Synthetic Extracellular Microenvironment for Modulating Stem Cell Behaviors

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ABSTRACT: The innate ability of stem cells to self-renew and differentiate into multiple cell types makes them a promising source for tissue engineering and regenerative medicine applications. Their capacity for self-renewal and differentiation is largely influenced by the combination of physical, chemical, and biological signals found in the stem cell niche, both temporally and spatially. Embryonic and adult stem cells are potentially useful for cell-based approaches; however, regulating stem cell behavior remains a major challenge in their clinical use. Most of the current approaches for controlling stem cell fate do not fully address all of the complex signaling pathways that drive stem cell behaviors in their natural microenvironments. To overcome this limitation, a new generation of biomaterials is being developed for use as three-dimensional synthetic microenvironments that can mimic the regulatory characteristics of natural extracellular matrix (ECM) proteins and ECM-bound growth factors. These synthetic microenvironments are currently being investigated as a substrate with surface immobilization and controlled release of bioactive molecules to direct the stem cell fate *in vitro*, as a tissue template to guide and improve the neo-tissue formation both *in vitro* and *in vivo*, and as a delivery vehicle for cell therapy *in vivo*. The continued advancement of such an intelligent biomaterial system as the synthetic extracellular microenvironment holds the promise of improved therapies for numerous debilitating medical conditions for which no satisfactory cure exists today.

KEYWORDS: stem cells, stem cell niche, differentiation, biomaterials, drug/protein delivery system, surface modification, substrate elasticity, topography

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Introduction

Disease, injury, and aging account for a significant number of clinical disorders. Current treatments vary with the type of tissue/organ affected, but all have limitations. Tissue engineering, which combines the disciplines of both materials science and life sciences to replace a diseased or damaged tissue or organ with a living functional engineered substitute, has emerged as a very promising therapeutic option.[1,2] A major consideration in tissue engineering is the architecture of scaffolds upon which seeded cells are directed to proliferate and differentiate to form a new tissue. Such tissue-engineered constructs composed of cell-seeded scaffolds are among the most promising approaches to generate functional replacement tissues.[3] To engineer a functional tissue construct, it is necessary to understand how specialized artificial scaffold templates and compositions affect cell behaviors, particularly stem cells, and use this information to direct the design of engineered tissues and organs.[2] Unfortunately, due to an incomplete understanding of the interactions between substrate materials and specific cell types, and the inability to control the complex signaling pathways elicited by these interactions, the ability to design functional tissue and organ substitutes has been limited.[4]

Tissue formation, homeostasis, and regeneration after disease or injury are critically dependent on stem cells, which offer potential in tissue engineering applications because of their unique capacity to self-renew and differentiate into multilineage cell types, such as neurogenic, osteogenic, chondrogenic, and myogenic cells, under appropriate stimuli. While there have been rapid advances in deciphering the signals and the underlying cellular pathways regulating stem cell fate, significant technical obstacles must be overcome before stem cells can be used safely and efficiently in patients. The greatest challenge remains the ability to control stem cells' fate outside of the cell's natural microenvironment or "niche". Stem cell niches are extracellular regulatory microenvironments that consist of a complex mixture of insoluble and soluble as well as short- and long-range extracellular matrix (ECM) proteins that regulate the behavior of the cells within that niche.[5,6] Different types of stem cells have their own characteristic niches. These environmental cues are interpreted by the stem cells,

which respond either by choosing a self-renewal pathway or differentiation. Outside their niche, adult stem cells quickly lose their developmental potential.[7] Hence, maintaining the native properties of stem cells outside their niche requires designing an artificial environment, or microenvironment, that can closely mimic the natural stem cell niche.

Recently, significant progress has been made in guiding stem cell differentiation *in vitro*, and has led to an understanding of the complex interplay of factors that control stem cell fate. Therefore, a significant focus has been the utilization of "functionalized" polymeric scaffolds as a means of controlling stem cell fate via physical, chemical, mechanical, and/or biological cues that are communicated to the cells. Biomaterials can play central roles in controlling stem cell fate due to their designable nature, where biophysical and biochemical signals can be incorporated to direct cell behavior and function.[8–10] The guidance provided by biomaterials may facilitate restoration of structure and function of damaged or dysfunctional tissues, both in acellular therapies, where materials induce ingrowth and differentiation of cells from healthy residual tissues in situ,[11–13] and cell-based therapies, where biomaterial scaffolds deliver cells and bioactive factors, which can induce morphogenesis in the targeted tissues *in vivo*.[1] Development of functionalized biomaterials has resulted in the establishment of synthetic microenvironments with near-physiologically precise delivery of stem cell regulatory signals that can modulate stem cell fate both *in vitro* and *in vivo*. This review attempts to highlight recent advances in the development of synthetic extracellular microenvironments for modulating stem cell behaviors, such as adhesion, proliferation, and differentiation, and efforts to design functional biomaterials to provide a proper microenvironment to promote stem cell growth for the purpose of tissue regeneration.

Interactions between Stem Cells and their Niche

Tissue dynamics in terms of disease pathology, as well as formation, function, and regeneration after damage, is the result of an intricate temporal and spatial coordination of numerous individual cell fate processes that are controlled by a myriad of signals originating from the extracellular microenvironment.[14] The stem cell niche is a dynamic ensemble of physicochemical and biological cues that provide vital information, and consists of three major components: cell–cell contacts, cell–substrate interactions, and cell–soluble factor interactions (Fig. 1).[15] The extracellular microenvironment surrounds cells and comprises the molecular signals; furthermore, it is a highly hydrated network consisting of three important components: (1) soluble macromolecules (growth factors, cytokines, and chemokines); (2) proteins on the surfaces of neighboring cells; and (3) insoluble hydrated macromolecules, which include fibrillar proteins such as collagens, non-collagenous glycoproteins such as elastin, laminin, or fibronectin, and hydrophilic proteoglycans with large glycosaminoglycan side chains. Although almost every cell is exposed to tissue-specific microenvironments, the nature and extent of stem cell–niche interaction depends on the tissue type, stage of development, physiological condition, and so on. One of the hallmarks of cell–niche interaction is the transfer of mechanical cues, such as stress, elasticity, and force, from the substrate to the cells (a process known as mechanotransduction) and the response of cells in shape, proliferation, or differentiation.[16] Hence, the ultimate decision of stem cells to perform specific functions, such as migration, proliferation, and differentiation, is a coordinated response to the biochemical or physical interactions from surrounding microenvironments.

Biomaterials as Synthetic Stem Cell Niche

The basic requirements of biomaterials for tissue engineering applications are biocompatibility, biodegradability, and the ability to be implanted without eliciting inflammatory responses that interfere with cellular function and tissue formation. Incompatible biomaterials are destined for an inflammatory response or foreign-body reaction that eventually leads to rejection and/or necrosis. Since biomaterials provide temporary mechanical support while the cells undergo spatial tissue organization, a suitable biomaterial should maintain adequate mechanical integrity to support tissue formation during early stages of development. Basically, biomaterials should (1) facilitate the localization and delivery of somatic cells to specific sites in the body, (2) maintain a three-dimensional architecture that permits the formation of new tissues, and (3) guide the development of new tissues with appropriate function.[3,17]

The aim of biomaterial-directed stem cell applications would be to mimic the properties of the respective physiological stem cell niche, both physically and biochemically. Many studies have demonstrated that modification of biomaterials can introduce specific biological responses in stem cells. The general approach involves sustaining adequate levels of signaling bioactive molecules to control stem cell behaviors.[18] For example, a study of the effect of the transforming growth factor beta-3 (TGF-β3) on stem cells for cartilage formation showed that transient TGF-β3 enhanced the mechanical properties of

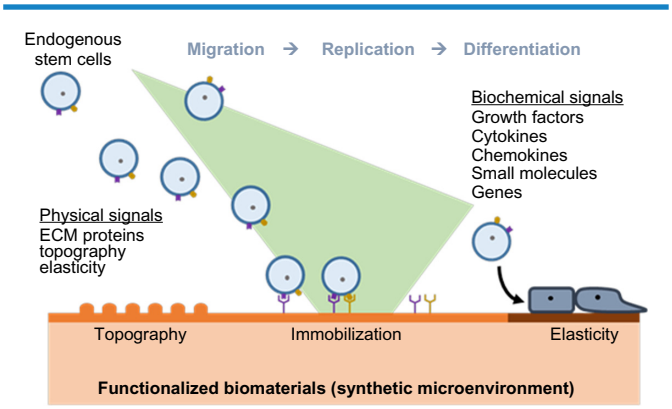

Figure 1. Schematic illustration of interactions between endogenous stem cells and synthetic microenvironment. Stem cells' fate in a particular microenvironment is regulated by intricate reciprocal molecular interactions with its surroundings.

synthetic cartilage.[19] Anseth et al designed synthetic hydrogels that contained a pendent peptide sequence Arg-Gly-Asp-Ser (RGDS), which is a photocleavable peptide sequence.[20] When these pendant peptide groups were cleaved in the culture using light, there was a dramatic increase in the production of glycosaminoglycan and type II collagen by mesenchymal stem cell (MSC) encapsulation, indicating a significant increase in chondrogenic differentiation. Use of a photocleavable ligand to initiate changes in a synthetic microenvironment (through time-varying release of stem cell-related factors) represents a novel approach toward modulating stem cells *in vitro*. In this section, we will focus on the design of functionalized biomaterials as a synthetic microenvironment to actively participate in tissue regeneration.

Biodegradable synthetic polymers. Biodegradable synthetic polymers offer a number of advantages for applications in tissue engineering and regenerative medicine. These biomaterials can be easily synthesized with reproducible quality and purity and fabricated into various shapes with desired bulk and surface properties. Specific advantages include the ability to tailor the mechanical properties and degradation kinetics of these materials to suit various applications. Poly(α-hydroxy acids), such as poly(glycolic acid) (PGA), poly(L-lactic acid) (PLLA), and their copolymer [poly(lactide-*co*-glycolide) (PLGA)] are the most widely used biodegradable synthetic polymers for tissue engineering applications.[21,22] These polymeric scaffolds have been shown to support the growth of human embryonic stem cells (ESCs) and promote three-dimensional (3-D) tissue-like organization.[23] Additionally, the use of appropriate biomolecular signals may allow differentiation of ESCs into a number of tissue types without altering the initial scaffold material.[23] Another type of biomaterial used to study adipogenesis, chondrogenesis, and osteogenesis of stem cells is the nanofibrous scaffold fabricated using poly(ε-caprolactone) (PCL).[24,25] The interconnected porous PCL scaffold created by electrospinning has been found to encourage cell proliferation and cell–cell interactions in both human MSCs and mouse ESCs.[26]

When mouse ESCs were encapsulated in a poly(ethylene glycol) (PEG) hydrogel and exposed to TGF-β, the resulting embryonic bodies (EBs) displayed regulation of chondrogenic markers.[27] In another study, chondrogenic differentiation of mouse ESCs in PEG-diacrylate hydrogels could be augmented by the addition of glucosamine.[28] Additionally, glucosamine was reported to increase the mechanical properties of the polymer scaffold by synthesizing new ECMs. Recently, Healy et al demonstrated short-term self-renewal and maintenance of human ESCs that were cultured in photo-cross-linked hydrogels made of poly(*N*-isopropylacrylamide-*co*-acrylic acid) [*p*(NIPAAm-*co*-AAc)] and an acrylated matrix metalloproteinase (MMP)-sensitive peptide Gln-Pro-Gln-Gly-Leu-Ala-Lys-NH_2 (QPQGLAK-NH_2).[29] Thus, modification of synthetic polymeric biomaterials with biological or chemical entities confers appropriate cellular response as well as tunable features such as mechanical properties, degradation rates, and scaffold porosities for cell infiltration and growth. This is critical for *in vitro* culture of stem cells and for their clinical applications.

Drug/protein delivery system. Progress in biomaterial functionalization has allowed enhanced cellular interactions via delivery of bioactive molecules from an implanted biomaterial scaffold.[30] Bioactive molecules, such as cytokines and growth factors, are powerful regulators of biological function, which include migration, proliferation, and differentiation. Incorporation of bioactive molecules into biomaterials is another approach to improving the outcome of cell-based therapies. The sustained release of bioactive molecules is an essential factor for controlling biological recognition within biomaterials to enhance cell survival, promote cell proliferation, or control cellular phenotype. The release of bioactive molecules from biomaterials can occur through a number of mechanisms, including diffusion-based release, degradation of the material, or cell-triggered release. These factors provide a significant degree of control over cells within and near the material by altering the cellular response to the bioactive material during tissue regeneration. To employ this technique, an understanding of the biological activities of these molecules is necessary. For example, the biological activity of growth factors is dependent not only on their presence in solution but also on their interactions with the surrounding microenvironment. Some growth factors are most effective when released over a prolonged period, whereas others are more effective when delivered in a bolus. Some factors are active while tethered to a material, whereas others are active only when they have been released from the biomaterial and are internalized into a cell. These considerations must be taken into account when designing a delivery system.[31]

Especially, the synthetic stem cell niche should provide an appropriate microenvironment that interacts with stem cells on the biomaterial surface and supports the proliferation and differentiation of the stem cells to form a desired tissue or a functional organ. For this purpose, it seems that multiple factors should be delivered to a target application due to the complexity of the microenvironment (Fig. 2A). Mooney and colleagues suggested a multiple protein delivery system for accelerating vascularization and tissue formation, because the development of tissues and organs is typically driven by the action of a number of growth factors such as vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF)-BB,[32] or VEGF and insulin-like growth factor-1 (IGF-1).[33] To efficiently deliver multiple factors, they developed a new polymeric system that allows the tissue-specific delivery of two or more growth factors, with controlled dose and rate of delivery. Controlling sustained release of bioactive molecules with different release kinetics enables effective tissue regeneration. In a recent study to demonstrate methods for sustained release of bioactive molecules over time, we have developed a dual protein delivery

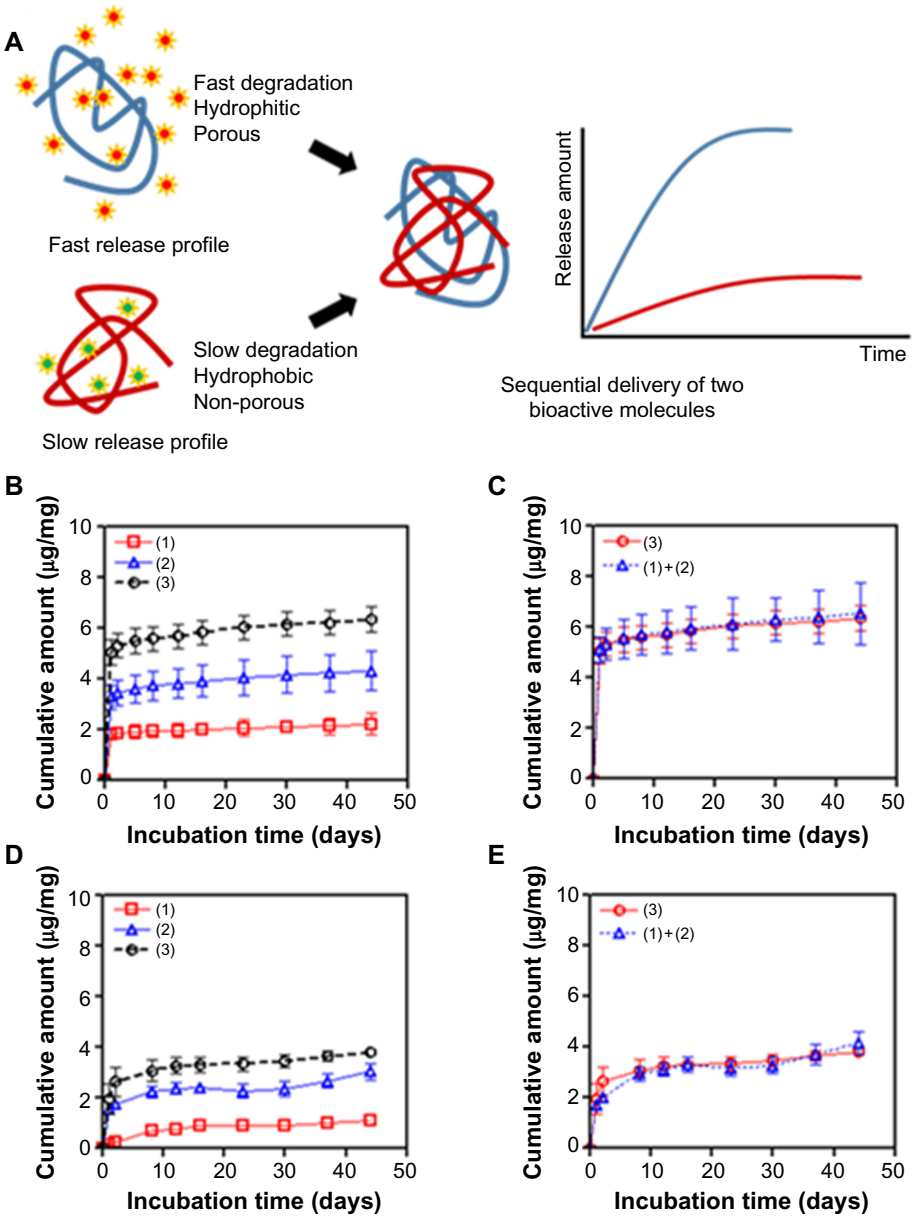

Figure 2. (**A**) Schematic illustration of different release profiles of two bioactive molecules resulting from different delivery strategies. Release profiles of dual protein delivery from the electrospun PLGA/pluronic F-127 (PF-127) scaffolds. Cumulative release amount of (**B** and **C**) BSA and (**D** and **E**) myoglobin from co-electrospun PLGA/PF-127 scaffolds. (1) PLGA-only + PLGA with 2 wt% protein (bovine serum albumin or myoglobin); (2) PLGA-only + PLGA/10% PF-127 with 2 wt% protein; and (3) PLGA with 2 wt% protein + PLGA/10% PF-127 with 2 wt% protein. (**C** and **E**) There was no significant difference between (1) + (2) and (3). This indicates that the co-electrospun scaffolds can deliver multiple factors with the designated release kinetics. Figure from Xu et al, 2013.[34] doi:10.1088/1748-6041/8/1/014104.

system based on electrospinning of PLGA with different hydrophilicities.[34] Release kinetics of bovine serum albumin (BSA) and myoglobin incorporated into the electrospun fibrous PLGA scaffolds (approximately 80% loading efficiencies the target proteins) were performed, and it was found that increase of the hydrophilicity of the scaffold by introduction of Pluronic F-127 dramatically increased the release kinetics of these proteins from the scaffolds (Fig. 2B–E). This is an example of a system that could be used for delivering multiple bioactive vehicles in a controlled manner for tissue engineering applications.

Surface immobilization. The interactions of cells with biomaterials are critically important for the successful outcome of tissue engineering applications. Thus, the behavior of cells grown on a biomaterial surface, including adhesion to the biomaterial substrate, development of appropriate cellular structures, proliferation, differentiation, and maintenance of proper cell function, must be investigated in order to mimic the native microenvironment. It is well known that adhesion and proliferation of different types of cells on biomaterial surfaces depend mostly on surface characteristics, such as wettability (hydrophilicity/hydrophobicity), chemistry, charge,

and so on.[35–39] Therefore, surface modification of biomaterials becomes an effective method of controlling the surface characteristics.[40] Table 1 lists synthetic biomaterials derived from the extracellular microenvironment with various chemical modifications for modulating stem cells *in vitro* for culture, expansion, differentiation, and potential applications.

Surface immobilization of bioactive molecules on biomaterials is essential to regulate cell differentiation and enhance the functionality of differentiated cells by providing adequate signaling.[41] In normal tissues, secreted growth factors or cytokines may be tethered to ECM components (proteoglycans), whereas receptor ligands are presented to stem cells at the surface of nearby support cells. In one study, covalent attachment of the fibroblast growth factor-2 (FGF-2) to a synthetic polymer stabilized the growth factor and increased its potency 100-fold compared to FGF-2 in solution. In response to the tethered FGF-2, ESCs exhibited increased proliferation and activation of extracellular signal-regulated kinase 1 [(ERK1, also known as mitogen-activated protein kinase 3 (MAPK3)], ERK2 (MAPK1), c-Jun N-terminal kinases (JNKs), and c-Fos transcription factor mediated signaling.[42] The function of receptor ligands associated with cell membranes is contingent on the mode of presentation. When attached to a material surface, rat JAG1 (ligand for the receptor Notch1) showed enhancement in Notch1 (a human gene encoding a single-pass transmembrane receptor) signaling and increased the differentiation of rat esophageal stem cells.[43] Both these examples demonstrate the importance of ligand presentation in stem cells' fate and function. Most approaches to identifying ECM molecules with biological relevance to stem cell regulation employ the use of ECM molecules tethered to biomaterials singly or in combination.

Mikos et al have demonstrated enhanced bioactivity of the biomaterial surface following attachment of bioactive molecules,[44] while other studies have demonstrated surface-dependent differences in integrin binding as a mechanism to regulate differential cellular responses to biomaterial surfaces and improve the performance of biotechnological culture supports.[45] Another study demonstrated that surface chemistry modification and the binding of integrin adhesion receptors (such as fibronectin, type I collagen) can activate signaling pathways in cells and direct cell cycle progression, gene expression, osteoblast survival, and matrix mineralization.[46] The mode of cell adhesion is found to be distinct for positive and negative charges. Konno et al studied the effects of electrostatic charge on ESCs by culturing them on polymers photoimmobilized with leukemia inhibitory factor (LIF).[47] In another study, titanium fiber mesh scaffolds were coated with Arg-Gly-Asp (RGD) peptides (a cell adhesive, integrin-binding peptide found in fibronectin and laminin). MSCs were shown to attach more strongly to these RGD-coated scaffolds.[48] It is known that conformational restriction of the RGD peptide can increase its integrin-binding affinity.[49] A study shows that the cyclic RGD peptide, CRGDC, conjugated to amine-modified tissue culture plates can support long-term culture of human ESCs.[50]

Substrate elasticity. A growing body of literature documents the profound impact of the biophysical attributes of

Table 1. Synthetic extracellular microenvironment derived biomaterials with various chemical modifications.

BIOMATERIALS	CHEMICAL MODIFICATION	CELLS USED	APPLICATIONS
Acryalamide/PEG	RGD peptide	NSCs	Neural tissue engineering
Oligo(PEG-fumarate)	Osteopointin-derived peptide	Rat MSCs	Osteoblast migration
PCL	Adipogenic promoting factors	Mouse ESCs	Cell propagation; adipogenesis
PEG-diacrylate	Fibronectin; RGD peptide; methacrylic acid	Murine MSCs	Differentiation into osteoblasts
PEG-diacrylate	RGD peptide	Human ESCs	Chondrogenesis
PEG-diacrylate	Glucosamine	Mouse ESCs	Chondrogenesis
PLLA	Polyaniline	NSCs	Neural tissue engineering
Poly (L-lactide-*co*-ε-caprolactone) (PLCL)	Fibronectin	Human ASCs	Cell attachment
Poly (*N*-isopropylacrylamideco-acrylic acid) [p(NIPAAm-*co*-AAc)]	Metalloproteinase sensitive peptide Gln-Pro-Gln-Gly-Leu-Ala-Lys-NH_2 (QPQGLAK-NH_2)	Human ESCs	Cell self-renewal and maintenance
PEG	Phosphoester group; Dexamethasone; Fibronectin	Human MSCs; human hematopoietic stem cells (HSCs); goat MSCs	Osteogenesis; cell adhesion; CD34+ cell proliferation
Poly (ethylene terephthalate) (PET)	Fibronectin	Human MSCs; human cord blood-derived HSCs	Cell seeding, proliferation, and aggregation
Single-walled carbon nanotubes (SWCNT)	Laminin	NSCs	Neural tissue engineering

the substrate on cellular behaviors, including cell proliferation, differentiation, phenotype, and function with substrate elasticity being identified as a cogent cue (Fig. 3A,B).[51–53] A number of studies have directed the effects of substrate elasticity on cellular function, extending from cell attachment and proliferation to stem cell differentiation.[54,55] Accordingly, elucidating the relationship between substrate elasticity and cell function is required to create *in vitro* microenvironments that could better mimic the *in vivo* condition.[56] Therefore, it is expected that biomechanical features of a 3-D microenvironment should play a role in regulating stem cell behaviors. In many studies, substrate elasticity has been shown to modulate the proliferation and differentiation of ESCs and certain types of adult stem cells. For example, adult neural stem cells (NSCs) cultured on a relatively stiff synthetic matrix gave rise primarily to glial cells, whereas on a softer matrix (closely mimicking a brain tissue) the predominant cell type observed were neurons.[57]

In another study, Trappmann et al reported stem cell differentiation in response to altered substrate elasticity. They hypothesized that the lack of responsiveness of epidermal stem cells might reflect the different microenvironments to which epidermal and mesenchymal cells are exposed *in vivo*. Human MSCs, cultured on a range of polyacrylamide (PAAm) hydrogels and analyzed for their spreading and differentiation into osteoblasts and adipocytes showed that stiff PAAm hydrogels stimulated osteogenic differentiation of the stem cells, while on soft PAAm hydrogels, the stem cells differentiated into adipocytes (Fig. 3C,D).[51] Also, in general, on these hydrogels the increasing elastic modulus corresponded with an increase in adhesive cell area and actin polymerization. These studies highlight the potent influence of the mechanical properties

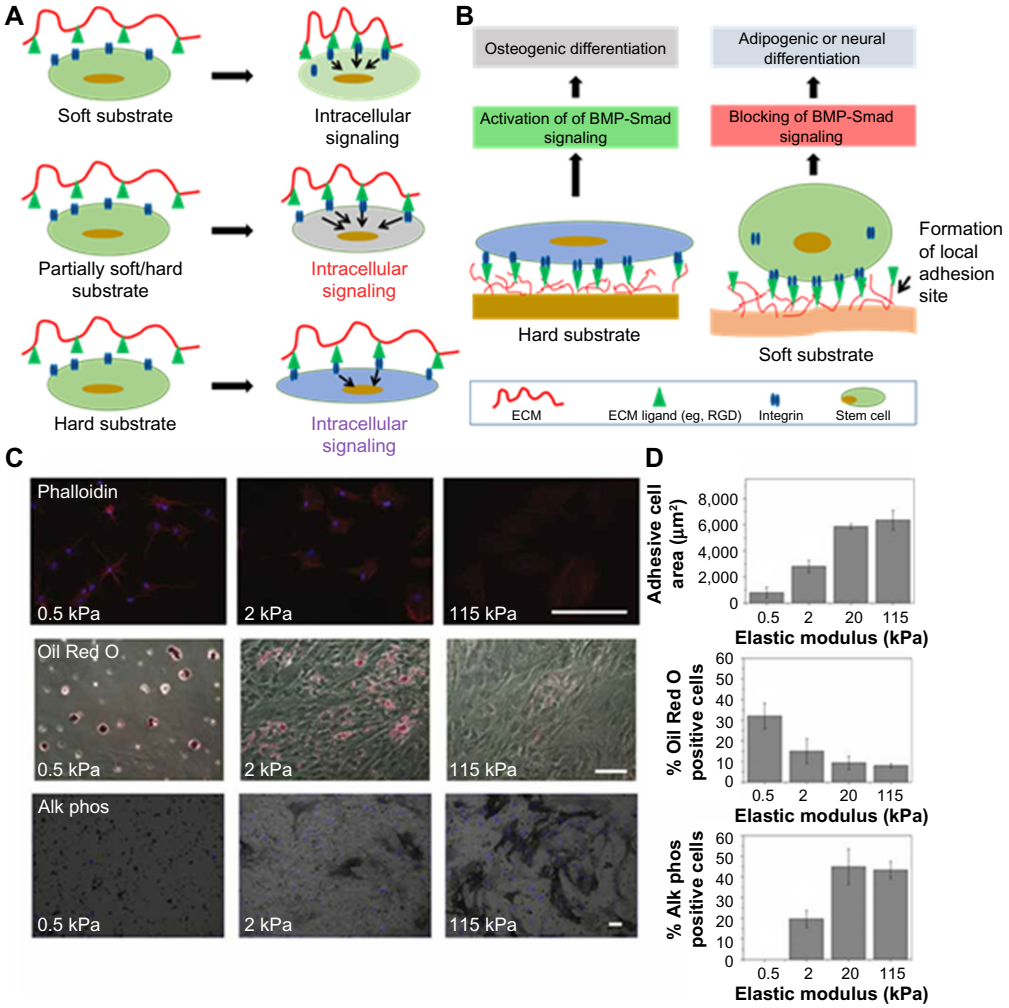

Figure 3. Schematic illustration of surface characteristics (ECM or synthetic microenvironment) on cell behavior. (**A**) Substrate stiffness (soft, hard, or intermediate) can regulate reorganization of integrin ligands on cell surfaces, thereby affecting intracellular signaling and cellular response. (**B**) Stem cell differentiation is dictated by substrate stiffness and can be used for deriving cells of different lineages, such as adipocytes or neural cells (soft substrate) or osteogenic cells (hard surface). (**C**) F-actin content (phalloidin staining; red), Oil Red O, and alkaline phosphatase (Alk Phos) staining on polyacrylamide (PAAm) hydrogel. Nuclei were counterstained with 4,6-diamidino-2-phenylindole (DAPI; blue). Scale bars, 200 μm. (**D**) Quantification of cell spreading and differentiation after 24 hours (F-actin) and 7 days (Oil Red O and Alk Phos) in culture on PAAm covalently functionalized with collagen; mean ± SD; $^*P < 0.05$ when compared with 115-kPa hydrogel. Reproduced with permission from © Nature Publishing Group (Trappmann et al, 2012).

of the matrix on stem cell fate and necessitate further exploration of the links between stem cell behavior and substrate elasticity.

Surface topography. Topographical cues generated by the ECM have significant effects upon cellular behaviors, including adhesion, proliferation, alignment, migration, and differentiation. Studies have shown that substratum topography has direct effects on the ability of cells to orient, migrate, and produce an organized cytoskeletal arrangement.[58] The topography of a native tissue matrix is a complex structure comprising pores, fibers, ridges, and other nanoscaled features. Fundamental understanding of cell–substrate interactions is important for tissue engineering applications and the development of medical implant devices. A surface patterning at the micro scale (1–500 μm) can be typically used for controlling cell/colony shape and positioning, while nanoscale patterning (1–100 nm) can be used to regulate cell–substrate interaction through control of integrin binding sites.[58] Since lateral dimensions ranging from tens of micrometers down to hundreds of nanometers are created, surface patterning is ideal for controlling cell shapes.[59] Chen and co-workers applied micro-contact printing to analyze the effect of stem cell shape on differentiation. They observed that human MSCs that adhere, flatten, and spread will differentiate along the osteogenic lineage, while a restriction in cell size by means of denser cultures or smaller micro-island size induces adipogenesis.[60]

Substrates with different chemically patterned shapes can be used to promote the differentiation of stem cells to distinct lineages. Modifications to cell shape, such as adhesive area, aspect ratio, and subcellular curvature, affect cytoskeletal tension, which ultimately leads to changes in gene and protein expression. Nanometer-scale topographical patterns with features such as pillars, grooves, and pits have been created for cellular studies.[58,61] The nano-topography of the ECM provides geometric cues to cells in the form of fiber diameter, length, and crosslinking patterns, as well as surface irregularities. Within the stem cell niche, cell shape is defined, in part, by the constraints imposed by the surrounding ECM on cells during development and in adulthood.[62,63] There is simple evidence suggesting that physical control of cell shape alone can act as a potent regulator of cell signaling and fate determination.[64] Using micro-patterned ECM islands that allow precise and reproducible control of the size of the cell attachment area, one particular study clearly demonstrated the influence of cell shape on cell function.[65] Single MSCs cultured on small islands adhered poorly, had a rounded morphology, and acquired an adipogenic fate, while on larger islands, these cells were adherent, spread out, exhibited increased focal adhesions and cytoskeletal reorganization, and acquired an osteogenic fate.[60] Furthermore, human ESCs cultured on spatially restricted islands yielded dense OCT4+ (octamer-binding transcription factor 4) pluripotent colonies, while on large islands, the same ESCs showed differentiation.[66]

Since stem cell niches *in vivo* have nanoscale topographies, nano-topography could be used to influence stem cell differentiation into neural lineages, including neurons, oligodendrocytes, and astrocytes. When Yim et al cultured human MSCs on micro-patterned and nano-patterned polydimethylsiloxane (PDMS) substrates that contained striped groove morphologies, 86.5% of cells aligned on nano-patterned surfaces, while no alignment was observed on unpatterned surfaces.[67] Also, neuronal gene markers, such as neurofilament light peptide (NFL), SOX2 (a transcription factor that is essential for maintaining self-renewal, or pluripotency, of undifferentiated ESCs), and tyrosine hydroxylase (TH), were significantly upregulated on nano-patterned surfaces, along with detection of microtubule-associated protein 2 (MAP-2) and TuJ-1 (neuron-specific class III beta-tubulin), both of which are mature neuronal markers. Synaptophysin expression was also detected when MSCs were cultured on nano-patterned surfaces in differentiation media (with retinoic acid), which suggests synapse formation in the cells.[67] These studies show nano-topography alone can induce significant upregulation of neuronal markers in human MSCs, thereby promoting them into the neuronal lineage.

Electrospinning technology has been widely employed to fabricate tissue-engineered scaffolds that mimic native ECM architecture. Thus, understanding of the interactions between electrospun fibrous scaffolds and mammalian cells is crucial to the successful production of target tissues and organs. Although many factors contribute to the successful generation of functional tissues, which include biomaterial selection and composition, this section will focus on the structural and morphological effects of electrospun nanofibers on cells. Cellular differentiation on electrospun fibers is closely related to cell–substrate interactions. NSC proliferation can be promoted using FGF–2.[68] In addition, NSC can be preferentially differentiated into the following cell types: neurons using retinoic acid and forskolin[69]; astrocytes using LIF and bone morphogenic protein (BMP)[70]; and oligodendrocytes with IGF or platelet-derived growth factor (PDGF).[71,72] The effect of topographical cues on NSC proliferation and differentiation is poorly understood. It remains unclear how topographical features (specifically, nanofiber diameter and alignment) influence stem cell proliferation and differentiation, and this is partially due to a lack of reliable methods for producing fibers with well-defined diameters. Systematic characterization of nano-topographical regulation of cell behavior is important in understanding and eventually engineering an artificial niche for *ex vivo* manipulation of stem cells. Christopherson et al demonstrated that fiber diameter is an important parameter that affects the adhesion, spreading, migration, proliferation, and lineage specification of NSCs under expansion and differentiation conditions.[73] As the fiber diameter increased, NSCs showed reduced migration, spreading, and proliferation in the presence of FGF-2 and serum-free medium. Accumulated evidence suggests that electrospun nanofibrous scaffolds

can partially mimic the topographical features of the natural ECM and influence cellular differentiation.

Biomaterials for Controlling Stem Cell Fate

Maintaining stem cells in an undifferentiated state and subsequently directing them to differentiate in a reliable and reproducible manner into specific cell types are key considerations in stem cell-based tissue engineering. ESCs can differentiate into any adult cell types,[74] while adult stem cells are restricted to certain lineages.[75] Both offer powerful new tools for regenerating a tissue as well as for advancing our understanding of early human development, pathophysiology, and epigenetics. The ability to exploit the power of stem cells has been limited by poor control over the complex signaling events that influence their differentiation. Recently, great progress has been made in the engineering of polymeric biomaterials that control stem cell fate.[76] For instance, ESCs are seeded on a feeder layer to maintain their undifferentiated state and to support their expansion; however, to support clinical applications of human ESCs, developing feeder-free culture conditions has become critical. Xu et al first reported a successful feeder-free culture for human ESCs.[77] Using culture dishes that were coated with collagen, laminin, and Matrigel™, they successfully demonstrated a feeder-free human ESC culture system in which undifferentiated cells could be maintained in 100% mouse embryonic fibroblast conditioned medium (supplemented with serum replacement and growth factors such as FGF) for at least 130 population doublings. Ying et al used a combination of BMPs and LIF to preserve the self-renewal, multilineage differentiation, and colonization properties of ESCs.[78] More studies have reported culture and expansion of ESCs using LIF in conjunction with biomaterials.[79,80] Hence, biomaterials-based expansion of human ESCs has become feasible. Similarly, large-scale culture of human ESCs in bioreactors has also become a possibility, and offers numerous advantages in terms of clinical application, such as a fully defined microenvironment, a disease transmission risk-free environment, and ease of scaling up.[81]

To enable tissue regeneration *in vivo*, an ideal biomaterial will have the following characteristics: (1) a mechanism for controlled matrix dissolution in response to tissue regeneration; (2) ligands for supporting migration and adhesion of cells from surrounding tissues; and (3) the capacity for delivery of bioactive factors that can attract endogenous stem and progenitor cells and induce their differentiation in a tissue-specific manner. New developments in biomaterial technologies are entertaining possibilities to modulate stem cells function *in vivo* (at a site of tissue damage). Biomaterials have been designed for the delivery of bioactive stem cell niche molecules *in vivo*. Targeted local delivery of bioactive molecules has been used as a stem cell niche that can respond to environmental signals such as cell-secreted proteases, or can be taken up by cells through endocytosis.[82,83] Some studies with cell transplantation have shown the possibility of externally supporting the formation of a heterotopic hematopoietic microenvironment. Using sub-ECs derived from human bone marrow stroma, which express a melanoma cell adhesion molecule (MCAM or CD146), it was shown that these cells were capable of forming a miniature bone structure.[84] In another example, formation of an active hematopoietic marrow (with stromal and hematopoietic compartments) was demonstrated when macroporous polymeric scaffolds preseeded with rat osteogenic cells were implanted. It was hypothesized that this particular scaffold design acted as a functional artificial niche with the capability to attract and retain endogenous hematopoietic precursor cells.

For the target-specific approach, a functionalized biomaterial has been developed that is sensitive to degradation by matrix metalloproteases (MMPs) or to plasmins. They also contained an integrin-binding ligand and the bone-inducing factor, BMP-2. When this biomaterial was implanted into bone defects, complete matrix remodeling was observed as well as new bone formation at the site of implantation.[85,86] One of the challenges for supporting tissue regeneration *in vivo* is the induction of blood vessel growth in the implanted scaffolds. Numerous pathologies arise due to lack of a functional vasculature; furthermore, establishing this vascularization remains a hurdle for the clinical success of tissue engineering therapies.[87] To overcome this limitation, synthetic biomaterials have been developed that can deliver angiogenic factors such as VEGF, TGFs, FGFs, and angiopoietins. In a study, such MMP-sensitive biomaterials, which also contained integrin-binding sites and were capable of delivering VEGF in a controlled manner, resulted in the formation of new blood vessels in the implants in animal models.[88]

Precise control of stem cell differentiation would be a determining factor for achieving the production of tissue-specific approaches. Cartilage defects are common features of joint diseases. In most current treatments, full function of the native cartilage is rarely restored.[89] A wide spectrum of natural and synthetic biomaterials has been evaluated to support chondrogenic differentiation of MSCs. Among synthetic polymers, use of autologous MSCs along with composite scaffolds made of PLGA, gelatin, hyaluronate, chondroitin, and the incorporation of two chondrogenic factors, dexamethasone, and TGF-β1, have proven useful in repairing full-thickness cartilage defects in rabbits.[90,91] In another study, improved hyaline-like cartilage was successfully regenerated when MSCs were used in PLGA scaffold composites that were pretreated with TGF-β3 before transplantation into rabbits.[48]

The central nervous system in mammals has evidently limited the regenerative capacity when lesions form as a result of trauma, stroke, neuropathological conditions, or neurodegenerative diseases such as Parkinson's disease.[92] Tissue engineering approaches using NSCs could provide a means to regenerate the damaged tissues of the central and peripheral nervous systems.[93] Li et al demonstrated that the use of NSCs with neurotrophin-3-chitosan scaffolds increases

the viability of these cells and enhances their differentiation into neurons.[94] In another study, Teng et al attempted to re-create a model for spinal cord by fabricating a bilayered scaffold with a biodegradable polymeric blend of PLGA and a block copolymer of PLGA-poly(L-lysine) with outer and inner microarchitectures that mimicked the white and gray matter of the spinal cord, respectively.[95] When NSCs were seeded in the inner layer of the scaffold and implanted into a lateral lesion of the rat spinal cord, the animals showed improved recovery of hind limb locomotory functions compared with controls.

Earlier attempts to modulate differentiation of ESCs into hepatocyte lineages involved the use of contact-printing methods to create a microarray system on silane-modified glass slides. Multiple circular protein spots of 500 μm diameter, consisting of FGF-2, hepatocyte growth factor (HGF), and BMP-4 mixed with fibronectin and collagen type I, were made.[96] When murine ESCs were cultured on these protein spots, they showed differentiation into hepatic lineage. Coculture of ESCs with hepatic stellate cells (HSCs) on these protein spots appeared to enhance hepatic differentiation, compared with ESC culture on the protein spots alone or with coculture without micro-patterning.[96] Hence, the micro-patterned protein spots seem to guide mouse ESCs into hepatic lineages with high efficiency.

Conclusions and Future Outlook

Biomaterials provide a sophisticated microenvironment for supporting growth and differentiation of stem cells. Within a physiological microenvironment, stem cells can be engineered to form various tissues and organs for numerous treatments. Improved understanding of tissue development, coupled with mimicking various cellular microenvironments using intelligent biomaterials, will be needed to better control stem cell differentiation. A major challenge in the advancement of biomaterials-based strategies in modulating stem cell behaviors lies not in the biomaterials field but rather in stem cell biology. The identification of markers that can specifically distinguish stem cells from their differentiated progeny has been cumbersome with many adult stem cell types. Synergy of work of cell biologists, materials scientists, and biomedical engineers will be needed to advance biomaterials and stem cell-based approaches to clinical use.

Currently, the specific molecular mechanisms controlling stem cell microenvironment and the signaling pathways that lead to efficient differentiation and tissue formation remain poorly understood. Knowledge of the complex stem cell niches and how these microenvironments direct stem cell fate is needed to incorporate bioactive factors into the biomaterial systems for better control of stem cell differentiation. Bio-instructive materials are valuable tools for unraveling the mechanisms of stem cell fate decisions in defined *in vitro* settings. Parallel analytical experiments, similar to high-throughput screenings of cell–matrix interactions, will be needed to delineate the multifactorial control of stem cells. Once identified, the relevant microenvironmental signals can be incorporated into biomaterials to facilitate expansion and differentiation of stem cells for therapeutic applications. Another overarching approach in understanding stem cell–biomaterials interactions and engineering stem cell microenvironments is the use of theoretical methods (such as computational modeling) and combinatorial experimental strategies (such as high-throughput analysis). Combining the use of "intelligent" biomaterials systems with advanced technologies, such as bioprinting, microfluidics, and time lapse, allows in situ analysis of cells in culture, which would provide invaluable insight for spatial control of stem cells *in vitro* and *in vivo*. Gaining a quantitative understanding of ECM signals from cell–biomaterials interaction studies will also drive the design of biologically inspired materials forward.

A better control of stem cells through biomaterials-based systems can enable advancement of engineered tissues toward clinical applications. Advancements in biomedical imaging technologies and increased use of computational analysis in mapping cell lineages will enhance understanding cell dynamics that direct cell fate and will allow researchers to carefully design biomaterial substrates to direct stem cell differentiation. Topography remains an untapped source of possibilities for guiding stem cell fate and will require basic studies to evaluate possible therapeutic applications. In the long term, new nanomaterials can serve as an important tool in directing stem cell fates. Toxicity is a major concern for any biomedical use of nanomaterials; therefore, additional research should be focused on improving the biocompatibility of multimodal nanocomposites, particularly for use in stem cell-based applications.

Using biomaterials to present multiple signals to control stem cell dynamics remains challenging. To this end, use of combinatorial approaches using both bioresponsive and time-sensitive delivery mechanisms might prove useful, and include the use of protecting groups, stimulus-sensitive linkers, and ligand-exposing mechanisms. Incorporating cell-specific chemotactic factors in a spatially controlled environment would enable hierarchical segmentation of biological signals. Examples include upregulating self-segregating molecules, such as cadherins, or incorporating potentially boundary-forming signals, such as the ephrins. Another approach would be to manipulate the transcription of bioactive factors in the stem cells directly. For example, a biomaterial could be used for the controlled delivery of bioactive molecules for manipulating the expression of transcription factors that regulate morphogen expression. Requirements of future biomaterials should include the ability to interact with and respond to their biological environment. The ability of biomaterials to sense biological demand or changes in their microenvironment will be critical to the development of "intelligent" biomaterials and would enable modulation of stem cell behaviors for a variety of therapeutic applications.

The human body is incredibly complex, and development of biomaterials and/or stem cell-based therapies will require

Table 2. Clinical trials using biomaterials and biomaterial-cell products.

TECHNOLOGY	SPONSOR	CONDITION/INTERVENTION	CLINICAL STUDY STATUS (START DATE)
Evaluation of efficacy and safety of autologous MSCs combined to biomaterials to enhance bone healing (OrthoCT1)	Institut National de la Santé Et de la Recherche Médicale (France)	Delayed union after fracture of humerus, tibial, or femur/ implantation of bone substitute plus autologous MSCs	Phase I/II (May 2013)
Safety and performance of macroporous biphasic calcium phosphate (MPBC) granules, combined with cellulosic-derived hydrogel in the osteonecrosis of femoral head	Service de chirurgie orthopédique et traumatologique- Hôpital Pellegrin (France)	Osteonecrosis/filling bones gap after aseptic osteonecrosis biopsy of femoral head	Phase II (February 2006)
The effect and mechanism of hyaluronan on the mucociliary differentiation of human respiratory epithelial cells	Far Eastern Memorial Hospital (Taiwan)	Respiratory system defect/ inducing mucociliary differentiation of human respiratory epithelial cells	Not known (January 2009)
Safety study of filler agent composed of autologous MSCs and hyaluronic acid (LipAge)	Cryopraxis Criobiologia Ltda. (Brazil)	Lipodystrophies aesthetics procedure/adipose tissue collection and transdermal injection	Phase I (November 2014)
Allogeneic tissue engineering (nanostructured artificial human cornea) in patients with corneal trophic ulcers in advanced stages, refractory to conventional (ophthalmic) treatment	Iniciativa Andaluza en Terapias Avanzadas–Fundación Pública Andaluza Progreso y Salud (Spain)	Corneal ulcer/evaluate the safety and feasibility of an allogeneic tissue engineered drug (nanostructured artificial human cornea) in patients with corneal trophic ulcers refractory to conventional treatment	Phase I/II (January 2014)
Clinical study of fiber-reinforced composite (FRC) implant to treat skull bone defects (Cranio-2)	Turku University Hospital (Finland)	Skull bone defect; craniofacial bone reconstruction/ reconstruction of skull bone defects and orbital floor defects.	Recruiting Patients (January 2013)

Information source: Clinicaltrials.gov website; last accessed: January, 2015.

careful evaluation of all aspects of stem cell behavior *in vitro* and *in vivo* to minimize the risks of unexpected negative effects of these products. Such studies will be helpful along the regulatory pathway for approval for clinical use and would increase the commercial value of stem cell-based therapies. Translating these biomedical advances to clinically useful products will fulfill the long-standing promise of tissue engineering and regenerative medicine to enhance the health and lives of patients. Table 2 lists selected biomaterials and biomaterial–cell products that are in various stages of clinical development.

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PC and SJL contributed equally to writing of the manuscript and agree with its contents. Both authors reviewed and approved of the final manuscript.

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