

Exploring and Engineering the Cell Surface Interface

Molly M. Stevens* and Julian H. George

Cells are inherently sensitive to local mesoscale, microscale, and nanoscale patterns of chemistry and topography. We review current approaches to control cell behavior through the nanoscale engineering of materials surfaces. Far-reaching implications are emerging for applications including medical implants, cell supports, and materials that can be used as instructive three-dimensional environments for tissue regeneration.

Deciding which protein to express, when to divide, when to specialize, and when to commit suicide are all ongoing processes that occur within cells. Genes must be activated in the correct sequence and synchrony in order to express the numerous proteins needed for proliferation and differentiation to hierarchical organization within organs. In addition to the intrinsic cell factors that regulate cell fate, extrinsic signals to the cell from the surrounding extracellular matrix (ECM) are essential in guiding it through distinct development paths.

Artificial biomaterial scaffolds designed to support cell and tissue growth have traditionally aimed on a macroscopic level to match the properties of the organs they are to replace without recreating the nanoscale detail observed in real organs (1). In the body, the nanoscale structure of the ECM provides a natural web of intricate nanofibers to support cells and present an instructive background to guide their behavior (2–6). Unwinding the fibers of the ECM reveals a level of detail unmatched outside the biological world. Each fiber hides clues that pave the way for cells to form tissues as complex as bone, liver, heart, and kidney. The ability to engineer materials to a similar level of complexity is becoming a reality (1, 7–12). This review aims to highlight recent developments in biomaterials that have taken the lead from nanoscale engineering in nature to create more biomimetic cellular environments.

The Cell Extracellular Matrix

Cells are inherently sensitive to their surroundings. Typically between 10 and 100 μm in diameter, cells respond

to environmental features at all length scales from the macro down to the molecular. The outer membrane of a typical cell is covered by specific carbohydrate structures and a forest of at least six different receptor systems that can be activated by interactions with adjacent cells, ligands in the surrounding ECM, and secreted

highly defined and specialized cell micro-environment, which is essential for correct tissue development and continued function.

The ECM takes a variety of forms in different tissues and at different stages of development in the same tissue (3, 13). Diversity arises through combinations of specific molecular interactions between numerous isoforms, ratios, and geometrical arrangements of collagens, elastins, proteoglycans, and adhesion proteins such as fibronectins and laminins. This creates an environment that is replete with informational cues. In addition to this, a

wealth of molecular mechanisms modulates the dissemination of this information. For example, the ECM plays a major role in regulating growth factor signaling, acting as a local reservoir for latent forms, and rapidly releasing and activating them on demand (14). Multiple motifs color each ECM protein. Encoded by specific amino acid sequences, these motifs target and bind specific cell surface receptors to trigger different intracellular signaling pathways. The transmembrane integrin receptors, with more than 20 members identified, are the most extensively characterized, and they recognize motifs such as Arg-Gly-Asp (RGD) within proteins of the ECM such as fibronectin and vitronectin (15). These receptors tether the cell cytoskeleton to the fibers of the ECM, forming local focal adhesions. When bound, integrins activate a cascade of intracellular signaling pathways, leading to changes in gene expression and affecting most aspects of cell behavior. They modify differentiation, proliferation, the further expression of ECM proteins, activation of growth factors, and the maintenance of survival signals to prevent apoptosis (programmed cell death) (16). Cell membrane receptors rarely act alone and frequently form part of multicomponent systems that allow for diverse signal integration, for example, between the growth factor and integrin signaling pathways (17, 18).

Engineering ECM ligands, such as RGD, into artificial surfaces enhances functionality in terms of cell behavior. The cell's response is

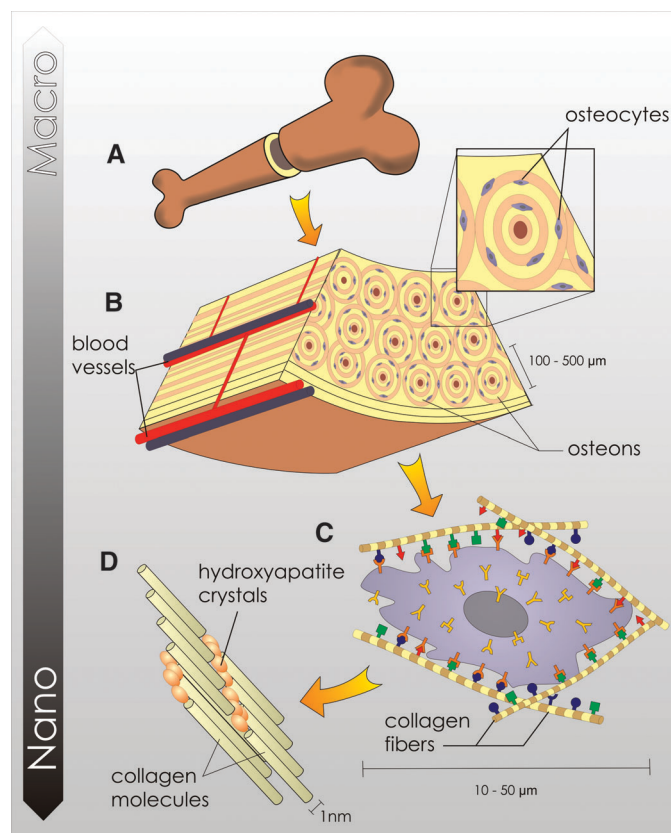


Fig. 1. Hierarchical organization of bone over different length scales. Bone has a strong calcified outer compact layer (A), which comprises many cylindrical Haversian systems, or osteons (B). The resident cells are coated in a forest of cell membrane receptors that respond to specific binding sites (C) and the well-defined nanoarchitecture of the surrounding extracellular matrix (D).

signaling molecules. Hundreds of different proteins play a role in the composite stimulation of cell receptors, which in turn determine a plethora of responses, including cell migration in the early embryo, coordinated organogenesis, and wound repair throughout adult life (2–4, 6). Collectively, these extrinsic factors make up a

Department of Materials and Institute for Biomedical Engineering, Imperial College of Science, Technology, and Medicine, Prince Consort Road, London SW7 2BP, UK.

*To whom correspondence should be addressed. E-mail: m.stevens@imperial.ac.uk

often biphasic (e.g., migratory response) and always specific to particular ligand surface densities and binding affinities (19–23). This response can be modulated by close colocalization of synergistic ligands. For example, a spacing of 4 nm between RGD and the synergy site Pro-His-Ser-Arg-Asn (PHSRN) will lead to increases in indicators of osteoblast cell function, such as metabolic activity and alkaline phosphatase production, but also lead to decreases in ECM production (20). The importance of RGD ligand clustering as a prerequisite to certain intracellular signaling pathways has been demonstrated *in vitro* on two-dimensional (2D) surfaces at the nanometer scale (15, 22). Control over ligand clustering and colocalization of multiple motifs (including growth factors) in 3D structures still poses challenges in materials design and synthesis. Nevertheless, advances in this area are likely to be highly relevant given that it is the precise spatial distribution of these motifs in tissues that determines many important aspects of cell behavior (24). Notably, the binding of integrins and the formation of focal adhesions, their structure, localization, and function in 3D tissues is substantially different from their binding and formation in 2D culture (24, 25). The 3D ECM environment *in vivo* strongly influences changes in cell shape that affect the differentiation process (13, 26). It is likely that cells require the full context of 3D nanofibrous matrix to maintain their phenotypic shape and establish natural behavior patterns.

Nanoscale Engineering at the Surface

Topographic reaction (i.e., reaction to the surface landscape) of cells to micrometer-range features such as grooves, ridges, and wells has been well established for decades (27). The fibers of the ECM and basement membrane (10 to 300 nm in diameter), their interconnecting nanopores, and hydroxyapatite crystals (4 nm) found in natural bone typically have nanoscaled dimensions (Fig. 1). Biomimetically driven studies are now exploring how the topography of a surface, if engineered with similar nanoscale structural features, can be used to control cell behavior.

Nanoscale alterations in topography elicit diverse cell behavior, ranging from changes in cell adhesion, cell orientation, cell motility, surface antigen display, cytoskeletal condensation, activation of tyrosine kinases, and modulation of intracellular signaling pathways that regulate transcriptional activity and gene expression (27). It is not only the scale of topography (5 nm to micrometer scale) that modulates cell behavior but also the type of ordered topography (e.g., ridges,

steps, grooves, pillars, and pits) and even their symmetry (e.g., orthogonal or hexagonal packing of nanopits) (28–35). At present, there is great disparity between the experimental approaches taken by different groups, making it difficult to compare data on nominally similar systems.

A noticeable early response of the cell to nanotopography is to increase its complement of filopodia and microspikes (effectively the “sensing” organelles of the cell), which may heighten the cell’s level of perception (29). In general, the presence of nanoscale features such as ridges, steps, and grooves increases cell attachment and proliferation, and ridges as thin as 70 nm guide cytoskeletal assembly (28, 31). Certain cell phenotypes show greater sensitivity to particular nanoscale features than others. For example, osteoblasts have been found to adhere preferentially to carbon nanofiber compacts in

bacterial infection. In a separate study, the inclusion of nanoscale features in titanium through acid etching enhanced osteoblast differentiation and growth-factor production (33).

The question of how cells detect and respond to nanofeatures is as yet unresolved (36). Nanoscale topology likely modulates the interfacial forces that guide cytoskeletal formation and membrane receptor organization in the cell, which in turn can modify intracellular signaling (29). Nanoscale surface features may also affect the adsorption and conformation of integrin binding proteins, changing the availability of binding sites and modifying integrin signaling (34). Several studies have established that similar scale nanofeatures may elicit similar biological effects independent of the underlying material chemistry. For example, there is close agreement between smooth muscle cell behavior

on both nanopatterned poly(methyl methacrylate) and poly(dimethylsiloxane), whereas the differing surface chemistry of the two polymers is unlikely to result in the same adsorption of proteins (35).

Nanoscale Scaffold Fabrication

The use of nanoscale material structuring to control cell behavior has important implications when designing new materials for tissue engineering. To regenerate tissue, engineered scaffolds play host to cells harvested from natural tissue. Conventionally, scaffolds have been designed macroscopically to have mechanical properties similar to natural tissue—hard scaffolds for bone (10) and elastic for bladder, veins, and arteries (37)—without the complexity and nanoscale detail observed in real organs at the level of the cell-matrix interaction. Approaches that progress toward the incorporation of

this level of detail may provide real benefits (Fig. 2). Simply increasing the nanoscale roughness of the scaffold pore walls has already been found to increase cell attachment, proliferation, and expression of matrix components (38).

Combining nanostructured scaffolds and the incorporation of biological signals into the scaffold fabric is likely to prove most rewarding. This can be achieved by building scaffolds from naturally derived biopolymers such as elastin and collagen. These constructs have nanoscale structure and borrow their binding sites from the ECM biopolymers, providing innate informational guidance. However, maintaining the quality and activity of harvested biopolymers throughout their extraction, processing, and remodeling is challenging, and some voice concern over the direct use of animal-derived material in a medical setting.

Synthetic materials such as biodegradable polymers offer a versatile alternative to naturally

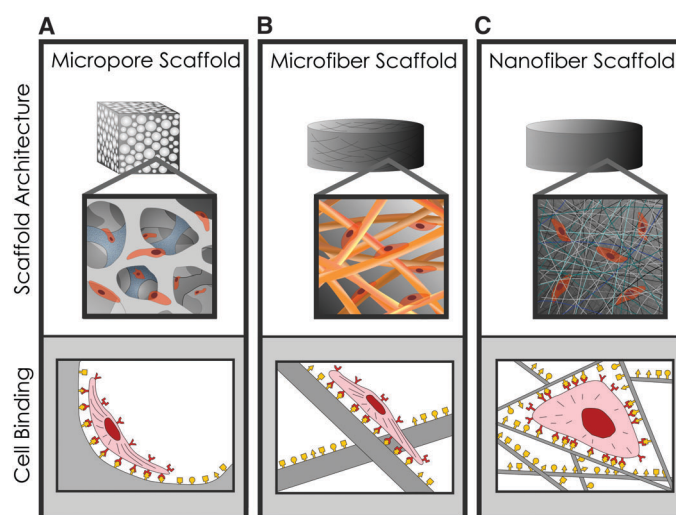


Fig. 2. Scaffold architecture affects cell binding and spreading. (A and B) Cells binding to scaffolds with microscale architectures flatten and spread as if cultured on flat surfaces. (C) Scaffolds with nanoscale architectures have larger surface areas to adsorb proteins, presenting many more binding sites to cell membrane receptors. The adsorbed proteins may also change conformation, exposing additional cryptic binding sites.

competition with chondrocytes, fibroblasts, and smooth muscle cells (30). These cell-specific discrepancies serve to highlight one of the main challenges faced in understanding cell-material interactions: Conclusions drawn from one cell type cannot be readily applied to another.

A powerful consequence of nanoscale engineering of the surface of materials is that topography alone can be used to elicit different responses from the same cell phenotype. For example, uroepithelial cells seeded on titanium surfaces engineered with well-defined nanometer topographies (hemispherical pillars or step edges) displayed modified cell morphologies and cytokine production to cells cultured on flat titanium controls with identical surface chemistry (32). The cells on the hemispherical pillars had a diminished release of the proinflammatory cytokine interleukin-6 and the chemokine interleukin-8, normally released in response to

derived biopolymers. Their mechanical properties can be highly tailored and they are easy to synthesize and shape. Many synthetic polymers already have established medical histories and are routinely used in medical implants, substantially increasing their applicability for regulatory approval (10). Nanofibrous scaffolds have been successfully and relatively simply produced from many synthetic and natural polymers through the technique of electrospinning to produce fibers with diameters ranging from a few nanometers to micrometers (39, 40). The nanofibers are continuous, potentially allowing for integrated manufacturing of 3D nanofiber matrices with high porosity, high spatial interconnectivity, and controlled alignment of fibers to direct cell orientation and migration (41, 42). Given the diversity of tissue-specific orientation of fibrils (parallel and aligned in tendon, concentric weaves in bone, orthogonal lattices in cornea, and meshlike in skin), this latter feature is yet to be fully exploited. The “biological” fine-tuning of these scaffolds toward particular cell types is of growing interest. Once challenges in materials design and solvent compatibility have been overcome, bioactive composite and core-shell fibers may be engineered to deliver growth factors, peptides, enzymes, drugs, and even DNA (42–44).

Thermally induced phase separation is another approach based on the thermodynamic demixing of polymer solution into polymer-rich and -poor phases. It has been used to produce a wide range of spongelike scaffolds or, in some instances, polymer nanofibers that mimic the size and scale of natural collagen fibers (45, 46). These new nanofibrous scaffolds promote cell attachment and proliferation and have been found to adsorb a wider spectrum and a greater quantity of integrin-binding proteins than do similar solid wall scaffolds (45).

Many natural biopolymers are assembled in multiple steps from the bottom up. The process of collagen self-assembly itself takes place over no fewer than nine separate steps (Fig. 3A). Synthetic nanoscale fabrication techniques cannot at present match nature’s ingenious ability, but much can be learned from natural systems. A number of groups have developed rationally designed polypeptide systems that self-assemble into nanoscaled fibers in aqueous media (47), such as the self-assembly of amyloid-like fibers (48). A few modifications through rational de novo design can drive peptides with alternating hydrophobic and hydrophilic residues to associate into small, self-complementary

β -sheet membranes. These further assemble into amyloid-like fibrils when suspended in an ionic solution of the correct pH (Fig. 3B) (9). Compared with electrospinning, the self-assembly approach can produce thinner fibrils, with diameters typically less than 10 nm, although these may cluster into thicker fiber bundles. When used in cell culture, the interwoven hydrogel structures physically surround cells in a manner similar to ECM and promote cell proliferation, active migration, and expression of ECM (9, 49).

An illustration of an artificially designed, amphiphilic self-assembling scaffold uses macro-

groups together. In effect, this system mimics the natural process by which collagen induces calcium, phosphate, and hydroxide to form hydroxyapatite crystallites within bone, which are necessary for bone’s structural rigidity. Similar amphiphilic macromolecules that use the IKVAV domain are under investigation to create hydrogel scaffolds for neural cell differentiation (51).

A Multidimensional Map

The many different types of information encoded into the extracellular environment combine to form a multidimensional map (Fig. 4). Cells use

this map to guide their activities and maintain their differentiation within tissues. Changes in signaling gradient across the map provide directionally encoded signals. Cell receptors distributed across the cell membrane perceive these signal gradients and respond with directional behavior such as migration and the expression of matrix components (52, 53). Different cells may even read the map in different ways, depending on their complement of receptors.

Each cell type fabricates its own network of precisely encoded ECM proteins to enable an exact tailoring of the structural properties and information content of each ECM environment throughout the body. As a consequence of the convoluted nature of protein folding, cryptic binding sites hidden within the structure of ECM molecules may be exposed through mechanisms that cleave or mechanically distort the proteins of the ECM (54, 55) (Fig. 4). [For example, several ECM proteins such as laminin 5, collagen, and tenascin-C possess multiple repeats of weak affinity epidermal growth factor-like ligands that are revealed after ECM modification and can function as cellular promigratory tracks (18, 56)]. As a result, information released dynamically by protein conformation change during tissue damage or deformation can direct cell behavior in response to

these events. This unmasking of cryptic sites is a tightly controlled process signifying the importance of cryptic ECM functions. Through the dynamic response to external events and elaborate reciprocal flow of signaling between the ECM backdrop and the cells that express it, the behavior of cells is coordinated into complex functional tissues (57).

Repainting the Map

Engineering these dynamic ECM mechanisms into biomaterials offers further control over cell behavior. By tailoring proteolytic cleavage, cell

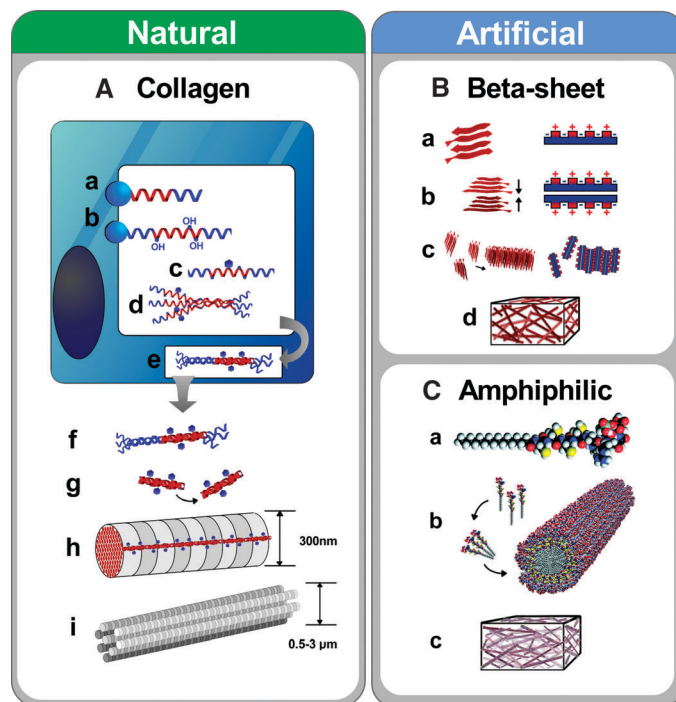


Fig. 3. Natural and artificial fiber self-assembly. (A) Natural assembly of collagen fibers: (a) Pro- α chains are synthesized in the lumen of the endoplasmic reticulum, where (b) hydroxylation of selected prolines and lysines occurs. (c) This is followed by glycosylation of selected hydroxylysines. (d) Three pro- α chains self-assemble and (e) form a procollagen triple helix. (f) These are secreted into the extracellular space, and (g) the protective propeptides are cleaved. (h) Finally, the procollagen self-assembles into fibrils, and (i) these aggregate into collagen fibers. (B) Amyloid-like assembly: (a) Beta-sheet membranes with hydrophilic and hydrophobic faces group by (b) burying their hydrophobic faces. In the correct ionic solution, (c) the hydrophilic faces attract and (d) the fibers self-assemble (9). (C) Amphiphilic peptide assembly: (a) Amphiphilic peptide design. (b) The peptides bury their hydrocarbon tails, (c) forming fibers in solution (50).

molecules that have thin hydrophobic alkyl tails covalently bonded to thick hydrophilic peptide head-groups (50) (Fig. 3C). In aqueous solution, these assemble into long nanocylinders as the hydrophobic tails form a core to shield themselves from the aqueous solution. The resulting interwoven gel provides a previously unexplored approach to conductive bone regeneration. The peptide head groups include a phosphorylated serine, encouraging hydroxyapatite nucleation and an RGD motif to aid bone cell adhesion and survival within the resulting scaffolds. Included cysteine thiol residues covalently bond the head

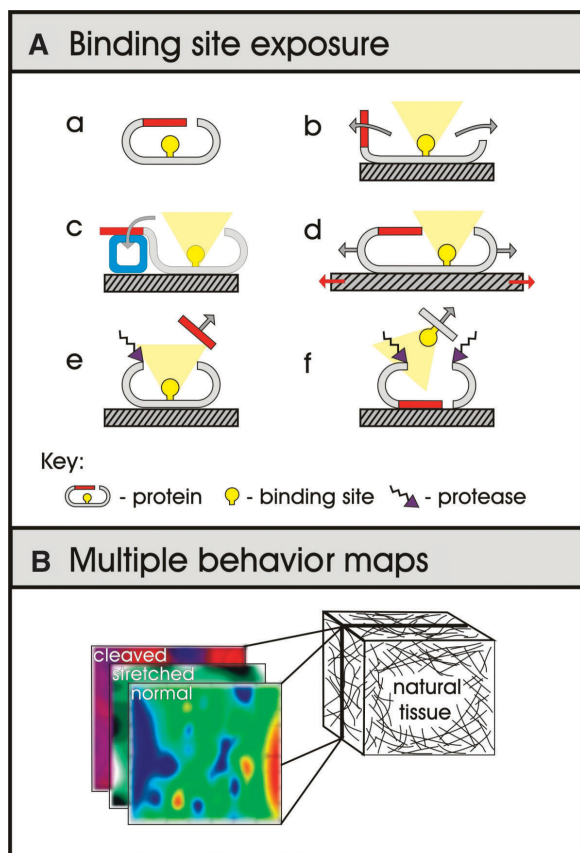


Fig. 4. Mechanisms of Cryptic Motif exposure. (A) (a) A representation of a protein structured with a hidden motif (yellow ball). Motifs can be exposed through conformation change due to (b) surface adsorption, (c) interaction with other proteins, and (d) mechanical distortion. (e) Proteolysis (purple arrow) can reveal hidden motifs and (f) may release them into the environment, enabling transport of signals away from the affected area. (B) A multidimensional tissue map. By providing different binding opportunities to cells in response to events that change protein conformation (such as stretching and cleaving), natural tissues effectively provide multiple behavioral maps, whose context is dependent on external events.

invasion can be encouraged and controlled. Designer oligopeptides that are recognized and cleaved by cell-secreted matrix metalloproteases have been successfully used to aid cell invasions into artificial poly(ethylene glycol)-based scaffolds (58) and to help induce in situ bone regeneration in combination with controlled release of recombinant human bone morphogenetic protein 2 (59). Elastin-like polymers, engineered with the use of recombinant DNA technologies, are also under investigation (60), with the aim of fabricating augmented fibrous scaffolds that promote cell invasion. As cells use proteolysis to migrate into these scaffolds, proteolytic fragments are released that present biologically active hexapeptide sequences to cell membrane receptors. Subsequent binding triggers signaling pathways that promote cell proliferation within the scaffold.

Future Directions

Nanostructuring of materials provides a powerful mechanism to encourage and direct cell behavior

ranging from cell adhesion to gene expression. Early findings have been greeted with excitement from the orthopedic industries where the promotion of one cell type over another, such as osteoblasts (bone-forming cells) over osteoclasts (bone-resorbing cells), to stimulate bone growth will be important in reducing aseptic loosening and failure of implants (10). Other applications easily envisaged are for biotechnological culture supports, implanted biosensors (61), neural interfaces (62), and other structural medical implants engineered with nanoscale features to reduce the likelihood of fibrous encapsulation and allow stronger interfacing with the host tissue for a longer period of time.

A key challenge is to capture the degree of complexity that is needed to functionally replicate the ECM of natural tissue. To this end, nanostructured scaffolds enriched with specific proteins are likely to emerge as strong contenders for the biocomposites of choice for tissue regeneration. Nevertheless, we are still a long way from recreating the molecular architecture of the ECM and the dynamic mechanisms by which information is revealed in response to challenges within the local environment. Given the diversity of cryptic motifs discovered within the proteins of the ECM, facile structure-based elucidation of these mechanisms seems unlikely.

Achieving effective temporal control over the signals that are presented to cells in 3D artificial matrices is still a key challenge in the optimization of outside-in signaling, as is the colocalization of cell signaling epitopes. Advances in the areas of fundamental matrix biology, nanofabrication, synthetic molecular self-assembly, recombinant DNA technologies (63), and printing technologies (64) will enable the generation of materials that can provide enhanced 3D tissue context maps of molecular and structural information.

References and Notes

1. B. D. Ratner, S. J. Bryant, *Annu. Rev. Biomed. Eng.* **6**, 41 (2004).
2. N. Zagris, *Micron* **32**, 427 (2001).
3. D. Gullberg, P. Ekblom, *Int. J. Dev. Biol.* **39**, 845 (1995).
4. M. Aumailley, B. Gayraud, *J. Mol. Med.* **76**, 253 (1998).
5. J. E. Scott, *J. Anat.* **187**, 259 (1995).
6. E. I. Wallner, Q. Yang, D. R. Peterson, J. Wada, Y. S. Kanwar, *Am. J. Physiol.* **275**, F467 (1998).
7. F. Rosso et al., *J. Cell. Physiol.* **203**, 465 (2005).
8. H. Shin, S. Jo, A. G. Mikos, *Biomaterials* **24**, 4353 (2003).
9. S. Zhang, *Nat. Biotechnol.* **21**, 1171 (2003).
10. L. L. Hench, J. M. Polak, *Science* **295**, 1014 (2002).
11. M. P. Lutolf, J. A. Hubbell, *Nat. Biotechnol.* **23**, 47 (2005).

12. D. G. Anderson, J. A. Burdick, R. Langer, *Science* **305**, 1923 (2004).
13. D. J. Behonick, Z. Werb, *Mech. Dev.* **120**, 1327 (2003).
14. J. Taipale, J. Keski-Oja, *FASEB J.* **11**, 51 (1997).
15. C. Bokel, N. H. Brown, *Dev. Cell* **3**, 311 (2002).
16. F. G. Giancotti, E. Ruoslahti, *Science* **285**, 1028 (1999).
17. G. E. Plopper, H. P. McNamee, L. E. Dike, K. Bojanowski, D. E. Ingber, *Mol. Biol. Cell* **6**, 1349 (1995).
18. K. T. Tran, L. Griffith, A. Wells, *Wound Repair Regen.* **12**, 262 (2004).
19. S. P. Palecek, J. C. Loftus, M. H. Ginsberg, D. A. Lauffenburger, A. F. Horwitz, *Nature* **385**, 537 (1997).
20. D. S. Benoit, K. S. Anseth, *Biomaterials* **26**, 5209 (2005).
21. B. G. Keselowsky, D. M. Collard, A. J. Garcia, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 5953 (2005).
22. G. Maheshwari, G. Brown, D. A. Lauffenburger, A. Wells, L. G. Griffith, *J. Cell Sci.* **113**, 1677 (2000).
23. W. L. Murphy, K. O. Mercurius, S. Koide, M. Mrksich, *Langmuir* **20**, 1026 (2004).
24. E. Cukierman, R. Pankov, D. R. Stevens, K. M. Yamada, *Science* **294**, 1708 (2001).
25. B. M. Gumbiner, *Cell* **84**, 345 (1996).
26. D. E. Discher, P. Janmey, Y.-l. Wang, *Science* **310**, 1139 (2005).
27. A. Curtis, C. Wilkinson, *Biochem. Soc. Symp.* **65**, 15 (1999).
28. A. I. Teixeira, P. F. Nealey, C. J. Murphy, *J. Biomed. Mater. Res.* **71A**, 369 (2004).
29. A. S. Curtis et al., *IEEE Trans. Nanobioscience* **3**, 61 (2004).
30. R. L. Price, K. Ellison, K. M. Haberstroh, T. J. Webster, *J. Biomed. Mater. Res. A* **70A**, 129 (2004).
31. R. L. Price, K. M. Haberstroh, T. J. Webster, *Med. Biol. Eng. Comput.* **41**, 372 (2003).
32. A.-S. Andersson et al., *Biomaterials* **24**, 3427 (2003).
33. O. Zinger et al., *Biomaterials* **26**, 1837 (2005).
34. T. J. Webster, L. S. Schadler, R. W. Siegel, R. Bizios, *Tissue Eng.* **7**, 291 (2001).
35. E. K. Yim et al., *Biomaterials* **26**, 5405 (2005).
36. A. Curtis, *IEEE Trans. Nanobioscience* **3**, 293 (2004).
37. Y. Wang, G. A. Ameer, B. J. Sheppard, R. Langer, *Nat. Biotechnol.* **20**, 602 (2002).
38. M. A. Pattison, S. Wurster, T. J. Webster, K. M. Haberstroh, *Biomaterials* **26**, 2491 (2005).
39. J. A. Matthews, G. E. Wnek, D. G. Simpson, G. L. Bowlin, *Biomacromolecules* **3**, 232 (2002).
40. W. J. Li, C. T. Laurencin, E. J. Caternon, R. S. Tuan, F. K. Ko, *J. Biomed. Mater. Res.* **60**, 613 (2002).
41. Y. Dzenis, *Science* **304**, 1917 (2004).
42. Z. Ma, M. Kotaki, R. Inai, S. Ramakrishna, *Tissue Eng.* **11**, 101 (2005).
43. J. Zeng et al., *Biomacromolecules* **6**, 1484 (2005).
44. Z. C. Sun, E. Zussman, A. L. Yarin, J. H. Wendorff, A. Greiner, *Adv. Mater.* **15**, 1929 (2003).
45. K. M. Woo, V. J. Chen, P. X. Ma, *J. Biomed. Mater. Res. A* **67**, 531 (2003).
46. F. Yang et al., *Biomaterials* **25**, 1891 (2004).
47. M. G. Ryadnov, D. N. Woolfson, *Nat. Mater.* **2**, 329 (2003).
48. C. M. Dobson, *Nature* **426**, 884 (2003).
49. J. Kinsiday et al., *Proc. Natl. Acad. Sci. U.S.A.* **99**, 9996 (2002).
50. J. D. Hartgerink, E. Beniash, S. I. Stupp, *Science* **294**, 1684 (2001).
51. G. A. Silva et al., *Science* **303**, 1352 (2004).
52. P. J. Van Haastert, P. N. Devreotes, *Nat. Rev. Mol. Cell Biol.* **5**, 626 (2004).
53. C. A. Parent, P. N. Devreotes, *Science* **284**, 765 (1999).
54. S. Schenk, V. Quaranta, *Trends Cell Biol.* **13**, 366 (2003).
55. F. X. Maquart, S. Pasco, R. Ramont, W. Hornebeck, J. C. Monboisse, *Crit. Rev. Oncol. Hematol.* **49**, 199 (2004).
56. C. S. Swindle et al., *J. Cell Biol.* **154**, 459 (2001).
57. C. D. Roskelley, A. Srebrow, M. J. Bissell, *Curr. Opin. Cell Biol.* **7**, 736 (1995).
58. M. P. Lutolf et al., *Proc. Natl. Acad. Sci. U.S.A.* **100**, 5413 (2003).
59. M. P. Lutolf et al., *Nat. Biotechnol.* **21**, 513 (2003).
60. A. Girotti et al., *J. Mater. Sci. Mater. Med.* **15**, 479 (2004).
61. J. T. Santini, M. J. Cima, R. Langer, *Nature* **397**, 335 (1999).
62. A. Curtis, *Nature* **416**, 274 (2002).
63. J. C. van Hest, D. A. Tirrell, *Chem. Commun. (Camb.)* **1897** (2001).
64. V. Mironov, T. Boland, T. Trusk, G. Forgacs, R. R. Markwald, *Trends Biotechnol.* **21**, 157 (2003).

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