Micropatterned Surfaces for Control of Cell Shape, Position, and Function

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The control of cell position and function is a fundamental focus in the development of applications ranging from cellular biosensors to tissue engineering. Using microcontact printing of self-assembled monolayers (SAMs) of alkanethiolates on gold, we manufactured substrates that contained micrometer-scale islands of extracellular matrix (ECM) separated by nonadhesive regions such that the pattern of islands determined the distribution and position of bovine and human endothelial cells. In addition, the size and geometry of the islands were shown to control cell shape. Traditional approaches to modulate cell shape, either by attaching suspended cells to microbeads of different sizes or by plating cells on substrates coated with different densities of ECM, suggested that cell shape may play an important role in control of apoptosis as well as growth. Data are presented which show how micropatterned substrates were used to definitively test this hypothesis. Progressively restricting bovine and human endothelial cell extension by culturing cells on smaller and smaller micropatterned adhesive islands regulated a transition from growth to apoptosis on a single continuum of cell spreading, thus confirming the central role of cell shape in cell function. The micropatterning technology is therefore essential not only for construction of biosurface devices but also for the investigation of the fundamental biology of cell−ECM interactions.

Introduction

The ability to control the placement of cells in an organized pattern on a substrate has become increasingly important for the development of cellular biosensor technology and tissue engineering applications (Singhvi et al., 1994; Hammarback et al., 1985; Healy et al., 1994). To build in circuitry to directly monitor the status of sensor cells on a device requires that the cells be properly positioned on the circuit. Similarly, automation technologies to perturb and observe cells serially requires that cells are positioned into addressable arrays that can be accessed, for example, by a motorized stage. Finally, tissue engineering applications require that cells can be specifically placed to generate organized structures, such as a neural network.

To address this need to provide strict control of cell−surface interactions, a method has been developed to fabricate "islands" of extracellular matrix (ECM) surrounded by nonadhesive regions, such that single cells would attach and spread only to the adhesive regions. This has been accomplished by microcontact printing patterns of self-assembled monolayers (SAMs) of alkanethiolates on gold, which have been used to control the interactions of surfaces with proteins (Prime and Whitesides, 1991, 1993; Mrksich and Whitesides, 1996). Previous work has shown that, while hydrophobic SAMs rapidly and irreversibly adsorb proteins and promote cell adhesion, SAMs that present ethylene glycol moieties effectively resist protein adsorption and cell adhesion (Prime and Whitesides, 1991, 1993). Thus, the pattern of these two SAMs presented on a substrate defines the pattern of ECM that adsorbs from solution onto the substrate, and an array of adhesive islands limits cell attachment to those islands (Singhvi et al., 1994; Mrksich et al., 1997). In addition, recent work has shown that the size and geometry of these islands control cell shape (Chen et al., 1997), enabling one to examine previously unapproachable questions regarding the mechanism by which ECM regulates cell function.

Past work suggests that cellular functions are regulated both by attachment of cells through cell surface integrin receptors (Hynes, 1992; Clarke and Brugge, 1995; Craig and Johnson, 1996) and by the subsequent physical spreading and flattening of cells against the substrate (Ingber, 1990, 1997). Because inhibition of angiogenesis (formation of new capillaries) and induction of capillary regression has been shown to block solid tumor growth (Ingber et al., 1990; Holmgren et al., 1994; O'Reilly et al., 1997), uncovering the mechanisms by which the ECM acts to regulate proliferation and apoptosis of capillary cells in particular has gained recent attention. Standard techniques, however, lack the ability to precisely control the adhesive microenvironment of cells that is necessary to clearly distinguish the role of

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S8756-7938(98)00031-9 CCC: $15.00
Published on Web 05/13/1998

cell shape from other aspects of cell adhesion. For example, previous work has shown that progressively increasing the density of ECM adsorbed to a substrate decreases apoptosis rates (Re et al., 1993) and promotes cell growth, or de novo synthesis of cellular DNA (Ingber, 1990). However, the approach cannot distinguish whether ECM density, or the resultant cell spreading on the substrate, is providing the signals for survival and growth. By using the micropatterning technology to control capillary endothelial cell spreading in the absence of confounding parameters such as ECM density, we set out to directly test whether cells can be switched between growth and apoptosis by altering cell shape (Chen et al., 1997). In this report, we describe our experimental methods in detail as well as additional evidence supporting the identification of such a switch.

Materials and Methods

Microfabrication of Patterned Substrates. We used microcontact printing techniques to fabricate substrates patterned with regions that adsorb ECM and regions that resist such adsorption, as previously described (Singhvi et al., 1994). Briefly, patterned substrates were prepared as follows (Figure 1A). In a clean room (100), silicon ⟨111⟩ wafers were cleaned, spin-coated with a 2-μm layer of poly(methyl methacrylate) photoresist, and baked. The wafers were exposed to high-energy UV light through a photolithographic mask containing the desired pattern (Figure 1A, i) using a standard mask aligner (Karl Suss). The wafers were developed and washed, leaving 2 μm thick photoresist where the UV was masked and naked silicon elsewhere. We prepared a poly(dimethylsiloxane) (PDMS) stamp from this silicon master by polymerizing prepolymer on top of the master (Figure 1A, ii). Substrates for cells were then prepared by electron beam physical vapor deposition of thin films of titanium (1.5 nm) and gold (12 nm) on glass cover slips (0.20 mm, No. 2, Corning). A cotton swab was wetted with a solution of hexadecanethiol ($HS(CH_2)_{15}CH_3$, 2 mM in ethanol) and dragged once across the face of a PDMS stamp molded from a silicon master as described above; the stamp was dried with a stream of nitrogen for 10 s and placed gently on a metallized glass slide with sufficient pressure to promote conformal contact between the stamp and the substrate (Figure 1A, iii). After 5 s, the stamp was removed from the substrate, taking care not to "double-stamp" the substrate. The slide was immersed immediately in a solution of the tris(ethylene glycol)-terminated alkanethiol in ethanol ($HS(CH_2)_{11}(OCH_2CH_2)_3OH$, 2 mM) for 30 min; the slide was removed, rinsed with ethanol, and dried with a stream of nitrogen (Figure 1A, iv). Hexadecanethiol was purchased from Aldrich and purified by silica gel column chromatography; the tris(ethylene glycol)-terminated alkanethiol was synthesized as described previously (Prime and Whitesides, 1993). The adsorption of protein on hydrophobic SAMs of hexadecanethiolate is usually rapid and irreversible. SAMs presenting oligomers of the ethylene glycol group are very effective at resisting the adsorption of protein. Therefore, when these substrate were immersed in 50 μg/mL of fibronectin (FN, Collaborative Biomedical) in PBS, FN rapidly adsorbed only to the stamped regions (Mrksich et al., 1997). After rinsing with PBS, the substrates were handled using standard cell culture techniques. The resolution of the original UV masks determines the quality of the final PDMS stamp (Figure 1B) and subsequent substrates. Masks were produced using high-resolution laser printing, step reduction methods, or electron beam etching.

Cell Culture. Prior to experiments, bovine capillary endothelial (BCE) cells were cultured in standard growth media under 10% CO_2 on gelatin-coated plastic 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). Human microvascular endothelial cells (HMVEC) were cultured in endothelial basal medium (EBM, Clonetics) containing 10% fetal calf serum, 1 μg/mL hydrocortisone; 10 μg/mL epidermal growth factor (EGF), 10 μg/mL bovine brain extract, 50 μg/mL gentamycin, and 50 μg/mL amphotericin-B. Experiments with HMVECS were carried out in the same medium containing 2% serum; BCE cells were studied in defined medium containing DMEM supplemented with basic fibroblast growth factor (5 ng/mL), human high-density lipoprotein (10 μg/mL), and transferrin (10 μg/mL).

Attaching ECM-Coated Beads to Suspended Cells. To attach cells to beads, endothelial cells were first suspended with trypsin and washed with experimental medium. Beads (Polysciences) coated overnight with 50 μg/mL of FN in 0.1 M carbonate buffer, pH = 9.4, were incubated with the cells at a 1:1 ratio (10^6 beads/mL) for 1 h to allow cells to attach to the beads. The mixture was then diluted 1:10 into 2% methylcellulose in experimental media to maintain them in suspension. Cells were recovered for analysis by fixing with 4% formaldehyde for 30 min, diluting the suspension with PBS, and spinning the samples in a centrifuge. Cells were resuspended in 1 mL of PBS and dried onto gelatin-coated slides at 40 °C. Samples were stained directly on the slides. Isolation of DNA from cells for apoptosis analysis was accomplished by diluting and spinning cell suspensions without fixing. The pellet was immediately frozen with liquid nitrogen.

Detection of Apoptosis. Samples were stained for DNA damage with the TUNEL method (TdT-mediated dUTP nick end labeling) and for nuclear condensation and fragmentation with DAPI (4′,6-diamidino-2-phenylindole). Samples were fixed in 4% formaldehyde for 30 min and washed with wash buffer (WB, 0.1% BSA in PBS). After permeabilization with 0.1% Triton X-100 in 0.1% sodium citrate buffer, samples were incubated with terminal deoxyribonucleic acid transferase enzyme in reaction buffer containing fluoresceinated dUTP for 1 h (Boehringer Mannheim). After washing with WB, samples were incubated with 3 μg/mL of DAPI in WB. Apoptosis was calculated as the percentage of individual cells that stained positively for TUNEL or showed evidence of nuclear condensation and fragmentation visualized with DAPI. A minimum of 200 cells were counted in each sample.

DNA degradation was assessed in certain conditions to confirm the presence of apoptosis. Cell pellets were washed twice with ice cold PBS, resuspended in TE (1 mM EDTA and 10 mM Tris-HCl, pH 7.4), and lysed with 0.5 mL of extraction buffer (0.5% SDS, 100 mM EDTA, and 10 mM Trix HCl, pH 8.0) with 0.5 mg/mL proteinase K. After incubating for 2 h at 50 °C, samples were extracted twice with 50% phenol/48% chloroform/2% isoamyl alcohol, and then nucleic acid was precipitated with 0.2 M NaCl with 2 volumes of cold ethanol. DNA was recovered by pelleting with high-speed centrifugation, washing with 70% ethanol, air-drying, and resuspending in TE with 20 μg/mL RNAse A. Samples were incubated for 1 h at 37 °C and separated on a 1% agarose gel containing ethidium bromide.

Detection of DNA Synthesis. Active DNA synthesis was detected by incorporation of the thymidine analogue

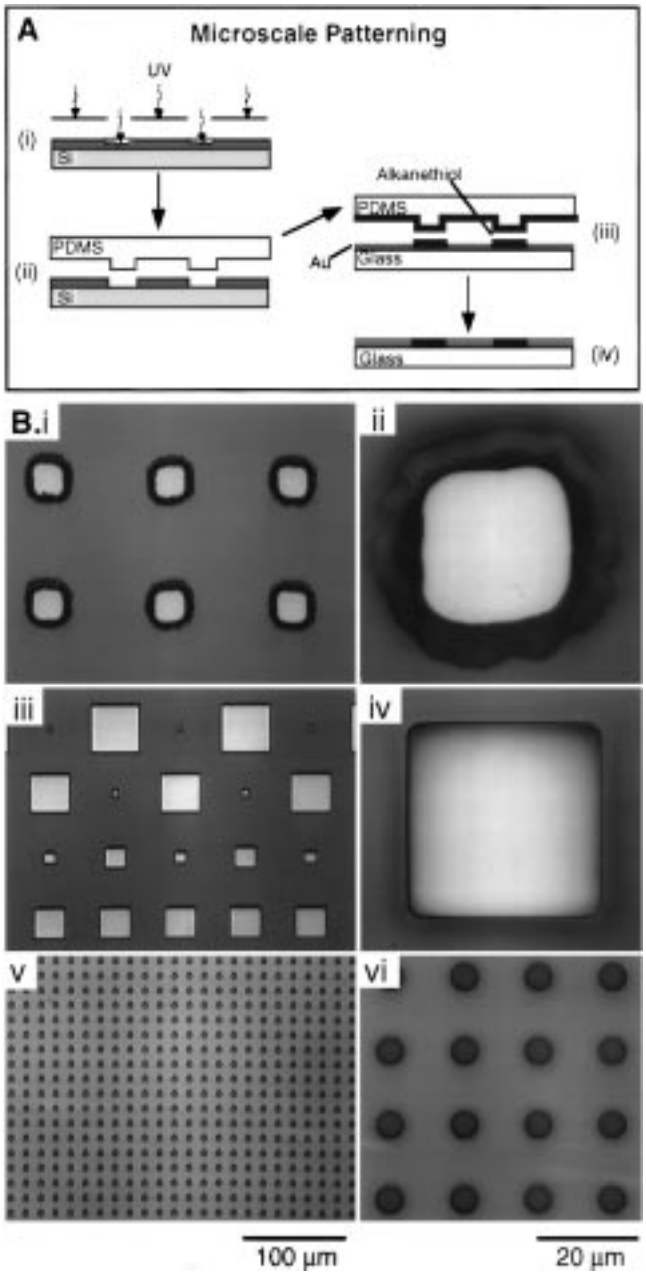

Figure 1. Schematic diagram (A) and examples of stamps (B) generated during the microcontact printing process. (A) Silicon wafer spin-coated with photoresist is exposed to UV light through a mask containing the desired pattern, such that the exposed regions are dissolved away in developing solution (i). A poly(dimethylsiloxane) (PDMS) stamp is fabricated by casting the prepolymer against the relief pattern (ii) to give a stamp having a complementary relief pattern. The stamp is "inked" with an alkanethiol and brought into conformal contact with a surface of gold (iii). A SAM of alkanethiolates is formed only at those regions where the stamp contacts the surface; the bare regions of gold remaining after the printing process can be modified with a different SAM by immersing the substrate in a solution of a second alkanethiol (iv). The stamped alkanethiol promotes protein adsorption and cell adhesion (e.g., hexadecanethiol) and the immersion thiol resists protein adsorption (e.g., oligo(ethylene glycol) undecanethiol). (B) PDMS stamps shown under light microscopy. The resolution of PDMS stamps depends on whether the masks were generated using high-resolution laser printing (i and ii), step reduction methods (iii and iv), or electron beam etching (v and vi). Micrographs show low (i, iii, and v) and high (ii, iv, and vi) magnification of features. (i and ii) 30-μm squares; (iii) 3−50 μm squares; (iv) 40-μm square; (v and vi) 5-μm circles.

5-bromodeoxyuridine (BrdU), which was added to the medium (Amersham kit). Cells were synchronized at the beginning of the cycle (G_o) by either serum deprivation (bovine cells, 0.5% calf serum) or exposure to lovastatin (human cells, 40 μM) for 36 h. Cells were then released from G_o during replating of cells onto patterned substrates by addition of growth factors (bovine cells) or mevalonate (human cells). BrdU was added either at the time of plating or during the S-phase of the cell cycle to allow incorporation. Cells to be stained for BrdU were fixed in 90% ethanol/5% H_2O/5% acetic acid for 30 min, washed in PBS, and incubated with primary mouse anti-BrdU antibody containing nuclease (provided at incubation concentration, Amersham) for 1 h at room temperature. Primary antibody was then detected using goat-anti-mouse antibody conjugated to fluorescein (Amersham) for 1 h at room temperature. After cells were cultured on different patterns for 24 h, the percentage of cells that incorporated BrdU was counted by fluorescence microscopy.

Determination of Cell Areas. Image processing software (BDS Image, Oncor) was used to calculate projected cell and nuclear areas from images grabbed from the microscope through a CCD camera. Projected cell area was determined from interactive tracing of cell edges of phase images.

Results

Controlling Cell Position and Spreading with Microfabricated Substrates. Using photolithography and microcontact printing to produce substrates containing islands coated with FN surrounded by nonadhesive regions (Figure 1), we explored how cell position and shape could be modulated by substrates containing different patterns of islands. Phase contrast microscopy studies demonstrated that bovine or human capillary cells attach specifically and precisely to the patterns generated, regardless of whether the adhesive regions were large areas allowing multiple cells to attach or small islands that contained only single cells (Figure 2). Cells attached to surfaces over minutes and spread over the next several hours, as is typical of these cells on standard tissue culture surfaces. When single cells attached to single islands of FN, it was found that cell spreading and shape could be precisely controlled by the size and shape of the islands, as long as the area of the island was equal to or less than the area of maximum spreading of the cell (approximately 3000 and 4000 μm^2 for bovine and human cells, respectively). Consistent with previous findings (Chen et al., 1997), even square edges (Figure 2A, right) could be generated in cells. Interestingly, even when cells were kept round by attaching to small circular islands (Figure 2), they continued to form active ruffling extensions at the FN−nonadhesive boundary, which would at first protrude several micrometers into the nonadhesive regions but rapidly retract within minutes of protrusion, indicating their attempt and inability to spread deep into these nonadhesive region.

However, when the nonadhesive spacing between islands was reduced, eventually cells were able to spread across multiple islands (Figure 2C). The thresholds at which this bridging occurred were at 10 and 20 μm spacings for bovine and human cells, respectively. This bridging ability appeared to be independent of the size of the adhesive island. The ability of human cells to bridge more easily may stem from their larger size (human to bovine cell volume ratio = 1.61 ± 0.31). This bridging phenomenon defines the upper limit of the density of islands per unit area that can be placed on a

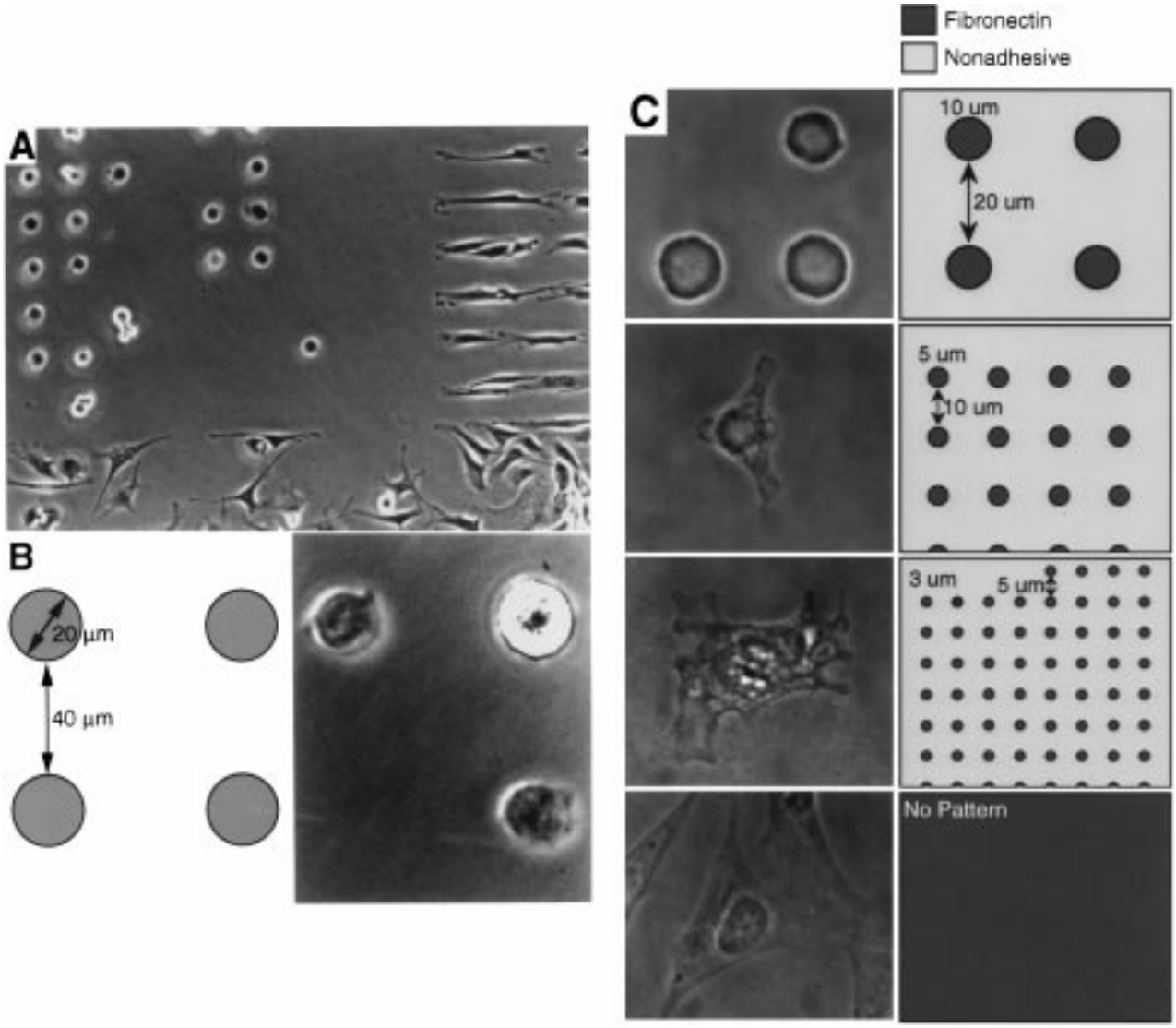

Figure 2. (A) Phase-contrast micrograph of bovine capillary endothelial cells cultured on a substrate containing fibronectin-coated circles 20 μm in diameter (top left region), lines 20 μm in width (top right region), and a large unpatterned region (bottom). (B) High magnification micrograph of cells on these circular islands. (C) Phase-contrast micrographs of bovine capillary endothelial cells cultured on a substrate containing fibronectin-coated circles separated by progressively shorter distances, demonstrating bridging of cells between multiple adhesive islands.

substrate where one cell attaches only to one island. All patterns used to control cell shape in this study therefore contained spacing distances larger than 20 μm. Cells were cultured with no deleterious effects on patterns that supported spreading for up to 10 days. All experiments conducted in this study were limited to 24 h.

Regulation of Apoptosis by Size of Attached Bead. Bovine adrenal or human pulmonary capillary endothelial cells placed in suspension rapidly underwent apoptosis (Figure 3A,B). Approximately half of the cells showed the characteristic hallmarks of DNA and nuclear damage within 24 h after releasing cells from their FN substrate. Attaching suspended cells to single, 10 μm diameter beads coated with fibronectin did not prevent the apoptosis from occurring. However, attaching to progressively larger beads (e.g., 25 μm diameter) led to increasing ability of the attached bead to prevent apoptosis in both cell lines. This increase in survival appeared to correlate with the ability of cells to spread and flatten against the surface of the bead. These results were confirmed by the presence of DNA degradation in suspended cells but not in cells attached to 25 μm diameter beads (Figure 3E). The presence or amount of serum in the media could not replace cell spreading as the survival signal (Figure 3C,D). On the contrary, increased serum appears to both increase the survival of spread cells and the apoptosis of round cells, consistent with previous descriptions of both life and suicide as active, energy-dependent processes (Williams, 1991). In addition, using two completely different approaches to modulate cell shape, attachment of beads to suspended cells (Figure 3C) and spreading cells on different densities of FN adsorbed to a flat substrate (Figure 3D), to arrive at the same finding that cell rounding induces cell suicide and cell spreading permits life provides further evidence that cell shape itself may act as a signal for cell function. However, several confounding factors inherent in each of the two approaches make these results difficult to interpret. Since small as compared to large beads appeared to be internalized by cells more quickly, it is unclear whether the modulation of apoptosis by bead size is a direct result of the length of time that an external adhesion actively signals into the cell or of the changes in cell shape. Similarly, low densities of FN may inherently cross-link and signal integrin receptors less efficiently than high densities, also providing a confounding

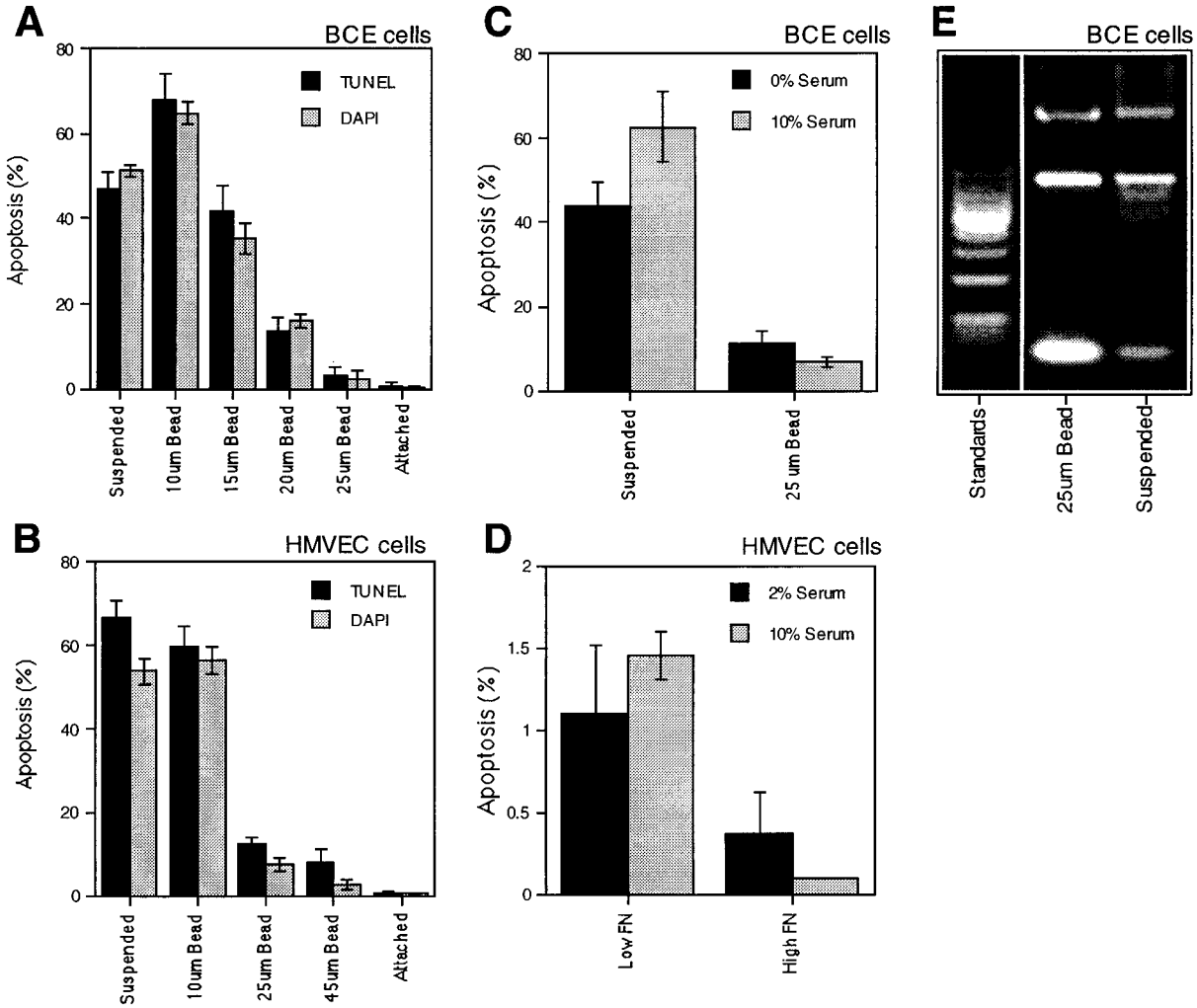

Figure 3. Effects of cell attachment to FN-coated beads of varying size on apoptosis. Apoptosis of bovine (A, C, and E) or human (B and D) endothelial cells attached for 24 h to FN-coated beads with indicated diameters. Apoptosis was measured as the percentage of cells positively identified for DNA fragmentation by either TUNEL (Boehringer-Mannheim) or DAPI staining (A−D) and confirmed with the presence of DNA laddering (E). Only single cells attached to single beads were scored. Graphed data represent between four and eight samples per condition repeated on two more separate occasions, where approximately 500 cells were counted per sample. *T*-tests were used to compare results from different conditions. Error bars indicate standard error of the mean. (Panel B is reprinted with permission from Chen et al. (1997). Copyright 1997 American Association for the Advancement of Science.

explanation for the increase in apoptosis rates. We therefore adopted the micropatterning approach to modulate cell shape in the absence of these confounding issues, by plating cells on different sizes of islands coated with a constant, high density of ECM.

Regulation of a Switch between Growth and Apoptosis by Cell Spreading. Bovine and human endothelial cells were attached to islands of different sizes (from 10 × 10 to 60 × 60 μm in length) to control cell spreading. Within 24 h after plating cells in the presence of saturating concentrations of growth factors, a switch from apoptosis to growth was observed as cells were spread on progressively larger islands (Figure 4). When cells were round (on 10 and 20 μm diameter circles), apoptosis rates were high while growth rates remained insignificant. Conversely, when cells were allowed to spread and flatten to their maximal amount, apoptosis was virtually undetectable while growth rates were high.

Discussion

Historically, the investigations of cellular responses to various adhesive environments were limited by a lack of control over substrate design. It was particularly difficult to generate substrates patterned with adjacent adhesive and nonadhesive regions. The results presented in this study demonstrate that microcontact printing of SAMs can be effectively used to generate patterned substrates to control cell distribution. In addition, the successful patterning of two different cell types in this study, as well as with hepatocytes (Singhvi et al., 1994), suggests that this technique can be generalized for any adhesion-dependent cell line, simply by changing the specific ECM molecule with which to coat substrates. This technology addresses many of the technical prerequisites for the development of interfaces between cells and microtechnology. Placing cells into arrays on a substrate allows automated systems to gain repeated access to specific single cells for manipulation and analysis, such as for the screening of large libraries of potential pharmacologic agents. Cell positioning is also critical for building cell-based biosensors where cells need to adhere to specific regions for monitoring and interfacing with on-site circuitry.

In examining the limits of cell position control, our results demonstrate that positioning of single cells onto distinct adhesive islands requires that the islands be separated by 20−40 μm, depending on cell type, indicat-

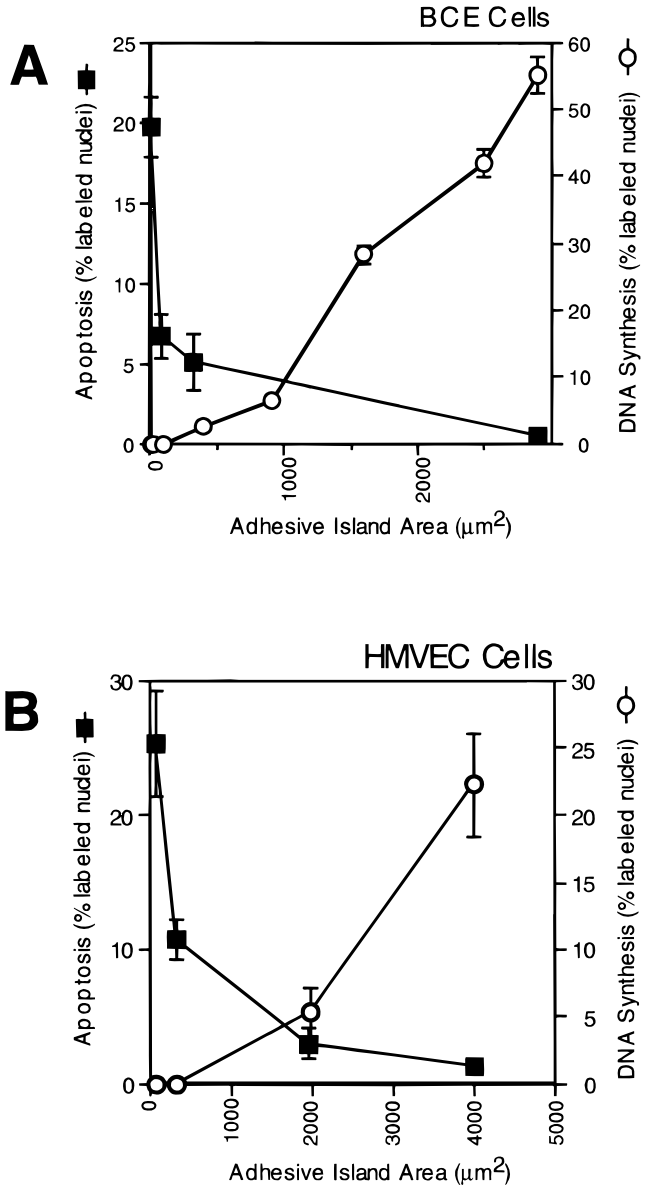

Figure 4. Effect of spreading on cell growth and apoptosis in bovine (A) and human (B) endothelial cells. Percentage of cells undergoing apoptosis or DNA synthesis were plotted as a function of projected cell area. Apoptosis was measured 24 h after plating. DNA synthesis detected by the incorporation of BrdU either over the first 24 h after plating (A) or between 20 and 24 h after plating, during S-phase (B). Panel A is reprinted with permission from Chen et al. (1997). Copyright 1997 American Association for the Advancement of Science.

ing that the inherent spreading properties of the cell limits the density of adhesive islands on a substrate. Such a finding is important not only to recognize in the future design of biosensor arrays but also in defining the required limits of resolution for patterning technology. For example, photolithography has been used to routinely produce patterns of defined surface chemistries with resolutions better than 1 μm. This technique has been used to directly photoablate proteins preadsorbed to a silicon or glass surface (Hammarback et al., 1985), expose protein-adsorbing regions of the substrate previously masked with photoresist (Bhatia et al., 1994), or covalently link preadsorbed protein onto a photosensitive group (Matsuda, 1995). However, such high resolutions appear to be unnecessary for these cell-positioning applications. In addition, significant costs are incurred to incorporate a clean room facility, which is generally recommended for photolithography, though not necessary (Hammarback et al., 1985; Lom et al., 1993). In comparison, while microcontact printing in special cases can match resolutions reached by conventional lithography, it generally produces patterns with resolutions of 1−2 μm, which is more than sufficient for cell-positioning applications (Xia et al., 1995). The even lower resolution requirements for more crude patterning applications can substantially reduce the cost for UV mask production by using the demonstrated high-resolution laser printing methods. Importantly, because microcontact printing relies on self-assembly in solution, it does not require a dust-controlled laboratory environment, allowing for the production of the stamps and substrates at relatively low costs.

SAMs of alkanethiolates on gold also provide an additional technical advantage over other patterning techniques: A major problem with many techniques is that the "nonadhesive" regions of the pattern are usually surfaces that actually promote protein adsorption and require passivation (blocking of adhesive sites) with a nonadhesive protein such as albumin. Over a period of days, however, cells are able to migrate onto these passivated regions, perhaps as a result of degradation of the albumin and deposition of ECM by cells. Several investigators have dealt with this issue by using photolithography to pattern siloxanes presenting perfluoro- and amino-terminated moieties, demonstrating preferential adhesion of cells to the amino-terminated siloxane without passivation of the perfluoro-terminated regions by albumin (Healy et al., 1994; Stenger et al., 1992; Britland et al., 1992; O'Neill et al., 1990; Kleinfeld et al., 1988). We have employed an alternative method taking advantage of the patterning of alkanethiols on gold, which has been well established to produce uniform surfaces with defined chemistry. Our results demonstrate that using ethylene glycol containing SAMs to render regions nonadhesive circumvents the need for a passivating agent like albumin, thus preventing cells cultured on patterned substrates from invading ethylene glycol regions. The durations of cell culture on these substrates match that of standard tissue culture plastic, demonstrating that the materials do not release toxic substances.

In addition to positioning cells with the patterning technology, we also demonstrate that the size and geometry of the adhesive islands control the shape of attached cells. Previous studies have suggested that cell shape per se may act to regulate cell functions (Folkman and Moscona, 1978; Ingber, 1990; Singhvi et al., 1994), presenting an opportunity to potentially program cells to hold specified functional states by directly controlling the cell shape through the appropriate patterned substrates. We therefore applied this technology to better define the historically controversial role of cell shape in cell function.

The finding that apoptosis increased with cell rounding, either by attaching to progressively smaller microbeads or to progressively lower densities of ECM coated on a substrate, suggested but could not confirm that cell shape acted directly to regulate cell survival. The microcontact printing technology provided the necessary tool to clearly demonstrate that cell spreading regulated a switch between growth and apoptosis programs within 24 h after plating cells onto patterns, in the presence of saturating concentrations of growth factors (Chen et al., 1997). In addition, the specific endothelial cell type and the presence or absence of serum both appear to modulate this shape-dependent switch but not to change the

general phenomenon. Cell shape has also been implicated to regulate differentiation in several cell types (Mooney et al., 1992; Singhvi et al., 1994; Watt et al., 1988; Ingber and Folkman, 1989), suggesting that geometry may act as a generalized fundamental regulator of cell function.

From the perspective of fundamental cell biology, these results suggest that the local differentials in growth and viability that are critical to the formation of complex tissue patterns may be generated by local changes in cell−ECM interactions. The existence of a geometric or mechanical control mechanism for switching between several cell fates points to a novel link between growth and apoptosis regulation. By sensing their degree of extension or compression, cells may be able to monitor local changes in cell crowding or ECM compliance and, thereby, couple changes in ECM extension to cell mass expansion within the local tissue microenvironment. Such a mechanism supports the in vivo observation that, during pharmacologic dissolution of the basement membrane of capillary beds, the resulting massive apoptosis and subsequent involution of the surrounding tissues is closely associated with capillary cell retraction and rounding (Ingber et al., 1986). Deregulation of such a switching mechanism may be a critical step in progression toward the uncontrolled growth of cells we know as cancer, when cells gain the ability to survive and grow independently of attachment to ECM (MacPherson and Montagnier, 1964; Stoker et al., 1968) and cell spreading (Wittelsberger et al., 1981; Tucker et al., 1981; Folkman and Greenspan, 1975). Understanding the mechanism by which changes in cell shape and cytoskeletal structure modulate apoptotic signaling by integrins also may open the window to rational design of new angiogenesis inhibitors and, hence, new forms of anticancer therapy.

In the context of cell culture technologies such as bioreactors and cellular engineering applications, the existence of cell shape regulation in cell function indicates that the adhesive microenvironment around cells can be carefully optimized in addition to soluble factors. Thus, the micropatterning technology will be critical not only to fundamental progress in understanding how cell adhesion and shape regulate different cellular responses but also in providing a means to control of these responses in vitro. And eventually, scaling these systems up to multicellular "tissues" in vitro will require the engineering of specific cell−cell and cell−ECM interactions easily accomplished by micropatterning technology.

Conclusion

The development of cell-based biosensors and bioreactors has demanded precision control of cellular position and function on surfaces. Microcontact printing of SAMs of alkanethiolates on gold provides a flexible and inexpensive technology to address this need. The technology can be used to pattern many cell types for long-term culture. In addition, the size and geometry of adhesive islands can dictate cell shape, which we demonstrate provides not only an essential tool to investigate the fundamental roles of cell geometry in growth and apoptosis but also a means by which to control cell function. Thus, at all stages of tissue engineering development, from basic research of single cells to the contruction of multicellular structures requiring complex cell−cell relationships, the ability to pattern cells will be critical.

Acknowledgment

This work was supported by grants from NIH (HL57669, CA55833, GM30367), the Defense Advanced Research Projects Agency, and the Office of Naval Research; postdoctoral fellowships from the American Cancer Society (M.M.) and the Swiss National Science Foundation (S.H.); and partial salary support (C.S.C.) from the Harvard-MIT Health Sciences Technology Program.

References and Notes

Bhatia, S. N.; Toner, M.; Tompkins, R. G.; Yarmush, M. L. Selective adhesion of hepatocytes on patterned surfaces. *Ann. N. Y. Acad. Sci.* **1994**, *745*, 187−209.

Britland, S.; Clark, P.; Connolly, P.; Moores, G. Micropatterned substratum adhesiveness: a model for morphogenetic cues controlling cell behavior. *Exp. Cell Res.* **1992**, *198*, 124−129.

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.

Clarke, E. A.; Brugge, J. S. Integrins and signal transduction pathways: the road taken. *Science* **1995**, *268*, 233−237.

Craig, S. W.; Johnson, R. P. Assembly of focal adhesions: progress, paradigms, and portents. *Curr. Opin. Cell Biol.* **1996**, *8*, 74−85.

Folkman, J.; Greenspan, H. P. Influence of geometry on control of cell growth. *Biochim. Biophys. Acta* **1975**, *417*, 217.

Folkman, J.; Moscona, A. Role of cell shape in growth control. *Nature* **1978**, *273*, 345−349.

Hammarback, J. A.; Palm, S. L.; Furcht, L. T.; Letourneau, P. C. Guidance of neurite outgrowth by pathways of substratum-adsorbed laminin. *J. Neurosci. Res.* **1985**, *13*, 213−220.

Healy, K. E.; Lom, B.; Hockberger, P. E. Spatial distribution of mammalian cells dictated by material surface chemistry. *Biotechnol. Bioeng.* **1994**, *43*, 792−800.

Holmgren, L.; O'Reilly, M. S.; Folkman, J. Dormancy of micrometastases: balanced proliferation and apoptosis in the presence of angiogenesis suppression. *Nat. Med.* **1995**, *1*, 149−153.

Hynes, R. O. Integrins: versatility, modulation, and signaling in cell adhesion. *Cell* **1992**, *69*, 11−25.

Ingber, D. E. Fibronectin controls capillary endothelial cell growth by modulating cell shape. *Proc. Nat. Acad. Sci. U.S.A.* **1990**, *87*, 3579−3583.

Ingber, D. E. Tensegrity: the architectural basis of cellular mechanotransduction. *Annu. Rev. Phys.* **1997**, *59*, 575−599.

Ingber, D. E.; Folkman, J. Mechanochemical switching between growth and differentiation during fibroblast growth factor-stimulated angiogenesis in vitro: role of extracellular matrix. *J. Cell Biol.* **1989**, *109*, 317−330.

Ingber, D. E.; Madri, J. A.; Folkman, J. A possible mechanism for inhibition of angiogenesis by angiostatic steroids: Induction of capillary basement membrane dissolution. *Endocrinology* **1986**, *119*, 1768−1775.

Ingber, D.; Fujita, T.; Kishimoto, S.; Sudo, K.; Kanamaru, T.; Brem, H.; Folkman, J. Synthetic analogues of fumagillin that inhibit angiogenesis and suppress tumour growth. *Nature* **1990**, *348*, 555−557.

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

Lom, B.; Healy, K. E.; Hockberger, P. E. A versatile technique for patterning biomolecules onto glass coverslips. *J. Neurosci. Methods* **1993**, *50*, 385−397.

MacPherson, I.; Montagnier, L. Agar suspension culture for the selective assay of cells transformed by polyoma virus. *Virology* **1964**, *23*, 291.

Matsuda, T.; Sugawara, T. Development of surface photochemical modification method for micropatterning of cultured cells. *J. Biomed. Mater. Res.* **1995**, *29*, 749−756.

Mooney, D.; Hansen, L.; Vacanti, J.; Langer, R.; Farmer, S.; Ingber, D. Switching from differentiation to growth in hepatocytes: control by extracellular matrix. *J. Cell. Phys.* **1992**, *151*, 497−505.

Mrksich, M.; Whitesides, G. M. Using self-assembled monolayers to understand the interactions of man-made surfaces with proteins and cells. *Annu. Rev. Biophys. Biomol. Struct.* **1996**, *25*, 55−78.

Mrksich, M.; Dike, L. E.; Tien, J.; Ingber, D. E.; Whitesides, G. M. Using microcontact printing to pattern the attachment of mammalian cells to self-assembled monolayers of alkanethiolates on transparent films of gold and silver. *Exp. Cell Res.* **1997**, *235*, 305−313.

O'Neill, C.; Jordan, P.; Riddle, P. Evidence for two distinct mechanisms of anchorage stimulation in freshly explanted and 3T3 swiss mouse fibroblasts. *J. Cell Sci.* **1990**, *95*, 577−586.

O'Reilly, M. S.; Boehm, T.; Shing, Y.; Fukai, N.; Vasios, G.; Lane, W. S.; Flynn, E.; Birkhead, J. R.; Olsen, B. R.; Folkman, J. Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. *Cell* **1997**, *88*, 277−285.

Prime, K. L.; Whitesides, G. M. Self-assembled organic monolayers: model systems for studying adsorption of proteins at surfaces. *Science* **1991**, *252*, 1164−1167.

Prime, K. L.; Whitesides, G. M. Adsorption of proteins onto surfaces containing end-attached oligo(ethylene oxide); a model system using self-assembled monolayers. *J. Am. Chem. Soc.* **1993**, *115*, 10714−10721.

Re, F.; Zanetti, A.; Sironi, M.; Polentarutti, N.; Lanfrancone, L.; Dejana, E.; Colotta, F. Inhibition of anchorage-dependent cell spreading triggers apoptosis in cultured human endothelial cells. *J. Cell Biol.* **1994**, *127*, 537−546.

Singhvi, R.; Kumar, A.; Lopez, G.; Stephanopoulos, G. N.; Wang, D. I. C.; Whitesides, G. M. Engineering cell shape and function. *Science* **1994**, *264*, 696−698.

Stenger, D. A.; Georger, J. H.; Dulcey, C. S.; Hickman, J. J.; Rudolph, A. S.; et al. Coplanar molecular assemblies of amino- and perfluorinated alkylsilanes: Characterization and geometric definition of mammalian cell adhesion and growth. *J. Am. Chem. Soc.* **1992**, *114*, 8435−8442.

Stoker, M.; O'Neill, C.; Berryman, S.; Waxman, V. Anchorage and growth regulation in normal and virus-transformed cells. *Int. J. Cancer* **1968**, *3*, 683.

Tucker, R. W.; Butterfield, C. E.; Folkman, J. Interaction of serum and cell spreading affects the growth of neoplastic and nonneoplastic fibroblasts. *J. Supramol. Struct. Cell. Biochem.* **1981**, *15*, 29.

Watt, F. M.; Jordan, P. W.; O'Neill, C. H. Cell shape controls terminal differentiation of human epidermal keratinocytes. *Proc. Nat. Acad. Sci. U.S.A.* **1988**, *85*, 5576−5580.

Whitesides, G. M.; Gorman, C. B. Self-assembled monolayers: Models for organic surface chemistry. In *Handbook of Surface Imaging and Visualization*; Hubbard, A. T., Ed.; CRC Press: Boca Raton, FL, 1995; pp 713−732.

Williams, G. T. Programmed cell death: Apoptosis and oncogenesis. *Cell* **1991,** *65*, 1097−1098.

Wittelsberger, S. C.; Kleene, K.; Penman, S. Progressive loss of shape-responsive metabolic controls in cells with increasingly transfomed phenotype. *Cell* **1981**, *24*, 859.

Xia, Y.; Mrksich, M.; Kim E.; Whitesides, G. M. Microcontact printing of siloxane monolayers on the surface of silicon dioxide, and its appliction in microfabrication. *J. Am. Chem. Soc.* **1995**, *117*, 9576−9577.

Accepted April 7, 1998.

BP980031M