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Strategies for Engineering the Adhesive Microenvironment

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Cells exist within a complex tissue microenvironment, which includes soluble factors, extracellular matrix molecules, and neighboring cells. In the breast, the adhesive microenvironment plays a crucial role in driving both normal mammary gland development as well tumor initiation and progression. Researchers are designing increasingly more complex ways to mimic the *in vivo* microenvironment in an *in vitro* setting, so that cells in culture may serve as model systems for tissue structures. Here, we explore the use of microfabrication technologies to engineer the adhesive microenvironment of cells in culture. These new tools permit the culture of cells on well-defined surface chemistries, patterning of cells into defined geometries either alone or in coculture scenarios, and measurement of forces associated with cell-ECM interactions. When applied to questions in mammary gland development and neoplasia, these new tools will enable a better understanding of how adhesive, structural, and mechanical cues regulate mammary epithelial biology.

KEY WORDS: cell adhesion; cell mechanics; cell shape; micropatterning; ECM architecture.

INTRODUCTION

Cells exist within a complex and ever-changing tissue microenvironment, which includes soluble factors such as cytokines, an extracellular matrix that contains adhesive proteins, and other neighboring cells. Cells actively sense and respond to these changes in their microenvironment, existing in a state of physiological equilibrium with it. A large body of data suggests that the adhesive microenvironment in particular plays a central role in driving both normal mammary gland development as well tumor initiation and progression (reviewed in *(1)*). Gaining new insights into the mechanisms by which cells detect such information within their microenvironment and respond to it will have major impact on our understanding of both normal and disease processes of mammary gland biology.

The mammary gland is a dynamic structure that undergoes numerous changes during the life of the female mammal, including cycles of proliferation by luminal epithelial cells, myoepithelial cells and stromal cells, differentiation, and apoptosis (reviewed in *(2)*). Consequently, both the glandular structure itself and the surrounding microenvironment are constantly being remodeled. Thus, the mammary gland is a dramatic example of how tissue architecture, or form, and biological function are tightly intertwined *(3)*. Disruption in the communication of mammary epithelial cells with their microenvironment can lead to changes in proliferation of the epithelial cells, as well as in the way that these cells interact with each other and their microenvironment, ultimately causing a breakdown in tissue architecture. Such are hallmarks of breast cancer progression. While it is clear that the adhesive microenvironment, including the extracellular matrix (ECM) organization, cell-matrix interactions and cell–cell interactions play

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Abbreviations used: ECM, extracellular matrix; EG, ethylene glycol; RGD, Arg-Gly-Asp; SAMs, self-assembled monolayers.

an important role in both normal mammary gland development and cancer, we still lack a complete understanding of the signaling pathways controlling these processes. Because so many aspects of the microenvironment change simultaneously with these processes, it is difficult to tease out causal relationships.

The information content in the adhesive microenvironment is encoded both in its composition and its organization on nanometer to micrometer scales *(4)*. At the molecular scale, the arrangement and mobility of ECM ligands can affect integrin clustering and adhesion formation *(5)*. For instance, in two-dimensional cell culture, the flexibility of immobilized fibronectin encourages the formation of fibrillar adhesions *(6)*. In contrast, when the fibronectin is covalently linked to the substrate, the formation of fibrillar adhesions is prevented and only classical focal adhesions form *(6)*. When fibroblasts are plated on three-dimensional extracellular matrices, they form a different type of focal adhesion structure than cells plated on immobilized two-dimenstional ECM *(7)*. These 3D matrix adhesions, like fibrillar adhesions, are also lost when the matrix is cross-linked or fixed. Thus, it is not only the composition of the matrix, but also its physical and mechanical properties—hydration, solvation, rigidity, microstructure—that regulates adhesion formation.

At the cellular and multicellular levels, the adhesive microenvironment can also have dramatic effects on tissue-specific cell function. When taken out of their physiological context and cultured in plastic tissue culture dishes, mammary epithelial cells, as well as other cell types, lose the cues that maintain their in vivo identity or phenotype, and dedifferentiate (reviewed in *(8)*). However, if the ECM is presented as a more malleable substrate, such as with a laminin or collagen gel, mammary epithelial cells will organize into three-dimensional structures that resemble mammary acini *(9,10)*. These structures will also develop physiological functions, such as milk protein production and secretion, when stimulated with lactogenic hormones *(9,11)*. Collectively, these studies show that the spatial presentation of adhesive cues can define the structure and three-dimensional organization of cells as well as their physiological, tissue-specific functions. Yet, despite an appreciation for the importance of these structural inputs, our understanding of the mechanisms by which adhesive cues regulate both normal and cancer cells have remained relatively incomplete, largely as a result of the paucity of tools to manipulate many of the different parameters that define the adhesive microenvironment.

Cell adhesion to the ECM involves the specific binding and clustering of integrins to immobilized ECM ligands, the spreading and extension of cells against the substrate, and the development of mechanical stresses at the adhesive interface caused by the contractile tension of the actin-myosin cytoskeleton (reviewed in *(5,12)*). Importantly, each of these processes of receptor ligation, changes in cell shape and structure, and changes in cell mechanics, appear to generate signals that regulate cell function. Similarly, both the presence of adhesion between neighboring cells, for example via cadherins, as well as the spatial positioning of cell–cell contacts appear to be transduced by cells into functional signals *(13)*. This greater appreciation for the complexity of the adhesion process results from, and has resulted in, the development of several technologies to address not only the control of adhesion receptor ligation but also the structural and mechanical signals encoded by adhesion.

Here, we provide a brief review of recent technical advances that allow investigators to generate well-defined interactions between cells and their local microenvironment in ways that allow one to better isolate which of the many cues within the microenvironment regulate which cellular responses. Most of these advances have been applied to the study of adhesion biology in nontransformed cells and have not yet been translated directly to cancer biology. Thus, one goal of this review is to highlight opportunities for potential advances in understanding the structure-function relationships in cancer biology. In particular, this review will focus on methods arising out of the semiconductor and surface chemistry worlds adapted to engineer biological microenvironments. We will describe strategies to control the specificity and spatial presentation of extracellular matrix ligands to cells, to control cell–cell adhesion, and to measure the mechanical forces generated by cell adhesion. Of note, we will not discuss the plethora of methods used to quantitatively measure cell adhesion, such as by fluorescence energy transfer methods, which have been reviewed in detail elsewhere *(14,15)*. Finally, we will describe areas of current and future work that are now emerging in the field of controlling cell adhesion, organization, and position, and discuss their potential impact in the study of cancer biology.

ENGINEERED SUBSTRATES TO CONTROL CELL ADHESION

Unlike exposure to soluble growth factor cues, the presentation of ECM ligands to cells has been historically difficult to control. Two relatively recent advances in surface engineering have begun to address the challenge of controlled cell adhesion: technologies to engineer substrates with defined surface chemistry, and technologies to control the spatial presentation of this surface chemistry on nanometer and micrometer length scales.

Generating Well-Defined Surface Chemistry

Providing cells with a well-defined substrate, though conceptually simple, is technically difficult. Most proteins, due to the presence of hydrophobic and hydrophilic amino acids within their sequences, tend to accumulate at surfaces ("surface active"). As a result, cell culture media constituents such as serum, which contains a complex mixture of proteins, differentially compete for adsorption to surfaces and leave a complex, ill-defined coating for experimental studies. Traditionally, investigators attempted to provide better defined surfaces by precoating cell culture substrates with a purified ECM protein, followed by blocking any remaining adsorption sites on the substrate with a nonadhesive protein such as albumin. Unfortunately, these surfaces are not inert. The adsorbed albumin is not stable, is vulnerable to degradation by cell-derived proteases, and also necessitates the use of serum-free culture medium to minimize unwanted protein adsorption due to exchange with the surface-bound proteins. Thus, although many chemistries have been developed to immobilize proteins on activated surfaces using the chemical moieties on amino acids *(16,17)*, perhaps the most important advance has been in the development of methods to block nonspecific protein adsorption. By immobilizing a blocking agent to an adhesive surface, one prevents the further adsorption of other proteins *(18)*. Hydrophilic polymers such as polyethylene oxide, polyethylene glycol, polyacrylamide, agarose, or mannitol have been used as blocking agents *(19,20)*. Thus, eliminating nonspecific, or undesired adhesion has been critical to the development of surfaces with bio-specific adhesion.

From a practical perspective, perhaps one of the most useful and flexible experimental systems to engineer surfaces for cell adhesion has been the alkanethiol self-assembled monolayers (SAMs) *(19,21)*. When a solution of alkanethiols (hydrocarbons terminated on one end with a sulfhydryl group) is exposed to a gold surface, they coordinate to the gold through the sulfur atom and self assemble into a highly organized molecular coating, as illustrated in Fig. 1(A) *(25)*. Because the alkanethiol is oriented on the surface such that the terminal group opposite the sulfhydryl group is exposed to the solution, the chemistry of the surface is essentially defined by the chemistry of this terminal functionality. Using alkanethiols with different terminal functionalities has provided a simple means to either resist or promote protein adsorption *(21)*. For instance, hydrophobic SAMs adsorb proteins and can thus promote cell adhesion, while SAMs that terminate in ethylene glycol moieties resist protein adsorption and therefore prevent cell adhesion *(19,21,26)*. To provide more quantitative control over integrin ligation, investigators have developed methods to produce alkanethiols terminated with adhesive peptides, such as Arg-Gly-Asp (RGD). Here, simply varying the mixture of RGD-terminated alkanethiol with ethylene glycol (EG)-terminated alkanethiols directly impacts the relative density of peptide chemisorbed onto the substrate.

With these newfound controls over surfaces, researchers have been able to study molecular aspects of cell adhesion previously not possible. For example, adherent cells deposit their own matrix when plated on surfaces. Because of this, it was not known if short segments of ECM such as the RGD sequence were sufficient to maintain long-term cell adhesion in the absence of augmentation by cell-deposited ECM. Roberts *et al.* tested this question by making use of the exceptional protein repellent characteristics of EG-terminated thiols *(23)*. Surfaces created with RGD-functionalized alkanethiols in a background of EG-terminated alkanethiol were able to resist endothelial cell-secreted ECM deposition, and still supported cell adhesion and survival for extended times (Fig. 1(B)). In another study, the alkanethiol SAMs were used to examine the role of steric hindrance to RGD accessibility in cell adhesion *(24)*. Using RGD-terminated alkanethiol in a bed of EG alkanethiols of varying length (by changing the number of EG repeats, thereby changing the exposure of the RGD moiety), they showed by a variety of measures that cell responses, such as spreading, strongly depend on the steric background against which RGD is presented (Fig. 1(C)) *(24)*. These studies illustrate the degree of control that is now possible in studying receptor-ligand-mediated adhesive interactions.

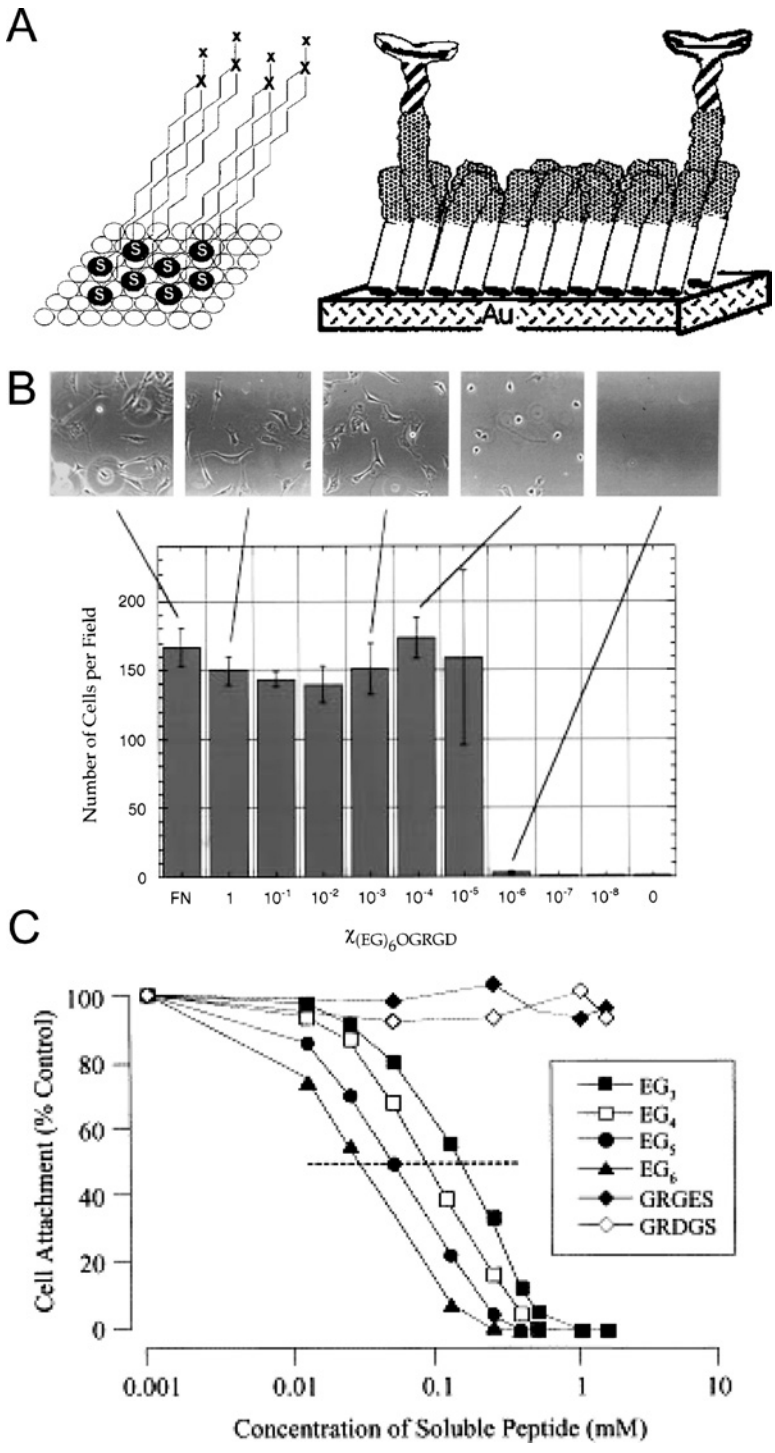

Fig. 1. Schematic representation of self-assembled monolayers (SAMs) of alkanethiols on gold (A) (from *(22)*, reprinted with permission). Surfaces created with varying concentrations of RGD-functionalized alkanethiol on a background of EG-terminated alkanethiol allow for cell adhesion, but variable amounts of cell spreading (B) (from *(23)*, reprinted with permission). RGD-functionalized alkanethiol on a background of EG-terminated alkanethiols of different lengths affects cell attachment capability (C) (from *(24)*, reprinted with permission).

Until now, these tools to present ECM ligands in well-defined systems have been used primarily in the reductionist tradition, to gain insights to the fundamental mechanisms of adhesion. Two unexplored opportunities exist here. First, only a few key receptor-ligand interactions have been extensively studied. A wealth of information remains untapped from the much larger array of less well-studied ligand-receptor pairs, many of which appear to become highly expressed in tumor cells. Because these surface engineered systems display virtually no background adhesion, even relatively weak adhesive interactions can be studied without interference from the usually more dominant components that would mask such interactions on more traditional substrates. Second, as our understanding of the tumor (and other) microenvironments continues to expand, one can adopt these surface engineering approaches to display combinatorially complex mixtures of adhesive ligands, again with some quantitative precision. Thus, both reductionist and constructionist strategies can be used cooperatively to gain some insight into the role of various ligands found within tumor matrices.

Engineering Patterns of Adhesive Regions Onto Substrates

While uniform control of the adhesiveness of a surface has been an important advance, the realization that the spatial heterogeneity of tissue structure is also critical to cell function has led to the development of new tools to engineer such heterogeneity into cell culture systems in a well-controlled fashion *(27)*. Generating surfaces with heterogeneous adhesiveness, where the regions of adhesive and nonadhesive chemistries are prescribed, is called "patterning." Patterning allows the direction of cell adhesion to desired regions of the substrate, and exclusion of cells from other parts, and comprises the most basic level of spatial engineering of adhesion. This class of techniques has been important in identifying how the structure of the microenvironment may be important in regulating cell function.

Early efforts to pattern cells used the deposition of palladium through a nickel mask, to make micrometer-sized adhesive squares of palladium on a nonadhesive background *(28,29)*. While significant insights into regulation of fibroblast migration *(27)*, neuronal guidance *(30)*, and glial cell proliferation *(31)* were obtained using this method, it had the disadvantage that the mechanism of adhesion was not well defined, and it required metal evaporation and specialized masks; hence, the technique was not easily adaptable for general use by the biological research community.

More recently, the advent of microcontact printing has addressed many of these shortcomings and has been widely adopted for patterning cell adhesion to surfaces. Microcontact printing uses rubber stamps containing the desired features to directly print chemistries or proteins onto substrates (Fig. 2(A)) *(36)*. Briefly, photolithography is used to generate an initial mold with an array of micrometer-sized features on a silicon wafer. A prepolymer of PDMS (polydimethylsiloxane) is then cured against this mold and peeled to reveal an elastomeric stamp containing the negative replica of the original mold. Stamps can then be inked with silanes, alkanethiols, or directly with ECM proteins *(37,38)*. When the elastomeric stamp is placed in contact with a surface, the inked material 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 *(26,39,40)*. 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 nonadhesive regions. While microcontact printing with alkanethiols lowers the barrier to micropatterning, it still requires equipment for coating of substrates with gold and requires special chemicals, such as specially modified alkanethiols. In contrast direct printing of proteins could become widely adopted in the biological community, because it is a general-purpose method to print proteins on standard cell culture substrates, without the need for specialized infrastructure *(41,42)*.

Using these surface micropatterning techniques, one can define adhesive and nonadhesive regions on a culture substrate with nearly 50-nm resolution. The sizes of features on the stamps is limited primarily by the method to generate the mold in which the stamp is cast. For example, using a standard high resolution laser printer, photographic film, or microfiche, one can typically generate features of 10 μm or larger with a standard drawing program. For higher resolution, most investigators rely on specialty processes.

These techniques have largely been used to study how the geometric presentation of ECM at the micrometer scale can affect cell function. For example, by culturing endothelial cells on islands of adhesive regions, such that single cells attach onto

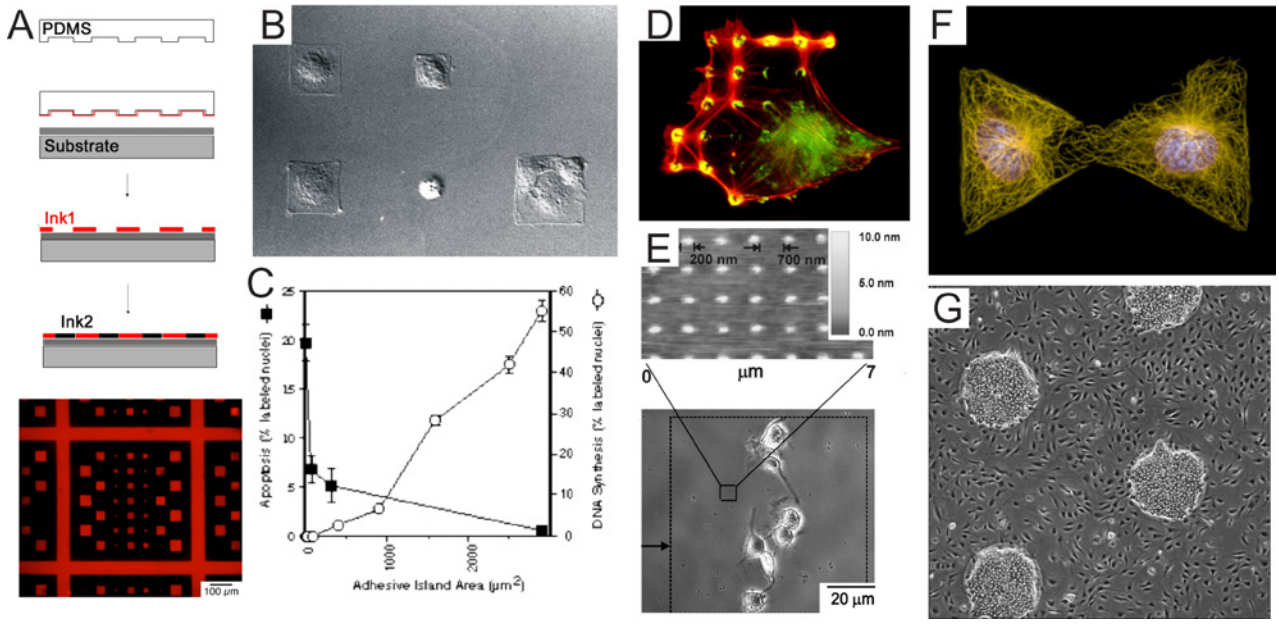

Fig. 2. Schematic outline of microcontact protein printing (A). Endothelial cells plated onto islands of ECM assume the geometry of the stamped region (B) (from *(32)*, reprinted with permission). Cell spreading regulates endothelial cell proliferation and apoptosis (C) (from *(32)*, reprinted with permission). Cells plated on substrates consisting of subcellular-sized islands can spread over multiple smaller islands, while their adhesion is still restricted to the stamped areas (D) (from *(33)*, reprinted with permission). Cells plated on 200-nm-sized ECM generated using dip-pen nanolithography (E) (from *(34)*, reprinted with permission). Cells seeded into bowtie shaped microwells (F) (from *(35)*, reprinted with permission). Coculture micropatterning of hepatocytes (circular structures) and supporting fibroblasts (G) (from S. Bhatia).

individual islands, one can directly constrain the degree to which cells adhere and spread against the substrate (Fig. 2(B)). Cells can even be forced to conform to unnatural shapes, such as squares and triangles *(43)*. This technology has allowed investigators to examine the role of cell shape in regulating cell function. In the context of endothelial cells, we have found that changes in the degree to which cells spread and flatten against a substrate appears to regulate cell proliferation, differentiation and apoptosis (Fig. 2(C)). Interestingly, while there is no doubt that cell adhesion can alter cell shape and structure, these series of studies show that cell shape can in turn regulate the formation of focal adhesions through inside-out mechanisms. These studies further highlight the complex interrelationships between the molecular events of cell adhesion (integrin ligation) and the global changes in cell structure, and how understanding the regulatory relationship between these processes will be critical to our models of adhesion-regulated cell function. While these tools have yet to be applied to mammary epithelial biology, much work has already demonstrated that cell structure and shape play important roles in normal mammary epithelial cell function. For example, it has been shown that plating mammary epithelia onto adhesive substrates causes the cells to spread and flatten against the substrate, lose their polarized organization, and downregulate mammary-specific gene expression *(8–10)*. In contrast, when different mammary epithelial cells both from primary and cell line cultures are more spherically shaped, they acquire a functionally differentiated phenotype *(44)*. In the context of breast cancers, it is thought that cells lose adhesion (and shape) dependent regulation during transformation. In vivo examination of solid breast tumors has revealed a dramatic disorganization of tissue architecture both at the cellular and multicellular levels *(45–47)*. For instance, cancerous mammary epithelial cells have abnormally shaped nuclei and altered cell–cell junctions (reviewed in *(48)*). As well, the gross acinar structure is atypical in breast cancer, losing normal lumenal epithelial organization and becoming apolar (reviewed in *(48)*). As these bioengineering tools become more sophisticated, it is likely that patterned surfaces could be used to quantitatively control adhesion signaling as a means to detect early

changes in carcinogenesis. For instance it is known that the progression of normal cells to a carcinogenic cancer is a multi-step process. Among the later events is the loss of adhesion-dependent proliferation. Because a loss of shape-dependent proliferation control likely precedes this adhesion-independent proliferation, aberrations in shape-dependent proliferation may serve as an early indicator of cancerous progression towards adhesion independence. Thus, using micropatterning techniques to control cell shape could enable a new screening tool to investigate early changes in carcinogenesis.

The adhesive features engineered on substrates are also being decreased to subcellular length scales, such that endothelial cells can spread over multiple smaller islands while their adhesion is still restricted to the stamped areas *(32)* (Fig. 2(D)). One question that has been inspired by this strategy is whether physically restricting the size of adhesive patches below the size of naturally occurring focal adhesions will alter adhesion formation, signaling, and ultimately cell function. Interestingly, recent studies using dip-pen nanolithography to generate patches of ligand down to 100 nm appear to not adversely affect cell adhesion in fibroblasts, in contrast to earlier predictions *(34)* (Fig. 2(E)). Surface scientists are now able to produce substrates where the position of individual protein ligands can be specified, allowing one to control the spacing between ligands with single nanometer precision. As these tools become available to the biological community, many questions concerning the molecular structure and defining features of adhesions will become accessible.

ENGINEERING CELL-TO-CELL INTERACTIONS

In addition to single-cell applications, patterned surfaces have also been used to study cells in multicellular scenarios. Controlling the shape and location of cells distributed on a patterned substrate at a multicellular scale allows one to investigate the effects of cell–cell contact in a well-controlled fashion. Traditional cell culture methods to change cell–cell contact rely on seeding cells at different densities. Seeding randomly introduces 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. (13)* developed a method to independently control cell–cell contacts and cell shape using a chip-based method.

In this technique, elastomeric stamps with bowtie-shaped features were used to create bowtie-shaped microwells. Endothelial 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) (Fig. 2(F)). 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 *et al. (13)* found that cell-cell contact caused a decrease in cell spreading that in turn inhibited cell proliferation. 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 length scale, Bhatia *et al. (49)* used a coculture micropatterning approach to show that liver cells enhance their function when in contact with supporting fibroblasts (Fig. 2(G)). 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 cocultured with fibroblasts demonstrated an increase in specific biochemical markers of hepatocyte differentiation, including urea and albumin secretion *(49)*. Furthermore, cells with quantitatively more heterotypic interactions retained hepatocyte-specific biochemistry, while hepatocytes that were not adjacent to fibroblasts did not retain the markers of hepatocyte differentiation *(50)*.

Heterotypic cell–cell contacts are also critical for breast tissue organization and function *(51)*. Xenograft models have been used to assess the role of such epithelial-fibroblast interactions in mammary gland development *(52)*. Such studies have injected mixed populations of human mammary epithelial cells and fibroblasts into nude mice and have demonstrated that acinar structures form only when mammary stroma is present. Such in vivo experiments

are greatly informative; however, the cells in such an environment are exposed to multiple factors that may affect their biology. Using more well-defined in vitro coculture systems may provide a complement to such studies. The epithelial-stromal interaction also plays a highly critical role in breast cancer initiation and progression (reviewed in *(53)*). Using well-defined in vitro coculture systems that maintain the differentiated phenotype of mammary epithelial cells and their surrounding stroma could address questions relating to the role of myoepithelial cells and fibroblasts in luminal epithelial cell biology. For instance, how are these interactions changed when epithelial cells become transformed? Interestingly, there is a strong relationship between the loss of expression of E-cadherin, a homotypic cell–cell adhesion molecule, and carcinogenesis *(54)*. While such studies suggest an important role for such homotypic interactions in growth control, we have little understanding of the underlying mechanisms. Because cell-to-cell communication is a critical cue for many physiological functions of cells, these tools will become increasingly important to standard biological experiments.

METHODS TO MEASURE THE FORCES OF CELL ADHESION

In addition to controlling the adhesive and structural inputs that cells experience in their surrounding microenvironment, these same surface patterning technologies are also being developed to measure the mechanical output of individual cells. Cell adhesion not only involves the binding of integrins and subsequent changes in cell shape, but also the accompanying generation of cytoskeletal tension. Because integrin-mediated adhesions grow and mature in response to mechanical stresses generated by cytoskeletal tension *(55,56)*, observing the changes in these forces is critical to understanding the dynamics of adhesion.

During the process of adhesion, cells exert nanonewton-scale contractile forces against their substrates *(57)*. Studies of these forces have relied principally on the culture of cells on soft materials such as uniformly cross-linked hydrogels or deformable elastic membranes *(55,58–62)*. Cells plated onto these surfaces generate contractile forces that pull on the membranes, causing deformations or wrinkles. Initial experiments using thin membranes demonstrated that cells exerted contractile forces through the formation of microscopic wrinkles in the membranes, but the wrinkles made quantification of these forces difficult *(66)*. Quantitative improvements came with the development of traction force microscopy *(63)*. Here, cells are plated onto a membrane that is prestressed to prevent wrinkling of its surface. Forces generated by the cells deform the membrane only slightly, so displacements are estimated with spherical beads embedded within the membrane or microfabricated regular arrays of fluorescent particles *(64)*. However, because the displacements depend in a nonlocal way on the applied forces, extracting the stress field produced by the cell requires inversion of an integral equation, which does not always lead to a unique solution *(65)*. In consequence, it has been necessary to adopt simplifying assumptions about the structure of the force field *(59,66,67)*.

An alternative approach used a microfabricated device to directly measure cell forces by embedding movable, horizontally mounted cantilevers into a micromachined device *(68)*. Cells plated onto the cantilevers deflected them as they migrated. Because the moveable unit in these devices (the cantilever) is mechanically decoupled from its surroundings, measurements of these deflections give a direct value for the local force generated at the cell surface. 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.

To address these limitations, Tan *et al. (69)* developed a microfabricated postarray detector (mPAD) that consists of vertical rather than horizontal cantilevers (Fig. 3(A) and (B)). The device consisted of a large array of closely spaced, vertical microneedles, where each microneedle acts as a deformable cantilever. A combination of soft lithography and replica molding as used to construct the array of vertical elastomeric cantilevers. Smooth muscle cells cultured onto the device attach and spread across the tips of multiple microneedles, and bend the posts as the cells probe the tips *(69)* (Fig. 3(C)). For small deflections, the posts behave like simple springs so that their deflections are directly proportional to the local forces applied by the attached cell (Fig. 3(D)—(F)). Because each post moves independently of its neighbors, its deflection directly reports the direction and magnitude of the local cell-generated force without the need for

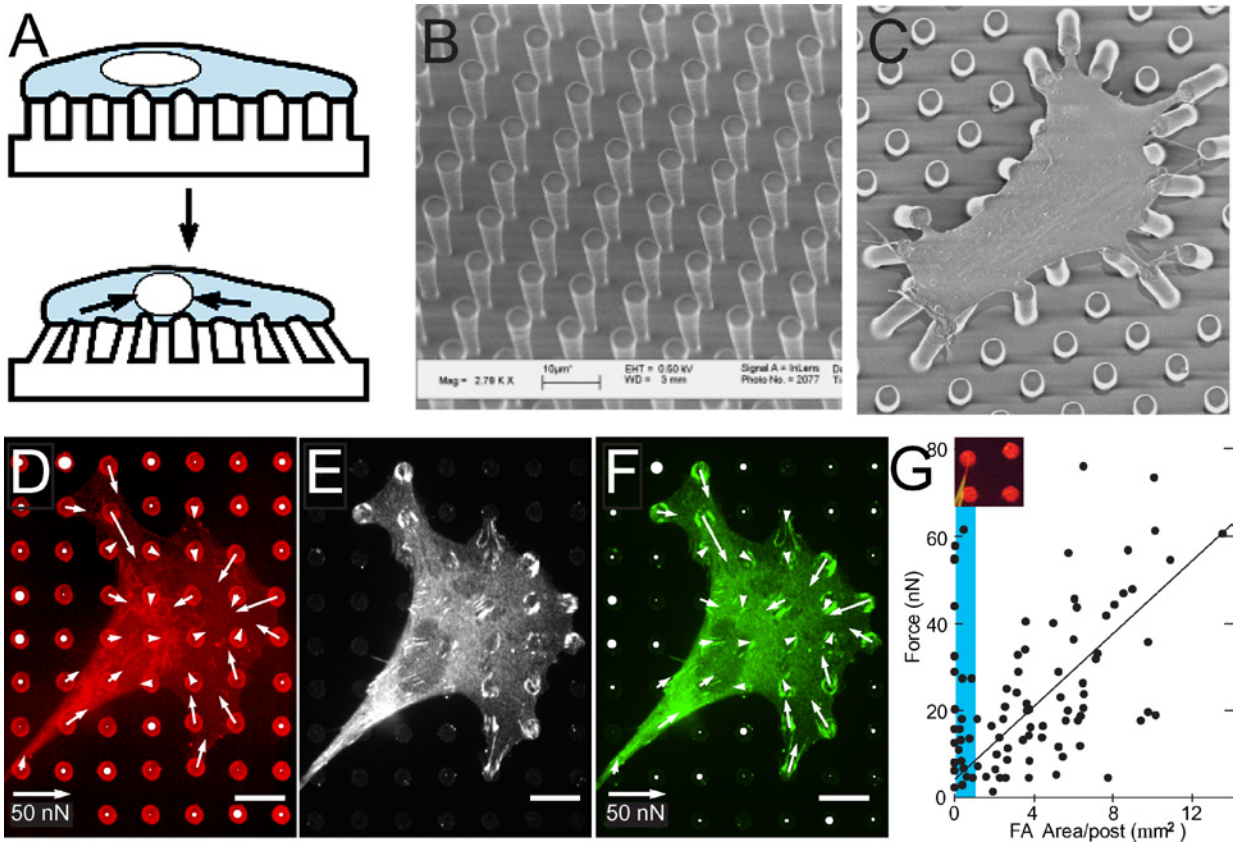

Fig. 3. Microfabricated postarray detectors (mPAD) (A and B). A vertical array of silicone microneedles were fabricated by replica-molding using PDMS. Coating the tops of the microneedles with ECM protein encouraged cells to attach specifically to the tips of microneedles (C). At the appropriate microneedle spacing, cells spread across multiple microneedles and mechanically deflect them as force is exerted on the underlying substrate (D, fibronectin; E, vinculin; F, force vectors). The magnitude of the force exerted by cells correlates with the size of adhesions (G). All panels from *(69)*, reprinted with permission.

a priori assumptions or complex calculations. 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 *(55,64)* (Fig. 3(G)). 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.

THE FUTURE OF ENGINEERED MICROENVIRONMENTS IN CANCER BIOLOGY

Much effort has been invested in studying 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. It has become increasingly evident that these physical cues are major regulators of normal cell physiology, as well as of the pathophysiology of cancers.

In this regard, the advent of tools to engineer well-defined adhesive microenvironments is revolutionary because these tools are enabling studies of the physical nature of cellular regulation in ways that were not previously possible. We can now begin to ask specific questions about how specific multicellular organizations, cell–cell interactions, cell architectures, and molecular organization of adhesions are transduced by cells to regulate the phenotypic states of cells. However, the journey into the interface between engineered microenvironments and cells has only just begun.

In contrast to the *in vivo* microenvironment, for example, of the mammary gland, current engineered systems are woefully simplistic. The spatial arrangement of cells of many origins, the complexity of the basement membrane, and the three-dimensional architecture schematically depicted (Fig. 4(A)) provide only a glimpse into the degree of complexity that must be infused into our engineered artificial microenvironments. Along these lines, several more sophisticated micropatterning technologies are currently being developed. To address the issue of cellular complexity, a few techniques have been demonstrated that place multiple cell types in specific arrangements with respect to one another. One example of this is to use rubber stencils sealed against a substrate, such that cells can be plated through holes in these stencils onto the substrate. By using stencils, or membranes, with holes of different sizes and placement, one can deliver several cell types to specific locations within a pattern (Fig. 4(B)). To address the shortcoming that current engineered surfaces are static, investigators are developing SAMs that can be triggered to actively capture or release adhesive ligands dynamically, while cells are cultured on the surface. This approach allows one to change the pattern of ECM on a surface, for example by capturing RGD peptide onto previously nonadhesive areas *(72)* (Fig. 4(C)). This technology holds much promise, particularly in the area of cell migration. Using the switchable SAMs would allow one to establish an initial pattern of cells and later release cells to migrate from these patterns. Other studies in cell migration have been aided by the use of microfabricated microfluidic channels which have been used to provide a chemotactic gradient to neutrophils. Because these channels can generate concentration gradients with arbitrarily specified profiles, they have allowed investigators to more rigorously investigate the transduction mechanisms of migratory cells in ways not previously possible *(73)*. While the studies on two-dimensional culture have been informative, perhaps the greatest unmet challenge is the development of engineered three-dimensional microenvironments with the same degree of control that has been accomplished in two-dimensional systems.

Nonetheless, it is clear that new tools to engineer the adhesive microenvironment will continue to impact our understanding of tissue physiology and

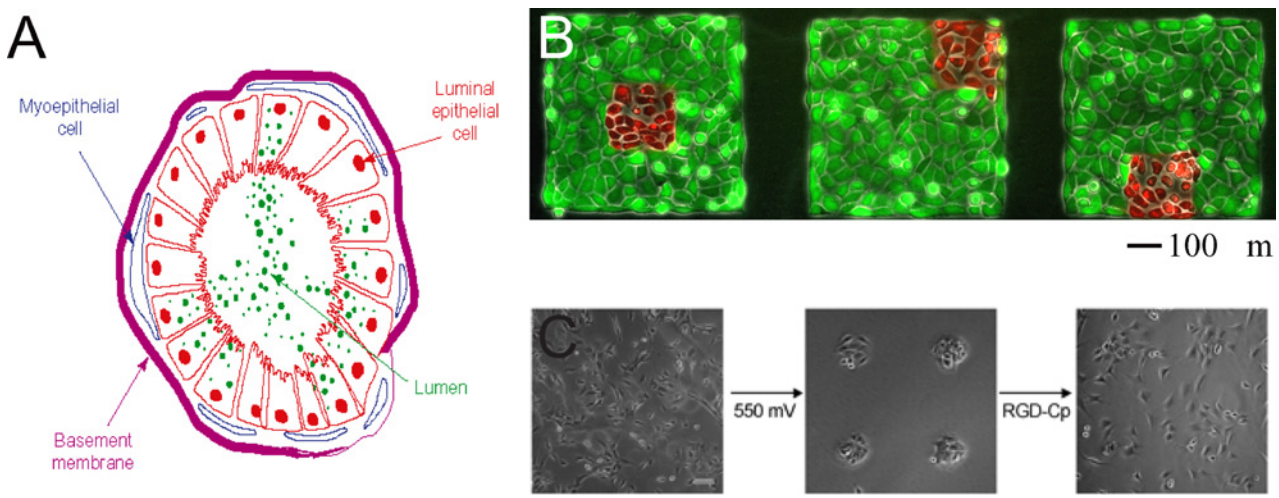

Fig. 4. Schematic drawing of a three-dimenstional mammary acinus (A) (from (1), reprinted with permission). Coculture of NRK cells (red) and fibroblasts (green) (B) using a multilevel stamp (from *(70)*, reprinted with permission). Cells plated on electroactive SAMs. Application of a potential to the underlying gold activates the surface and enables the linkage of RGD peptides to previously nonadhesive regions, so that cells can migrate into the newly adhesive regions (C) (from *(71)*, reprinted with permission).

cancer progression. The tools available to the community today already can enable a better understanding of how adhesive, structural, and mechanical cues regulate mammary epithelial biology. Ultimately, the realization of the potential of these engineered systems depends on the degree to which the biological and engineering communities continue to build toward the common goal of understanding cell and tissue pathophysiology.

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