Adult Stem Cells

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Engineering the In Vitro Cellular Microenvironment for the Control and Manipulation of Adult Stem Cell Responses

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1. INTRODUCTION

Stem cells have generated a great deal of excitement as a potential source of cells for transplantation because of their ability to self-renew and differentiate into functional cells of various tissues *(1–3)*. Stem cells can be derived from multiple stages of development as well as numerous adult tissues. Adult tissues are an attractive and readily accepted source of stem cells because such cells have demonstrated efficacy in multiple types of cellular therapeutics *(4,5)* and can be directly obtained from individual patients, thereby eliminating the difficulties associated with tissue rejection. Despite this enormous potential, the use of adult stem cells has been limited, primarily because of the inability to identify these rare cells from the heterogeneous tissue populations *(6)* and to expand populations of cells that retain stem cell properties in vitro.

Historically, many adult tissues were thought incapable of regeneration. However, cells with regenerative capability have been detected in most adult tissues, including liver *(7–9)*, intestine *(10)*, retina *(11)*, skin *(12)*, muscle *(13)*, neural *(14)*, mammary glands *(15)*, and others. Although extensive documentation of the properties of many of these cells with respect to their stem cell characteristics (i.e., individual cells with the capacity for extended self-renewal and multilineage differentiation) is under way, taken together, it is clear that adult tissues may provide an untapped source of cells for cellular therapies.

Numerous studies suggest that the proliferative and differentiative potential of tissue-specific stem cells changes during ontogeny *(16–18)* and is dependent on intrinsic factors such as telomere shortening *(19)* and genetic stability *(20)*. The ability to measure changes in the developmental potential of stem cells is limited by the inability to fingerprint such cells genetically

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(21) and by the properties of the assays used to detect them (such as tissue homing *[22]*). Despite these limitations, it is well recognized that adult stem cells in vivo have a proliferative potential much beyond the lifespan of the organism. For example, a single hematopoietic stem cell (HSC) not only can reconstitute hematopoiesis in primary recipients by contributing to both lymphoid and myeloid cells *(23,24)*, but also can reconstitute secondary and tertiary hosts *(25–27)*. Although less-rigorously analyzed (to a large extent because of the lack of transplantation assays based on tissue repopulation), adult stem cells from other tissues also have extensive regenerative capacities *(10,12,13)*. Despite the apparent intrinsic capability of adult stem cells for extensive self-renewal, efforts to grow these cells in culture have failed to recapitulate their in vivo potential.

The interest in adult stem cells has been elevated by recent reports that some adult stem cells, or stem cell populations, may be capable of crossing lineage boundaries by differentiating into cells with unexpected developmental properties. For example, bone marrow-derived cells have been reported to give rise to different types of muscle cells, such as from unfractionated bone marrow *(28–30)* or enriched HSC-like cells *(31,32)*; liver cells, such as from unfractionated rodent *(33,34)* and human bone marrow *(35,36)*, or enriched HSC-like cells *(34,37)*; lung *(38)* and neuronal cells, including neurons detected in vivo *(39,40)* and in vitro *(41,42)*; and astroglia and microglia *(43,44)*. Strong evidence of the multiorgan generating capability of bone marrow-derived stem cells has been demonstrated in the ability of a single cell to reconstitute hematopoiesis in primary and secondary recipients as well as to differentiate into apparently functional epithelial cells of the liver, lung, intestine, and skin *(45)*.

Stem cells not derived from bone marrow may also have developmental capacities outside their tissue of origin *(46,47)*, although recent reports have led investigators to question many of these early results *(48–51)*. Even with the uncertainties regarding the intrinsic potential of adult stem cells, the ability of cells of one tissue to give rise to differentiated cells of another tissue (either because of broader differentiation capacity or because the tissue in question contains multiple types of stem cells) may be of great therapeutic potential and could provide alternative adult stem cell sources that are readily accessible (i.e., peripheral blood, skin, or fat-derived stem cells).

This chapter highlights some of the main bioengineering challenges in the development of adult stem cell-based therapies also; methods to control the self-renewal and differentiation of adult stem cells and to create clinically relevant bioprocesses are discussed. Particular emphasis is given to analyzing these techniques in the context of well-established adult stem cell

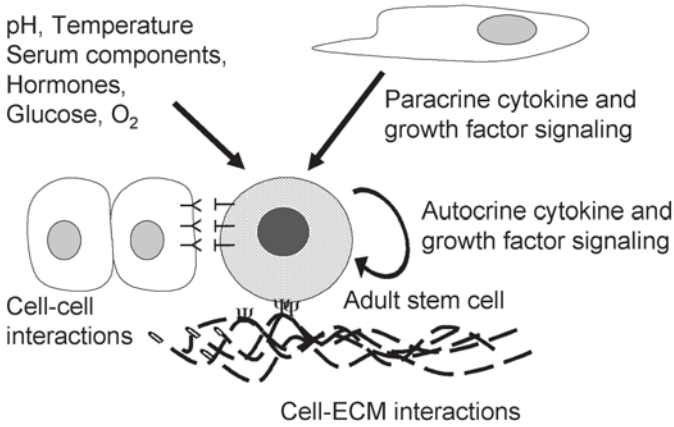

Fig. 1. Adult stem cell niche. The microenvironment of adult stem cells is regulated through a complex network of paracrine and autocrine soluble signals as well as cell–cell and cell–ECM bound signals. Physicochemical parameters such as pH, temperature, oxygen, perfusion, and mechanical stimuli can also influence cell fate decisions.

systems and the effect of the cellular microenvironment on the responses of such cells.

2. STEM CELL MICROENVIRONMENT

In vivo, stem cells reside in a complex microenvironment characterized by their local geometry (structural and physicochemical), by specific types of surrounding tissue cells, and by soluble and extracellular matrix (ECM) components *(52,53)*. The properties of this microenvironment are dynamic, depend on the specific tissue, and are affected by factors such as vascularization and loading (Fig. 1). Importantly, the analysis and understanding of the role of the microenvironment on stem cell responses should be motivated by more than the desire simply to mimic the in vivo milieu. The in vivo microenvironment dynamically exposes cells to positive and negative regulators of specific stem cell responses; the selective application of these regulatory mechanisms during in vitro culture will ultimately depend on the type of cell response to be elicited and the ability to control dominant (i.e., response-determining) culture parameters.

2.1. Cytokines and Growth Factors

Cytokines and growth factors are important regulators of the tissue microenvironment. They are produced by stem cells or their neighboring cells in an autocrine or paracrine manner and often combine with other

microenvironmental components to elicit nonlinear responses, for instance, threshold-based *(54)* or synergistic responses. Due in part to difficulties in the quantitative identification of most tissue-specific stem cells and their immediate derivatives, defining cytokine networks that allow for controlled self-renewal and differentiation of these cells has been challenging. So far, most experiments have studied the response of putative stem cells to individual or limited numbers of combinations of cytokines. Few studies have rigorously analyzed and optimized the effect of multiple cytokines, as well as interactions between these cytokines, on stem cell responses.

Factorial experiments are one approach to overcome some of these limitations and quantitatively analyze the effect of cytokine interactions on stem cell responses *(18,54–58)*. By analyzing complex interactions between various cytokines, relationships commonly missed by conventional dose–response approaches are detectable. For example, with respect to HSCs, this type of analysis has been helpful in defining self-renewal and differentiation factors *(59)*, a threshold cytokine concentration effect on self-renewal and differentiation *(54)*, and changes in cell's cytokine responsiveness with ontogeny *(18)*.

These and other results (*see* ref. *60* for a review) have led to the identification of molecules thought important for the regulation of HSC fate and have led to the development of feeder-free (and serum-free) culture systems. Feeder-free cultures provide more control in studying the effects of specific signals and are desirable in clinical applications. Briefly, these studies have identified a "cocktail" of cytokines containing stem cell factor (SCF), flt-3 ligand (FL), and interleukin 11 (IL-11) family of cytokines *(61)* (with or without the addition of thrombopoietin, TPO) *(62)* and revealed much about the mechanisms of cytokine action on stem cells.

For example, even though SCF has been shown to be critical for the maintenance and expansion of HSCs *(63)*, it cannot by itself maintain HSCs in vitro *(64)*. SCF acts synergistically with various growth factors, including TPO, FL, and IL-11, to induce proliferation and maintenance of myeloid and lymphoid progenitors *(65)*. FL has also been shown to synergize with a wide variety of hematopoietic cytokines (in particular, SCF and the IL-11 family of cytokines) to stimulate the proliferation, self-renewal, and differentiation of HSCs *(66)*.

Despite these results, a definitive cocktail that leads to reproducible expansion of HSCs has not yet been developed; illustrating the underlying complexity in cytokine networks *(60)* and pointing to the need to develop more effective in vitro culture technologies.

Significant progress has recently been made with respect to growing other types of adult stem cells in cytokine-supplemented media. For example, cells with characteristics of neural stem cells (NSCs) have been expanded as neurospheres in well-defined culture conditions *(67)*. Typically, epithelial growth factor (EGF) *(68,69)* and fibroblast growth factor 2 (FGF-2) *(70–72)* are used to propagate the early tissue dissociates containing NSCs. The presence of these growth factors seems to prevent the differentiation of NSCs and allows their continual proliferation. These properties have allowed the creation of bioreactors, which have been used to expand neurosphere-forming cells *(73)*.

Multipotential adult stem cells have been isolated from human and murine skin *(74)*. Cultures derived from these cells plated clonally and maintained for many passages at low cell densities generated both neuroectodermal (neurons and glia cells) and mesodermal (smooth muscle and adipocytes) tissues. Interestingly, the propagation of these cells seems to depend, among other things, on the addition of EGF and FGF to the culture, conditions similar to those defined for NSCs *(68,75)*. It remains to be seen whether these so-called multipotent adult progenitor cells can be isolated directly from specific tissues or arise as a product of in vitro culture.

In addition to identifying the types of growth factors and cytokines important in the control of adult stem cell growth and differentiation, it is becoming well recognized that their (relative) concentrations *(56)*, mode of presentation *(76)*, and order of application *(77)* also play an important role in eliciting particular responses from stem cells. The significant increase in the complexity of experiments investigating these parameters clearly indicates the need for quantitative, systems biology approaches as a tool in analyzing such interactions. These types of approaches have been used to analyze the cross-talk between two independent ligand-activated signaling pathways *(78)* and may one day be useful for the analysis of individual candidate stem cells *(79,80)*.

To study the "effective concentration" of growth factors present in the microenvironment of an individual cell, it is necessary to understand important mediating steps, such as the transport properties of the ligand in the cellular vicinity, the complexities of ligand–receptor binding interactions, the role of ECM binding on ligand availability and ligand–receptor complex internalization *(81)*, and the downstream consequences of signaling activation *(82)*. Clearly, to signal through a receptor, a ligand must reach its receptor on the cell membrane. This step could provide a significant barrier to signaling and is dependent on a number of parameters, such as the degradation and diffusion rates of the soluble ligand, ligand interactions with the

ECM molecules, mixing and turbulence of the surrounding fluid, and other parameters in the microenvironment. Bioengineering approaches can be used to engineer proteins with modified stability and diffusive properties *(83,84)* to optimize transport to the cells. Protein engineering approaches have also been used to design and select for *(85)* proteins with modified affinity for their particular receptor *(81,86)*.

Significant deviations in supplemented growth factor concentrations can occur throughout the culture period as a result of receptor–ligand complex internalization and degradation by cells *(54,81,87)*. For example, a cell-associated depletion of growth factors and cytokines in both hematopoietic *(54)* and embryonic stem (ES) cell *(88)* cultures has been observed. Significant progress has been made in understanding and manipulating the mechanisms that underlie ligand possession by cells *(89)*. This information has been applied to optimize biological responses of T cells to engineered and mutant IL-2 proteins *(87,90)*. These strategies, along with protein engineering techniques *(91,92)*, can also be used to develop receptor–ligand complexes that dissociate in the acidic environment of the endosome, thus allowing for higher ligand recycling rates *(93)*, an approach that has been shown to enhance the "effective" concentration of the ligand in the vicinity of the cell greatly *(89,93)* and may lead to decreased exogenous requirements of cytokines and growth factors.

Another approach to growth factor supplementation that holds significant promise for the modulation of stem cell responses is the design of ligand–receptor complexes that cannot be internalized and thus may allow the delivery of controlled and sustained stimulation to the cells. This can be achieved by immobilizing proteins to various surfaces and scaffolds. To achieve this goal, techniques ranging from direct protein adsorption to covalent linkage of aldehyde-containing surface groups to amine base side chains, as utilized in protein–protein interaction arrays *(94)*, can be used. These simple strategies, however, may be limited because nonspecific adsorption of serum proteins may "mask" the immobilized proteins. To overcome this potential limitation, a linking molecule (such as polyethylene oxide, PEO) may be used to tether the growth factor to a surface or ECM. This approach has been successful in covalently binding EGF to PEO *(95)*, for which the tethered EGF elicited the deoxyribonucleic acid (DNA) synthesis of hepatocytes at rates similar to that of its soluble counterpart and significantly greater than that of adsorbed EGF at comparable surface concentrations. Significant challenges exist in the implementation of these technologies, both in terms of the biomaterial design strategies and in terms of the underlying biological mechanisms that need to be mimicked *(96–101)*.

2.2. Cell–Cell and Cell–Extracellular Matrix Interactions

In vivo cells are typically in direct contact with surrounding cells and ECM. ECM is a dynamic assembly of interacting molecules that recognizes and regulates cell function in response to endogenous and exogenous stimuli *(102)*. ECM is produced by cells and consists of collagens, proteoglycans, adhesive glycoproteins, and glycoasaminoglycans and associated bound protein modulators of cell function. Along with providing a framework in which cells form tissues, ECM directly modulates cell attachment, shape, morphology, migration, orientation, and proliferation. ECM also serves as a reservoir for various growth factors. It has been proposed that the existence of matrix is essential for the activity of many growth factors (such as hepatocyte growth factor [HGF], transforming growth factor-β [TGF-β], and acidic and basic FGF) *(103)*. The complex combination of signals provided by the ECM to adult stem cells likely provides the cell with information unique to the tissue of origin and is important for the regulation of stem cell self-renewal, differentiation, and homing.

In the bone marrow, HSC interactions with adhesion molecules (e.g., CD34, stem cell antigen 1 [Sca-1], selectins, and various integrins) on the vascular endothelium have been reported to aid in cell homing during hematopoietic reconstitution experiments and to regulate cellular trafficking during homeostasis (for review, see ref. *104*). Cell adhesion molecules have also been shown to be present in other types of stem cells. For example, mesenchymal stem cells (MSCs) were first isolated based on their adherence (albeit poorly characterized) to tissue culture surfaces *(105)*.

Cell–cell and cell–ECM interactions have been shown to greatly influence the self-renewal and differentiation of stem cells *(106,107)*. An example of the importance of cell–cell and cell–matrix interactions in the modulation of adult stem cell fate is the maturation of intestinal crypt stem cells *(10,108–113)*. These cells give rise to epithelial cells that line the gastrointestinal tract and typically lie in the base of test-tube-like structures *(114)*. As cells move toward the luminal pole, they go through a series of differentiation and proliferation steps so that the pole is occupied by short-lived functional cells. Mathematical *(115)* and experimental *(108)* studies suggested that as few as four to six stem cells are sufficient in maintaining homeostasis for each crypt. Once these cells leave a stem cell niche within the base of the structure, they are induced to differentiate. This differentiation is thought to be regulated by a variety of signals, including cell–cell and cell–ECM signals.

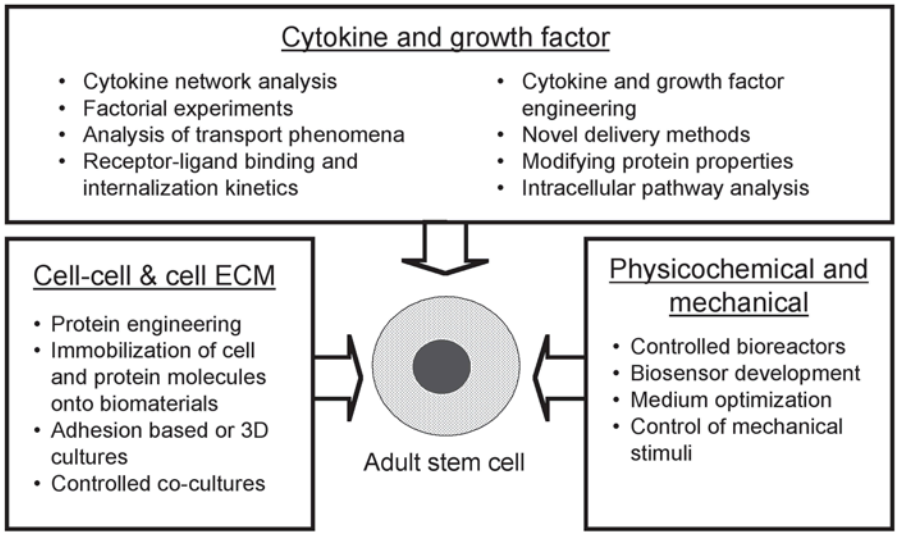

Fig. 2. Bioengineering methods to control, mimic, and analyze stem cell niche. A number of engineering approaches have been developed to control the immediate microenvironment of adult stem cells. These techniques aim to control the interactions between tissue stem cells and stimuli selected for their potential role in the design of stem cell-based bioprocesses.

A number of ECM proteins and receptors have been reported to be expressed differentially in the stem cell region of the crypt *(116)*. However, even though numerous molecules (such as Notch) mediated by cell–cell and cell–ECM interactions are suspected, the molecular mechanisms that induce this behavior remain elusive (for review, *see* ref. *117*). Further insight into understanding such signals will facilitate the design of culture technologies that mimic critical aspects of the in vivo microenvironment and facilitate better control over stem cell responses in vitro (Fig. 2).

2.3. Physicochemical Parameters

Physicochemical properties such as pH and oxygen and glucose concentrations are another important aspect of the stem cell niche. Changes in such parameters have been shown to be critical in both embryonic development and ES cell differentiation *(118,119)*, as well as adult stem cell regulation *(120)*. Low oxygen concentration has been linked to the activation of transcriptional factors such as hypoxia-inducible factor *(121)*, which in turn regulates the expression of signaling molecules, such as erythropoietin and vascular endothelial growth factor (VEGF), cytokines that influence stem and progenitor cell behavior. In hematopoietic precursors, low oxygen ten-

sion increases the size and frequency of hematopoietic colonies in semisolid media *(122,123)*.

However, the exact role of oxygen in the development of HSCs is yet to be determined, let alone the role of oxygen in the function of other tissue-derived stem cells. For example, it has been observed that, with low oxygen concentrations, HSC and progenitor numbers seem to be maintained *(124,125)*, whereas HSC expansion has been shown to occur at higher oxygen concentrations *(126,127)*. For neurosphere-forming cells, low oxygen concentrations inhibit cellular proliferation *(73)*.

In addition, it has been demonstrated that oxygen tension is also important in the regulation of MSCs *(128,129)*. These studies suggested that MSC proliferation and myogenic and bone differentiation are enhanced in physiological oxygen concentrations; higher oxygen concentrations induce adipocyte differentiation.

Clearly more research is required to completely understand the effects of oxygen on progenitor vs more differentiated cell populations. Different tissue microenvironments are comprised of widely varying physicochemical properties, and these may play a direct or indirect role in the observed functional differences between cells seeded in different tissues.

Another microenvironmental cue that may influence the in vivo and in vitro responses of stem cells is mechanical stimuli. It has long been known that mechanical forces play an important role in the development and maintenance of vascular, muscle, and bone tissues *(130–132)*. Mechanical stimuli may initiate mechanotransductive signaling pathways that are still largely unresolved *(133)*. The effect of these forces on the differentiation of stem cells is under study. For example, compressing marrow-derived stromal cells thought to contain MSCs encourages bone development; stretching MSCs immobilized in a matrix encourages tendon and cartilage formation *(134)*. However, the mechanisms by which mechanical stimuli affect the differentiation and self-renewal of mesenchymal or other adult stem cells largely remain to be determined.

3. MODELS OF STEM CELL BEHAVIOR

The design and implementation of models predictive of cell responses should facilitate the rapid investigation of a large number of "experimental" conditions and lead to a more in-depth understanding of the biological mechanisms that control stem cell behavior. Numerous models have been developed to describe the behavior of stem cells and to predict self-renewal and differentiation of these cells. In fact, mathematical modeling of stem cell behavior is as old as the concept of stem cells *(135)*.

Most established models have typically tried to develop an understanding of the way that stem cells respond to changes in the cellular microenvironment by imposing either stochastic or deterministic constraints onto the results of in vitro and in vivo experiments. Significant evidence exists that both stochastic and directive mechanisms play important roles in the regulation of stem cell responses, and that the particular mechanism (i.e., threshold-based responses; see ref. *136*) used may be dependent on the tissue system/cellular microenvironment.

For example, in hematopoiesis, several studies suggest that exposure to growth factors may not be obligatory for the differentiation of primitive cells, and that at least under certain conditions, the identity of the differentiated cell population may be intrinsically determined *(137)*. Particularly interesting in this regard is the recent demonstration that coexpression of multiple lineage-restricted genes precedes commitment in multipotent progenitors *(138–141)*. This multilineage "priming" process is consistent with the flexibility in the gene expression profiles seen during osteoprogenitor development and implies that the commitment of a multipotent cell to a particular pathway may reflect the stabilization of a particular subset of expressed genes *(142)*. The stabilization process may occur in a stochastic manner in the absence of a particular instructive signal. Conversely, the commitment of an undifferentiated cell may proceed through an instructive signal that stabilizes a particular set or subset of expressed transcription factors. Although it is not clear whether all adult stem cell types utilize the same underlying mechanisms for the control of their responses, many in vitro studies supported the existence of this two-level (stochastic in the absence of signal, directive in the presence of signal) regulatory mechanism *(138,143–146)*.

The above-described low-level expression of multiple transcription factors may also be at the root of at least some stem cell plasticity phenomena *(147)*. In this case, plastic stem cells may also express (either "stochastically" or as a result of ligand-mediated upregulation) transcription factors associated with cells of other tissues, and exposure to particular tissue microenvironments may directly or indirectly (through survival mechanisms) elicit this novel differentiation capacity.

Mathematical descriptions of stochastic differentiation mechanisms have typically utilized Monte Carlo simulations to mimic the probabilistic nature of stem cell responses. For example, Till et al. found that their experiments on colony-forming unit spleen (CFU-S) cell self-renewal were best described in computer simulations when the probability of self-renewal was fixed at 0.6 *(135)*. They also explained the colony size distributions using the same approach, although others have suggested that heterogeneity in the

transplanted population is also consistent with such differences *(146)*. Since then, the stochastic models have been extended to the hematopoietic progenitor cells *(148,149)*, NSCs *(150–152)*, and intestinal crypt stem cells *(112,115)*.

Deterministic models typically incorporate external conditions to derive kinetic data that describe the growth (and differentiation) rates of populations of cells. These models have been used to explain the ex vivo expansion of hematopoietic cells *(153)*. For example, Peng et al. developed a kinetic description of single-lineage hematopoietic cell expansion based on self-renewal responses to cytokine supplementation, the growth rates of different progenitor cell populations, and mature cell death *(154)*. The model is consistent with experimental observations of cytokine-supplemented hematopoietic cultures *(154)* and predicted a self-renewal probability of 0.62 to 0.73 under these conditions.

Mackey and colleagues used deterministic modeling approaches to develop multicompartment models to reveal control mechanisms that may be at the root of some types of hematopoietic disease *(155,156)*. Stem cell growth has also been mathematically described by defining cell growth in terms of the proliferation responses of subpopulations of cells *(157)*, in some cases taking into account symmetric and asymmetric division by defining differentiation as a state that is attained after a certain number of symmetric mitotic cycles have occurred *(158)*. Although stochastic and deterministic models utilize different approaches, both can be applied and fit to experimental data (and thus are somewhat limited in their ability to provide new insight into the mechanisms that regulate stem cell responses).

Using ES cells as a model stem cell system, we are developing novel models that incorporate the known variability in receptor expression between individual cells into a deterministic cell population-based model *(136)*. This generalized model illustrates how quantitative variations in ligand–receptor interactions, arising from interactions of the cell with its microenvironment, can result in alteration in cell fate choices. Our approach is distinct from stochastic models of stem cell differentiation control, which typically assume that cell fate processes are random and are best described by statistical probability distributions. This comprehensive approach, which attempts to incorporate molecular events in the description of macroscopic cellular behavior (i.e., ligand concentration and receptor expression to generate predictions of self-renewal) should be valuable to adult stem cell models *(55,88,136)*.

To be useful, mathematical models should not only be consistent with the observed data, but also be able to predict new experimental observations

and to determine system-controlling parameters. Statistical models that aim to analyze stem cell gene expression and to correlate such information with stem cell hierarchy will also be useful in revealing common mechanisms between stem cell types. We have started to explore such an approach on clones of osteoblast progenitors to determine which genes are expressed as they develop into mature bone. Our analysis indicated that the adult progenitor cells can use several developmental routes to get to the same end stage *(142)*. This unexpected plasticity in the genetic paths used to generate the same stable differentiated state may likewise be a property of multiple stem cell systems. The modeling of highly complex molecular interactions and gene regulatory networks has already been successfully applied to predict system behavior in intracellular signaling networks comprised of hundreds of components *(159–162)*. The application of these and other approaches to stem cell systems should prove fruitful.

4. DEVELOPING SCALABLE STEM CELL BIOREACTORS

In addition to developing strategies to control and manipulate the cellular microenvironment, bioengineers must devise bioprocesses to implement this microenvironmental control at a clinically relevant scale. For some stem cell-based applications, current bioreactors must be scaled up to industrial size units (>10 L), and others (such as purified HSC) may require much smaller volumes (i.e., <100 mL); each poses significant process control challenges. Bioreactors can be designed with two goals: generation of large quantities of differentiated cells and expansion of transplantable stem cells. The former may find their implementation in the treatment of acute disease and injury (such as acute liver failure or burns), and the latter may be useful for the treatment of chronic disorders (such as diabetes or gene therapy for sickle cell anemia). A number of culture systems have been developed for the production of stem cell-based therapeutics. These include stirred or attachment-based culture techniques *(163)*.

Stirred cultures have a number of advantages, such as scalability, culture homogeneity, and simplicity *(163)*. Therefore, parameters such as oxygen tension, cytokine concentration, and serum components may be easily regulated in these cultures *(164–166)*. Stirred bioreactors have been successfully used to culture hematopoietic *(164)*, neural *(73)*, and bone marrow populations capable of reading out as fibroblast and bone progenitors (MSCs) adult stem cells *(165)*. Suspension culture systems may also be useful for controlling the ratios between differentiated and undifferentiated cells during in vitro culture. In addition, inhibitory signals, generated by the differentiated progeny (reviewed in ref. *60*) may regulate the yield of stem cells in such

cultures. Mathematical models *(167)* have predicted and experiments have confirmed *(168)* that the control of this differentiated subpopulation dynamic can be used to influence culture output *(168)*.

Despite the simplicity of stirred cultures, these cultures may not be suitable for all types of adult stem cells. For example, epithelial progenitor cells may require three-dimensional (3-D) signals for expansion or directed differentiation. In such cases, the use of culture conditions that enhance adhesion-based interactions may be important. Some investigators have combined these requirements with suspension culture systems, for example, using simulated microgravity conditions that result in the maintenance of in vivo-like gene expression *(169)* and cellular organization *(170–173)*.

Adhesion-based cultures are typically used to create bioprocesses that have characteristics of the in vivo microenvironment. These may involve the use of scaffolds or beads as the templates onto which progenitor cells grow *(174)* and often utilize feeder or stomal cells as delivery vehicles for stimulatory signals (thereby overcoming the difficulties associated with insufficient knowledge of factors that influence stem cell self-renewal and differentiation). Bioengineering approaches for positioning anchorage-dependent cells on surfaces with control over size and spatial arrangements (cellular "micropatterning"; *see* refs. *175–178*) can create a high level of complexity in the cocultures and may be useful for the analysis of stem cell behavior under defined conditions and geometries.

These and other microfabrication techniques (reviewed in ref. *179*) may become important tools in creating bioreactors that mimic in vivo conditions. Soft lithography and photolithography techniques have become widely available tools for biological applications *(179)*. The particular advantage of these techniques is evident in biological applications that require length scales of 10 μm or greater *(180)*.

Microfluidics may be used in combination with these techniques to control the delivery of cytokines and growth factors to cells *(179)*. Microfluidic systems take advantage of the laminar flow of fluids within narrow channels (<100 μm) to allow for the formation of concentration gradients of soluble factors and therefore allow for direct control of cell responses at length scales that are developmentally relevant *(179,181)*.

Microfabricated bioreactors may also be used to study and expand stem cells under perfused conditions *(182,183)*. Such cultures maintain differentiated phenotypes of hepatocytes in vitro *(183)*; however, their feasibility in expanding stem cells has yet to be determined.

A critical property of ideal stem cell bioreactors is the ease of periodic medium replacement. Replenishing medium not only eliminates nutrient and

cytokine depletion and end product inhibition, but also allows the transient presentation of signals specific to the differentiation stage of the cells. The use of such signaling techniques has been particularly important in inducing the differentiation of ES cells into hepatocytes by exposing the cells to transient conditions that mimic the embryonic development *(184)*. Such techniques may provide a valuable tool in adult stem cell therapies. Furthermore, metabolic properties such as cell-specific glucose consumption and lactate production increase and inhibitory factors such as medium acidification decrease in fresh medium *(185)*.

5. CONCLUSIONS

To utilize adult stem cells fully in cell therapy applications, understanding the molecular cues that regulate their behavior is crucial. The lack of suitable in vitro models has hindered stem cell research and limited much experimentation to in vivo models. The challenge is to design controlled systems that will deliver proper microenvironmental cues at optimal doses. Bioengineering approaches, including the modeling, analysis, and manipulation of microenvironmental cues, as well as the design of novel bioreactors, should facilitate the generation of therapeutically significant amounts of stem cells. Differentiated human tissues may provide a basis for the detailed understanding of the molecular mechanisms that control stem cell responses.

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REFERENCES

1. Fuchs, E., and Segre, J. A. (2000). Stem cells: a new lease on life. Cell 100, 143–155.
2. Weissman, I. L. (2000). Stem cells: units of development, units of regeneration, and units in evolution. Cell 100, 157–168.
3. Watt, F. M., and Hogan, B. L. (2000). Out of Eden: stem cells and their niches. Science 287, 1427–1430.

4. Barrett, A. J., and Treleaven, J. (1998). The Clinical Practice of Stem-Cell Transplantation. Oxford, UK: Isis Medical Media, Mosby-Year Book.
5. Green, H. (1989). Regeneration of the skin after grafting of epidermal cultures. Lab Invest 60, 583–584.
6. Nordon, R., and Schindhelm, K. (1999). Ex Vivo Cell Therapy. San Diego, CA: Academic Press.
7. Michalopoulos, G. K., and DeFrances, M. C. (1997). Liver regeneration. Science 276, 60–66.
8. Sell, S. (1978). Distribution of alpha-fetoprotein- and albumin-containing cells in the livers of Fischer rats fed four cycles of N-2-fluorenylacetamide. Cancer Res 38, 3107–3113.
9. Thorgeirsson, S. S. (1996). Hepatic stem cells in liver regeneration. FASEB J 10, 1249–1256.
10. Potten, C. S., and Loeffler, M. (1990). Stem cells: attributes, cycles, spirals, pitfalls and uncertainties. Lessons for and from the crypt. Development 110, 1001–1020.
11. Tropepe, V., Coles, B. L., Chiasson, B. J., et al. (2000). Retinal stem cells in the adult mammalian eye. Science 287, 2032–2036.
12. Watt, F. M. (1998). Epidermal stem cells: markers, patterning and the control of stem cell fate. Philos Trans R Soc Lond B Biol Sci 353, 831–837.
13. Seale, P., and Rudnicki, M. A. (2000). A new look at the origin, function, and "stem-cell" status of muscle satellite cells. Dev Biol 218, 115–124.
14. Gage, F. H. (2000). Mammalian neural stem cells. Science 287 (5457), 1433–1438.
15. Ormerod, E. J., and Rudland, P. S. (1986). Regeneration of mammary glands in vivo from isolated mammary ducts. J Embryol Exp Morphol 96, 229–243.
16. Potten, C. S., Martin, K., and Kirkwood, T. B. (2001). Ageing of murine small intestinal stem cells. Novartis Found Symp 235, 66–79; discussion 79–84, 101–104.
17. De Haan, G., and Van Zant, G. (1999). Genetic analysis of hemopoietic cell cycling in mice suggests its involvement in organismal life span. FASEB J 13, 707–713.
18. Zandstra, P. W., Conneally, E., Piret, J. M., and Eaves, C. J. (1998). Ontogeny-associated changes in the cytokine responses of primitive human haemopoietic cells. Br J Haematol 101, 770–778.
19. Rufer, N., Brummendorf, T. H., Kolvraa, S., et al. (1999). Telomere fluorescence measurements in granulocytes and T lymphocyte subsets point to a high turnover of hematopoietic stem cells and memory T cells in early childhood. J Exp Med 190, 157–167.
20. Peters, A. H., O'Carroll, D., Scherthan, H., et al. (2001). Loss of the Suv39h histone methyltransferases impairs mammalian heterochromatin and genome stability. Cell 107, 323–337.
21. Phillips, R. L., Ernst, R. E., Brunk, B., et al. (2000). The genetic program of hematopoietic stem cells. Science 288, 1635–1640.
22. Danet, G. H., Lee, H. W., Luongo, J. L., Simon, M. C., and Bonnet, D. A. (2001). Dissociation between stem cell phenotype and NOD/SCID repopulat-

ing activity in human peripheral blood CD34(+) cells after ex vivo expansion. Exp Hematol 29, 1465–1473.
23. Szilvassy, S. J., Humphries, R. K., Lansdorp, P. M., Eaves, A. C., and Eaves, C. J. (1990). Quantitative assay for totipotent reconstituting hematopoietic stem cells by a competitive repopulation strategy. Proc Natl Acad Sci U S A 87, 8736–8740.
24. Dick, J. E., Magli, M. C., Huszar, D., Phillips, R. A., and Bernstein, A. (1985). Introduction of a selectable gene into primitive stem cells capable of long-term reconstitution of the hemopoietic system of W/Wv mice. Cell 42, 71–79.
25. Keller, G., and Snodgrass, R. (1990). Life span of multipotential hematopoietic stem cells in vivo. J Exp Med 171, 1407–1418.
26. Lemischka, I. R., Raulet, D. H., and Mulligan, R. C. (1986). Developmental potential and dynamic behavior of hematopoietic stem cells. Cell 45, 917–927.
27. Iscove, N. N., and Nawa, K. (1997). Hematopoietic stem cells expand during serial transplantation in vivo without apparent exhaustion. Curr Biol 7, 805–808.
28. Wakitani, S., Saito, T., and Caplan, A. I. (1995). Myogenic cells derived from rat bone marrow mesenchymal stem cells exposed to 5-azacytidine. Muscle Nerve 18, 1417–1426.
29. Makino, S., Fukuda, K., Miyoshi, S., et al. (1999). Cardiomyocytes can be generated from marrow stromal cells in vitro. J Clin Invest 103, 697–705.
30. Ferrari, G., Cusella-De Angelis, G., Coletta, M., et al. (1998). Muscle regenera-tion by bone marrow-derived myogenic progenitors. Science 279, 1528–1530.
31. Bittner, R. E., Schofer, C., Weipoltshammer, K., et al. (1999). Recruitment of bone-marrow-derived cells by skeletal and cardiac muscle in adult dystrophic mdx mice. Anat Embryol (Berl) 199, 391–396.
32. Gussoni, E., Soneoka, Y., Strickland, C. D., et al. (1999). Dystrophin expression in the mdx mouse restored by stem cell transplantation. Nature 401, 390–394.
33. Petersen, B. E., Bowen, W. C., Patrene, K. D., et al. (1999). Bone marrow as a potential source of hepatic oval cells. Science 284, 1168–1170.
34. Theise, N. D., Badve, S., Saxena, R., et al. (2000). Derivation of hepatocytes from bone marrow cells in mice after radiation-induced myeloablation. Hepatology 31, 235–240.
35. Alison, M. R., Poulsom, R., Jeffery, R., et al. (2000). Hepatocytes from non-hepatic adult stem cells. Nature 406, 257.
36. Theise, N. D., Nimmakayalu, M., Gardner, R., et al. (2000). Liver from bone marrow in humans. Hepatology 32, 11–16.
37. Lagasse, E., Connors, H., Al-Dhalimy, M., et al. (2000). Purified hematopoietic stem cells can differentiate into hepatocytes in vivo. Nat Med 6, 1229–1234.
38. Pereira, R. F., Halford, K. W., O'Hara, M. D., et al. (1995). Cultured adherent cells from marrow can serve as long-lasting precursor cells for bone, cartilage, and lung in irradiated mice. Proc Natl Acad Sci U S A 92, 4857–4861.

39. Brazelton, T. R., Rossi, F. M., Keshet, G. I., and Blau, H. M. (2000). From marrow to brain: expression of neuronal phenotypes in adult mice. Science 290, 1775–1779.
40. Mezey, E., Chandross, K. J., Harta, G., Maki, R. A., and McKercher, S. R. (2000). Turning blood into brain: cells bearing neuronal antigens generated in vivo from bone marrow. Science 290, 1779–1782.
41. Sanchez-Ramos, J., Song, S., Cardozo-Pelaez, F., et al. (2000). Adult bone marrow stromal cells differentiate into neural cells in vitro. Exp Neurol 164, 247–256.
42. Woodbury, D., Schwarz, E. J., Prockop, D. J., and Black, I. B. (2000). Adult rat and human bone marrow stromal cells differentiate into neurons. J Neurosci Res 61, 364–370.
43. Eglitis, M. A., and Mezey, E. (1997). Hematopoietic cells differentiate into both microglia and macroglia in the brains of adult mice. Proc Natl Acad Sci U S A 94, 4080–4085.
44. Kopen, G. C., Prockop, D. J., and Phinney, D. G. (1999). Marrow stromal cells migrate throughout forebrain and cerebellum, and they differentiate into astrocytes after injection into neonatal mouse brains. Proc Natl Acad Sci U S A 96, 10,711–10,716.
45. Krause, D. S., Theise, N. D., Collector, M. I., et al. (2001). Multi-organ, multilineage engraftment by a single bone marrow-derived stem cell. Cell 105, 369–377.
46. Galli, R., Borello, U., Gritti, A., et al. (2000). Skeletal myogenic potential of human and mouse neural stem cells. Nat Neurosci 3, 986–991.
47. Jackson, K. A., Mi, T., and Goodell, M. A. (1999). Hematopoietic potential of stem cells isolated from murine skeletal muscle. Proc Natl Acad Sci U S A 96, 14,482–14,486.
48. Ferrari, G., Stornaiuolo, A., and Mavilio, F. (2001). Bone-marrow transplantation failure to correct murine muscular dystrophy. Nature 411, 1014–1015.
49. Morshead, C. M., Benveniste, P., Iscove, N. N., and van der Kooy, D. (2002). Hematopoietic competence is a rare property of neural stem cells that may depend on genetic and epigenetic alterations. Nat Med 8, 268–273.
50. Terada, N., Hamazaki, T., Oka, M., et al. (2002). Bone marrow cell adopt the phenotype of other cells by spontaneous cell fusion [advance on-line publication]. Nature.
51. Ying, Q., Nichols, J., Evans, E. P., and Smith, A. G. (2002). Changing potency spontaneous fusion [advance on-line publication]. Nature.
52. Koller, M. R., Manchel, I., and Palsson, B. O. (1997). Importance of parenchymal:stromal cell ratio for the ex vivo reconstitution of human hematopoiesis. Stem Cells 15, 305–313.
53. Koller, M. R., Bender, J. G., Miller, W. M., and Papoutsakis, E. T. (1993). Expansion of primitive human hematopoietic progenitors in a perfusion bioreactor system with IL-3, IL-6, and stem cell factor. Biotechnology (N Y) 11, 358–363.
54. Zandstra, P. W., Jervis, E., Haynes, C. A., Kilburn, D. G., Eaves, C. J., and Piret, J. M. (1999). Concentration-dependent internalization of a cytokine/

cytokine receptor complex in human hematopoietic cells. Biotechnol Bioeng 63, 493–501.
55. Zandstra, P. W., Lauffenburger, D. A., and Eaves, C. J. (2000). A ligand-receptor signaling threshold model of stem cell differentiation control: a biologically conserved mechanism applicable to hematopoiesis. Blood 96(4), 1215–1222.
56. Zandstra, P. W., Conneally, E., Petzer, A. L., Piret, J. M., and Eaves, C. J. (1997). Cytokine manipulation of primitive human hematopoietic cell self-renewal. Proc Natl Acad Sci U S A 94, 4698–4703.
57. Koller, M. R., Oxender, M., Brott, D. A., and Palsson, B. O. (1996). flt-3 ligand is more potent than c-kit ligand for the synergistic stimulation of ex vivo hema-topoietic cell expansion. J Hematother 5, 449–459.
58. Audet, J., Miller, C. L., Rose-John, S., Piret, J. M., and Eaves, C. J. (2001). Distinct role of gp130 activation in promoting self-renewal divisions by mitogenically stimulated murine hematopoietic stem cells. Proc Natl Acad Sci U S A 98, 1757–1762.
59. Petzer, A. L., Hogge, D. E., Landsdorp, P. M., Reid, D. S., and Eaves, C. J. (1996). Self-renewal of primitive human hematopoietic cells (long-term-culture-initiating cells) in vitro and their expansion in defined medium. Proc Natl Acad Sci U S A 93, 1470–1474.
60. Madlambayan, G. J., Rogers, I., Casper, R. F., and Zandstra, P. W. (2001). Controlling culture dynamics for the expansion of hematopoietic stem cells. J Hematother Stem Cell Res 10(4), 481–492.
61. Audet, J., Zandstra, P. W., Eaves, C. J., and Piret, J. M. (1998). Advances in hematopoietic stem cell culture. Curr Opin Biotechnol 9, 146–151.
62. Yagi, M., Ritchie, K. A., Sitnicka, E., Storey, C., Roth, G. J., and Bartelmez, S. (1999). Sustained ex vivo expansion of hematopoietic stem cells mediated by thrombopoietin. Proc Natl Acad Sci U S A 96, 8126–8131.
63. Bodine, D. M., Seidel, N. E., Zsebo, K. M., and Orlic, D. (1993). In vivo admin-istration of stem cell factor to mice increases the absolute number of pluripotent hematopoietic stem cells. Blood 82, 445–455.
64. Li, C. L., and Johnson, G. R. (1994). Stem cell factor enhances the survival but not the self-renewal of murine hematopoietic long-term repopulating cells. Blood 84, 408–414.
65. Petzer, A. L., Zandstra, P. W., Piret, J. M., and Eaves, C. J. (1996). Differential cytokine effects on primitive ($CD34^+CD38^-$) human hematopoietic cells: novel responses to Flt3-ligand and thrombopoietin. J Exp Med 183(6), 2551–2558.
66. Audet, J., Miller, C. L., Rose-John, S., Piret, J. M., and Eaves, C. J. (2001). Distinct role of gp130 activation in promoting self-renewal divisions by mitogenically stimulated murine hematopoietic stem cells. Proc Natl Acad Sci U S A 98, 1757–1762.
67. Gritti, A., Parati, E. A., Cova, L., et al. (1996). Multipotential stem cells from the adult mouse brain proliferate and self-renew in response to basic fibroblast growth factor. J Neurosci 16, 1091–1100.
68. Reynolds, B. A., and Weiss, S. (1992). Generation of neurons and astrocytes

from isolated cells of the adult mammalian central nervous system. Science 255, 1707–1710.
69. Reynolds, B. A., Tetzlaff, W., and Weiss, S. (1992). A multipotent EGF-responsive striatal embryonic progenitor cell produces neurons and astrocytes. J Neurosci 12, 4565–4574.
70. Gensburger, C., Labourdette, G., and Sensenbrenner, M. (1987). Brain basic fibroblast growth factor stimulates the proliferation of rat neuronal precursor cells in vitro. FEBS Lett 217, 1–5.
71. Richards, L. J., Kilpatrick, T. J., and Bartlett, P. F. (1992). De novo generation of neuronal cells from the adult mouse brain. Proc Natl Acad Sci U S A 89, 8591–8595.
72. Ray, J., Peterson, D. A., Schinstine, M., and Gage, F. H. (1993). Proliferation, differentiation, and long-term culture of primary hippocampal neurons. Proc Natl Acad Sci U S A 90, 3602–3606.
73. Kallos, M. S., and Behie, L. A. (1999). Inoculation and growth conditions for high-cell-density expansion of mammalian neural stem cells in suspension bioreactors. Biotechnol Bioeng 63, 473–483.
74. Toma, J. G., Akhavan, M., Fernandes, K. J., et al. (2001). Isolation of multipotent adult stem cells from the dermis of mammalian skin. Nat Cell Biol 3, 778–784.
75. Carpenter, M. K., Cui, X., Hu, Z. Y., et al. (1999). In vitro expansion of a multipotent population of human neural progenitor cells. Exp Neurol 158, 265–278.
76. Rathjen, P. D., Toth, S., Willis, A., Heath, J. K., and Smith, A. G. (1990). Differentiation inhibiting activity is produced in matrix-associated and diffusible forms that are generated by alternate promoter usage. Cell 62, 1105–1114.
77. Neildez-Nguyen, T. M., Wajcman, H., Marden, M. C., et al. (2002). Human erythroid cells produced ex vivo at large scale differentiate into red blood cells in vivo. Nat Biotechnol 20, 467–472.
78. Fambrough, D., McClure, K., Kazlauskas, A., and Lander, E. S. (1999). Diverse signaling pathways activated by growth factor receptors induce broadly overlapping, rather than independent, sets of genes. Cell 97, 727–741.
79. Billia, F., Barbara, M., McEwen, J., Trevisan, M., and Iscove, N. N. (2001). Resolution of pluripotential intermediates in murine hematopoietic differentiation by global complementary DNA amplification from single cells: confirmation of assignments by expression profiling of cytokine receptor transcripts. Blood 97, 2257–2268.
80. Brail, L. H., Jang, A., Billia, F., Iscove, N. N., Klamut, H. J., and Hill, R. P. (1999). Gene expression in individual cells: analysis using global single cell reverse transcription polymerase chain reaction (GSC RT-PCR). Mutat Res 406, 45–54.
81. Lauffenburger, D. A., and Linderman, J. J. (1996). Receptors: Models for Binding, Trafficking, and Signaling. New York: Oxford University Press.
82. Starr, R., Willson, T. A., Viney, E. M., et al. (1997). A family of cytokine-inducible inhibitors of signalling. Nature 387, 917–921.
83. Lumelsky, N., Blondel, O., Laeng, P., Velasco, I., Ravin, R., and McKay, R.

(2001). Differentiation of embryonic stem cells to insulin-secreting structures similar to pancreatic islets. Science 292, 1389–1394.
84. Pluen, A., Boucher, Y., Ramanujan, S., et al. (2001). Role of tumor–host interactions in interstitial diffusion of macromolecules: cranial vs subcutaneous tumors. Proc Natl Acad Sci U S A 98, 4628–4633.
85. Wittrup, K. D. (2001). Protein engineering by cell-surface display. Curr Opin Biotechnol 12, 395–399.
86. Saga, T., Neumann, R. D., Heya, T., et al. (1995). Targeting cancer micrometastases with monoclonal antibodies: a binding-site barrier. Proc Natl Acad Sci U S A 92, 8999–9003.
87. Fallon, E. M., Liparoto, S. F., Lee, K. J., Ciardelli, T. L., and Lauffenburger, D. A. (2000). Increased endosomal sorting of ligand to recycling enhances potency of an interleukin-2 analog. J Biol Chem 275, 6790–6797.
88. Zandstra, P. W., Le, H. V., Daley, G. Q., Griffith, L. G., and Lauffenburger, D. A. (2000). Leukemia inhibitory factor (LIF) concentration modulates embryonic stem cell self-renewal and differentiation independently of proliferation. Biotechnol Bioeng 69, 607–617.
89. Lauffenburger, D. A., Fallon, E. M., and Haugh, J. M. (1998). Scratching the (cell) surface: cytokine engineering for improved ligand/receptor trafficking dynamics. Chem Biol 5, R257–R263.
90. Fallon, E. M., and Lauffenburger, D. A. (2000). Computational model for effects of ligand/receptor binding properties on interleukin-2 trafficking dynamics and T cell proliferation response. Biotechnol Prog 16, 905–916.
91. Maynard, J., and Georgiou, G. (2000). Antibody engineering. Annu Rev Biomed Eng 2, 339–376.
92. Chirumamilla, R. R., Muralidhar, R., Marchant, R., and Nigam, P. (2001). Improving the quality of industrially important enzymes by directed evolution. Mol Cell Biochem 224, 159–168.
93. French, A. R., Tadaki, D. K., Niyogi, S. K., and Lauffenburger, D. A. (1995). Intracellular trafficking of epidermal growth factor family ligands is directly influenced by the pH sensitivity of the receptor/ligand interaction. J Biol Chem 270, 4334–4340.
94. MacBeath, G., and Schreiber, S. L. (2000). Printing proteins as microarrays for high-throughput function determination. Science 289, 1760–1763.
95. Kuhl, P. R., and Griffith-Cima, L. G. (1996). Tethered epidermal growth factor as a paradigm for growth factor-induced stimulation from the solid phase. Nat Med 2, 1022–1027.
96. Wehrle-Haller, B., and Weston, J. A. (1995). Soluble and cell-bound forms of steel factor activity play distinct roles in melanocyte precursor dispersal and survival on the lateral neural crest migration pathway. Development 121, 731–742.
97. Kurosawa, K., Miyazawa, K., Gotoh, A., et al. (1996). Immobilized anti-KIT monoclonal antibody induces ligand-independent dimerization and activation of Steel factor receptor: biologic similarity with membrane-bound form of Steel factor rather than its soluble form. Blood 87, 2235–2243.

98. Miyazawa, K., Williams, D. A., Gotoh, A., Nishimaki, J., Broxmeyer, H. E., and Toyama, K. (1995). Membrane-bound Steel factor induces more persistent tyrosine kinase activation and longer life span of c-kit gene-encoded protein than its soluble form. Blood 85, 641–649.
99. Doheny, J. G., Jervis, E. J., Guarna, M. M., Humphries, R. K., Warren, R. A., and Kilburn, D. G. (1999). Cellulose as an inert matrix for presenting cytokines to target cells: production and properties of a stem cell factor-cellulose-binding domain fusion protein. Biochem J 339(Pt. 2), 429–434.
100. Nakashima, T., Kobayashi, Y., Yamasaki, S., et al. (2000). Protein expression and functional difference of membrane-bound and soluble receptor activator of NF-kappaB ligand: modulation of the expression by osteotropic factors and cytokines. Biochem Biophys Res Commun 275, 768–775.
101. Barille, S., Collette, M., Thabard, W., Bleunven, C., Bataille, R., and Amiot, M. (2000). Soluble IL-6R alpha upregulated IL-6, MMP-1 and MMP-2 secretion in bone marrow stromal cells. Cytokine 12, 1426–1429.
102. Slater, M. (1996). Dynamic interactions of the extracellular matrix. Histol Histopathol 11, 175–180.
103. Flaumenhaft, R., and Rifkin, D. B. (1991). Extracellular matrix regulation of growth factor and protease activity. Curr Opin Cell Biol 3, 817–823.
104. Rafii, S., Mohle, R., Shapiro, F., Frey, B. M., and Moore, M. A. (1997). Regulation of hematopoiesis by microvascular endothelium. Leuk Lymphoma 27, 375–386.
105. Friedenstein, A. J., Gorskaja, J. F., and Kulagina, N. N. (1976). Fibroblast precursors in normal and irradiated mouse hematopoietic organs. Exp Hematol 4, 267–274.
106. Prosper, F., and Verfaillie, C. M. (2001). Regulation of hematopoiesis through adhesion receptors. J Leukoc Biol 69, 307–316.
107. Ploemacher, R. E., Mayen, A. E., De Koning, A. E., Krenacs, T., and Rosendaal, M. (2000). Hematopoiesis: gap junction intercellular communication is likely to be involved in regulation of stroma-dependent proliferation of hemopoietic stem cells. Hematology 5, 133–147.
108. Potten, C. S., and Loeffler, M. (1987). A comprehensive model of the crypts of the small intestine of the mouse provides insight into the mechanisms of cell migration and the proliferation hierarchy. J Theor Biol 127, 381–391.
109. Potten, C. S. (1998). Stem cells in gastrointestinal epithelium: numbers, characteristics and death. Philos Trans R Soc Lond B Biol Sci 353, 821–830.
110. Evans, G. S., and Potten, C. S. (1991). Stem cells and the elixir of life. Bioessays 13, 135–138.
111. Potten, C. S. (1991). The role of stem cells in the regeneration of intestinal crypts after cytotoxic exposure. Prog Clin Biol Res 369, 155–171.
112. Paulus, U., Potten, C. S., and Loeffler, M. (1992). A model of the control of cellular regeneration in the intestinal crypt after perturbation based solely on local stem cell regulation. Cell Prolif 25, 559–578.
113. Li, Y. Q., Roberts, S. A., Paulus, U., Loeffler, M., and Potten, C. S. (1994). The crypt cycle in mouse small intestinal epithelium. J Cell Sci 107 (Pt 12), 3271–3279.

114. Potten, C. S. (1986). Cell cycles in cell hierarchies. Int J Radiat Biol Relat Stud Phys Chem Med 49, 257–278.
115. Loeffler, M., Birke, A., Winton, D., and Potten, C. (1993). Somatic mutation, monoclonality and stochastic models of stem cell organization in the intestinal crypt. J Theor Biol 160, 471–491.
116. Marshman, E., Booth, C., and Potten, C. S. (2002). The intestinal epithelial stem cell. Bioessays 24, 91–98.
117. Simmons, P. J., Levesque, J. P., and Zannettino, A. C. (1997). Adhesion molecules in haemopoiesis. Baillieres Clin Haematol 10, 485–505.
118. Gassmann, M., Fandrey, J., Bichet, S., et al. (1996). Oxygen supply and oxygen-dependent gene expression in differentiating embryonic stem cells. Proc Natl Acad Sci U S A 93, 2867–2872.
119. Sauer, H., Rahimi, G., Hescheler, J., and Wartenberg, M. (2000). Role of reactive oxygen species and phosphatidylinositol 3-kinase in cardiomyocyte differentiation of embryonic stem cells. FEBS Lett 476, 218–223.
120. McAdams, T. A., Miller, W. M., and Papoutsakis, E. T. (1997). Variations in culture pH affect the cloning efficiency and differentiation of progenitor cells in ex vivo haemopoiesis. Br J Haematol 97, 889–895.
121. Wang, G. L., Jiang, B. H., Rue, E. A., and Semenza, G. L. (1995). Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. Proc Natl Acad Sci U S A 92, 5510–5514.
122. Broxmeyer, H. E., Cooper, S., Lu, L., Miller, M. E., Langefeld, C. D., and Ralph, P. (1990). Enhanced stimulation of human bone marrow macrophage colony formation in vitro by recombinant human macrophage colony-stimulating factor in agarose medium and at low oxygen tension. Blood 76, 323–329.
123. Bradley, T. R., Hodgson, G. S., and Rosendaal, M. (1978). The effect of oxygen tension on haemopoietic and fibroblast cell proliferation in vitro. J Cell Physiol 97, 517–522.
124. Cipolleschi, M. G., Dello Sbarba, P., and Olivotto, M. (1993). The role of hypoxia in the maintenance of hematopoietic stem cells. Blood 82, 2031–2037.
125. Ivanovic, Z., Belloc, F., Faucher, J. L., Cipolleschi, M. G., Praloran, V., and Dello Sbarba, P. (2002). Hypoxia maintains and interleukin-3 reduces the pre-colony-forming cell potential of dividing CD34(+) murine bone marrow cells. Exp Hematol 30, 67–73.
126. Koller, M. R., Emerson, S. G., and Palsson, B. O. (1993). Large-scale expansion of human stem and progenitor cells from bone marrow mononuclear cells in continuous perfusion cultures. Blood 82, 378–384.
127. Palsson, B. O., Paek, S. H., Schwartz, R. M., et al. (1993). Expansion of human bone marrow progenitor cells in a high cell density continuous perfusion system. Biotechnology (N Y) 11, 368–372.
128. Csete, M., Walikonis, J., Slawny, N., et al. (2001). Oxygen-mediated regulation of skeletal muscle satellite cell proliferation and adipogenesis in culture. J Cell Physiol 189, 189–196.
129. Lennon, D. P., Edmison, J. M., and Caplan, A. I. (2001). Cultivation of rat marrow-derived mesenchymal stem cells in reduced oxygen tension: effects on in vitro and in vivo osteochondrogenesis. J Cell Physiol 187, 345–355.

130. Li, C., and Xu, Q. (2000). Mechanical stress-initiated signal transductions in vascular smooth muscle cells. Cell Signal 12, 435–445.
131. Burger, E. H., and Klein-Nulen, J. (1999). Responses of bone cells to biomechanical forces in vitro. Adv Dent Res 13, 93–98.
132. Fisher, A. B., Chien, S., Barakat, A. I., and Nerem, R. M. (2001). Endothelial cellular response to altered shear stress. Am J Physiol Lung Cell Mol Physiol 281, L529–L533.
133. Ingber, D. E. (1997). Integrins, tensegrity, and mechanotransduction. Gravit Space Biol Bull 10, 49–55.
134. Pittenger, M. F., Mackay, A. M., Beck, S. C., et al. (1999). Multilineage potential of adult human mesenchymal stem cells. Science 284, 143–147.
135. Till, J. E., McCulloch, E. A., and Siminovitch, L. (1964). A stochastic model of stem cell proliferation, based on the growth of spleen colony-forming cells. Proc Natl Acad Sci U S A 51, 29.
136. Viswanathan, S., Benatar, T., Rose-John, S., Lauffenburger, D. A., and Zandstra, P. W. (2002). Ligand/receptor signaling threshold (LIST) model accounts for gp130-mediated embryonic stem cell self-renewal responses to LIF and HIL-6. Stem Cells 20, 119–138.
137. Mayani, H., Dragowska, W., and Lansdorp, P. M. (1993). Cytokine-induced selective expansion and maturation of erythroid vs myeloid progenitors from purified cord blood precursor cells. Blood 81, 3252–3258.
138. Fairbairn, L. J., Cowling, G. J., Reipert, B. M., and Dexter, T. M. (1993). Suppression of apoptosis allows differentiation and development of a multipotent hemopoietic cell line in the absence of added growth factors. Cell 74, 823–832.
139. Hu, M., Krause, D., Greaves, M., et al. (1997). Multilineage gene expression precedes commitment in the hemopoietic system. Genes Dev 11, 774–785.
140. Liu, L., and Roberts, R. M. (1996). Silencing of the gene for the beta subunit of human chorionic gonadotropin by the embryonic transcription factor Oct-3/4. J Biol Chem 271, 16,683–16,689.
141. Enver, T., and Greaves, M. (1998). Loops, lineage, and leukemia. Cell 94, 9–12.
142. Madras, N., Gibbs, A. L., Zhou, H., Zandstra, P. W., and Aubin, J. E. (2002). Modeling stem cell development by retrospective analysis of gene expression profiles in single progenitor-derived colonies. Stem Cells, 20(3), 230–240.
143. Mayani, H., Dragowska, W., and Lansdorp, P. M. (1993). Lineage commitment in human hemopoiesis involves asymmetric cell division of multipotent progenitors and does not appear to be influenced by cytokines. J Cell Physiol 157, 579–586.
144. Ogawa, M. (1993). Differentiation and proliferation of hematopoietic stem cells. Blood 81, 2844–2853.
145. Humphries, R. K., Eaves, A. C., and Eaves, C. J. (1981). Self-renewal of hemopoietic stem cells during mixed colony formation in vitro. Proc Natl Acad Sci U S A 78, 3629–3633.
146. Kimmel, M., and Axelrod, D. E. (1991). Unequal cell division, growth regulation and colony size of mammalian cells: a mathematical model and analysis of experimental data. J Theor Biol 153, 157–180.

147. Lemischka, I. (2001). Stem cell dogmas in the genomics era. Rev Clin Exp Hematol 5, 15–25.
148. Francis, G. E., and Leaning, M. S. (1985). Stochastic model of human granulocyte-macrophage progenitor cell proliferation and differentiation. I. Setting up the model. Exp Hematol 13, 92–98.
149. Nakahata, T., Gross, A. J., and Ogawa, M. (1982). A stochastic model of self-renewal and commitment to differentiation of the primitive hemopoietic stem cells in culture. J Cell Physiol 113, 455–458.
150. Le Douarin, N. M., and Dupin, E. (1993). Cell lineage analysis in neural crest ontogeny. J Neurobiol 24, 146–161.
151. Yakovlev, A. Y., Mayer-Proschel, M., and Noble, M. (1998). A stochastic model of brain cell differentiation in tissue culture. J Math Biol 37, 49–60.
152. Boucher, K., Yakovlev, A. Y., Mayer-Proschel, M., and Noble, M. (1999). A stochastic model of temporally regulated generation of oligodendrocytes in cell culture. Math Biosci 159, 47–78.
153. Fokas, A. S., Keller, J. B., and Clarkson, B. D. (1991). Mathematical model of granulocytopoiesis and chronic myelogenous leukemia. Cancer Res 51, 2084–2091.
154. Peng, C. A., Koller, M. R., and Palsson, B. O. (1996). Unilineage model of hematopoiesis predicts self-renewal of stem and progenitor cells based on ex vivo growth data. Biotechnol Bioeng 52, 24–33.
155. Milton, J. G., and Mackey, M. C. (1989). Periodic haematological diseases: mystical entities or dynamical disorders? J R Coll Physicians Lond 23, 236–241.
156. Haurie, C., Dale, D. C., and Mackey, M. C. (1998). Cyclical neutropenia and other periodic hematological disorders: a review of mechanisms and mathematical models. Blood 92, 2629–2640.
157. Sherley, J. L., Stadler, P. B., and Stadler, J. S. (1995). A quantitative method for the analysis of mammalian cell proliferation in culture in terms of dividing and non-dividing cells. Cell Prolif 28, 137–144.
158. Yakovlev, A. Y., Boucher, K., Mayer-Proschel, M., and Noble, M. (1998). Quantitative insight into proliferation and differentiation of oligodendrocyte type 2 astrocyte progenitor cells in vitro. Proc Natl Acad Sci U S A 95, 14,164–14,167.
159. Haugh, J. M., Schooler, K., Wells, A., Wiley, H. S., and Lauffenburger, D. A. (1999). Effect of epidermal growth factor receptor internalization on regulation of the phospholipase C-gamma1 signaling pathway. J Biol Chem 274, 8958–8965.
160. Bhalla, U. S., and Iyengar, R. (1999). Emergent properties of networks of biological signaling pathways. Science 283, 381–387.
161. Weng, G., Bhalla, U. S., and Iyengar, R. (1999). Complexity in biological signaling systems. Science 284, 92–96.
162. Schoeberl, B., Eichler-Jonsson, C., Gilles, E. D., and Muller, G. (2002). Computational modeling of the dynamics of the MAP kinase cascade activated by surface and internalized EGF receptors. Nat Biotechnol 20, 370–375.

163. Zandstra, P. W., and Nagy, A. (2001). Stem cell bioengineering. Annu Rev Biomed Eng 3, 275–305.
164. Zandstra, P. W., Eaves, C. J., and Piret, J. M. (1994). Expansion of hematopoietic progenitor cell populations in stirred suspension bioreactors of normal human bone marrow cells. Biotechnology (NY) 12(9), 909–914.
165. Baksh, D., Davies, J. E., and Zandstra, P. W. (2003). Adult human bone marrow-derived progenitor cells are capable of adhesion-independent survival and expansion. Exp Hematol 31(8) 723–732.
166. Cashman, J. D., Clark-Lewis, I., Eaves, A. C., and Eaves, C. J. (1999). Differentiation stage-specific regulation of primitive human hematopoietic progenitor cycling by exogenous and endogenous inhibitors in an in vivo model. Blood 94, 3722–3729.
167. Furusawa, C., and Kaneko, K. (2000). Origin of complexity in multicellular organisms. Phys Rev Lett 84, 6130–6133.
168. Sandstrom, C. E., Bender, J. G., Papoutsakis, E. T., and Miller, W. M. (1995). Effects of $CD34^+$ cell selection and perfusion on ex vivo expansion of peripheral blood mononuclear cells. Blood 86, 958–970.
169. Hammond, T. G., Lewis, F. C., Goodwin, T. J., et al. (1999). Gene expression in space. Nat Med 5, 359.
170. Molnar, G., Schroedl, N. A., Gonda, S. R., and Hartzell, C. R. (1997). Skeletal muscle satellite cells cultured in simulated microgravity. In Vitro Cell Dev Biol Anim 33, 386–391.
171. Freed, L. E., Langer, R., Martin, I., Pellis, N. R., and Vunjak-Novakovic, G. (1997). Tissue engineering of cartilage in space. Proc Natl Acad Sci U S A 94, 13,885–13,890.
172. Ingram, M., Techy, G. B., Saroufeem, R., et al. (1997). Three-dimensional growth patterns of various human tumor cell lines in simulated microgravity of a NASA bioreactor. In Vitro Cell Dev Biol Anim 33, 459–466.
173. Margolis, L., Hatfill, S., Chuaqui, R., et al. (1999). Long term organ culture of human prostate tissue in a NASA-designed rotating wall bioreactor. J Urol 161, 290–297.
174. Mitteregger, R., Vogt, G., Rossmanith, E., and Falkenhagen, D. (1999). Rotary cell culture system (RCCS): a new method for cultivating hepatocytes on microcarriers. Int J Artif Organs 22, 816–822.
175. Bhatia, S. N., Toner, M., Tompkins, R. G., and Yarmush, M. L. (1994). Selective adhesion of hepatocytes on patterned surfaces. Ann N Y Acad Sci 745, 187–209.
176. Bhatia, S. N., Yarmush, M. L., and Toner, M. (1997). Controlling cell interactions by micropatterning in co-cultures: hepatocytes and 3T3 fibroblasts. J Biomed Mater Res 34, 189–199.
177. Bhatia, S. N., Balis, U. J., Yarmush, M. L., and Toner, M. (1998). Microfabrication of hepatocyte/fibroblast co-cultures: role of homotypic cell interactions. Biotechnol Prog 14, 378–387.
178. Bhatia, S. N., Balis, U. J., Yarmush, M. L., and Toner, M. (1999). Effect of cell-cell interactions in preservation of cellular phenotype: cocultivation of hepatocytes and nonparenchymal cells. FASEB J 13, 1883–1900.

179. Whitesides, G. M., Ostuni, E., Takayama, S., Jiang, X., and Ingber, D. E. (2001). Soft lithography in biology and biochemistry. Annu Rev Biomed Eng 3, 335–373.
180. Folch, A., and Toner, M. (2000). Microengineering of cellular interactions. Annu Rev Biomed Eng 2, 227–256.
181. Takayama, S., Ostuni, E., LeDuc, P., Naruse, K., Ingber, D. E., and Whitesides, G. M. (2001). Subcellular positioning of small molecules. Nature 411, 1016.
182. Powers, M. J., and Griffith, L. G. (1998). Adhesion-guided in vitro morphogenesis in pure and mixed cell cultures. Microsc Res Tech 43, 379–384.
183. Griffith, L. G., Wu, B., Cima, M. J., Powers, M. J., Chaignaud, B., and Vacanti, J. P. (1997). In vitro organogenesis of liver tissue. Ann N Y Acad Sci 831, 382–397.
184. Hamazaki, T., Iiboshi, Y., Oka, M., et al. (2001). Hepatic maturation in differentiating embryonic stem cells in vitro. FEBS Lett 497, 15–19.
185. Caldwell, J., Locey, B., Clarke, M. F., Emerson, S. G., and Palsson, B. O. (1991). Influence of medium exchange schedules on metabolic, growth, and GM-CSF secretion rates of genetically engineered NIH-3T3 cells. Biotechnol Prog 7, 1–8.