Using Lab-on-a-chip Technologies to Understand Cellular Mechanotransduction

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I. Introduction

We are living in the age of micro and nanotechnology. The electronics industry owes its rapid expansion over the past several decades to its ability to invent new approaches to make things ever smaller. Using similar techniques, the fabrication of small structures is being applied in other disciplines, particularly in the areas of chemistry and biology, enabling scientists to ask questions in ways not previously possible. The first non-electronic microfabricated chips were used in analytical chemistry, where miniaturized assays were developed to perform gas and liquid chromatography (Manz et al., 1990a; Manz et al., 1990b). In chemistry, as in the field of electronics, miniaturization enhanced the performance of these techniques, but it also had the added benefits of smaller reagent consumption, portability, and parallel construction for high throughput applications. These same benefits have pushed micro-systems into the realm of biological chemistry, and have resulted in such developments as micro-PCR (Northrup, 1993), flow cytometry (Sobek, 1993), and DNA sequencing (Woolley and Mathies, 1995).

Today, variations of these lab-on-a-chip techniques are commonplace in the biological laboratory. One dramatic example is the chip-based cDNA microarray. In cDNA microarrays, DNA is immobilized onto a solid platform such that different DNA sequences are addressed to specific spots on the array. A sample is passed over the array, such that matching sequences of DNA in the sample hybridize to the immobilized cDNA array. In the process, thousands of different cDNAs can be assessed simultaneously for their relative abundance. This strategy is now widely used in gene expression profiling applications, where 20,000 to 40,000 genes can be simultaneously analyzed in a single experiment, and has immeasurable impact on biological research (Xiang and Chen, 2000).

While chip-based assays have enabled enormous advances in biochemistry, many insights into the biological function of organisms and their component tissues comes from the direct observation of individual living cells. To address this need, lab-on-a-chip methods have recently been extended beyond standard biochemical assays to include direct cell culture on chips. These tools are enabling novel experiments to assess the biology of whole, living cells, such as those that assay for cell migration, polarization, or

reorganization. Remarkably, as compared to traditional biochemical assays, an entirely different set of benefits offered by microchip approaches is defining a niche for cell-chip technologies in living cell assays. In particular, these systems appear to be unique in enabling investigators to control the local physical and chemical environment around cells, and in doing so, are re-defining our understanding of how cells function.

In their normal setting, cells exist in a complex micro-environment in which they must adapt and react to cues present in their surroundings. These cues may be both soluble (growth factors and cytokines) and insoluble (adhesion and mechanical forces) (Figure 1).

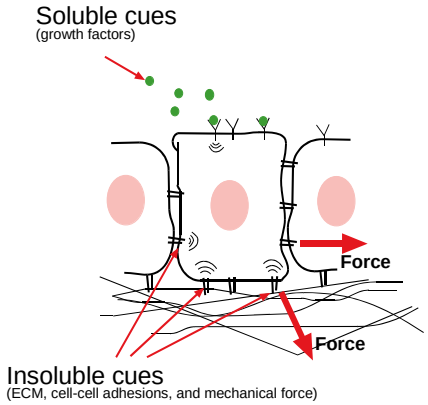

Figure 1: Schematic representation of cells in culture. In the cellular microenvironment, cells respond to soluble cues such as growth factors as well as insoluble cues that are adhesive and mechanical in nature. Insoluble cues include attachment of cells to the surrounding extracellular matrix or to other cells, and other external forces that act on cells. Cooperation between all of these cues ultimately governs the way a cell will behave.

While much effort has been dedicated to understanding the cellular response to soluble signals, less is known about how cells mechanically sense and transduce signals from insoluble cues (collectively referred to as "mechanotransduction"). Both classes of cues are critical for cell survival and function, but in contrast to soluble cues, insoluble cues have been experimentally hard to control. To address these challenges, chip-based microfabrication technologies recently have been developed to control or engineer the physical microenvironment of cells in culture and are now being used to study mechanotransduction. This chapter will focus on the lab-on-a-chip based methods developed to control the physical input contributed by the microenvironment and to measure the output response of cells to such microenvironments. A brief primer on the current understanding of cellular mechanotransduction will be presented, followed by a discussion of current

microengineering approaches that have been developed to study these questions.

II. Cell mechanotransduction

Cells require exposure to many factors in order to function properly; both soluble mitogens such as growth factors and also physical attachment to the extracellular matrix (ECM) are needed. Cell adhesion in culture both to the underlying ECM substrate (cell-ECM) and to other cells (cell-cell) regulates signaling cascades that govern many cell behaviors, including cell proliferation, apoptosis, polarity, motility, and differentiation (Bershadsky et al., 2003; Chen CS, 2004; Katsumi et al., 2004). Understanding how cells sense and respond to these adhesive and mechanical cues is an area of intense study.

Cell adhesion to the ECM is mediated by transmembrane receptors (integrins) that mechanically connect the cell to the ECM (Ingber, 2003). Integrins bind to the ECM through their extracellular domains and associate with a large number of proteins on their cytoplasmic tails (Geiger et al., 2001) (Figure 2).

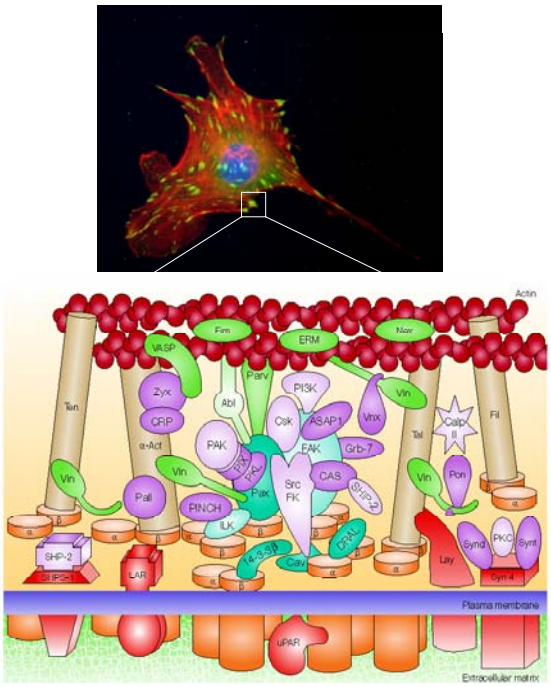

Figure 2: Bovine Pulmonary Artery Endothelial Cell (BPAEC) co-stained to show actin stress fibers (red) and focal adhesions (green). One focal adhesion is schematically highlighted to show some of the molecular details. Schematic from Geiger et al., 2001, reprinted with permission.

When cells initially attach to the ECM, the agglomeration of cytoplasmic proteins forms transient structures known as focal complexes. As these structures experience tension generated through the actin cytoskeleton, they grow larger and become focal adhesions (Geiger et al., 2001; Riveline et al., 2001). Mature focal adhesions contain a large number of different proteins (~50) having diverse functions. Some of these proteins such as vinculin, paxillin and talin are thought to function as scaffolding proteins, and are important for the stabilized anchoring of the actin cables to the focal adhesions, while others such as focal adhesion kinase (FAK) have catalytic activity and function to propagate intracellular signals through various signal transduction pathways (Geiger and Bershadsky, 2001). The large numbers of molecules that associate within focal adhesions demonstrate the molecular complexity of these structures and position them as unique biochemical and mechanical signaling hubs.

The growth and development of focal adhesions is dependent on mechanical forces, which can be either internally generated or externally applied. Internally, forces generated through contractility of the actin cytoskeleton play a major role in adhesion integrity. For instance, when cytoskeletal tension is relaxed with drugs that inhibit actomyosin activity, a rapid loss of focal adhesions soon follows (Chrzanowska-Wodnicka and Burridge, 1996; Folsom and Sakaguchi, 1999) (Figure 3, F,G).

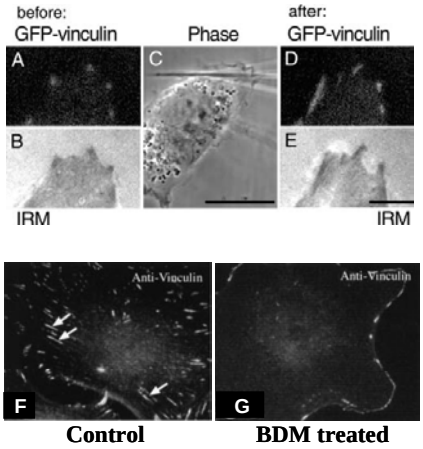

Figure 3: Focal adhesions are mechano-sensitive structures. With application of external force (micropipette pulling, see panel C), adhesions grow in size. Panels A and B show focal adhesions (as marked by GFP-vinculin) before external force application, while panels D and E show the same adhesions after force application (Panels A through E from Riveline et al., 2001, reprinted with permission). Panels F and G demonstrate focal adhesion shrinkage with decreased cytoskeletal tension. Cells were treated with 20mM BDM to inhibit myosin ATPase activity and stained with an antibody against vinculin. Panels F and G from Folsom and Sakaguchi, 1999, reprinted with permission.

In addition to the internally generated forces within the cell, adhesion maturation can also be achieved by externally applied physical forces (Choquet et al., 1997; Riveline et al., 2001) (Figure 3, A-E). ECM coated beads that are attached to the apical side of a cell initially form a nascent focal contact. Using laser tweezers to physically pull on the bead causes these contacts to mature into focal adhesions (Choquet et al., 1997). Such experiments demonstrate the mechanical nature of focal adhesions and highlight their role as mechanosensors. Because cell adhesion itself stimulates cell contraction and the mechanical stress caused by such contractions in turn alters the physical nature and biochemical activity of the adhesions, it is now clear that cell adhesion and cell mechanics are tightly coupled. As a result, our understanding of how cells respond to adhesion and to mechanical forces are inherently linked.

Because cells are mechanically coupled to their environment, changes in the extracellular matrix or cell mechanics can dramatically change cell behavior. For example, hepatocytes, mammary epithelial cells, capillary endothelial cells and fibroblasts in culture can be switched from a growth state to a differentiated, non-proliferating state by modifying the stiffness or adhesivity of the ECM in a manner that causes cell rounding (Ingber and Folkman, 1989; Mooney et al., 1992; Streuli et al., 1991). Human mesenchymal stem cells can be directed to either adipogenic or osteogenic lineages simply by controlling cell shape and thereby altering cell mechanics (McBeath R, 2004). Varying the compliance of the underlying substrate can influence the rate and direction of cell migration (Gray et al., 2003; Lo et al., 2000). In fact, cells will preferentially migrate to stiffer areas, a process called durotaxis. In each of these examples the presence of soluble factors between different experimental conditions is the same; the only differences are in the mechanical environment. This point emphasizes the importance of the mechanical environment in cellular systems, as cells may behave in very different ways depending on physical cues in their microenvironment.

The response of cells to mechanical input is critical in governing cell behavior not only in cell culture, but extends to the physiology of whole organisms as well. In vivo mechanical forces play a major role in development, tissue maintenance, wound healing, angiogenesis, and metastasis (Ingber, 2003). Particularly important is the role of mechanics in embryogenesis. Epithelial branching morphogenesis as seen in the developing lung and salivary gland can be altered by altering the mechanical cues surrounding these structures (Fata et al., 2004; Spooner and Faubion, 1980). Other examples of mechanotransduction in organisms are the alignment of endothelial cells in response to the fluid shear stress of flowing blood, and growth and remodeling of bone in response to mechanical loading, such as weight bearing exercise (Carter et al., 1987; Davies, 1995; Davies et al., 1994; Duncan, 1995; Resnick et al., 1997).

Because of the strong in vitro and in vivo evidence that cell mechanics governs cell behavior, it is not surprising that many different human diseases may arise from abnormalities in the mechanical environment surrounding cells or the ability of cells to properly sense and respond to these forces. In cancer metastasis, tumor cells must dramatically change their physical interactions with surrounding cells and ECM in order for them to break away, begin migrating, invade blood vessels, extravasate, and grow at distant sites (Ingber, 2002; Sternlicht et al., 2000; Sternlicht et al., 1999). This is a dramatic example of how cells in a pathological state cease to obey the normal physical restraints of their environment, with serious detrimental consequences. Other diseases such as hypertension or asthma are strongly correlated with physical perturbations (Masumoto et al., 2001; Waters et al., 2002). Arterial muscle cell hypercontractility results in the vasoconstriction and increased vascular resistance that elevates blood pressure in hypertension, while pulmonary muscle cell hypercontractility constricts the airways in asthma. Drugs that cause smooth muscle cell relaxation are effective treatments for hypertension and asthma. The treatments of many diseases are likely to depend in part upon targeting mechanical processes. Understanding cell mechanotransduction will not only provide us with a more complete understanding of cell behavior, but will also establish new opportunities for the treatment of diseases whose pathologies have a basis in physical perturbations.

III. Lab on a chip technology to investigate mechanobiology

Mechanotransduction is clearly a central component to the regulation of cell function both in culture and in the larger physiological context of the entire organism. A first step to understanding how cells respond to their mechanical environment is to use cell culture systems that can precisely control the adhesive and mechanical environment of cells. Traditionally, control of the soluble environment has been straightforward. Varying the concentration of a specific soluble signal in the culture media affects the degree of receptor signaling and is the primary tool for manipulating soluble cues. Controlling the adhesive and mechanical environment in precise or well-defined ways has been more difficult. Lab-on-a-chip approaches have been developed to manipulate the physical environment of cells in a well-controlled fashion (input signals) as well as being used to measure the physical responses (output behaviors) of cells under various experimental conditions. Applying these tools to study cell biology is leading to new discoveries that traditional cell culture has not achieved, and is proving to be specifically well suited to the study of cell mechanotransduction.

Controlling input

Lab-on-a-chip technologies can be used to study many aspects of cell function, but they are particularly well-suited to the investigation of cell-substrate and cell-cell interactions. For cell adhesion to the ECM, it has become evident that the integrin-ECM binding interaction, the spreading and flattening of the whole cell against a substrate, and the changes in cell mechanics that ensue may each provide distinct signals that regulate cell behavior. To understand how each of these parameters is independently detected and transduced in cells, methods are being developed to independently manipulate these parameters. Traditionally, investigators have coated cell culture surfaces with different densities of ECM to control cell adhesion. At low ECM density, cells attach loosely on the surface, remaining somewhat spherical (Ingber, 1990). At high ECM density, cells attach strongly and flatten and spread extensively on the surface (Ingber, 1990). Thus, this type of approach could not distinguish between the effects that are due to changes in cell shape from those which stem from the exposure of cells to different densities of ECM. Recently, chip-based technologies have emerged which allow for the fine control of surface chemistry in such a way that cell shape and ECM density can be decoupled.

There are two technologies to controlled cell adhesion that are important for this chapter: the ability to create surfaces with defined surface chemistry, and the ability to pattern adhesive and non-adhesive regions such that placement and spreading of cells can be defined. Self-assembled monolayers (SAMs) have been used as model surfaces to develop the appropriate defined surface chemistries (Kane et al., 1999; Prime and Whitesides, 1991). When sulfhydryl terminated hydrocarbons called alkanethiols are exposed to a surface of gold, they coordinate to the gold through the sulfur atom and self assemble into a highly organized molecular coating (Figure 4).

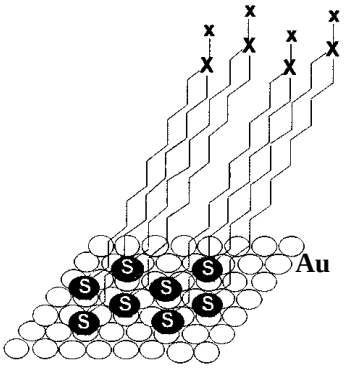

Figure 4: Schematic representation of alkanethiols and self-assembled monolayers (SAMs). On gold surfaces the alkanethiols coordinate and self assemble into a highly organized molecular surface. Adapted from Whitesides et al., 2001.

Various SAMs can be used to either resist or promote protein adsorption. For instance, hydrophobic SAMs adsorb proteins and can thus promote cell adhesion and spreading on surfaces coated with these regions, while SAMs that terminate in ethylene glycol moieties resist protein adsorption and therefore prevent cell adhesion (Kane et al., 1999; Palegrosdemange et al., 1991; Prime and Whitesides, 1991). When segregated into different regions on a single substrate, cell adhesion-resistant and adhesion-promoting domains can be arbitrarily and specifically arranged. Thus, the pattern of the two SAMs presented on the surface defines the pattern of ECM that is adsorbed from solution onto the surface.

Techniques such as photolithography and microcontact printing are used to produce substrates that are patterned with micrometer sized features such that cell placement and cell spreading geometry can be tightly controlled (Chen et al., 1997; Singhvi et al., 1994) (Figure 5 A-D). Microcontact printing uses physical stamps rather than direct lithographic approaches (Kumar et al., 1994). Briefly, photolithography is used to generate an array of micrometer-sized features on a silicon wafer. A pre-polymer of PDMS (polydimethylsiloxane) is then cured against this mold (master) and peeled to reveal an elastomeric stamp containing the negative replica of the original master. Stamps can then be inked with silanes, alkanethiols, or directly with ECM proteins (Bernard et al., 2001; Corey et al., 1996; Healy et al., 1994). When the elastomeric stamp is placed in contact with a surface, the inked protein is transferred to the receiving surface. Unstamped regions can be blocked by various substances that resist protein adsorption such as ethylene glycol-terminated alkanethiols or detergents (Amiji and Park, 1992; Bohner et al., 2002; Palegrosdemange et al., 1991). When cells are plated onto these surfaces, they specifically adhere to the adhesive regions (those coated with ECM proteins) and are blocked from attaching to or spreading into the non-adhesive regions (those blocked with detergents or ethylene glycol-terminated alkanethiols).

Using these surface micropatterning techniques, one can directly control the geometry of the cells based on features defined in the elastomeric stamp. Cells can even be forced to conform to unnatural shapes such as squares and triangles (Chen et al., 1999) (Figure 5 E). When the adhesive features are decreased in size and spaced closely together, cells can spread over multiple smaller islands, while their adhesion is still restricted to the stamped areas (Chen et al., 1997). Collectively, these technologies provide spatial control of the adhesiveness of a surface, and allow investigators to arbitrarily constrain the shape of cells as well as the specific arrangement of their location on the surface.

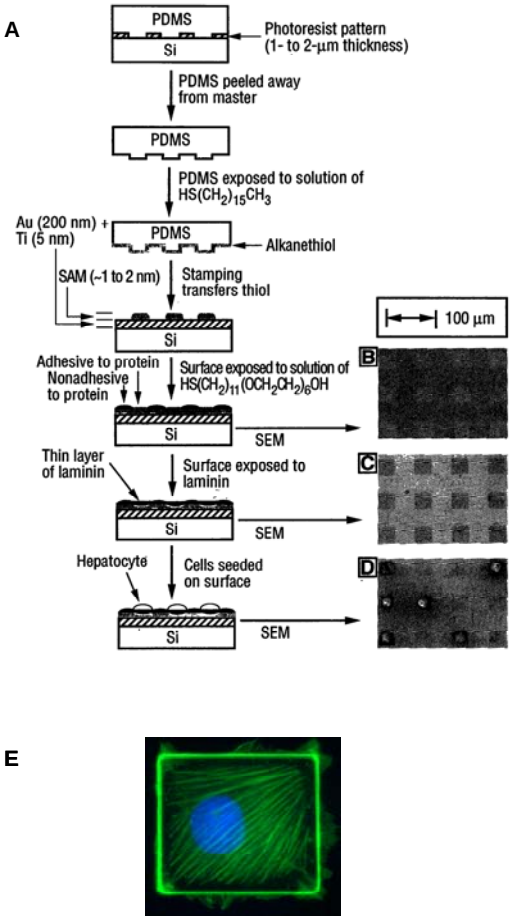

Figure 5: Schematic outline of microcontact protein printing. Briefly, an elastomeric stamp is produced by casting a prepolymer of polydimethylsiloxane (PDMS) onto a photolithographically generated master. Following curing of the polymer and stamp removal, the stamp is inked with desired alkanethiol, stamped onto a substrate, remaining regions are blocked, and ECM protein is adsorbed to the adhesive regions. Panels A through E from Singhvi et al., 1994, reprinted with permission. Cells seeded onto these patterns assume the geometry of the stamped features (Panel E). Panel E from Chen et al., 1999, reprinted with permission.

Controlling input – uses for single cell investigations

Spatially engineering the adhesive environment using micropatterning has allowed for many new ways to investigate cell function. Early work by Ingber's group using this micropatterning approach crystallized the notion that controlling cell shape (geometry) could control cell function (Chen et al., 1997; Singhvi et al., 1994). In these studies, they found that restricting cell spreading using small adhesive islands of fibronectin caused a general inhibition of cell proliferation (Figure 6).

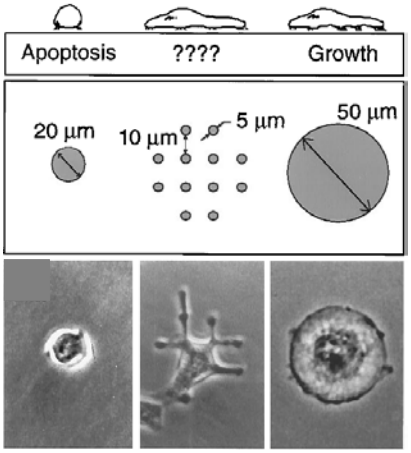

Figure 6: Cell spreading regulates cell function. Microcontact printing was used to control the spread area of endothelial cells. Cells that were plated on small islands of ECM were not able to spread and were blocked in the G1 phase of the cell cycle. Cells plated on the same area of ECM, but spaced out over multiple smaller islands were able to proliferate like other well-spread cells. Figure from Chen et al., 1997, reprinted with permission.

Here, even in the presence of saturating concentrations of soluble growth factors, the ability of cells to progress through the cell cycle depended directly on the degree of cell spreading, not on the amount of ECM binding (Huang et al., 1998). These findings suggested the necessity of a physical cue for proliferation that was not dependent on ligand occupancy of either growth factor or integrin receptors. It has since been found that cell shape also acts as a cue that regulates cell survival, differentiation, and migration in many cell types (McBeath R, 2004; Palecek et al., 1997; Parker et al., 2002; Spiegelman and Ginty, 1983; Thomas et al., 2002). Cells appear to detect their shape through a mechanical mechanism: when the actin cytoskeleton of cells is disrupted, spread cells that normally would proliferate are arrested. When round cells are stimulated with growth factors, they respond with appropriate MAPK signaling, much like their well-spread counterparts (Huang et al.,

1998). In other words, the shape of the cell appears not to affect soluble signaling but rather modulates a mechanical signal imparted by the actin cytoskeleton. Understanding the mechanical signals highlighted by these patterning experiments has become a major focus in cell biology.

Additional micropatterning experiments have substantiated the idea that cell geometry alone can directly regulate cell mechanics and cell function. By controlling cell geometry through adhesive patterning, the overall organization of the actin cytoskeleton is affected, as well as the localization of focal adhesions and extent of focal adhesion formation (Chen et al., 2003; Parker et al., 2002). In square shapes for instance, cells re-orient their stress fibers along the axis of highest force (the diagonal of the square) and also localize their adhesions to these regions. In addition, lamellipodia form preferentially from the corners of the square, the regions that experience the highest tractional forces (Parker et al., 2002). Because lamellipodia are an important component of cell migration and generally serve to lead the cell in the direction of migration, cell shape may determine the distribution of internal mechanical forces and thereby prescribe the direction that cells migrate.

Micropatterning of single cells is being used in many different applications. Based on these studies of ECM geometry and cell shape, it is now clear that geometric cues have far-reaching, global effects on cell behavior. Because traditional culture, in which cells are randomly seeded on a dish, cannot control the size, shape, and orientation of individual cells, there may be a wide disparity in the behavior of cells within the population. Thus, micropatterning can be used to eliminate the variability associated with traditional cell culture. By generating large arrays of single cells that are precisely the same size and shape, a uniform population of cells may be obtained and assessed by various methods, including biochemical and immunocytochemical approaches. Large arrays of micropatterned cells may also potentially be addressed such that the locations of individual cells are indexed for longitudinal studies. This approach is currently being explored by Cellomics, Inc. for the automation of drug screening and procedures in toxicology. In all, single cell control of adhesion is being used in numerous ways by biologists both for controlling cell position and cell adhesion signals.

Controlling input – uses for multicellular investigations

In addition to single cell applications, lab-on-a-chip technologies have also been used to study cells in multicellular scenarios. Controlling how cells organize allows one to investigate the effects of cell-cell contact in a well-controlled fashion. Traditional cell culture methods rely on random cell seeding, which causes wide variations in local cell density across the dish and results in cells of many different sizes and shapes that also have varying extents of cell-cell contacts. In fact, the increased crowding itself causes cells

to change their shape and become more rounded. Nelson et al. developed a method to independently control cell-cell contacts and cell shape using a chip-based method (Nelson and Chen, 2002) (Figure 7).

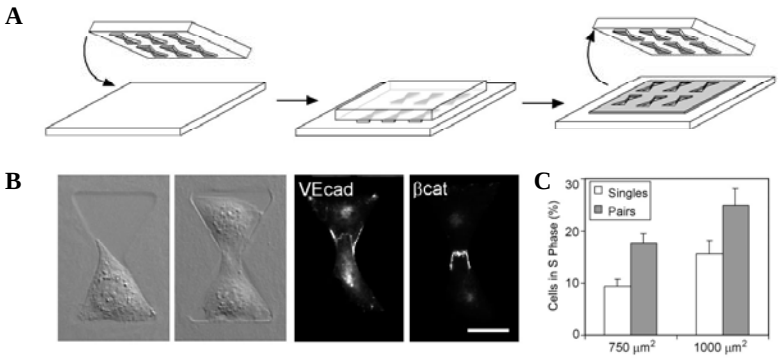

Figure 7: Cell-cell contact can be controlled using an agarose microwell patterning method. Panel A shows a schematic for this method. Following sealing of PDMS stamps sealed against a glass slide, agarose is wicked underneath and stamps are removed. Microwells can be coated with ECM protein and cells seeded into wells (Panel B). Cells in microwells making contact with neighboring cells exhibit normal adherens junctions containing VE-cadherin and β-catenin (Panel B). In these experiments, the presence of cell-cell contact increased the level of cell proliferation above that of single cells. Adapted from Nelson and Chen, 2002.

In this technique, elastomeric stamps with bowtie-shaped features were sealed against a glass slide. Agarose was flowed under the stamp and upon peeling of the stamp, bowtie shaped microwells were created. Cells seeded onto these substrates either filled the patterns as singles (one cell filling half of the bowtie) or pairs (one cell filling each half of the bowtie). In this way, cells of the same shape and size, but either containing or lacking a cell-cell contact could be compared. By independently varying cell shape and cell contacts in a scenario that traditional cell culture could not achieve, Nelson and colleagues found that cell-cell contact caused a decrease in cell spreading that in turn inhibited cell proliferation (Nelson and Chen, 2002). Surprisingly, they also discovered that cell-cell contact increased cell proliferation when cell spreading was held constant. Thus, these new microengineering approaches have radically changed experimental design to allow for alternative ways to approach unresolved controversies in cell biology.

On a larger scale, Bhatia et al. used micropatterning approaches to show that liver cells enhance their function when in contact with supporting fibroblasts (Bhatia et al., 1999) (Figure 8).

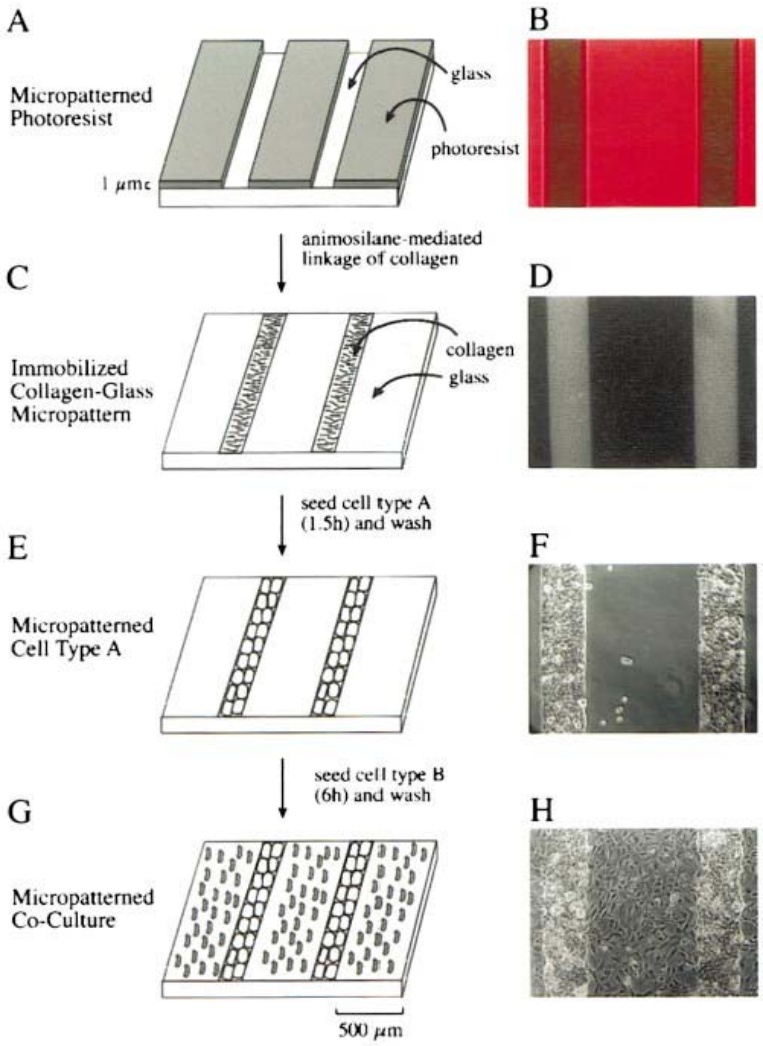

Figure 8: Schematic drawing of micropatterned co-culture method. Briefly, collagen was immobilized on a photolithographically generated substrate. Hepatocytes were seeded onto the substrates. Subsequently, fibroblasts were added to generate micropatterned co-cultures. Figure from Bhatia et al., 1999, reprinted with permission.

In this system, substrates were photolithographically patterned to control the adsorption of collagen I. Primary hepatocytes plated onto these patterns attached only to the areas covered with the collagen. Following attachment of hepatocytes, fibroblasts were plated and attached to the remaining free space. By manipulating the size and shapes of these patterns, heterotypic (fibroblast-liver cell) interactions could be spatially controlled. In these studies, hepatocytes co-cultured with fibroblasts demonstrated an increase in specific biochemical markers of hepatocyte differentiation, including urea and albumin secretion (Bhatia et al., 1999). Furthermore, using this micropatterning approach, the amount of hepatocyte to fibroblast area (area of heterotypic interaction) could be varied such that hepatocytes were exposed to more or less heterotypic interactions. In these manipulations, cells with more heterotypic interactions retained hepatocyte-specific biochemistry, while hepatocytes that were not adjacent to fibroblasts did not retain the markers of hepatocyte differentiation (Bhatia et al., 1998; Bhatia et al., 1999; Bhatia et al., 1997). These studies demonstrate that micropatterning approaches can be used not only to study cell-cell interactions, but also how the geometry of these interactions affect cell biology. Because cell-to-cell communication is a critical cue for many physiological functions of cells, such as in neuronal communication, epithelial-stromal interactions, and tumor-host communications, these tools will become increasingly important to standard biological experiments.

Controlling input - complex micropatterning for cell applications

While some micropatterning tools are quite mature in their technological development and application to biology, others are only just being realized. One limitation to standard micropatterning is that it is static in nature. That is, once the ECM proteins are patterned onto the substrate and the cells are seeded there can be no release from this pattern. Surface chemistries using electrochemically active alkanethiols developed by the Mrksich group have allowed for ways to change a pattern once it has been initially stamped (Yousaf et al., 2001a; Yousaf et al., 2001b) (Figure 9). After cells are initially plated in a patterned array, the application of a short voltage pulse can change the oxidation state of the electrochemically responsive alkanethiol. This permits the capture of an RGD peptide, a common adhesive ligand, from solution onto previously non-adhesive areas, thereby converting these switched regions into cell-adhesive areas. Such technologies will be important for assessing how cells respond to a changing and evolving ECM environment.

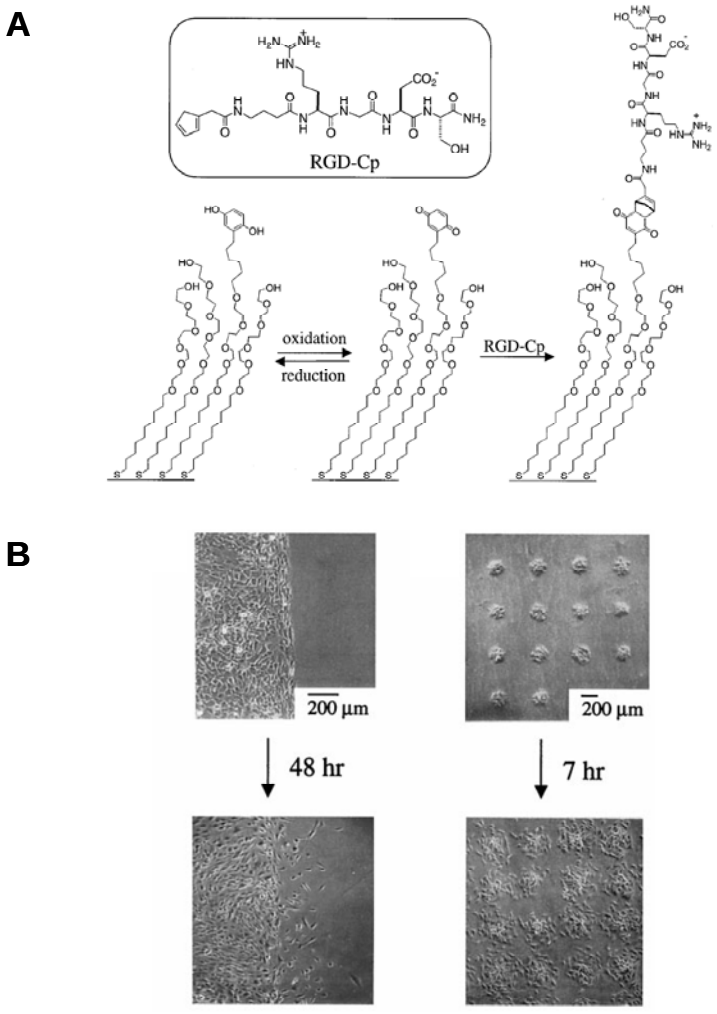

Figure 9: Chemical schematic for substrates that can be electrically switched to allow for cell attachment (Panel A). Application of a potential to the underlying gold activates the surface and enables the linkage of RGD peptides to previously non-adhesive regions of the surface, so that cells are able to migrate into newly switched adhesive surfaces (Panel B). Adapted from Yousaf et al., 2001b.

Another modification to initial microcontact printing strategies was the development of a multilevel stamp capable of creating multiple differently stamped regions. Traditionally, microcontact printing generates stamped regions that are binary in nature – that is, regions are either stamped or not and can either have cells attached or not. Tien and colleagues fabricated multilevel elastomeric stamps that generate surfaces with more than two regions of functional material (Tien et al., 2002) (Figure 10). Compressing the stamp to different degrees allows different levels within the stamp to contact the surface. Thus, complex patterns of two or more different proteins or chemistries can be printed on the surface at once. This technique also offers the potential to pattern multiple cell types next to each other.

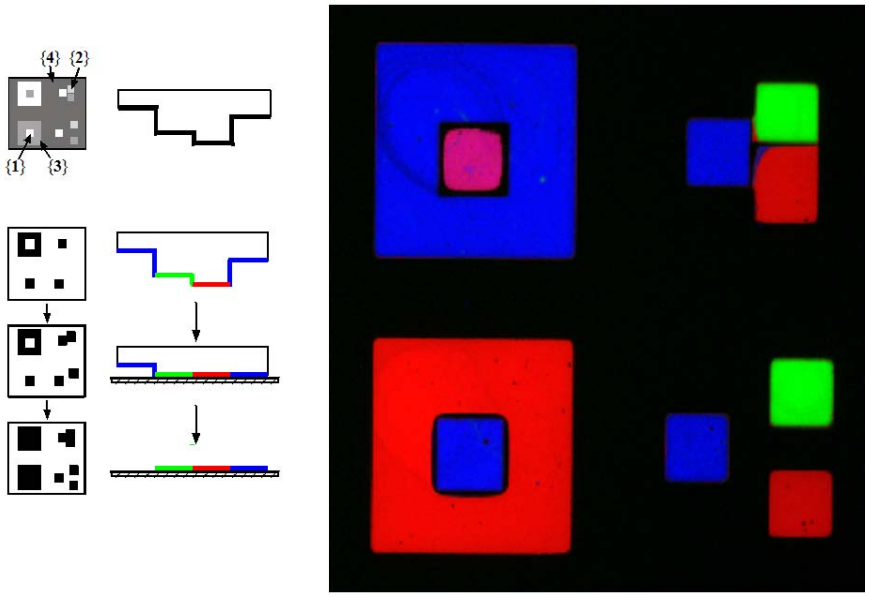

Figure 10: Schematic outline of patterning with a four-level PDMS stamp. Application of increasing pressure to the stamp causes the stamp to collapse allowing for sequential or step-wise contact of stamp with substrate surface (Panel A). Panel B shows a fluorescence image of three labeled proteins stamped with this method. Adapted from Tien et al., 2002.

Chemical patterning methods are one means to pattern cell on chips, but there are others. Among these are optical patterning methods and dielectrophoretic methods. Optical methods have been used to position individual cells with great precision (Ashkin et al., 1987) (Figure 11A), however, such 'laser tweezers' are currently difficult to array, so most applications to date use a single beam to place cells one at a time. Recent advances, however, have used fiber optics to split a laser beam into many

paths, thereby forming an array of optical tweezers (Biran et al., 2003). This type of strategy is likely to be expanded into array-based assays in the near future. Magnetic forces can also be used to physically move cells to the desired locations. Photolithographic techniques can be used to create arrays of micron-scale magnets to generate traps for single cell capture. However, in order for the cells to be patterned onto the traps, they must be magnetized themselves. One method for such magnetization is through the use of magnetic nanowires, which can be taken up by the cells (Hultgren et al., 2003; Reich et al., 2003). Yet another method to pattern cells is through electrical forces. Dielectrophoresis, which is the movement of uncharged particles in a non-uniform electrical field (Jones, 1995; Pohl, 1978), can be used in a chip-based strategy to pattern cells (Figure 11B).

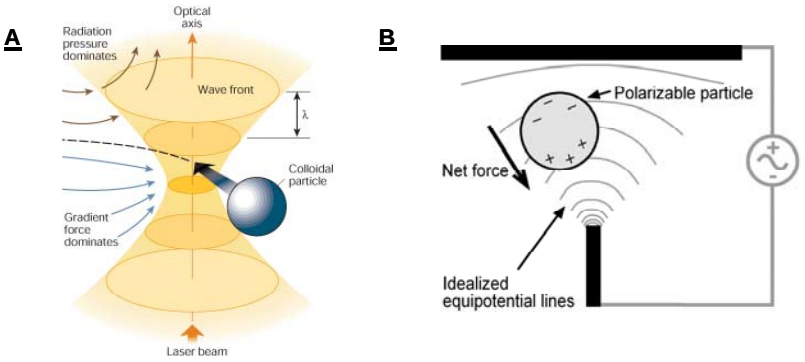

Figure 11: Optical and electrical cell patterning methods. Optical tweezers use a laser beam to trap particles such as cells (Panel A, from Grier, 2003, reprinted with permission). Electrical fields can also be used to trap cells (Panel B, from Gray et al., 2004, reprinted with permission.

Here, the cells are effectively uncharged particles that can be placed onto predetermined arrays of 'traps' in a non-uniform electrical field (Gray et al., 2004; Pethig, 1996; Voldman et al., 2001). Once cells are trapped by the dielectric forces, the electrical field can be turned off and cells will attach and spread. This method may be used for examining the biology of single cells, but has also been expanded to allow for the examination of arrays of cells (Gray et al., 2004). This technology can be also be combined with traditional chemical patterning methods to give added complexity to experimental systems (Gray et al., 2004). Thus, using a combination of micropatterning techniques, cells can be patterned into multicellular arrays that cannot be achieved with random seeding. Cell patterning by DEP also provides another method for studying the interaction of multiple different cell types. Here, one

cell type can be initially trapped; subsequently, a different set of traps can be activated in the presence of a second cell type, allowing for the placement of two or more cell types in desired proximity to each other. This opens up new venues for studying cell-cell interactions and co-culture systems.

Measuring output

In addition to controlling the adhesive and mechanical inputs that cells experience in their surrounding microenvironment, lab-on-a-chip technologies can also be used to measure the mechanical output of individual cells. This is particularly useful in the area of cell mechanics where devices have been made which can be used to study the forces that cells exert on their underlying substrate. Researchers have been devising ways to measure the forces that cells exert on their underlying substrate for decades. Toward this end, much progress was made using deformable membranes (Balaban et al., 2001; Burton and Taylor, 1997; Chrzanowska-Wodnicka and Burridge, 1996; Lee et al., 1994; Lo et al., 2000; Pelham and Wang, 1997; Riveline et al., 2001). Cells plated onto these surfaces pull on the membranes causing deformations or wrinkles. Complex algorithms were developed to calculate the magnitude and distribution of these forces (Balaban et al., 2001; Dembo et al., 1996).

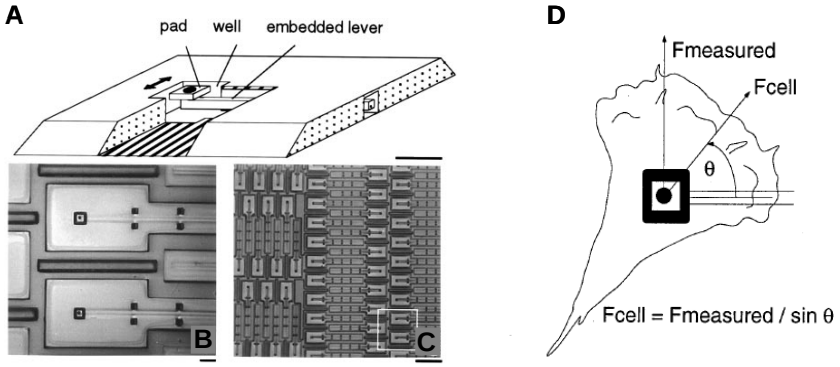

Figure 12: Microfabricated cantilever for traction force measurement. Panel A shows a drawing of the micromachined device, while Panels B and C show two different magnifications of the device. Force is calculated by dividing the measured force by the sine of the angle the cell makes with the cantilever (Panel D). Adapted from Galbraith and Sheetz, 1997.

While useful, this overall approach could not provide unique solutions to the cell force problem (Beningo and Wang, 2002; Dembo et al., 1996; Schwarz et al., 2002). To address this shortcoming, Galbraith et al. developed a chip-based strategy to directly measure cell forces by embedding movable,

horizontally mounted cantilevers into a micromachined device (Galbraith and Sheetz, 1997) (Figure 12). Cells plated onto the cantilevers deflected them as they migrated. Although the calculation of cell forces was straightforward based on cantilever deflection, this technique could only measure the deflection along one axis of the cell and thus generated only a component of the entire cellular force measurement. Building on this concept, Tan et al. used a microfabricated post array detector (mPAD) that consists of vertical rather than horizontal cantilevers (Tan et al., 2003) (Figure 13).

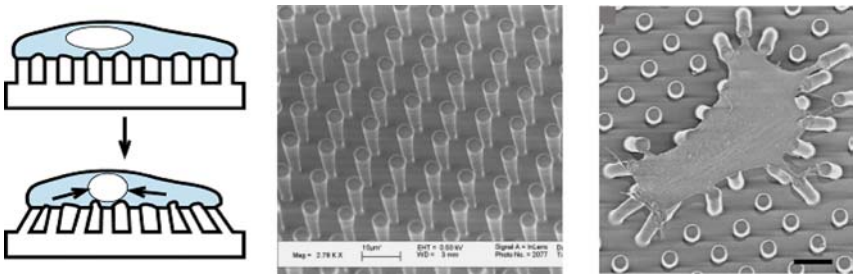

Figure 13: Microfabricated post-array-detectors (mPAD) to measure traction forces. A vertical array of silicone microneedles were fabricated by replica-molding using PDMS. Coating the tips of the microneedles with ECM protein encouraged cells to attach specifically to the tips of microneedles. At the appropriate microneedle spacing, cells spread across multiple microneedles and mechanically deflect them as force is exerted on the underlying substrate. Adapted from Tan et al., 2003.

This mechanosensing device overcame some of the previous limitations in force measurement. A combination of soft lithography and replica molding were used to construct an array of vertical elastomeric cantilevers that deflect in response to traction forces. Based on known physical parameters of the posts, deflections can be measured and force measurements derived. Because the posts are only several micrometers in diameter, cells attach to and spread across multiple posts, allowing these force measurements to be calculated with subcellular resolution. Additionally, because each post deflects independently, a unique solution is reported. Thus, the calculation of such forces is relatively straightforward. Using this system, Tan and colleagues found that intracellular force generation in a cell varied with cell spreading such that well spread cells exerted more average force per post than their less spread counterparts. They also confirmed earlier studies that the magnitude of the force exerted by cells correlated with the size of adhesions formed by cells attaching to the ECM-coated posts (Balaban et al., 2001; Chrzanowska-Wodnicka and Burridge, 1996; Sawada and Sheetz, 2002). This study further strengthened the link between cell shape, cell mechanics, and cell-ECM

adhesion control and highlighted the importance of controlling each of these parameters when studying cellular mechanotransduction. In addition, by varying the height, width, and shape of the posts, the mechanical stiffness of the underlying substrate could be altered. Thus, this system could also be used to vary the mechanical environment, while still maintaining uniform material properties and thus uniform surface chemistry.

IV. Conclusions and future lab on a chip technologies in cell biology

In biology, much effort has been put into understanding the role of soluble cues in cell behavior. While this has resulted in the accumulation of a large body of data delineating various signal transduction pathways that control cell behavior, the story is hardly complete. Most of these data did not take into account the physical microenvironment that surrounds cells and it has become increasingly evident that these physical cues are major regulators of cell function. Lab-on-a-chip technologies are revolutionary in this regard because they are enabling us to examine the physical nature of cells in ways that were not previously possible. We can now begin to ask specific questions wherein we can manipulate the physical microenvironment of cells in a well-controlled fashion and determine how these interventions affect cell function. These physical interventions can ultimately be coupled with studies using soluble factors to gain insight into how these two aspects of biology are coordinated by the cell into a single biological response.

While being able to control single cells, lab-on-a-chip applications also offer many exciting possibilities for studying multicellular systems. These methods may allow us to dissect the contribution of cell-cell interactions in a very controlled way. Advances in the capability to pattern multiple cell-types will allow for more intricate studies of complex arrangements of cells. Future applications of such technologies hold promise in the area of tissue microengineering, an emerging field that builds on the technologies described in this chapter to create small multicellular arrays that may recapitulate the functions of normal tissues (Bhatia, 1999). Such ‘microscale’ tissue constructs show promise as implantable devices. Incorporated into the body, these microscale tissue constructs could expand into larger, more complex tissues. These emerging technologies offer great potential in the area of organ transplantation and tissue regeneration.

Ultimately, the technologies described in this chapter will realize their full potential only after they are integrated into larger systems. For example, the approaches to measure cell forces could be incorporated into microfluidics systems to yield microsensor biochips that could be useful in drug discovery efforts or for cell-based biosensor systems. Cell-based arrays could be integrated into high throughput drug screening assays that would reflect more physiological responses to drug toxicity and efficacy. Analogous chip-based cell systems will be developed for use as diagnostic biosensors, for example

to monitor physiological changes induced by exposure to environmental toxins or pathogens. Thus, the journey into the interface between microsystems and cells has only just begun.

References:

Amiji, M., and K. Park. 1992. Prevention of protein adsorption and platelet adhesion on surfaces by PEO/PPO/PEO triblock copolymers. *Biomaterials*. 13:682-92.

Ashkin, A., J.M. Dziedzic, and T. Yamane. 1987. Optical Trapping and Manipulation of Single Cells Using Infrared-Laser Beams. *Nature*. 330:769-771.

Balaban, N.Q., U.S. Schwarz, D. Riveline, P. Goichberg, G. Tzur, I. Sabanay, D. Mahalu, S. Safran, A. Bershadsky, L. Addadi, and B. Geiger. 2001. Force and focal adhesion assembly: a close relationship studied using elastic micropatterned substrates. *Nat Cell Biol*. 3:466-472.

Beningo, K.A., and Y.L. Wang. 2002. Flexible substrata for the detection of cellular traction forces. *Trends Cell Biol*. 12:79-84.

Bernard, A., D. Fitzli, P. Sonderegger, E. Delamarche, B. Michel, H.R. Bosshard, and H. Biebuyck. 2001. Affinity capture of proteins from solution and their dissociation by contact printing. *Nat Biotechnol*. 19:866-9.

Bershadsky, A.D., N.Q. Balaban, and B. Geiger. 2003. Adhesion-dependent cell mechanosensitivity. *Annu Rev Cell Dev Biol*. 19:677-95.

Bhatia, S.N., U.J. Balis, M.L. Yarmush, and M. Toner. 1998. Probing heterotypic cell interactions: hepatocyte function in microfabricated co-cultures. *J Biomater Sci Polym Ed*. 9:1137-60.

Bhatia, S.N., U.J. Balis, M.L. Yarmush, and M. Toner. 1999. Effect of cell-cell interactions in preservation of cellular phenotype: cocultivation of hepatocytes and nonparenchymal cells. *Faseb J*. 13:1883-900.

Bhatia SN, Chen CS. 1999. Tissue Engineering at the Micro-Scale. *Biomedical Microdevices.* 2:131-144.

Bhatia, S.N., M.L. Yarmush, and M. Toner. 1997. Controlling cell interactions by micropatterning in co-cultures: hepatocytes and 3T3 fibroblasts. *J Biomed Mater Res.* 34:189-99.

Biran, I., D.M. Rissin, E.Z. Ron, and D.R. Walt. 2003. Optical imaging fiber-based live bacterial cell array biosensor. *Analytical Biochemistry*. 315:106-113.

Bohner, M., T.A. Ring, N. Rapoport, and K.D. Caldwell. 2002. Fibrinogen adsorption by PS latex particles coated with various amounts of a PEO/PPO/PEO triblock copolymer. *J Biomater Sci Polym Ed*. 13:733-46.

Burton, K., and D.L. Taylor. 1997. Traction forces of cytokinesis measured with optically modified elastic substrata. *Nature*. 385:450-4.

Carter, D.R., T.E. Orr, D.P. Fyhrie, and D.J. Schurman. 1987. Influences of mechanical stress on prenatal and postnatal skeletal development. *Clin Orthop*:237-50.

Chen, C.S., J.L. Alonso, E. Ostuni, G.M. Whitesides, and D.E. Ingber. 2003. Cell shape provides global control of focal adhesion assembly. *Biochem Biophys Res Commun*. 307:355-61.

Chen, C.S., C. Brangwynne, and D.E. Ingber. 1999. Pictures in cell biology: squaring up to the cell-shape debate. *Trends Cell Biol*. 9:283.

Chen, C.S., M. Mrksich, S. Huang, G.M. Whitesides, and D.E. Ingber. 1997. Geometric control of cell life and death. *Science*. 276:1425-8.

Chen CS, T.J., Tien J. 2004. Mechanotransduction at Cell-Matrix and Cell-Cell Contacts. *Annu Rev Biomed Eng*.

Choquet, D., D.P. Felsenfeld, and M.P. Sheetz. 1997. Extracellular matrix rigidity causes strengthening of integrin-cytoskeleton linkages. *Cell*. 88:39-48.

Chrzanowska-Wodnicka, M., and K. Burridge. 1996. Rho-stimulated contractility drives the formation of stress fibers and focal adhesions. *J Cell Biol*. 133:1403-15.

Corey, J.M., B.C. Wheeler, and G.J. Brewer. 1996. Micrometer resolution silane-based patterning of hippocampal neurons: Critical variables in photoresist and laser ablation processes for substrate fabrication. *Ieee Transactions on Biomedical Engineering*. 43:944-955.

Davies, P.F. 1995. Flow-mediated endothelial mechanotransduction. *Physiol Rev*. 75:519-60.

Davies, P.F., A. Robotewskyj, and M.L. Griem. 1994. Quantitative studies of endothelial cell adhesion. Directional remodeling of focal adhesion sites in response to flow forces. *J Clin Invest*. 93:2031-8.

Dembo, M., T. Oliver, A. Ishihara, and K. Jacobson. 1996. Imaging the traction stresses exerted by locomoting cells with the elastic substratum method. *Biophysical Journal*. 70:2008-2022.

Duncan, R.L. 1995. Transduction of mechanical strain in bone. *ASGSB Bull*. 8:49-62.

Fata, J.E., Z. Werb, and M.J. Bissell. 2004. Regulation of mammary gland branching morphogenesis by the extracellular matrix and its remodeling enzymes. *Breast Cancer Res*. 6:1-11.

Folsom, T.D., and D.S. Sakaguchi. 1999. Disruption of actin-myosin interactions results in the inhibition of focal adhesion assembly in Xenopus XR1 glial cells. *Glia*. 26:245-59.

Galbraith, C.G., and M.P. Sheetz. 1997. A micromachined device provides a new bend on fibroblast traction forces. *Proc Natl Acad Sci U S A*. 94:9114-8.

Geiger, B., and A. Bershadsky. 2001. Assembly and mechanosensory function of focal contacts. *Curr Opin Cell Biol*. 13:584-92.

Geiger, B., A. Bershadsky, R. Pankov, and K.M. Yamada. 2001. Transmembrane crosstalk between the extracellular matrix--cytoskeleton crosstalk. *Nat Rev Mol Cell Biol*. 2:793-805.

Gray, D.S., J.L. Tan, J. Voldman, and C.S. Chen. 2004. Dielectrophoretic registration of living cells to a microelectrode array. *Biosensors & Bioelectronics*. 19:771-780.

Gray, D.S., J. Tien, and C.S. Chen. 2003. Repositioning of cells by mechanotaxis on surfaces with micropatterned Young's modulus. *J Biomed Mater Res*. 66A:605-14.

Grier, D.G. 2003. A revolution in optical manipulation. *Nature*. 424:810-6.

Healy, K.E., B. Lom, and P.E. Hockberger. 1994. Spatial-Distribution of Mammalian-Cells Dictated by Material Surface-Chemistry. *Biotechnology and Bioengineering*. 43:792-800.

Huang, S., C.S. Chen, and D.E. Ingber. 1998. Control of cyclin D1, p27(Kip1), and cell cycle progression in human capillary endothelial cells by cell shape and cytoskeletal tension. *Mol Biol Cell*. 9:3179-93.

Hultgren, A., M. Tanase, C.S. Chen, G.J. Meyer, and D.H. Reich. 2003. Cell manipulation using magnetic nanowires. *Journal of Applied Physics*. 93:7554-7556.

Ingber, D.E. 1990. Fibronectin controls capillary endothelial cell growth by modulating cell shape. *Proc Natl Acad Sci U S A*. 87:3579-83.

Ingber, D.E. 2002. Cancer as a disease of epithelial-mesenchymal interactions and extracellular matrix regulation. *Differentiation*. 70:547-60.

Ingber, D.E. 2003. Mechanobiology and diseases of mechanotransduction. *Ann Med*. 35:564-77.

Ingber, D.E., and J. Folkman. 1989. Mechanochemical switching between growth and differentiation during fibroblast growth factor-stimulated angiogenesis in vitro: role of extracellular matrix. *J Cell Biol*. 109:317-30.

Jones, T.B. 1995. Electromechanics of particles. Cambridge University Press, Cambridge ; New York. xxii, 265 pp.
Kane, R.S., S. Takayama, E. Ostuni, D.E. Ingber, and G.M. Whitesides. 1999. Patterning proteins and cells using soft lithography. *Biomaterials*. 20:2363-76.
Katsumi, A., A.W. Orr, E. Tzima, and M.A. Schwartz. 2004. Integrins in mechanotransduction. *J Biol Chem*. 279:12001-4.
Kumar, A., H.A. Biebuyck, and G.M. Whitesides. 1994. Patterning Self-Assembled Monolayers - Applications in Materials Science. *Langmuir*. 10:1498-1511.
Lee, J., M. Leonard, T. Oliver, A. Ishihara, and K. Jacobson. 1994. Traction forces generated by locomoting keratocytes. *J Cell Biol*. 127:1957-64.
Lo, C.M., H.B. Wang, M. Dembo, and Y.L. Wang. 2000. Cell movement is guided by the rigidity of the substrate. *Biophys J*. 79:144-52.
Manz, A., N. Graber, and H.M. Widmer. 1990a. Miniaturized Total Chemical-Analysis Systems - a Novel Concept for Chemical Sensing. *Sensors and Actuators B-Chemical*. 1:244-248.
Manz, A., Y. Miyahara, J. Miura, Y. Watanabe, H. Miyagi, and K. Sato. 1990b. Design of an Open-Tubular Column Liquid Chromatograph Using Silicon Chip Technology. *Sensors and Actuators B-Chemical*. 1:249-255.
Masumoto, A., Y. Hirooka, H. Shimokawa, K. Hironaga, S. Setoguchi, and A. Takeshita. 2001. Possible involvement of Rho-kinase in the pathogenesis of hypertension in humans. *Hypertension*. 38:1307-10.
McBeath R, P.D., Nelson CM, Bhadriraju K, Chen CS. 2004. Cell Shape, Cytoskeletal Tension, and RhoA Regulate Stem Cell Lineage Commitment. *Developmental Cell*. 6:1-20.
Mooney, D., L. Hansen, J. Vacanti, R. Langer, S. Farmer, and D. Ingber. 1992. Switching from differentiation to growth in hepatocytes: control by extracellular matrix. *J Cell Physiol*. 151:497-505.
Nelson, C.M., and C.S. Chen. 2002. Cell-cell signaling by direct contact increases cell proliferation via a PI3K-dependent signal. *FEBS Lett*. 514:238-42.
Northrup, M., Ching MT, White, RM, Watson, RT. 1993. *Transducers '93*:924-926.
Palecek, S.P., J.C. Loftus, M.H. Ginsberg, D.A. Lauffenburger, and A.F. Horwitz. 1997. Integrin-ligand binding properties govern cell migration speed through cell-substratum adhesiveness. *Nature*. 385:537-40.
Palegrosdemange, C., E.S. Simon, K.L. Prime, and G.M. Whitesides. 1991. Formation of Self-Assembled Monolayers by Chemisorption of Derivatives of Oligo(Ethylene Glycol) of Structure $HS(CH_2)_{11}(OCH_2CH_2)_m$-OH on Gold. *Journal of the American Chemical Society*. 113:12-20.
Parker, K.K., A.L. Brock, C. Brangwynne, R.J. Mannix, N. Wang, E. Ostuni, N.A. Geisse, J.C. Adams, G.M. Whitesides, and D.E. Ingber. 2002. Directional control of lamellipodia extension by constraining cell shape and orienting cell tractional forces. *Faseb J*. 16:1195-204.
Pelham, R.J., Jr., and Y. Wang. 1997. Cell locomotion and focal adhesions are regulated by substrate flexibility. *Proc Natl Acad Sci U S A*. 94:13661-5.
Pethig, R. 1996. Dielectrophoresis: using inhomogeneous AC electrical fields to separate and manipulate cells. *Crit Rev Biotechnol*. 16:921-930.
Pohl, H.A. 1978. Dielectrophoresis : the behavior of neutral matter in nonuniform electric fields. Cambridge University Press, Cambridge ; New York. xii, 579 pp.
Prime, K.L., and G.M. Whitesides. 1991. Self-assembled organic monolayers: model systems for studying adsorption of proteins at surfaces. *Science*. 252:1164-7.
Reich, D.H., M. Tanase, A. Hultgren, L.A. Bauer, C.S. Chen, and G.J. Meyer. 2003. Biological applications of multifunctional magnetic nanowires (invited). *Journal of Applied Physics*. 93:7275-7280.

Resnick, N., H. Yahav, L.M. Khachigian, T. Collins, K.R. Anderson, F.C. Dewey, and M.A. Gimbrone, Jr. 1997. Endothelial gene regulation by laminar shear stress. *Adv Exp Med Biol*. 430:155-64.

Riveline, D., E. Zamir, N.Q. Balaban, U.S. Schwarz, T. Ishizaki, S. Narumiya, Z. Kam, B. Geiger, and A.D. Bershadsky. 2001. Focal contacts as mechanosensors: externally applied local mechanical force induces growth of focal contacts by an mDia1-dependent and ROCK-independent mechanism. *J Cell Biol*. 153:1175-86.

Sawada, Y., and M.P. Sheetz. 2002. Force transduction by Triton cytoskeletons. *J Cell Biol*. 156:609-15.

Schwarz, U.S., N.Q. Balaban, D. Riveline, A. Bershadsky, B. Geiger, and S.A. Safran. 2002. Calculation of forces at focal adhesions from elastic substrate data: The effect of localized force and the need for regularization. *Biophysical Journal*. 83:1380-1394.

Singhvi, R., A. Kumar, G.P. Lopez, G.N. Stephanopoulos, D.I. Wang, G.M. Whitesides, and D.E. Ingber. 1994. Engineering cell shape and function. *Science*. 264:696-8.

Sobek, D., Young, AM, Gray ML, Senturia, SD. 1993. An investigation of microstructures, Sensors, Actuators, Machines, and Systems. *Micro Electro Mechanical Systems, Proceedings*:219-224.

Spiegelman, B.M., and C.A. Ginty. 1983. Fibronectin modulation of cell shape and lipogenic gene expression in 3T3-adipocytes. *Cell*. 35:657-66.

Spooner, B.S., and J.M. Faubion. 1980. Collagen involvement in branching morphogenesis of embryonic lung and salivary gland. *Dev Biol*. 77:84-102.

Sternlicht, M.D., M.J. Bissell, and Z. Werb. 2000. The matrix metalloproteinase stromelysin-1 acts as a natural mammary tumor promoter. *Oncogene*. 19:1102-13.

Sternlicht, M.D., A. Lochter, C.J. Sympson, B. Huey, J.P. Rougier, J.W. Gray, D. Pinkel, M.J. Bissell, and Z. Werb. 1999. The stromal proteinase MMP3/stromelysin-1 promotes mammary carcinogenesis. *Cell*. 98:137-46.

Streuli, C.H., N. Bailey, and M.J. Bissell. 1991. Control of mammary epithelial differentiation: basement membrane induces tissue-specific gene expression in the absence of cell-cell interaction and morphological polarity. *J Cell Biol*. 115:1383-95.

Tan, J.L., J. Tien, D.M. Pirone, D.S. Gray, K. Bhadriraju, and C.S. Chen. 2003. Cells lying on a bed of microneedles: an approach to isolate mechanical force. *Proc Natl Acad Sci U S A*. 100:1484-9.

Thomas, C.H., J.H. Collier, C.S. Sfeir, and K.E. Healy. 2002. Engineering gene expression and protein synthesis by modulation of nuclear shape. *Proc Natl Acad Sci U S A*. 99:1972-7.

Tien, J., C.M. Nelson, and C.S. Chen. 2002. Fabrication of aligned microstructures with a single elastomeric stamp. *Proc Natl Acad Sci U S A*. 99:1758-62.

Voldman, J., R.A. Braff, M. Toner, M.L. Gray, and M.A. Schmidt. 2001. Holding forces of single-particle dielectrophoretic traps. *Biophysical Journal*. 80:531-541.

Waters, C.M., P.H. Sporn, M. Liu, and J.J. Fredberg. 2002. Cellular biomechanics in the lung. *Am J Physiol Lung Cell Mol Physiol*. 283:L503-9.

Whitesides, G.M., E. Ostuni, S. Takayama, X. Jiang, and D.E. Ingber. 2001. Soft lithography in biology and biochemistry. *Annu Rev Biomed Eng*. 3:335-73.

Woolley, A.T., and R.A. Mathies. 1995. Ultra-high-speed DNA sequencing using capillary electrophoresis chips. *Anal Chem*. 67:3676-80.

Xiang, C.C., and Y. Chen. 2000. cDNA microarray technology and its applications. *Biotechnol Adv*. 18:35-46.

Yousaf, M.N., B.T. Houseman, and M. Mrksich. 2001a. Turning On Cell Migration with Electroactive Substrates We are grateful for support of this work by DARPA and the National Institute of Health (GM 54621). This work used facilities of the MRSEC supported by the National Science Foundation (DMR-9808595). M. M. is a Searle

Scholar and an A. P. Sloan Fellow. B. T. Houseman is supported by MD/PhD Training Grant HD-09007. *Angew Chem Int Ed Engl*. 40:1093-1096.

Yousaf, M.N., B.T. Houseman, and M. Mrksich. 2001b. Using electroactive substrates to pattern the attachment of two different cell populations. *Proc Natl Acad Sci U S A*. 98:5992-6.