Selective Deposition of Proteins and Cells in Arrays of Microwells

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This paper describes a method for depositing proteins and cells selectively into microwells fabricated in poly(dimethylsiloxane)—an elastomeric organic polymer. The arrays of microwells were generated using soft lithography. The surface *between* the wells was coated with bovine serum albumin, and the surface *within* the wells was coated with fibronectin (FN)—an adhesive extracellular matrix protein. Fluorescence staining of FN using antibodies indicated that the protein had adsorbed to the wells selectively. Exposure of the FN-coated wells to a suspension of bovine capillary endothelial cells resulted in attachment and spreading of the cells only in the wells. This method for patterning cells was demonstrated with wells of several diameters (≤ 100 μm) and depths (≤ 50 μm). It should be useful for (i) the development of sensors based on arrays of cells, (ii) screening combinatorial libraries of ligands on cells, and (iii) testing the effect on cells of compounds and samples relevant to problems in environmental chemistry, pharmacology, medicinal chemistry, research biology, and biodefense.

Introduction

This paper describes a method to pattern proteins and cells by depositing them into arrayed microwells. To fabricate these arrays, we molded poly(dimethylsiloxane) (PDMS, a transparent, inert elastomer) against masters that had been formed photolithographically.[1–3] We exploited the pinning of drop edges at steps in this contoured surface to deposit bovine serum albumin (BSA) selectively on the planar surfaces between wells. In a separate step, we deposited the extracellular matrix (ECM) protein fibronectin (FN) selectively on the interior surface of the wells (Figure 1). BSA-coated surfaces resisted the adhesion of cells; FN promoted the adhesion of cells to surfaces. Substrates fabricated in this manner directed the selective adhesion of bovine capillary endothelial (BCE) cells to the surfaces of the wells. The volume of the wells could be controlled by changing their depth and diameter.

A number of methods in biochemistry require a technique for patterning single cells with a high degree of spatial selectivity. Many laboratories are trying to take advantage of the high sensitiviy of living cells in sensing units for biosensor applications; in these systems, the cells must be located precisely on the circuitry of the device.[4–10]

In tissue engineering, it may be useful to pattern different kinds of cells on different areas of a substrate in order to build defined architecture into multifunctional tissues.[11] Automated technologies for high-throughput screening require the placement of cells in well-ordered arrays that can be addressed individually. Basic studies of cellular function and metabolism will also benefit from the ability to control the microenvironment of patterned cells and to perturb them individually. Assays aimed at identifying the phenotype of a cell in a population of heterogeneously transfected cells might be simplified if individual cells were localized in a well; the production of a fluorescently labeled gene product could be detected in the well rather than remaining unidentified in solution.

Although cell-based assays are commonly available, applications involving patterned single cells have been limited by technological problems, e.g., the selective delivery of small volumes of liquid to a well with a 50 μm diameter, the placement of cells on a defined grid, and the prevention of nonselective adhesion and cell migration. Improving the technology to generate regular arrays of cells will make it possible to develop (i) analytical systems based on single cells for the detection of toxic agents, (ii) systems for high-throughput screening of combinatorial libraries and gene products, (iii) research tools to study the effect of the adhesive environment on the behavior of a cell, and (iv) new methods for the study of cellular function and metabolism at the level of single cells and individually isolated groups of cells.

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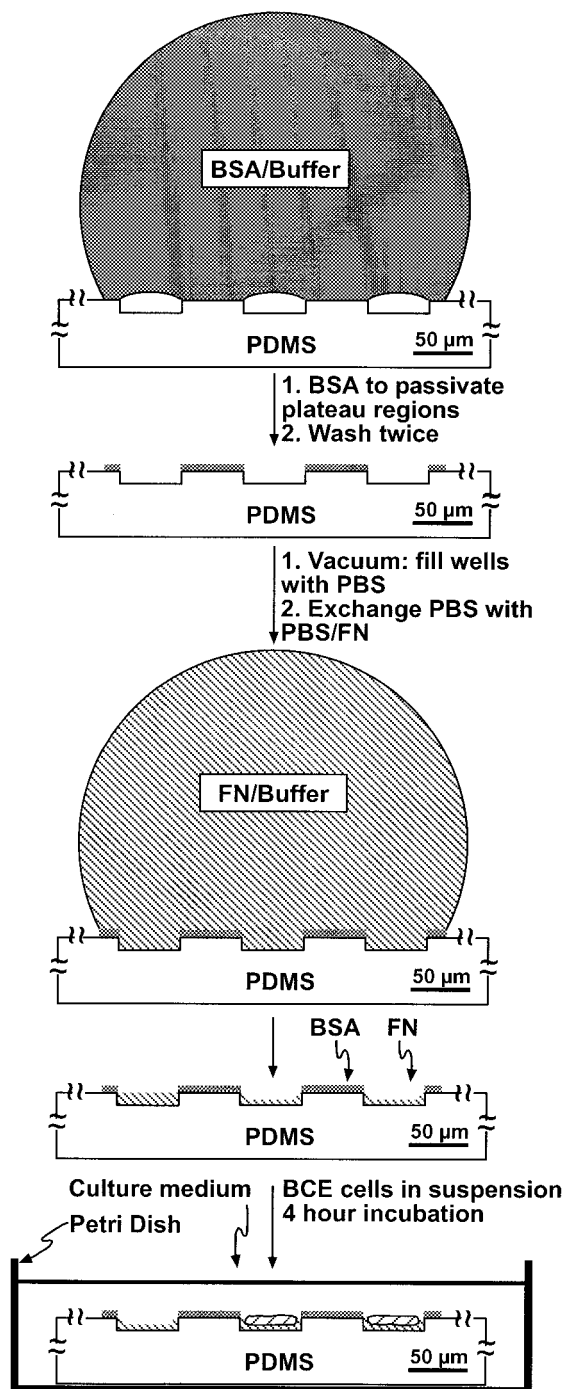(11) Langer, R.; Vacanti, J. P. *Science* **1993**, *260*, 920–926.

Figure 1. Schematic diagram describing the delivery of FN to the wells and the deposition of cells in the wells; see Experimental Section for details. In the first step, a drop of BSA in phosphate-buffered saline (PBS, 1 mg/mL) is placed on top of a stamp for 1 h to allow the protein to adsorb on the surface between wells. After the substrate was washed with PBS and kept under house vacuum for 30 s, a solution of FN in PBS (50 μg/mL) was placed on top of the stamp and incubated for 1 h to allow this protein to coat the interior surface of the wells. Substrates prepared in this manner were then exposed to a suspension of BCE cells. Cells attached selectively to the FN that adsorbed in the wells.

To pattern single mammalian cells onto a substrate, the best strategy is often to pattern adhesive ECM proteins onto that substrate. Current methods for patterning ECM proteins use self-assembled monolayers (SAMs) of al-kanethiolates on gold or of alkyltrichlorosiloxanes on silicon. Previously, we have shown that mammalian cells can adhere to flat[12,13] or contoured[12] gold surfaces pat-

terned with SAMs.[3,14] Earlier work on patterning cells focused on their interaction with SAMs of alkylsilox-anes.[15-18] Photolithographic patterning of siloxanes allowed the definition of patterns of functional groups that were recognized nonspecifically by various types of cells; complex biological ligands, however, are not compatible with these photolithographic methods. Microcontact printing has also been used to directly print patterns of proteins onto surfaces.[19,20] You et al.[21] coated arrays of large wells (1-mm diameter) nonselectively with ECM proteins; mink lung cells were forced into the wells from a suspension by dragging a flat piece of PDMS across the array of wells. Parce et al. have previously used gravitational sedimentation to deposit cells in arrays of 50 μm wells that were fabricated using silicon micromachining.[6] We have also developed a method for patterning cells onto surfaces using elastomeric membranes as resists against the adsorption of proteins and the adhesion of cells.[22]

The method described here patterns cells conveniently on inexpensive, optically transparent polymeric supports. PDMS is elastomeric; this property allows it to be stretched or deformed and fitted to nonplanar surfaces. The contoured PDMS substrates are fabricated from reusable masters by replica molding: this procedure is operationally straightforward, requires no special equipment, and can be carried out in a conventional laboratory. The size and shape of the wells can be defined easily using rapid prototyping[2,23] (for wells > 30 μm in diameter) or standard photolithography (for sizes between 1 and 30 μm). We delivered BSA to the surfaces between the wells selectively by trapping bubbles of air in the wells when coating the surface. FN solution was then delivered to the wells in a step that involved brief exposure to vacuum to remove any residual air bubbles. In the second coating step, FN adsorbed only to the interior surface of the wells because the surface between the wells was protected by the adsorbed BSA. Cells adhered selectively to the surfaces that were coated with FN—that is, the interior of the wells. The simplicity and the rapidity of this method make it attractive for applications in biotechnology and biology.

Experimental Section

Fabrication of Arrays of Microwells. PDMS substrates were prepared as described previously by Jackman et al.[24] Briefly, a master consisting of posts of photoresist supported on a silicon

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Table 1. Advancing Contact Angles (deg) of Various Fluids on Flat PDMS Substrates[a]

	$\theta_{a(PDMS)}$ ($\pm 5°$)	filling		
		25(5)	50(1.3)	50(5)
distilled water	111	−	−	−
fibronectin/PBS (50 µg/mL)	109	−	−	−
BSA/PBS (1 mg/mL)	100	−	−	−
ethylene glycol	92[b]	±[c]	±[c]	±[c]
tri(ethylene glycol)	76[b]	+	+	+
butan-1-ol	36[b]	+	+	+
ethanol	31[b]	+	+	+

[a] The columns under "filling" report whether the tested liquids filled wells with the specified dimensions. "+" indicates wells that are filled by the liquid, and "−" is used to denote cases when filling was not observed. [b] Obtained from ref 21. [c] EG does not fill the wells immediately; over minutes, the trapped bubbles escape (see text).

wafer was prepared photolithographically; the height of the posts, which corresponds to the depth of the wells, was controlled by the choice of the photoresist and the spinning rate. The chrome masks used for photolithography had circular features with diameters between 25 and 50 µm and spacing between 50 and 75 µm; masks were prepared by Advanced Reproductions, North Andover, MA. The PDMS (Dow Corning, Sylgard 184) was cured against these masters at 60 °C for 2 h and peeled away from the silicon wafers to give an array of wells molded into the surface of the polymer. Substrates were then cut to the desired size (typically 1−2 cm²) and washed with ethanol and distilled water before use in cell culture. For simplicity, we refer to the different types of wells using a code; for example, 50(1.3) refers to wells with a diameter of 50 µm and a depth of 1.3 µm.

Contact Angle Measurements. The contact angles of various solutions on PDMS were measured using a contact angle goniometer (Rame-Hart, Mountain Lakes, NJ). Advancing (θ_a) and receding contact angles were measured for three separate drops on each substrate by delivering/withdrawing aliquots (3 × 5 µL) of solution with a micropipet (Matrix Technologies, MicroElectrapette). Each entry in Table 1 is the average of the three measurements. Occasionally, the drops pinned on heterogeneities on the surface of the polymer; in such cases, the values of the contact angles deviated from the average by more than 50% and new measurements were taken on another region of the surface. The receding contact angles of solutions that contained proteins could not be measured reliably and are not reported. The proteins adsorbed to the PDMS on the time scale of the experiment; the adsorbed layer remained hydrated and it did not allow the edge of the drop to recede.

Microscopy. The air bubbles trapped inside the wells were imaged on a Leica DM IRB inverted microscope. Images were collected with a CCD camera (Sony, Iris) connected to a Scion Corp. CG-7 frame grabbing card mounted in a Power Macintosh 8500. Confocal images of bubbles inside the wells were collected with a Leica DM RBE equipped with a TCS 4D confocal scanner. Phase contrast and fluorescence micrographs of cells and stained proteins were taken with a 35 mm camera connected to a Zeiss Axiophot microscope.

Selective Adsorption of FN inside the Wells (Figure 1). Delivery of BSA to the surface between the wells, and of FN to the interior surface of the wells, was accomplished using a two-step procedure. PDMS stamps were placed inside sterile Petri dishes. In the first step, a drop of BSA (Intergen Company, Purchase, NY; 1 mg/mL) in phosphate-buffered saline (PBS) buffer was placed on top of a substrate for 1 h to allow the protein to deposit on the surface between wells. The liquid trapped air bubbles inside the wells; these air bubbles protected the interior surface of the wells from contact with the solution containing BSA. We carefully avoided shaking or vibrating the substrate to avoid dislodging these bubbles accidentally. The substrate was then washed gently with PBS three times. Brief exposure (30 s) of the substrates to house vacuum (ca. 400 mm Hg) ensured that all the bubbles escaped from the wells. We exchanged the buffer with a solution of FN (Collaborative Biomedical, 50 µg/mL) in buffer by rapidly aspirating the buffer and placing the solution of FN on top of the array of microwells; during this procedure,

we avoided drying the sample to prevent the formation of bubbles and damage to the adsorbed BSA. The adsorption of FN to the PDMS was then allowed to proceed at room temperature for 1 h.

Cell Culture. Bovine adrenal capillary endothelial (BCE) cells were cultured under 10% CO_2 on Petri dishes (Falcon) coated with gelatin in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% calf serum, 2 mM glutamine, 100 µg/mL streptomycin, 100 µg/mL penicillin, and 1 ng/mL basic fibroblast growth factor (bFGF). Prior to incubation with the polymeric substrates, cells were dissociated from culture plates with trypsin-EDTA and washed in DMEM containing 1% BSA (BSA/DMEM).[25] The cells were placed on the substrates in chemically defined medium (10 µg/mL high-density lipoprotein, 5 µg/mL transferrin, 5 ng/mL bFGF in BSA/DMEM)[26] and incubated in 10% CO_2 at 37 °C.[25] A typical incubation time was 4 h, and cells were routinely cultured for up to 48 h in the microwells. Cells can also be patterned in serum-containing medium for 48 h after being seeded in serum-free medium.

Staining of FN and Cells on Polymeric Substrates. The substrates coated with FN were exposed to 4% paraformaldehyde (v/v) in PBS buffer (PFA) for 20 min, washed with PBS, and then immersed in a solution of rabbit anti-human fibronectin IgG (Sigma, 5 µg/mL) for 1 h. The substrates were rinsed twice with PBS containing 0.1% (w/v) BSA and 0.1% (w/w) Triton X-100 and placed in contact with 100 µL of Texas Red-labeled goat anti-rabbit IgG (Amersham Life Sciences, 50 µg/mL) for 1 h, rinsed, and then mounted with Fluoromount-G (Southern Biotechnology, Inc.) onto microscope slides.

Substrates that contained cells patterned on FN in wells were fixed with PFA for 20 min, washed with methanol for 1 min, and stained with Coomassie Blue (5 mg/mL in 40% v/v methanol, 10% v/v acetic acid, and 50% v/v water) for 30 s; they were then rinsed with distilled water and dried in air.

Results

Trapping Air Bubbles in Microwells. When drops of aqueous solutions with values of $\theta_a > 90°$ were placed on top of a substrate that presented wells and allowed to spread, air was trapped in the wells. Figure 2A shows the appearance of an array of wells covered with a drop of solution of BSA (1 mg/mL) in PBS buffer ($\theta_a = 100°$). Wells filled with air were much brighter than the rest of the substrate when imaging the system in reflection mode on an inverted microscope (light coming from the "bottom" of the sample) using light of high intensity (100 W Hg arc lamp). The difference between the index of refraction of PDMS and air (ca. 0.4) is higher than the difference between PDMS and buffer (ca. 0.05); hence, the wells that were filled with air reflected more light and appeared brighter than the rest of the sample. We allowed liquids with values of $\theta_a > 90°$ to cover the entire surface of the substrates by detaching the air from the wells with a brief exposure of the system to house vacuum for 30 s; the bubbles in the wells expanded and detached when vacuum was applied, and the wells filled when vacuum was released. Wells that were filled with liquid were not brighter than the rest of the surface (Figure 2B). We confirmed the results obtained with optical microscopy using confocal microscopy. Figure 2C shows a confocal image of a well filled with air; the image was obtained in reflection mode (light coming from the "top" of the sample). We observed a complex optical diffraction pattern produced by the light that is reflected from the air-filled cavity (Figure 2D). We made indistinguishable observations with all types of wells when distilled water or PBS buffer was used to cover the substrates. We also trapped air in the wells when the substrates were placed *upside down, on top* of a drop of liquid. Liquids such as ethanol and butan-

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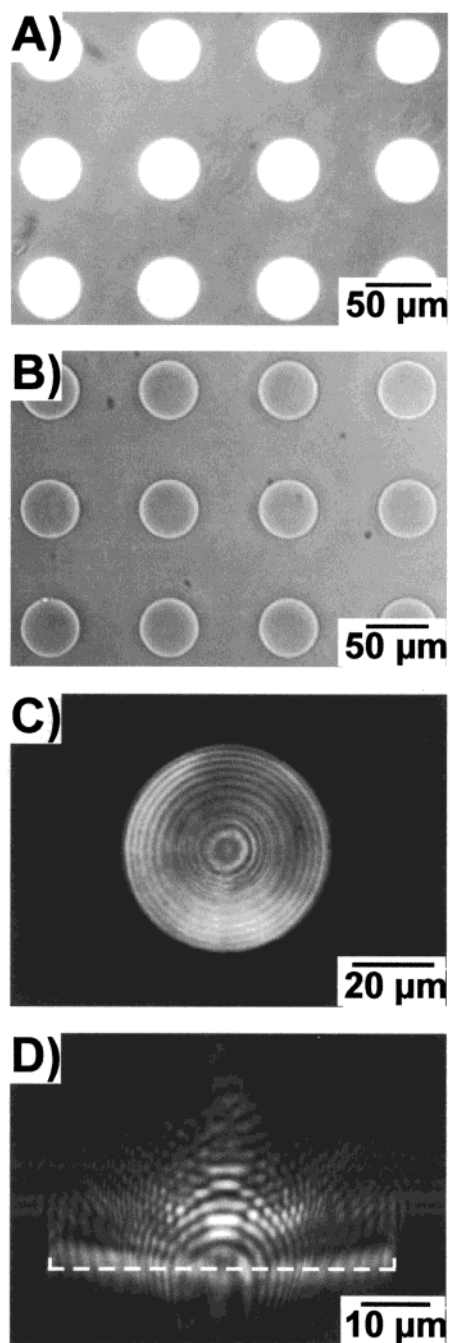(26) Ingber, D. E. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 3579−3583.

Figure 2. Visualization of air bubbles trapped in 50(1.3) wells. We made similar observations with 25(5) wells. The images in A and B were collected on an inverted microscope using a 100-W Hg arc lamp; only 50% of the light reflected from the substrate was sent to the camera. In this configuration, light illuminated the samples from the bottom and the CCD camera collected light that was reflected from the samples. The images in C and D were collected on a confocal microscope (in reflection mode) on stamps that were exposed to a drop of PBS containing fluorescently labeled BSA (1 mg/mL); a glass coverslip was placed on the drop to seal the sample. (A) A drop of BSA solution in PBS buffer was placed on top of a stamp presenting wells The wells were empty as indicated by the lensing effect. (B) The same stamp as in (A) with wells that were filled after being exposed to house vacuum for 30 s. The wells no longer appeared bright because the solid−liquid interface was continuous, unlike that in (A). (C) Confocal image (*XY* plane) *in reflection mode* of a well covered with a drop of fluorescent BSA. In reflection mode, fluorescence from the BSA cannot be detected; the observed pattern, therefore, arises from constructive interference of light reflected in the well. (D) Vertical confocal slice (*xz* plane) through an empty well prepared as in (C). The dotted line represents the outline of the well that was imaged in transmission.

1-ol had values of $\theta_a < 90°$ on PDMS and filled the wells (Table 1). When ethylene glycol (EG, $\theta_a = 92 \pm 5°$) covered the wells, air was trapped in the wells; these bubbles escaped within minutes. EG entered the wells because its value of θ_a on PDMS is almost 90°.

The phenomenon by which certain liquids trap air in wells on a surface can be explained using the Laplace−Young equation to define θ_a (eq 1); γ is the interfacial

$$\cos \theta_a = \frac{\gamma_{sv} - \gamma_{sl}}{\gamma_{lv}} \tag{1}$$

tension of the solid−vapor (sv), solid−liquid (sl), and liquid−vapor (lv) interfaces.[27] The solid−vapor interface of the PDMS has lower energy ($\gamma_{SV} \sim 21$ dyn/cm) than the liquid−vapor interface of the drops of liquid (γ_{LV} of water ~ 73 dyn/cm). The solutions used in this study had values of $\theta_a > 90°$, implying that the values of γ_{sl} are high. Under such conditions, it is thermodynamically favorable for the area of all interfaces with the liquid to be minimized. Hence, a drop of liquid that contacts the flat regions of the stamp, but does not fill the wells, forms a system of lower free energy than one in which liquid contacts the entire surface of the stamp.

Cassie derived an equation (eq 2) to describe the contact

$$\cos \theta_b = f_1 \cos \theta_a - f_2 \tag{2}$$

angle of a liquid on a contoured surface by taking into account the areas of the solid−liquid and liquid−vapor interfaces.[28] In eq 2, θ_b is the apparent contact angle, θ_a is the contact angle measured on the flat surface, f_1 is the area of the solid−liquid interface, and f_2 is the area of the liquid−vapor interface. Porosity causes the surface of a material on which a liquid has $\theta_a > 90°$ ($<90°$) to appear more hydrophobic (hydrophilic) than a flat surface of the same material.[28] Cassie and Shuttleworth used eq 2 to predict that a droplet of liquid with $\theta_a > 90°$ would trap air inside pores in a surface.[28,29] This prediction has been confirmed experimentally by us and by many others.[28−30] The derivations that we have described assume that the contribution of gravitational force to the wetting behavior is minor;[28−32] this assumption seems valid since the measured values of contact angles on porous surfaces are in agreement with the predicted ones.[28−30]

A drop of liquid with a high value of θ_a on PDMS pins at the edge of a well until its contact angle on the wall of the well reaches the advancing value; pinning of liquid drops at discontinuities has been observed before (Figure 3).[30,33,34] Liquids with a value of $\theta_a > 90°$ reach the other edge of the well before the angle between the liquid and the vertical wall reaches θ_a; the edge of the liquid, therefore, advances over the face of the well as described in Figure 3 and traps air inside the wells. Previous studies of the behavior of liquids on textured surfaces were motivated by the design of textiles with improved resist-

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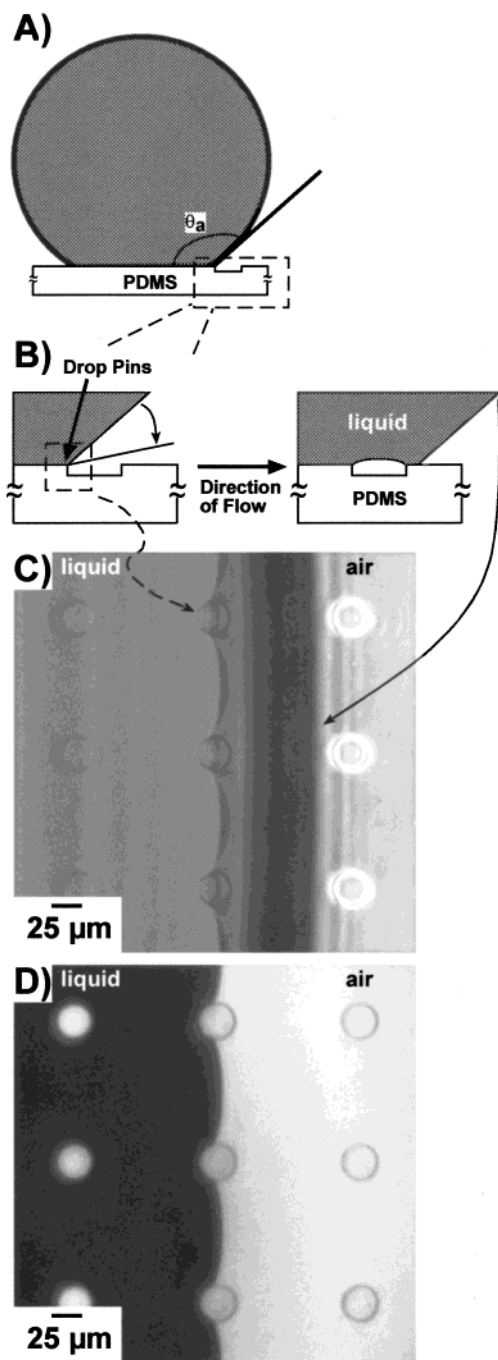
Figure 3. (A) Schematic representation of a drop of solution of BSA on an array of microwells. (B) Enlargement of the schematic that shows the drop pinned at the edge of a well. As the advancing front approaches the equilibrium advancing contact angle on the wall of the well, the drop touches the other wall of the well and does not fill it. (C) Visualization of the advancing front of a drop of PBS containing BSA (1 mg/mL) on an array of 25(5) wells. The drop was advancing from left to right across the image. The edge of the drop was imaged in transmission mode. The advancing front of the drop can be seen at the surface of the stamp (dashed arrow), while above the stamp, the drop "leans over" the contact point with the surface because θ_a > 90°; the dark region indicated by the solid arrow is the part of the drop not in contact with the surface. The dashed arrow also points to a well that is covered by, but not filled by, liquid. (D) The same region as in (C) imaged in reflection mode using a 100-W Hg arc lamp. The wells covered by liquid are empty as indicated by the bright reflectance, while the solvent front appears as a dark contour at the surface.
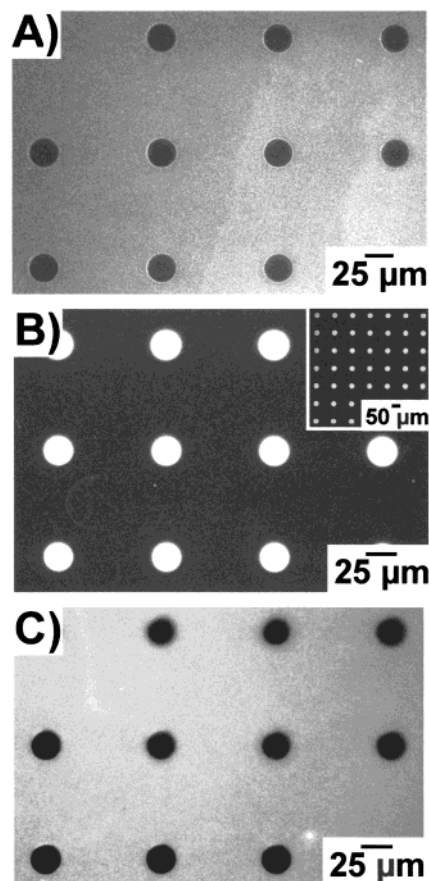

Figure 4. Fluorescence imaging of proteins deposited in 25(5) wells. The lighter regions of the photographs correspond to fluorescence from the proteins that were labeled fluorescently or stained immunofluorescently. (A) Fluorescently labeled BSA was delivered to the regions of the substrate that separated the wells by placing a drop of solution in PBS on the substrate; after incubation with BSA, substrates were washed with PBS buffer instead of a solution of FN. BSA adsorbed only to the regions between the wells. (B) BSA and fibronectin were deposited on the polymeric substrate following the method described in Figure 1. FN was stained immunofluorescently as described in the Experimental Section. The inset shows 41 wells coated selectively with FN. (C) Fluorescence micrograph of wells that were incubated with a drop of FN, rinsed, and immunofluorescently stained. The protein did not adsorb to the wells but only to the flat areas between wells.

ance to penetration of water.[28,29] In other work, we found that liquids with values of the receding contact angle (θ_r) on PDMS < 90° filled wells as drops of the liquids *receded* on the arrays.[24] The findings and the methods described in this paper apply to liquids that *advance* on the arrays of microwells (θ_a > 90°).

Patterning Proteins in Microwells. The behavior of liquids on contoured surfaces formed the basis of a method for patterning the adsorption of proteins onto PDMS substrates having surfaces embossed with microwells. The surface *between* the wells was first coated with BSA by placing a solution of the protein in PBS on the substrate for 1 h at room temperature. In this interval, air trapped in the wells protected their surfaces from contact with the solution of BSA (Figure 2A); the trapped air effectively acted as a resist against the adsorption of protein. We confirmed this result by performing the same incubation with a solution of fluorescently labeled BSA (Figure 4A). Following the adsorption of BSA on the regions between the wells, the substrates were rinsed with PBS and exposed to house vacuum for 30 s to remove the air bubbles from
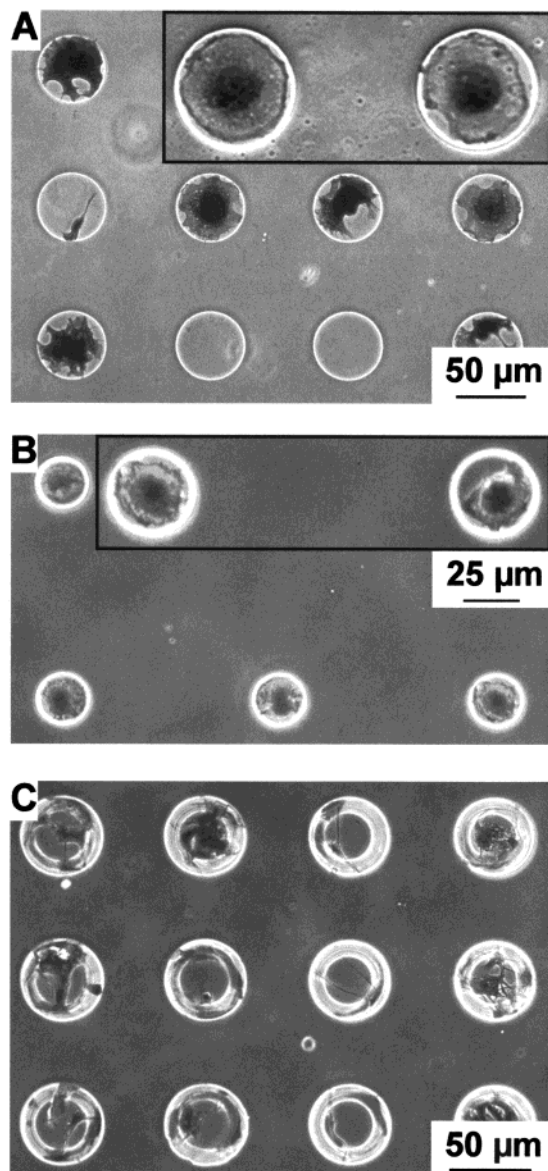
Figure 5. BCE cells immobilized on arrays of wells. The polymeric substrates coated with BSA and FN, following the method described in Figure 1, were incubated with a suspension of BCE cells (ca. $10^{-4}/\mu m^2$) for 4 h. The cells were fixed with PFA and stained with Coomassie blue. (A) Cells adhered selectively to 50(1.3) wells. The inset shows a detailed image of two cells that spread to cover the entire surface of wells. (B) 25(5) wells containing cells. The inset shows a detailed picture of two patterned cells. (C) Under the microscope, the 50(50) wells diffract light more strongly than the 25(5) or 50(1.3) wells; hence, the 50(50) wells have a doughnut-like shape. The walls of the 50(50) wells present the cells with an adhesive area that is much larger than that presented by the 25(5) and 50 (1.3) wells.[35] Cells in 50(50) wells adhered to the walls of the wells as well as to the bottom of the wells. The diffraction caused by the height of the wells makes it difficult to see the cells that are attached to the wall. Dark areas in the bright diffraction rings are cells attached to the walls of the wells.

the wells and ensure that liquid filled them. The PBS was then exchanged with a solution of FN (50 μg/mL) in buffer. FN adsorbed on those hydrophobic regions of the surface not already covered with BSA, that is, the surface of the wells; the adsorbed BSA protected the regions between the wells from the adsorption of FN. Immunofluorescence staining of FN confirmed that this protein was delivered and adsorbed to the wells selectively (Figure 3B). A drop of FN left on top of an uncoated PDMS substrate allowed
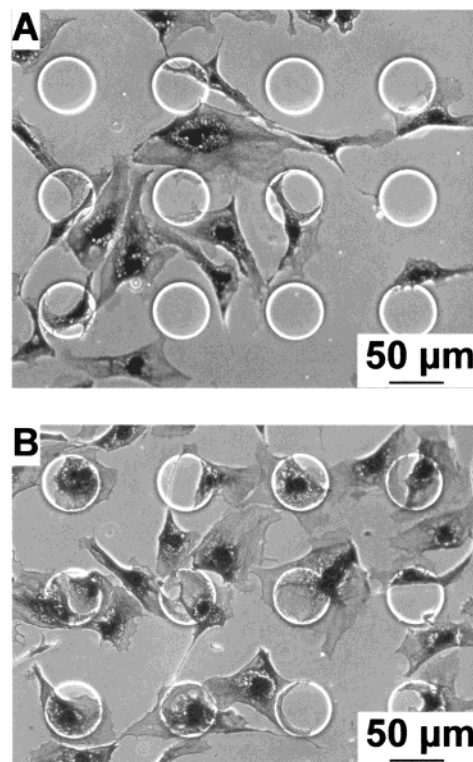

Figure 6. (A) *Wells* (*BSA*); *between wells* (*FN*): BCE cells adhered to the surface of an array of 50(1.3) wells that were coated with FN; the wells were coated with BSA. Cells extended across the wells, but they did not adhere to the interior surface of the wells. (B) *Wells* (*FN*); *between wells* (*FN*): Cells adhered to the surfaces of the wells and the plateau between wells for substrates in which both surfaces were coated with FN. Cells did not show preferential adhesion on the surface of or between the wells.

the protein to adsorb *only* to the regions of the array between the wells (Figure 4C).

Selective Attachment of Cells in Microwells. In an array presenting wells coated with FN, separated by surfaces coated with BSA, cells selectively adhered to the wells (Figure 5). We used BCE cells suspended in DMEM enriched with growth factor, lipoprotein, transferrin, and BSA. In a typical experiment, the PDMS substrate, patterned with proteins as described above, was immersed in 4 mL of solution that contained 10^5 cells. Cells attached in ca. 70% of the 50(1.3) wells (Figure 5A). The occupancy of cells in the 25(5) wells was lower (40%) (Figure 5B).

The cells in suspension reached the surface of the substrate by gravitational sedimentation; once on the surface, cells rolled under the influence of motion in the liquid. Cells generally adhered to the adhesive island of FN presented at the bottom of wells with depths between 1.3 and 5 μm. The depth of the wells prevented the cells from binding to the neighboring islands of FN; cells occasionally "bridged" two shallow (1.3 μm deep) wells. When wells were used that were 50 μm deep, the walls of the wells presented an adhesive surface that supported the adhesion of whole cells rather than just parts of cells (Figure 5C); it was also possible to find multiple cells in the same 50(50) well. In 50(50) wells, a large fraction of cells adhered preferentially to the walls of the wells.[35]

Using the method described here, we prepared substrates in which the wells were coated with BSA and the surface between the wells was coated with FN. Cells adhered mostly to the surface between the wells on such substrates; since the majority of the surface was adhesive,

the cells were, however, capable of extending across a well without adhering to its sides or bottom (Figure 6A). It was not possible to pattern the cells only on the surface *between* the wells. When all surfaces of the array of wells were coated with FN, cells adhered to the bottom of wells and to the surface between the wells (Figure 6B).

Discussion

Engineering the interface between living cells and artificial devices requires controlling the topography of the surface and the pattern and density of different types of molecules immobilized on the surface. One important step in applications involving patterned cells, therefore, consists of patterning adhesive proteins onto islands that are separated by nonadhesive borders. For some applications, it would be advantageous if the environment of each patterned cell could be isolated and controlled to allow the delivery of different substances to the individual cell.

The system we describe in this paper is based on the fabrication of arrays of microwells that are (i) chemically compatible with biological media, (ii) hydrophobic, thus promoting the adsorption of proteins, (iii) optically transparent at visible wavelengths, to allow microscopic examination of cells, (iv) mechanically robust, and not brittle, (v) low in fluorescence background, and thus compatible with immunofluorescence techniques that are used to visualize nuclei and ECM proteins, (vi) easily fabricated, and (vii) permeable to O and CO_2. We used PDMS to fabricate all the substrates described here, but our method for patterning proteins and cells is compatible with substrates fabricated in any hydrophobic material.

PDMS is also the material of choice for the fabrication of microscopic structures by soft lithography.[2] These straightforward methods can be used to replicate features down to 1 μm using masters prepared either by e-beam writing, by photolithography, or by using high-resolution acetate films as masks (for features > 30 μm)—a technique that we refer to as "rapid prototyping".[24] Soft lithographic techniques that are applied to an elastomeric material make separation of master and replicas straightforward. PDMS can also be used as a master to produce relief structures in the surfaces of polymers that are not elastomeric; the separation of two materials that are not elastomeric is technically more challenging than a separation that involves soft materials.

To coat the surface of the wells selectively with a protein, we took advantage of the behavior of fluids on contoured surfaces to pattern proteins onto such substrates. Our method does not require patterning the chemical reactivity of a surface. A liquid with $\theta_a > 90°$ traps air bubbles in

arrayed microwells. Using this method, we deposited BSA to the surface between wells; subsequently, other solutions of proteins could be delivered to the wells selectively by exposing to vacuum the assembly formed by the array of microwells and the solution.

We used these observations to develop a method that achieves efficient patterning of ECM proteins and BCE cells on contoured polymeric substrates. This method has several advantages: (1) Preparing the substrates is easier than evaporating gold on glass and patterning the noble metal with alkanethiols or alkyltrichlorosiloxanes; PDMS can be cast against masters of photoresist numerous times without damaging the master. (2) The PDMS stamps can be prepared and stored for >1 year without changes in performance. (3) This method does not require the synthesis and purification of alkanethiols. (4) Patterning of cells is achieved by a method that uses only the properties of liquids on contoured surfaces, rather than one that physically places the cells in the wells. (5) This method is applicable to contoured substrates made of a variety of polymers. Its only requirement is that it uses aqueous solutions and arrays of wells made with hydrophobic polymers.

The method has a few disadvantages: (1) It does not allow the molecular-level control of the properties of the interface that is possible with SAMs. (2) It does not allow the constraint of the wells to be released after patterning the cells onto any substrate; the ability to release cells from constraints is possible using a membrane method described previously and is useful in studying cell spreading.[22]

Conclusions

We have presented a method for coating the interior surface of wells in arrays with FN and the surface between the wells with BSA. Substrates prepared in this manner direct the selective adhesion of cells to the interior of the wells. The method is easy to implement because it relies on the behavior of a fluid on a contoured surface. This method could form the basis of systems that make it possible to isolate the environment of each cell in the array. Using this system, it would be straightforward to address wells individually using automated devices (such as ink-jet printer heads or FACS machines); these types of devices should be able to address microscopic arrays of single cells by delivering appropriate reagents for high-throughput analyses.

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(35) In the case of the 50(50) wells, the ratio of the area of the wall of the well to the area of the bottom of the well is 4; hence, cells adhere preferentially to the walls of the wells. The area of the wall of a 50(50) well is $2\pi RL$, where R is the radius of the well and L is the height. The area of the bottom of the well is πR^2. The ratio of the surface of the wall to that of the bottom of the well is $2\pi RL/\pi R^2 = 2L/R$. For the 50(50) wells, $2L/R = 4$.