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Designing synthetic materials to control stem cell phenotype

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The micro-environment in which stem cells reside regulates their fate, and synthetic materials have recently been designed to emulate these regulatory processes for various medical applications. Ligands inspired by the natural extracellular matrix, cell–cell contacts, and growth factors have been incorporated into synthetic materials with precisely engineered density and presentation. Furthermore, material architecture and mechanical properties are material design parameters that provide a context for receptor–ligand interactions and thereby contribute to fate determination of uncommitted stem cells. Although significant progress has been made in biomaterials development for cellular control, the design of more sophisticated and robust synthetic materials can address future challenges in achieving spatiotemporal control of cellular phenotype and in implementing histocompatible clinical therapies.

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Introduction
Stem cells are defined by their capacities for self-renewal and differentiation into one or more cell lineages [1,2]. Without tight regulation or control of these properties, any derivative cell population, will exhibit a range of heterogeneous phenotypes, yielding artifacts that may complicate the development of cell therapies and pharmaceuticals. Recent work demonstrates that biomaterials (i.e. matrices, scaffolds, culture substrates) can present key regulatory signals that combine with other environmental and genetic influences to create synthetic micro-environments that control stem cell fate (Box 1). It can be argued that many of the promising therapeutic applications of stem cells will require instructive materials that exert active control over stem cell phenotype. Such materials may be designed for stem cell expansion and differentiation *ex vivo*, tissue regeneration via implantation with stem cells, or implantation alone to direct endogenous stem cell behavior. This review will discuss fundamental material properties that will be required to control stem cell function for any of these applications (Box 2).

Natural versus synthetic materials
Natural niches direct stem cell behavior *in vivo* to orchestrate the processes of tissue development, homeostasis, and physiological remodeling as well as injury recovery throughout life [3]. Components of native stem cell niches [e.g. extracellular matrix (ECM) proteins such as collagen and laminin, as well as proteoglycans such as heparan sulfate] can be isolated and used to create micro-environments that direct stem cell behavior *in vitro*, typically in combination with a cocktail of exogenous soluble factors in the culture media [4–6]. Like the *in vivo* niche, these natural materials engage cell surface receptors as well as provide a physical environment to regulate cell function. However, natural materials suffer from high lot-to-lot variability [7], high contamination potential [7], and xenogeneic protein components that may elicit an immune response upon implantation [8,9].

Synthetic material systems can be specifically designed to interact with cells on different length scales (e.g. molecular, cellular, and macroscopic) and thereby mimic the elements of natural stem cell niches [10–12]. By contrast with their natural counterparts, synthetic materials offer the potential for improved control, repeatability, safety, and scalability.

A broad variety of synthetic materials has been designed and created to direct stem cell phenotype. *Natural polymers*, typically elements of mammalian ECM or structural components from other organisms (e.g. alginate or chitosan), can be chemically, thermally, or physically processed to alter their chemistry, mechanics, degradation, and biological performance [13,14]. However, these modified materials suffer from many of the same problems of repeatability, safety, and scalability of their natural polymer parents. *Synthetic polymers* offer a wide

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Box 1 Glossary. See also http://www.isscr.org/glossary.

- *Cell fate determinant*: any molecular entity (e.g. hormones, small molecules, proteins, small RNAs, epigenetic factors) that controls the precise timing and output of cell phenotype.

- *Cell differentiation*: progressive restriction of the developmental potential and increasing specialization of function that takes place during the development of the embryo and leads to the formation of specialized cells, tissues, and organs.

- *Embryonic stem cell*: pluripotent cell lines derived from early embryos before formation of the germ layers.

- *Epigenetic*: reversible, heritable changes in gene regulation that occur without a change in DNA sequence (e.g. chemical modification of DNA or its surrounding proteins).

- *Modulus*: an intrinsic property of the material relating stress and strain. For elastic materials, the elastic modulus (i.e. Young's modulus, E) is the ratio of stress to strain (for small strains). For viscoelastic materials, the complex dynamic shear modulus, G^*, along with the loss angle, is often used to represent the relations between the oscillating stress and strain and depends on the loading rate.

- *Niche*: cellular micro-environment providing support and stimuli necessary to sustain self-renewal or controlled differentiation.

- *Potency*: the range of fate commitment options available to a cell.

- *Phenotype*: an ostensible property of a cell, such as its differentiation fate, that is determined by its genotype, history, and environment.

- *Presentation*: the manner in which a ligand appears at the interphase between a material and a cell, including mobility, valency, conformation, and orientation.

- *Self-renewal*: cycles of division that repeatedly generate at least one daughter equivalent to the mother cell with latent capacity for differentiation.

- *Stem cells*: cells that have the capacity both to self-renew (make more stem cells by cell division) as well as to differentiate into mature, specialized cells.

- *Stiffness*: resistance of an elastic body to deflection or deformation by an applied force; an extrinsic material property that depends on the material geometry and boundary conditions.

- *Viscoelasticity*: time-dependent mechanical properties characterizing materials exhibiting both storage (elastic solid) and loss (viscous fluid) characteristics.

Box 2 Self-renewal and differentiation are highly context dependent

Mechanisms controlling self-renewal and differentiation in stem cells orchestrate the expansion and diversification of cell types during an organism's development [1]. Self-renewal of a stem cell requires, after cell division, the absence of differentiation in at least one of the daughter cells. Importantly, regulation of these processes is highly context dependent, such that determinants of cell fate inside (i.e. intrinsic) and outside (i.e. extrinsic) of the stem cell act in concert. Intrinsic determinants include a wide array of epigenetic and genetic factors [59], whereas extrinsic determinants include soluble and solid-phase ligands, cell–cell contacts, and architectural and mechanical properties of the cellular environment [1,40••,52••]. These various determinants converge in highly complex and dynamically interconnected developmental signaling networks involving second messenger and protein signaling cascades, transcription factors, and epigenetic patterning. Therefore, for stem cells in culture, the molecular history of previous signals such as during cell harvesting and expansion, as well as the soluble factors in culture media, may impact the way cells respond to signals presented from biomaterials.

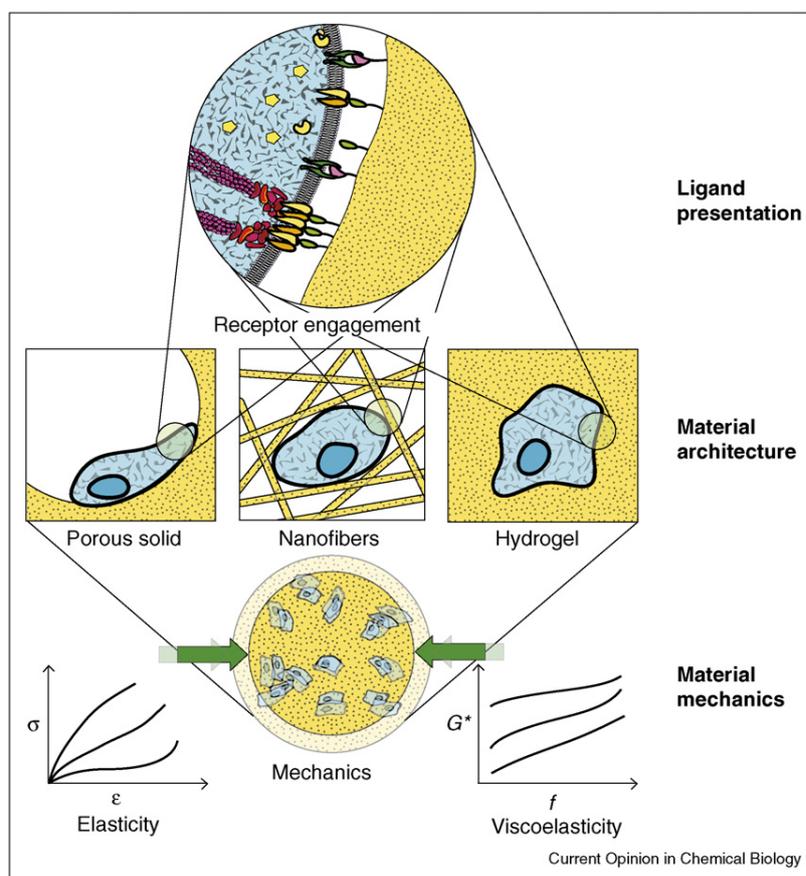---

Synthetic micro-environment design parameters

Regardless of which class is utilized, materials must be processed and functionalized for specific therapeutic applications. In particular, material properties important for controlling stem cell behavior include ligand identity, presentation, and density, as well as material architecture and mechanical properties (Figure 1). Effectively engineering these design parameters will yield materials that create an architecture that resembles their native environment, have controlled mechanical properties that enable adhesion and the development of contractility in the cellular cytoskeleton, and present ligands that direct intracellular signaling and gene expression (Table 1).

Ligand identity, presentation, and density

Ligands modulate stem cell phenotype in a manner dependent on their identity (i.e. specificity), mode of presentation, and density [20•,26,27•,28–31]. Self-renewal and differentiation mechanisms have been shown to be sensitive to numerous ligands and combinations of ligands: adhesion ligands from the ECM [26,32,33,35•], ligands presented from neighboring cells [27•], and immobilized growth factors [28,34]. Synthetic peptide ligands are often used in place of large proteins or protein fragments because of their stability and ease of synthesis, isolation, and conjugation to materials [16••,20•,35•]. For example, Gelain *et al.* used self-assembling amphiphilic peptides to determine the effect of a variety of peptide ligand sequences on mouse adult neural stem cell differentiation.

Once a ligand or set of ligands is selected for a specific biomaterials application, it must be conjugated to the material for proper presentation (e.g. surface immobilization, polymer modification, or creation of ligand macromers); the subject of many investigations with

range of controlled chemistries and mechanical properties because of the variety of available monomers and co-polymer structures [15••,16••,17]. Popular synthetic polymer types include polyacrylamides, polyacrylates, polyethers, polyesters, polyhydroxy acids, polyfumarates, and polyphosphazenes. *Self-assembling peptides*, *peptide-amphiphiles*, and *genetically engineered proteins* allow incorporation of specific cell-engaging motifs into rationally designed chemical biology assemblies [18,19•,20•]. *Inorganic materials* have been used to mimic the osteogenic niche [21–23], whereas *hybrids* and *composites* combine the aforementioned classes to create unique, application-specific matrices [21,24,25].

Figure 1

Design parameters for engineering synthetic stem cell materials. (a) Ligand identity, density, and presentation from the material surface dictate interactions with cell surface receptors to alter cytoskeletal linkages and intracellular signaling pathways. (b) Receptor–ligand interactions are further modulated by material architectures, which provide a two-dimensional (e.g. flat surfaces, microporous solids) or three-dimensional (e.g. nanofibers, hydrogels) micro-environment for cellular engagement. (c) Also, the elastic and viscoelastic properties of the material determine the interplay between cell and material mechanics. Collectively, these parameters define the context for stem cell self-renewal and differentiation in a similar fashion to their native niches. Graphs schematically depict mechanical properties: elastic properties via a stress (σ)–strain (ε) plot and viscoelastic properties via a complex modulus (G^*)–frequency (f) plot.

Table 1

Engineered synthetic stem cell materials

Parameter	Stem cell	Synthetic material	Reference
Ligand identity	Mouse adult neural stem cells	RADA16 self-assembling peptide nanofibers	[20•]
	Human mesenchymal stem cells	Polyethylene glycol phosphate hydrogel	[29]
	Rat esophageal epithelial stem cells	Polyhydroxyethyl methacrylate hydrogel	[27•]
	Human embryonic stem cells	Polycellulose acetate microfibers	[28]
Ligand density	Rat mesenchymal stem cells	Oligo polyethylene glycol fumarate hydrogel	[26]
	Rat adult neural stem cells	Polyacrylamide-*co*-polyethylene glycol/acrylic acid hydrogel	[35•]
Material architecture	Mouse embryonic stem cells	RADA16 self-assembling peptide nanofibers	[48]
	Human embryonic stem cells and mouse embryonic fibroblasts	Polydimethylsiloxane microwells	[47]
	Rat mesenchymal stem cells	Peptide-amphiphile nanofibers	[46]
	Rat preadipocytes	Polyethylene glycol hydrogel	[44]
	Mouse mesenchymal stem cells	Polyamide electrospun nanofibers	[43•]
Material mechanics	Human mesenchymal stem cells	Polyacrylamide hydrogel	[52••]
	Human embryonic stem cells	Poly N-isopropylacrylamide-co-acrylic acid/polyacrylic acid hydrogel	[16••]

non-stem-cell types [36,37]. Peptide and protein ligands are typically conjugated to materials or material building blocks via primary amines (the amino terminus or lysines) or sulfhydryl groups (cysteines). In addition, spacer arm length and chemistry can in general be tuned to alter ligand availability and activity. Furthermore, the secondary and tertiary structures of native macromolecules frequently present ligands in a specific spatial conformation that promotes binding to receptors, and it is thus desirable to mimic such conformations in synthetic materials by using cyclic peptides or other ligand structures [37]. Recent work has shown that the density of the selected ligand strongly influences the downstream stem cell response [26,33,35•]. For example, Saha et al. demonstrated the effect of peptide and mixed peptide densities on neural stem cells using modular biomimetic interpenetrating network (IPN) hydrogels. IPNs presenting >5.3 pmol/cm^2 of an integrin-binding RGD-containing peptide sequence from bone sialoprotein supported both self-renewal and differentiation similar to laminin, whereas an IKVAV-containing peptide from laminin did not support attachment or influence differentiation.

Material architecture

The manner in which a material is organized and structured on the microscale and nanoscale, or 'material architecture,' is known to modulate cell signaling and organization. At the cellular scale, ligand engagement, molecular diffusion, and force transmission are dictated by the geometry of the cellular interface with the material, the neighboring cells, and the surrounding aqueous micro-environment [38,39]. In addition, at larger scales (>10^2 μm), material architecture determines bulk mechanical properties, possible cell seeding methods, cell migration, and nutrient and waste exchange.

Both two-dimensional (2D) and three-dimensional (3D) material architectures have been used for stem cell culture. In traditional 2D culture systems, signaling and diffusion are inherently asymmetric. Still, such culturing platforms can effectively present ligands to stem cells [4,34,35•,40••], are straightforwardly produced and seeded with cells [15••], and may be readily scaled up for therapeutic applications. For 3D scaffolds and regenerative implants, three predominant architectures have been used: porous solids, nanofibers, and hydrogels (Figure 1). Stem cells have been cultured within interconnected microporous 3D solids with cell porosities greater than the cell diameter [28,41], such that the materials signal effectively as 2D surfaces. Nanofibrous scaffolds present a 3D nanostructured topology that resembles the fibrillar ECM proteins in vivo [42,43•], whereas hydrogels simulate the hydrated structural aspect of native ECMs [44,45]. Three-dimensional cell encapsulation can be achieved through in situ formation of materials around stem cells [46], a general biomaterials approach recently reviewed elsewhere [36].

Stem cells have also been influenced by employing material architecture to control engagement with the material and neighboring cells [40••,43•,47,48]. For example, recent work used microwell architectures to grow and contain small clusters of stem cells, which were found to be less prone to differentiation [47]. Other work suggests that material architecture can be designed to operate in conjunction with biological ligands to determine their ultimate effect on stem cell phenotype [48,49]. Garreta et al. used mouse embryonic stem cell engagement with a 3D nanofibrous architecture to alter cell surface receptor and cytoskeletal spatial arrangement and, in turn, ligand signaling. In addition, techniques that can spatially pattern ligands have recently been applied to create 'niches' with reigospecific chemistry for stem cell adhesion and engagement, in both 2D [40••] and 3D [50]. The former study organized cell adhesion at the 1–100 μm^2 length scale and thereby demonstrated that cell spreading regulates mesenchymal stem cell differentiation.

Material mechanical properties

The mechanics of a material, determined primarily by its composition, water content, and structure, affect intermolecular and intramolecular forces and stress distributions. Common methods of altering the mechanical properties of biomaterials include modulating molecular composition and connectivity, thermal processing, and creating reinforced and porous composites. Previous studies using differentiated cell types have demonstrated that the mechanical properties of a material affect cell behaviors such as proliferation and migration. In particular, adhesion ligands, which bind to integrins and other cell surface receptors, serve as mechanical transducers between the external material and the internal cytoskeleton of the cell, allowing cells to sense and respond to the stiffness of their substrates. Tensional homeostasis with the micro-environment thereby induces cellular cytoskeletal organization [51] and contraction [49] and alters gene regulatory pathways [51,52••].

Recent work with human stem cells indicates that the elastic modulus of a culture material can alter or maintain stem cell phenotype [16••,52••]. Engler et al. suggest that contractile forces in the cytoskeleton must be developed by actin–myosin molecular motor action for phenotypic differentiation. They further indicate that human mesenchymal stem cells differentiate into cells of the tissue type that matches the stiffness of the environment in which they are cultured. In the work on human embryonic stem cells, Li et al. propose that the soft mechanical properties of their hydrogels establish a cellular context to promote stem cell self-renewal.

A less considered aspect of cell material mechanics is viscoelasticity. Natural tissues and cells themselves are viscoelastic [53], and cells may probe their environments

at several physiologically relevant frequencies [54]. Further work is needed to characterize the effect of material mechanics, in particular substrate viscoelasticity, on stem cell self-renewal and differentiation mechanisms.

Conclusions and future directions

Stem cells respond with exquisite sensitivity to cell-extrinsic signals, many of which can be engineered into synthetic materials. Emerging work in this field indicates that five key design parameters influence stem cell behavior in a biomaterial: *ligand identity*, *presentation*, and *density*; *material architecture*; and *material mechanical properties*. Together, these material properties coordinate the interplay between intrinsic and extrinsic determinants of stem cell fate to produce a desired phenotype.

However, progress is still required to improve the static and dynamic properties of materials. Materials' design will benefit from new methods to *independently* tune the parameters of multifunctional scaffolds and matrices. In addition, 3D *in vitro* culture will likely be used to further mimic and study *in vivo* physiological phenomena. Spatial patterning of 3D materials [50] and cells [55] will facilitate such studies. Because signaling dynamics affect phenotype commitment of stem cells [1,2], further control over the spatiotemporal properties of materials will be required. Controlled release methods with programmed material degradation, to engineer ligand release kinetics for example, are beginning to be used in conjunction with stem cells [29]. Cellular architecture and matrix infiltration can also be dynamically controlled by enzymatic material degradation [16**,44,56–58].

Even with significant progress in these directions, particular challenges exist when applying these materials to practical stem cell applications. In regenerative medicine applications, materials will need to be designed to modulate or elude the immune response *in vivo* beyond the current passive nonfouling approaches, as well as direct stem cells to do the same. Finally, even low frequency culturing artifacts from genetic and epigenetic instability or material fluctuations could be amplified in scaling from bench-top to industrial culture systems. Massively produced culture substrates and micro-environments will need to be uniform and could be engineered to select for cells with proper genomes and epigenetic patterning. Overcoming these challenges in the large-scale production of cell substrates and culture systems will be required before the clinical potential of stem cells can be realized.

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