

# Engineering biomaterials to control cell function

In this review, we highlight some of the recent advances in understanding how environmental cues, presented through cellular adhesions, can regulate cellular processes such as proliferation and differentiation. We discuss how these findings may impact design considerations for new materials in biology.

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Understanding the interactions between cells and materials is important for the development of new materials for biological applications<sup>1</sup>. To study cell biology, cells are typically removed from their host organism and cultured on a plastic culture dish – an environment unlike their natural, physiological environment<sup>2</sup>. Thus, cells tend to lose many of their normal functions and often dedifferentiate for reasons that are not well understood. Recognizing the microenvironmental cues that affect cellular phenotype and function will contribute to our general understanding of cells, as well as provide direct approaches to engineer artificial tissue for medical applications<sup>3,4</sup>.

The current strategy for developing tissue-engineered constructs involves combining cells with a scaffold. The scaffold provides the initial structural integrity and organizational backbone for cells to organize and assemble into a functioning tissue<sup>5</sup>. The ability to control cell position and function directly within these artificial environments is critical to the eventual success of this industry. Therefore, biologists and materials scientists alike seek to understand how the local interactions between

cells and their surrounding microenvironment can regulate cellular behavior. Tools taken from traditional materials engineering are now being adopted to create spatially and structurally defined biological microenvironments, and are providing important new insights into how cells probe their surroundings<sup>6</sup>. These studies will not only give an insight into the basic understanding of how cells function within our bodies in both normal and pathological conditions, but will also contribute to the development of new medical therapies.

Cells are embedded within a complex and dynamic microenvironment consisting of the surrounding extracellular matrix (ECM), growth factors, and cytokines, as well as neighboring cells (Fig. 1). Cell adhesion to the ECM scaffolding involves physically connecting to the ECM proteins through specific cell surface receptors. Integrins are the major transmembrane receptors responsible for connecting the intracellular cytoskeleton to the ECM<sup>7–10</sup>. Binding of integrins to ligands decorating the ECM induces integrins to cluster into focal adhesions. These adhesive processes trigger a cascade of intracellular signaling events that can lead to changes in cellular behaviors, such as growth,

migration, and differentiation<sup>11,12</sup>. Since materials derived from natural ECM, such as collagen, provide natural adhesive ligands that promote cell attachment through integrins, they have been an attractive starting point for engineering biomaterials (materials for biological applications)<sup>13</sup>. However, a major drawback of collagen and other biological materials is that our ability to control their chemical and physical properties is limited. The discovery of short peptide sequences that initiate cellular adhesion, such as arginine-glycine-aspartic acid (RGD)<sup>14</sup>, have allowed scientists to develop biologically inert synthetic polymers onto which these adhesive peptides can be conjugated<sup>15-19</sup>. This method enables independent control of materials chemistry and substrate adhesivity. Furthermore, growth factors can be conjugated to the same synthetic polymers, allowing the presentation of such diffusible molecules to cells in a spatiotemporally controlled manner<sup>20-22</sup>. Thus, specifying the chemical environment of cells is a well-established method of controlling cellular adhesion and growth.

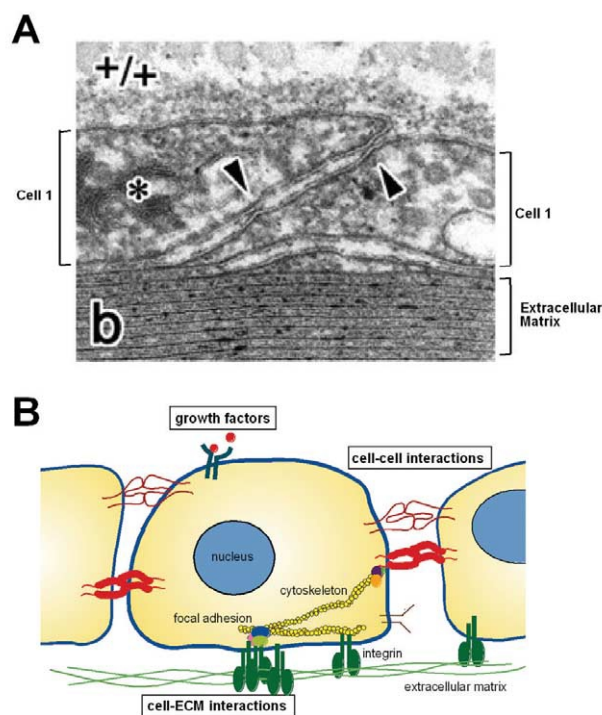
While much effort in developing new materials for biological applications has been focused on chemical properties, biologists have long observed that cells are also sensitive to their physical environment. Early studies of chick heart fibroblasts demonstrated that the curvature of a cylindrical substrate affects cellular alignment and migration<sup>23</sup>. As with investigating the effects of chemical cues on cells, the main

challenge in studying structural cues is to manipulate one factor precisely without changing other environmental factors. Here, we review several aspects of the physical microenvironment that affect cellular behavior. In particular, we will discuss the effects of geometric presentation of adhesive ligands, substrate stiffness, and externally applied mechanical forces on cellular adhesion and proliferation or differentiation. We will present new tools derived from materials-engineering technologies that have been used to isolate the effects of these structural and mechanical cues.

## Geometric presentation of adhesive cues

Initial studies of cell adhesion used fairly crude methods to vary the degree of cellular adhesion, for example by varying the density of adhesive ligands coated on the whole culture surface<sup>24,25</sup>. These techniques, which are widely used and accepted by biologists, do not control the spatial arrangements of cells or adhesive ligands. For example, the only way to control the degree of cell-cell juxtaposition (and adhesion) is by varying the cell density of the entire culture. Engineering tools were needed to control the geometric presentation of adhesive ligands; these techniques have progressed over the past decade and are now accessible to many cell biologists. Soft-lithography methods, derived from microfabrication technologies in the semiconductor industry, have been used to control ligand placement and configuration on the surface, allowing precise control of both cell-ECM and cell-cell adhesion<sup>26,27</sup>. While photolithography can generate submicron-sized features (limited by the wavelength of light), patterning adhesive ligands on the cellular scale only requires features that are tens of microns in size. Thus, soft lithography has been easily tailored to the study of cells by adapting the method to a number of common tissue-culture materials<sup>28</sup>. Briefly, a poly(dimethyl siloxane), or PDMS, rubber stamp is made using a photolithographically generated Si master. The stamp is coated with ECM proteins, such as collagen or fibronectin, and the proteins are then stamped onto the cell-culture surface to yield a pattern dictated by the rubber stamp. When the unstamped regions are blocked with a nonadhesive, cells adhere and spread onto the micron-sized adhesive islands and are prevented from spreading onto the nonadhesive regions (Fig. 2). By engineering where the adhesive ligands are presented on the surface, one thus defines the position of cells in the culture. By controlling the shape and size of the islands, one determines the shape and degree to which cells spread and flatten against the substrate. These patterns are viable for several days to weeks, depending on the type of nonadhesive material used<sup>29</sup>, enabling both short- and long-term biological studies.

While it has long been postulated that cell spreading or shape influences a variety of cellular behaviors, including migration,



**Fig. 1** Cells are embedded within a complex microenvironment. (A) Electron micrograph of two cells (arrows indicate junctions). (B) Schematic of a cell and its surroundings.

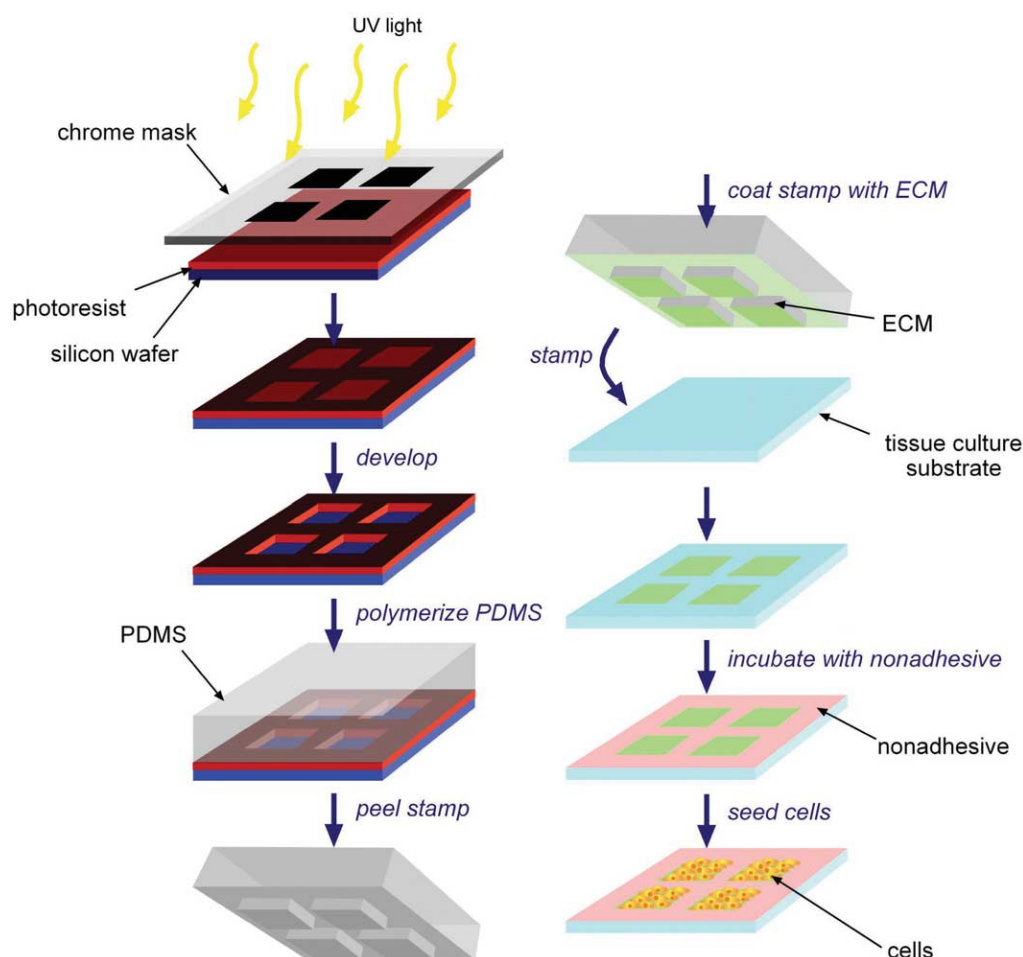


Fig. 2 Schematic of micropatterning process. PDMS stamps are generated by photolithography (left column) and, using these stamps, ECM proteins are printed onto tissue-culture substrates upon which cells are seeded (right column).

proliferation, and differentiation, micropatterning techniques have provided the key tool to demonstrate that cellular architecture is an integral mechanism by which cells regulate their behavior (Fig. 3). Studies of proliferation show that the degree of cell spreading controls proliferation<sup>30</sup>. A recent study demonstrated that cell shape can direct the fate of mesenchymal stem cells (MSC) – adult stem cells that are derived from bone marrow and can differentiate into a number of mesenchymal lineages, including bone, fat, muscle, and cartilage<sup>31</sup>. While soluble factors are commonly used to differentiate these cell types into different lineages, this study demonstrated that cell shape influences differentiation independently of the soluble environment. These cellular responses to cell spreading or shape appear to be regulated by forces generated through the cytoskeleton and cell-ECM adhesions<sup>32,33</sup>. Furthermore, examination of cells that are patterned on different polygonal shapes reveals oriented actin filaments and directed cell migration<sup>34,35</sup>. These studies suggest that the spatial arrangement of adhesive cues around the cell and mechanical structures within the

cell are intricately linked. Although it has been demonstrated that cell shape has important consequences in several biological outputs, including proliferation and differentiation, there are likely to be countless other proteins, signaling pathways, and cellular functions that are affected. Understanding how physical parameters, such as cell shape or cytoskeletal structures, are linked to biochemical processes has now become a major focus of the biological research community. The integration of biological tools with engineering technologies has unleashed a plethora of questions for biologists and biomedical engineers to answer together.

Similar lithographic approaches have been used to pattern cells at a multicellular scale simply by creating island sizes that are either larger or in a particular geometry suited for multiple cells<sup>27,36–38</sup>. For many organs, the alignment and arrangement of cells is critical to the overall function of that organ. In the context of heart tissues, the alignment of the myocytes that generate contractile force is critical to the proper conduction of electrical currents and mechanical coordination of the

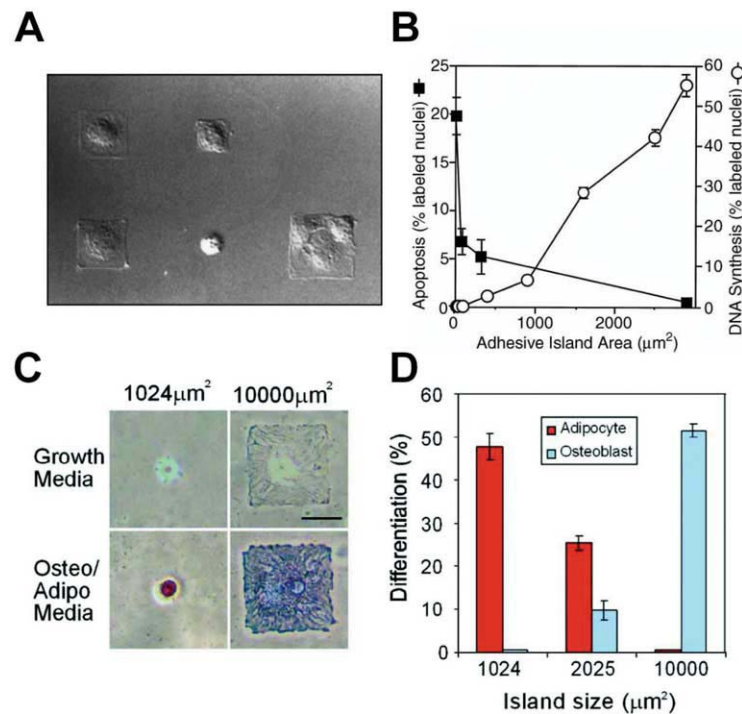


Fig. 3 Cell spreading affects proliferation and differentiation. (A) Bovine pulmonary artery endothelial cells patterned on different-sized islands, and (B) the growth or apoptotic response of these cells. (Reprinted with permission from<sup>30</sup>. © 1997 American Association for the Advancement of Science.) (C) Mesenchymal stem cells patterned on small and large islands stained for fat (Oil Red O, left) or bone (alkaline phosphatase, right), and (D) the quantified differentiation of these cells. (Reprinted with permission from<sup>31</sup>. © 2004 Elsevier.)

tissue. At a smaller scale, to maintain control of both cell-ECM and cell-cell adhesion between just two cells in culture, a bowtie-shaped structure has been used in which each cell typically fills exactly one half of the bowtie and a contact is formed across the middle (Fig. 4A)<sup>27</sup>. Patterning larger groups of cells on larger islands has revealed that cells have the ability to sense their local environment on a multicellular scale. Multiple cells patterned on large squares have a distinct growth pattern that depends on their position within the square island (Fig. 4B)<sup>39</sup>, demonstrating important feedback from multicellular structure to the

behavior of individual cells. While such shapes, geometries, and techniques are generally not seen in the normal life of a cell, these experiments have uncovered many important insights into the inner workings of these cells. By isolating specific adhesive and structural cues, such studies have demonstrated the importance of these material properties in influencing cellular behavior.

Microfabrication tools have been valuable in exploring the fundamental biology of how cells respond to the adhesive cues in their microenvironment, but there are also many applications. As we

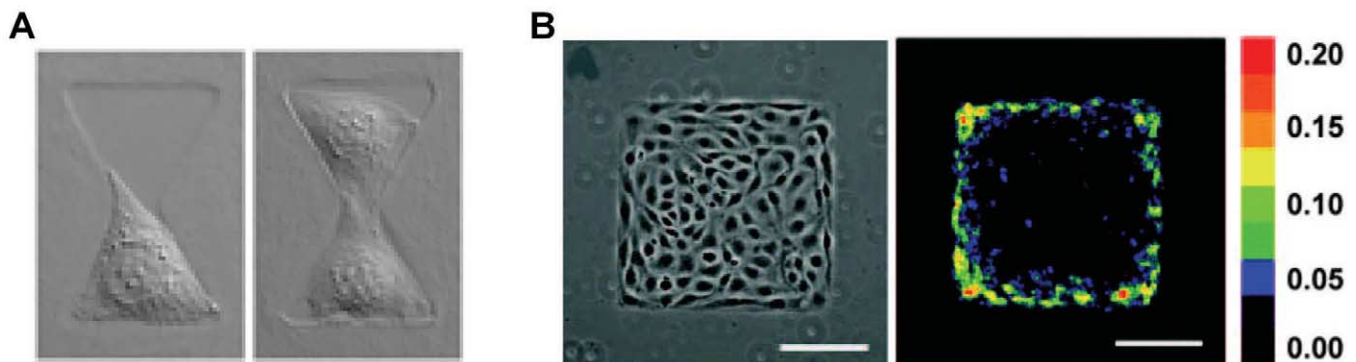


Fig. 4 Patterning of multicellular aggregates. (A) Single (left) and pair (right) of bovine pulmonary artery endothelial cells patterned in bowtie-shaped microwells. (Reprinted with permission from<sup>75</sup>. © 2004 American Society for Cell Biology.) (B) Phase image (left) and proliferation index (right) of cells patterned onto a large island of fibronectin. (Reprinted with permission from<sup>39</sup>. © 2005 National Academy of Sciences.)



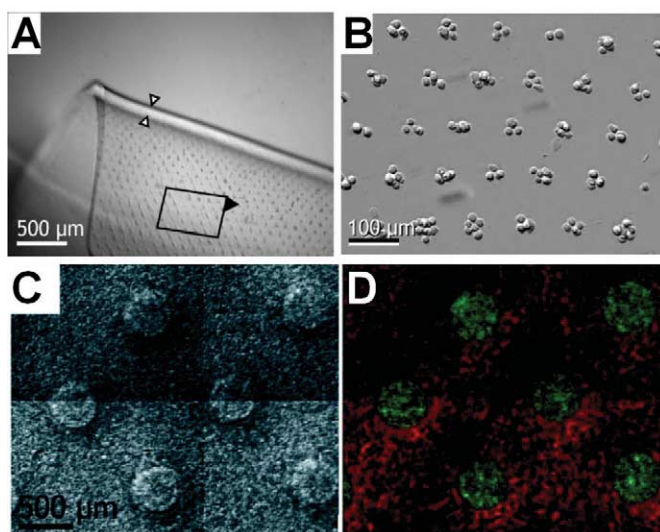


Fig. 5 (A and B) Fibroblasts patterned by dielectrophoresis into clusters separated by 100  $\mu\text{m}$  within a 100  $\mu\text{m}$  thick poly(ethylene glycol) hydrogel. (C and D) Red- and green-labeled liver cells patterned within a 250  $\mu\text{m}$  thick hydrogel using photopatterning. (Reprinted with permission from<sup>41</sup>. © 2005 Royal Society of Chemistry.)

understand more about how cells interact with their adhesive environment, we can direct cell position or fates in tissue-engineered constructs or cellular therapies. In more advanced systems, multiple cells or cell types can be placed in an ordered configuration. Proof of this concept has been achieved using liver cells, where a co-culture of hepatocytes and fibroblasts leads to improved hepatocyte function compared with hepatocytes cultured alone<sup>40</sup>. While patterning technologies have mostly been achieved in two-dimensional cultures, micropatterning in three dimensions will be necessary to create constructs that are useful for engineering larger tissues. A recent study has demonstrated that cells can be patterned within a three-dimensional matrix either by using electrical forces or by photopolymerization of the hydrogel (Fig. 5)<sup>41</sup>. While micropatterning has been useful in an academic setting, great strides are essential for these techniques to be used in an industrial setting. Ensuring that cell health is not compromised and scaling-up for larger scale production will both be necessary for evolving such methods into practical technologies for clinical applications.

## Material stiffness

Although most of our understanding of adherent cells is derived from experiments on cells cultured on very hard surfaces – plastic culture dishes or glass substrates – tissues within our bodies have a variety of different stiffnesses (defined as the Young's modulus, or elasticity, of a material). Bone tissue is very stiff (~18 000 Pa), but brain tissue is soft (~2500 Pa). When diseases occur, the stiffness of tissues and the matrix

surrounding the cells is often altered. For example, scar tissue and tumor samples generally have a higher stiffness (~4000 Pa for breast tumors) compared with their normal, healthy tissue counterparts (~150 Pa for mammary glands)<sup>42</sup>. These observations have led to the supposition that surrounding tissue stiffness might impact cellular behavior.

Just as a gymnast performing a tumbling routine prefers a spring-loaded floor rather than foam cushions or a concrete floor, a cell also favors a certain mechanical environment to execute its own acrobatics. As a cell binds to a substrate and forms adhesions, forces are generated from the cytoskeleton to these adhesive bonds, allowing the cell to spread. The stiffness of the substrate determines the magnitude of these forces and the extent of cell spreading that ensues<sup>43,44</sup>. As it tugs on its surroundings, a cell can create a larger force at the adhesion if the substrate is stiff, but not soft. It is thought that cells strengthen their linkages to the ECM proportionally to the apparent rigidity of the substrate through the clustering of integrins and the formation of focal adhesions<sup>45,46</sup>.

A simple method typically used to alter the stiffness of a natural polymer is to change its concentration, since the elasticity of semiflexible biopolymers that form viscoelastic networks has been reported to be proportional to the concentration squared<sup>47</sup>. While this method is easy to execute, it is not clear whether the resulting changes in cellular behavior are caused by changes in substrate stiffness or changes in chemical composition (e.g. adhesive ligand density). In order to isolate the effects of substrate stiffness without changing material chemistry, several groups have employed synthetic polymers. Polymers such as polyacrylamide or poly(ethylene glycol), which are not conducive to cell attachment or protein adsorption, can be made with a wide range of stiffnesses by changing the crosslinking density. Conjugating or coating with an adhesive ligand renders the material adhesive to cells<sup>48</sup>. These substrates have reasonable chemical and mechanical specificity. To achieve three-dimensional environments of varied stiffnesses, combinations of natural and synthetic polymer constructs have been used. For example, natural polymer gels can be attached to polyacrylamide gels of different stiffnesses<sup>49</sup> or released from a substrate entirely<sup>50</sup>. However, the composition of the matrix is again not well defined. A more defined three-dimensional stiffness matrix would be comprised of a synthetic polymer with varied crosslinker densities and conjugated with a specific density of adhesive peptides. The investigation of different materials that can be used to control stiffness has only recently begun, so there are likely to be many alternative ways to achieve materials with spatially controlled three-dimensional stiffness properties that have not yet been explored.

Substrate stiffness appears to regulate proliferation and differentiation depending on cell type (Fig. 6)<sup>51</sup>. Myocytes differentiate

and form striations on substrates with intermediate stiffnesses, but not on substrates with stiffnesses that are too high or too low<sup>49</sup>. Endothelial cells on soft substrates form capillaries or tube-like structures, but tend to be more spread and proliferate on rigid substrates<sup>25,52</sup>. Neurons prefer to grow and form branches on a soft substrate compared with a stiff substrate<sup>53,54</sup>. Remarkably, the stiffnesses of materials on which these observations were made *in vitro* correlate with physiological stiffnesses of these tissues. Furthermore, normal fibroblasts have the ability to sense different stiffnesses but transformed fibroblasts (cells derived from normal cells but treated so they can proliferate indefinitely in culture and are hence malignant) do not<sup>55</sup>. These data strongly suggest that pathologies result from both changes in tissue stiffness and a loss in the cells' ability to sense their surroundings.

Although the concept that cells can sense the mechanics of their underlying substrate has been gaining acceptance, the molecular basis is still not well understood. A more detailed model of how different cells

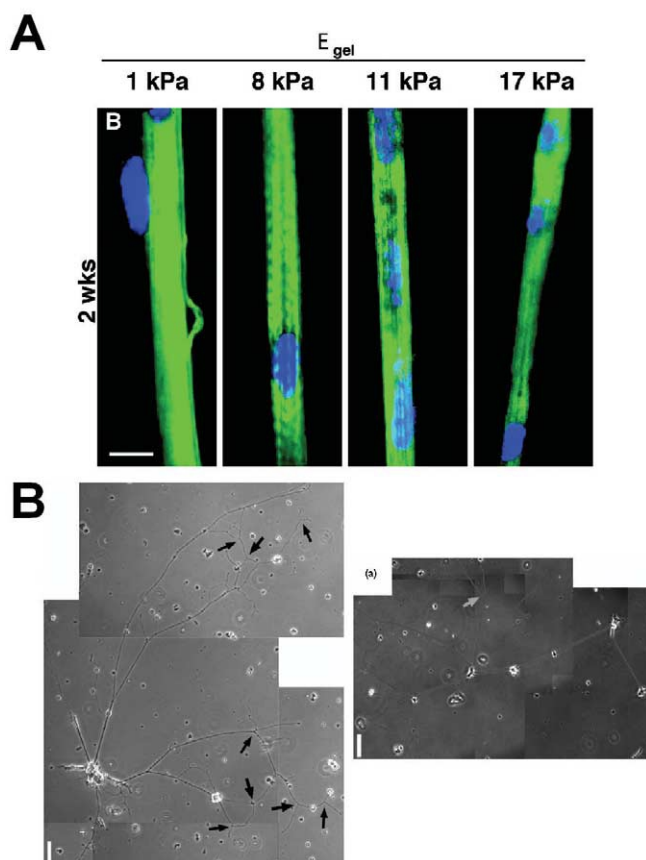
respond to the stiffness of their surroundings, and how these functions go awry during disease, will be valuable when developing new biomaterials. It has been demonstrated that cells respond dynamically to spatial gradients of stiffness<sup>48,56</sup>, and it is likely that cells will also respond differently to dynamic changes in stiffness. While it is understood that biomaterials that are too stiff or too soft may cause undesirable proliferation or differentiation and the eventual futility of a biomedical device, the design of new and 'smarter' materials will probably require spatially and temporally controlled stiffnesses. Reaching these goals will undoubtedly require collaborative efforts and significant cross communication between biologists and engineers.

## Externally applied mechanical forces

Since substrate stiffness acts as a passive influence on cellular mechanics, it is not surprising that active mechanical forces also affect cellular adhesion and intracellular tension. In the body, forces have known functions in the maintenance of healthy tissues, and aberrant forces often lead to pathological conditions. For example, bone and cartilage tissues are subject to compressive forces and the endothelial cells that line the walls of blood vessels experience shear stress and stretch forces. Loss of compressive loading of the skeleton, such as microgravity, often leads to degradation of bone and cartilage, and enhanced shear levels and turbulent flow are associated with vascular diseases at these locations, such as hypertension and atherosclerosis.

Early studies of cellular mechanotransduction used uniformly applied mechanical forces on cells cultured on deformable silicone membranes. These studies suggested that cellular adhesions and an intact cytoskeleton were required for cells to respond to mechanical forces<sup>57,58</sup>. Recent studies have demonstrated that forces applied directly on adhesions cause changes in adhesive structure and intracellular signaling. More specifically, pulling small, nascent adhesions with micron-sized beads or pipettes causes the assembly of adhesion components, and thus adhesion growth, maturation, and strengthening (Fig. 7A)<sup>59,60</sup>. Stretch has also been observed to increase integrin affinity to the ECM<sup>61</sup>. These studies suggest that mechanical structures within the cell support mechanical forces transduced from the outside of the cell and through the cell membrane. The changes in molecular signaling that ensue also feed back to alter the forces felt at the adhesions. The molecular mechanism that determines how these forces are transmitted into biochemical signals is still being unraveled, and likely involves the coordination of many molecules and signaling pathways.

The sensitivity of cells to mechanical forces is not limited to pulling or tensile forces, nor is it restricted to mechanosensing at cell-ECM adhesions. In addition to pulling or tensile forces, cells can sense a wide array of mechanical forces, including shear flow (Fig. 7B)<sup>62</sup>. In many



**Fig. 6** Cells respond differently to substrate stiffness. (A) Myoblasts cultured within collagen gels on polyacrylamide gels of indicated stiffnesses. Striations are most prominent on intermediate-stiffness substrates. (Reprinted with permission from<sup>49</sup>. © 2004 The Rockefeller University Press.) (B) Neurites cultured on polyacrylamide gels show greater extension and branching on soft (left) compared with stiff (right) substrates. (Reprinted with permission from<sup>54</sup>. © 2002 Lippincott Williams & Wilkins.)

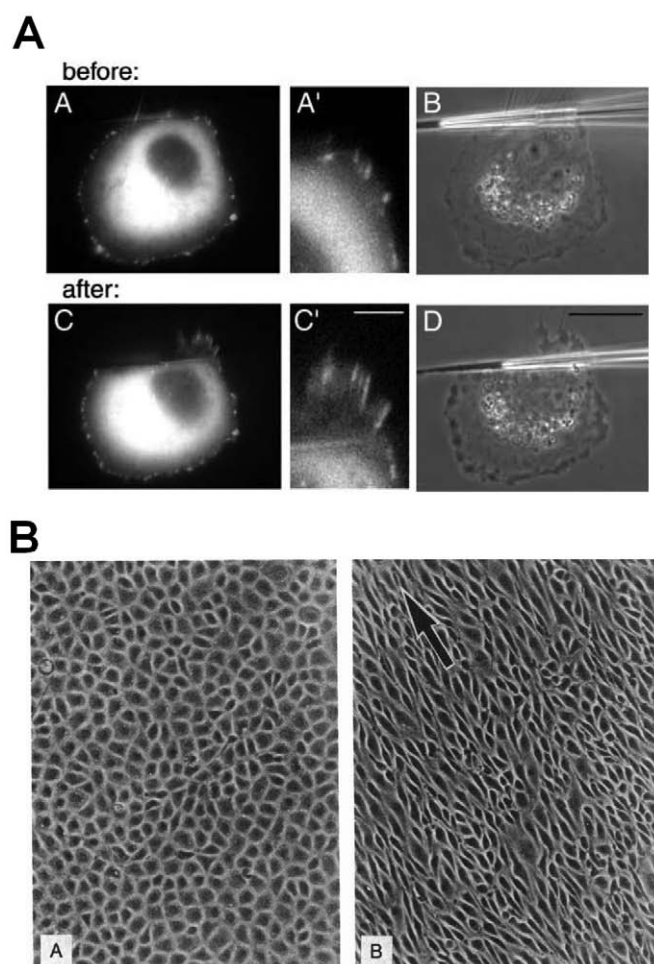


Fig. 7 Cellular response to externally applied forces. (A) Focal adhesion growth with translation of the micropipette. (Reprinted with permission from<sup>60</sup>. © 2001 The Rockefeller University Press.) (B) Endothelial cells align in response to fluid flow (arrow indicates direction of flow). (Reprinted with permission from<sup>76</sup>. © 1986 National Academy of Sciences.)

cases, cell-ECM adhesions have been implicated in mechanosensing, but cell-cell junctions, as well as proteins and carbohydrate molecules on the apical, or top, surface of the cell, have also been shown to be required for endothelial cells to respond to shear stress<sup>63,64</sup>. It is thought that forces sensed at these locations are transmitted through the cytoskeleton to cell-ECM adhesions. Many studies have indicated that mechanical forces activate intracellular signaling pathways, such as mitogen activated protein kinase (MAPK) or nuclear factor  $\kappa$ B (NF $\kappa$ B) signaling, and upregulation of these pathways is dependent on cell-ECM adhesions<sup>57,61,65</sup>. Mechanical forces induce numerous biological outputs, including reorganization of the surrounding matrix and increased tube formation of vascular cells<sup>66</sup>, as well as enhanced differentiation of endothelial cells from their precursors<sup>67</sup>.

An environment suited to growing functionally relevant, structurally and metabolically useful tissues to replace diseased organs must consist

not only of the appropriate passive structural and mechanical cues, but also the necessary actively applied forces<sup>68</sup>. Niklason *et al.*<sup>69</sup> have demonstrated that blood vessels cultured under physiological levels of pulsatile flow have greater strength than vessels cultured in a static environment. Understanding how external forces control cellular behavior may help to design new cell culture systems that will encourage cell growth and differentiation. However, there are still several major challenges that lie ahead. One challenge is to downsize and control the application of mechanical forces precisely. While localized forces can be applied to single cells using micropipettes or microbeads, these methods are tedious and difficult to apply to many cells at once. Culture environments with applied forces that are spatially defined on a cellular scale would give more precise control of cellular function. There are also challenges in designing scaffold materials that can withstand the magnitude of these applied forces without damage and fatigue. Thus, the integration of materials and mechanics will ultimately determine the success of a cell culture environment.

## Future directions and conclusions

A critical paradigm in the field of materials science is that structure and mechanics are critically linked to function at all length scales. It is only now being appreciated that the same paradigm is true for biological systems. We have reviewed several features of the physical cellular microenvironment that influence cellular mechanics and behaviors such as proliferation and differentiation. This list, however, is by no means all inclusive. Cells are known to respond to many other physical cues, such as electrical and magnetic forces, mechanical pressures, and substrate porosity or topology, particularly at the micro- or nanoscale since this is the length scale of the ECM fibers in which they are embedded<sup>70</sup>.

Many of the studies we have described examine cells cultured on a two-dimensional surface. However, investigators have shown that adhesions of cells in three-dimensional membranes have some distinct characteristics compared with the adhesions of cells on a flat substrate<sup>71</sup>. A major challenge will be to design tools that enable the controlled presentation in three dimensions of the different microenvironmental cues discussed here.

Finally, cells exist within a dynamically changing environment, with chemical and physical cues that are constantly shifting. A full appreciation of how cells exist within the body and interact with biomaterials requires an understanding of how cells respond to these dynamic cues. Current strategies toward this goal include switchable substrates, which allow toggling between adhesive and nonadhesive environments<sup>72,73</sup>, or dynamic patterning methods, which enable the positioning of multiple cell types with not only spatial but also temporal control<sup>41,74</sup>. Typically, electromagnetic forces are used to position either

the cells themselves or the molecules onto which the cells adhere. Ensuring the compatibility of these methods with cell viability is critical to their use in clinical applications.

A key step for the future of biomaterials will be to integrate biochemical cues with structural cues to generate highly defined microenvironments for optimal cellular functions. Technologies that have been exploited to isolate physical cues may also be used to combine many different physical cues. For instance, photolithography techniques that are used to pattern adhesive ligands as well as substrate

stiffnesses may be used to present spatially organized adhesion and stiffness cues simultaneously. The synergy of chemical properties with physical properties presented here may have a powerful impact on the design of new biomaterials. [mt](#)

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