

## Bioinspired Materials for Controlling Stem Cell Fate

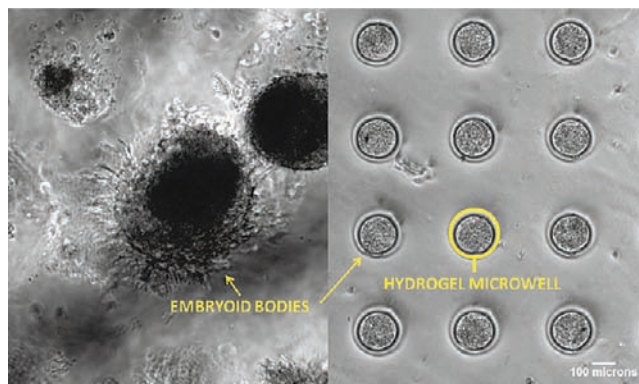
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### CON SPECTUS

**A**lthough researchers currently have limited ability to mimic the natural stem cell microenvironment, recent work at the interface of stem biology and biomaterials science has demonstrated that control over stem cell behavior with artificial microenvironments is quite advanced. Embryonic and adult stem cells are potentially useful platforms for tissue regeneration, cell-based therapeutics, and disease-in-a-dish models for drug screening. The major challenge in this field is to reliably control stem cell behavior outside the body. Common biological control schemes often ignore physicochemical parameters that materials scientists and engineers commonly manipulate, such as substrate topography and mechanical and rheological properties. However, with appropriate attention to these parameters, researchers have designed novel synthetic microenvironments to control stem cell behavior in rather unnatural ways.



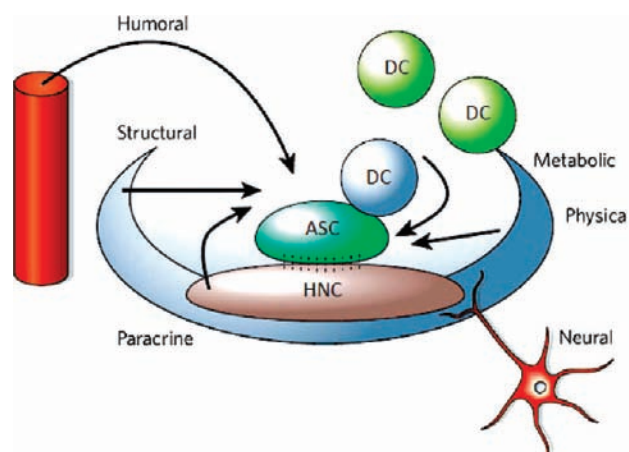
In this Account, we review synthetic microenvironments that aim to overcome the limitations of natural niches rather than to mimic them. A biomimetic stem cell control strategy is often limited by an incomplete understanding of the complex signaling pathways that drive stem cell behavior from early embryogenesis to late adulthood. The stem cell extracellular environment presents a miscellany of competing biological signals that keep the cell in a state of unstable equilibrium. Using synthetic polymers, researchers have designed synthetic microenvironments with an undisturbed array of cell signals, both specific and nonspecific, that are motivated by rather than modeled after biology. These have proven useful in maintaining cell potency, studying asymmetric cell division, and controlling cellular differentiation.

We discuss recent research that highlights important biomaterials properties for controlling stem cell behavior, as well as advanced processes for selecting those materials, such as combinatorial and high-throughput screening. Much of this work has utilized micro- and nanoscale fabrication tools for controlling material properties and generating diversity in both two and three dimensions. Because of their ease of synthesis and similarity to biological soft matter, hydrogels have become a biomaterial of choice for generating 3D microenvironments. In presenting these efforts within the framework of synthetic biology, we anticipate that future researchers may exploit synthetic polymers to create microenvironments that control stem cell behavior in clinically relevant ways.

### 1. Introduction

Embryonic stem cells (ESCs) can differentiate into any adult cell type,<sup>1</sup> while adult stem cells (ASCs) are restricted to certain lineages.<sup>2</sup> Both offer pow-

erful new tools for regenerating lost tissue as well as advancing our understanding of early human development, pathophysiology, and epigenetics. Our ability to exploit the power of stem cells has



**FIGURE 1.** Control elements of the stem cell niche. The adult stem cell (ASC) can receive both long- and short-range paracrine and endocrine signals, neural input, and architectural, mechanical, and trophic cues from the ECM. Cell–cell communication can occur between the ASC and heterologous niche cells (HNC) or daughter cells (DC). Reprinted by permission from Nature Publishing Group, ref 5, Copyright 2006.

been limited by poor control over the complex signaling events that influence their differentiation. Recently there has been great progress in engineering polymeric biomaterials that control stem cell fate.<sup>3</sup> The insight they provide on stem cell behavior *in vivo* depends on how closely they mimic the naturally occurring stem cell microenvironment, or “niche”. But it may not be true that *only* by reconstituting elements of the natural niche can we truly derive therapeutic benefits from stem cells. A biomimetic<sup>4</sup> approach to creating synthetic microenvironments comes with formidable challenges because there is much we do not yet know about the natural stem cell microenvironment. Therapeutically, it may be more useful to take a bioinspired<sup>4</sup> approach to design, where the synthetic niche acts on the stem cells in an unnatural way to achieve a therapeutic goal.

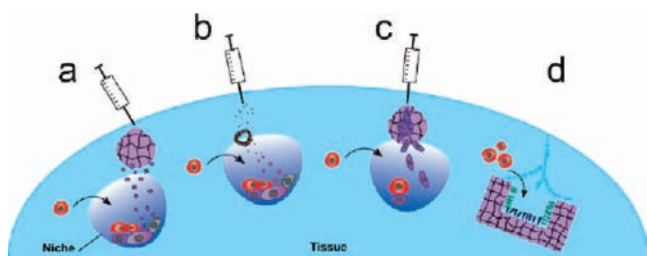
The stem cell niche is a dynamic ensemble of physico-chemical and biological cues that provide the cell with vital decision making information. It can be broken down into three major components: cell–cell contacts, cell–extracellular matrix (ECM) interactions, and cell–soluble factor interactions.<sup>5</sup> These components can be broken down further. For example, the ECM provides topographical, mechanical, and biochemical input to the stem cell. Cell–cell communication can be heterologous (e.g., neural input) and homologous, such as with daughter cells. Likewise, soluble factors can be endocrine or paracrine in origin (Figure 1). Modeling these components *ex vivo* can be a difficult feat, while integrating them in a controlled way has proven to be exceedingly complex. While all of these components may control *in vivo* stem cell differentiation, they all may not be

necessary *in vitro*. In this Account, we demonstrate that we can compensate for a lack of natural materials in our design of synthetic microenvironments by exerting greater control over the natural materials present or by engineering synthetic materials with enhanced signaling properties. In this way, we can create synthetic stem cell niches that are more *bioinspired* than *biomimetic*<sup>6</sup> and potentially more efficient than those observed in nature.

## 2. Engineering the Stem Cell Niche: Mimicking What We Know

In order to appropriately model the *in vivo* stem cell niche, we must first know all necessary niche components along with individual and synergistic effects on cell behavior. Due to a lack of this knowledge, many of the *in vitro* methods currently used to control stem cell behavior are poor biomimics. A large amount of the work up until now has been based on single stem cell types exposed to a range of experimental conditions, without regard for cell source or interspecies variations. ESCs potentially provide a more uniform platform to study cell–material interactions. But there have been documented differences between ESC lines in the same species.<sup>7</sup> ASCs may be more attractive to use therapeutically, for both ethical and immunological reasons, but they also present great challenges. For example, ASCs can undergo lineage transitions that we are only beginning to understand, such as transdifferentiation<sup>8</sup> and malignant transformation.<sup>9</sup> The fact that naturally occurring ASC niches are as varied as the stem cells they nurture adds an additional layer of complexity. Furthermore, synthetic materials with similar properties may have dissimilar effects on stem cells of different species or with different degrees of potency.<sup>10</sup> Thus, before any feasible model is developed for the rational design of biomimetic stem cell niches, further attention to cell source variation will be needed.

Stem cells may be crucial in regenerating lost or damaged tissue beyond the human body’s natural ability to heal itself. But there is an inherent causality dilemma in trying to design biomimetic niches to control stem cell behavior. The natural niche is developmentally derived from stem cell activity, whereas adult stem cell activity is directed by the niche. It is unclear exactly when during development ASC niches are established and by which cell types. But the microenvironments where these cells reside have certain similarities, such as their close proximity to basement membranes and vasculature and relative protection from damage.<sup>11</sup> Their numbers, along with their resident cells, also decline with age.<sup>11</sup> This



**FIGURE 2.** Biomaterials can be used to modulate the natural stem cell microenvironment: (a) local delivery of bioactive niche components or inhibitory or stimulatory molecules from a solid (injectable) biomaterial scaffold; (b) targeting the niche via micro- or nanoparticles that carry and deliver bioactive molecules to manipulate the niche; (c) local delivery of support cells to augment or manipulate stem cell fates *in vivo*; cell delivery could be facilitated using (injectable) biomaterial carriers that likely improve the survival and engraftment of the transplanted cells; (d) implanted, multicomponent artificial niche that could possibly attract stem cells to populate it. Reprinted with permission from ref 3. Copyright 2009 Wiley-VCH.

process is accelerated by damage and disease, further limiting the body's natural regenerative capacity. Biomaterials could potentially alleviate this decline through the targeted delivery of bioactive compounds and cells or by providing endogenous stem cells with additional niches (Figure 2).

The pharmaceutical field began with a focus on understanding the activity of naturally occurring therapeutic compounds and eventually moved toward studying unnatural drugs with greater therapeutic potential. The same shift is occurring in the stem cell community. Good evidence of this is the recent identification of (–)-indolactam, a small molecule that directs ESCs toward a pancreatic lineage.<sup>12</sup> Biomaterials have continued this shift by giving the stem cell researcher new ways to control the major components of the stem cell milieu.

### 3. Synthetic Polymer Microenvironments: Design Considerations

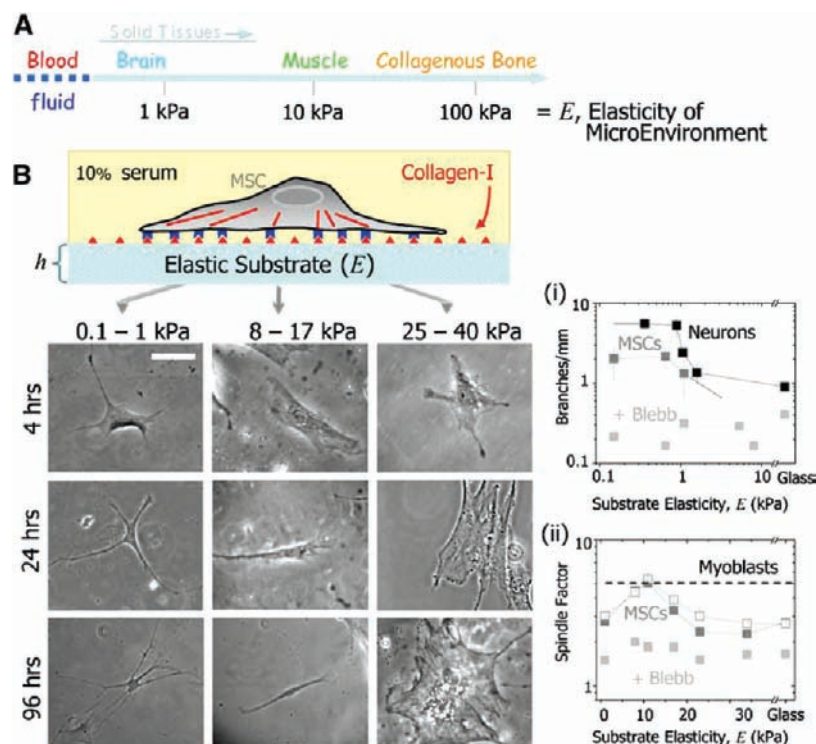
Many of the new biomaterials used to develop synthetic stem cell microenvironments are based on synthetic polymers. These can be procured from commercial sources or freshly synthesized. They come with ample control over size and shape and possess tailorable material properties. Synthetic polymer production can also be more easily scaled up than bioderived materials. A wide variety of synthetic polymers have been evaluated as cell substrates and scaffolds for tissue regeneration, with varying degrees of success.<sup>13</sup> Before these materials can be effectively used in artificial microenvironments an integrated expertise in both stem cell biology and polymer science is needed. This includes an understand-

ing of the mutual interplay between cells and materials and how they may change each other's properties. Many of the works that we have reviewed here show that material properties can be considered biological signals. Therefore the limited range of properties inherent in many biomaterials needs to be considered before they are used.

**3.1. Modeling the Extracellular Matrix.** The most basic function of synthetic microenvironments is to act as a physical substrate for stem cell attachment and migration, similar to the natural ECM. The natural ECM is a heterogeneous, self-assembled network of biological macromolecules with structural hierarchy. The molecular constituents of the ECM bind specifically to cells, other matrix molecules, and soluble factors with spatiotemporal control. Stem cells can bind to different components of the ECM either directly or via intermediate factors with varying adhesion affinity. The ECM can influence stem cell behavior via specific interactions with cells, such as with certain adhesive ligands, or nonspecifically through its physicochemical properties.

The diverse biological functions of the ECM are still being uncovered. But lack of knowledge has not limited the success of synthetic ECM analogues. Gerecht and co-workers<sup>14</sup> used one constituent of the ECM present during the early stages of embryogenesis, hyaluronic acid (HA), to develop microenvironments that inhibited human ESC differentiation. This was achieved by encapsulating the ESCs in a photopolymerized HA hydrogel, a configuration with homogeneity and structural coherence otherwise not encountered during embryogenesis. Other groups have also controlled stem cell behavior by reconfiguring single elements of the natural ECM, such as collagen,<sup>15</sup> fibronectin, and laminin,<sup>16</sup> or by utilizing cooperative effects of multiple ECM molecules. For example, Flaim and co-workers<sup>17</sup> analyzed combinatorial mixtures of ECM molecules for cooperative control over murine ESC differentiation and rapidly identified key mixtures with synergistic properties. Nakajima et al.<sup>18</sup> screened combinatorial mixtures of ECM proteins combined with immobilized growth factors for controlling neural stem cell differentiation, uncovering synergistic effects between certain matrix components and soluble factors. This work demonstrates the rigorous and potentially protracted empirical work needed to find the right niche for the right stem cell, particularly for the stem cell researcher without access to microfabrication technology. One way around this is to use bioderived ECM as starting materials, such as small intestine submucosa,<sup>19</sup> or Matrigel,<sup>20</sup> which have both proven superior to synthetic substrates in certain applications. However, these materials are generally xenogenic and prone to batch-to-batch inconsistency. Also, as





**FIGURE 3.** Substrate elasticity directs the differentiation of MSCs: (A) the range of elastic modulus,  $E$ , for select tissues; (B) MSCs placed on substrates with varied stiffness are initially small and round but overtime change morphology according to substrate elasticity; (i) cell branching per length of primary mouse neurons, MSCs, and blebbistatin-treated MSCs, and (ii) spindle morphology of MSCs, blebbistatin-treated MSCs, and mitomycin-C treated MSCs (open squares) compared with C2C12 myoblasts (dashed line). Reprinted with permission from ref 21. Copyright 2006 Elsevier.

poorly understood materials, they provide little mechanistic information for controlling stem cells.

**3.1.1. Synthetic Extracellular Matrices.** What is fascinating about many of the synthetic biomaterials that have been developed to control stem fate is both how much and how little they mimic the natural ECM. For example, poly(ethylene glycol) (PEG) hydrogels are a popular biomaterial for encapsulating stem cells. They mimic the natural ECM in their ability to imbibe large amounts of water and can be tailored to possess mechanical properties similar to various natural tissue types. But these nonionic and covalently cross-linked networks are very different from the self-assembled polyelectrolytes that comprise the bulk of natural ECMs. Likewise, synthetically degradable biomaterials often have nonbiologically controlled degradation rates, whereas the turnover of the natural ECM is largely dictated by needs of the resident cell types. Similarly, inorganic surfaces and other highly crystalline materials are substrates that stem cells generally do not contact in vivo.

There are those biomaterials that control stem cell behavior primarily because of their biomimicry. For example, Engler et al.<sup>21</sup> showed that mesenchymal stem cells (MSCs) differentiate into tissues that most closely match the mechanical prop-

erties of the polyacrylamide substrate upon which they were cultured (Figure 3). Thus, MSCs that were cultured on stiff (bonelike) gels differentiated into osteoblasts, those that were cultured on medium stiffness (muscle-like) gels differentiated into muscle cells, and those that were cultured on compliant gels (neural-like) differentiated into neural cells. Due to the heterogeneity of MSCs, it is unclear whether the same cell type responded differently to substrate stiffness or stiffness resulted in the differential growth of precommitted progenitor cells. Benoit et al.<sup>22</sup> modeled the unique chemical environments present in various tissue types and the potential cues these provide to resident stem cells. In their work, PEG hydrogels were doped with small amounts of pendant carboxyl groups to mimic glycosaminoglycans in cartilage, phosphate groups for their role in bone mineralization, or *tert*-butyl groups to mimic the lipid-rich environment in adipose tissue. True to their model, these gels were successfully able to direct the differentiation of human MSCs down chondrogenic, osteogenic, or adipogenic pathways respectively.

While isolated ECM proteins and mixtures thereof can provide a more controlled ECM mimic, a more reductionist approach to controlling stem cell fate is to use individual peptide sequences and epitopes derived from ECM molecules or

to model the basic physicochemical properties of the ECM itself. In this way, ECM signaling cues can be utilized without the redundancy and complexity of the natural material. Hybrid microenvironments that consist of both natural and synthetic components can allow certain biological cues to be used in otherwise non-natural ways. A good example of this is the use of substrates with covalently attached peptide sequences, such as matrix metalloproteinase sensitive peptide sequences,<sup>23</sup> within stem cell microenvironments. This allows for “cell-demanded” matrix degradation without the additional signaling cues present in many of the natural proteins from which these peptides are derived. Recently Martino et al.<sup>24</sup> were able to isolate peptide fragments from fibronectin that were able to enhance the osteogenic differentiation of human MSCs compared with the full-length protein. The peptide showed increased specificity for integrin  $\alpha_5\beta_1$  in contrast to the relatively promiscuous integrin binding of fibronectin, which provides both pro- and antidifferentiative cues in the natural MSC niche. Certain peptides can be designed to self-assemble in situ to form synthetic microenvironments. They can be designed to assemble into 3D ECM-like supramolecular structures but with greater control over both architecture and cell signaling. They are an attractive alternative to poorly understood substrates such as Matrigel. In certain cases, they have been shown to perform as well or better. Gelain et al.<sup>25</sup> designed self-assembling peptide scaffolds with preprogrammed cell adhesion, differentiation, and bone marrow homing motifs for controlling neural stem cells (NSC) differentiation compared with Matrigel. The peptide scaffold was similar to Matrigel in its ability to induce neuronal differentiation but caused a different and more uniform gene expression. Silva et al.<sup>26</sup> have also engineered amphiphilic, self-assembling peptides of particular interest in stem cell differentiation. These are comprised of a peptide component and a lipophilic hydrocarbon tail. In physiological conditions, these molecules undergo an ordered arrangement of entangled nanofibers, similar to the nanofibrous architecture of the native ECM. These peptides can be tailor-made with biological specificity. When these were designed to display the laminin-derived IKVAV peptide sequence, they proved effective at directing mNSCs toward a neuronal fate.

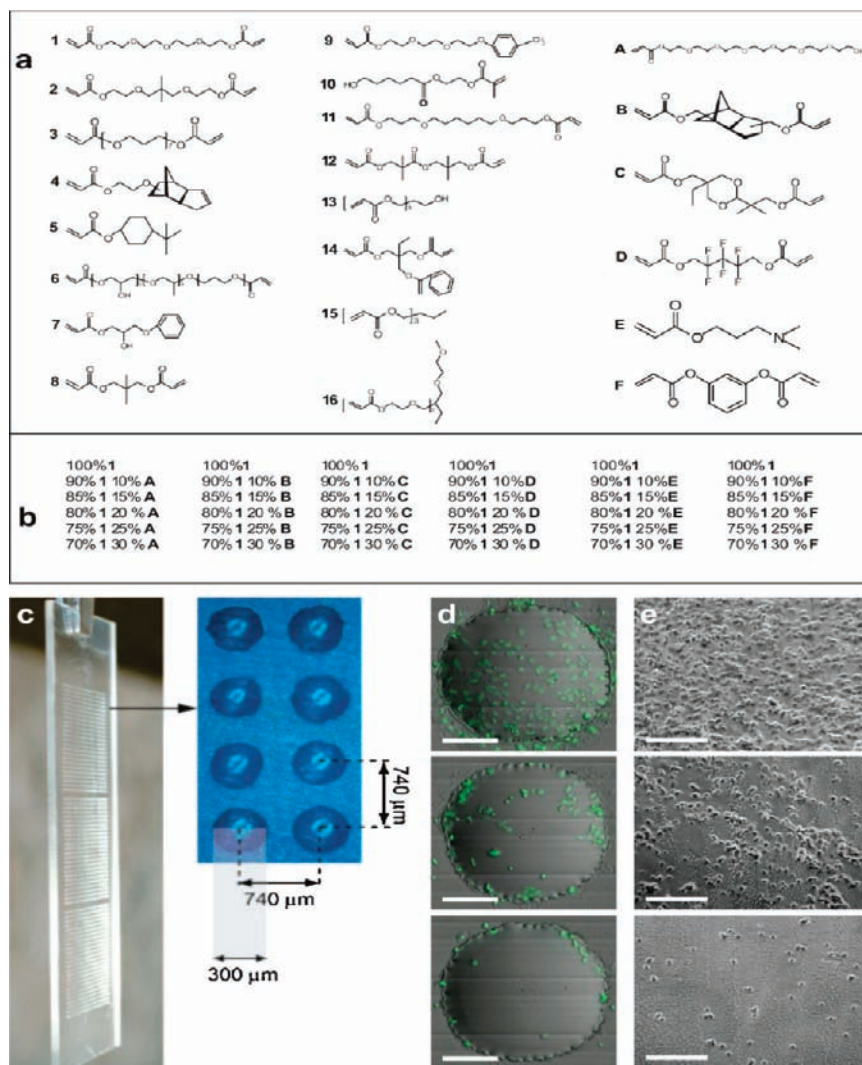
### 3.2. Combinatorial and High-Throughput Screening.

Combinatorial and high-throughput screening (CHTS) can be extremely useful for selecting polymers for biomedical applications, particularly where there is a lack of mechanistic information. Combined with micro- and nanofabrication, CTHS can be performed using small amounts of sample and at the cellular size scale. For example, Langer and co-workers<sup>27,28</sup> used

CTHS to identify polymers that supported the attachment and proliferation of MSCs and ESCs (Figure 4). Others have similarly used CTHS to analyze stem cells exposed to libraries of synthetic polymers, mixtures of ECM proteins, and soluble factors.<sup>29–31</sup> To date, most of the work done investigating stem cell–biomaterial interactions using CTHS has been conducted using 2D surfaces. This limits the amount of mechanistic information that can be derived from the results of these studies and therefore makes it difficult to determine how closely they model the natural stem cell niche.

**3.3. Hydrogel Microenvironments.** Hydrogels have become a biomaterial of choice for 3D stem cell work. These materials are used as substrates, scaffolds, and encapsulants for stem cells due to their tissue-like tunable material properties. These networks can be held together via physical or chemical cross-links, can be made biodegradable, and can be engineered to controllably deliver soluble factors.<sup>32</sup> While hydrogel cross-linking is controllable, it may not be straightforward to modify other gel properties independently. The swelling ratio, mesh size, and mechanical properties of hydrogels all can vary with cross-linking, and changes in these properties have been correlated with changes in stem cell behavior.<sup>21,33</sup>

Covalently cross-linked hydrogels offer greater structural integrity and can be easily synthesized using methods such as photopolymerization,<sup>34</sup> thermally initiated polymerization,<sup>33,35</sup> and Michael addition.<sup>24,36</sup> Hydrogel niches can also be self-assembled (i.e., physically cross-linked) from polymers such as polysaccharides and custom-made peptides.<sup>32</sup> Care should be taken in the fabrication of hydrogel niches formed for the purpose of stem cell encapsulation or in situ polymerization. In case of free radical polymerization, the chemical environment around the cell changes drastically throughout the course of the reaction. This process has been shown to negatively affect the viability, proliferation, and differentiation of ASCs during encapsulation.<sup>37</sup> In many cases, it is unclear how close the reaction comes to completion or how many residual active groups are leftover. This deserves further attention in light of recent work, which showed that low concentrations of small functional groups can affect the lineage commitment of encapsulated MSCs.<sup>22</sup> An alternative approach is to preform porous hydrogels capable of stem cell infiltration. Recently such a method was developed using gaseous CO<sub>2</sub> as a porogen.<sup>38</sup> These PEG scaffolds possessed interconnected pores ranging in size from 100 to 600  $\mu\text{m}$ .



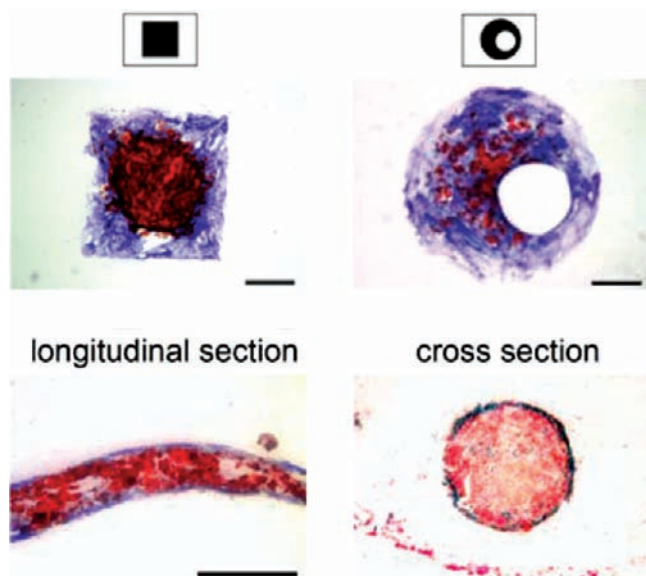
**FIGURE 4.** Biomaterial array designed for high-throughput analysis of hESC–substrate interactions: (a) monomers used for array synthesis; (b) combinations for the major monomer **1** with monomers **A–F**; (c) a polymer microarray in triplicate with a close-up of eight spots; (d) merged fluorescence and laser scattering images showing three representative cell attachments (high, intermediate, low) on the polymer spots (scale bar = 100  $\mu\text{m}$ ); (e) reproduced large-scale stem cell adhesion on polymer film (scale bar = 200  $\mu\text{m}$ ). Reprinted with permission from ref 28. Copyright 2009 Wiley-VCH.

#### 4. Geometric Control of Stem Cell Fate

A spatial relationship is known to exist between stem cells and their niche. The microarchitecture of the *in vivo* stem cell niche can maintain concentration gradients of growth factors, modulate cell–cell adhesion, and determine proximity to the vasculature or basement membrane. For example, MSCs are known to show a gradient pattern of differentiation depending on their distance from the endosteal surface.<sup>39</sup> Similarly, intestinal epithelial cells display a crypt-to-villus pattern of differentiation,<sup>40</sup> and rodent incisor stem cells show a apical to incisal pattern.<sup>41</sup> Recently, micro- and nanofabrication schemes have allowed for the design of artificial microenvironments that may reductively shed light on these mechanisms and offer novel ways to control stem cell fate.<sup>42,43</sup> Some of the first studies in controlling

behavior of individual cells on surfaces were performed by immobilizing cells on micropatterned substrates that were coated with regions of adhesive and nonadhesive molecules. By use of this technology, it was determined that primary cells on smaller surfaces tended to undergo apoptosis while those that were patterned on larger substrates tended to proliferate.<sup>44</sup> Similar studies on ASCs demonstrated that changes in cell substrate geometry caused changes in cytoskeletal tension, which influenced their differentiation.<sup>21,45</sup> Recently micropatterned microenvironments have been used to control the differentiation of stem cells exposed to a mixture of prodifferentiative signals. Ruiz et al.<sup>45</sup> cultured MSC sheets on micropatterns of controlled shape and exposed them to a mixture of pro-osteogenic and pro-adipogenic morphogens. The cells showed a pattern of differentia-

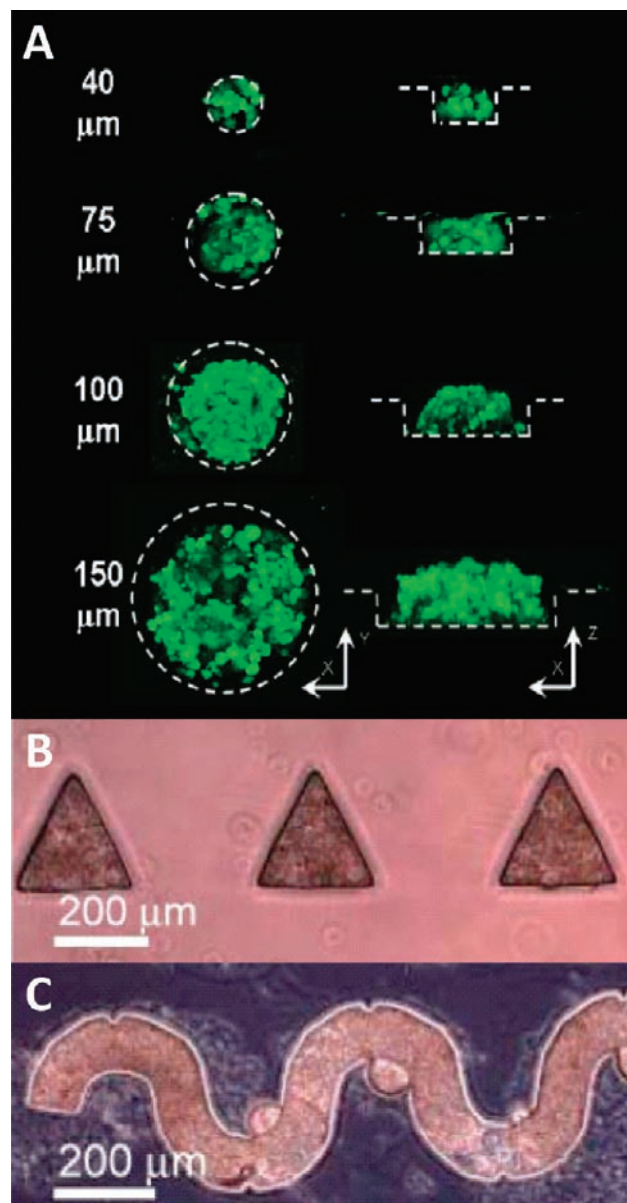




**FIGURE 5.** Spatially controlled differentiation of mesenchymal stem cells into bony (blue) and fatty tissue (red). Planar cell adhesive micropatterns, such as a square (top left) or an offset annulus (top right) provide controlled regions of high and low cytoskeletal stress, thereby influencing differentiation. Scale bar = 250  $\mu\text{m}$ . In the bottom panels, multicellular 3D constructs differentiate into a fatty core surrounded by bony tissue, similar to natural long bones. Reprinted with permission from ref 45. Copyright 2008 Wiley-VCH.

tion dictated by their spatial arrangement and corresponding cytoskeletal stress. The authors used this mechanism to create multicellular stem cell constructs that mimicked the architecture of normal bone, a bony tube filled with a fatty core (Figure 5).

Geometric control is also useful for controlling cell–cell interactions. For example, the aggregation of ESCs in vitro into embryoid bodies (EB) is a preliminary step toward differentiation. One issue with culturing embryoid bodies using conventional methods is poor control over EB formation and a resulting broad range of sizes. Spatial control over the formation of EBs can lead to a more homogeneous, and thereby more efficiently controlled, differentiation. Karp et al.<sup>46</sup> developed microfabricated hydrogel microwells as a way to control the shape of EBs. Their approach resulted in synthetic microenvironments that enhanced the differentiation of ESCs and significantly reduced variability in the expression of differentiation markers. They were also able to pattern EBs into shapes that do not naturally occur, such as triangles and curves (Figure 6). The biological implications of this have yet to become clear. In another approach, Carpenedo et al.<sup>47</sup> designed morphogen-releasing spherical templates to both control the shape and direct the differentiation of EBs. In their work, embryonic stem cells were cultured on biodegradable microspheres loaded with retinoic acid. This resulted in the



**FIGURE 6.** Microfabricated culture wells designed to control the shape of embryoid bodies derived from murine ESCs: (A) confocal microscopy images of fluorescently labeled EBs within microwells 40–150  $\mu\text{m}$  in diameter; the technique was also used to create EBs with synthetic shapes, such as triangles (B) and curves (C). Reprinted with permission from ref 46. Copyright 2007 The Royal Society of Chemistry.

homogeneous differentiation of cystic spheroids with a biepithelial morphology (Figure 7). This approach could be useful for applications such as regenerating intestinal tissue for the treatment of short bowel syndrome, where a hollow epithelial construct is required prior to implantation.

## 5. The Right Timing

It is well understood that time varying cues are important components of the stem cell niche, particularly in the inher-





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**Robert Langer** is the David H. Koch Institute Professor at MIT. He has authored over 1050 articles, has over 750 issued or pending patents, and holds 11 honorary doctorates. He has received over 150 awards including the Charles Stark Draper Prize, considered the engineering equivalent of the Nobel Prize; the Albany Medical Prize, the nation's largest medical prize; the National Medal of Science; and the American Chemical Society's (ACS) Polymer Chemistry, Applied Polymer Science, and Chemistry of Materials Awards. He has been elected to the National Academy of Science, the Institute of Medicine of the National Academy of Science, and the National Academy of Engineering.

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#### FOOTNOTES

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