Engineering the Stem Cell Microenvironment

Christian M. Metallo, Jeffrey C. Mohr, Christopher J. Detzel, Juan J. de Pablo, Bernard J. Van Wie, and Sean P. Palecek

Department of Chemical and Biological Engineering, University of Wisconsin–Madison, 1415 Engineering Drive, Madison, Wisconsin 53706, and Department of Chemical Engineering and Bioengineering, Washington State University, Pullman, Washington 99164-2710

Multipotent stem cells in the body facilitate tissue regeneration, growth, and wound healing throughout life. The microenvironment in which they reside provides signals that direct these progenitors to proliferate, differentiate, or remain dormant; these factors include soluble molecules, the extracellular matrix, neighboring cells, and physical stimuli. Recent advances in the culture of embryonic stem cells and adult progenitors necessitate an increased understanding of these phenomena. Here, we summarize the interactions between stem cells and their local environment, drawing on in vivo observations and tissue culture studies. In addition, we describe novel methods of characterizing the effects of various environmental factors and review new techniques that enable scientists and engineers to more effectively direct stem cell fate.

Stem Cells and Their Fate

Pluripotent stem cells possess an extraordinary proliferative capacity and the unique ability to differentiate into other cell types. Having the potential to provide a seemingly limitless supply of functional cells for therapeutic and discovery-based applications, these cells have generated great excitement in the scientific (and political) communities. Their properties also make them important tools in the fields of developmental and cancer biology. However, applications that effectively use stem cells require complex environments that induce specific, reproducible cellular outcomes. Designing and implementing processes that control stem cell growth and plasticity will be an enormous challenge for cell culture engineers in the next decade.

The term “stem cell” is often broadly applied to totipotent embryonic stem cells as well as lineage-committed adult progenitors. Most biological systems in multicellular organisms experience high cell turnover rates and require a steady supply of specialized cells to function properly. Epidermal cells terminally differentiate and slough off the skin to maintain barrier function and protect the underlying tissues (1). Hematopoietic cells, which include platelets, erythrocytes, white blood cells, and lymphocytes, have limited lifespans in the body and must regenerate rapidly to sustain their functions (2). Developing embryos contain cells that must proliferate over a fairly short gestation period to generate complex tissues and organs, and there are regions in the adult brain that continue to produce neurons and glia throughout life (3). These tissues all include cells capable of propagating extensively when needed or becoming dormant, depending on the environmental stimuli provided to the cells. The unifying characteristic of adult progenitors and embryonic stem cells is their plasticity, i.e., the ability to self-renew, differentiate terminally, or remain quiescent.

Stem cell fate choice is controlled by a complex set of signals dictated by the cellular microenvironment. Several components contribute to the stem cell niche, including soluble factors present in the surrounding tissue or culture media, extracellular matrix (ECM) or cell substrate, the biophysical environment, and nearby cells that can elicit cell-to-cell signaling (Figure 1). These factors converge via a multitude of intracellular signaling pathways that ultimately govern whether a cell divides, differentiates, or dies. Although organisms have evolved exquisite control systems to manage these stem cell populations, these processes can go awry, leading to uncontrolled cell growth and tumor formation or cell death (4). Though efforts have recently increased to gain a mechanistic understanding of stem cell fate choice, little is known regarding the combinatorial effects of multiple input signals present in most culture systems. This fact highlights the need for engineering approaches (i.e., modeling and high-throughput methods) to better identify and characterize how specific environmental signals affect stem cells.

As stem cell technologies transition from the research lab to clinical applications, there will be an increasing need for robust culture systems that consistently control stem cell growth and differentiation. In contrast to current industrial cell culture processes, which often use immortalized, tumorigenic cell lines to produce purified biological products, stem-cell-based processes must reproducibly generate large amounts of functional cells or tissues, and the multistep mechanisms involved will require complex, well-controlled systems. These goals can only be accomplished by exploiting the interactions of stem cells with their microenvironment. In this review we will examine the key components of the stem cell microenvironment as they relate to both adult progenitor cells and embryonic stem cells. Emerging technologies that are capable of elucidating specific cellular interactions will be discussed, and the role of these phenomena in current and future culture systems will be explored.

Soluble Factors in the Microenvironment

Owing to the ease and rapidity with which they can be manipulated in culture, soluble factors are the best-characterized environmental signals impacting stem cell behavior. Given the
multitude of stem cell signaling molecules described in the literature, we will focus on the most prominent factors involved in self-renewal and differentiation of example stem cell lines. Basic fibroblast growth factor (bFGF or FGF2) is functional in a wide variety of stem cell lineages and mediates signaling events in several pathways, including Akt (5) and MAPK (6). bFGF is necessary for the maintenance of neural stem cell populations in the mouse brain (7) and can enhance neural progenitor proliferation in vitro in several species (8); in the hematopoietic lineage, angiogenesis is mediated by bFGF (9). Finally, bFGF has emerged as one of the key factors responsible for maintaining human embryonic stem cells (hESCs) in the undifferentiated state (10, 11), as high concentrations are required for their successful derivation and culture in defined conditions (12). Although bFGF mediates a wide range of downstream pathways, it is not yet clear whether this factor acts as a negative regulator of differentiation or simply promotes rapid stem cell proliferation. bFGF has also been found to mediate various phenotypes in mesenchymal stem cells (MSCs). MSCs respond in a proliferative manner to the addition of bFGF to culture medium and also favor an osteogenic lineage (70). There is an expanding list of FGF-related peptides that have been characterized, many of which are important in lineage-specific stem cell maintenance or embryo development (13).

Bone morphogenetic protein (BMP)/transforming growth factor-β (TGF-β) superfamily members induce a variety of responses in different stem cell lineages. BMPs act synergistically with leukemia inhibitory factor (LIF) in murine embryonic stem cells (mESCs) to prevent differentiation (14). However, BMP-4 can induce apoptosis and/or differentiation in murine neural precursors (15) and has been shown to stimulate hESC differentiation to various cell types through SMAD1/5 signaling (16–18). TGF-β1 and Activin A activate SMAD2/3 signaling to prevent differentiation of hESCs, though these events are not necessary for maintenance of undifferentiated mESCs (18). Though great strides have been made in our understanding of mESC culture, this and other key differences between the mouse and human have been identified, emphasizing the importance of hESC research for regenerative medicine applications and for understanding human development. TGF-β superfamily ligand also affects MSCs, typically directing differentiation toward a specific lineage. Depending on dose and culture conditions the cells preferentially express markers indicating osteogenesis or chondrogenesis (72–74).

Other soluble factors implicated in development and stem cell mobilization can be used to direct differentiation to specific cell types. All-trans retinoic acid (RA) is a strong differentiation agent involved in embryo development and organogenesis (19, 20). This compound can prevent the terminal differentiation of epidermal stem cells (21) and is used in vivo to induce differentiation of cancerous blood cells in acute myeloid leukemia (22). In vitro, RA can direct neural stem cells to become neurons, and these effects depend on concentration and the developmental stage of application (23). In addition to many other functions, sonic hedgehog (Shh) regulates tissue patterning during development and progenitor proliferation (24, 25). Shh can act in conjunction with β-catenin to induce new hair follicle formation in the epidermis (26) or stimulate neurogenesis in the adult hippocampus (25). Computational and theoretical analyses of Shh-mediated neural progenitor proliferation have identified positive and negative feedback loops in this regulatory network (27, 28), demonstrating the inherent complexity of stem cell fate decisions.

Soluble, secreted factors clearly play an important role in stem cell growth and differentiation, and while these molecules can easily be added or removed from culture systems, the complexity of the underlying mechanisms must be resolved before they can be included in stem cell processes. Although mathematical modeling has improved our understanding of these responses (29, 30), experimental methods must be used to elucidate species, lineage, concentration, location, and time-dependent effects. Empirical approaches to process development have been successful but time-consuming (31); novel methods of rapidly screening factors affecting stem cell differentiation are now under development. Soen et al. have generated an array-based system capable of characterizing combinatorial and dose-dependent responses of human neural precursors to various ligands, adhesion molecules, and ECM components (32). An interesting aspect of this work is that the screened factors were immobilized to the ECM to facilitate screening, as opposed to

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**Figure 1.** Stem cell interactions with various inputs from the microenvironment. Soluble factors, ECM, intercellular contacts, and biophysical forces synergize to influence stem cell fate. The plasticity of progenitor cells enables them to self-renew, differentiate, remain quiescent, or enter apoptosis.
being in soluble form, a characteristic which has been shown to increase activity for the Delta-1-Notch system (33). High-throughput methods such as this will enable engineers to decipher the complex interactions that direct stem cell fate and more rapidly design culture/differentiation schemes.

The Extracellular Matrix

Lineage-specific adult progenitors are localized to particular regions of tissue, and while the specific ECM components responsible for stem cell maintenance are often unknown, the restricted nature of these populations demonstrates the importance of the surrounding matrix. A clear example of ECM interactions with stem cells occurs in the basal layer of the interfollicular epidermis (IFE). These keratinocytes express specific integrins (α6 and β1) (34, 35), maintaining contact with the dermal compartment or basement membrane, which contains fibroblasts that secrete growth factors and ECM proteins. A small population of these epidermal stem cells proliferates throughout life to maintain the skin’s barrier function by replenishing basal cells and producing cells for stratification. It is thought that separation from the basement membrane transduces signals within the cell that initiate terminal differentiation (1).

Hematopoietic stem cells (HSCs), which give rise to myeloid and lymphoid blood cells, primarily reside in the bone marrow, though some may be present in the peripheral blood. Many stromal cell types are present in the bone marrow, secreting the growth factors and ECM proteins necessary for maintaining the HSC niche (36). Adult neural progenitors are located in two regions of the brain, the dentate gyrus and subventricular zone (SVZ) (37), and contact the basal lamina, which is made up of laminin and collagen-1 ECM proteins and thought to concentrate cytokines (38). Cultivation of undifferentiated hESCs in the absence of feeders requires Matrigel to be used as a substrate (39). Recent efforts to derive and culture hESCs in defined conditions have employed laminin, a major component of Matrigel, though this method lacks robustness and is subject to lot-to-lot variation (12) (unpublished observations).

Currently, the most common stem cell substrates are complex, undefined mixtures. As a result, recent efforts have focused on generating synthetic matrices capable sustaining stem cell growth or differentiation. Array-based methods have been successfully used to identify polymers (40), proteins (32), and peptides (41) that may be used as defined ECMs capable of directing stem cell fate. Another approach used to manipulate the ECM microenvironment involves culturing cells in confined areas. Various methods have been described in which stem cells are encapsulated in alginate (42–44) or agarose beads (45), and many differentiation schemes have been developed that employ three-dimensional polymer scaffolds (46) or hydrogels (47, 48, 72, 75–77). A promising technique used in hESC cultures, which do not exhibit contact-dependent quiescence, is the use of three-dimensional microwells (49, 50). Mohr et al. used self-assembled monolayer patterning to present non-adhesive tri-(ethylene glycol) to prevent cellular outgrowth. hESCs remain undifferentiated for several weeks when cultured in microwells, though cell proliferation is not inhibited, suggesting that local or global cell density may regulate self-renewal. Confocal microscopy images of Oct-4 expression demonstrate that cells remain undifferentiated and confined to the wells (Figure 2). This method reproducibly generates hESC arrays of specific sizes that can be used directly for analysis or for the production of uniform cell aggregates.

Although in vivo studies have laid the foundation of knowledge regarding stem cell-ECM interactions, multifactorial strategies are necessary to decipher how ECM components interact with each other and soluble factors to regulate stem cell phenotype. An increased understanding of these complex environments will enable engineers to design novel, instructive matrices for use in stem cell processes, with the goal of ultimately identifying fully defined substrates to generate completely defined culture systems.

Interacellular Interactions

In addition to exogenous molecules in the microenvironment, stem cells are also responsive to neighboring cells in the niche. In vivo and in vitro studies on the spatial organization of stem cells have identified various systems in which stem cell fate is affected by cell-to-cell contacts. For example, adult neural progenitors in the SVZ maintain contact with ependymal cells that line the lateral ventricle (36), and in the bone marrow HSCs that lose contact with osteoblasts begin to differentiate (51). Epithelial cells form adherens junctions with their nearest neighbors, and components of this complex mediate cell signaling events through the MAPK or LeF1/β-catenin pathways (52, 53). As a result, changes in cell density or loss-of-function mutations in adherens junction components (α-catenin, β-catenin, E-cadherin) can initiate cell division, mediating growth or possibly tumor formation (54). In vitro studies have demonstrated efficient differentiation of ESCs to hematopoietic progenitors using coculture with a stromal cell line (55), and spatial analysis of mESC aggregates has detected radial variations in stem cell marker expression, which led to the identification of an autoregulatory system mediated via an autocrine gp130 ligand (56). Furthermore, stimulation of cell-cell interaction by pellet culture has been shown to facilitate MSC differentiation toward chondrocytes (78), suggesting that 3D matrix culture and intercellular communication play a significant role in regulating cell differentiation.

Although many cell-mediated control mechanisms have been characterized, difficulty in regulating these interactions complicates their usage in stem cell culture processes. As stated above, some ESC differentiation schemes have employed coculture, though the inclusion of tumorigenic cell lines is undesirable. Recently, novel methods of generating spatially oriented cocultures have been developed using layer-by-layer deposition of bioactive molecules (57), and these systems may be particularly useful for directed differentiation of stem cells.
in non-therapeutic applications, although any processes incorporating different cell types must allow for the subsequent isolation of the desired cells. Intercellular interactions may have limited use in engineering strategies, but it should be noted that any differentiation scheme involving cell colonies or aggregates (i.e., embryoid bodies) implicitly include intercellular interactions as different lineages arise.

The Biophysical Environment

Tissues in the body are subjected to a wide variety of biophysical environments depending on their location and function. Whereas the brain experiences minimal forces, muscles and bones must withstand significant mechanical loading, and the vasculature is exposed to constant fluid flow. Given that tissue progenitors must proliferate and differentiate in these physically dynamic environments, recent efforts have focused on understanding how stem cells detect and respond to phenomena such as physical forces and substrate compliance. Application of fluid shear stress and/or mechanical strain is known to affect vascular cell (endothelial cells and smooth muscle cells) gene expression and morphology, and when researchers cultured peripheral blood endothelial progenitors under fluid shear, the cells responded by increasing proliferation and endothelial marker expression. Similar results were obtained using predifferentiated Flk-1+ mESCs in a slightly different apparatus. Among other cell types, mesenchymal stem cells (MSCs) can differentiate into smooth muscle cells, an important component of the vascular wall. MSCs subjected to cyclic uniaxial strain showed enhanced proliferation and upregulated smooth muscle marker expression. Interestingly, this phenomenon is dependent upon cellular orientation: the response of MSCs aligned parallel to the strain was somewhat different than that of perpendicularly aligned cells. Mechanical forces such as hydrostatic pressure and compressive loading have been shown to enhance chondrogenesis in MSCs. When subjected to cyclic hydrostatic pressure, MSC-derived chondrocytes exhibited an increase in chondrogenic markers such as collagen and glycosaminoglycan over non-loaded controls. Cyclic compressive loading of cultured MSCs induced a chondrogenic lineage without the application of a soluble growth factor; concurrent stimulation with TGF-β1 and compressive strain led to further increases in chondrogenic protein production. Highlighting the complex nature of these effects when applied to different cell types, results reported by Saha et al. indicate that cyclic strain inhibits hESC differentiation. However, it is important to note that these cellular responses must always be evaluated in the context of other microenvironmental factors such as culture media and ECM.

Another recent study has investigated the effects of matrix elasticity on MSC lineage specification. By manipulating the cross-linking density of polyacrylamide gels, Engler et al. were able to generate substrates of varying compliance. Amazingly, lineage specification correlated well with in vivo matrix elasticities. Neuronal markers were expressed on compliant substrates, myoblast markers were induced on substrates that mimicked muscle, and osteoblast-specific factors were observed on the most rigid, bone-like matrices. Furthermore, these effects were demonstrated both in the presence and absence of specific lineage-inducing media.

Manipulation of the biophysical environment offers an exciting new tool for stem cell engineers to more efficiently direct stem cell fate. These phenomena may be particularly useful in ESC differentiation processes, which must first undergo germ layer specification before differentiating into specific somatic cell types. At this time, however, mechanical induction of cells is limited to anchorage-dependent culture systems that are difficult to scale up. Regardless, the biophysical response of stem cells and their derivatives must be considered during development of any tissue engineering processes.

Monitoring and Controlling the Stem Cell Microenvironment

Given the complexity of stem cell fate control systems, much can still be learned through observations of in vivo stem cell trafficking. For example, halogenated nucleotide incorporation enables scientists to identify where cycling cells are localized. Another approach is to generate organisms or cells containing stem cell promoter-driven green fluorescent protein; this approach has been successfully used in the hematopoietic and epithelial lineages and may be of use in the purification of specific cell types from differentiated cultures.

As engineers learn more about how the microenvironment directs stem cell fate decisions, these factors can be incorporated into culture processes and exploited to better control progenitor growth and differentiation. However, each aspect of the microenvironment is not distinct: soluble factors can bind to and interact with the ECM, surrounding cells can modify the ECM, and soluble factors can modulate the cellular response to mechanical forces. As more factors are identified, additional degrees of freedom will arise, so combinatorial methods involving multiple inputs from Figure 1 must be developed to completely characterize these systems. An additional challenge facing stem cell culture engineers involves maintaining scalability. Although systematic processes containing multiple cell types may differentiate stem cells more efficiently, subsequent purification steps may become more complicated. Novel reactor systems are able to exert more control over the microenvironment, but more traditional approaches should also be investigated. The plasticity and proliferative capacity of stem cells provides them with the potential to greatly impact the biotechnology industry; however, these attributes complicate the design of efficient, scalable culture processes. Engineering approaches must therefore be applied to these technologies to better characterize stem cell fate decisions and more effectively develop robust culture systems.

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References and Notes


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