Introduction
The concept that the behavior of a stem cell can be modulated by factors in its immediate vicinity arose several decades ago in studies of spleen colony-forming cells, which were later appreciated to be hematopoietic stem and progenitor cells (HSPCs) [1]. It was hypothesized that these HSPCs and their progeny were distinct cell populations that possessed an ‘age structure’; such that once the progeny left their stem cell niche during developmental ‘aging’, their stem-like qualities were lost, and entry into a new niche promoted differentiation into a more mature, lineage-committed cell type. Subsequent work with Drosophila germ stem cells [2] and other systems demonstrated that the niche is a region that regulates stem cell fate decisions by presenting the cell with specific repertoires of soluble and immobilized extracellular factors. It is increasingly appreciated that many of these signals are biophysical in nature, particularly biochemical factors that are spatiotemporally modulated, mechanical cues, and electrostatic cues. Over the past several years, numerous examples in which the first two of these properties in particular have been shown to play key regulatory roles have emerged.

Spatial organization of cues in the niche
Many factors that are often thought of as soluble are known to harbor matrix-binding domains that immobilize them to the solid phase of tissue. For example, fibroblast growth factors, platelet-derived growth factors (PDGFs), transforming growth factors (TGFs), vascular endothelial growth factors (VEGFs), Hedgehogs, and numerous cytokines contain heparin-binding domains [3-6]. Immobilization of such factors to the extracellular matrix (ECM) often modulates their activity by promoting sustained signaling via inhibiting receptor-mediated endocytosis [7], increasing their local concentration and establishing concentration gradients emanating from the source [8], and otherwise modulating the spatial organization of factors in a manner that affects signaling. As an example, compared with soluble VEGF, VEGF bound to collagen preferentially activates VEGFR2, associates with β1 integrins, and promotes the association of all of these molecules into focal adhesions [9]. There are also strong examples of synthetic systems that harness these phenomena, the first of which involved tethering epidermal growth factor to immobilized poly(ethylene oxide) (PEO) to prolong growth factor signaling in rat hepatocyte cultures [10]. A subsequent study showed that immobilization of Sonic hedgehog (Shh) onto interpenetrating polymer network surfaces, along with the integrin- engaging peptide arginine-glycine-aspartagine (RGD),
induced potent osteoblastic differentiation of bone
marrow-derived mesenchymal stem cells (MSCs),
whereas soluble Shh enhanced proliferation [11]. As
another example, crosslinking heparin-binding peptides
to fibrin gels along with neurotrophic factor 3 (NT-3) and
PDGF resulted in neuronal and oligodendrocytic differ-
etiation of mouse neural stem cells (NSCs) with inhibi-
tion of astrocytic differentiation [12]. Finally, immobi-
lization of leukemia inhibitory factor (LIF) to a synthetic
polymer surface supported mouse embryonic stem cell
(mESC) pluripotency for up to two weeks in the absence
of soluble LIF, indicating the advantage of substrate
functionalization in lowering cell culture reagent costs
and facilitating future multifactorial cell fate screening
experiments [13].

Immobilization of cues to the solid phase – that is, the
ECM or the surface of adjacent cells or both – also offers
the opportunity to modulate the nanoscale organization
in which these factors are presented (Figure 1). Growing
evidence has indicated that ligand multivalency, or the
number of ligands organized into a nanoscale cluster, can
exert potent effects on cell behavior [14-17]. For example,
seminal work using a synthetic system to present clusters
of ECM-derived adhesion ligands showed that the spatial
organization of ECM cues can also impact cell responses.
Specifically, on surfaces functionalized with the integrin
adhesion ligand YGRGD in various states of valency,
fibroblast attachment did not vary as a function of ligand
valency, yet substrates bearing highly clustered or
multivalent peptides required significantly lower ligand
densities to induce cell spreading and migration [18]. In
recent work that explored the behavior of MSCs in a
three-dimensional (3D) hydrogel functionalized with
RGD peptides, investigators who used a fluorescence
resonance energy transfer technique found that the cells
apparently reorganized the peptides into clusters upon
integrin binding [19].

The role of ligand clustering also extends to growth
factors and morphogens. The morphogen Hedgehog and
its family member Shh, best known for their role in tissue
patterning during development, have been shown to
require nanoscale clustering to achieve long-range para-
crine signaling [20]. Additionally, transforming growth
factor-beta (TGF-β) is able to induce distinct differential
signaling by activating either a homomeric or a hetero-
meric form of its receptor, which needs to be dimerized
or tetramerized before signaling can occur [21]. Further-
more, cell membrane-bound ligands (for example, Delta/
Jagged that activate the Notch receptor and ephrins that
activate corresponding Eph receptors) often require oligo-
merization to transduce biochemical signaling cascades
[22,23]. The creation of synthetically clustered, or multi-
valent, ligands offers a useful tool to study basic biological
aspects of receptor clustering as well as a reagent to
better control stem cell self-renewal or differentiation.
For example, Shh has been chemically conjugated to the
long polymer chain hyaluronic acid at varying stochio-
metric ratios to produce a range of multivalent forms of
Shh, and higher-valency Shh bioconjugates exerted
progressively higher potencies in inducing the osteogenic
differentiation of a primary fibroblast line with MSC
characteristics [24]. This concept was recently extended
to create highly active and multivalent versions of ligands
that are naturally integral membrane proteins (A Conway,
T Vazin, N Rode, KE Healy, RS Kane, DV Schaff er,
unpublished data).

In addition to spatial regulation of cues at the nano-
scale, microscale features in the niche can play key roles.
Fibrous ECM proteins such as collagen and fibronectin
are present throughout the NSC niche, raising the
hypothesis that cells may respond to ECM surface
topography. One interesting demonstration of this idea
showed that rat NSCs cultured on laminin-coated
synthetic polyethersulfone fibers of 280 or 1,500 nm in
diameter preferentially differentiated into oligodendro-
cytes or neurons, respectively. It has also been shown
that culturing MSCs atop vertically oriented nanotubes
of 70 to 100 nm in diameter (but not less than 30 nm) is
sufficient to induce their differentiation into osteoblasts
[25]. In an analogous study, culturing MSCs on nanopits
of 100 nm also induces osteogenesis but only if the pits
are anisotropic, or disordered [26]. Recently, the cyto-
skeletal scaffolding protein zyxin was shown to play an
important role in the response of human MSCs to surface
nanotopography [27]. Specifically, MSCs expressed zyxin
at lower levels when plated on a polydimethylsiloxane
(PDMS) surface patterned with a 350-nm grating, which
resulted in smaller and more dynamic focal adhesions
and increased directional migration of the cells along the
gratings.

In addition to nanoscale features, cell-cell interactions
at the microscale affect behavior. Specifically, the assembly
of stem cells themselves into multicellular aggregates
exerts strong influences on cell self-renewal or differ-
etiation, as the cells actively secrete factors and modu-
late local biological transport properties in ways that
impact their neighbors. For example, several groups have
created controlled 3D culture systems to generate human
embryonic stem cell (hESC) embryoid bodies (EBs) – or
cell clusters – of defined sizes. These involved centrifugal-
forced aggregation [28] as well as microfabricated PDMS
wells surrounded with functionalized protein-resistant
self-assembled monolayers [29]. These methods pro-
duced more consistent sizes than EB suspensions, and in
the latter example a tighter distribution of EB volume
was accompanied by a higher level of expression of the
pluripotency marker Oct-4. In another key study, hESC
culture inside microfabricated poly(ethylene glycol)
(PEG) wells yielded EBs from 40 to 450 μm in diameter [30,31]. Greater endothelial cell differentiation was observed in smaller EBs (150 μm), which was shown to be due to higher Wnt5a expression, whereas larger EBs (450 μm) enhanced cardiogenesis as a result of higher Wnt11 expression. Interestingly, another group used microcontact printing of adhesive islands on two-dimensional substrates to control hESC colony size and showed that smaller hESC colonies became more endoderm-biased, whereas larger colonies exhibited greater differentiation into neural lineages [32]. Within the endoderm-biased colonies, cardiogenesis was found to be more pronounced in larger EBs as opposed to the neural-biased colonies, which had higher levels of cardiogenesis in smaller EBs. Collectively, these results demonstrate that spatial organization of molecules and cells can play critical roles in modulating stem cell fate and can therefore serve as important tools to exert exogenous control over these processes.

Mechanoregulation in the niche
The mechanical properties of tissues have been studied for a number of decades. In the 1950s, it was observed that cells of the mesenchyme grow preferentially toward regions that are under higher mechanical stress, indicating a fundamental contribution of mechanical properties to biological function [33,34]. Aberrant tissue-elastic mechanical properties have also been shown to play a pathological role in certain cases, such as causing increased contractility of arterial resistance vessels within hypertensive rats, leading to elevated blood pressure and eventual heart failure [35]. There is a strong rationale for why mechanical properties may also modulate stem cell behavior. Tissues in the body range over several orders of magnitude in stiffness, from the softness of adipose to the toughness of bone, hinting at the possible importance of mechanics in maintaining different adult organs. In addition, there is local heterogeneity within individual tissues, as it has been shown, for example, that the hippocampus – a brain region that harbors adult NSCs – spatially varies in stiffness, as assessed by atomic force microscopy [36]. These various differences are not captured in the hard tissue culture surfaces typically used for in vitro study.

Engler and colleagues [37], in pioneering work, demonstrated that substrate elastic modulus affects stem cell lineage commitment, in which MSCs cultured on polyacrylamide substrates of varying elastic moduli differentiated into cell types characteristic of tissues with the corresponding stiffness: neurons, myoblasts, and osteoblasts. A later study extended this concept to another stem cell type by showing that NSCs cultured on variable modulus substrates differentiate preferentially into neurons on softer substrates and astrocytes on harder materials [38]. Recently, it was shown that soft substrates enhance the ability of human embryonic and human-induced
pluripotent stem cells to differentiate into neural lineages [39].

The finding that increased matrix rigidity can modulate cell differentiation has also been extended to analysis of the epithelial-mesenchymal transition (EMT) of both murine mammary gland cells and canine kidney epithelial cells, where more rigid substrates promoted EMT via upregulating the Akt signaling pathway [40]. In addition to differentiation on a single stiffness, durotaxis – the ability of cells to migrate in response to a stiffness gradient – and mechanosensitive differentiation can be integrated. For example, upon seeding of MSCs on a surface with a gradient in stiffness, cells migrated preferentially toward the stiffer region of the gel and then differentiated according to the local stiffness [41]. Finally, stem cells can, in turn, strongly influence their mechanical environment. MSCs cultured on non-linear strain-stiffening fibrin gels have been shown, upon application of local strain via cytoskeletal rearrangement and cell spreading, to globally stiffen the gel [42]. This effect led to long-distance cell-cell communication and alignment, thus indicating that cells can be acutely responsive to the non-linear elasticity of their substrates and can manipulate this rheological property to induce patterning.

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, or satellite cells, to undergo self-renewal in culture. Upon implantation, cells isolated from muscle and grown on soft substrates were able to expand and contribute to muscle to a much greater extent than stem cells cultured on stiff surfaces [43]. Furthermore, mESC self-renewal is promoted on soft substrates, accompanied by downregulation cell-matrix tractions [44].

Mechanobiologists have begun to elucidate mechanisms by which stem cells undergo mechanoregulation, building on advances with non-stem cells. Several mechanotransductive proteins involved with producing traction forces via cytoskeletal rearrangements are thought to be implicated in translating mechanical signals into changes in gene expression in stem cells [37,45,46]. For example, it has been shown that inhibition of myosin II diminishes the effect of ECM stiffness on MSC differentiation [37]. Furthermore, decreasing ECM stiffness decreases RhoA activity and subsequent calcium signaling in MSCs [47]. Recent work also indicates that Rho GTPases, specifically RhoA and Cdc42, enable NSCs to adjust their own stiffness as a function of the substrate modulus and thereby regulate the cells’ stiffness-dependent differentiation into either astrocytes or neurons in vitro and potentially in vivo [46]. Furthermore, an important study demonstrated that the transcriptional coactivator YAP undergoes nuclear localization in MSCs on higher-stiffness substrates, thereby narrowing the gap in our understanding of how microenvironmental mechanical properties may ultimately modulate gene expression and, as a result, cell differentiation [48]. Finally, while mechanosensitive stem cell behavior has been demonstrated on several materials in addition to the original polyacrylamide, recent work broaches another possible mechanism for cell behavior on different stiffnesses. Specifically, investigators found that MSCs exhibited different behavior on polyacrylamide but not PDMS gels of variable modulus, and additionally found that the porosity of the polyacrylamide but not the PDMS gels varied with stiffness. This raised the intriguing possibility that differences in ECM conjugation – specifically the number of anchoring points of collagen to the gel surface – could subsequently affect integrin binding and thereby modulate cell responses [49]. This possibility should be explored further, potentially in comparison with findings that NSCs and MSCs on polyacrylamide-based materials behave similarly as a function of modulus for materials presenting either ECM proteins [37,46] or simple RGD peptides [19,38].

In addition to the static mechanical properties of cells and surrounding tissue, dynamic biomechanical processes can regulate stem cell function. For instance, stress and strain from local tissue contraction and expansion, including processes such as contraction of muscle, tendons, and ligaments as well as cyclic deformation of tissue surrounding vasculature and the lungs, are prevalent in vivo. Furthermore, organismal development is a highly dynamic process that exposes cells and structures to mechanical forces. In Drosophila embryos, for example, compression of cells induces expression of Twist, a protein involved with regulating germ layer specification and patterning [50]. Similarly, in zebrafish, tensile strains were shown to regulate gastrulation during early development [51]. Such basic studies extend to mammalian stem cells. For example, cyclic strain of lung embryonic MSCs stimulates expression and nuclear localization of tension-induced/inhibited protein-1 (TIP-1) and inhibits expression of TIP-3, thereby promoting myogenesis and inhibiting adipogenesis [52]. Cyclic stretching also inhibits differentiation of hESCs through upregulation of Nodal, Activin A, and TGFβ1 [53]. Differential effects of equiangular versus uniaxial strain have also been observed, with equiangular primarily downregulating smooth muscle cell promoting factors in MSCs and uniaxial upregulating them [54].

Even temporal variation of the ECM on slower timescales may play a role in regulating stem cell function [55]. For example, matrix metalloproteinases (MMPs), enzymes that remodel the ECM through cleavage of key constituent proteins, can modulate stem cell differentiation. Interestingly, it has been shown that, in response to two injury-induced chemokines, SDF-1 and VEGF,
NSCs in the subventricular zone of the lateral ventricles in the adult rodent brain differentiated into migratory cells that secreted MMPs at elevated levels [56]. Blocking the expression of these proteins inhibited differentiation of the NSCs, indicating that the cells require matrix remodeling to proceed with their differentiation and subsequent migration into injured areas of the brain. MSCs localized to bone marrow have also been shown to secrete MMPs to facilitate infiltration of sites of tissue damage, inflammation, or neoplasia before undergoing differentiation [57]. In addition to experiencing a decrease in ECM integrity, cells can experience ECM stiffening (for example, an approximately 10-fold increase in stiffness during cardiac maturation). Young and Engler [58] created a hyaluronic acid poly(ethylene glycol) hydrogel that could undergo stiffening over a two-week period and found that pre-cardiac cells within the gel underwent a significantly higher increase in maturation – both expression of muscle markers and assembly into muscle fibers – than corresponding cells seeded on static hydrogels. The development of hydrogels in which crosslinks are photosensitive has enabled investigators to vary stiffness in time and space, powerful capabilities that will enable further advances in the field [59,60].

Another form of dynamic stress is shear flow, most often associated with the circulatory system. The earliest study of shear on stem cell fate determined that flow promotes maturation and capillary assembly of endothelial progenitor cells [61]. Subsequent studies showed that shear flow can induce differentiation of other stem cell types, including endothelial cell specification from murine embryonic MSCs [62] and vascular endothelial cell lineage commitment from ESCs [63,64]. Each of these properties and parameters of the niche (summarized in Table 1).

Table 1. Examples of biophysical regulation within the stem cell niche

| Biophysical property | Stimulus                        | Cell type                                      | Response                                                                 | References |
|----------------------|---------------------------------|------------------------------------------------|--------------------------------------------------------------------------|------------|
| Ligand-substrate immobilization | VEGF, EGF; Shh; NT-3, PDGF, LIF, SCF | Human endothelial cells; rat hepatocytes; rat MSCs; hESC-derived NPCs; mESCs | VEGFR2 activation; DNA synthesis; osteoblast differentiation; decreased osteogenesis; STAT3/MAPK activation | [9], [10], [11]; [12], [13] |
| Multivalent presentation | Galactose, RGD; Hh; TGF-β; Shh | Eschscholzia californica; mouse fibroblasts; Drosophila; mink lung epithelial cells; mouse embryonic C3H10T1/2 cells | Chemotaxis; motility/adhesion; patched activation; endocytosis; osteogenic differentiation | [16], [18], [20], [21], [24] |
| Surface topography   | 70- to 100-nm nanotubes; nanotopographical disorder; 350-nm gratings; decreased collagen-anchoing sites | hMSCs; hMSCs; hMSCs; human epidermal stem cells | Osteoblast differentiation; bone ECM formation; decreased zyxin/increased motility; increased differentiation | [25], [26], [27], [49] |
| Physical orientation of stem cells | 450-μm cell cluster size/150-μm cell cluster size; decreased cell colony size | mESCs; hESCs | Cardiogenesis/endothelial cell differentiation; increased endodermal differentiation | [31], [32] |
| Elastic modulus      | Soft/hard matrix; decreased substrate stiffness; increased ECM stiffness; decreased/increased matrix rigidity; substrate stiffness gradient; soft hydrogel substrates; soft substrates | hMSCs; rat NPCs; hPSCs; murine mammary gland cells; hMSCs; mMuSCs; mESCs | Neurogenesis/osteogenesis; increased neuronal differentiation; increased cell and colony spreading; TGFβ1-induced apoptosis/EMT; migration up stiffness gradient; self-renewal and in vivo regeneration; homologous self-renewal and downregulated cell tractions | [37], [38], [39], [40], [41], [43], [44] |
| Dynamic mechanical forces | Local cell traction on non-linear elastic fibrin gel; cell compression; cell-cortex tension; stretch-induced TIP-1/TIP-3 expression; cyclic biaxial strain; equiaxial/uniaxial strain; dynamic hydrogel stiffening; shear stress; soft stress; laminar shear stress; fluid shear stress | hMSCs; Drosophila germ cells; zebrafish; lung EEMCs; hESCs; human bone marrow MSCs; chicken cardiomyocytes; hEPCs; mEMCs; mESCs; mEMCs | Global matrix stiffening; Twist protein expression; progenitor-cell sorting; myogenesis/adipogenesis; increased TGFβ1/Activin A/Nodal expression; SM α-actin and SM-22α downregulation/upregulation; increased cardiac maturation; proliferation, differentiation, and vascular tube formation; endothelial differentiation; epigenetic histone modification and cardiovascular lineage programming; differentiation into vascular endothelial cells | [42], [50], [51], [52], [53], [54], [58], [61], [62], [63], [64] |
Conclusions
Understanding the properties and effects of each complex component of a local stem cell microenvironment is an essential step toward understanding the stem cell itself. In particular, the ability of a stem cell to respond to spatiotemporally varying biochemical cues and distinct mechanical and physical stimuli within its surroundings is being increasingly recognized and will continue to be elucidated in the years to come. The effect of substrate stiffness on stem cell fate has been increasingly appreciated in recent years, and other facets of the niche’s solid phase – including spatial organization in the presentation of biochemical information, electrostatics [65], and biomechanical transport [66] – will increasingly be investigated. While technological limitations in the ability to control, quantify, and image these properties currently exist, advances in super-resolution microscopy may be combined with stem cell research to enable considerable progress [67].

Furthermore, an appreciation of these interactive processes in natural tissue may greatly aid the development of stem cell therapies to treat numerous human diseases. For example, this basic knowledge may enable therapeutic modulation of endogenous stem cells via alterations in the niche as well as offer opportunities to create more effective large-scale culture systems and bio-reactors to expand and differentiate stem cells. Furthermore, the creation of in vitro cell and tissue equivalents of therapeutically relevant organs, enabled by the technological advances and optimized model culture systems, will enable both basic and therapeutic investigations of human disease biology. Therefore, as is evidenced by an increasing number of important studies, a blend of biology, chemistry, physics, and engineering can empower progress in both basic and translational directions.

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