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REVIEW

Biophysical regulation of stem cell behavior within the niche

Anthony Conway and David V Schaffer*

Abstract

Stem cells reside within most tissues throughout the lifetimes of mammalian organisms. To maintain their capacities for division and differentiation and thereby build, maintain, and regenerate organ structure and function, these cells require extensive and precise regulation, and a critical facet of this control is the local environment or niche surrounding the cell. It is well known that soluble biochemical signals play important roles within such niches, and a number of biophysical aspects of the microenvironment, including mechanical cues and spatiotemporally varying biochemical signals, have also been increasingly recognized to contribute to the repertoire of stimuli that regulate various stem cells in various tissues of both vertebrates and invertebrates. For example, biochemical factors immobilized to the extracellular matrix or the surface of neighboring cells can be spatially organized in their placement. Furthermore, the extracellular matrix provides mechanical support and regulatory information, such as its elastic modulus and interfacial topography, which modulate key aspects of stem cell behavior. Numerous examples of each of these modes of regulation indicate that biophysical aspects of the niche must be appreciated and studied in conjunction with its biochemical properties.

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 that 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.

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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-asparagine (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 differentiation of mouse neural stem cells (NSCs) with inhibition of astrocytic differentiation [12]. Finally, immobilization 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 paracrine signaling [20]. Additionally, transforming growth factor-beta (TGF-β) is able to induce distinct differential signaling by activating either a homomeric or a heteromeric form of its receptor, which needs to be dimerized or tetramerized before signaling can occur [21]. Furthermore, cell membrane-bound ligands (for example, Delta/Jagged that activate the Notch receptor and ephrins that activate corresponding Eph receptors) often require oligomerization to transduce biochemical signaling cascades [22,23]. The creation of synthetically clustered, or multivalent, 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 stochiometric 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 Schaffer, unpublished data).

In addition to spatial regulation of cues at the nanoscale, 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 oligodendrocytes 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 cytoskeletal 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 differentiation, as the cells actively secrete factors and modulate 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 produced 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)

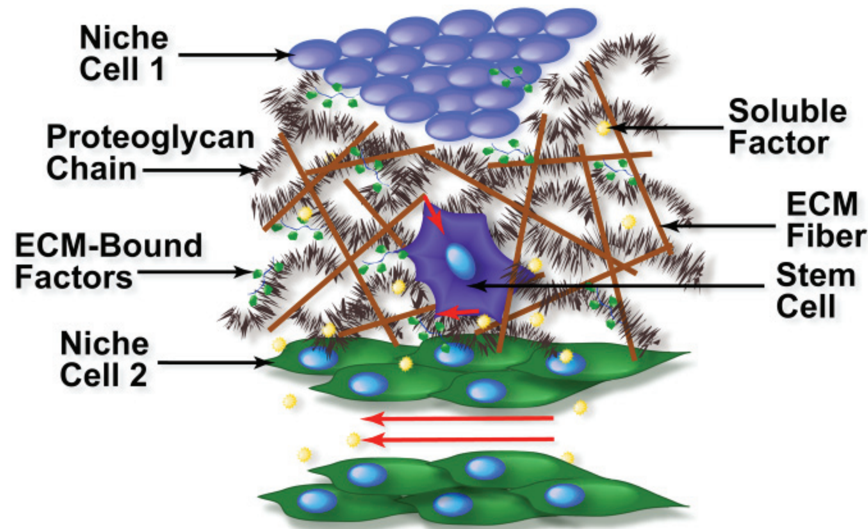

Figure 1. Mechanical and biophysical interactions in the stem cell niche. The native microenvironment, or niche, in which a stem cell resides can be highly complex, consisting of various cell types, extracellular matrix (ECM) molecules, and growth factors. Proteoglycans and ECM proteins bind and immobilize otherwise soluble growth factors, providing functional sites for cell binding as well as mechanical stability of the space surrounding a stem cell in its niche. ECM fibers and neighboring niche cells provide mechanical support and stimuli (short red arrows) to influence stem cell fate. The degree of 'crosslinking' of the various ECM molecules also affects the pore size in the niche, dictating the rate of diffusion of soluble factors as well as the ability of niche cells to infiltrate nearby space. Finally, flow through local vasculature (long red arrows) mechanically shears endothelial and other cells (green), which may in turn affect nearby stem cells.

(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]. Cyclical stretching also inhibits differentiation of hESCs through upregulation of Nodal, Activin A, and TGFβ1 [53]. Differential effects of equiaxial versus uniaxial strain have also been observed, with equiaxial 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,

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 astrogensis; STAT3/MAPK activation	[9]; [10]; [11]; [12]; [13]
Multivalent presentation	Galactose; RGD; Hh; TGF-β; Shh	*Escherichia coli*; 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-anchoring 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; TGF-β1-induced apoptosis/EMT; migration up stiffness gradient; self-renewal and *in vivo* regeneration; homogeneous 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; shear stress; laminar shear stress; fluid shear stress	hMSCs; *Drosophila* germ cells; zebrafish; lung EMCs; hESCs; human bone marrow MSCs; chicken cardiomyocytes; hEPCs; mEMCs; mESCs; mESCs	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]

ECM, extracellular matrix; EGF, epidermal growth factor; EMC, embryonic mesenchymal cell; EMT, epithelial-mesenchymal transition; hEPC, human endothelial progenitor cell; hESC, human embryonic stem cell; Hh, hedgehog; hMSC, human mesenchymal stem cell; hPSC, human pluripotent stem cell; LIF, leukemia inhibitory factor; MAPK, mitogen-activated protein kinase; mEMC, mouse embryonic mesenchymal cell; mESC, mouse embryonic stem cell; mMuSC, mouse muscle stem cell; MSC, mesenchymal stem cell; NPC, neural progenitor cell; NT-3, neurotrophic factor 3; PDGF, platelet-derived growth factor; RGD, arginine-glycine-asparagine peptide; SCF, stem cell factor; Shh, sonic hedgehog; SM, smooth muscle; STAT3, signal transducer and activator of transcription 3; TGF-β, transforming growth factor-beta; TIP, tension-induced/inhibited protein; VEGF, vascular endothelial growth factor; VEGFR2, vascular endothelial growth factor receptor 2.

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) offers opportunities to control cell fate for downstream therapeutic application.

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 biomolecular 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 bioreactors 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.

This article is part of a thematic series on *Physical influences on stem cells* edited by Gordana Vunjak-Novakovic. Other articles in the series can be found online at http://stemcellres.com/series/physical

Abbreviations

3D, three-dimensional; EB, embryoid body; ECM, extracellular matrix; EMT, epithelial-mesenchymal transition; hESC, human embryonic stem cell; HSPC, hematopoietic stem and progenitor cell; LIF, leukemia inhibitory factor; mESC, mouse embryonic stem cell; MMP, matrix metalloproteinase; MSC, mesenchymal stem cell; NSC, neural stem cell; PDGF, platelet-derived growth factor; PDMS, polydimethylsiloxane; RGD, arginine-glycine-asparagine; Shh, sonic hedgehog; TGF, transforming growth factor; TIP-1, tension-induced/inhibited protein-1; VEGF, vascular endothelial growth factor.

Competing interests

DVS is an inventor on a patent involving polyvalent ligands as potent activators of cellular signalling, and holds founder's stock in a company that is developing this commercially. AC declares no competing interests.

Authors' contributions

AC wrote the manuscript with helpful feedback and editing by DVS.

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