Dynamic Substrates for Cell Biology

Milan Mrksich

Abstract

The development of dynamic substrates that can modulate the behavior of adherent cells is important for fundamental studies in cell biology, applications in biomaterials, and engineering microsystems that combine cellular and material components. This review outlines several strategies based on physical transduction schemes (including electrical, photochemical, thermal, and mechanical forces) for designing interfaces that are active and can signal changes in the behavior of attached cells.

Keywords: *biointerfaces, biomaterials, cell biology, substrates.*

Introduction

An important goal of materials science is the development of interfaces that integrate the functions of living cells and materials. The development of materials that serve as substrates for adherent cells is important in a range of basic and applied programs.[1] In basic research, substrates are used to study the adhesion of cells to the extracellular matrix (the protein scaffold that serves to organizes cells in tissue) and the processes by which this matrix directs cell function. In applied programs, materials are used to direct tissue compatibility in biomaterials and are now undergoing development to direct the differentiation of stem cells.

A significant effort during the past 20 years has produced a variety of methods for modifying the surfaces of materials to promote cell adhesion.[2] These methods are mostly based on modifying materials with polymers or self-assembled monolayers that in turn provide ligands that promote the adhesion of cells. Ligands are either directly immobilized on the modifying layers or are introduced indirectly when proteins in the contacting biological fluid adsorb to the surface. In both cases, cell adhesion is mediated by the interaction between receptors on the cell surface and peptide ligands on the material (Figure 1). Further, many researchers have contributed methods that can pattern the immobilization of ligands and therefore exert control over the shapes, sizes, and positions of cells on a substrate.[3] Hence, it is now reasonably straightforward to modify the properties of a broad range of materials to provide cell adhesion.

But these tailored interfaces fail to capture many of the important properties of the interface that joins a cell to its environment. In particular, the interactions between cells and the protein matrix are highly dynamic and undergo biochemical modifications to alter the ligands displayed to a cell and the mechanical properties of the matrix, both of which are important in influencing the activities of the cells. The development of dynamic, synthetic substrates that can similarly alter the ligands presented to a cell would provide unprecedented opportunities in fundamental cell biology. Further, the development of materials that can modulate cell adhesion (by directing cell growth, organizing multiple cell types into complex patterns, and releasing cultured cells) would have immediate application in tissue engineering and other cell-based technologies. This article describes recent work in developing dynamic substrates with these properties, the applications for which these materials are important, and current challenges in this field.

The early reports of dynamic substrates that influence cell adhesion can be categorized according to the physical stimuli used to manipulate the properties of the substrate and the surface chemistries used to introduce these functions. This article includes examples of dynamic substrates that are prepared by many methods, but places an emphasis on the use of self-assembled monolayers. The discussion is organized according to the transduction schemes used to influence cells and begins with a description of electrical and electrochemical processes that have been used in this context. The review also addresses transduction schemes that have not been directly applied to dynamic substrates for cell biology and notes the opportunities for developing those schemes.

Electrical Transduction

Cells possess an intrinsic electrical activity and can be influenced by electrical potentials that are applied to the underlying substrate.[4] Several empirical studies have demonstrated the effects of electric fields on cell behavior, including a report that applied fields could direct the migration of human keratinocytes, a cell derived from skin tissue.[5] The mechanisms by which electric fields influence cell migration (and other aspects of cell behavior) are not yet understood. These effects may be caused, in part, by electrostatic forces between the substrate and the charged species in the solution (i.e., ions, molecules, and proteins) that in turn alter the composition of the medium surrounding the cell, leading to changes in the local pH, ionic strength, and metabolite concentration both outside and inside the cell. One report, for example, showed that the axis of cell division could be controlled by the imposition of fields 150 V mm^{-1} in magnitude and suggested the underlying mechanism might derive from the redistribution of membrane-bound proteins and polarization of the protein cytoskeleton of the cell.[6] Alternatively, the fields (and associated field gradients) might exert a more direct effect by altering the

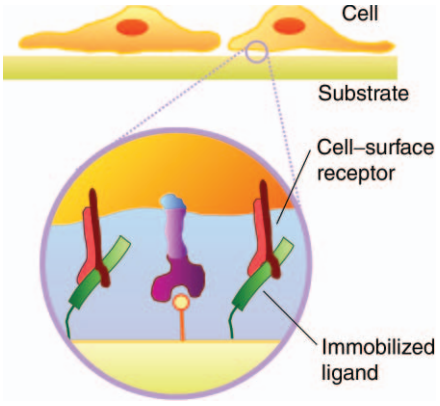

Figure 1. Most cells are adherent and must attach and spread on a matrix. The interaction of a cell with the substrate relies on the binding of cell–surface receptors with ligand proteins that are adsorbed to the material.

structures and activities of transmembrane and intracellular proteins. Many ion channels, for example, are voltage-gated and can be activated by a change in transmembrane potential.[7]

These same transduction strategies have proven useful in the reverse direction, by using electrodes to measure electrical properties of the cell membrane. One strategy, for example, uses microelectrode arrays to measure changes in the activities of cellular ion channels, resulting in biosensors that use living cells as the sensing component (Figure 2).[8] These sensors are the first examples of microsystems that combine living and non-living components, using cells as living sensors in place of non-living, materials-based sensors that are difficult to engineer. For example, a cell will exhibit a characteristic response to a chemical or biological warfare agent even if the agent has been modified to escape detection by conventional methods.

Electrochemical Transduction

A second class of electrical transduction strategies is based on using the applied potentials to cause redox reactions at the interface, thereby directly altering the structures of electroactive groups confined to the interface. Langer and Ingber used a conducting polymer substrate to control the shape and growth of mammalian cells.[9] They found that aortic endothelial cells cultured on polypyrrolium films coated with fibronectin spread normally and synthesized DNA. Electrochemical reduction of the organic film (to the neutral state) resulted in the arrest of cell extension and DNA synthesis without adversely affecting cell viability. These same substrates were later used to stimulate the outgrowth of nerve cells.[10] For both examples, the mechanisms by which oxidation and reduction of the polymer substrate influence cell behavior remain uncharacterized.

Our research group has pursued a related strategy to prepare dynamic substrates that provide even more control over the ligand–receptor interactions between cells and substrates.[11] Our approach is based on monolayers of alkanethiolates on gold, which present appropriately designed electroactive groups that can be selectively modified in response to applied potentials.

An early example demonstrated an electroactive monolayer that could be switched to turn on the immobilization of ligands and subsequently promote the migration and growth of cells.[12] This dynamic property is based on the Diels–Alder cycloaddition reaction of benzoquinone groups on the monolayer with a cyclopentadiene group conjugated to the Arg-Gly-Asp peptide (Figure 3). The reactivity of the monolayer can be modulated by the electrochemical reduction of the benzoquinone to the corresponding hydroquinone, which is not reactive toward the diene. A monolayer was patterned using microcontact printing[13] into circles of hexadecanethiolate, with the intervening regions presenting the hydroquinone group mixed with penta(ethylene glycol) groups. Cell addition to the substrate resulted in the attachment and growth of cells only on the circular regions. On application of an electrical potential to the gold (typically, 220 mV for 10 s), the hydroquinone groups were converted to benzoquinone groups, which then reacted with the peptide-diene conjugate and resulted in cell migration from the patterned regions. This example demonstrates that the electroactive monolayers can indeed be designed to influence the behavior of attached cells *in situ* and in real time.

A similar strategy was used to design substrates that can selectively release tethered ligands.[14,15] One example was based on the use of an O-silylhydroquinone group to tether a peptide ligand to the monolayer. Cells attached efficiently to monolayers presenting an Arg-Gly-Asp ligand. Electrochemical oxidation of this group gave the corresponding benzoquinone, with concomitant hydrolysis of the silyl ether and release of the tethered ligand.

This example also illustrates a strategy for creating surfaces that combine multiple electrochemical activities. The benzoquinone group that is generated in the electrochemical oxidation can subsequently be used to immobilize a second ligand by way of the Diels–Alder reaction. This example also establishes that the electrochemical treatment does not produce unwanted effects on the monolayer—it does not, for example, compromise the inertness of the film—and hence provides a means to selectively release a particular ligand even when multiple ligands are presented on the substrate. One drawback with these methods is that they require a substantial effort in synthetic chemistry to prepare the monolayer substrates. A recent report offers a simple version of electroactive self-assembled monolayers, wherein an inert monolayer can be electrochemically desorbed and in turn switches a surface from a state that prevents cell attachment to a state that promotes cell adhesion and migration.[16]

These examples are general in that they can be adapted to manipulate the presentation of a range of ligands that interact with cell–surface receptors. These examples are also significant because they illustrate a molecular-level route that gives unprecedented control in engineering surface properties. In a similar approach, Willner has demonstrated a strategy to electrically modulate the conversion of glucose to gluconic

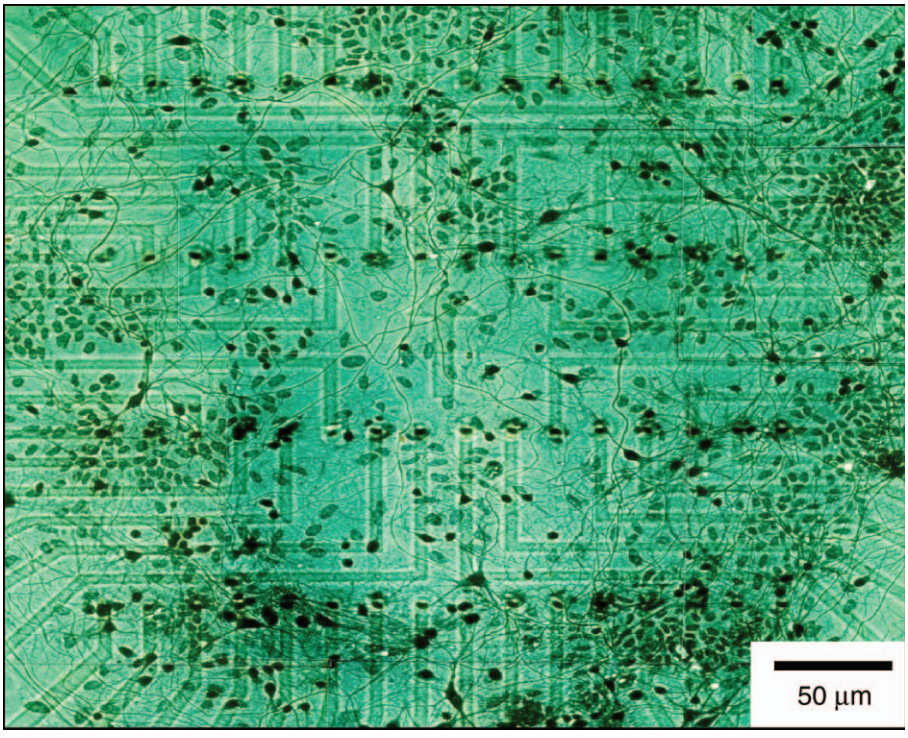

Figure 2. Optical micrograph of neuronal cells cultured on a microelectrode array. The electrodes detect changes in cellular activities associated with exposure to neurotoxins.

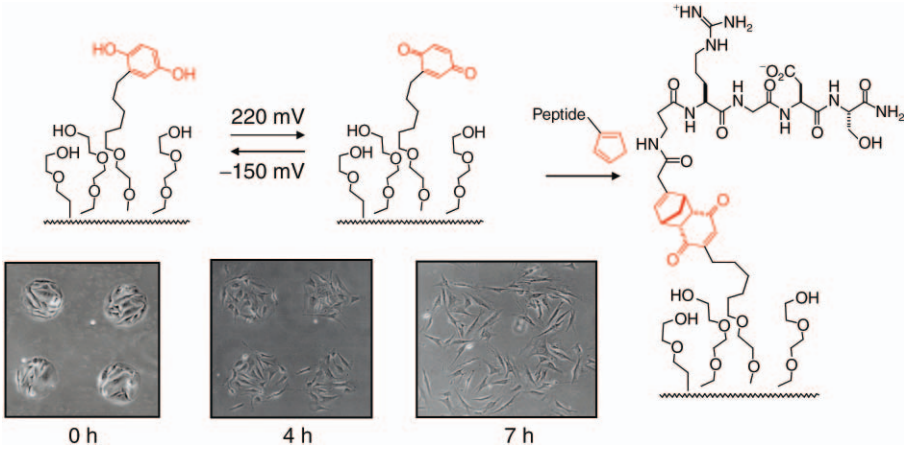

Figure 3. Design of a substrate that electrically turns on the immobilization of a ligand. An electrical potential converts a hydroquinone group to benzoquinone, which serves to immobilize a peptide ligand. The dynamic surfaces can initiate the migration of cells that are originally confined to 200 μm circular regions on the substrate.

acid by the enzyme glucose oxidase.[17] The molecule flavin adenine dinucleotide (FAD) was attached to a self-assembled monolayer and could bind to apo-glucose oxidase to give an active enzyme. The turnover of the enzyme, however, required electrochemical regeneration of the cofactor. This example demonstrates a sophisticated integration of enzyme activity and electrical processes.

One additional recent report is worthy of mention. Nishizawa et al. demonstrated a strategy wherein a conductive probe is used to locally generate the oxidizing agent HBrO (from KBr), which acts on an albumin-coated substrate to render these regions cell-adhesive. While the mechanism of surface modification has not been characterized, this example is significant because it enables wide generality in controlling, in real time, the substrate regions that support cell attachment.[18]

Photochemical Transduction

Molecular approaches that are similar to those already discussed can be used to engineer dynamic substrates that respond to light. Instead of developing molecular groups that undergo specific redox reactions, molecules are designed to undergo photochemical reactions that lead to a change in the activity of an immobilized ligand. Such photoactive substrates have not yet been used to dynamically influence cell behavior, but several related examples of photo-modulated ligands establish the feasibility of these approaches.

Willner has engineered a monolayer that presents a semisynthetic nitrospiropyran-FAD cofactor that can be reversibly modulated to control the binding of apo-glucose oxidase (as described in the previous section).[19] The FAD cofactor was designed with the photoactive group such that it could be optically switched between a structure that binds apo-glucose oxidase to give active enzyme and a structure that cannot bind apo-glucose oxidase. Isomerization of the spiropyran upon illumination with light at 370 nm results in an active enzyme that oxidizes glucose. Illumination of this substrate with light at 475 nm, however, reverses the photochemical isomerization and again results in an inactive enzyme. This example establishes the compatibility of monolayer substrates with photochemical influences to dynamically alter the biological activity of the substrates. Two additional examples of photochemical control over monolayer substrates include the development of a surface that releases nitric oxide[20] and a surface that can be activated for Diels–Alder-mediated immobilization of ligands.[21]

Thermal Transduction

A significant body of work during the past five years has established a class of polymer-modified substrates that modulate their interactions with cells in response to changes in temperature. These methods are illustrated in a report by Okano et al. that uses the thermally responsive polymer poly(*N*-isopropylacrylamide).[22,23] At room temperature, this polymer is in an extended, solvent-swelled conformation, but when heated to 32°C, the polymer undergoes a phase transition to yield a collapsed morphology that excludes solvent. This collapsed morphology is a good substrate for attached cell culture, whereas the unheated polymer is inert to cell attachment, and therefore, the cell attachment and cell release from the substrate can be controlled with a change in temperature around the phase transition of the acrylamide. This substrate is significant because it enables a simple, nondestructive method for releasing cells from culture substrates. It also avoids the use of the protease trypsin to release adherent cells, since this protease can cause unwanted degradation of cell–surface proteins.

Chilkoti et al. have pursued an analogous strategy that uses polypeptides as the polymeric material. Much of this work uses a peptide that is derived from elastin and undergoes a thermally induced phase transition between states that promote or prevent protein adsorption.[24] These biopolymers could also be patterned at submicron scales to provide surfaces that manipulate the presentation of proteins.[25]

Mechanical Transduction

Adherent cells exert forces on their underlying substrates and rely on mechanical coupling to maintain normal cellular functions.[26,27] Several biological studies have demonstrated that the resulting mechanical tension in the cellular cytoskeleton can have important influences on cell behavior. Ingber, for example, has shown that mechanical forces between the cell and the substrate can affect many cellular functions, including cell growth and death.[28] A combination of experimental and simulation studies are beginning to provide an understanding of the effects of substrate deformation on the actin cytoskeleton of adherent cells.[29] Endothelial cells and human skin fibroblasts were predicted and shown to orient their actin filaments uniformly at an angle of 60° with respect to the stretching direction.

Studies of the mechanotransduction schemes have not yet provided an understanding at the molecular level of the coupling of mechanical forces and biological activity. Nonetheless, there is an opportunity to apply microfabrication techniques (and more broadly, the set of tools used in fabricating MEMS, or microelectromechanical systems) to investigate the use of mechanical perturbations on a cell. Galbraith and Sheetz prepared a micromachined substrate that had 5904 cantilevers, each with an area of approximately 10 μm^2, to map out the pattern of forces between a migrating cell and the substrate.[30] Chen prepared a polymeric substrate with an array of posts that could spatially resolve the lateral forces exerted on the substrate by the cell.[31] This work revealed that the migrating cell applies forces on the substrate at its leading edge. The work is significant because it demonstrates the use of MEMS for *in situ* monitoring (and influencing) of cells.

Comparison of Transduction Strategies

The strategies reviewed in this article differ in the complexity of methods required to engineer the substrate and the sophistication with which cell behavior can be dynamically controlled. Because of these differences, each strategy may be best suited to particular applications. Strategies that use electrochemical or photochemical stimuli provide the most thorough control of the substrate properties because the dynamic properties derive from specific chemical reactions at the interface. These methods are also well suited for integrating the substrate with molecular pathways within the cells, because they can be used to modulate the activities of ligands with which cellular receptors interact. The choice of electrochemical or photochemical strategies will, of course, be determined by the context of the application. For substrates that include integrated circuits or other conductive components, the electrochemical methods are desirable because they avoid the need to introduce optical elements.[32] For applications that use optical or fluorescence microscopies to image cells, the photochemical strategies are most appropriate and avoid the need to install the electrical elements on a substrate. Both strategies, however, have the disadvantage of requiring a substantial investment in chemistry to design and synthesize the active substrates.

Strategies that use electrical and thermal means to influence cells are intrinsically more straightforward to apply than those that use electrochemical and photochemical means, but they provide less generality in controlling cell behavior. This limitation stems from the multitude of effects that electric field or temperature changes can have on a cell. The responses of a cell to these dynamic substrates are shaped by many processes in the cell, and the molecular pathways by which cells respond are only partly understood. Clearly, much fundamental biology remains to be learned before these strategies can be rationally adapted to control cell behavior. The same is true of strategies that use mechanical forces to influence cell behavior. But these latter strategies are exciting because of the sophistication in MEMS technology that can now be applied to cell-based devices.

Applications for Dynamic Substrates

The dynamic substrates described in this article are still at an early stage of development and have not yet been implemented in routine applications. As is common with many new biotechnologies, the first "applications" will be directed to fundamental studies in cell biology. Dynamic substrates that can alter the presentation of ligands to an attached cell, for example, will generate immediate opportunities for studies of cell adhesion, migration, and differentiation. These fundamental studies will likely give way to applications in tissue engineering and stem cell maturation. Indeed, the thermally active polymeric substrates have already been demonstrated by the growing and harvesting of multicellular tissues (Figure 4).[33] On the longer horizon, the development and demonstration of several classes of dynamic substrates will, in turn, motivate the design of a range of cell-based microsystems that are currently beyond prediction.

The development of dynamic substrates (and, more broadly, of engineered interfaces between cells and functional materials) is at a very early stage. An important aim of further work developing dynamic substrates is to increase the biological relevance of the model systems. Much of our work has used substrates that present peptide ligands taken from the extracellular matrix. Whereas the peptides do capture some of the functions of the natural matrix, it is clear that they do not mimic other functions of the full proteins. This need may be addressed through the development of immobilization chemistries for protein domains and the elaboration of these methods to bring them under electrochemical control.[22,23]

A second opportunity is the development of three-dimensional culture systems which more closely capture the properties of the matrix by presenting ligands to the entire surface of a cell. Hubbell has been a leader in developing bioactive gel matrices.[24] The most direct route to incorporate dynamic functions into these gels will rely on photochemical routes to trigger the gel. Photochemical methods also offer the prospect for spatio-temporal control of the gel properties by activating ligands in discreet regions of a material. This spatially directed activation can be accomplished either by patterning the ligand precursor in the gel or by using a confocal source to illuminate designated volume elements of the gel.

Summary

This review provides a perspective on the design of materials that can modulate their interactions with adherent cells. Early research in this field has demonstrated several strategies that hold promise for engineering active interfaces between cells and materials and now provides unprecedented opportunities for addressing fundamental and applied problems in biology. These examples will serve as a foundation for a new thrust that bridges materials science, chemistry, and biology and advances the engineering of interfaces that join living and non-living structures.

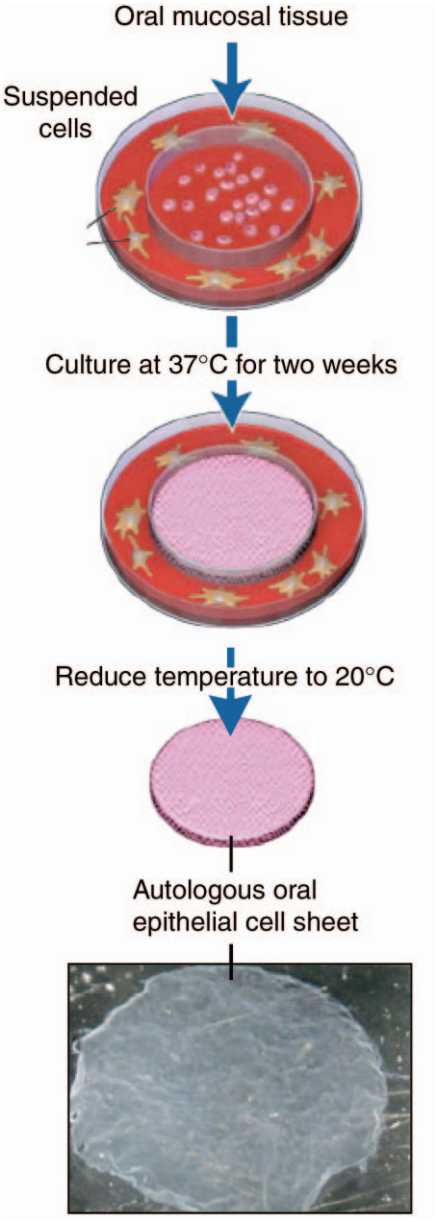

Figure 4. Thermally responsive polymer substrates were used to culture epithelial cells harvested from a patient. After the cells grow into sheets, the cultures are removed to a lower temperature, whereupon the polymer layer releases the cells. The resulting tissue can then be surgically implanted.

Acknowledgments

Our work in developing dynamic substrates has been supported by the U.S. Army, the National Science Foundation, and the National Institutes of Health.

References

1. R. Langer and D.A. Tirrell, *Nature* **428** (2004) p. 487.
2. U. Hersel, C. Dahmen, and H. Kessler, *Biomaterials* **24** (2003) p. 4385.
3. D.R. Jung, R. Kapur, T. Adams, K.A. Giuliano, M. Mrksich, H.G. Craighead, and D.L. Taylor, *Crit. Rev. Biotechnol.* **21** (2001) p. 111.
4. C.D. McCaig and M. Zhao, *BioEssays* **19** (1997) p. 819.
5. K.Y. Nishimura, R.R. Isseroff, and R. Nuccitelli, *J. Cell Sci.* **109** (1996) p. 199.
6. M. Zhao, J.V. Forrester, and C.D. McCaig, *Proc. Natl. Acad. Sci. USA* **96** (1999) p. 4942.
7. S.C. Hinnah, K. Hill, R. Wagner, T. Schlicher, and J. Soll, *EMBO J.* **16** (1997) p. 7351.
8. J.J. Pancrazio, J.P. Whelan, D.A. Borkholder, W. Ma, and D.A. Stenger., *Ann. Biomed. Eng.* **27** (1999) p. 697.
9. J.Y. Wong, R. Langer, and D.E. Ingber, *Proc. Natl. Acad. Sci. USA* **91** (1994) p. 3201.
10. C.E. Schmidt, V.R. Shastri, J.P. Vacanti, and R. Langer, *Proc. Natl. Acad. Sci. USA* **94** (1997) p. 8948.
11. M. Mrksich, *Chem. Soc. Rev.* **29** (2000) p. 267.
12. M.N. Yousaf, B.T. Houseman, and M. Mrksich, *Angew. Chem. Int. Ed.* **40** (2001) p. 1093.
13. M. Mrksich, L.E. Dike, J. Tien, D.E. Ingber, and G.M. Whitesides, *Exp. Cell Res.* **235** (1997) p. 305.
14. W.S. Yeo, C.D. Hodneland, and M. Mrksich, *ChemBioChem* **2** (2001) p. 590.
15. W.S. Yeo, M.N. Yousaf, and M. Mrksich, *J. Am. Chem. Soc.* **125** (2003) p. 14994.
16. X. Jiang, R. Ferrigno, M. Mrksich, and G.M. Whitesides, *J. Am. Chem. Soc* **125** (2003) p. 2366.
17. R. Blonder, I. Willner, and A.F. Bückmann, *J. Am. Chem. Soc.* **120** (1998) p. 9335.
18. H. Kaji, M. Kanada, D. Oyamatsu, T. Matsue, and M. Nishizawa, *Langmuir* **20** (2004) p. 16.
19. R. Blonder, E. Katz, I. Willner, V. Wray, and A.F. Bückmann, *J. Am. Chem. Soc.* **119** (1997) p. 11747.
20. S. Sortino, S. Petralia, G. Compagnini, S. Conoci, and G. Condorelli, *Angew. Chem. Int. Ed.* **41** (2002) p. 1914.
21. W.S. Dillmore, M.N. Yousaf, and M. Mrksich, *Langmuir* **20** (2004) p. 7223.
22. A. Kushida, M. Yamato, C. Konno, A. Kikuchi, Y. Sakurai, and T. Okano, *J. Biomed. Mat. Res.* **45** (1999) p. 355.
23. M. Ebara, M. Yamato, T. Aoyagi, A. Kikuchi, K. Sakai, and T. Okano, *Biomacromolecules* **5** (2004) p. 505.
24. N. Nath and A. Chilkoti, *Adv. Mater.* **14** (2002) p. 1243.
25. J. Hyun, W.K. Lee, N. Nath, A. Chilkoti, and S. Zauscher, *J. Am. Chem. Soc.* **126** (2004) p. 7330.
26. C.S. Chen, J. Tan, and J. Tien, *Annu. Rev. Biomed. Eng.* **6** (2004) p. 275.
27. G. Bao and S. Suresh, *Nature Mater.* **2** (2003) p. 715.
28. J.R. Sims, S. Karp, and D.E. Ingber, *J. Cell Sci.* **103** (1992) p. 1215.
29. J.H.-C. Wang, *J. Theor. Biol.* **202** (2000) p. 33.
30. C.G. Galbraith and M.P. Sheetz, *Proc. Natl. Acad. Sci. USA* **94** (1997) p. 9114.
31. J.L. Tan, J. Tien, D. Pirone, D.S. Gray, and C.S. Chen, *Proc. Nat. Acad. Sci. USA* **100** (2003) p. 1484.
32. J.M. Cooper, *Trends Biotechnol.* **17** (1999) p. 226.
33. K. Nishada, M. Yamato, Y. Hayashida, K. Watanabe, K. Yamamoto, E. Adachi, S. Nagai, A. Kikuchi, N. Maeda, H. Watanabe, T. Okano, and Y. Tano, *N. Eng. J. Med.* **351** (2004) p. 1187.
34. C.D. Hodneland, Y.-S. Lee, D.-H. Min, and M. Mrksich, *Proc. Natl. Acad. Sci. USA* **99** (2002) p. 5048.
35. W.L. Murphy, K.O. Mercurius, S. Koide, and M. Mrksich, *Langmuir* **20** (2004) p. 1026.
36. J.A. Hubbell, *Curr. Opin. Biotechnol.* **14** (2003) p. 551. □