IDENTIFICATION AND MODULATION OF BIOPHYSICAL SIGNALS THAT CONTROL STEM CELL FUNCTION AND FATE

David V. Schaffer

INTRODUCTION

Stem cells are defined by two hallmark properties: the ability to self-renew, or divide while maintaining themselves in an immature state, and the capacity to differentiate into one or more specialized cell types. By virtue of these properties, stem cells play central roles in the development and maintenance of tissues throughout our body, and the biomedical field has increasingly been exploring their potential in cell replacement therapies for treating human disease or injury. In particular, stem cells can theoretically be harvested, expanded and differentiated in culture, and implanted for tissue regeneration. Alternatively, endogenous pools of stem cells can potentially be modulated for tissue repair. However, both gaining a deeper understanding of their natural biological functions, as well as tapping into their promise as next generation therapeutics, requires fundamental knowledge of how stem cell behavior is controlled, and specifically the processes of self-renewal and differentiation.

Populations of stem cells reside within specialized regions of developing and adult tissues that continuously present them with regulatory cues, and this repertoire of signals is collectively referred to as the stem cell niche [1]. These include small molecules; proteins and other components of the extracellular matrix (ECM), i.e. the solid phase material that enmeshes most cells in the body; small proteins that may be soluble or immobilized to the ECM; and signals presented from the surface of neighboring cells (Figure 1).

Due to many successful efforts in genetics, developmental biology, and cell biology, it is well recognized that biochemical cues within the niche play critical roles in regulating stem cell function. However,

biology encodes regulatory information to cells not only in the binary absence or presence of a given molecule, but also in numerous biophysical aspects of such regulatory signals. These include tissue mechanics, topographical features, electrostatics, biological transport phenomena, and spatiotemporal variation in each of these cues.

A major difficulty in studying and manipulating these biophysical properties of the niche is that they are not monogenic, i.e. they depend on the properties of many molecules and genes. However, an emerging theme in stem cell research is to use engineered systems in cell culture – ranging from synthetic materials to microfluidic devices – to systematically vary these biophysical properties, i.e. to provide an "x-axis" in a manner that is not currently possible with genetic approaches. While there are inherent challenges with this paradigm – including establishing the *in vivo* relevance of findings, as well as integrating engineering and biology approaches to explore the underlying mechanisms – these engineering studies have broadened the field's view of the stem cell niche [2,3]. Furthermore, because of the complexity of their endogenous niches, stem cells are exceedingly difficult to control in culture. Therefore, each biophysical property offers a new opportunity to engineer synthetic systems and materials to control stem cell function for regenerative medicine applications.

MECHANOREGULATION OF STEM CELL FUNCTION

There are many mechanical features and processes of tissues that could potentially regulate cell function, including elasticity, viscosity, strain, and others. In landmark work, it was demonstrated that the lineage choice of differentiating mesenchymal stem cells (MSCs) is strongly influenced by elastic modulus of the surrounding material – i.e. the linear proportionality constant between its strain and stress – such that cells developed into neuron-like cells on soft hydrogels, myoblasts on intermediate stiffnesses, and osteocytes on harder substrates [4]. In subsequent work, neural stem cells (NSCs) were shown to preferentially differentiate into neurons when cultured on soft materials and astrocytes on hard materials [5]. Recently, human embryonic stem cell and induced pluripotent stem cell differentiation into neural lineages, but not

self-renewal, has been shown to be mechanosensitive (Figure 2) [6]. In addition to differentiation, modulus can influence stem cell self-renewal. For example, it was shown that substrate stiffness strongly impacts the ability of muscle stem cells to undergo self-renewal in culture, and subsequently capacity ability to undergo reimplantation into muscle [7].

In parallel, there have been advances in understanding molecular mechanisms by which extracellular forces regulate cellular behavior, which at their essence require a mechanical cue to be converted into a biochemical signal that drives cell fate decisions. ECM protein structures, cell adhesion receptors, the intracellular network of structural proteins known as the cytoskeleton, and key proteins in the nucleus may serve as mechanosensors.

In addition to the stiffness of the cellular microenvironment, shear flow and cyclic strain have both been implicated in regulating the self-renewal and/or differentiation of several classes of stem cells. Collectively, these studies have established the mechanical properties of the stem cell niche as a prominent regulator of fate choice, as well as offered the promise that mechanical aspects of synthetic materials can be manipulated to better control stem cell fate choice in culture.

TOPOGRAPHICAL AND SHAPE FEATURES OF THE STEM CELL NICHE

In addition to providing resident stem cells with a mechanical milieu, niches offer features that can alter the shape of a cell. On the microscale, ECM and neighboring cells can modulate and even constrain the surface area or volume available to, and therefore the shape of, a cell in a manner important for its function. Likewise, on the nanoscale, ECM proteins often assemble into fibers and other structural features that modulate the topographical features that an adherent cell experiences. Advances in lithography and in materials science have enabled investigators to investigate the effects of these features on stem cell behavior [8].

In seminal work, microcontact printing was used to pattern adhesive islands of different sizes onto a surface [9]. When MSCs were seeded onto these substrates, it was found that large islands that enabled cells to spread subsequently promoted osteogenic (bone cell) differentiation, whereas small islands that did not permit substantial cell spreading instead promoted adipogenic (fat cell) differentiation. There has been progress in both extending this principle to other fate choices and in elucidating its underlying mechanisms.

In addition to microenvironmental properties that alter cell shape on the micron scale, topographical cues – such as the organization of the ECM into fibers – offer a cell with features that can modulate its shape at the nanometer scale. In early work in this area, culturing NSCs on microgrooves patterned into polystyrene led to significantly higher extents of neuronal differentiation compared to flat surfaces [10]. Another study explored the effects of electrospun fibers of polyethersulfone with different dimensions on the NSC behavior and found that fibers of small dimension promoted differentiation into one major central nervous system cell type (oligodendrocytes), whereas larger fibers increased differentiation into neurons. These studies have yielded insights into mechanisms by which structural features in the niche can regulate cell function, and again offer potential opportunities to design biomimetic culture systems that can better control stem cell behavior.

ELECTRIC FIELDS

The role of electrophysiology in the cardiovascular and nervous systems is well appreciated, and a growing body of work has explored the possibility that electric fields may regulate the function of stem cells from these tissues. In initial work, heart muscle precursors became aligned with the direction of an electric field, exhibited a substantial increase in contractile amplitude, and expressed higher levels of various cardiac protein markers compared to cells that were not electrically stimulated [11]. Electric fields have subsequently been shown to promote the maturation and differentiation of skeletal muscle precursors, neural precursors, and embryonic stem cells.

CONCLUSIONS AND FUTURE DIRECTIONS

The application of the physical sciences and engineering to stem cell research has contributed significantly toward the development of culture systems to conduct reductionist biology, i.e. to elucidate the basic effects of a biophysical property on cell regulation. Such investigations will greatly benefit from future technological advances, particularly in the development of novel materials whose properties can be varied spatiotemporally to mimic tissue heterogeneity and development. Furthermore, there are considerable additional opportunities for "analysis by synthesis," i.e. engineering systems to emulate and thereby investigate more features of the cellular microenvironment.

Another major need in the field is the development of scaleable, safe, and reproducible stem cell culture systems for biomedical translation. Many current culture systems employ complex and poorly defined protein mixtures (e.g. serum, matrix) to recreate the complexity of the niche. Basic progress in our understanding of key biochemical and biophysical cues can be integrated toward the development of advanced, defined, synthetic culture systems that are in some ways less complex or complicated than current systems. Finally, a major challenge in the application of stem cells for tissue engineering and repair is poor cell survival upon implantation into a site of injury or disease, and engineered systems and materials have increasingly integrated biological information to mimic the natural properties of tissue and thereby enable cells to better integrate into their new niche. The integration of biology, physical sciences, and engineering is thus poised to greatly advance stem cell biology and medicine.

REFERENCES

1 Scadden, D. T. The stem-cell niche as an entity of action. *Nature* **441**, 1075-1079 (2006).

2 Keung, A. J., Kumar, S. & Schaffer, D. V. Presentation counts: microenvironmental regulation of stem cells by biophysical and material cues. *Annual review of cell and developmental biology* **26**, 533-556, doi:10.1146/annurev-cellbio-100109-104042 (2010).

3 Discher, D. E., Mooney, D. J. & Zandstra, P. W. Growth factors, matrices, and forces combine and control stem cells. *Science* **324**, 1673-1677, doi:10.1126/science.1171643 (2009).

4 Engler, A. J., Sen, S., Sweeney, H. L. & Discher, D. E. Matrix elasticity directs stem cell lineage specification. *Cell* **126**, 677-689 (2006).

5 Saha, K. *et al.* Substrate Modulus Directs Neural Stem Cell Behavior. *Biophysical Journal* **95**, 4426-4438, doi:10.1529/biophysj.108.132217 (2008).

6 Keung, A. J., Asuri, P., Kumar, S. & Schaffer, D. V. Soft microenvironments promote the early neurogenic differentiation but not self-renewal of human pluripotent stem cells. *Integrative biology : quantitative biosciences from nano to macro*, doi:10.1039/c2ib20083j (2012).

7 Gilbert, P. M. *et al.* Substrate Elasticity Regulates Skeletal Muscle Stem Cell Self-Renewal in Culture. *Science* **329**, 1078-1081, doi:10.1126/science.1191035 (2010).

8 Kolind, K., Leong, K. W., Besenbacher, F. & Foss, M. Guidance of stem cell fate on 2D patterned surfaces. *Biomaterials* **33**, 6626-6633, doi:10.1016/j.biomaterials.2012.05.070 (2012).

9 McBeath, R., Pirone, D. M., Nelson, C. M., Bhadriraju, K. & Chen, C. S. Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment. *Developmental cell* **6**, 483-495 (2004).

10 Recknor, J. B., Sakaguchi, D. S. & Mallapragada, S. K. Directed growth and selective differentiation of neural progenitor cells on micropatterned polymer substrates. *Biomaterials* **27**, 4098-4108, doi:10.1016/j.biomaterials.2006.03.029 (2006).

11 Radisic, M. *et al.* Functional assembly of engineered myocardium by electrical stimulation of cardiac myocytes cultured on scaffolds. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 18129-18134, doi:10.1073/pnas.0407817101 (2004).

FIGURES

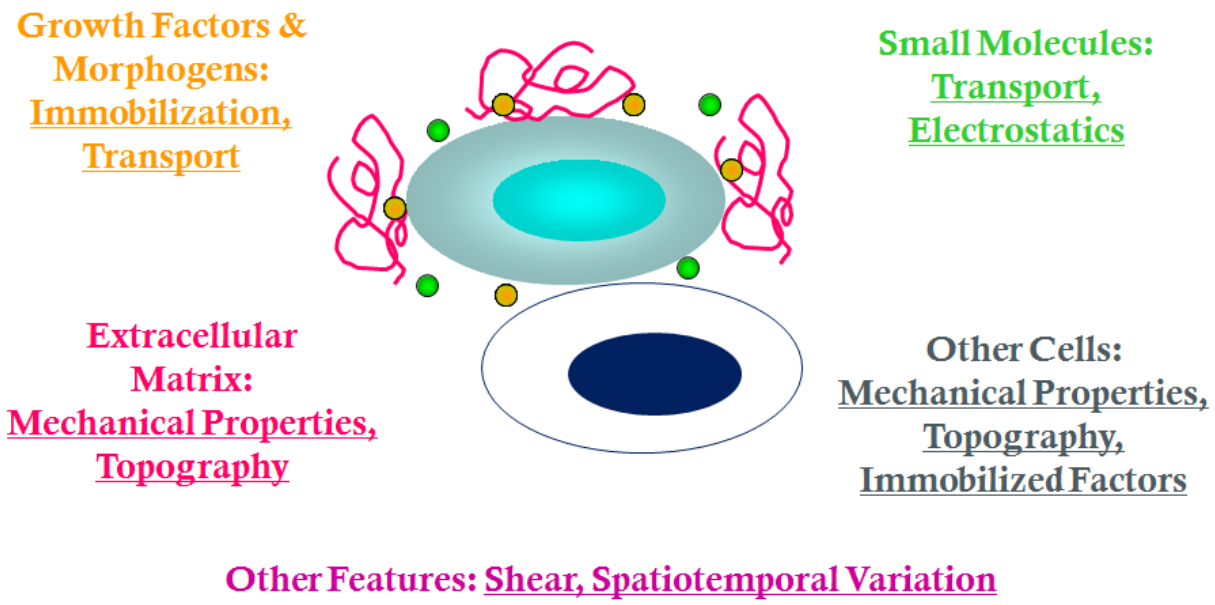

Figure 1. Schematic of the stem cell niche. Soluble small molecules, soluble and immobilized proteins, extracellular matrix components, and intercellular components collaborate to regulate stem cell behavior. In addition, there are numerous physical and engineering principles that modulate the manner in which these components present information, including mechanical properties, spatial organization and temporal variation in the presentation of cues, topographical features of the niche on the nanoscale and microscale, mass transport properties, and electrostatics.

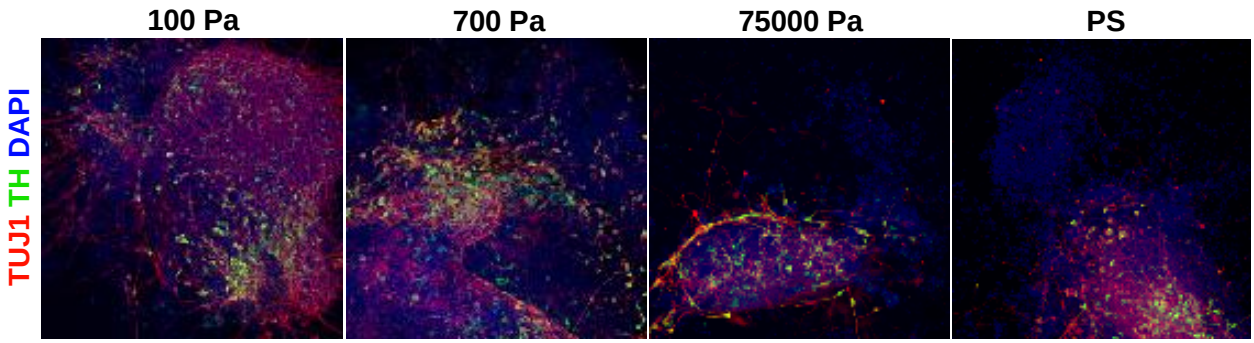

Figure 2. Mechanical cues regulate human embryonic stem cell differentiation. Human embryonic stem cells were differentiated into dopaminergic neurons, the cell type that is lost in patients with Parkinson's Disease. During the initial phase of differentiation, cells were cultured on polymeric hydrogels of various elastic moduli. The softer materials, coincidentally with stiffnesses characteristic of the brain, led to significantly higher numbers of dopaminergic neurons. Red is the neuronal marker TUJ1, green is the neuronal marker tyrosine hydroxylase, and blue is the nuclear dye DAPI. PS is polystyrene. Borrowed from [6].