with respect to sensing elements, 2) the chemical interaction (both attractive and repulsive) between the surfaces of the materials used in the biosensors and biomolecules and cells, and 3) the local environment of the biological system, in such a way that the biological components remain active. The materials used in creating this interface must also be compatible with both the optical system (that is, they must be transparent, have the correct index of refraction, and form adequate mechanical seals) and the biological system (that is, they must be non-toxic, be selective in their molecular recognition, and have appropriate surface composition). These criteria are met by the materials used in soft lithography.

The examples that we use in this chapter concentrate on systems of the class that is shown in Figure 1 in which molecules, cells, and media can all, in principle, be patterned. These systems—instrumented micro-cell culture systems—are immediately useful in fundamental studies of cell biology. They also have the potential to act as sophisticated biosensors and analytical systems. Both cell-based assays, which require repeated examination of individual cells, and biosensors, which rely on the collective observation of multiple cells, will benefit from accurate control of cell location. Biosensors and combinatorial screens may also require surfaces that display specific ligands in a surface that otherwise minimizes non-specific interactions with proteins or cells. Control of the bio/materials interface is also key to solving an important, long-term problem: the design of hybrid systems that combine (or allow direct communications between) living and non-living systems.

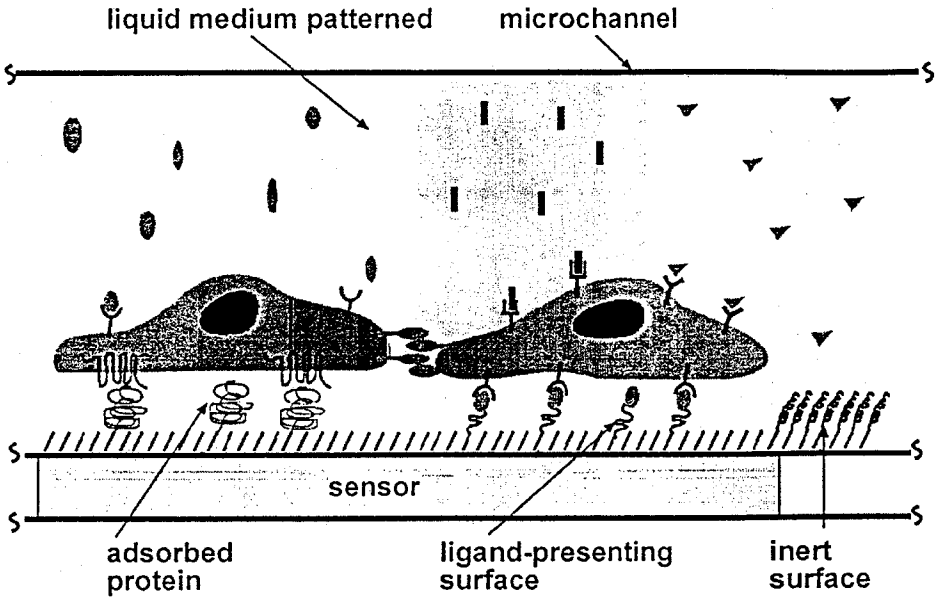


Figure 1. Controlling the interactions of a cell with its environment using soft lithography. Most of the environmental features sensed by the cell can be patterned using soft lithography or devices fabricated using soft lithography: the surface on which proteins adsorb or that presents ligands (patterning by microcontact printing); the identity of neighboring cells (membrane-based patterning or patterning using three-dimensional microfluidic systems); the composition of the extracellular medium (laminar flow patterning in microchannels).

1.2. Soft lithographic methods of microfabrication

Controlling the environment experienced by individual cells or groups of cells requires control over the composition of both the surface and the medium on relevant length scales (micrometers for single cells and millimeters to centimeters for groups of cells). We have developed a set of techniques that we call "soft lithography" that is an alternative to photolithography, and that can be used to create microstructures, and to control the surface chemistry of synthetic materials, with a spatial resolution of microns. Soft lithographic techniques are inexpensive, procedurally simple, and do not require stringent control over the laboratory environment. These techniques can be used to pattern both planar and non-planar substrates, and also to pattern the cell culture medium.

In soft lithography, elastomeric stamps, microfluidic channels, and membranes prepared by casting or spin coating the liquid prepolymer of an appropriate elastomer against a master that has a patterned relief structure (Figure 2). Most of the research based on soft lithography has used poly(dimethylsiloxane)

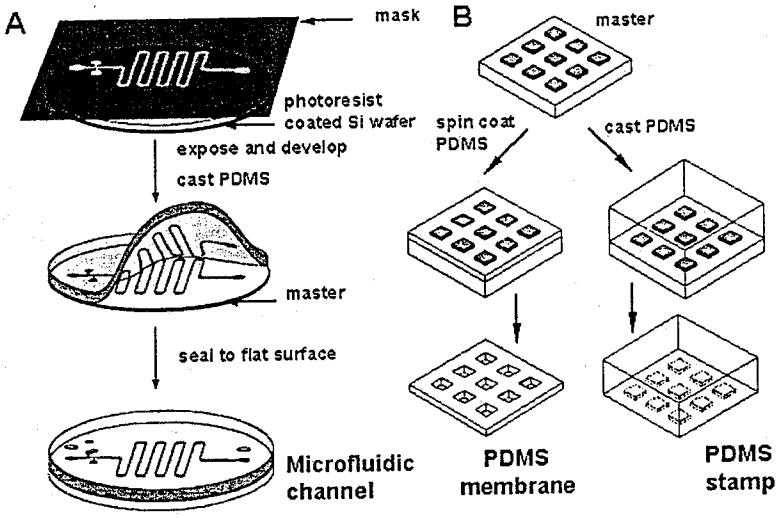


Figure 2. Schematic diagrams of the soft lithographic approach of fabricating microfluidic channels (A), membranes, and stamps (B). A) A transparency prepared on a high resolution printer (5000 dpi) is used as a photomask. Epoxy photoresist is spun onto a silicon wafer, exposed, and developed to create a master structure. Many (> 100) negative copies of the structure on the master can be formed by molding the structure into poly(dimethylsiloxane) (PDMS), an elastomeric polymer. To form a closed channel, the PDMS mold is sealed to a flat surface either covalently by oxidizing the surfaces in a low temperature plasma or non-covalently by applying pressure. B) Fabrication of a membrane (left) and a stamp (right) from a master. To form a membrane, PDMS is spun onto the master in a thin layer such that the features on the master create holes that tranverse the entire thickness of the layer. On a stamp, the negative of the features on the master are molded in bas-relief on one surface (McDonald et al., 2000; Xia and Whitesides, 1998; Whitesides and Stroock, 2001).

(PDMS) as the elastomer, because PDMS is biocompatible, permeable to gases (and can thus be used for cell culture), and inexpensive. PDMS also has good optical characteristics; the cured polymer is transparent from 235 nm to the near infrared (Wu and Whitesides, 2001) and can make tight, weakly scattering seals around embedded optical elements such as optical fibers (Chabinyc et al., 2001). The interfacial properties of PDMS can be readily modified by plasma oxidation and silanization (Chaudhury and Whitesides, 1991). PDMS structures can often be used many times in transferring patterns (we have used the same PDMS stamp in microcontact printing approximately 100 times over a period of several months without any noticeable degradation in its performance), and each master can be used to make a large number of stamps or membranes. The access to photolithographic equipment required (to fabricate masters) in soft lithography is therefore minimal.

An advantage of soft lithography as a method for patterning cells is that, at the feature sizes required for this application (typically, 2-500 μm), it is often possible to make photomasks using procedures that are significantly more rapid and less expensive than those commonly used to make chrome masks for conventional photolithography. For the fabrication of masters having feature sizes greater than or equal to 20 μm, masks can be generated by the high-resolution laser printing of patterns (generated using computer programs such as Freehand or AutoCAD) onto flexible transparencies (Qin et al., 1996). The masks can be made in a few hours at a cost as low as $0.25 per square inch. For feature sizes between 10 μm and 20 μm, the optical reduction of images printed onto transparencies generates patterns in microfiche (Deng et al., 1999). Microfiche is then used as the photomask. For feature sizes between 2 μm and 20 μm, a relatively inexpensively approach is to use commercial laser writing to fabricate masters from which PDMS stamps can be molded (Grzybowski et al., 1998). For feature sizes between a few hundred nanometers and 20 μm, there are more specialized techniques (Wu and Whitesides, 2001; Love et al., 2001b). The capability to produce features larger than 20 μm rapidly and inexpensively allows researchers to prototype and produce small numbers of simple microstructures and microsystems. This capability, which we call "rapid prototyping", has minimized the barriers to the use of lithographic techniques by biochemists.

Soft lithography also facilitates the fabrication of complex structures such as three-dimensional (3D) networks of channels (Love et al., 2001a; Anderson et al., 2000). Figure 3 shows an example of a multi-level network of microchannels made by stacking a stamp and a membrane (Chiu et al., 2000). The elastomeric character of PDMS enables the simple integration of a variety of thin organic materials such as filters and dialysis membranes (Ismagilov et al., 2001; Chiu et al., 2001).

1.3. Molecular control of interfaces

A necessary ingredient for the control of the bio-material interface is a versatile strategy for adding organic functionality to synthetic substrate. We have made extensive use of self-assembled monolayers (SAMs) for this purpose (Dubois and Nuzzo, 1992; Whitesides and Gorman, 1995; Whitesides et al., 1996; Wilbur and Whitesides, 1999; Folch and Toner, 2000; Mrksich, 2000). SAMs are organized organic monolayer films that provide molecular-level control over the composition and properties of the interface. Most studies of SAMs have involved monolayers of alkanethiolates on gold and silver. A benefit of working with SAMs of thiols on thin metal layers is compatibility with surface plasmon resonance (SPR) techniques (Sigal et al., 1996, 1997; Lahiri et al., 1999b; Chapman et al., 2000). SAMs of alkanethiolates on gold and silver are also

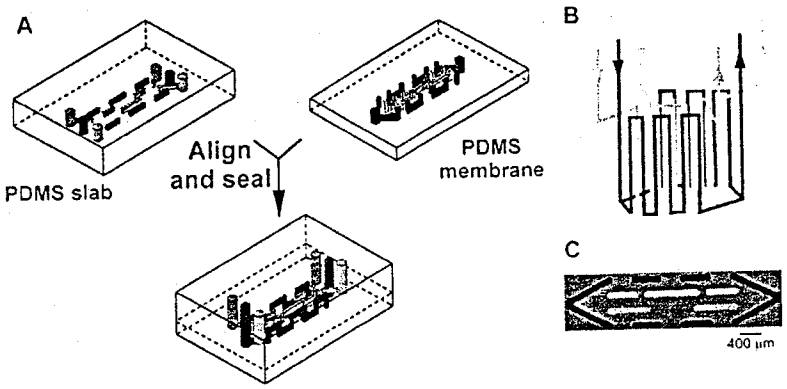


Figure 3. Three-dimensional microfluidic network for applying reagents to a surface in a discontinuous pattern, a technique called 3D MIMIC. A) Scheme for the fabrication of 3D-microfluidic stamp. The slab and the membrane are formed with different masters (cf. Figure 2B). The membrane contains segments of channel that will be in contact with the surface that is to be patterned. Vertical vias in the membrane connect the lower channels (in contact with surface) to the upper layer of channels in the slab. The aligned stack of the slab and the membrane are sealed (non-covalently) to the surface that is to be patterned. B) Schematic illustration of the fluid paths in the 3D network. C) Silicon oxide surface that has been etched with a network of channels such as in (A) and (B) in which different concentrations of etchant (hydrofluoric acid) were run through the three independent channels. Different shades of the etched regions correspond to different thicknesses of the oxide after etching (Chiu et al., 2000).

compatible with microcontact printing, a powerful technique for creating chemical patterns with micron-scale resolution (cf. Section 1.4) (Wilbur et al., 1994; Xia and Whitesides, 1998).

Figure 4 outlines three methods developed in our group for attaching ligands covalently to surfaces with controlled orientation and density (Lahiri et al., 1999a, 1999b; Roberts et al., 1998; Yan et al., 1997). Control over the density of groups presented at a surface can be achieved by forming a SAM from a solution containing a mixture of alkanethiols, although phase segregation in the monolayer might affect the surface properties of certain mixed SAMs. In Figures 4A and 4B, the ligand is presented in a background of SAMs terminated with oligomers of ethylene glycol (OEG); SAMs that present OEG resist the non-specific adsorption of protein (Prime and Whitesides, 1991); such surfaces are called "inert". The presentation of ligands in an inert background is important for performing quantitative, low-noise binding assays at surfaces (Lahiri et al., 1999b).

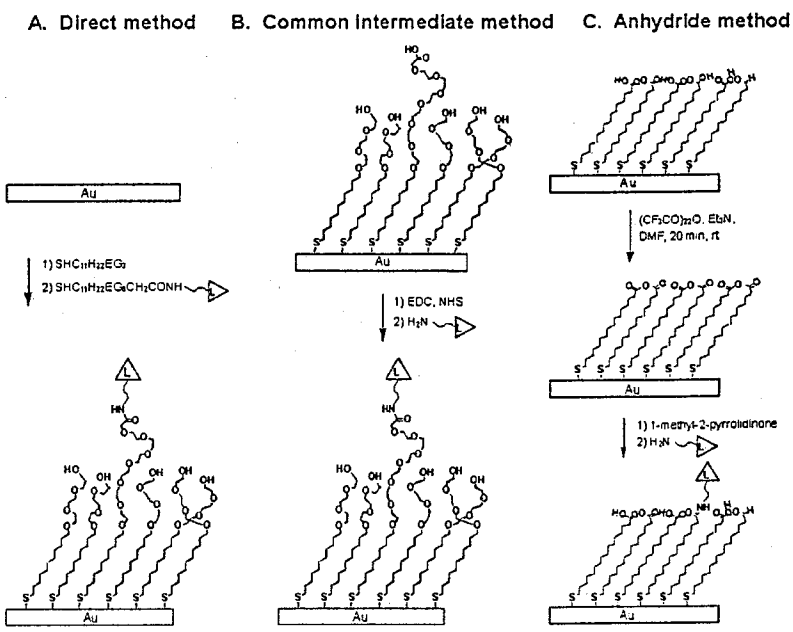


Figure 4. Generation of SAMs presenting specific ligands: A) Immersion of a gold substrate in a solution containing a mixture of an alkanethiol terminating in oligo(ethylene glycol) groups (OEG), and a second alkanethiol terminating in a ligand of interest, generates a surface presenting the desired ligand (Roberts et al., 1998). B) A mixed SAM is formed by the immersion of a gold substrate in a solution containing a mixture of EG_3OH-terminated and EG_6-OCH_2COOH-terminated alkanethiols. Activation of the carboxylic acid groups using N-hydroxysuccinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), followed by a reaction with an amine-terminated ligand, generates a surface presenting that ligand (Lahiri et al., 1999a, 1999b). C) A surface presenting interchain carboxylic acid anhydrides is generated by treating SAMs that present terminal carboxylic acid groups with trifluoroacetic anhydride. Reacting these activated SAMs with amine-terminated ligands results in a surface presenting a 1:1 mixture of the desired ligand and carboxylic acid groups (Yan et al., 1997).

1.4. Patterning molecules and cells on synthetic surfaces

The types of microstructures (Section 1.2) and surface chemistry (Section 1.3) described in the proceeding sections can be combined to form powerful techniques for patterning organic chemical functionality, biomolecules, and even living cells on surfaces with micron-scale precision. In this section, we outline the use of three such techniques: microcontact printing (μCP) (Wilbur et al., 1994; Xia and Whitesides, 1998), membrane patterning (MEMPAT) (Ostuni et al., 2000), and three-dimensional micromolding in capillaries (3D MIMIC) (Chiu et al., 2000). Microcontact printing is a technique that uses the relief pattern on the surface of an elastomeric PDMS stamp to form patterns of SAMs on the

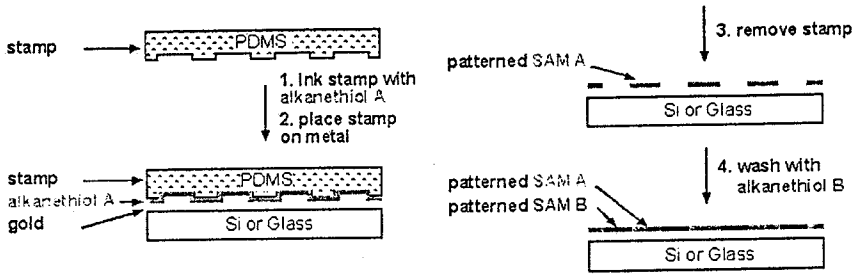


Figure 5. Procedure for patterning SAMs by microcontact printing: A stamp is inked with an alkanethiol and placed on a gold (or silver) surface; the pattern on the stamp is transferred to the gold by the formation of a SAM on the regions that contacted the substrate. The bare areas of the gold are exposed to a different alkanethiol to generate a surface patterned with a SAM that presents different chemical functionalities in different regions (Xia and Whitesides, 1998).

surfaces of substrates (Figure 5). Patterned SAMs generated by microcontact printing can be used to control the adsorption of proteins on surfaces. López et al. (1993) first used microcontact printing to pattern gold surfaces into regions presenting oligo(ethylene glycol) groups in a background of methyl groups. Immersion of the patterned SAMs in solutions of proteins resulted in the adsorption of proteins only on the methyl-terminated regions. These systems have subsequently been extended to other experiments in cell biology (see Section 3.2) (Chen et al., 1997; Mrksich et al., 1997; Mrksich, 2000).

While microcontact printing is a technique that has sufficient resolution to allow the patterning of single cells, in its simplest configuration, it does not allow any changes in the pattern or shape of adsorbed cells. MEMPAT and 3D MIMIC are complementary techniques that not only allow the patterning of cells—individually or in groups—on arbitrary substrates, but also allow studies of the spreading or migration of cells from their initial pattern.

MEMPAT makes use of elastomeric membranes—free-standing PDMS films that have through-membrane pores—to pattern proteins and cells on a variety of substrates including plastics and glass (Figure 6) (Folch and Toner, 2000; Ostuni et al., 2000). Bringing an elastomeric membrane into contact with a substrate restricts access of a solution of protein or a suspension of cells to those regions of the substrate exposed through the pores. The deposition of proteins, or the attachment of cells, is therefore restricted to these exposed regions of the substrate. The patterned cells are constrained by the walls of the pores in the membrane. The ability to remove the constraints imposed by the membrane by peeling it away from the substrate, and to observe the subsequent spreading or

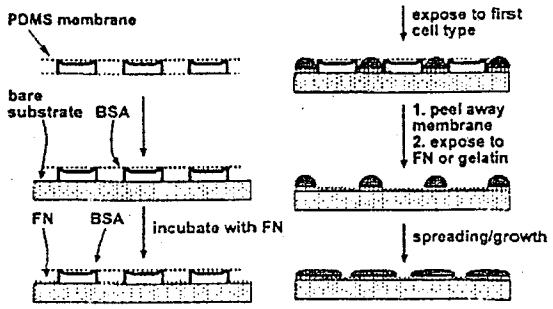


Figure 6. Schematic diagram that describes the use of MEMPAT for plating cells onto a substrate with well defined position and shape. The surface of the membrane and the walls of its holes are coated with bovine serum albumin (BSA). The membrane is placed on a clean surface (e.g., Petri dish) and exposed to a solution of fibronectin (FN). After rinsing with a solution of a phosphate buffer solution, the membrane and the substrate are covered with a suspension of cells for 24 h. The membrane can be removed without damaging the cells and the protected areas of the substrate can be modified by the adsorption of an adhesive protein that allows the patterned cells to spread (Ostuni et al., 2000).

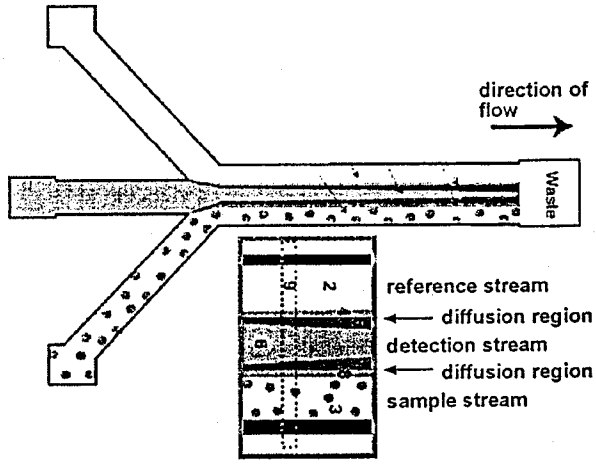


Figure 7. Schematic diagram of a "T-Sensor" based on the diffusional mixing between three laminarly flowing streams in a microchannel (Weigl and Yager, 1999). The analyte of interest (e.g., an enzyme) is introduced in the sample stream (bottom). A solution of indicator (e.g., a substrate that becomes fluorescent upon interaction with the enzyme) flows in the detection stream (center). The third stream contains a reference solution. Diffusive mixing of the solutions occurs at the interface of the streams. The product of this mixing (e.g., a fluorophore) will be localized in the diffusively mixed region between the streams.

migration of the cells as a function of the composition of the substrate and the cellular environment, may be exploited in several areas of cell biology. 3D

MIMIC allows the generation of arbitrary and discontinuous patterns of proteins or cells on planar substrates (Figure 3). We had previously developed a soft lithographic technique called MIMIC (micromolding in capillaries) (Kim et al., 1995) for fabricating three-dimensional structures by allowing solutions to flow into microfluidic channels. The use of MIMIC was limited to relatively simple, continuous patterns. To overcome some of the limitations of MIMIC, we developed an analogous technique that makes use of three-dimensional microfluidic systems (Anderson et al., 2000) for patterning (3D MIMIC). Figure 3C illustrates the use of 3D MIMIC to create a discontinuous pattern of etched regions in a silicon oxide layer on silicon. The same methods can be used with solutions of proteins and suspensions of cells to deliver these elements to surfaces in complex, discontinuous patterns (cf. Section 3.2.2).

1.5. Laminar flow in microfluidic systems

Networks of microchannels can be used to control the location of fluids on the micron scale. This spatial control can be extended by taking advantage of the laminar character of flows in microchannels (Takayama et al., 1999; Kenis et al., 1999). Since microfluidic channels are small (100 μm in cross-sectional dimension), the flow of liquids in them is usually laminar (Bird, 1960); adjacent streams of different composition mix into one another only by diffusion. This characteristic of laminar flows has been exploited by Weigl and Yager (1999) for continuous chemical analysis of solutions flowing in a microchannel (Figure 7).

Laminar flows in microchannels also allow the chemical composition of solutions and surfaces to be patterned on scales smaller than the channel itself; we refer to this method of patterning as "laminar flow patterning" (Kenis et al., 2000). Figure 8 demonstrates the use of laminar flow patterning for the electroless deposition of a silver wire on the center of the floor of a microchannel (Kenis et al., 1999). Laminar flow patterning can be extended to biological systems and allows for control of both cells and the cellular environment—that is, it allows control over the nature of molecules that are deposited on the substrate, the nature and position of neighboring cells, and the composition of the extracellular medium. Laminar flow patterning makes it possible to pattern the fluid culture medium itself (cf. Section 3.2.3).

Networks of microchannels can be used to generate complicated patterns in the composition of flowing solutions. A strategy for forming gradients in the concentration of solutes in a flow of buffer in a microchannel is shown in Figure 9: A small number (six in the case shown) of solutions of different composition are injected into a network of channels; in the network, streams of the solutions are allowed to divide and mix into one another to form a larger number (24 in the case shown) of streams of solutions of intermediate composition; at the outlet of the network, the multiple streams are allowed to recombine in single channel across which there is, as a result of this "combination", a gradient in the

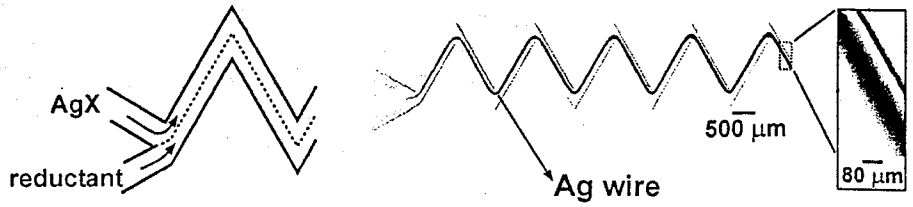


Figure 8. Demonstration of patterned surface chemistry achieved with laminar flowing streams in a microchannel. A silver wire deposited in a zigzag channel at the laminar flow interface between solutions containing the components of an electroless silver plating solution (Kenis et al., 1999).

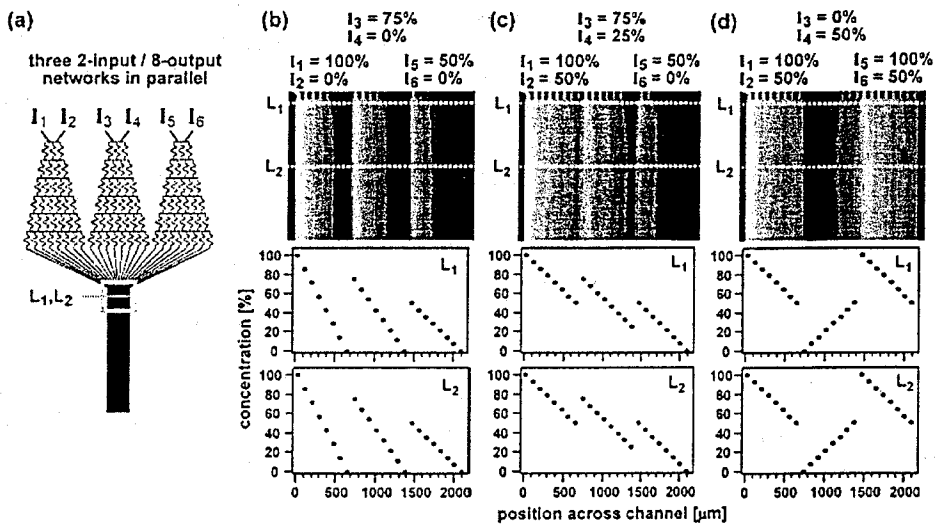


Figure 9. Gradients of solute made with a microfluidic network. a) Schematic diagram of network that transforms 6 input solutions into 24 output solutions that contain intermediate concentrations of solute in the incoming streams. The 24 streams flow laminarly in a broad outlet channel (2160 μm wide). b-d) Demonstration of three types of gradients that can be formed in the outlet channel using fluorescein solutions. On top are fluorescent micrographs of streams flowing in the outlet channel. Below, plots show the corresponding fluorescence intensity profile across the outlet channel at the beginning of the channel (L1, white dotted line) and 800 μm downstream from the junction (L2, white dotted line). The fluorescein concentration of the solutions introduced into the inlets of the microfluidic device is shown above the individual gradients (Dertinger et al., 2001).

concentration of the solutes from the original solutions; under laminar flow conditions, this gradient propagates along the channel with only diffusional

broadening of the gradient profile (Jeon et al., 2000; Dertinger et al., 2001). The gradient in the combined stream can be used to study cell behaviors such as chemotaxis. In the case in which the solutes adsorb on walls of the channel, we have found that the gradient in the combined stream led to gradients of qualitatively similar form in the concentration of the species that are bound to the wall (unpublished results).

2. History

2.1. Self assembled monolayers

The history of ordered molecular monolayers is long, but only in the past few decades have convenient methods emerged that allow for the formation of high quality films on solid substrates. Early work on monolayers was based on the Langmuir-Blodgett method in which the film is formed at the liquid-air interface and subsequently transferred to a solid support (Ulman, 1991). This method can lead to films with a high degree of molecular ordering, but the process is complicated and prone to errors. Silanes offer a flexible way to bring organic functional groups to solid surfaces that present hydroxyl groups (Grushka, 1974). The molecules in silane layers are only partially ordered, and silane films are prone to degradation in aqueous buffer due to the hydrolysis of silicon-oxygen-silicon bonds. In 1985, Allara and Nuzzo discovered the self-assembly of disulphides on metal surfaces (Allara and Nuzzo, 1985); this discovery led to work with thiols on metals. Long-chain (greater than 10 carbons) alkane thiols form SAMs with crystalline order that are very stable in aqueous medium. SAMs of thiols have been extensively characterized by our group and others (Dubois and Nuzzo, 1992; Whitesides and Gorman, 1995).

2.2. History of patterned surface chemistry

Until recently, there were few methods available to pattern the chemical groups presented on a surface with micron-scale resolution: photo-labile groups presented a surfaces could be patterned with exposure through a photomask (Wollman et al., 1994); photolithography could also be used to define patterns in photoresist that would act as a mask for the deposition of metals, oxides, and organics (Muller and Kamins, 1986). The development of SAMs of thiols on metals opened the possibility of using traditional patterning methods such as writing and stamping to create patterns of well ordered molecular layers with sub-micron-scale resolution (Kumar et al., 1992, 1994; Wilbur et al., 1994; Piner et al., 1999).

2.3. History of microfluidics

The initial development of microfluidics (Manz et al., 1991) used fabrication techniques adapted from the electronics industry. With these techniques, channel structures are formed in hard materials such as glass and silicon using photolithography followed by etching; the channels are typically sealed by anodic bonding (Kovacs, 1998). These steps are slow, expensive, and require a cleanroom environment. Hard plastics are also used (see for example, Micronics, www.micronics.net). Most of the early work in microfluidics focused on using electroosmotic flow (Harrison et al., 1993; Jacobson et al., 1994). Over the past few years, we and others have been developing alternative methods based on soft lithography to fabricate microfluidic devices (Delamarche et al., 1997; Duffy et al., 1998; Beebe et al., 2000; Quake and Scherer, 2000) (Figures 2 and 3). These methods, which use PDMS as the principle material, are simple, are inexpensive, and can be performed in a standard laboratory environment. The mechanical flexibility of PDMS makes it appropriate for the fabrication of the movable components that are often required for the control of pressure-driven flows (Unger et al., 2000).

2.4. History of patterning cells

Early work on patterning cells on synthetic substrates was done using silanes and photolithography (Kleinfeld et al., 1988); this process required multiple steps.

The development of μCP simplified the process of patterning cells. We have employed μCP to pattern extracellular matrix proteins and to control the position, shape, and function of single living cells (Singhvi et al., 1994; Mrksich et al., 1997; Chen et al., 1997; Dike et al., 1999; Kane et al., 1999; Takayama et al., 2000). Others working in the area of cell patterning include the groups of Toner (Folch and Toner, 2000) and Shakesheff (Patel et al., 1998). The use of cells in sensors has been developed by the groups of Stenger and Kovacs (Jung et al., 1998; Pancrazio et al., 1998).

3. State of the Art

3.1. Microfluidic systems fabricated using soft lithography

3.1.1. Microfluidic channels and components. Soft lithography provides a method for fabricating almost any system of channels that might be needed for microfluidics (Figure 2, cf. Section 1.2) (McDonald et al., 2000). For example, channels made in PDMS using soft lithography support electroosmotic flow; these channels can be used for capillary electrophoresis (Duffy et al., 1998). Soft

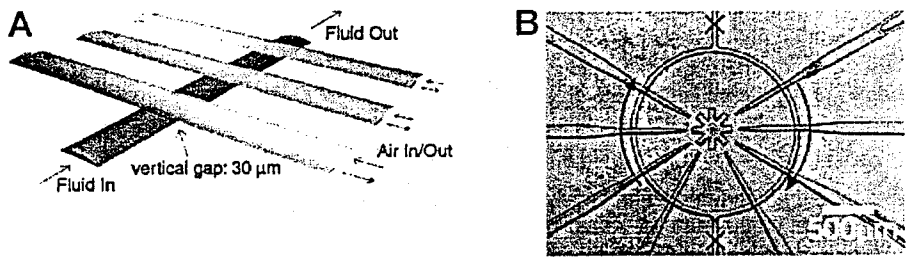


Figure 10. Rotary pump made with soft lithography in PDMS. A) Schematic diagram of a peristaltic pump based on pneumatic valves that take advantage of the elastomeric character of PDMS. The air pressure in each upper channels is controlled independently; high pressure in one of the upper channels locally deforms the lower channel and restricts flow. When actuated sequentially, the three valves act as a peristaltic pump. B) Flow ring. Six valves are activated sequentially to drive fluid around the circle when the input and output channels are blocked. The recirculating flow can be used to increase the residence time of flow in the channel for mixing or for the completion of a slow chemical reaction (Unger et al., 2000; Quake and Scherer, 2000).

lithography also simplifies the fabrication of three-dimensional channels (Figure 3) (Anderson et al., 2000); the Beebe group used soft lithography to fabricate a active components, such as pumps and valves, that are required in microfluidic systems. Chou and Quake have designed a valve and a peristaltic pump using a multilayer structure made from PDMS (Figure 10) (Unger et al., 2000; Quake and Scherer, 2000). Soft lithographic methods make the registration and integration of multiple layers (at the 50 μm scale) simple (Love et al., 2001b). The elastomeric character of PDMS allows the valve to be actuated with small changes in the pressure in the gas-filled channels.

3.1.2. Integrated microfluidic devices. Soft lithographic methods also facilitate the integration of multiple materials (e.g., PDMS, glass, organic membranes, polymer tubing, and metal films) and non-fluidic components (e.g., lenses, optical fibers, and electrodes) into a single device. An important integration process that is simplified by soft lithography is the connection of external tubing with on-chip microchannels for the introduction and collection of samples. With soft lithography, these connections are made by simply boring holes in the PDMS and press-fitting tubing into these holes; this press-fit seal can withstand several bars of pressure (McDonald et al., 2000).

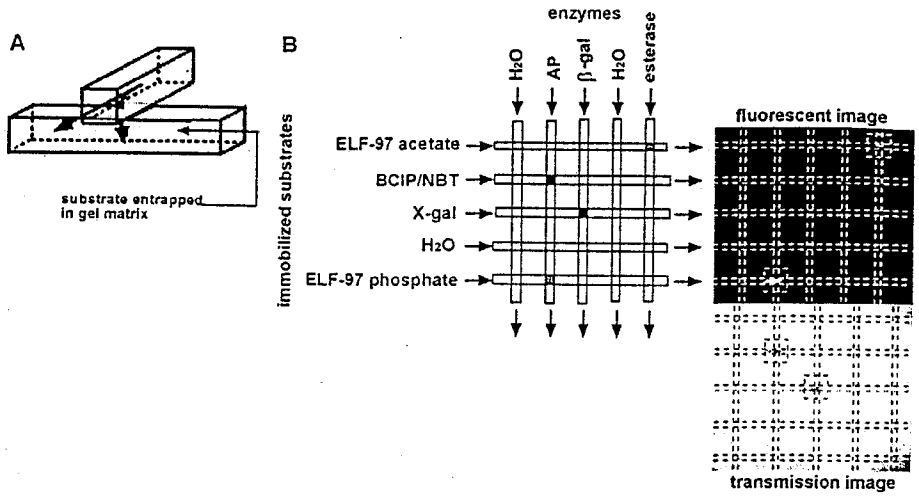


Figure 11. Parallel detection using an array of crossing microfluidic channels. A) Schematic drawing of a 1×1 array of microfluidic channels in which a poly(carbonate) membrane (0.1 μm pores) separates the two channels at the point at which they cross. The bottom channel contains a substrate entrapped in a gel matrix. B) Detection of enzymatic activity using fluorometric (top) and colorimetric (bottom) methods in a 5×5 array of crossing channels. Substrates for enzymes were immobilized in a 1.25% agarose gel in the lower (horizontal) set of channels. Solutions of different enzymes were allowed to flow by gravity in the upper (vertical) set of channels. The ELF-97-linked substrates release a fluorescent molecule upon cleavage by an enzyme; these products are visualized under UV illumination (top). BCIP/NBT and X-gal form precipitates upon cleavage; these products are visualized by optical adsorption (bottom).

Figure 11 shows a simple optical biosensor that allows for five tests to be performed on five solutions in parallel. In this device, a poly(carbonate) membrane is sealed between two layers of PDMS, each of which contains a set of microchannels; the membrane allows for diffusive (not convective) exchange of molecules between the channels in the regions in which they cross (Ismagilov et al., 2001). The bottom channels contain substrates for the enzymes of interest in an agarose gel. The sample solutions are allowed to flow in the upper channels. An enzyme is detected when it diffuses from an upper channel into the lower channel and acts on one of the substrates to form either a precipitate or a fluorescent molecule. This type of hybrid system (PDMS-thin film-PDMS) is easy to fabricate using soft lithography because PDMS conforms and seals around the intervening layer.

Figure 12 shows an integrated microfluidic device for the separation and detection of fluorescently-labeled proteins; an optical fiber, optical filter, and micro avalanche photo diode (μAPD) are integrated with the microchannel (Chabinyc et al., 2001). The fiber is molded into the slab of PDMS that contains

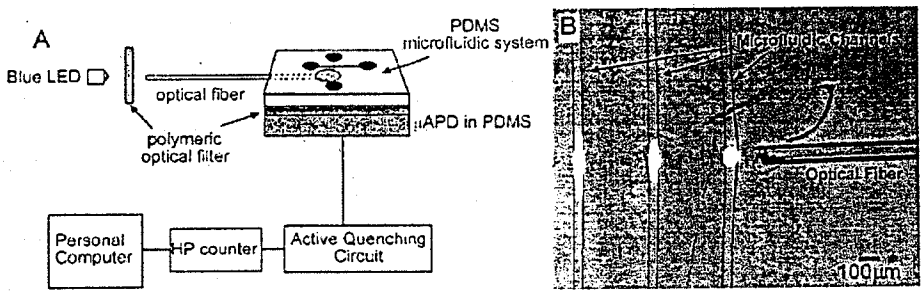


Figure 12. Microfluidic chip in PDMS for capillary electrophoresis with integrated optical fiber and micro avalanche photo diode (μAPD) detector. A) Schematic diagram of experimental setup. The excitation light was provided by a blue LED off-chip. This light was coupled into an optical fiber that was molded into the PDMS chip. Both the excitation and the detection light were filtered with inexpensive polymeric optical filters. The chip has three levels: a slab of PDMS that contains the channel and the fiber, a polymeric optical filter, and a array of μAPDs embedded in a slab of PDMS. The signal from the μAPD was processed off-chip. B) Micrograph of microchannel and optical fiber. The serpentine microchannel was filled with fluorescein. The image shows the size of the detection volume (~25 nL) and that the light is coupled from the fiber into the PDMS with minimal scattering (Chabinyc et al., 2001).

the channel. Figure 11B illustrates the clean optical coupling that is achieved between the fiber and the PDMS. A sheet of polymeric filter is sealed (non-covalently) between the slab of PDMS and the μAPD that is embedded in PDMS; the filter eliminates stray excitation light. The PDMS makes conformal contact with the filter so the interface between the PDMS and the filter is optically smooth.

3.2. Controlling the cellular environment using soft lithography

3.2.1. Spatially constrained cell culture using μCP. The ability to pattern SAMs by microcontact printing, and the resulting control over the adsorption of adhesive proteins (for example, the extracellular matrix proteins fibronectin, vitronectin, and laminin), enables the patterning of cells on substrates (Figure 13). Mrksich et al. (1997) used microcontact printing to pattern gold substrates into regions comprising SAMs capped with oligo(ethylene glycol) groups that resist the adsorption of proteins and regions comprising SAMs capped with methyl groups that adsorb proteins. After immersing the substrates in a solution of fibronectin, bovine capillary endothelial cells were found to attach only to the methyl-terminated, fibronectin-coated regions of the patterned SAMs.

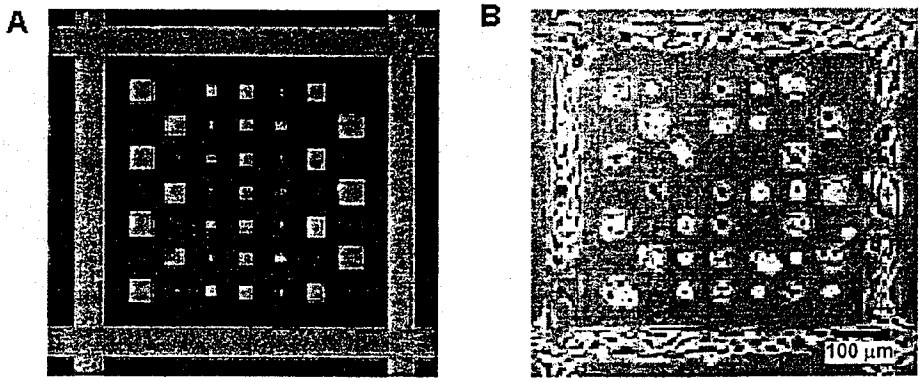


Figure 13. Patterned cells on a surface patterned with micro-contact printing. A) A gold surface was patterned into regions of hexadecanethiolate and undecanethiolate terminated with tri(ethylene glycol). Fibronectin (bright) adsorbed on the hydrophobic squares of hexadecanethiolate but not on the tri(ethyleneglycol)-terminated alkanethiolate (dark). Patterned substrates were soaked in a solution of fibronectin, fixed using paraformaldehyde, and immersed in a solution of anti-human fibronectin IgG and then rinsed. The substrates were then placed in contact with a solution of Texas Red®-labeled goat anti-rabbit IgG and mounted in fluoromount-G. B) Bovine capillary endothelial (BCE) cells patterned by culturing on a substrate presenting hydrophobic squares of varying sizes that were coated with fibronectin prior to incubation with cells using the procedure described in (A)) (Chen et al., 1997).

The ability to engineer the properties of the interface between mammalian cells and their substrates using microcontact printing has been useful in understanding the effect of cell shape on cell behavior. Singhvi et al. (1994) used the ability to control cell shape by microcontact printing to investigate the effect of cell shape on cell function. They plated primary hepatocytes on substrates (patterned by microcontact printing) presenting square and rectangular islands of laminin surrounded by non-adhesive regions. Cells attached preferentially to the laminin-coated regions, and in most cases, conformed to the shape of the island. The size and shape of cells could therefore be manipulated by changing the size and shape of the adhesive islands, without changing the density of the adhesive protein laminin. The synthesis of DNA was highest on unpatterned surfaces, where the cells could spread without restriction, and a decrease in the size of the cells led to a progressive reduction in DNA synthesis. For the smallest islands (< 1600 μm^2), less than 3 % of the adherent cells entered the DNA synthesis phase of the cell cycle. The size of the cells also affected the differentiated function of hepatocytes, as reflected by the concentration of secreted albumin in the culture supernatant: albumin secretion rates increased as the size of the adhesive island was decreased. This study demonstrated that cell shape could influence cell growth and protein secretion independent of any changes in the density of the adhesive protein laminin. Studies of cells grown on micropatterned substrates

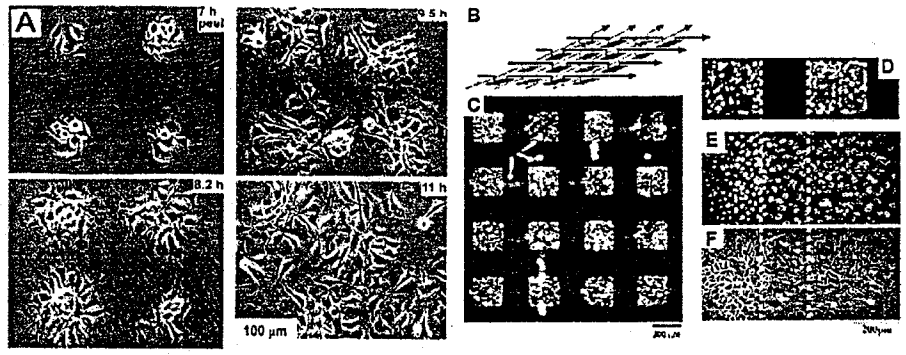


Figure 14. A) Optical micrographs of bovine capillary endothelial cells patterned using MEMPAT on a bacteriological petri dish (see Figure 6 for experimental details). The top left frame shows the state of the cells just after the membrane was removed (7 hours after cells were plated). The other frames show the spreading of the unconstrained cells in the hours following the removal of the membrane. (Ostuni et al., 2000) B) Schematic representation of the channel network used to pattern cells by 3D MIMIC (see Figure 3 for generic structure of channels). C) Fluorescence micrograph of human bladder cancer cells (ECVs; labeled (bright) with 5-chloromethylfluorescein diacetate) and bovine capillary endothelial cells (BCEs; labeled (dim) with 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine); the cells were patterned on alternating squares of a checkerboard motif. The cells were cultured for 42 h before the fluorescence micrograph was taken. The channel structure is still on the surface. D) A picture of a confluent layer of cells before the 3D microfluidic stamp was removed. E-F) Pictures taken in fluorescence (E) and in phase contrast (F) show the spreading and growth of the two cell types after the removal of the PDMS stamp. The pictures in (E) and (F) were taken 20 h after removal of the stamp. The three images in D-F are registered; the dotted lines show the relative orientation of the patterns. The BCEs spread more rapidly than the ECVs by a factor of 2-3 (Chiu et al., 2000).

have also indicated that the constrained size and shape of a cell influences whether it lives or dies (undergoes apoptosis) (Chen et al., 1997) as well as its differentiated state (Singhvi et al., 1994; Dike et al., 1999).

3.2.2. Patterned cell culture with variable spatial constraints. MEMPAT and 3D MIMIC offer the possibility of plating cells in regions of well defined size, shape, and location (cf. Section 1.4). Furthermore, with both methods the spatial constraint on the cell(s) imposed by the membrane or channel can be released by peeling the membrane (MEMPAT) or channel network (3D MIMIC) away from the substrate to allow the cells to spread and migrate. Figure 14A shows the movement of bovine capillary endothelial cells across the surface after their release from the regions defined by the holes in a PDMS membrane (see Figure 6 for experimental details) (Ostuni et al., 2000). The rate and degree of the spreading of cells could be used as a simple indicator of the state of cells as they interact with their environment in an cell-based effect sensor.

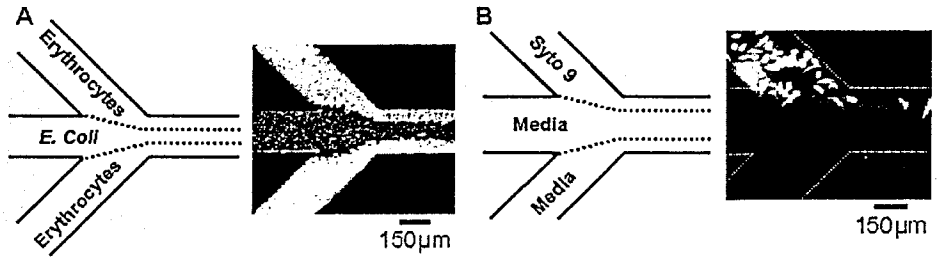


Figure 15. Patterned plating of cells from laminar flow. A) Patterning different cell types on the floor of a single microchannel. Chick erythrocytes and *E. coli* were deposited selectively in their designated lanes by patterned flow of cell suspensions. Adherent cells were visualized with a fluorescent nucleic acid stain (Syto 9). B) Patterning the delivery of a stain to bovine capillary endothelial cells in a microchannel. A suspension of bovine capillary endothelial cells was introduced into channels that were pre-treated with fibronectin and allowed to attach and spread. After removing non-adherent cells by washing with medium, Syto 9 was allowed to flow through one of the inlets as medium was allowed to flow through the other two (Takayama et al., 1999).

3D MIMIC makes it possible to deposit different cell types in close proximity (~ 200 μm) to one another. This technique may therefore be useful in exploring interactions between different types of cells, and valuable in exploring processes such as morphogenesis, angiogenesis, and differentiation. In the experiment shown in Figures 14B-F, 3D MIMIC is used to create a culture of two cell types (cancer cells and capillary endothelial cells) that are relevant for the study of angiogenesis (Chiu et al., 2000). To achieve an alternating pattern of the two types of cell (Figure 14C), suspensions of cells were allowed to fill the two orthogonal sets of channels in the 3D microfluidic network that is shown schematically in Figure 14B (cf. also Figure 3). The cells were allowed to settle and attach to the substrate for 42 hours with the microfluidic network still sealed against the surface. Figures 14D-F show the evolution of cells after the microfluidic network was removed. This type of experiment could be useful for studying the effect of angiogenic factors released by the cancer cells.

3.2.3. Controlling the total environment of a cell with laminar flow patterning.
Laminar flow patterning allows for the patterned deposition of cells and the patterned delivery of reagents to cells that are already present on the walls of a microchannel. Figure 15A illustrates patterning of multiple cell types (erythrocytes and *E.coli* in this case) on the floor of a microchannel (Takayama et al., 1999). In this experiment, streams of the suspensions of the different types of cell were introduced into the channel through independent inlets. As these streams join in the main channel, they flow laminarly along side one another; the populations of the cells remain segregated in streams and they attach to the floor in the distinct regions covered by the different streams. This technique is a

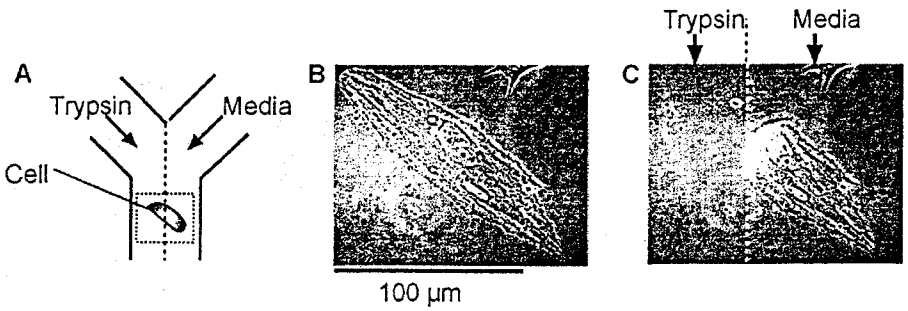


Figure 16. PARTCELL. Patterned flow of trypsin over a bovine capillary endothelial cell on the floor of a microchannel. A) The scheme shows the geometry of the channel. B) Micrograph that shows the cell before treatment with trypsin. C) Micrograph that shows the partially detached cell after treatment (Takayama et al., 1999).

simple alternative to 3D MIMIC to plate different cell types in close proximity to one another for studies of cell interaction.

Figure 15B illustrates patterning of the culture medium itself. In this experiment, a single type of cell was plated uniformly on the floor of the microchannel by allowing a suspension to flow through all three inlets. Subsequently, a stream of medium containing a fluorescent stain (Syto-9) was allow to flow through only one of the three inlets of the channel as regular media was allowed to flow through the other inlets. In the main channel, the stain remained localized to a third of the width of the channel as the streams of media flowed laminarly along side one another. As is seen in the micrograph, the only cells that were stained were those covered by the stream that carried the stain. This experiment demonstrates the selective delivery of a reagent to only part of a small population of cells. In this experiment, the untreated cells could act as an internal control or the other inlets could be used to selectively deliver another reagent to a distinct or overlapping sub-population of the cells.

For large cells (~ 100 μm), laminar flow patterning can be used to deliver reagents selectively to parts of a single cell; we call this technique PARTCELL (Figure 16) (Takayama et al., 1999). In the experiment shown, a suspension of cells was allowed to flow through the entire channel and attach to its floor. Subsequently, distinct streams of medium containing trypsin (a protease that cleaves the attachments of the cells to the surface) and medium without trypsin were allowed to flow through the two inlets of the channel. Figures 16B and 16C show a cell that spanned the interface between the two laminarly flowing streams. In the region covered by the stream that contained trypsin, the attachments that held the cell to the surface were cleaved; the cell retracted from this region. PARTCELL provides the new capability of interacting with a cell on the cellular scale in a non-destructive way (Takayama et al., 2001).

4. Advantages and Limitations

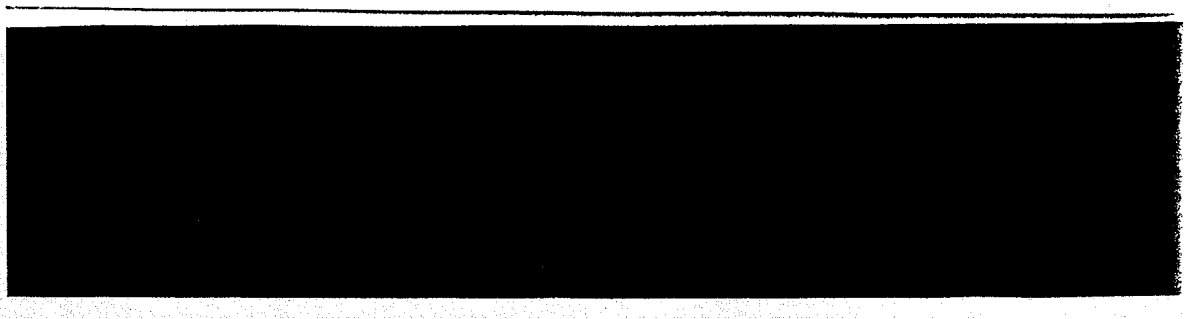
The central advantages of soft lithography as compared to conventional microfabrication methods are its simplicity and its broad compatibility with both organic and inorganic systems. The methods described in this chapter can be used with a minimum of specialized equipment and in a typical laboratory environment (not a cleanroom). The abilities to make many copies of a microstructure by molding, and copies of a chemical pattern by stamping, are useful outcomes of soft lithography. The use of SAMs of thiols allows chemical modification of substrates that are compatible with both electrical (e.g., electrochemical) and optical (e.g., SPR and microscopy) measurements.

Soft lithography offers a simple means of fabricating complicated microstructures that integrate organic (filters, membranes, gels), inorganic (glass and silicon surfaces, optical elements, electrodes), and biological elements (medium, proteins, living cells). PDMS, the core material in these systems, has a number of useful properties including low cost, low toxicity, transparency from the visible into the near ultraviolet, chemical inertness, versatile surface chemistry, mechanical flexibility, and durability. PDMS forms conformal seals with most smooth surfaces and can be covalently sealed to itself and to glass after a short (one minute) oxidation step in a low temperature plasma. Soft lithographic methods facilitate the fabrication of microfluidic systems in PDMS. The use of flows in these microfluidic devices to deliver reagents to surfaces with micron-scale resolution is particularly interesting for work with living cells; the flows are non-destructive and the walls of the channels are gas permeable.

One limitation of soft lithographic methods comes from the incompatibility of PDMS with many organic solvents; solvents such as dichloromethane and tetrahydrofuran penetrate PDMS and cause it to swell. The use of micro-contact printing has been most successful with thiols on metals. Some work has been done with silanes on silicon oxide, but the procedure is more complicated and the resolution is not as high as with thiols (Jeon et al., 1997). PDMS structures might not be durable enough for certain industrial applications; the low cost of these devices means that they can often serve in "one-time-use" applications.

5. Potential for Improving Biosensor Performance

Soft lithographic methods can be used in both the fabrication and the operation of biosensors. Many of the structural, mechanical, and optical elements of biosensors could be fabricated simply and inexpensively in PDMS using soft lithographic techniques. For example, an array of micro-lenses could be molded into one side of a thin slab of PDMS that contains a network of microchannels in relief on the other side. If aligned with the channels, the array of lenses could be used to focus the light from a flood illumination source onto small regions of the



underlying fluidic network (Wu and Whitesides, 2001). Furthermore, this slab could be aligned and sealed to another slab of PDMS in which an array of optical detectors was embedded.

Waveguides and photonic structures could also be fabricated and integrated into sensors using soft lithographic methods (Schueller et al., 1999; Yang et al., 2000). Waveguides can act as highly sensitive detectors of binding at surfaces: the evanescent field of the light in a waveguide can excite fluorescence in molecules bound to the surface of the guide; the emission light is then coupled back into the guide with an efficiency that depends, in part, on the geometry of the guide (Golden et al., 1992). Using soft lithography, this geometry could be controlled precisely.

Self-assembled monolayers of thiols on gold-coated glass are an attractive system for use with SPR detection. SAMs of thiols are particularly useful for assays of specific binding of proteins to ligands at the surface. Mixed SAMs of thiols that are terminated with a ligand of interest and thiols that are terminated with oligo(ethylene glycol) offer a effective platform for binding assays; in this system, the degree of specific binding can be tuned by changing the concentration of ligand-terminated thiol and non-specific binding is very low. These are both important characteristics in applications in biosensing.

We believe that soft lithographic techniques will be particularly important for the development of cell-based effect sensors in which the detection of an agent is based on the response of a living cell. For example, microcontact printing or MEMPAT could be used to plate cells in an initial condition (e.g. spatially constrained) from which they evolve in a known way as a function of their environment. Laminar flow patterning could be used to treat a single culture of cells or even a single cell in parallel with different solutions of interest. This method has the advantage of having a built-in control.

6. Acknowledgments

Work from both the GMW group and the Ingber group was support by NSF DMR 9809363 (MRSEC). The Whitesides group was also supported by DARPA, NIH GM 30367, and NSF ECS 9729405. The Ingber group was also supported by NIH CA 45548 and NIH CA 55833. A number of our co-workers have been responsible for the work reported here. Although they are listed in the references, we are especially grateful to Milan Mrksich, Shu Takayama, Robert Chapman, Emanuele Ostuni, Chris Chen, Lin Yan, and Joydeep Lahiri for major contributions to the program.

7. References

Allara, D. L. and R. G. Nuzzo, 1985, Langmuir 1, 45.

Anderson, J. R., D. T. Chiu, R. J. Jackman, O. Cherniavskaya, J. C. McDonald, H. Wu, S. H. Whitesides and G. M. Whitesides, 2000, Anal. Chem. 72, 3158.

Beebe, D. J., J. S. Moore, Q. Yu, R. H. Liu, M. L. Kraft, B. H. Jo and C. Devadoss, 2000, Proc. Natl. Acad. Sci. USA 97, 13488.

Bird, R. B., 1960, Transport Phenomena, Wiley, New York.

Chabinyc, M. L., D. T. Chiu, J. C. McDonald, A. D. Stroock, J. F. Christian, A. M. Karger and G. M. Whitesides, 2001, Anal. Chem. 73, 4491.

Chapman, R. G., E. Ostuni, S. Takayama, R. E. Holmlin, L. Yan and G. M. Whitesides, 2000, J. Am. Chem. Soc. 122.

Chaudhury, M. K. and G. M. Whitesides, 1991, Langmuir 7, 1013.

Chen, C. S., M. Mrksich, S. Huang, G. M. Whitesides and D. E. Ingber, 1997, Science 276, 1425.

Chiu, D. T., N. L. Jeon, S. Huang, R. Kane, C. J. Wargo, I. S. Choi, D. E. Ingber, and G. M. Whitesides, 2000, Proc. Natl. Acad. Sci. USA 97, 2408.

Chiu, D. T., E. Pezzoli, H. Wu, A. D. Stroock, and G. M. Whitesides, 2001, Proc. Natl. Acad. Sci. USA 98, 2961.

Delamarche, E., A. Bernard, H. Schmid, B. Michel and H. Biebuyck, 1997, Science 276, 779.

Deng, T., J. Tien, B. Xu and G. M. Whitesides, 1999, Langmuir 15, 6575.

Dertinger, S. K. W., D. T. Chiu, N. L. Jeon and G. M. Whitesides, 2001, Anal. Chem. 79, 1240.

Dike, L. E., C. S. Chen, M. Mrksich, J. Tien, G. M. Whitesides and D. E. Ingber, 1999, In Vitro Cell. Dev. Biol.-Anim. 35, 441.

Dubois, L. H. and R. G. Nuzzo, 1992, Annu. Rev. Phys. Chem. 43, 437.

Duffy, D. C., J. C. McDonald, O. J. A. Schueller and G. M. Whitesides, 1998, Anal. Chem. 70, 4974.

Folch, A. and M. Toner, 2000, Annu. Rev. Biomed. Eng. 2, 227.

Golden, J. P., L. C. Shriver-Lake, G. P. Anderson, R. B. Thompson and F. S. Ligler, 1992, Opt. Eng. 31, 1458.

Grushka, E., 1974, Bonded Stationary Phases in Chromatography, Ann Arbor Science Publication, Ann Arbor, 937 pp.

Grzybowski, B. A., R. Haag, N. Bowden and G. M. Whitesides, 1998, Anal. Chem. 70, 4645.

Harrison, D. J., K. Fluri, K. Seiler, Z. Fan, C. S. Effenhauser and A. Manz, 1993, Science 261, 895.

Ismagilov, R. F., J. M. K. Ng, P. J. A. Kenis, and G. M. Whitesides, 2001, Anal. Chem. 73, 5207.

Jacobson, S. C., R. Hergenroder, L. B. Koutny and J. M. Ramsey, 1994, Anal. Chem. 66, 1114.

Jeon, N. L., S. K. W. Dertinger, D. T. Chiu, I. S. Choi, A. D. Stroock and G. M. Whitesides, 2000, Langmuir 16, 8311.

Jeon, N. L., K. Finnie, K. Branshaw and R. G. Nuzzo, 1997, Langmuir 13, 3382.
Jo, B. H., L. M. Van Lerberghe, K. M. Motsegood and D. J. Beebe, 2000, J. Microelectromech. Syst. 9, 76.
Kane, R. S., S. Takayama, E. Ostuni, D. E. Ingber and G. M. Whitesides, 1999, Biomaterials 20, 2363.
Kenis, P. J. A., R. F. Ismagilov, S. Takayama, G. M. Whitesides, S. Li and H. S. White, 2000, Acc. Chem. Res. 33, 841.
Kenis, P. J. A., R. F. Ismagilov and G. M. Whitesides, 1999, Science, 285, 83.
Kim, E., Y. Xia and G. M. Whitesides, 1995, Nature 376, 581.
Kleinfeld, D., K. H. Kahler and P. E. Hockberger, 1988, J. Neurosci. 8, 4098.
Kovacs, G. T. A., 1998, Micromachined Transducers Sourcebook, CB/McGraw-Hill, Boston, 911 pp.
Kumar, A., H. A. Biebuyck, N. L. Abbott, and G. M. Whitesides, 1992, J. Am. Chem. Soc. 114, 9188.
Kumar, A., H. A. Biebuyck and G. M. Whitesides, 1994, Langmuir 10, 1498.
Lahiri, J., L. Isaacs, B. Grzybowski, J. D. Carbeck and G. M. Whitesides, 1999a, Langmuir 15, 7186.
Lahiri, J., L. Isaacs, J. Tien and G. M. Whitesides, 1999b, Anal. Chem. 71, 777.
López, G. P., H. A. Biebuyck, R. Härter, A. Kumar and G. M. Whitesides, 1993, J. Am. Chem. Soc. 115, 10774.
Love, J. C., J. R. Anderson and G. M. Whitesides, 2001a, MRS Bull. 26, 523.
Love, J. C., D. B. Wolfe, H. O. Jacobs and G. M. Whitesides, 2001b, Langmuir 17, 6005.
Manz, A., D. J. Harrison, E. M. J. Verpoorte, J. C. Fettinger, H. Ludi and H. M. Widmer, 1991, Chimia 45, 103.
McDonald, J. C., D. C. Duffy, J. R. Anderson, D. T. Chiu, H. Wu and G. M. Whitesides, 2000, Electrophoresis 21, 27.
Mrksich, M., 2000, Chem. Soc. Rev. 29, 267.
Mrksich, M., L. E. Dike, J. Tien, D. E. Ingber and G. M. Whitesides, 1997, Exp. Cell Res. 235, 305.
Muller, R. S. and T. I. Kamins, 1986, Device Electronics for Integrated Circuits, 2nd ed., Wiley, New York, 524 pp.
Ostuni, E., R. Kane, C. S. Chen, D. E. Ingber and G. M. Whitesides, 2000, Langmuir 16, 7811.
Pancrazio, J. J., P. P. Bey, D. S. Cuttino, J. K. Kusel, D. A. Borkholder, K. M. Shaffer, G. T. A. Kovacs and D. A. Stenger, 1998, Sens. Actuators B-Chem. 53, 179.
Patel, N., R. Padera, G. H. W. Sanders, S. M. Cannizzaro, M. C. Davies, R. Langer, C. J. Roberts, S. J. B. Tendler, P. M. Williams and K. M. Shakesheff, 1998, Faseb J. 12, 1447.
Piner, R. D., J. Zhu, F. Xu, S. Hong and C. A. Mirkin, 1999, Science 283, 661.
Prime, K. L. and G. M. Whitesides, 1991, Science 252, 1164.
Qin, D., Y. Xia and G. M. Whitesides, 1996, Adv. Mater. 8, 917.
Quake, S. R. and A. Scherer, 2000, Science 290, 1536.

Roberts, C., C.S. Chen, M. Mrksich, V. Martichonok, D.E. Ingber and G.M. Whitesides, 1998, J. Am. Chem. Soc. 120, 6548.

Schueller, O. J. A., X.-M. Zhao, G.M. Whitesides, S.P. Smith and M. Prentiss, M., 1999, Adv. Mater. 11, 37.

Sigal, G. B., C. Bamdad, A. Barberis, J. Strominger and G.M. Whitesides, 1996, Anal. Chem. 68, 490.

Sigal, G. B., M. Mrksich and G.M. Whitesides, 1997, Langmuir 13, 2749.

Singhvi, R., A. Kumar, G.P. Lopez, G.N. Stephanopolous, D.I.C. Wang, G.M. Whitesides and D.E. Ingber, 1994, Science 264, 696.

Takayama, S., R.G. Chapman, R. Kaneand G.M. Whitesides, Eds., 2000, Patterning of Cells and Their Environment, Academic Press, San Diego, 209 pp.

Takayama, S., J.C. McDonald, E. Ostuni, M.N. Liang, P.J.A. Kenis, R.F. Ismagilov and G.M. Whitesides, 1999, Proc. Natl. Acad. Sci. USA 5545.

Takayama, S., E. Ostuni, P. LeDuc, K. Naruse, D.E. Ingber and G.M. Whitesides, 2001, Nature 411, 1016.

Ulman, A., 1991, An Introduction to Ultrathin Organic Films Langmuir-Blogett to Self-Assembly, Academic Press, Boston, 442 pp.

Unger, M. A., H. Chou, T. Thorsen, A. Schererand S.R. Quake, 2000, Science 288, 113.

Weigl, B. H. and P. Yager, 1999, Science 283, 346.

Whitesides, G. M., A.J. Black, P.F. Nealey and J.L. Wilbur, 1996, In The Robert A. Welch Foundation 39th Conference on Chemical Research on Nanophase Chemistry, Houston, TX, 109 pp.

Whitesides, G. M. and C.B. Gorman, Eds., 1995, Self-Assembled Monolayers: Models for Organic Surface Chemistry, CRC Press, Boca Raton, 713 pp.

Whitesides, G. M., E.S. Ostuni, S. Takayama, X. Jiang and D.E. Ingber, 2001, Ann. Rev. Biomed. Eng. 3, 335.

Whitesides, G. M. and A.D. Stroock, 2001, Physics Today 54, 42.

Wilbur, J. L., A. Kumar, E. Kim and G.M. Whitesides, 1994, Adv. Mater. 6, 600.

Wilbur, J. L. and G.M. Whitesides, 1999, In Nanotechnology, Ed., G. Timp, Springer-Verlag, New York, 331 pp.

Wollman, E. W., D. Kang, C.D. Frisbie, I.M. Lorkovic and M.S. Wrighton, 1994, J. Am. Chem. Soc. 116, 4395.

Wu, M. H. and G.M. Whitesides, 2001, Appl. Phys. Lett. 78, 2273.

Xia, Y. and G.M. Whitesides, 1998, Angew. Chem. Int. Ed. Engl. 37, 550.

Yan, L., C. Marzolin, A.Terfort, A. and G.M. Whitesides, 1997, Langmuir 13, 6704.

Yang, P., G. Wirnsberger, H. Huang, S.R. Cordero, M.D. McGehee, B. Scott, T. Deng, G.M. Whitesides, B.F. Chmelka, S.K. Buratto and G.D. Stucky, 2000, Science 287, 465.