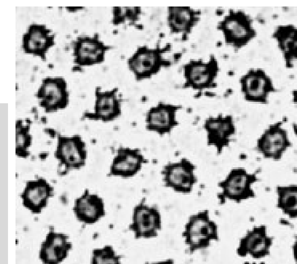
Micropatterned Environments in Cell Biology**

By *Srivatsan Raghavan* and *Christopher S. Chen**



Microfabrication and materials-science-based approaches provide a powerful new set of tools to control the spatial organization and temporal presentation of cellular cues. In this progress report, we highlight recent advances in the technological development and application of these tools to study how cells sense and respond to their microenvironment.

1. Introduction

Spatiotemporal signals are an important regulator of cellular behavior. During development, these signals govern the orchestration of cell proliferation, migration, and differentiation events that eventually give rise to the highly distinctive structures and functions of various tissues and organ systems. For example, progenitor neurons must seek out appropriate muscle targets, form contacts with muscle cells, and reinforce these connections as they differentiate into motor neurons. This targeted search is directed via adhesion cues that act as highways, and gradients of attractive and repellant guidance factors, both surface-bound and soluble.[1] Neurons that do not follow these cues fail to target the muscle. Lacking any positive reinforcing signals from muscle cells, these neurons eventually die. Such a complex sequence of events depends upon the appropriate spatial and temporal coordination of the numerous interactions between cells and their environment: namely, cells must interact with and respond to neighboring cells, the surrounding extracellular matrix (ECM), soluble factors, and other local physical forces. Although much progress has been made in understanding whether the presence or absence of many of these cues (such as adhesion or growth factors) affects cell function, very little progress has been made in explicitly understanding how the spatial (geometric) or temporal presentation of these cues regulates cells.

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There are many cues that have a spatial or geometric component. For adhesive cues, cells attach to surfaces using specialized receptors. This binding to the surface causes cells to tailor their behavior accordingly. Interestingly, adhesion is not merely an on–off signal; the *area* of adhesion is important. Folkman and Moscona showed that cells that attach, spread, and flatten over a larger area of a substrate proliferate more than poorly spread cells.[2] Within the context of cell migration, cells packed in a monolayer can sense unoccupied edges and migrate to fill in any remaining spaces or defects. Early studies of neurons demonstrated that parallel adhesive lines could direct axons to grow along these lines.[3] Although these early studies demonstrated the importance of local spatial cues, progress was limited because of an inability to clearly identify and decouple these geometric parameters from other signals. These experimental limitations pointed to a need for improved methods of specifying the spatial arrangement of cells and their ligands.

Practically speaking, cellular responses to these cues have been difficult to study because of our inability to present defined spatial and temporal stimuli. In order to address these questions, new tools based upon microfabrication technology and materials science are being developed that allow greater control over the spatial organization and temporal presentation of the cellular microenvironment. This progress report will describe new developments in the application of soft and hard lithography-based microfabrication tools to study spatiotemporal cellular cues. More specifically, we will highlight recent technological developments that have enabled insights into how cells sense and respond to the local matrix and neighboring cells. We will first briefly describe the development of tools to spatially control cell adhesions. Because this set of technologies is now quite mature and reviewed in detail elsewhere,[4,5] the review will then focus on newer, less well-developed applications in this arena. Recent advances have

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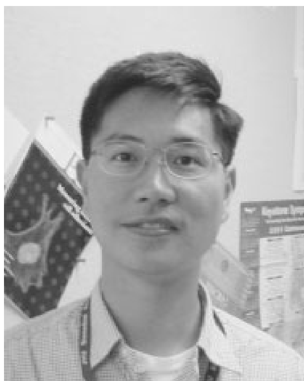
demonstrated that we are only beginning to realize the importance of these cues, and the application of microfabrication approaches to study cell biology has opened up many new and interesting avenues for research.

2. Patterning Approaches to Study Cell–ECM Interactions

Several tools have been developed in the last decade to study the role of spatial signaling in cell biology. Two basic technologies were needed to spatially organize cells—control over surface chemistry to render specific regions adhesive or non-adhesive, and the ability to pattern these chemistries onto a surface. Alkanethiolates provided a key tool that advanced both needs.[6–11] These compounds self-assemble into highly ordered, semi-crystalline monolayers when exposed to a coinage-metal surface. By controlling the terminal group of the alkane chain, properties such as the surface hydrophobicity can be tuned to specific needs.[12–15] For example, forming self-assembled monolayers (SAMs) from hexadecanethiol results in a highly hydrophobic surface; proteins such as fibronectin, an ECM molecule, adsorb onto such hydrophobic surfaces from aqueous solution, thereby enabling the subsequent adhesion of cells. In comparison to traditional coating methods, SAMs are advantageous because they can be patterned on a surface. Specific regions of the surface can be made adhesive while others are made non-adhesive. Combining this with microfabrication techniques developed in the semiconductor industry enabled the generation of micrometer-scale patterns, similar to the size scale of cells.

This geometric control over surface chemistry allowed investigators to study cell adhesion as a spatial cue. Poly(dimethyl siloxane) (PDMS) stamps containing the desired features were used to transfer SAMs to a surface in specified patterns, and remaining regions were rendered inert with an oligo(ethylene glycol)-terminated SAM. Using patterned surfaces containing adhesive "islands" of different shapes and sizes, several investigators demonstrated that the degree of cell spreading against a substrate is an important regulator of cell function.[16–19] By seeding cells onto islands of different sizes such that one cell attached onto each island, cells spread to the size and shape of the printed patterns (Fig. 1A). Well-spread cells on the larger islands proliferated[17] (Fig. 1B) while limiting adhesion area induced differentiated function.[16] Physiologically, these findings suggest that when cells are packed onto a surface with little room, they perform specialized tissue-specific functions, but when given room to spread, they reduce functions in order to proliferate. These data correlate with in-vivo observations that, following liver damage, liver cells at the wound edge proliferate until they have regenerated and filled in the extra space.[20,21] These and more recent studies clearly established that geometric considerations are important, and that cell shape directly regulates many cell functions.[22–25] In all, the demonstrated importance of using patterning to control cell function, together with the realization by tissue engineers that the spatial organization of cells is critical to tissue function, has provided a strong im-



Christopher S. Chen, M.D., Ph.D., is an Assistant Professor in the Department of Biomedical Engineering and in the Oncology Center at Johns Hopkins University. His laboratory uses semiconductor manufacturing tools to control interactions between mammalian cells and their microenvironment in order to identify the underlying mechanisms by which cells coordinate with each other to build tissues. In recognition of his work, Dr. Chen has been awarded the Presidential Early Career Award for Scientists and Engineers, and the Office of Naval Research Young Investigator Award. He serves on the Board of Trustees for the Society for BioMEMS and Biomedical Nanotechnology. He received his A.B. in Biochemistry from Harvard, M.S. in Mechanical Engineering from M.I.T., and Ph.D. in Medical Engineering and Medical Physics from the Harvard–M.I.T. Health Sciences and Technology Program. He earned his M.D. from the Harvard Medical School.

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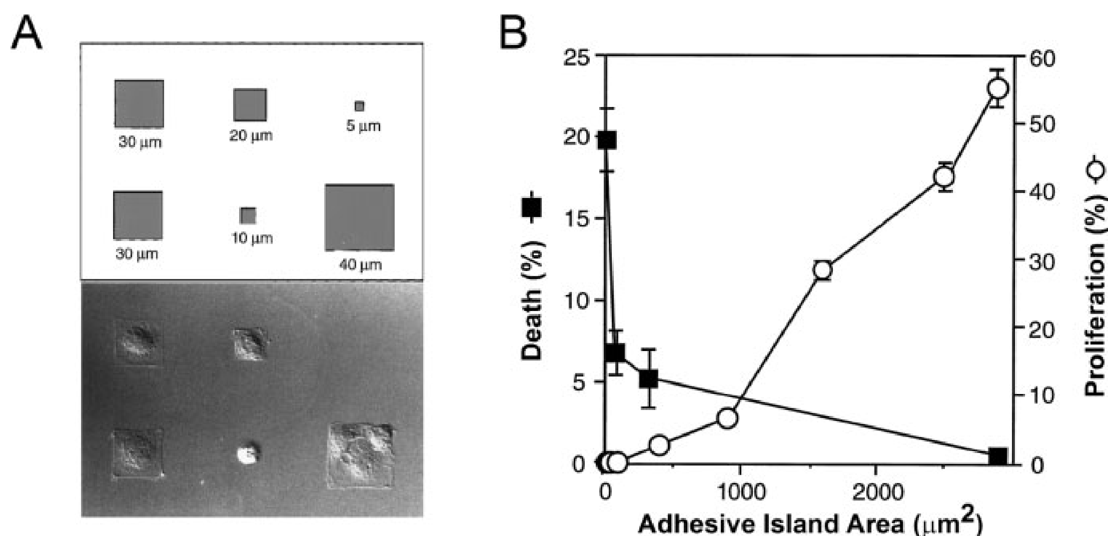

Figure 1. Microcontact printing to control cell spreading and proliferation. A) Schematic of initial pattern and differential interference contrast image of capillary endothelial cells cultured on square adhesive islands of different sizes. B) Plot of cell proliferation and programmed cell death as a function of island area. Adapted with permission from [17]. Copyright 1997, American Association for the Advancement of Science.

petus for the development of additional tools to aid in furthering our understanding of how cells interact with and respond to their microenvironment.

In recent years, scientists have developed many other techniques to facilitate cell patterning. In general, these new techniques to pattern proteins and cells are numerous, quite mature, and have been reviewed extensively elsewhere.[4,5,26] Therefore they will only be briefly reviewed here for historical continuity. Although SAMs provided an elegant and customizable method of controlling the spatial presentation of adhesion cues, they have not been easily accessible to most biologists due to the need for gold-coated surfaces and custom-synthesized reagents. Protein printing is a recent development that enables biologists to directly stamp ECM ligands of interest in patterns onto PDMS, glass, or polystyrene surfaces (Fig. 2A).[27,28] The remaining regions are rendered inert to protein adsorption using commercially available poly(ethylene glycol)-based surfactants and polymers.[29] Alternatively, physical barriers are used to restrict cells spatially. Agarose, a hydrogel that resists protein adhesion, can be wicked under a PDMS stamp and cured; subsequent removal of the stamp generates wells with agarose walls (Fig. 2B).[30] Gray et al. have used dielectrophoretic forces generated by electrodes to pattern arrays of cells.[31] This method actively traps cells in registration with a patterned ECM, and permits increased cell density, improved pattern fidelity, and more complex arrangements of cells (Fig. 2C). In addition, multiple cell types can be patterned onto the same surface using this technique. Tools utilizing magnetic and optical forces to actively trap cells in patterned arrays are also being developed,[32–36] and will provide important additions to the currently available technologies.

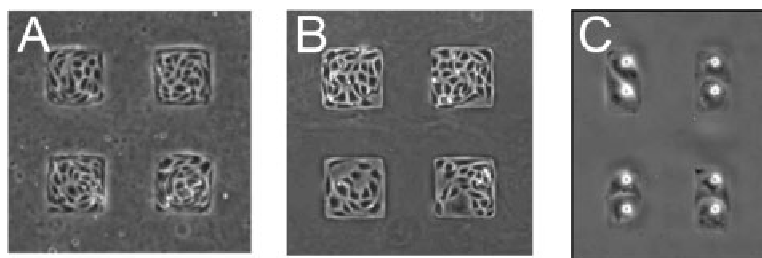

Figure 2. Patterning capillary endothelial cells by different methods. A) Substrate prepared by printing fibronectin and blocking with poly(ethylene glycol)-based polymer. B) Substrate prepared by creating agarose wells and coating with fibronectin. Parts (A,B) reprinted in part with permission from [62]. Copyright 2003, American Chemical Society. C) Cells trapped using dielectrophoresis. Bright spots are gold electrodes. Reprinted with permission from [31]. Copyright 2004, Elsevier.

3. Patterning Approaches to Study Cell–Cell Interactions

In addition to geometric control over cell shape, micropatterning methods have been used to investigate cell–cell interactions. Bhatia et al. used these techniques to produce spatially defined co-cultures of hepatocytes and fibroblasts.[37–39] These microfabrication approaches enabled a quantitative investigation into the extent of heterotypic interactions needed to induce liver-specific functions. Hepatocytes and fibroblasts were co-cultured using a lithographic masking technique to spatially localize collagen on the surface; hepatocytes preferen-

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tially adhered to the collagen, while fibroblasts attached to the remaining areas (Fig. 3A). As net hepatocyte–fibroblast contact area was increased by increasing the number of hepatocyte regions while decreasing their size, the hepatocytes exhibited enhanced liver-specific function, as monitored by albumin secretion and urea synthesis (Fig. 3B).[39] Interestingly, images of the hepatocytes in these configurations revealed that these functional improvements were limited to the interface between hepatocyte and fibroblast regions, and only penetrated a limited distance into the larger hepatocellular aggregates (Fig. 3C); this key insight directed future studies to focus on the hepatocyte–fibroblast interactions in driving liver function, and has had important ramifications for engineering liver-tissue constructs. In a counterpart study, the hepatocyte aggregate adhesion area was preserved while the fibroblast/hepatocyte cell ratio was varied.[38] Under these conditions, increased numbers of fibroblasts improved hepatocyte function. These studies suggest that both the area of heterotypic contact and the net ratio of mesenchymal (fibroblast) to parenchymal (hepatocyte) cells are important regulators of hepatocyte function. These studies demonstrated the utility of applying microfabrication approaches to gain quantitative insight into important variables regulating heterotypic interactions, and point the way towards improving technologies to address these types of questions.

In the cell–cell interaction field, it has been difficult to decouple geometric effects from effects induced by cell–cell contacts. Historically, investigators have thought that cell–cell contact inhibits proliferation ("contact inhibition") during monolayer culture.[40] However, these effects are confounded by simultaneous changes in cell spreading as cells become crowded for space. In order to decouple spreading effects from cell–cell contact mediated effects, Nelson and Chen studied cell–cell interactions using microfabrication approaches to better regulate the degree of cell spreading.[30,41] Cells were seeded at different densities onto microcontact-printed SAM islands of specified areas coated with fibronectin (Fig. 4A). In this fashion, the number of cells per island could be varied, thereby altering the number of cell–cell contacts independently of the extent of cell spreading. At each degree of cell spreading, the presence of additional cell–cell interactions caused an increase in proliferation (Fig. 4A). Although net island size and number of cells per island could be controlled using this SAM-based approach, there was significant variability in the degree of cell spreading and cell–cell contacts within each island. In other words, the extent of spreading and number of contacts for *each* cell were not directly specified. In order to surmount this problem and further decouple these geometric signals, a bow-tie-shaped agarose barrier was developed to isolate pairs of cells while controlling cell spreading and specifying the location of the contact (Fig. 4B). These studies demonstrated that the specific presence of VE (vascular

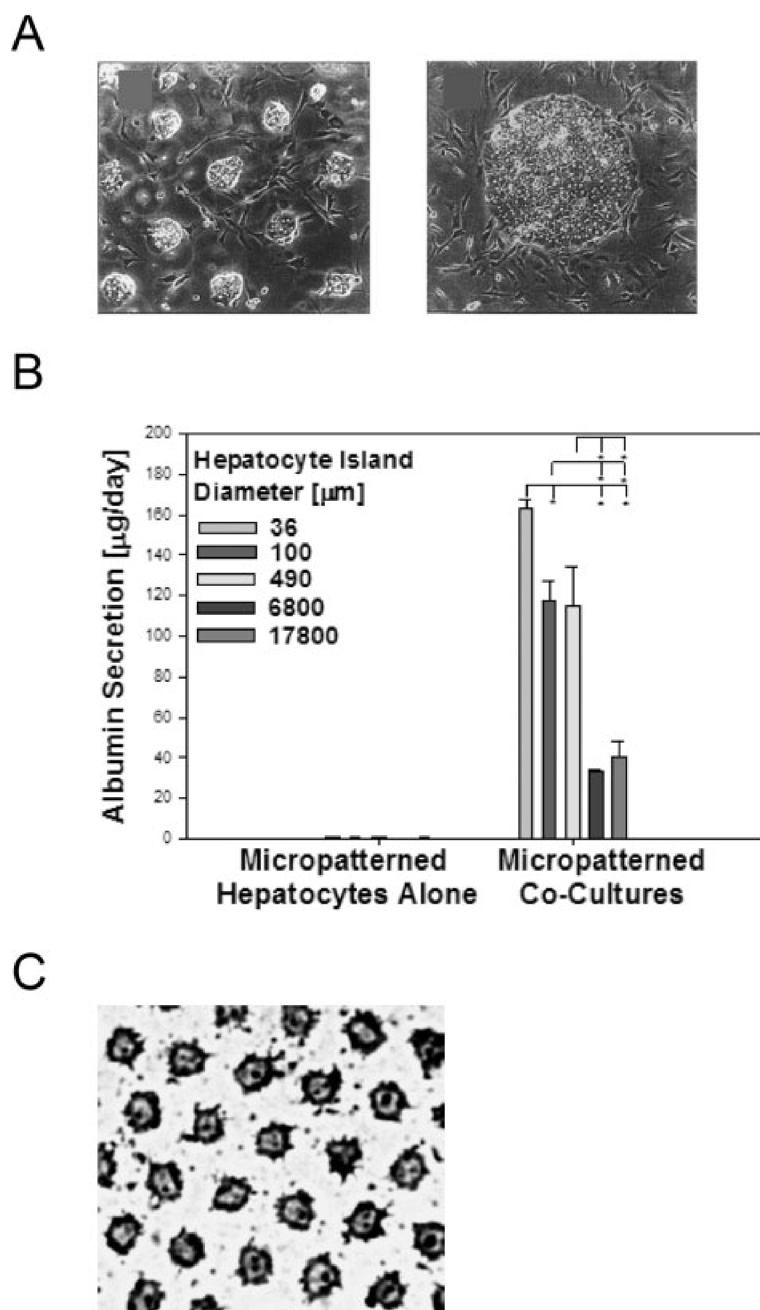

Figure 3. Microfabrication to control cell–cell interactions. A) Hepatocytes cultured on circular collagen islands of different areas surrounded by fibroblasts. Adapted from [63]. B) Effects of cell–cell interaction upon liver-specific function (albumin secretion). C) Immunohistochemical staining of intracellular albumin (dark regions) in hepatocytes cultured on circular islands with surrounding fibroblasts. Increased albumin production was primarily observed at the interface between hepatocytes and fibroblasts, and only penetrated a limited distance into the circular hepatocellular aggregates. Parts (B,C) courtesy of Dr. Sangeeta Bhatia, University of California, San Diego. CA.

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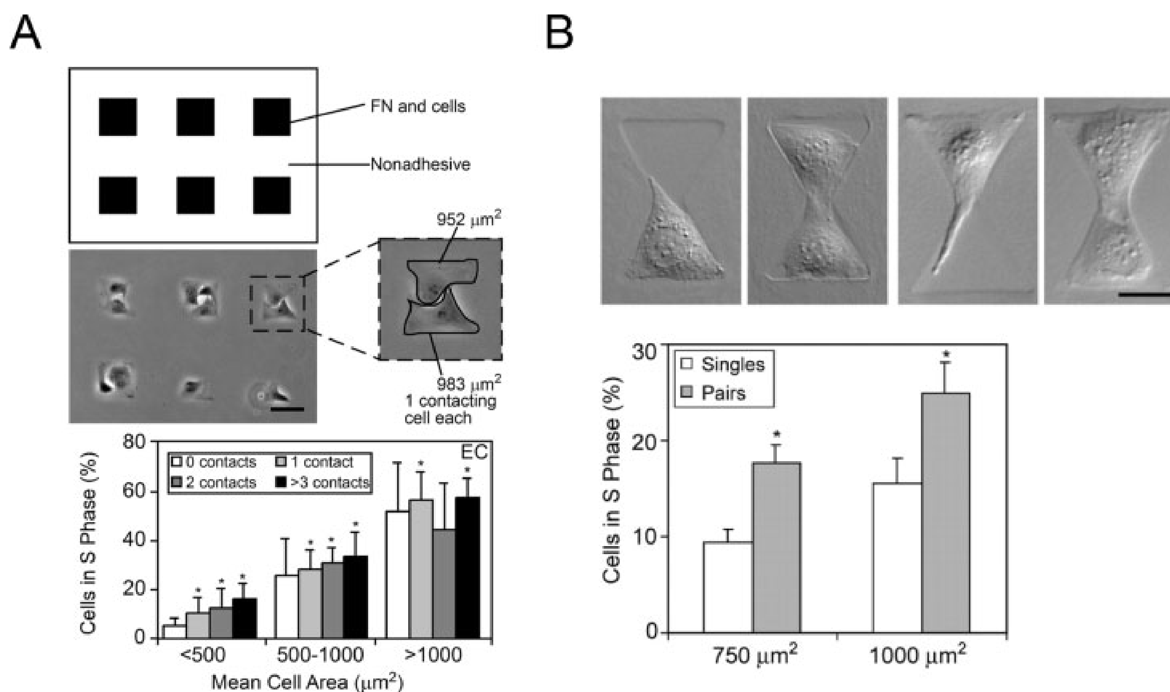

Figure 4. Using patterning approaches to study cell–cell interactions. A) Capillary endothelial cells cultured on square SAM islands coated with fibronectin. Proliferation as a function of cell area and number of contacts is graphed. Adapted from [30]. B) Capillary endothelial cells cultured in bow-tie-shaped agarose wells. Proliferation as a function of cell spreading and number of contacts is shown. Adapted from [41] with permission from Company of Biologists, Ltd.

endothelial) –cadherin-mediated contacts stimulated this pro-growth response relative to isolated control cells. Interestingly, cell–cell contacts have downstream effects upon cytoskeletal pathways, analogous to cell–matrix interactions.[41] In addition, cell–cell and cell–matrix interactions influence each other, since cells contacting other cells in these bow-tie patterns had smaller and fewer focal adhesions than cells without contacts.[42] The mechanisms underlying this crosstalk are still under active investigation, with micropatterning approaches providing general tools to separate these spatially dependent variables.

While these initial studies demonstrated the importance of spatial control in studying cell-to-cell contact-mediated cues and how they determine cell function and behavior, researchers are only beginning to explore the full potential of microfabrication tools in these areas. Investigating co-cultures of different cell populations is often limited by the fact that populations migrate and mix with time, hindering our ability to study long-term interactions. In the fibroblast–hepatocyte system developed by Bhatia et al., the liver cells are initially restricted to their proper locations by the collagen patterning, but over time the fibroblasts produce collagen that allows for population reorganization. The agarose-based bow-tie system has its own limitations as well. The technique is very good for patterning single cells or pairs of cells, but more complex patterns become difficult because cellular seeding within the wells is random. The application of active positioning systems to place cells in more complex arrangements may provide a more general strategy for studying multicellular interactions. Although these techniques require more difficult combinations of hard and soft-lithographic approaches, they will be critical to the study of cell–cell communication as investigators begin to unravel the myriad of interactions between cells that characterize the multicellular physiology of higher organisms.

4. Measurement of Subcellular Forces

Patterning studies investigating how cells respond to spatial cues have suggested that cells sense their environment by mechanically probing it.[18,19] When a cell adheres to a substrate, it not only spreads and flattens against the surface but also contracts using its actin–myosin cytoskeleton.[43] This contraction results in stresses experienced by focal adhesions, which are then thought to transduce these stresses into regulatory signals.[44,45] Thus, cells use this system to sense and respond to changes in the mechanics of their environment. While it has been difficult to study this mechanosensory system, several methods have now been developed for this purpose. We will discuss some of these approaches in the following section.

The earliest method of measuring subcellular forces involved plating cells upon flexible substrates such as silicone elastomers and monitoring the surface wrinkling due to cell contraction.[43] Using polyacrylamide sheets with embedded fluorescent tracking particles, Oliver and co-workers devel-

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oped traction-force microscopy techniques that led to significant advances in estimating the magnitudes of forces exerted by cells.[46–48] In a recent study, Balaban et al. extended this approach by using micropatterned, textured PDMS substrates to measure how forces exerted by cells feed back upon focal adhesion assembly.[49] PDMS substrates containing regularly spaced micrometer-scale depressions, elevations, or embedded beads of fluorescent photoresist enabled substrate deformations due to cellular contraction to be easily tracked (Figs. 5A,B). Importantly, the array of tracking particles permitted the computation of forces at individual focal adhesions. Using this approach, the size and protein content of the focal adhesions were found to be linearly correlated with the force exerted by the cell at those adhesions. When actomyosin contractility, and consequently intracellular tension generation, was disrupted with a pharmacologic inhibitor, focal adhesion size and protein content rapidly diminished in response to the decrease in exerted force, indicating a feedback relationship between the forces exerted by the cell and its adhesions. These studies began to illustrate the importance of cytoskeletal force generation as a cellular regulatory and signaling mechanism, and permitted more systematic quantitative evaluation of these effects.

Traction-force microscopy techniques typically require significant computational analysis to generate a force field, since deformations in the substrate propagate throughout the surface. To circumvent these limitations, Galbraith and Sheetz developed a micromachined cantilever device to directly measure subcellular forces as cells migrated over a surface.[50] As cells move across the surface, they exert forces upon silicon-based micrometer-sized cantilevers embedded within the surface (Figs. 5C,D). By mechanically isolating each cantilever, force profiles were more easily analyzed without the confounding effects of forces propagating through the substrate. This technique was especially useful in dynamically measuring the forces at different subcellular regions of a migrating cell, and helped to distinguish between different models of cell migration. In this case, forces at the leading and trailing edges of a migrating fibroblast pointed toward the center, suggesting that these cells move by pulling themselves forward, with the tail retracting until the rear adhesions are severed.

In an approach that combined the advantages of the preceding methods, Tan et al. fabricated a microneedle array device from PDMS (Fig. 5E).[51] The tips of these microneedles were patterned with protein using microcontact printing (Fig. 5G), while the rest of the device was rendered inert by adsorption of a poly(ethylene glycol)-based polymer. This device enabled the correlation of subcellular force distribution with cell shape, and allowed independent variation of substrate mechanics and surface chemistry. Previous approaches typically adjusted substrate mechanics by manipulating polymer crosslink density, but these alterations could also simultaneously affect surface chemistry and adhesivity. In this microneedle device, substrate stiffness can be controlled without altering surface chemistry by changing the microneedle diameter and length (Fig. 5E). In an advance over the unidirectional force measurements capable with the micromachined device previously described, multidirectional forces across the entire cell surface are easily estimated based upon the deflection of each free-standing, mechanically isolated post (Fig. 5F). With increased spreading, cells formed more actin stress fibers and exerted more force per post. Furthermore, global cellular geometry regulated cellular contractile responses to external stimuli; spread cells treated with lysophosphatidic acid (LPA), an inducer of contractility, were able to exert increased forces upon the posts, whereas unspread cells were not as capable of responding. Together, these studies begin to connect force generation by cells with the intracellular mechanisms that lead to changes in global cellular behavior.

All in all, the field of cellular mechanosensation is only beginning. With each advance in our study of cell mechanics, new technologies have been developed to further the next step. As more investigators apply new approaches to increase mechanical control—through active devices, better materials, and higher resolution substrates—the field will become clearer. These technological advances will extend our ability to both present active stimuli as well as quantify cellular force generation, permitting a more robust understanding of the mechanisms underlying how cells probe, interact with, and respond to their mechanical environment.

5. Approaches to Control Dynamic Stimuli

Cells in vivo are not only exposed to spatially encoded signals, but these signals continually change over time. For example, after injury numerous cytokines are suddenly and transiently released to promote the wound-healing process, and the ECM must be remodeled as the wounded region is reconstructed. Cells at the wound edge respond to these local signals by migrating into the wound to repair it. These processes are highly dynamic events, and additional novel techniques are needed to study them. Microcontact printing presents static surface stimuli; once cells are adherent, it is difficult to subsequently alter the matrix environment. In addition, although concentrations of soluble factors can be changed by removing and replacing the culture media on a global scale, it is difficult to present continuously time-varying concentrations of these factors and investigate the cellular responses. In the following section we discuss recent developments in the microfabrication of fluidic devices and novel surface chemistries that enable rapid alteration of the local cellular microenvironment, thereby permitting a more rational approach to studying dynamic cellular responses.

Laminar flow microfluidics platforms can be used to pattern cell attachment onto a surface, to control the spatial deposition of proteins onto a surface, and to manipulate the composition of the fluidic environment surrounding attached cells with little extra equipment beyond the microfabricated PDMS mold.[52] Using numerous inlets and outlets, multiple streams of fluid can be passed within the same channel with minimal mixing between input streams (Figs. 6A,B). This per-

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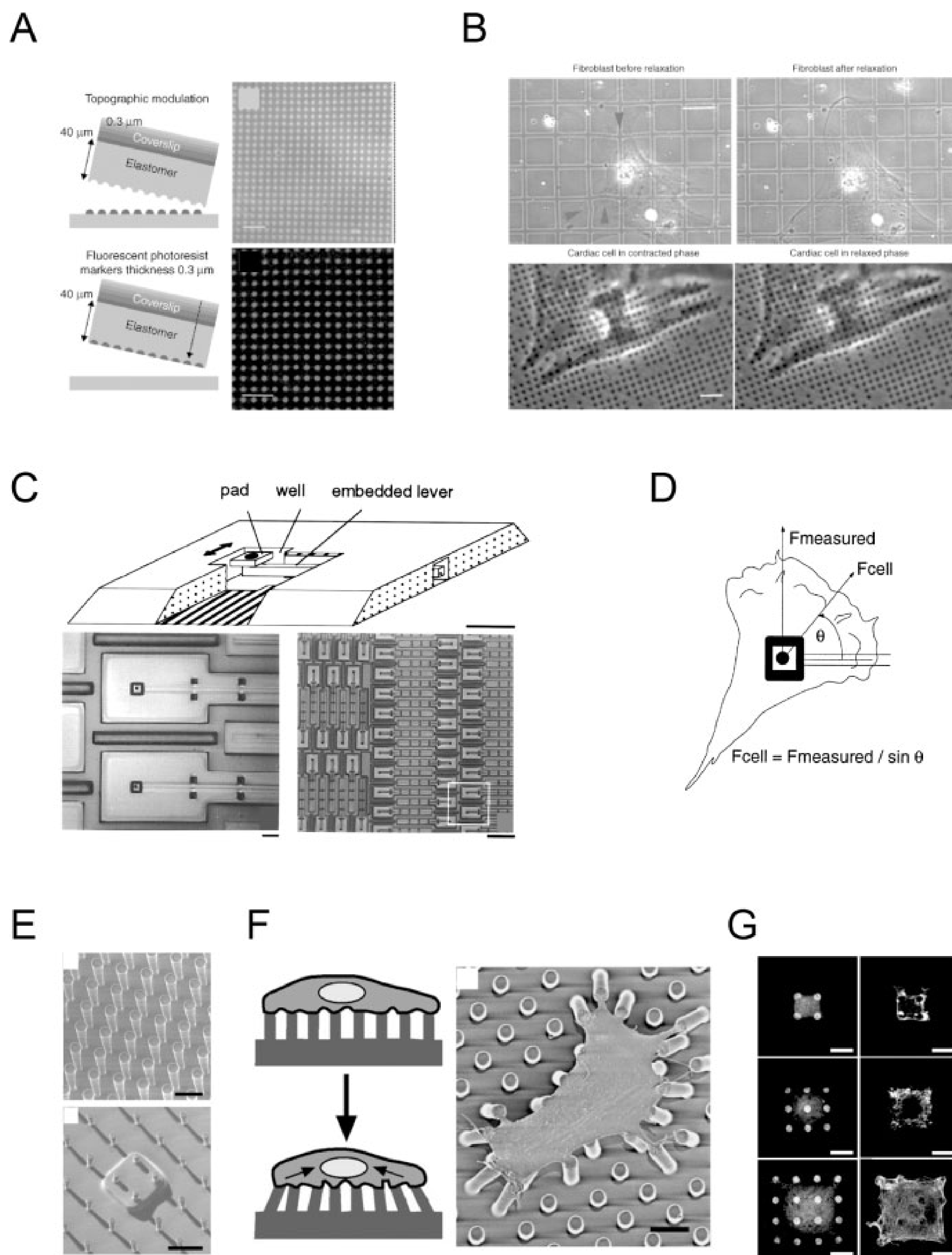

Figure 5. Microfabrication approaches to measure cellular force generation. A) Micropatterned substrates for traction-force microscopy. The upper panels show PDMS substrates with micrometer-scale depressions, while the lower panels show PDMS substrates with embedded fluorescent photoresist particles. B) Contraction of a fibroblast (upper panels) and a cardiac cell (lower panels). Substrate deformations are indicated by arrowheads. Parts (A,B) adapted with permission from [49]. Copyright 2001, Nature Publishing Group. C) Microfabricated cantilever device. The upper panel shows a cutaway drawing of the device. The lower panels are close-ups of the pads (left) and an array of cantilevers (right). D) Schematic illustration of force computation from cantilever deflections. Parts (C,D) adapted with permission from [50]. Copyright 1997, National Academy of Sciences, USA. E) Scanning electron micrographs of microneedle array device. Lower panel illustrates a method to alter post stiffness by reducing their height. F) Schematic illustration and scanning electron micrograph of smooth muscle cell on microneedles. G) Using microcontact printing to pattern adhesion onto microneedles. Immunohistochemical staining of fibronectin squares of different areas stamped onto tops of posts (left panels). Immunohistochemical staining of actin cytoskeleton of smooth muscle cells adherent to these posts. Parts (E–G) adapted with permission from [51]. Copyright 2003, National Academy of Sciences, USA.

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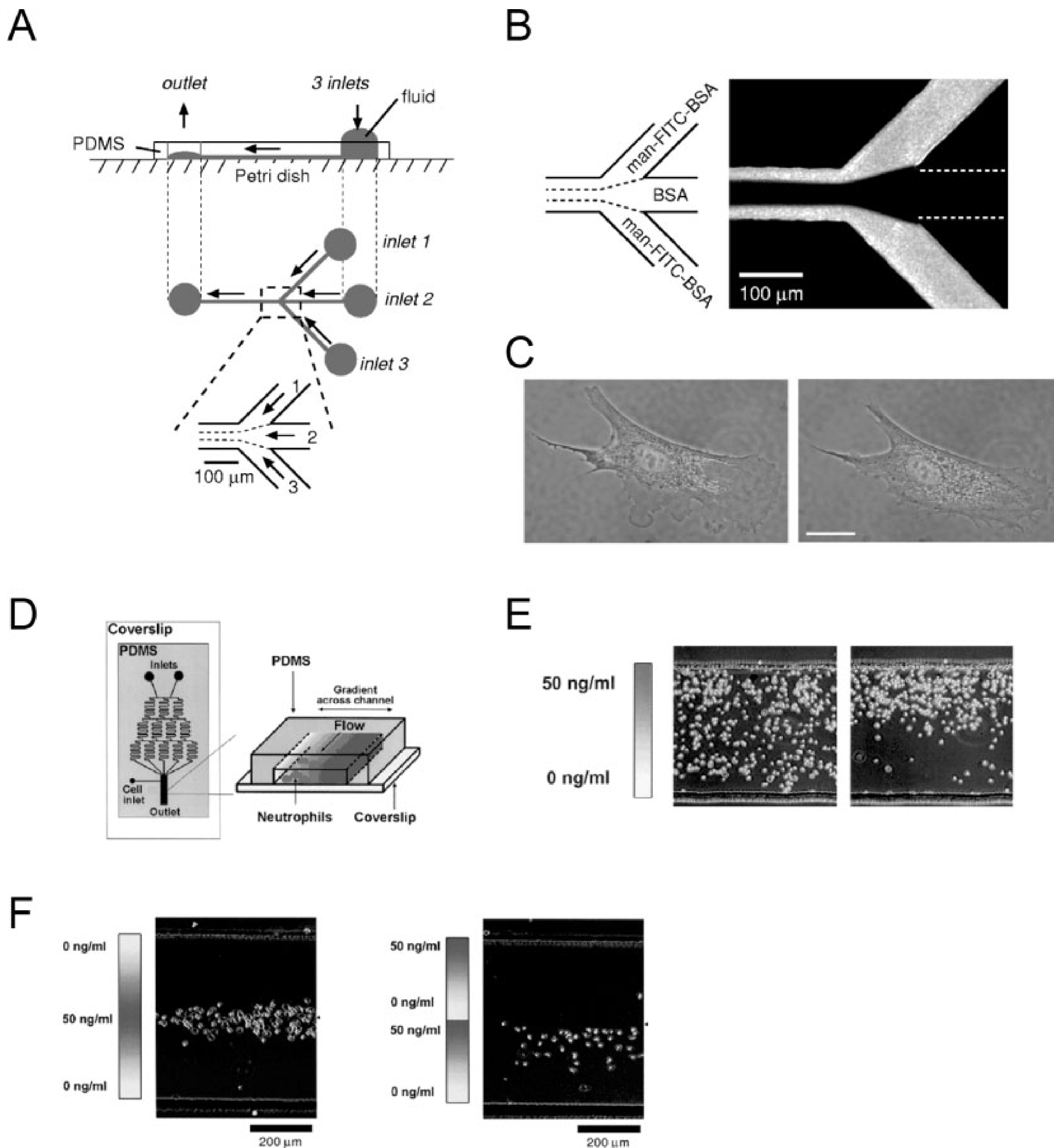

Figure 6. Microfluidics platforms to control the cellular environment. A) Schematic illustrating laminar flow through channels. B) Schematic illustration and fluorescence image of three solutions flowing through channel. Parts (A,B) adapted with permission from [52]. Copyright 1999, National Academy of Sciences, USA. C) Treatment of each half of a cell with different mitochondrial dyes. Left panel shows cell immediately after treatment, while right panel shows cell 2.5 h later, when mitochondria have mixed within the cell. Adapted with permission from [53]. Copyright 2001, Nature Publishing Group. D) Schematic illustration of device to generate fluidic gradients. E) Neutrophils migrate towards higher concentrations of interleukin-8. Left panel shows initial arrangement of cells, while right panel shows cells 90 min later. F) "Hill" (left panel) and "cliff" (right panel) gradients. Neutrophils originated at the bottom of the channel and migrated towards the peak of the gradient. Parts (D–F) adapted with permission from [55]. Copyright 2002 Nature Publishing Group.

mits the adsorption of different proteins from the different input solutions onto adjacent regions of the surface. Cells can also be cultured on a surface, and different regions of the culture can subsequently be exposed to different soluble stimuli through the laminar flow streams. These techniques were applied to treat two halves of a single cell with different mitochondrial dyes in order to follow the subsequent intermixing of subcellular mitochondrial populations (Fig. 6C).[53,54] In a similar experiment, a cytoskeletal disrupting agent was applied to a portion of a cell, and the subsequent rearrangement of the rest of the cell was tracked.[53,54] These methods present a powerful approach to gain insight into how cells respond to both spatial and temporal modulation of the soluble environment that bathes cells.

One major advantage with microfluidics is the ease with which concentration gradients of molecules can be generated. Historically, stable spatial gradients of soluble factors have been very difficult to generate. Jeon et al. developed a platform where adjacent laminar streams are intentionally mixed to produce a new third stream at half the concentration of the contents of the first two streams.[55] Sequential iterations eventually gave rise to a smooth gradient across the fluidic channel (Fig. 6D). This approach was utilized to study neutrophil chemotaxis in gradients of interleukin-8, a chemoattractant. In addition to generating linear gradients (Fig. 6E), more complex mixing arrangements produced "hill" or "cliff" concentration profiles (Fig. 6F). In all these cases, neutrophils migrated toward higher concentrations of interleukin-8. Dertinger et al. used a similar platform to generate surface gradients of adsorbed laminin and observed that neurons specify axon extension based upon the direction of increasing laminin concentration.[56] The advent of these devices gives fine spatial control over surface-bound stimuli and spatiotemporal control over soluble cues so that both steady state and dynamic cellular responses can be studied.

Dynamically modifiable surface chemistries have provided a novel approach to present cells with time-variant surface-bound stimuli. The traditional assay used to investigate cellular responses to the exposure of new ECM, often in the context of wound healing, involves scraping a monolayer of cells and monitoring wound closure.[57] However, this is a highly invasive technique, and the newly exposed surface is not well characterized. In an alternative approach, Jiang et al. showed that cells migrate out of microcontact-printed SAM islands after the application of a voltage pulse.[58] Cells were initially patterned by stamping hexadecanethiol and rendering surrounding regions inert with an oligo(ethylene glycol)-terminated SAM. After application of a voltage pulse, the oligo(ethylene glycol)-terminated SAM desorbed from the surface, allowing proteins from solution to adsorb onto those regions and permitting subsequent cell migration (Fig. 7A); this approach was used in a screening assay to test the efficacy of various cell migration inhibitors. Yousaf et al. pioneered an elegant approach to dynamically tailor surface ligands by doping the non-adhesive SAM surface with redox reactive capture groups.[59] Upon application of a low-voltage pulse, novel linker moieties react with chemical partners dissolved in the immersing solution to enable different synthetic ligands such as arginine–glycine–aspartate (RGD), a common adhesion tripeptide, to attach or detach from the surface (Fig. 7B).[59–61] These methods provide an alternative strategy to pattern multiple cell types by sequentially switching the adhesivity of different regions of the surface (Fig. 7C).

As more tools to dynamically alter the cellular microenvironment are developed, we are beginning to appreciate the importance of time-varying stimulatory cues in regulating cell function. These tools will play a critical role in elucidating these phenomena by providing researchers with the ability to spatiotemporally control the presentation of soluble cues to cells while simultaneously controlling cell–ECM interactions in real-time. As these techniques become more refined, more complex scenarios can be simulated in these in-vitro settings and correlated to in-vivo situations.

6. Future Directions

In recent years, we have seen the advent of the application of microfabrication technologies to study fundamental questions in cell biology. Starting from early applications using SAMs and microcontact printing to spatially control cell adhesion, the field has branched out into diverse arenas such as the study of cell–cell interactions, cellular force measurements, and the introduction of time-variant stimuli to cells. With each of these advances, new technologies have been invented that have expanded our capabilities. A variety of techniques have been developed to spatially position cells, ranging from surface chemical modification to constructing physical barriers. In addition, several different approaches to measuring cellular contraction and associated responses have been devised. Exciting new methods involving microfluidics platforms and dynamically modifiable surface chemistries are enabling greater temporal control over soluble and surface-bound stimuli. This report has only touched upon a few of the many different technologies that have been developed for applications in cell biology, but these examples illustrate the growing importance of these types of approaches to further our understanding of cellular behavior in response to spatiotemporal and microenvironmental cues.

In the future, the integration of different microfabrication-based approaches will be necessary to investigate increasingly complex scenarios. For example, incorporating fluidics platforms or dynamic surface chemistries with measurement technologies such as the microneedle array device will improve our understanding of dynamic cellular responses to multiple stimuli. In addition, while single-cell behavior must be understood, most important physiologic processes are multicellular. Indeed, it is thought that emergent behaviors such as the formation of specialized multicellular structures (for example, the hollow tubes of the vascular system) cannot be understood in single-cell systems. Extending microfabrication approaches

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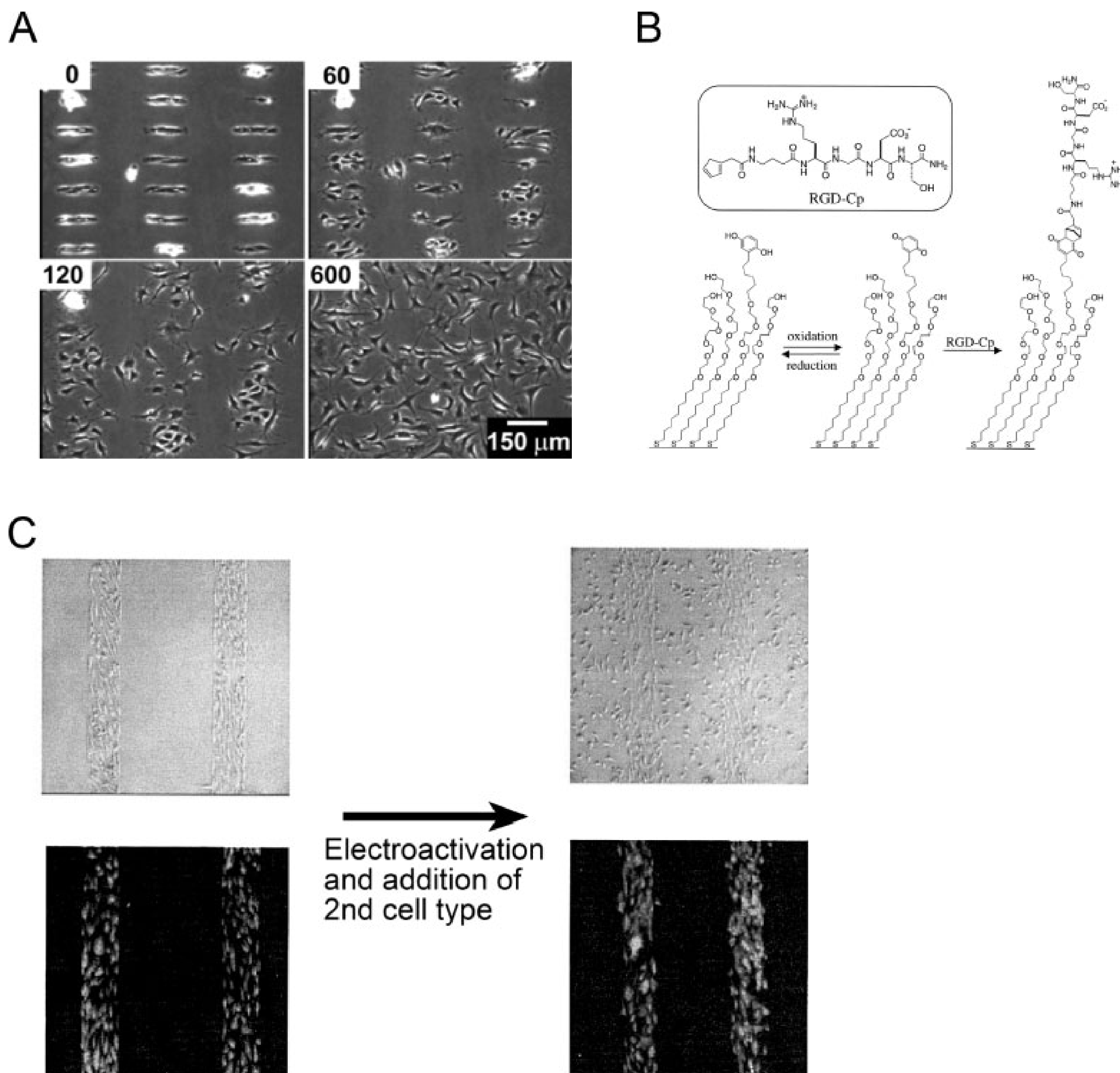

Figure 7. Dynamic substrates to modify the ECM environment. A) Electrochemical desorption of oligo(ethylene glycol)-terminated SAMs enables cells to migrate out of patterns. Numbers indicate time [min] after activation. Reprinted with permission from [58]. Copyright 2003, American Chemical Society. B) Reaction scheme for the electroactive attachment of RGD to previously inert regions. C) Using electroactive substrates to pattern the adhesion of multiple cell types. Left panels show the initial population of fluorescently labeled fibroblasts adhering only to the initially adhesive regions of the pattern. Right panels depict addition of unlabeled fibroblasts after electrochemically activating the surface. The cell-adhesion ligand RGD reacts with the surface, permitting the adhesion of a second cell type. The labeled cells are shown to persist in the original pattern. Parts (B,C) adapted with permission from [59]. Copyright 2001, National Academy of Sciences, USA.

to study how populations of cells react to spatiatemporal cues presents new challenges, and additional studies into how these biological mechanisms propagate through cell populations are needed to gain insight into the paradigms that govern developmental and other morphogenetic processes.

Thus far, we have been able to spatially define the cellular environment on two-dimensional (2D) substrates. However, because many tissues are organized in a three-dimensional (3D) architecture, developing new methods of fabricating in 3D will be essential in future studies. Controlling ligand presentation and cellular position within 3D systems poses new challenges. Designing the scaffolding in which to embed cells also presents different opportunities. For example, because cells are capable of sensing and responding to the mechanical properties of a substrate, scientists will need to be able to tune the mechanics of substrate materials. The ability to control ligand chemistry, matrix mechanics, and cellular organization within 3D in-vitro systems would provide a powerful tool to

systematically study cellular behaviors and their underlying mechanisms in a more in-vivo-like environment.

Microfabrication-based strategies provide the tools to address fundamental biological questions about the spatiotemporal and microenvironmental regulation of cell behavior as well as design improved tissue constructs and devices that have significant clinical impact. As the importance of spatiotemporal cues becomes increasingly apparent, the creative application of microfabrication techniques will provide novel insights into unanswered problems in cell biology.

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