Review

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Engineering strategies to emulate the stem cell niche

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The stem cell niche is an anatomical site that contains a reservoir of multipotent stem cells (SCs) that can maintain normal tissue, or replenish injured or aged cell populations, in response to mechanisms that regulate whether they should remain quiescent, undergo self-renewal, or differentiate. The choice among these hallmark SC behaviors is governed by intricate soluble and ''solid phase'' signals that are systemic or presented by the local niche cells. In this review, we discuss the progress achieved in understanding the mechanisms and principles that govern microenvironmental regulation of SC behavior, and focus on novel approaches that have been developed to synthesize this basic information to engineer creative strategies for harnessing and controlling SCs *ex vivo* and *in vivo*.

Introduction

Over the past decade, stem cell (SC)-based therapies have evolved as promising new approaches for the treatment of a variety of degenerative diseases. Major progress has been achieved in identifying candidate cell populations including adult SCs, or the differentiated progeny of embryonic and pluripotent SCs, which can potentially be transplanted to replace damaged cells or repair cellular function. Strategies have also been developed to stimulate endogenous adult SCs to enhance repair of damaged tissue [1].

However, for successful translation of SC-based therapeutics to the clinic, numerous challenges remain, including gaining precise control over SC expansion and differentiation. This review provides a general description of the key components of adult SC niches that regulate these behaviors, and discusses how this basic information can be used as a foundation to engineer artificial SC microenvironments. Emulation of the properties of the natural SC niche will increase our understanding of endogenous SC-based repair mechanisms, as well as enhance our ability to channel the plasticity of SCs toward regenerative medicine applications.

SC niche: lessons from *Drosophila* model systems

The concept of a regulatory microenvironment that surrounds SCs in adult tissues was proposed in the late 1970s [2]. Ten years later, the first in-depth *in vivo* characterization of SC-specific niches was carried out by Allan Spradling's group in studies of germline stem cells (GSCs) in *Drosophila*. Since then, studies of SC niches in simpler experimental organisms such as *Drosophila* have emerged as a valuable avenue for elucidating regulatory niche signals. The studies by Spradling and colleagues have demonstrated that extracellular signals presented by constituent cells of the GSC niche aid in establishing homeostasis between SC self-renewal versus differentiation [3]. In fact, this niche also has the capacity to reprogram cells, as it has been shown that an experimentally-vacated ovarian GSC niche is a stable structure capable of inducing cell division of foreign surrounding somatic SCs that give rise to ovarian follicle cells, and of dedifferentiating ectopic follicle progenitor cells in relatively early stages of differentiation [4]. These and numerous other findings have suggested that SC fate and function could potentially be controlled by engineered *in vitro* systems that artificially recapitulate elements of the niche.

The *Drosophila* system also has implicated many familiar classes of signals that regulate SC self-renewal and differentiation, which must be considered in understanding and engineering mammalian niches. For example, Zhdanov and coworkers [5] have described an intriguing kinetic model of signal propagation in the ovary SC niche and have suggested that each GSC employs at least 700 receptors to receive soluble signals from its microenvironment. Other important and familiar classes of signals in *Drosophila* GSC niches include extracellular matrix (ECM) molecules and molecules such as cadherins, that mediate cell–cell adhesion, which are indispensable for the functional capabilities of the SC niche [6,7].

Importantly, as many classes of niche signals are conserved evolutionarily between invertebrates and vertebrates, important findings that have been made possible from using *Drosophila* as a genetic model system also shed light on the complex nature of mammalian SC microenvironments, with clinical and translational implications [8]. Examples of these microenviroments include hematopoietic stem cell (HSC), neural stem cell (NSC), intestinal, and epithelial niches.

Common hallmarks of mammalian SC niches

Although investigating SC niches in mammals is more challenging than in lower organisms, because of their greater biological and experimental complexity, the knowledge gained from invertebrate model systems has enabled significant progress in the delineation of interactions between SCs and their microenvironment in vertebrate systems. Here, we discuss important principles and common elements of mammalian SC niches, including cell–cell,

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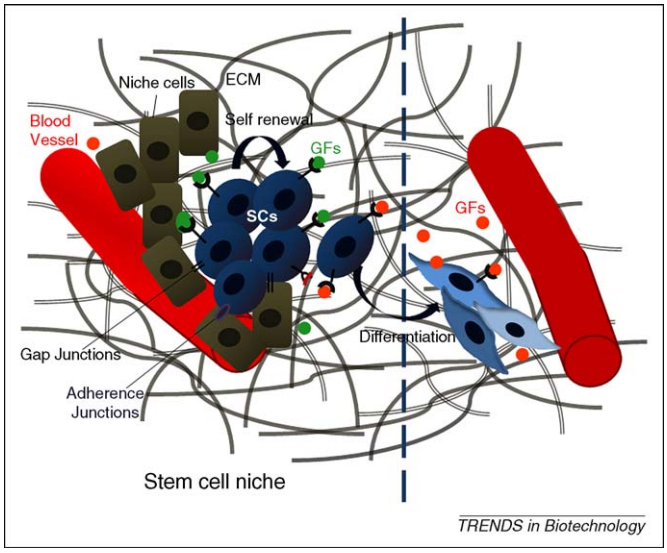

Figure 1. Schematic depiction of common elements of the SC niche regulatory network in mammalian organs. Soluble factors, cell membrane or ECM-tethered signaling molecules, ECM components, and physical contacts between SCs and niche constituent cells are the main elements that control the behavior of SCs in their microenvironment. Endothelial-cell-mediated signaling in the vascular niche is also thought to be important in regulation of self-renewal and differentiation.

cell–ECM, and diffusible signaling factor mediated signal transduction (Figure 1), which might assist in engineering biomimetic systems for controlling growth and differentiation of SCs *in vitro*.

Direct cellular contact between stem and niche cells

There is an emerging understanding of the importance of cellular contacts and organizations in SC niches. Similar to *Drosophila* GSC niches [6,7], physical contacts between mammalian SCs and their surrounding cells, either through adherence or gap junctions, are key elements in the native SC microenvironment. For example, adhesive interactions between neighboring SCs, as well as between SCs and adjacent cells, can be mediated by cadherins, a family of homophilic adhesion receptors, which are essential for anchoring SCs to their niche [2] and for directing self-renewal and maintenance of NSCs [9]. Also, osteoblasts provide an adhesive attachment for HSCs through E-cadherin-mediated interactions and serve as a critical component of the HSC niche [10]. Furthermore, Ephrin and Notch receptors and their ligands are other classes of integral membrane proteins that are involved in cell-contact-mediated signaling between SCs and their surroundings [10–12]. For example, Ephrin-mediated cellular contact between NSCs and their neighboring cells has been proposed to modulate signaling involved in neurogenesis and NSC self-renewal in the adult brain [11,12]. Analogously, in the hematopoietic niche, cell–cell signaling between HSCs and osteoblasts that is mediated by Notch activation has been implicated in supporting HSC self-renewal and function [13].

Physical contact between stem and niche cells is also known to regulate their mobilization to and from the niche. For instance, sophisticated *in vivo* imaging approaches have demonstrated recently that HSCs become activated and transit from the bone marrow to the blood stream at specialized vascular microdomains, in which there is an interaction between the HSCs and vascular endothelial cells, which suggests that the endothelial cells function as a niche that attracts HSCs [14]. In addition, it has been suggested that adherence of HSCs to osteoblasts is crucial in maintaining HSCs in a quiescent state [15]. However, it should be noted that it is not understood completely how the signals from the vascular and osteoblastic niches interact in order to convey the necessary information to HSCs [16].

Comparable to HSCs, there is increasing evidence that NSC niches are defined by the presence of several key cell populations. For example, in the adult brain, NSCs can be found in the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) of the dentate gyrus of the hippocampus [17]. It has been found that adult hippocampal NSC proliferation occurs in a vascular niche [18], and that adult hippocampal NSC differentiation is promoted by contact with astrocytes [19]. Recent elegant studies have dissected the functional interactions of the vascular niche with NSCs and have demonstrated that the endothelial component of the vasculature is responsible for enhanced proliferation of mouse embryonic and adult NSCs, through enhanced generation of junctional contacts between the NSCs [20], which further implies that cellular organization and density are important to consider in the design of *in vitro* culture systems.

Similar to the neurogenic niches in the hippocampus, neurovascular interaction has been observed in the SVZ. Recently, it has been illustrated that adult NSCs in the SVZ are highly polarized and make connections with the endothelial cells of blood vessels through long basal processes, while also extending short apical process that make connections with the ependymal cells that line the surface of the walls of the lateral ventricles [21]. In addition to cells of the vasculature and ventricular lining, these adult NSCs contact one another, as well as astrocytes and differentiating neuroblasts, and are likely to receive signals from each other.

Engagement of SCs with the ECM

Niche ECM components, such as laminin, fibronectin and collagen, provide a physical framework and instructive signals that regulate SCs. For example, there is a substantial body of evidence that the engagement of integrin receptors, which are expressed widely by NSCs, with niche ECM molecules influences cellular behavior [22]. It also has been demonstrated that β-integrins that regulate the maintenance of NSCs might indirectly activate other cell surface receptors that transduce information to NSCs [23,24], which indicates that integrin ligands most likely serve as key signals in regulating the signaling environment of NSCs [25].

Shen and coworkers [26] have proposed that NSC contacts with vasculature occur primarily through integrins that bind the laminin present in blood vessels. Moreover, landmark studies by Mercier and coworkers [27,28] have served to describe the most neurogenic regions of the SVZ in the adult brain as dense laminin-rich extravascular basal lamina structures, termed fractones, which further signifies the importance of integrin ligands in the NSC niche.

Glycosaminoglycans are another major ECM component believed to play important regulatory roles in SC

niches. For example, hyaluronic acid (HA) is highly expressed in bone marrow stromal cells and on the surface of HSCs, and furthermore, has been associated directly with regulation of hematopoeisis in the HSC niche [29], which suggests that HA might serve as an appropriate component of biomaterials that emulate the structure of the native HSC niche *ex vivo*. In the NSC niche, HA activity has been associated with NSC quiescence [30], which suggests that this ECM molecule also has a use in the synthesis of systems to modulate NSC behavior *in vitro*.

Soluble and immobilized signaling factors within the SC niche

Small protein signaling factors are another important niche element that regulates SC function [31,32]. However, it should be emphasized that although many *in vitro* studies have investigated these cytokines and growth factors as soluble factors [33], recent studies have demonstrated that the immobilization of these cues to the ECM plays an important role in mediating their biological effects [34]. Presentation of signaling molecules in an immobilized fashion alters their local effective concentration, bioavailability, and stability, and thereby modulates their effects on target cells. In one striking example, NSC-proliferative regions in the SVZ are situated in proximity to regions, in which growth factors including basic fibroblast growth factor-2 are concentrated by heparan sulfate proteoglycan (HSPG) [27], which indicates that synthetic proteoglycan mimetic materials might constitute an appropriate milieu for activation of matrix-binding factors that influence neural and other type of SCs.

Lipid modification of proteins that contribute to membrane association or otherwise modulate solubility is another means to limits the motility of signaling proteins. Examples of signaling proteins that are known to undergo such modifications include Sonic hedgehog (Shh) and Wingless (Wnt) proteins [35–37], which both have important regulatory functions in the neural and hematopoietic SC niches [38–41].

Many growth factors also influence cells in a soluble form. For example, diffusible inhibitory factors that are secreted from bone-marrow adipocytes have recently been shown to suppress the proliferation of hematopoietic progenitor cells within the HSC niche [42]. However, it should be noted that spatial and/or dynamic regulation of the soluble concentrations of bioactive molecules can play a role in their effects. For example, many soluble morphogens are known to influence SC fate in a concentration-dependent fashion under strict temporal constraints, which presents a challenge in experimentally controlling SC behavior with precision [43]. As an example, neural cells respond to changes in Shh concentration and to duration of Shh-mediated signaling [44]. Another complex strategy involved in SC fate restriction and tissue patterning is the establishment of opposing gradients of specific signals. For instance, opposing actions exerted by Shh and bone morphogenetic or Wnt proteins are known to play a prominent role in the specification of the ventral neural tube cell types during central nervous system development [45]. Thus, in certain cases, synthetic microenvironments will need to be designed with the capacity to control the temporal and spatial availability of bioactive molecules.

Moreover, it has been suggested that small molecules such as neurotransmitters play a role in the regulation of SC function in their niches [46–48]. A landmark study by Katayama *et al*. has demonstrated that neurotransmitters of the sympathetic nervous system are important for the migration of HSCs from their niche [46], and neurotransmitters such as gamma-aminobutryic acid have been shown to regulate adult neurogenesis [47,48], which indicates that addition of such molecules to biological *in vitro* niche models could assist in a more accurate emulation of the native SC niche.

In principle, SCs in their niches make decisions to either remain in a quiescent state, undergo self-renewal, or to exit the niche through exposure to a complex ensemble of localized cues from their microenvironment, as well as systemic signals. These signals are often actively coordinated and presented in a temporally and spatially regulated manner to ensure a balance in SC populations and behavior that are present in many of our organs. Engineered *in vitro* systems can aid in the analysis and control of SC differentiation for potential therapeutic applications and can serve as tools to decipher how the various niche components interact with one another to provide the SCs with the appropriate signals.

Biomimetic strategies to control stem cell behavior

Synthetic approaches to create artificial SC niches have great potential in creating suitable *in vitro* biological systems for adult or pluripotent SC culture and their conversion to specific cell types of interest [49–51]. These systems have emulated numerous strategies employed by the SC niche, including biochemical and mechanical cell–matrix interactions, soluble and immobilized factors, and to an extent regulable cell–cell contacts. Furthermore, these engineering strategies based on adult SC niches have been applied successfully to SCs of embryonic origin to control their plasticity and cell fate decisions.

ECM-mimetic and 3D biomaterials

Extensive efforts have been devoted to constructing biomaterials that resemble natural ECMs, which are gel-like structures that contain a hydrated network of glycosaminoglycan chains and protein polymers. Hydrogels are highly hydrated networks of natural or synthetic polymers which resemble the natural ECM, and are therefore excellent candidates for culturing SCs [52]. Hydrogels made of natural polymers, including alginate, HA and collagen, have been used to culture and differentiate embryonic and adult SCs [52]. As demonstrated by studies in hematopoietic and neural SC niches, HA is a potent biomaterial in retaining SCs in an undifferentiated state *in vitro* [29,30]. An important advantage in using HA networks in tissue engineering is that its physical properties can be tailored easily to simulate the microenvironments of distinct cell types [53].

Some hydrogels derived from natural materials contain inherent cell recognition sites that allow them to interact with the encapsulated cells and thus can be gradually degraded by natural cellular enzymes. Nevertheless,

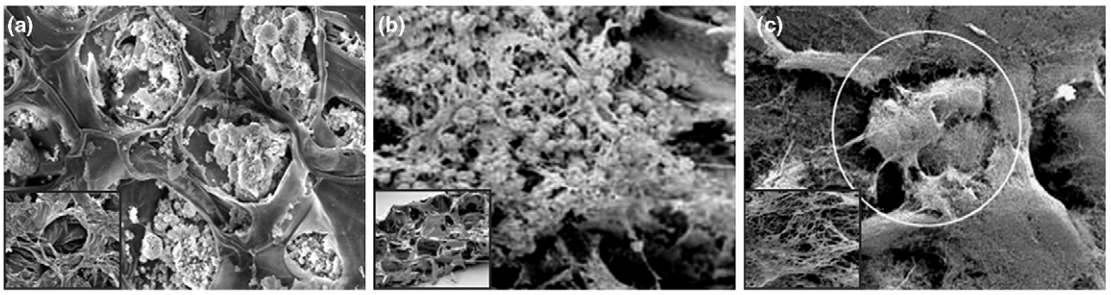

Figure 2. Examples of 3D biodegradable scaffolds for culture of multipotent SCs. Scanning electron micrographs of **(a)** differentiating hESCs in porous alginate constructs [62] and **(b)** poly(lactic-co-glycolic acid)/poly(l-lactic acid) polymeric hydrogels [63]. **(c)** Adult mouse NSCs (within the circle) embedded in self-assembling peptide nanofiber scaffolds [64]. Inserts show lower magnification views of the structure of the scaffold in each image.

limitations to such materials, including difficulties with reproducible sources of materials, immunogenicity, potential pathogen contamination, and poor control over mechanical properties, have encouraged the development of synthetic biomaterials as cellular substrates. Synthetic hydrogels have been generated by polymerization of monomers with a wide range of properties. Poylacrylamide/acrylic acid copolymers are an example of a synthetic biomaterial that has been engineered as highly tunable scaffolds to influence the behavior of SCs. Importantly, proteolytic sensitivity of these biomaterials can be conferred by using small peptides with enzyme-specific cleavage sequences as cross-linkers in polymer synthesis [54].

Inspired by the understanding of the composition of the ECM in different SC niches, numerous studies have focused on incorporating ECM-biomimetic elements into synthetic materials. For example, presentation of integrin-binding arginine-glycine-aspartic acid (RGD) motifs has been implicated in enhanced osteogenesis of mesenchymal SCs [55]. Specific integrin-mediated engagement with RGD bioactive epitopes also has been shown to promote adhesion of NSCs to polyacrylamide/PEG hydrogels [56] and to aid in supporting the short-term self-renewal of human embryonic stem cells (hESCs) [57]. A study by Silva and colleagues [58] has illustrated that nanofibers composed of peptides that carry the neurite-promoting laminin epitope IKVAV could promote neuronal lineage restriction of NSCs at the expense of gliogenesis, which lends support to the notion of the crucial role of ECM molecules in lineage restriction of NSCs.

In addition to presenting cell adhesion motifs, the ECM provides cells with mechanical signals that regulate their behavior. Engler and colleagues have illustrated that lineage restriction of mesenchymal SCs to neurogenic, myogenic, or osteogenic lineages is highly dependent on the rigidity of culture substrates [59]. Saha and colleagues have provided further evidence of the physical effects of the microenvironment on SC fate, by demonstrating that NSCs can be directed to undergo neurogenesis or gliogenesis, by altering the mechanical properties of a modular hydrogel cell culture system [56]. These findings highlight the need for engineering mechanically tunable synthetic biomaterials that correspond to the physiological stiffness of various tissues for desired lineage restriction of SCs.

Furthermore, the dimensionality of cell culture systems makes a profound difference in cellular signaling, gene expression, and behavior [60,61]. Cells *in vivo* are exposed to adhesive contacts in all three dimensions, yet cells in culture almost exclusively are exposed to 2D substrates. Hence, there is an arguable need to expand culture systems to a third dimension to mimic the spatial organization of SCs and instructive cues within their niche. Porous alginate 3D matrices, for example, have been associated with enhanced proliferation of embryonic, mesenchymal and neural SCs [52]; encouraged more heterogeneous differentiation of hESCs in the form of embryoid bodies (Figure 2a); and resulted in enhanced vascularization in differentiating hESC aggregates [62]. Three-dimensional hydrolytically degradable polyethylene glycol (PEG) and poly (lactic-co-glycolic acid)/poly(l-lactic acid)-based hydrogels (Figure 2b) are also among the commonly used scaffolds for cultivation and differentiation of SCs [63].

Based on advances in understanding the self-assembly of peptides, engineering of artificial nanofiber protein scaffolds has opened a new paradigm in development of biomimetic materials (Figure 2c) [64]. Nonetheless, although the nanometer scale architecture of this type of scaffold resembles the biological ECM more closely, these systems might not offer the conformational flexibility and tunability of the mechanical properties of polymeric hydrogels.

Biomimetic presentation of signaling proteins

The simplest way to introduce growth factors to polymeric scaffolds is to add them directly to the liquid medium. However, this approach does not enable spatiotemporal control of their presentation, which as previously described, is a key aspect of SC niches. To overcome this limitation, and potentially gain access to new means to regulate SC behavior, it is possible to undertake a more biomimetic approach by introducing HSPGs, such as heparin, which function as bioactive reservoirs for signaling factors [65], into scaffolds. Motivated by the properties of HSPGs in NSC niches [28], Willerth and associates [66] have incorporated heparin into fibrin scaffolds and have utilized this system for controlled delivery of neurotrophic factors, which has resulted in increased neural differentiation of ESCs. In another study, heparin-functionalized PEG gels have been used as a delivery vehicle for bone morphogenetic protein 2 and fibronectin, which has led to efficient osteogenic differentiation of human mesenchymal SCs [67]. A similar affinity-based delivery system has been developed for long-term retention of

Table 1. Examples of strategies for presentation or delivery of bioactive molecules.

Strategy	Biomaterial	Bioactive molecule	Application	Ref.
Affinity-based systems for delivery of biomolecules				
Glycosaminoglycan/polysaccharide-growth factor interactions	Fibrin scaffolds containing heparin	Shh NT-3 PDGF	Neural differentiation of mouse embryonic SCs	[66]
	PEG hydrogels functionalized with heparin	BMP2 FN	Osteogenic differentiation of human mesenchymal SCs	[67]
	Benzylaminated dextran-modified hydrogels	TGF-β1	–	[68]
Immobilization of biomolecules				
Chemical conjugation	Interpenetrating polymer networks (functionalized with bsp-RGD peptides)	Shh	Osteogenic differentiation of rat mesenchymal SCs	[70]
Magnetic bead complex system (antibody–receptor coupling)	–	TGF-β3	Chondrogenic differentiation of rat mesenchymal SCs	[71]
Magnetic bead complex system (streptavidin–biotin binding and antibody-antigen coupling)	–	DLL4	T-cell differentiation from mouse bone marrow HSCs	[72]
Controlled delivery of biomolecules				
Degradable carriers	PLGA nano- and microspheres	CNTF	Neural differentiation of mouse NSCs	[73]
Bioresponsive degradable hydrogels	Metalloproteinase-sensitive PEG-based hydrogels	BMP2	Bone regeneration *in vivo*	[74]

Shh, sonic hedgehog; NT-3, neurotrophin-3; PDGF, platelet-derived growth factor; BMP2, bone morphogenetic protein 2; FN, fibronectin; TGF-β, transforming growth factor-beta; DLL4, delta-like 4; CNTF, ciliary neurotrophic factor; PEG, Poly(ethylene glycol); PLGA, poly(lactic-co-glycolic acid)

transforming growth factor (TGF)-β1 [68]. Alternatively, biomaterials can be functionalized with heparin by engineering hydrogels to contain artificial proteins that present heparin binding sites [69].

Likewise, as previously mentioned, tethering of biomolecules to surfaces leads to higher local concentrations and permits spatial organization or clustering of factors, which might also modulate signaling. Therefore, direct immobilization of signaling factors has been an area of active development in engineering biomaterials for culture of SCs. For instance, surface-immobilized Shh [70] and immobilized TGF-β3 using a magnetic bead complex system [71] have been shown to enhance osteogeneic differentiation and chondrogenesis of bone-marrow-derived mesenchymal SCs, respectively, as compared to cultures that contain only the soluble form of these factors.

For delivery of soluble growth factors, it is possible to use microbeads or degradable vehicles within the scaffolds used to culture SCs. One of the advantages of such systems is that the temporal release kinetics of signaling biomolecules can be controlled by the design of the material [72]. Another important parameter that can control their release kinetics is the size of the delivery vehicles [73].

Bioresponsive materials are another system that has been developed for sequential delivery of growth factors to manipulate SC function in a biomimetic manner. In an elegant study by Lutolf *et al*. [74], responsive PEG-based hydrogels have been fabricated by using building blocks of peptide sequences that are sensitive to proteolytic degradation by cell-derived matrix metalloproteinases. This molecularly engineered scaffold has been degraded successfully by human fibroblast cells and is suitable for delivery of bioactive molecules [74]. Furthermore, to emulate the SC perivascular niche environment, many studies have attempted the controlled delivery of angiogenic factors [75,76], to promote vascularization within constructs of biodegradable polymeric scaffolds [77]. Examples of strategies that have been employed for presentation or delivery of bioactive molecules are illustrated in Table 1.

Devices for SC culture and analysis

Other parameters that are particularly challenging to investigate *in vivo* include cell–cell contacts, oxygen gradients, and nutrient transfer; therefore, novel *in vitro* systems that gain control over these properties offer the opportunity to study their basic effects in SC biology, as well as to gain more precise control over SC behavior for biotechnological application. Technical advances in the development of bioreactors have contributed to development of biological systems that offer enhanced regulation of numerous parameters, such as oxygen levels and nutrient transport, and can be used in general to modulate the molecular, mechanical, and other cues necessary to reconstruct different SC niches *in vitro* on a larger scale that is more suitable for future clinical applications [78]. In addition, micromechanical devices that recently have been engineered to control direct cell–cell interactions can be applied to study cell-contact-mediated signaling in SC cultures. One example of such a system is microfabricated silicon comb structures, which can be separated and brought into close contact for precise cellular positioning to study the communication between different cell types [79].

To further analyze the behavior of SCs, emerging microscale technology has been used to design 3D microfluidic devices that imitate very closely the vasculature *in vivo* and provide control over gradients of soluble growth factors and of mechanical parameters relevant for the SC microenvironment. Important features of these devices include electrical stimulation of excitable cells (Figure 3a) [80], imaging of cell behavior in response to environmental cues (Figure 3b) [81], and precise quantification of ligand concentration gradients needed to direct lineage-specific differentiation of SCs (Figure 3c) [82]. An interesting study by Chung *et al*. [83] have illustrated that human NSCs can

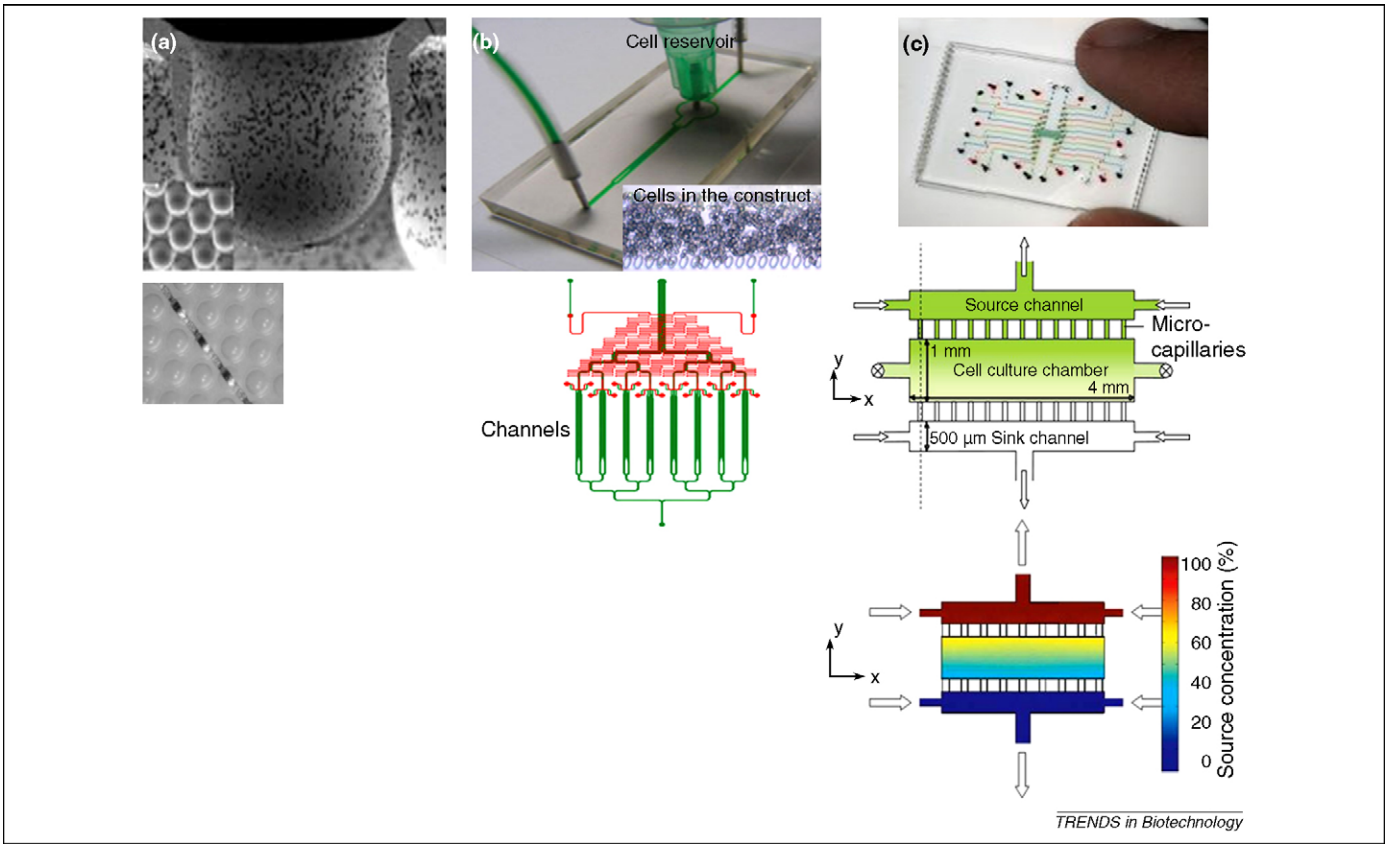

Figure 3. Examples of microscale devices used for cultivation and analysis of SCs. **(a)** Polymer-based chip scaffolds equipped with 3D round micro-containers (upper view) functionalized with a gold conductor microelectrode (lower view), which allows for electrical stimulation of excitable cells [80]. **(b)** Transparent microdevice system that allows for imaging of cell behavior in microfluidic channels in response to ligand concentration gradients [81]. **(c)** Microfluidic chip for the study of cells that are sensitive to shear stresses (upper view), which has been fabricated with microcapillaries, as illustrated in the middle panel, to minimize fluid convection within the cell culture chamber. This chip can also generate a stable concentration gradient in two or three dimensions, which can be quantified easily, as shown in the illustration below [82].

survive in microfluidic devices for more than one week, and that differentiation of these cells is highly dependent on growth factor concentration gradients. Different flow configurations and cell densities in combination with 2D or 3D biomaterials also have been employed in controlled growth and development of hESCs in complex microbioreactors and microfluidic systems [84].

Concluding remarks and future perspectives

The reconstitution of SC niches or biological systems with functional equivalents of specific tissues is clearly a challenging task. Recent studies of *Drosophila* GSCs and various mammalian SC niches have begun to unravel the regulatory mechanisms involved in the complex cellular interactions of SCs with their microenvironment. A greater understanding of the biology of SCs and their microenvironments has inspired researchers to develop biomaterials and technologies that represent increasingly accurate replicas of SC niches *ex vivo*, as well as microfabricated systems that offer unprecedented control over key parameters. Engineering such systems holds great promise for multifaceted control of SC behavior that might enable us to expand and differentiate SCs *ex vivo*, enhance the survival and functional integration of their progeny post-transplantation, or even recruit endogenous adult SCs to sites of injury.

Furthermore, research in human embryonic and induced pluripotent SCs will continue to grow. Although the molecular relationship between hESCs and the inner cell mass of the embryo is not straightforward [85], and pluripotent cells are transient entities in the embryo, principles and approaches learned from studying and emulating other SC niches will aid in the development of fully artificial microenvironments, to control the long-term self-renewal and differentiation of human pluripotent stem cells [57,86]. In the coming 5–10 years, the rapidly expanding field of engineering artificial SC niches is anticipated to have an impact on the biotechnology industry, with further progress in deciphering the complex network of niche signals and in the engineering of artificial microenvironments to control SCs for numerous applications. The key to success in this field will be, however, to integrate the building blocks of these complex systems, including the physical properties of the scaffold structure, the spatial and temporal control of bioactive factor delivery, and the presentation of key ECM motifs, into modular and scalable platforms for biotechnological and clinical translation.

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