# Cellular Micropatterns on Biocompatible Materials

**Albert Folch and Mehmet Toner***

Center for Engineering in Medicine and Surgical Services, Massachusetts General Hospital, Shriners Burns Hospital, and Harvard Medical School, Boston, Massachusetts 02114

We present a method to produce micropatterns of cells on tissue culture substrates. A network of deep elastomeric microchannels defining the desired pattern is sealed onto the surface of interest, and a protein template is created by injecting sub-milliliter quantities of protein solution into the microchannels. Protein adsorbs only on the areas that were exposed to the microflow. After the channels are flushed and the elastomer is removed, cells attach only on the protein template. Micropatterns of collagen or fibronectin were used to selectively adhere cells on various biomedical polymers and on heterogeneous or microtextured substrates. Since the bare substrate areas remain apt for seeding other, more adhesive cell types such as fibroblasts, we were able to create micropatterned co-cultures. Our method allows for inexpensive patterning of a rich assortment of biomolecules, cells, and surfaces under physiological conditions.

## Introduction

Tissue function is modulated by the spatial organization of cells on a sub-millimeter scale. For this reason, artificial replication of cellular microstructures is important in understanding, measuring, and simulating their in vivo functions in the laboratory. Biotechnological applications of synthetic cellular patterns range from tissue engineering and wound healing to single-cell assays and biosensors for toxic compounds. However, geometrically selective attachment of cells on surfaces is an ongoing technological challenge. Early studies utilized spider webs (Harrison, 1914), grooved surfaces (Weiss, 1959; Rovensky et al., 1971), shadow-evaporated metallic lines (Carter, 1965), or scratched extracellular matrix (ECM) protein patterns (Rovasio et al., 1983) to guide cell migration, attachment, and/or spreading. These studies could not address the structural dimensions and precise repeatability over large areas found in living tissue. Recently, silicon microfabrication techniques (Ristic, 1994) and advances in surface chemistry have allowed for micropatterning of self-assembled monolayers (SAMs) (Laibinis et al., 1991; Ulman, 1991; Kumar and Whitesides, 1993; Kumar et al., 1994) of organic molecules which promote or inhibit cell adhesion on substrates otherwise homogeneously adherent to cells (Kleinfeld et al., 1988; Clark et al., 1992; Stenger et al., 1992; Singhvi et al., 1994; Bhatia et al., 1997; Chen et al., 1997). Unfortunately, the specialized SAM chemistries, e.g. functionalized alkylsiloxanes on silicon oxide or alkanethiols on gold (Ulman, 1991), are not easily combined with most tissue culture or implantable ("biocompatible") materials (Allara, 1995; Ratner, 1995) such as polymers or heterogeneous surfaces and, most importantly, can result in undesirable biointeraction (Schaffner et al., 1995; Tidwell et al., 1997).

* Address correspondence to this author at Shriners Research Center Bldg., 1400 West, One Kendall Square, Cambridge, MA 02139. Telephone: (617)374-5611. Fax: (617)374-5665. E-mail: mtoner@sbi.org.

Other schemes for cell micropatterning based on cytoscribing (Klebe, 1988), thermoresponsive polymers (Takezawa et al., 1990; Ito et al., 1997), or irradiation of proteins (Letourneau, 1975) or polymers (Matsuda et al., 1990; Rohr et al., 1991; Lee et al., 1993, 1994) have been proposed as well. We present an alternative strategy based on physically blocking protein adsorption on selected areas of a substrate by means of a removable and reusable elastomeric mask. As a result, the technique presented here uniquely allows for patterning, without chemical alteration of the substrate, polystyrene petri dishes and other biocompatible polymers, as well as heterogeneous surfaces containing a metallic or biomolecular micropattern.

## Materials and Methods

**Microfabrication.** Photoresist (1-$\mu$m-thick) patterns on 0.5-$\mu$m-thick $SiO_2$ on Si were defined by conventional photolithography (Ristic, 1994) and transferred to the $SiO_2$ layer by HF etch (5 min). The $SiO_2$ pattern was used to mask a 20−30-$\mu$m-deep Si plasma etch (plasma parameters: 250 W, 350 mTorr, 100 sccm $SF_6$, 10 sccm $CCl_4$, 10 sccm He, 1 cm gap). The Si master was coated with ~50 Å of Cr and ~500 Å of Au to prevent adhesion of the elastomer. The poly(dimethylsiloxane) (PDMS) precursor and curing agent (Sylgard 184, Dow Corning) were mixed with a thinning fluid (Dow Corning 200) (10:1:1), poured over the master in a container (forming a ~7-mm-thick layer), put under low vacuum (~12 Torr) to evacuate the bubbles from the microtrenches, and cured at 65 °C for 12 h (8-h overcure to avoid toxic bleaching). The cured PDMS replica, constituting a microfluidic network of microchannels ($\mu$Chs), was gently peeled off the master and the container by hand.

**Substrate Preparation.** We used human plasma fibronectin (Sigma) in sodium carbonate buffer (pH = 9.6) and collagen type I from Lewis rat tail tendons in HCl 0.1 mM (pH = 4 to prevent gel formation inside the $\mu$Chs). Dunn et al. described the collagen and rat hepatocytes isolations (Dunn et al., 1991). The $\mu$Chs

S8756-7938(98)00037-X CCC: $15.00 
Published on Web 05/20/1998

were rinsed with acetone and ethanol and blow-dried with $N_2$ between each use. We either perfused the $\mu$Chs in sterile conditions or sterilized the protein-patterned substrates with 70% ethanol or with overnight UV light exposure. We found the latter the most practical sterilization procedure.

**Immunofluorescence**. Collagen-patterned substrates were incubated in 1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) (blocking solution) for 30 min, then in rabbit anti-rat collagen antibody (BioDesign, 100:1 dilution in blocking solution) for 1 h; after being rinsed thoroughly with PBS, they were incubated in CY3-labeled goat anti-rabbit IgG (Jackson Immunoresearch, 500:1 dilution in blocking solution) for 1 h.

**Cell Culture**. Prior to cell seeding, the surfaces were rinsed with deionized water or PBS and incubated at 37 °C or room temperature for 30 min in 0.05% BSA to increase attachment selectivity (Bhatia et al., 1997). In addition to the polymers polystyrene (PS) (Falcon, either plasma-treated #3002 or untreated #1007), polycarbonate (PC) (Nalgene), poly(methyl methacrylate) (PMMA) (Glasflex), and PDMS, we also studied poly(methylpentene) Petri dishes (Nalgene), poly(vinyl chloride) sheets (Fisher), and UV-cured polyurethane films (Summers Optical), to which cells did not adhere. These polymers may need pretreatment such as surface hydroxylation by $O_2$ plasma (Curtis et al., 1986) to promote protein and/or cell adhesion. To demonstrate selective cell seeding, hepatocytes were allowed to attach for 1 h in serum-free medium (shaking every 15 min virtually eliminates background attachment); then the substrates were rinsed twice with serum-free medium to remove nonadhered hepatocytes. The same seeding/rinse sequence was repeated twice. Finally, the hepatocytes were cultured in medium containing 10% fetal bovine serum (Bhatia et al., 1997). For micropatterned co-cultures, 3T3-J2 fibroblasts were added 24 h after hepatocyte seeding and allowed to attach for another 24 h as described by Bhatia et al. (1997, 1998).

## Results and Discussion

**Elastomeric Microchannels.** In our method, a network of deep (~20−30 $\mu$m) elastomeric $\mu$Chs sealed onto the surface of interest is perfused with sub-milliliter quantities of an ECM protein solution. Thus protein adsorbs on the areas exposed to the microflow and is blocked in the areas where the elastomer contacts the surface. Essentially, we implement, in a microfluidic environment, noncovalent procedures for protein adsorption. These procedures are simple, they do not compromise the specific cell functions associated with the rich chemical complexity of polymeric surfaces (Tidwell et al., 1997), and they have been used in cell culture for decades on a wide variety of materials.

Recently, others have filled shallow (~1.5 $\mu$m) $\mu$Chs by capillary action to pattern curable polymers (Kim et al., 1995, 1996) and immunoglobulins (Delamarche et al., 1997). However, capillarity patterning is limited to lengths on the order of a few millimeters and, more importantly, it results in protein gradients due to depletion of protein adsorbing on the $\mu$Ch walls (Delamarche et al., 1997). Deeper $\mu$Chs yielding smaller pressure drops (Potter and Foss, 1982) are needed to pattern larger areas and reach steady-flow, nondepleting conditions. Our method is schematized in Figure 1. We used an anisotropic $SF_6/CCl_4$-based plasma etch (Ristic, 1994) to fabricate 20−30-mm-deep vertical-sidewall trenches of various shapes, widths, and lengths on a Si wafer (Figure 1a). We followed a simple replication procedure (Kumar and Whitesides, 1993; Kumar et al., 1994) to mold the $\mu$Chs in PDMS from the Au-coated Si master (Figure 1b). A post glued to the center of the Si pattern resulted in a via hole in the PDMS (Figure 1c) for liquid injection or suction. The molding procedure leaves the Si master intact; therefore, it can be used repeatedly. Upon dry contact with a smooth surface, the $\mu$Chs self-seal reversibly (Kim et al., 1995, 1996; Delamarche et al., 1997) and can be perfused with various solutions over large areas (up to tens of square centimeters). The elastomer, by virtue of its inert nature, does not appear to leave debris or toxic remains on the substrate or to degrade over time. Thus it can be reused virtually indefinitely.

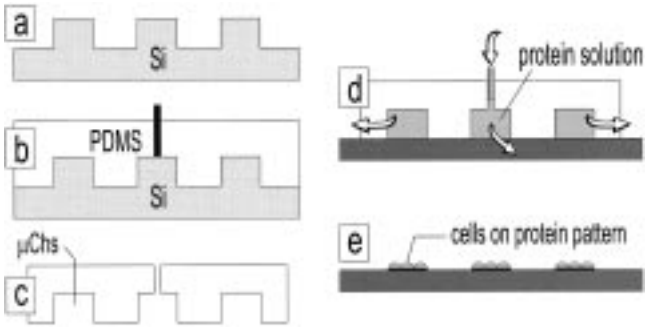


**Figure 1.** Cross-section schematics of our cell patterning method: (a) Si master fabrication; (b and c) replica molding of PDMS from the Au-coated master (Au layer not depicted); (d) protein patterning by injecting protein solution into the $\mu$Chs; (e) cell seeding after elastomer removal, resulting in selective cell attachment only on the protein template.

**Micropatterning**. The minimum achievable line width, the maximum patternable area, and the maximum injection pressure depend on the sealing pressure and $\mu$Ch geometry and pattern and must, therefore, be determined empirically for each application. As shown below, we straightforwardly achieved single-cell line widths. We typically inject <1 mL (~0.1 mL/min) of collagen (0.2 mg/mL) or fibronectin (10 mg/mL) solution for ~5 min to create the ECM protein template (Figure 1d). To prevent protein solution from spreading onto adjacent areas, the $\mu$Chs are flushed with deionized water or saline solution before being separated from the surface. Cells adhere preferentially to the protein template (Figure 1e). Note that this method is compatible with nondenaturing as well as sterile conditions. The fact that the $\mu$Chs are continuously perfused (as opposed to filled by capillary action (Delamarche et al., 1997)) prevents depletion of protein from the solution and ensures that the protein coverage is uniform throughout the perfused area, as demonstrated by indirect immunofluorescence staining (Figure 2). The exact protein coverage (presently undergoing characterization in our laboratory) is not crucial to the current study, but it is known to affect adhesion strength (Truskey and Pirone, 1990; Garcia et al., 1997), growth, and differentiation (Mooney et al., 1992) and could, in principle, be adjusted by varying the surface hydroxylation (Curtis et al., 1986) and the time of exposure to the protein solution as well as its temperature, pH, ionic strength, and concentration (Lee and Ruckenstein, 1988). Slight local variations in protein coverage may result from parabolic flow velocity vector distribution (typical of channel fluidics) (Potter and Foss, 1982), from anomalies in the microflow around edges, particles, or trapped bubbles or (in the case of collagen) from gel nucleation.

**Biocompatible Surfaces**. Our method is readily compatible with off-the-shelf polymeric substrates com-

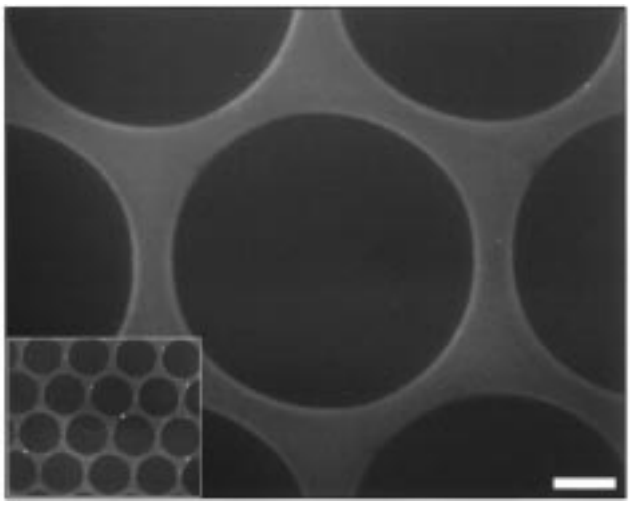

**Figure 2.** Indirect immunofluorescence visualization of collagen (light gray) pattern on polystyrene (dark gray) Petri dish (inset: larger area). Scale bar is 100 $\mu$m.

monly used in biology laboratories. These include PS or PC Petri dishes, medical-grade PMMA disks, or PDMS films. Traditional substrates for cell patterning such as glass and Si or Au-coated glass could also be patterned. As a model, we demonstrated selective adhesion of rat hepatocytes onto collagen or fibronectin templates. Cells were "selectively attached" if they were not washed away during the rinse following the 1-h seeding periods in serum-free medium (see Materials and Methods). Seeding in serum-containing medium results in destruction of the collagen or fibronectin patterns, probably due to nonselective adsorption of serum proteins onto the polymeric surface (Hubbell, 1995). Figure 3 depicts hepatocyte patterns on some combinations of the proteins and polymers mentioned above (see caption for details) 12−24 h after seeding. In general, we did not observe significant differences in attachment selectivity (during the 1-h seeding periods) or attachment/spreading dynamics (during the following 24 h) on the protein-patterned polymers: on all materials, the protein template was covered within ~15 min with cells which did not detach when the dish was shaken; after ~6 h, cells had flattened on all materials. This indicates similarities in the properties of the surface "seen" by the hepatocyte, namely in surface roughness, collagen/fibronectin coverage, and/or adsorption of other molecular species from the serum-free medium on the bare polymer areas. (It is possible, however, that long-term hepatocyte function, under current evaluation, is influenced by the underlying morphology or composition of these substrates.) In all materials, cells could be constricted to lines 20 $\mu$m wide (single-cell width), resulting in elongated cellular geometries (e.g., Figure 3a,c).

**Micropatterned Co-cultures**. Preferential adhesiveness is highly dependent on cell type. For example, fibroblasts adhere to both the background and the protein-coated areas. Since the bare polymer areas are apt for seeding another, more adhesive cell type such as fibroblasts, the technique allows for micropatterned hepatocyte/fibroblast co-cultures, as demonstrated in Figure 4. Bhatia et al. have shown that, on covalently immobilized collagen patterns on glass, this micropatterned co-culture system is particularly convenient for studying cell−cell heterotypic interactions in vitro (Bhatia et al., 1997, 1998). The advantage of leaving bare areas for seeding a second cell type can be a disadvantage for other applications where constraining cell shape is

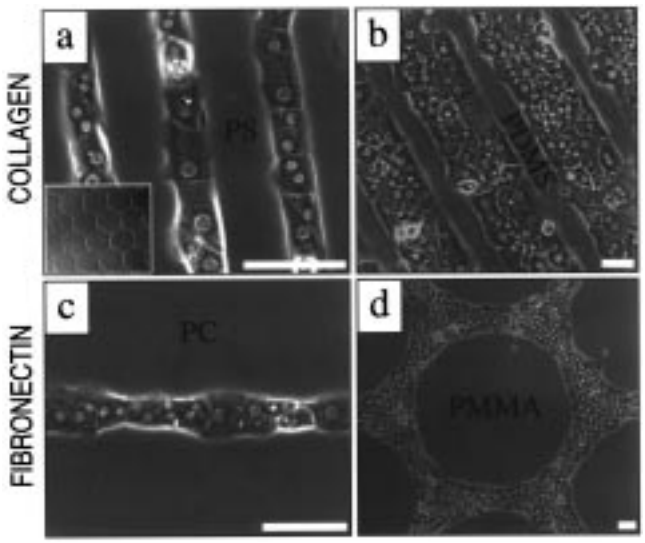


**Figure 3.** Phase-contrast optical micrographs of hepatocyte micropatterns on various polymers 12−24 h after seeding. The hepatocytes only adhered to the underlying collagen (a and b) or fibronectin (c and d) template created on (a) PS Petri dish (inset: another pattern, 2.4 mm × 2 mm area); (b) PDMS sheet; (c) PC Petri dish; and (d) PMMA disk. Position of the initials marks the bare polymer area. Scale bar is 50 $\mu$m.

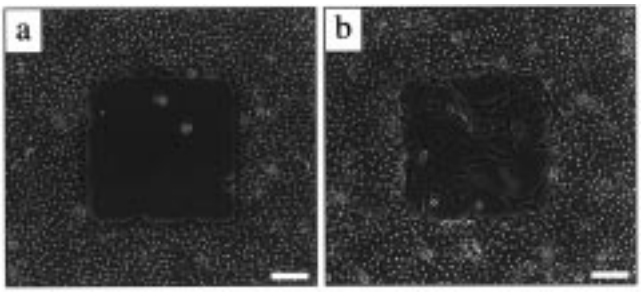


**Figure 4.** Phase-contrast optical micrographs showing a micropatterned co-culture of hepatocytes and 3T3-J2 fibroblasts (a) before and (b) 12 h after fibroblast seeding. Fibroblasts were seeded 24 h after hepatocyte seeding. Scale bar is 100 $\mu$m.

important (Carter, 1967; Singhvi et al., 1994; Chen et al., 1997). With time (>24 h), the hepatocytes start spreading out of the patterns, likely attributable to adsorption/diffusion of endogenously secreted ECM proteins (Odenthal et al., 1992).

**Heterogeneous Surfaces.** Another unique feature of our technique is that it allows for patterning of nonhomogeneous substrates. Interestingly, cells seeded on protein patterns overlaying metal (Ti, Cr, Au) microelectrodes on glass behaved in strikingly different ways depending on the metal. Cell micropatterns overlapped Cr/glass or Au/glass areas with equal conformity (Figure 5a). In contrast, cells did not attach to Ti and had difficulty attaching to the narrow (~10 $\mu$m) glass stripes and their spreading was slowed at the glass/Ti edges (Figure 5a, inset), presumably due to poor protein adsorption on the Ti areas. In addition, it is possible to create patterns of fibronectin on collagen-patterned substrates. As shown in Figure 5b, attachment and spreading as well as compliance to the pattern were identical for both ECM proteins. We highlight that the collagen-coated areas retain their function as a cell adhesion promoter and their immunoreactivity even after being dried and brought in contact with the elastomer.

**Microtextured Surfaces.** Alternatively, the $\mu$Chs themselves constitute a microtextured surface suitable

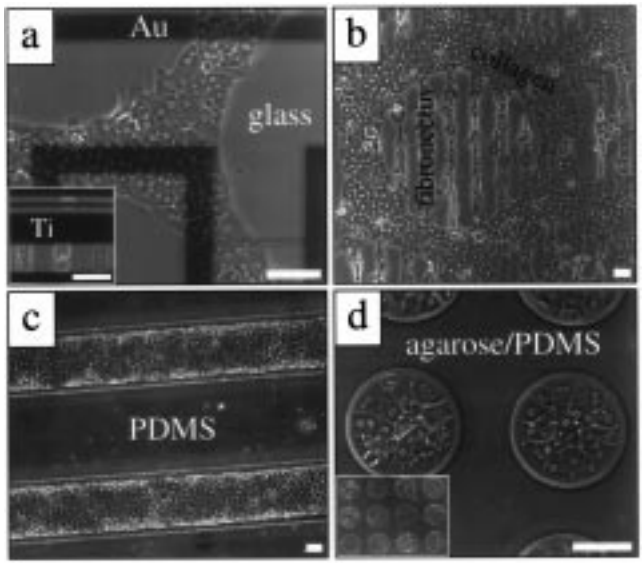


**Figure 5.** Hepatocyte micropatterns on nonhomogeneous surfaces: (a) collagen patterns on Au/glass and (inset) Ti/glass microelectrodes (glass areas appear brighter); (b) 20-mm-wide fibronectin lines crossing a mosaic of collagen circles (same pattern as in Figure 3d) on PS; (c) inside collagen-coated PDMS trenches; (d) fibronectin-coated PDMS islands surrounded by agarose-filled PDMS trenches (inset: larger area). Scale bar is 50 $\mu$m.

for selective cell seeding (Mrksich et al., 1996). Indeed, all three walls of a $\mu$Ch are rendered adhesive to cells when perfused with protein solution. When hepatocytes were seeded on PDMS $\mu$Chs which had been perfused with a protein solution, cells attached selectively to the bottom and sidewalls of the trenches and not on the elevated bare PDMS areas (Figure 5c). Cells do not attach to PDMS (and that is valid for the inside of the trenches as well as the elevated areas) *unless* it is coated with protein (see Figure 3b). This constitutes a versatile approach for cellular patterning in the vertical dimension. We stress that the ability to produce micropatterns of cells on moldable polymers is of paramount importance in the artificial simulation of complex three-dimensional (3D) cellular microstructures, such as liver sinusoids or blood capillaries.

**Cellular "Islands".** Note that, although our method only allows for connected patterns, it can generate disconnected patterns when used in combination with a heterogeneous surface capable of differential adhesion such as Ti/glass. Cellular "islands" can also be created by a variation of our technique which uses agarose as a cell adhesion repellent. Hot liquid agarose (1% w/w in water at ~80 °C) injected into the $\mu$Chs solidifies within the $\mu$Chs when cooled to room temperature; when separated from the sealing surface, the $\mu$Chs constitute a PDMS surface with agarose-filled trenches. After being incubated (37 °C) in protein solution for 30 min, cells attach to the elevated PDMS areas surrounded by agarose (Figure 5d). Note how hepatocyte spreading conforms to the shape of the island.

In conclusion, we have been able to make micropatterned cell cultures using ECM protein templates on a variety of tissue culture materials. Our protein patterning method is compatible with nondenaturing and physiological conditions, does not present toxic side effects, and can be extended to other biomolecules that spontaneously adsorb on surfaces from aqueous solution. Furthermore, it does not require routine access to microfabrication facilities. Due to its low cost, versatility, and 3D capabilities, the method has strong implications in basic cell biology and biotechnology, encompassing tissue engineering, electrophysiology, bioartificial devices or implants, and biosensors.

## Acknowledgment

We are thankful to U. Balis, S. Bhatia, O. Hurtado, L. Horowitz, J. Voldman, and M. Yarmush for insightful comments and to W. Jastromb, C. Pligavko, and S. Beshad for hepatocyte and collagen isolations and media preparation. This study was partially funded by the Shriners Hospitals for Children and NIH. We microfabricated the Si masters at the Microsystems Technology Laboratory (MIT).

## References and Notes

Allara, D. L. Critical Issues in Applications of Self-Assembled Monolayers. *Biosens. Bioelectron.* **1995**, *10*, 771.

Bhatia, S. N.; Yarmush, M. L.; Toner, M. Controlling cell interactions by micropatterning in co-cultures: hepatocytes and 3T3 fibroblasts. *J. Biomed. Mater. Res.* **1997**, *34*, 189−199.

Bhatia, S. N.; Balis, U. J.; Yarmush, M. L.; Toner, M. Microfabrication of Hapatocyte/Fibroblast Co-cultures: Role of Homotype Cell Interactions. *Biotechnol. Prog.* **1998**, *14*, 378−387.

Carter, S. B. Principles of cell motility: the direction of cell movement and cancer invasion. *Nature* **1965**, *208*, 1183−1187.

Carter, S. B. Haptotactic islands: a method of confining single cells to study individual cell reactions and clone formation. *Exp. Cell. Res.* **1967**, *48*, 189−193.

Chen, C. S.; Mrksich, M.; Huang, S.; Whitesides, G. M.; Ingber, D. E. Geometric control of cell life and death. *Science* **1997**, *276*, 1425−1428.

Clark, P.; Connolly, P.; Moores, G. R. Cell guidance by micropatterned adhesiveness in vitro. *J. Cell Sci.* **1992**, *103*, 287−292.

Curtis, A. S. G.; Forrester, J. V.; Clark, P. Substrate hydroxylation and cell adhesion. *J. Cell Sci.* **1986**, *86*, 9−24.

Delamarche, E.; Bernard, A.; Schmid, H.; Michel, B.; Biebuyck, H. Patterned delivery of immunoglobulins to surfaces using microfluidic networks. *Science* **1997**, *276*, 779−781.

Dunn, J. C.; Tompkins, R. G.; Yarmush, M. L. Long-term in vitro function of adult hepatocytes in a collagen sandwich configuration. *Biotechnol. Prog.* **1991**, *7*, 237−245.

Garcia, A. J.; Ducheyne, P.; Boettiger, D. Cell adhesion strength increases linearly with adsorbed fibronectin density. *Tissue Eng.* **1997**, *3*, 197−206.

Harrison, R. G. The reaction of embryonic cells to solid structures. *J. Exp. Zool.* **1914**, ***17***, 521−544.

Hubbell, J. A. Biomaterials in tissue engineering. *Bio/Technology* **1995**, *13*, 565−576.

Ito, Y.; Chen, G. P.; Guan, Y. Q.; Imanishi, Y. Patterned immobilization of thermoresponsive polymer. *Langmuir* **1997**, *13*, 2756.

Kim, E.; Xia, Y. N.; Whitesides, G. M. Polymer Microstructures Formed By Molding in Capillaries. *Nature* **1995**, *376*, 581.

Kim, E.; Xia, Y. N.; Whitesides, G. M. Micromolding in capillaries: Applications in materials science. *J. Am. Chem. Soc.* **1996**, *118*, 5722.

Klebe, R. J. Cytoscribing: a method for micropositioning cells and the construction of two- and three-dimensional synthetic tissues. *Exp. Cell Res.* **1988**, *179*, 362−373.

Kleinfeld, D.; Kahler, K. H.; Hockberger, P. E. Controlled outgrowth of dissociated neurons on patterned substrates. *J. Neurosci.* **1988**, *8*, 4098−4120.

Kumar, A.; Whitesides, G. M. 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.* **1993**, *63*, 2002.

Kumar, A.; Biebuyck, H. A.; Whitesides, G. M. Patterning Self-Assembled Monolayers—Applications in Materials Science. *Langmuir* **1994**, *10*, 1498.

Laibinis, P. E.; et al. Comparison of the Structures and Wetting Properties of Self- Assembled Monolayers of Normal-Alkanethiols On the Coinage Metal-Surfaces, Cu, Ag, Au. *J. Am. Chem. Soc.* **1991**, *113*, 7152.

Lee, S. H.; Ruckenstein, E. Adsorption of proteins onto polymeric surfaces of different hydrophilicities—a case study with bovine serum albumin. *J. Colloid Interface Sci.* **1988**, *125*, 365−379.

Lee, J. S.; et al. Selective adhesion and proliferation of cells on ion-implanted polymer domains. *Biomaterials* **1993**, *14*, 958−960.

Lee, J. S.; et al. Micropatterning of Cultured-Cells On Polystyrene Surface By Using an Excimer-Laser. *Appl. Phys. Lett.* **1994**, *65*, 400.

Letourneau, P. C. Cell-to-substratum adhesion and guidance of axonal elongation. *Dev. Biol.* **1975**, *44*, 92−101.

Matsuda, T.; Inoue, K.; Sugawara, T. Development of micropatterning technology for cultured cells. *Am. Soc. Artif. Intern. Organs Trans.* **1990**, *36*, M559−562.

Mooney, D.; et al. Switching from differentiation to growth in hepatocytes: control by extracellular matrix. *J. Cell. Physiol.* **1992**, *151*, 497−505.

Mrksich, M.; et al. Controlling cell attachment on contoured surfaces with self-assembled monolayers of alkanethiolates on gold. *Proc. Natl. Acad. Sci. U.S.A* **1996**, *93*, 10775−10778.

Odenthal, M.; et al. Rat hepatocytes in primary culture synthesize and secrete cellular fibronectin. *Exp. Cell Res.* **1992**, *203*, 289−296.

Potter, M. C.; Foss, J. F. *Fluid mechanics*; Great Lakes Press: Michigan, 1982.

Ratner, B. D. Surface modification of polymers: chemical, biological and surface analytical challenges. *Biosens. Bioelectron.* **1995**, *10*, 797−804.

Ristic, L., Ed. *Sensor technology and devices*; Artech House: Boston, MA, 1994.

Rohr, S.; Scholly, D. M.; Kleber, A. G. Patterned growth of neonatal rat heart cells in culture. Morphological and electrophysiological characterization. *Circ. Res.* **1991**, *68*, 114−130.

Rovasio, R. A.; Delouvee, A.; Yamada, K. M.; Timpl, R.; Thiery, J. P. Neural crest cell migration: requirements for exogenous fibronectin and high cell density. *J. Cell. Biol.* **1983**, *96*, 462−473.

Rovensky, Y. A.; Slavnaja, I. L.; Vasiliev, J. M. Behaviour of fibroblast-like cells on grooved surfaces. *Exp. Cell Res.* **1971**, *65*, 193−201.

Schaffner, A. E.; Barker, J. L.; Stenger, D. A.; Hickman, J. J. Investigation of the factors necessary for growth of hippocampal neurons in a defined system. *J. Neurosci. Methods* **1995**, *62*, 111−119.

Singhvi, R.; et al. Engineering cell shape and function. *Science* **1994**, *264*, 696−698.

Stenger, D. A.; et al. Coplanar Molecular Assemblies of Aminoalkylsilane and Perfluorinated Alkylsilane—Characterization and Geometric Definition of Mammalian-Cell Adhesion and Growth. *J. Am. Chem. Soc.* **1992**, *114*, 8435.

Takezawa, T.; Mori, Y.; Yoshizato, K. Cell culture on a thermoresponsive polymer surface. *Bio/Technology* **1990**, *8*, 854−856.

Tidwell, C. D.; et al. Endothelial cell growth and protein adsorption on terminally functionalized, self-assembled monolayers of alkanethiolates on gold. *Langmuir* **1997**, *13*, 3404.

Truskey, G. A.; Pirone, J. S. The effect of fluid shear stress upon cell adhesion to fibronectin- treated surfaces. *J. Biomed. Mater. Res.* **1990**, *24*, 1333−1353.

Ulman, A. *An introduction to ultrathin organic films: from Langmuir−Blodgett to self-assembly*; Academic Press: Boston, MA, 1991.

Weiss, P. Cellular dynamics. *Rev. Mod. Phys.* **1959**, *31*, 11−20.

Accepted April 10, 1998.

BP980037B