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# Engineering physical microenvironment for stem cell based regenerative medicine

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**Regenerative medicine has rapidly evolved over the past decade owing to its potential applications to improve human health. Targeted differentiations of stem cells promise to regenerate a variety of tissues and/or organs despite significant challenges. Recent studies have demonstrated the vital role of the physical microenvironment in regulating stem cell fate and improving differentiation efficiency. In this review, we summarize the main physical cues that are crucial for controlling stem cell differentiation. Recent advances in the technologies for the construction of physical microenvironment and their implications in controlling stem cell fate are also highlighted.**

## Introduction

Regenerative medicine has rapidly evolved during the past decade and opened up a new avenue to meet the demands for tissue and/or organ transplantation in clinics [1], where stem cells have drawn considerable attention owing to their unique capability to differentiate into desired cell lineage and to self-renew. For example, stem cells have been widely explored to repair defective and damaged tissues such as cartilage [2], heart [3] and neural tissues [4]. Apart from organ transplantation, the specific cell lineages derived from stem cells also provide reliable cell sources for drug discovery and development (e.g. target identification/validation and safety/metabolism studies). For example, physiologically relevant hepatocytes, derived from stem cells, as opposed to primary hepatocytes, can be grown in a large scale and have better applications in toxicity tests [5]. Therefore, there is a great need to grow a large number of undifferentiated stem cells and to differentiate them into targeted cell lineages, which remains elusive.

Constant efforts have been made to control the differentiation of stem cells and to gain new knowledge of the underlying mechanisms. Accumulating evidence has indicated that the fate of stem cells is highly affected by the microenvironment (also called niche) where they are located. In physiological milieu, stem cells encounter complex stimulations (e.g. physical, chemical and biological cues) from surrounding cells and extracellular matrix (ECM), which have significant effects on fate determination [6–8]. For instance, stem cell factor (SCF) expressed by neighbor cells is a key constituent that maintains the pluripotency of hematopoietic stem cells [6]. Thus, engineering stem cell microenvironment would benefit the production of stem cells and subsequent differentiation into cells of interest for biomedical and clinical applications.

Although it is well accepted that biological and chemical cues (e.g. hormones, growth factors, and small chemicals) can significantly influence cell functions [9–11], more and more evidence has also shown that physical cues, for example mechanical properties of growing substrate [12], topographical cues [13] and tension force [14], also play an important part in controlling the fate of stem cells. Recently, with the development of nano- and micro-engineering technologies [15], reconstructing 3D physical microenvironment *in vitro* with a spatiotemporal control becomes feasible. 3D artificial constructs can mimic the native physical

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environment to some extent and thus hold great promise to facilitate controlling stem cell fate in a directed manner when combined with the presence of chemical and biological cues.

Although several good reviews have been published on the topic of interactions between stem cells and physical cues [16–22], most of them addressed the effects of material property on the stem cell fate, namely cell–substrate interaction where they are commonly uniform or static. Few review articles focus on engineering approaches that can manipulate the physical microenvironment *in vitro* accurately and dynamically. In this review, we mainly aim to introduce the state-of-the-art technologies for engineering complex physical microenvironment with a focus on the physical factors that affect stem cells *in vivo*. Specifically, we first summarized the physical cues that can be potentially used to regulate stem cell fate. Then we discussed how to engineer a complex microenvironment with consideration of the important physical cues.

### *Physical microenvironment of stem cells*

Cells *in vivo* are exposed to a broad variety of physical cues depending on their functions and locations. For instance, neurons bear minimal mechanical loadings, muscle cells usually experience significant forces and endothelial cells are under shear stress induced by blood flow. According to the nature of physical cues in the ECM, we divided them into three categories including matrix stiffness, mechanical force and topology. Besides, we emphasized the presentation of these cues in a spatiotemporally dynamic manner (Fig. 1).

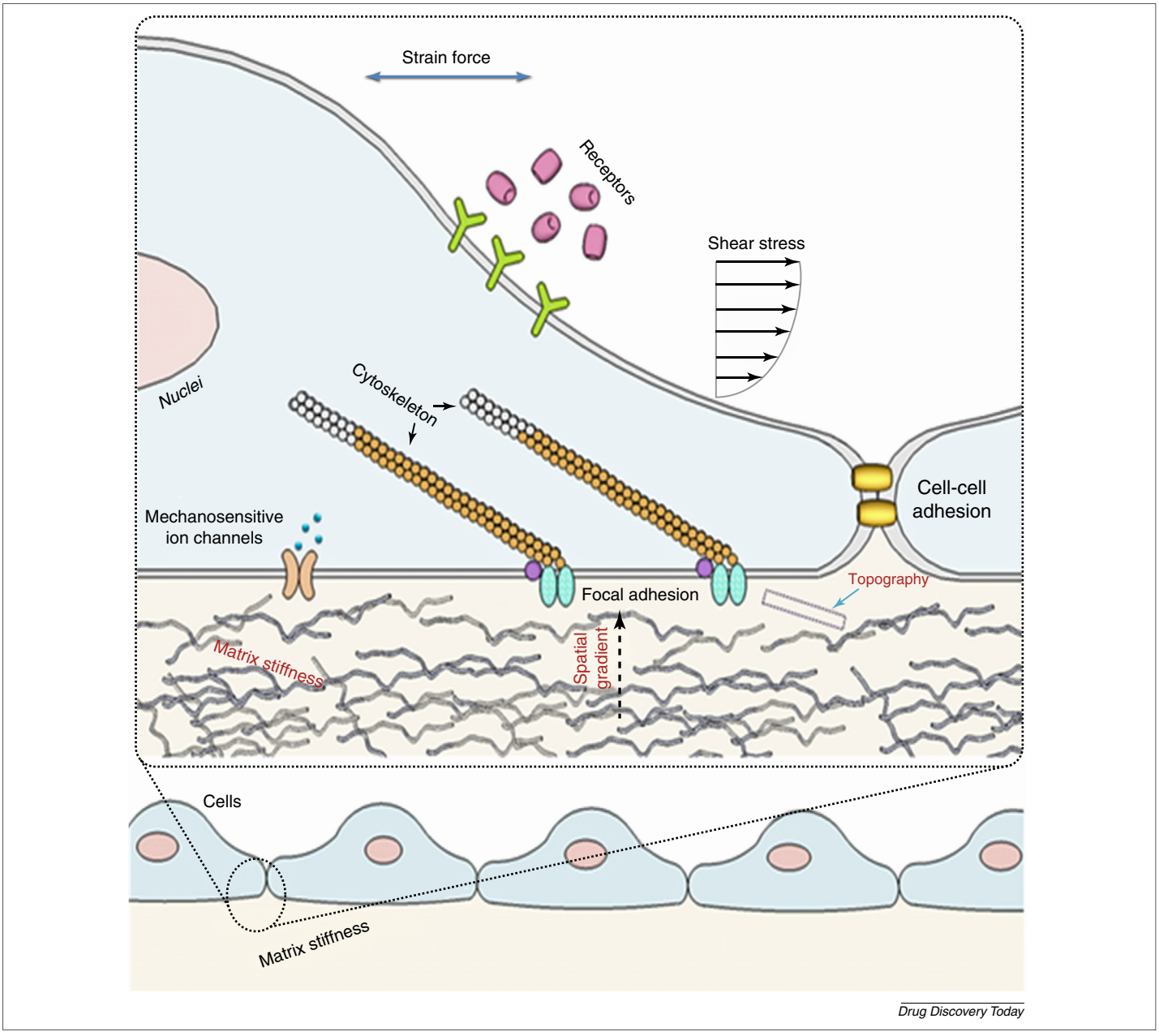


**FIGURE 1**

Native physical microenvironment and mechanosensors of stem cells. The stem cells *in vivo* are subjected to a broad variety of physical cues, including matrix stiffness, mechanical forces (e.g. strain force and shear stress) and topography, mostly in a spatiotemporally dynamic manner (spatial gradients).

### Matrix stiffness

Matrix stiffness is defined as the degree that an extracellular scaffold resists deformation. Tissues *in vivo* possess a broad range of mechanical properties, and are tailored to function at varying mechanical demands. For example, adipose tissue is a soft cushion for vital organs, whereas bone is a rigid protector and mechanical support for body. The homeostasis of stiffness within a tissue is important for its biological functions, whereas its alterations are usually associated with dysfunction. Thereby, the varying stiffness of ECM within different tissues is crucial to differentiate stem cells into specific cell lineages. Additionally, matrix stiffness is of great importance during embryogenesis *in vivo*. For instance, during the gastrulation of *Xenopus laevis* the convergence and extension movements can occur only if the notochord and mesoderm are stiff enough to withstand buckling [23,24]. The involuting marginal zone becomes stiffer and thus does not deform or collapse during gastrulation [25], indicative of the significance of stiffness to cell function.

### Mechanical forces

Mechanical forces are also a vital stimulus during embryogenesis and throughout life [26]. The forces at the cellular level can be classified into two categories, namely internal forces and external forces [27]. Internal forces are defined as a contractile force arising from the cellular actomyosin cytoskeleton, whereas external forces refer to the force acting from the outside of cells. Although internal forces are also important for cell functions, we will not discuss it here because it is beyond the scope of this review in the perspective of engineering cell microenvironment. Physiological actions such as blood flow, muscular movement, gravity bearing and other processes generate different external forces to cells, such as compressive forces, stretch forces and shear stress. These mechanical forces are also found to be crucial to determine the fate of stem cells *in vitro*. For instance, shear stress has been found to drive the differentiation of embryonic stem cells (ESCs) toward vascular endothelial cells [28], whereas the stretching of mesenchymal stem cells (MSCs) results in upregulation of specific markers as seen in smooth muscle cells [29]. Therefore, mimicking the mechanical forces that stem cells experience *in vivo* is desirable to control the fate of stem cells.

### Topography

Native ECM presents various geometrically defined physical boundaries through composition and structure (i.e. topographies). The components of the ECM can be arranged into structures such as fibers and sheets that support cells and regulate their function [30–34]. Take intestinal mucosa for example, it consists of epithelial folds (i.e. villi) with a dimension of 400–500 μm [35,36] and epithelial invaginations (i.e. intestinal crypts) with dimensions of 100–200 μm. The basement membranes under the intestinal mucosa are composed of 50-nm-thick collagen fibers. Nanoscale structures (e.g. collagen fibers) interact with cell receptors and affect protein clustering and organization, whereas microscale structures change the curvature of the cell membrane [37]. Both of these structures can affect cytoskeleton assembly, alter internal forces and influence stem cell behaviors [37]. *In vitro*, the topography of the extracellular microenvironment can affect the responses of stem cells during the process of attachment, migration, differentiation and formation of new tissues [19].

### Spatiotemporal dynamics

Biophysical and biochemical signals can not only play an important part in controlling cell functions but also significantly affect tissue development and regeneration via forming dynamic concentration gradients in a spatial–temporal manner [38,39]. For instance, investigations of zebrafish embryogenesis uncovered the underlying spatial and temporal dynamics of molecular gradients (e.g. retinoic acid and the Ntla transcription factors) during embryonic development [40,41]. In addition, the gradient of some small molecules such as $H_2O_2$ generated during wound formation in zebrafish helps recruit leukocytes to the wound zone [42]. The effect of the dynamic microenvironment on cell behavior has been studied *in vitro*. Mechanical force gradients were also observed in the micropatterned epithelial monolayer. Such a force gradient drives cell motions and the propagation of the gradient (termed mechanical wave) plays a central part in epithelial expansion during the development of organ shape [43]. In addition, the spatiotemporal microenvironment can also regulate cell behavior at micro- and/or nanometer scales. Alignment of humans mesenchymal stem cells (hMSCs) is sensitive to the dynamically and reversibly changed topographies achieved through strain-responsive buckling patterns on polydimethylsiloxane, which demonstrated the importance of dynamic topography [44]. Besides, it is well known that cells grown on substrates with a stiffness gradient will migrate to stiffer areas [45], indicative of the importance of mechanical gradients.

## Approaches for engineering physical microenvironment to control the fate of stem cells

Studies on stem cells over the past two decades have shown that engineering the physical microenvironment could facilitate addressing the challenges in controlling the stem cell fate. A variety of approaches have been developed to create microenvironment *in vitro* including material-based approaches, mechanical-force-based approaches and micro- and/or nano-fabrication-based approaches (Fig. 2).

### Material-based approaches

With advances in material science, a variety of materials including polymers, ceramics and metals have been developed to match the diverse elasticity of tissues *in vivo*, mimicking the physical microenvironment where stem cells are surrounded (Fig. 2).

*Polymers*. With advances in polymer science, natural and synthetic polymers with tunable properties have been developed, providing more options for the control of stem cell fate [46]. The mechanical properties (e.g. stiffness) of polymers can be tuned from 0.1 kPa to 1 MPa, making it attractive for tissue engineering and regenerative medicine. The natural polymers commonly have relatively lower stiffness (0.01–100 kPa) than synthetic polymers (10 kPa to 1 MPa), therefore they are more suitable to mimic soft niches. In addition, many of these natural polymers (such as hyaluronic acid and chondroitin sulfate) exist *in vivo* and play an important part in stem cell differentiation. However, there are still some challenges associated with most natural polymers when used *in vivo*, including weak mechanical properties and potential immunoreaction risks.

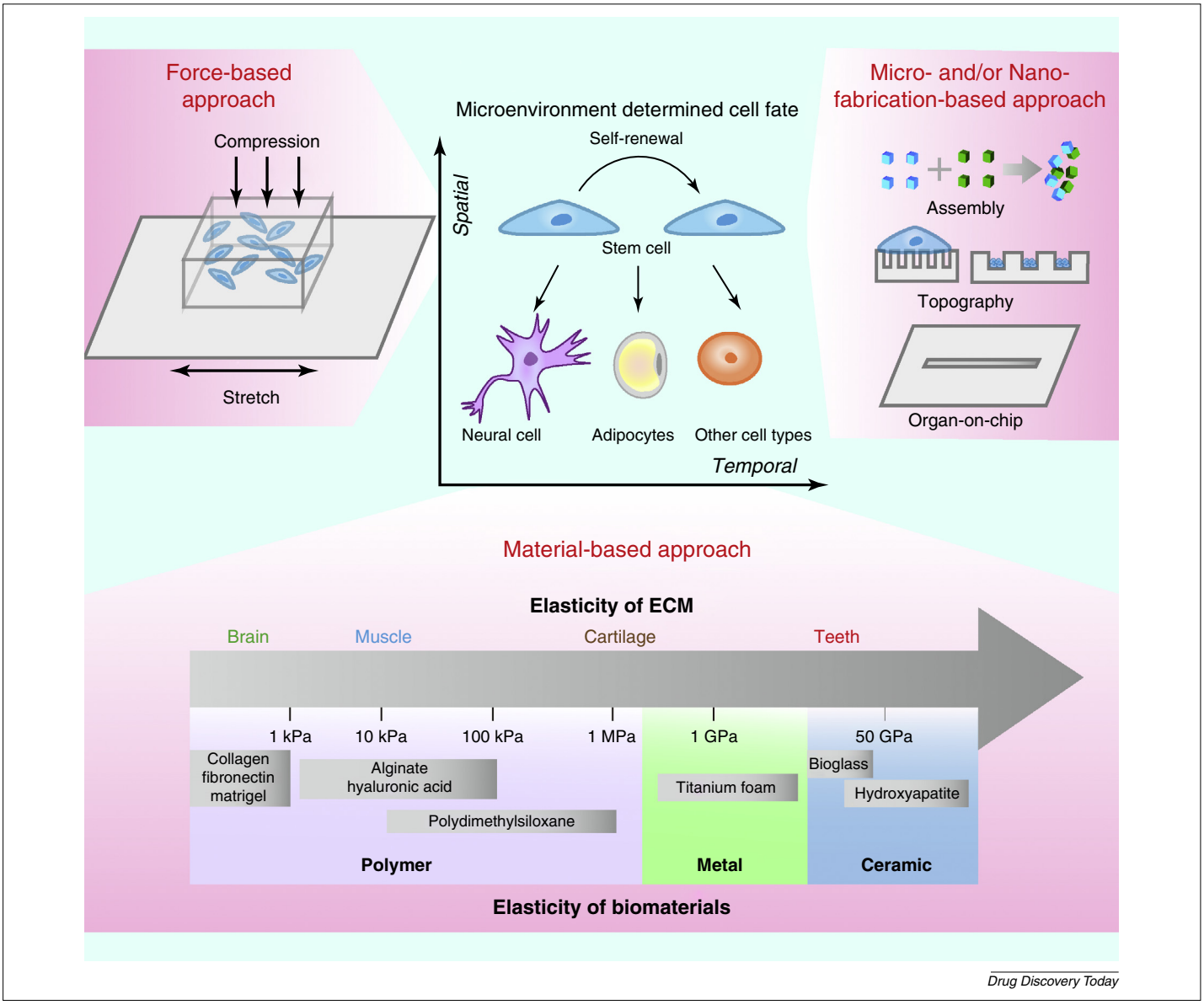


**FIGURE 2**

Schematic representation of approaches for controlling stem cell fate with physical cues. The stem cell fate (i.e. self-renewal and differentiation) is affected by spatiotemporal physical microenvironment. There are three approaches to engineering physical microenvironment *in vitro* including material-based approaches, force-based approaches and micro- and/or nano-fabrication-based approaches. Abbreviation: ECM, extracellular matrix.

*Ceramics and metals*. Owing to high mechanical properties, ceramics and metals exhibit as good substrates for the osteogenic differentiation of stem cells. The most commonly used ceramics include calcium phosphate ceramics, bioactive glass and hydroxyapatite. When cultured on the surface of calcium phosphate ceramics, MSCs displayed a stable osteoblastic phenotype with the formation of apatite in the ECM [47]. Hydroxyapatite is a naturally occurring ceramic mineral found in bones, and it has been widely investigated as a bone substitution. This kind of materials can adsorb proteins strongly, and thus benefit the adhesion, proliferation and differentiation of MSCs [48]. Bioactive glass, which is composed of phosphate oxide, calcium oxide, sodium oxide, calcium oxide and silicon dioxide, has a high compatibility with bone tissues and it is usually used as defect fillers. MSCs grown on this material demonstrated an osteoblastic phenotype with mineralized ECM, indicative of the promoted differentiation of MSCs into osteoblasts [49]. Titanium is another type of material that has been widely used in dental and orthopedic surgeries owing to its good biocompatibility and inertness. Titanium substrates (i.e. titanium dish) can favor stem cell adhesion, proliferation and differentiation [50]. Embryonic bodies (EBs) were also observed to form effectively in 3D titanium scaffolds with obvious cell–matrix interactions [51].

*Regulation of stem cell fate by substrate stiffness*. Engler *et al*. laid the foundation of how physical cues direct stem cell differentiation by culturing hMSCs on hydrogel substrates with different stiffness [52]. The proteins and transcription profiles were analyzed to reflect the impacts of stiffness on stem cell fate. Stem cells expressed significant neural markers on softer materials (0.3 kPa), whereas osteogenic markers were observed on a rigid substrate

(35 kPa). Stiff hydrogel substrates enhance the growth and development of force sensors (focal adhesion). These sensors transfer the cell–substrate force into the cell signal pathway and then adjust cell–ECM interaction via actin–myosin contractions. As a result, cells grown on a stiffer hydrogel substrate presented a more highly tensed state. The generated forces on the cell actin cytoskeleton contributed to regulating the differentiation of stem cells into an osteogenic lineage. Subsequent studies also illustrated the importance of substrate stiffness on stem cell fate [53,12,54]. Further, it was found that a substrate with a proper stiffness was crucial to maintain the 'stemness'. For instance, muscle stem cells grown on a rigid Petri dish lose their pluripotency, resulting in decreased regenerative capability in their progenitors. To address this challenge, hydrogel-coated plastic dishes with different stiffness (2, 12 and 42 kPa) were used to culture muscle stem cells. On softer hydrogel substrates the number of muscle stem cells increased twice after a week, whereas the number remained constant when cultured on a rigid Petri dish, indicating an enhanced cell survival and proliferation by soft hydrogels [53].

### Mechanical-force-based approaches

*Cyclic strain*. Cyclic strain can be applied to stem cells *in vitro* and affect their differentiation pathways. This effect depends on the strain amplitudes, frequencies, load means and cell types. Commonly, stem cells are cultured on a flexible membrane (which can be coated with various proteins or not), on which uniaxial or biaxial strains are applied at a constant frequency. For instance, the differentiation behavior of MSCs under cyclic strains has been widely investigated using this system. MSCs encountering a 5–10% uniaxial stretch showed a typical myogenic phenotype accompanied with the expression of myogenic proteins (e.g. smooth muscle actin) [55–57]. By contrast, such a phenotype was not observed when the applied strains were lower than 1% or higher than 15%, suggesting the importance of strain magnitude during MSC differentiation [58]. In addition, different cell types such as adipose-derived stem cells responded differently to a similar strain (10%) [59]. Uniform biaxial stretch was found to enhance osteogenic differentiation of MSCs with an increased expression of osteogenic-specific markers [60]. Cyclic compression was usually achieved by loading a pressure on 3D hydrogels encapsulating stem cells. For example, dynamic compression of a MSC-laden 3D agarose hydrogel was used to study the mechanical responses of stem cells. Under mechanical stimulus, an increase in aggrecan and collagen II transcriptional activity was observed, indicating that a chondrogenic differentiation was induced by mechanical compression [61].

*Shear stress*. Shear stress can be created either by a stir-based method [62] or pump-based method [63]. In the stir-based method stem cells are seeded and then attached to a substrate of interest. The apparatus for stress creation consists of a rotating disk driven by a motor and a stage to adjust the distance between cells and the disk. The shear stress can be controlled through angular velocity of the disk and cell positions. In a pump-based method, a pump and a parallel plate apparatus are used to create shear stress. The configuration of a parallel apparatus (such as height and width) and the velocity of fluid are the determining factors to the final shear stress applied to the cells. Based on these platforms, the effects of fluid shear stress on stem cell functions have been widely studied [64–67]. For instance, two days after the shear stress was applied, an increased expression of endothelial markers and formation of vessel-like structures were observed for mouse ESCs, indicating that shear stress promotes the differentiation of mouse ESCs toward the endothelial-like phenotype [63]. These findings implicate that the design of bioreactors, accompanied with complex shear stress, is important for a scale production of stem cells and targeted differentiation.

### Micro- and nano-fabrication-based approaches

Emerging micro- and/or nano-scale engineering technologies offer unprecedented opportunities for the creation of cell microenvironment *in vitro* that recapitulates the crucial cues *in vivo*, such as spatiotemporal physical and chemical gradients, surface topography and dynamic mechanical microenvironment. Here, we summarize three kinds of strategies that have been used to engineer complex stem cell niches: bottom-up assembly, topography patterning and organ-on-a-chip.

*Bottom-up assembly*. The bottom-up approach was firstly proposed to construct intricate microstructural features of the cell microenvironment by designing specific structural features on microscale modules [68,69]. Emerging methods in recent years hold great potentials to engineer heterogeneous physical cell milieu (Fig. 3). For instance, an electrostatic-force-based platform has been developed recently to assemble microgels into various patterns with a control over final architectures [70]. By incorporating biomaterials with positively and negatively charged hydrogels, the biomaterials with opposite charges are attracted to each other (Fig. 3a), which could be used to assemble biomaterials with different physical properties. To improve the recognition efficiency between microgels, DNA was used as a glue to direct the self-assembly of microgels into prescribed structures [71]. Owing to the high recognition efficiency of DNA, 50 distinct microgels were assembled into 25 predesigned pairs in a simple mixing process (Fig. 3b), demonstrating the capability of multiplexing microgel assembly in a single system. Additionally, another multilayer photolithography was developed to engineer digitally specified 3D spatial confinement on stem cells [72]. By switching multiple masks with microscale controls, ECM components and cell types can be modulated easily (Fig. 3c). Particularly, ESCs and two other types of cells were aligned to mimic the complex process of myocardium regeneration. Based on a similar principle, heterogeneous differentiation of EBs was investigated through the fabrication of two kinds of hydrogels around a single EB [73]. Moreover, the paramagnetic property of microgels was revealed, and the microgels were manipulated temporally and spatially without the need for other magnetic components (e.g. magnetic nanoparticles) (Fig. 3d) [74]. Taken together, the rapid development of bottom-up assembly methodologies provides a simple, low-cost and highly accurate way to recreate stem cell niches *in vitro*, especially with asymmetrical architectures.

*Topography patterning*. Nano- and micro-patterned surfaces have gained increasing importance in the design of biomaterials for regenerative medicine, as reviewed [19,75]. Numerous technologies, such as electron beam and nanoimprint lithography, have been developed to recapitulate the topography *in vivo* and modulate the cell function *in vitro* [76]. For example, the electron beam lithography has been used to fabricate an assay of nanopits that

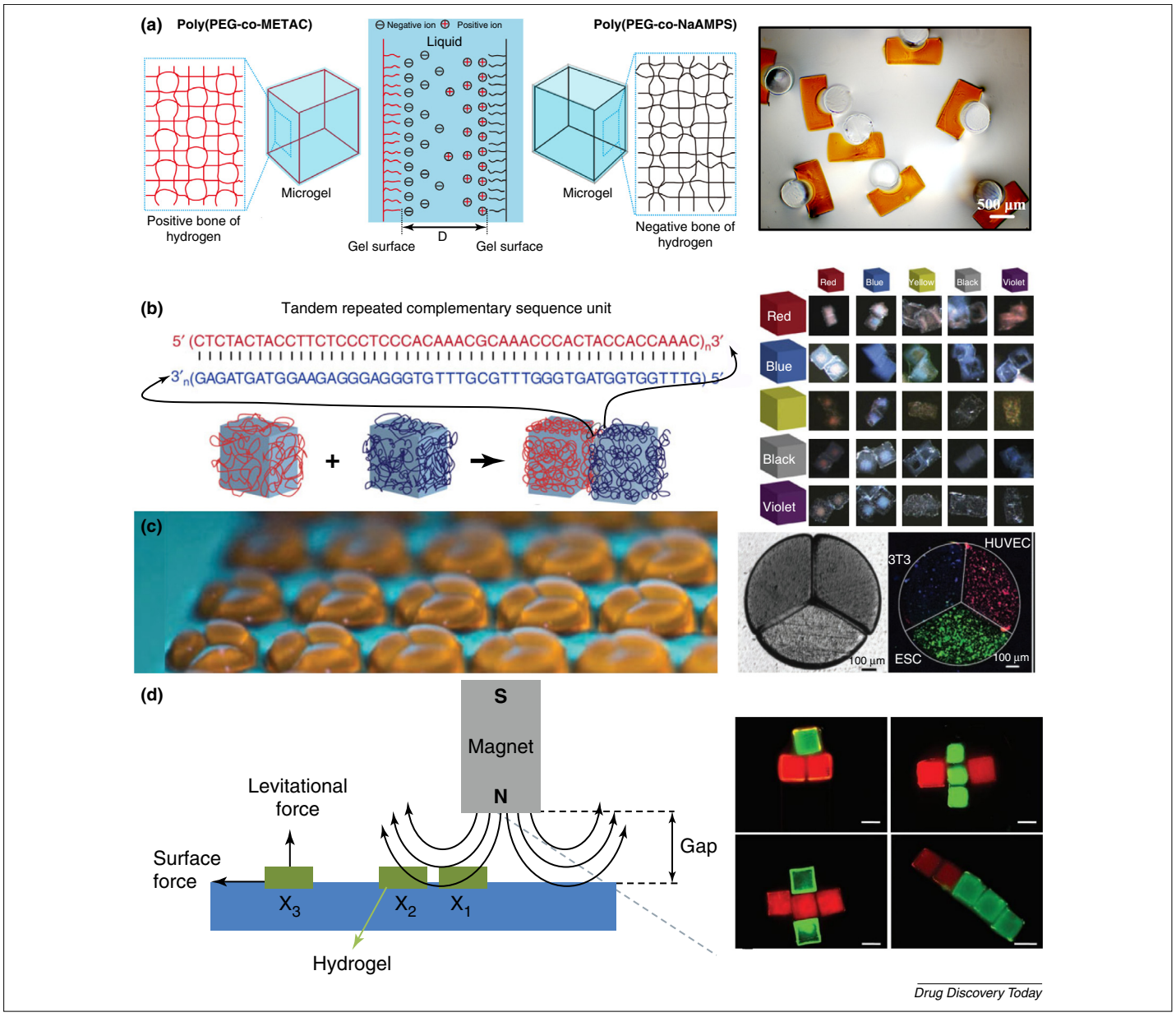


**FIGURE 3**

Bottom-up assembly of physical microenvironment *in vitro*. **(a)** Assembly of microgels based on electrostatic force [70]; **(b)** DNA-glue-based assembly of microgels with high recognition efficiency [71]; **(c)** construction of heterogeneous microenvironment for embryonic stem cells (encapsulated in microgels) by multilayer photolithography [72]; **(d)** paramagnetic levitational assembly of microgels [74].

allowed the maintenance of multipotency of MSCs [77]. More recently, some effective microfabrication methods have been developed to avoid the use of expensive and complex nanofabrication techniques. Reactive ion etching was combined with standard photolithography and used for patterning nanoarchitecture on glass substrates with precise control [78]. The features of nanoarchitectures (i.e. shape, diameter, height, and distribution) are the key regulators for various cell behaviors, including cell adhesion, proliferation, self-renewal and differentiation. Microscale topography can also regulate the behaviors of stem cells (Fig. 4a). Microscale contact patterning of adhesive proteins (e.g. fibronectin) to a nonadhesive surface makes it possible to control the 2D cell geometry [79] and study its effects on the commitment of stem cells into different linages. The geometry parameters such as shape, area, aspect ratio and curvature significantly affect the differentiation commitment of stem cells. Take human MSCs for example, they tend to differentiate into adipocytes when having a small adhesion area (~1000 $\mu m^2$), whereas they tend to differentiate into osteoblasts when having a larger adhesion area (~5000 $\mu m^2$) [80]. 3D structures, for example microgroove [81], micropost [82] and microwell [83], are also important to direct the differentiation of stem cells. For example, the size of EBs can be controlled using microwells with designed dimensions, which has been shown to affect the WNT signalling pathway and subsequent differentiation [84].

*Organ-on-a-Chip*. Organ-on-a-Chip is defined as the reconstitution of native tissues within a microfluidic device that aims to study the physiology of a specific organ or to develop disease

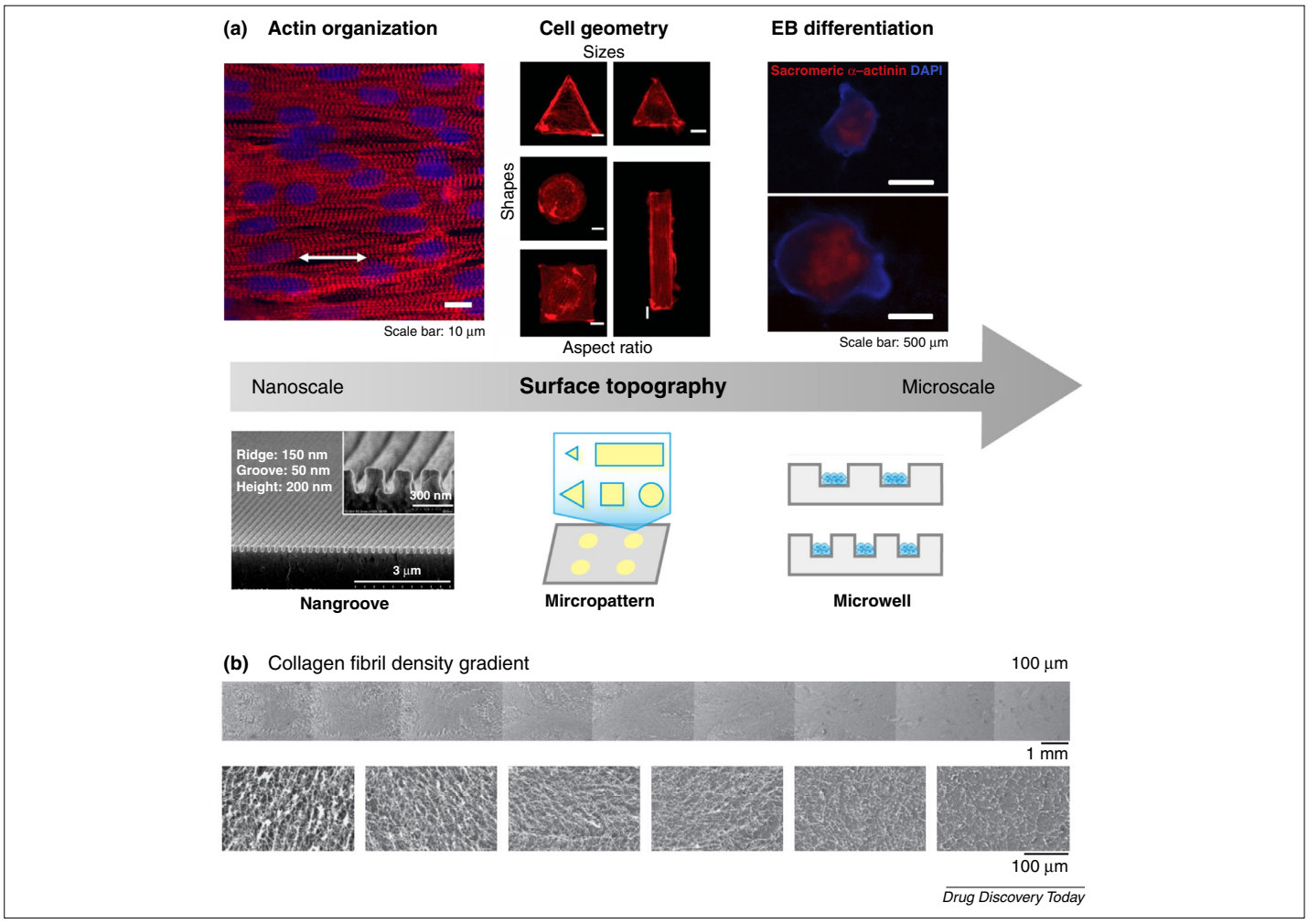


**FIGURE 4**

Topography engineering and microfluidic technologies for recapitulation of physical cues in stem cell niche. **(a)** Engineering topography in cell microenvironment from nanoscale to microscale [79,83,127]. **(b)** Collagen fibril density gradient generated from microfluidic device [90]. Abbreviations: DAPI, 4′,6-diamidino-2-phenylindole; EB, embryonic bodies.

models *in vitro* [85]. With the rapid development of microfluidic technologies [86–88], mounting evidence shows that the microfluidic platform is a powerful tool to engineer physical niches of cells including flow-induced shear stress and cyclic strain [85,89]. Besides, microfluidic devices can be used to create spatial gradients in physical and biochemical aspects. Flow convection in a microchannel has been used to generate gradients of polymers, cells, particles and molecules, where the fluid was pumped fast while alternating flow directions (i.e. pumped and withdrawn) [90]. For instance, a density gradient of collagen fibril (Fig. 4b) was achieved by pumping a collagen solution at a higher concentration (3.8 mg/ml) into a channel embedded with a collagen solution with a lower concentration (0.5 mg/ml) with alternating flow. The gradient of cell-adhesion ligand (Arg-Gly-Asp-Ser) was also generated based on the similar principle to study the cell–material interactions [91]. 3D gradients of cell density within a collagen hydrogel were generated using a staggered herringbone microfluidic mixer [92]. Using this method, linear, exponential and other geometrical gradients could be potentially achieved through different microfluidic designs. Opposing gradients of two cell types including stem cells and osteoblasts were generated in 3D collagen hydrogels that can potentially be used to mimic the bone marrow microenvironment and to study the effect of stromal cell (i.e. osteoblasts) gradient on stem cell behaviors. Another 3D stiffness gradient within a hydrogel was established in a tube using two mixing pumps to study the effects of 3D stiffness gradient on the stem cell fate [93]. MSCs cultured in softer regions had a higher proliferation rate compared with those in stiffer regions [93].

*State-of-the-art biojet technologies*. Although the aforementioned approaches have been used to recreate physical microenvironment of stem cells for years, they are far from any clinical usage because of tedious pre-processing steps and low throughput [94,95]. Conventional cell printing approaches such as inkjet technology and laser-directed writing have shown intriguing abilities to mimic various physiological situations during the past decades [96–102]. However, they are suffering from the limited spatial resolution and the shortage of sufficient biological assessment [103]. The emerging newly developed biojet technologies have recently led to many significant findings in regenerative medicine and have undergone complete biological assessment, indicating a great

possibility in clinical application. Here, we briefly introduce these technologies including cell electrospinning, bio-electrospraying and aerodynamically assisted biojetting and threading.

- *Cell electrospinning and bio-electrospraying*. Electrospinning and electrosprays basically exploit a potential difference between two charged electrodes to draw a liquid jet that either generates continuous fibers or droplets, respectively [104,105]. The basic principle of this process relies on the movement of charged liquid in the electric field existing between two charged electrodes. The charging liquid is driven by an electric force, exiting a needle toward the grounded electrode. Compared with conventional cell printing approaches, these technologies can fabricate droplets and fibers at a nanometer scale (~50 nm) and they are compatible with large concentrations of materials in suspension, or liquid with high viscosity (~10,000 mPas). Besides, these two technologies have been well evaluated and developed by Jayasinghe's group at University College London in technological and biological views [106–115]. First, they have shown that these two technologies can work with a broad range of cell types from stem cells to whole blood cells and demonstrated their ability to control cell spatial distribution within droplets or fibers. Second, the effects of this fabrication process on cell function have been studied at the cellular and molecular levels, and the feasibility of fabricated construct for translation was demonstrated in mice. Owing to the vast perspective in synthetic organotypic tissue engineering, these technologies are now known as bio-electrospraying (BES) and cell electrospinning (CE). It has been validated that BES and CE are capable of handling heterogeneous cell populations at high cell densities and of controlling cell distribution in 3D. In addition, BES and CE can directly handle complex multicellular organisms without altering their biological developments (such as *Danio rerio* and *Drosophila melanogaster* at their early development stage) [116,117]. Moreover, studies have shown their capability to construct various cell-bearing structures that can potentially be used in clinical application. For the sake of complete assessment of any possibly missing cellular aspects during previous *in vitro* studies, these cell-laden structures are engrafted into mice to form a wide range of tissues, which demonstrated that these two technologies are completely inert to the cell function [109].
- *Aerodynamically assisted biojetting and threading*. Aerodynamically assisted biojetting (AABJ) is a very versatile technique, which has widespread biological applications such as printing cells and tissues. In this system, droplets are squeezed out from an exit orifice of a chamber by a pressure differential generated through either a gas or liquid. Specifically, a high pressure within the chamber is initially generated relative to the atmosphere. Then, the medium reserved in designed needles is drawn into a liquid filament under a high pressure, exiting the orifice. Over the past decade, AABJ has been used to handle a wide range of cells and whole organisms, and the functional studies have also been investigated *in vivo*. For instance, AABJ-treated splenic cells are capable of homing to lymph nodes after transplantation into mice, indicating that AABJ does not alter splenic cells functionally [118]. However, to date, this technique is still under further evaluation (explored with other animal models) before it can enter preclinical studies [119–121].

### *Concluding remarks and future perspectives*

The regulation of stem cell fate *in vivo* remains largely unknown. The investigation of this topic requires a multidisciplinary convergence including biology, chemistry, engineering, physics and material science. Mounting evidence demonstrates that the fate of stem cells is not only controlled by heredity but also by the microenvironment. The ideal microenvironment is a combination of various cues in a spatiotemporal context, including specific ECM proteins, appropriate stiffness and force, and adequate topography, among others. It is challenging to guide stem cell behaviors by engineering only physical microenvironment, because biological cues are also profound in regulating the differentiation of stem cells. However, research in physical microenvironment is deeply helpful to understand the behaviors of stem cells and to design materials and/or bioreactors for regenerative medicine. Recent advances in micro- and/or nanoengineering technologies endow the ability to recapitulate the complexity of the native stem cell microenvironment such as heterogeneity and physical and chemical gradients, which makes it possible to study their roles in stem cell differentiation and to provide useful platforms for a broad range of biomedical applications.

Most current studies on physical microenvironment were performed using a 2D model where cells are cultured in monolayers. It is well known that stem cells reside in a 3D microenvironment *in vivo* and that a 2D system cannot recapitulate the innate characteristics of stem cells. For cells grown on 2D hydrogels the stiffness of substrate can affect cell adhesion, spreading and fate. In addition to stiffness, stem cells can also be influenced by geometric constraints on cell adhesion, leading to limited tension generation and cell spreading. So far, how stem cells respond to 3D physical cues still largely remains unclear. Emerging studies have shown that stem cells behaved differently in 3D physical niches. For instance, the morphology of MSCs was independent of matrix stiffness and remained rounded throughout the differentiation process when MSCs were encapsulated into nondegradable alginate hydrogels [122]. MSCs migrated on a 2D substrate with a stiffness gradient [123], whereas no migration was observed in matrix with a 3D gradient [93]. Therefore, the investigation of stem cell behaviors in 3D physical niches is desirable in the future with the aid of emerging approaches for engineering 3D microenvironment.

The dynamic properties of 3D microenvironment (i.e. spatiotemporal context) also play a significant part during embryonic development and throughout the whole life. To date, several studies have shown that stem cell behaviors can be regulated by the dynamic changes of 3D microenvironment [124–126]. For instance, the phenotypes of hMSCs encapsulated in hyaluronic acid hydrogels can be regulated from osteogenesis to adipogenesis by changing the ratio of mixed hydrogels [124]. This study indicates that the traction force rather than the monomer of hydrogel mediates the fate of stem cells encapsulated in a 3D nondegradable hydrogel, providing insights into how stem cells interact with their surroundings in 3D milieu and highlighting the significance of degradability in the 3D microenvironment. However, the mechanism of how the dynamic microenvironment affects stem cell fate is still unknown. Therefore, future research is needed to design exquisite and dynamic 3D microenvironments so as to

unravel further the function of biochemical and biophysical cues and subsequently to induce targeted stem cell differentiation.

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