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Engineering cell microenvironment using novel functional hydrogels

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ABSTRACT

Cell microenvironment plays critical roles in regulating cellular activities both spatially and temporally. Engineering cell microenvironment using hydrogels has attracted increasing attention given their native-mimicking properties. In particular, developing hydrogels with specific functions has recently emerged as novel biocomposites to enable the regulation of cell microenvironment from a variety of aspects such as the biological, mechanical and electrical microenvironment. In this review, the state-of-the-art methods for the preparation and application of several novel functional hydrogels are presented, including magnetic hydrogels, photoresponsive hydrogels, conductive hydrogels, thermoresponsive hydrogels, molecule-response hydrogels, as well as tough and stretchable hydrogels. In particular, the applications of these functional hydrogels for engineering cell microenvironment are also reviewed. Concluding remarks and perspectives for the future development of functional hydrogels are addressed.

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1. Introduction

Cells *in vivo* are situated in a three-dimensional (3D) complicated microenvironment composed of various biological, physical and chemical cues [1,2]. These biological, physical and chemical microenvironments play critical roles in regulating cellular activities (e.g., proliferation, migration, differentiation) both spatially and temporally [3–5]. Therefore, it is of great importance to engineer cell microenvironment *in vitro* to reconstruct the native cellular behaviors and functions for various applications, such as tissue engineering and regenerative medicine. For this, hydrogels have attracted increasing attention given their native-mimicking properties including biological adhesion, biodegradation, biocompatibility and high permeability that allow molecules of different sizes to transport out of and into [6–9]. However, the limitations of the conventional hydrogel systems in controllability, actuation, response and mechanical properties [10–12] make it challenging to engineer complex cell microenvironment *in vitro* to mimic the native microenvironment [13].

With advances in polymer science, various novel functional hydrogels have recently been developed through functionalizing the conventional hydrogels with certain special properties (e.g., magnetic response, photo response, electrical conductivity). These special properties endow hydrogels with additional potentials and widen the scope of their applications in engineering cell microenvironment (Fig. 1). For instance, the mechanical environment of hydrogels with embedded magnetic nanoparticles can be

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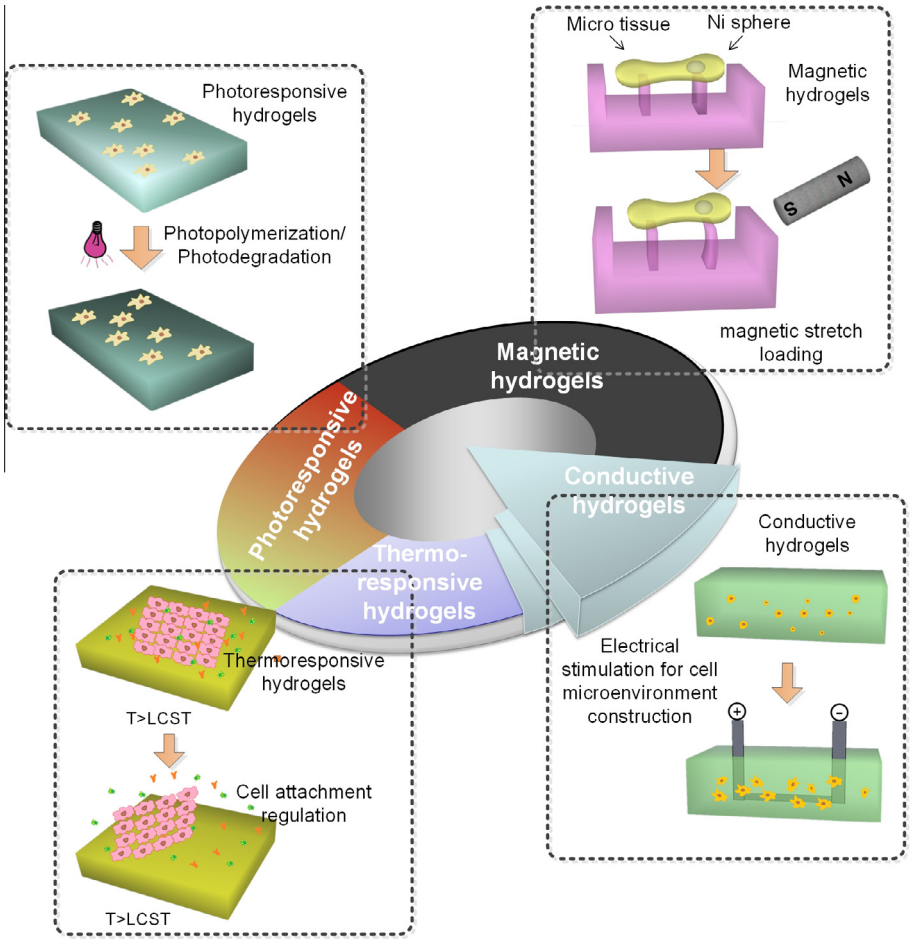

Fig. 1. Schematic illustration of engineering cell microenvironment using novel functional hydrogels, including magnetic hydrogels to manipulate mechanical microenvironment through magnetic stretch loading, conductive hydrogels to change the electrical microenvironment via electrical stimulation, photoresponsive hydrogels to manipulate the mechanical microenvironment through photo-degradation, and thermoresponsive hydrogels to manipulate the biological microenvironment by changing the temperature.

easily manipulated through the hydrogel deformation by using an external magnetic field (MF) [14]. Conductive nanomaterials have been incorporated into cell-encapsulating hydrogels, especially for cardiac tissue and neuronal regeneration, to facilitate the electrical signal transport and thus enhance the cellular function via electrical stimulation [15]. Thus, the developments of functional hydrogels enable the construction and manipulation of complex cell microenvironment *in vitro* with more controllability.

Although there exist several reviews on different types of functional hydrogels, they mostly emphasize the hydrogel materials and the synthesis methods [16–20]. In this review, we aim to present an overview of recent studies on the novel functional hydrogels with a focus on their applications in engineering cell biological, physical and chemical microenvironments. Several types of functional hydrogels, including magnetic hydrogels, photoresponsive hydrogels, conductive hydrogels, and other functional hydrogels will be addressed. In each section, we will start with a brief introduction of the hydrogels followed by some examples of their applications in engineering the cell microenvironment.

2. Functional hydrogels for engineering cell microenvironment

2.1. Magnetic hydrogels for engineering cell microenvironment

Magnetic hydrogels have recently emerged as a novel kind of hydrogels for their active and controllable responsive properties to an external MF with widespread applications, such as tissue remodeling, drug delivery systems, enzyme immobilization, local hyperthermia therapy and soft actuators [14]. Magnetic hydrogels are commonly composed of magnetic micro/nano particles (MPs) (e.g., Fe_3O_4, γ-Fe_3O_4 and $CoFe_2O_4$), enabling their quick response to an external MF. The responsive properties are influenced by several factors, including the type, concentration, size and even the spatial distribution of MPs [21,22]. Several

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approaches have been developed to encapsulate MPs in hydrogels. One of the strategies is to initially disperse the MPs in an aqueous phase to avoid oxidization and aggregation, and then mix the MP solution with the hydrogel precursors followed by a polymerization process, resulting in the encapsulation of the MPs in the hydrogels. Such a blending strategy is easy to process as the encapsulation and preparation of MPs are realized separately. However, it is difficult to obtain a uniform magnetic nanoparticles dispersion in the hydrogel network using this method, and the magnetic nanoparticles tend to diffuse out of the hydrogels when immersed in a liquid solution, leading to a reduced response to an external MF. Another approach to prepare magnetic hydrogels is to generate covalent bonds between the polymer chains/crosslinker and the MPs by grafting functional groups onto the MPs surface. Such a strategy would facilitate the realization of uniform magnetic nanoparticles dispersion and decrease the chance of nanoparticles diffusion, thus overcoming the potential toxic effects of the diffused nanoparticles on the cells [23–25]. These magnetic hydrogels have demonstrated great potentials in enhancing cell growth either through the stabilization of growth factors or other biological agents bound to MPs or through mechanical stimulus via the interactions between MNPs and an alternating magnetic field (AMF). Magnetic hydrogels also find great applications in drug delivery, enzyme immobilization and cancer therapy.

Owing to the sensitive and controllable responsive properties of magnetic hydrogels to an external MF, many attempts have been made to engineer 3D cell microenvironment *in vitro* by using magnetic hydrogels, which is important for engineering complex tissue constructs and studying mechanobiology in a 3D environment [26]. One approach to achieve a spatiotemporal control of 3D cell microenvironment is to assemble the microscale cell-encapsulating magnetic hydrogels (M-gels) to form more complex cellular structures [17] or manipulate untethered M-gels that encapsulate heterogeneous components facilitated with hydrogel configurations under an external MF [17,18] Such M-gels are commonly composed of functional MPs with superparamagnetic property and configurability, which can be actuated by a system composed of magnets (static or dynamic MF). The magnetic force is applied directly to the MPs within the M-gels to induce translation. For instance, we recently reported a magnetic assembly strategy by utilizing a permanent magnet to assemble the M-gels and form complex tissue constructs *in vitro* [17]. In our study, the M-gels in an assembly chamber were exposed to an external MF by placing the sheet magnets in parallel. The M-gels were subsequently assembled into multi-row patterns, which retained their shape when continuously exposed to MF and remained intact configuration even after the MF was removed. 3D microgel constructs with multilayer spherical structures were easily obtained via assembling the M-gels onto the tip of the magnetic rod through a layer-by-layer approach (Fig. 2). Other complex 3D constructs, such as 3D flexible surfaces (e.g., arc and dome shapes), were also fabricated. Since the MPs were encapsulated into the M-gels without chemical bonding, the encapsulated MPs can be released from the photo-crosslinked gelatin methacrylate hydrogels with the hydrogel biodegradation [27]. Other methods have also been developed to fabricate complex tissue constructs in both 2D and 3D by manipulating M-gels. For example, an untethered magnetic micro-robot system composed of neodymium–iron–boron (NdFeB) particles and a polyurethane binder was fabricated and actuated through eight electromagnets (Fig. 3). Microgels in the assembly chamber were then pushed by micro-robots controlled by a house-made magnetic actuated system. Thus, a temporal and spatial control of the manipulation and assembly of the microscale cell-encapsulating hydrogels into reconfigurable heterogeneous materials in 2D and 3D settings can be achieved, without compromising the cell behaviors including

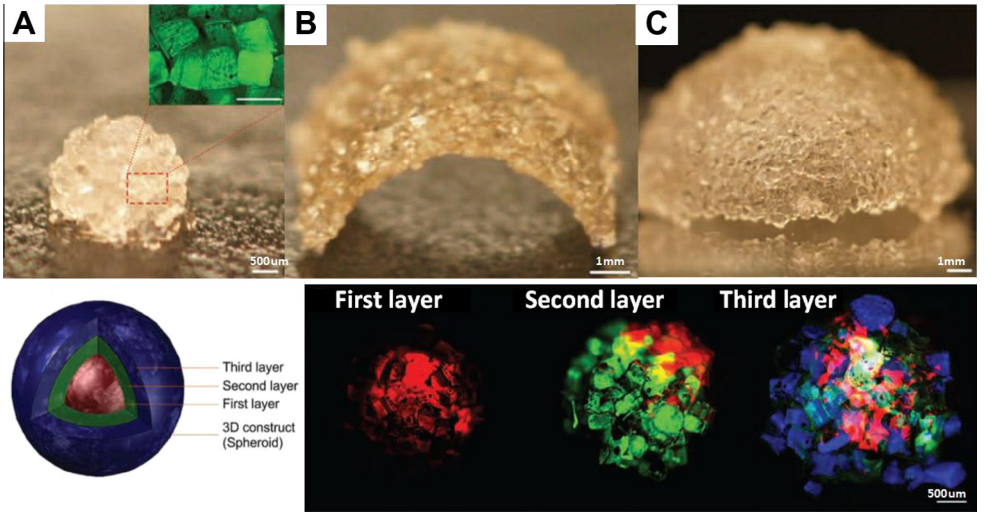

Fig. 2. 3D assembly of magnetic micro-scaled hydrogels by a MF. (A) Magnified image of the assembled single-layer construct; (B–C) images of arc- and dome-shaped constructs through a flexible surface and magnetic assembly; (D) merged fluorescent images of three-layer spheroids [17].

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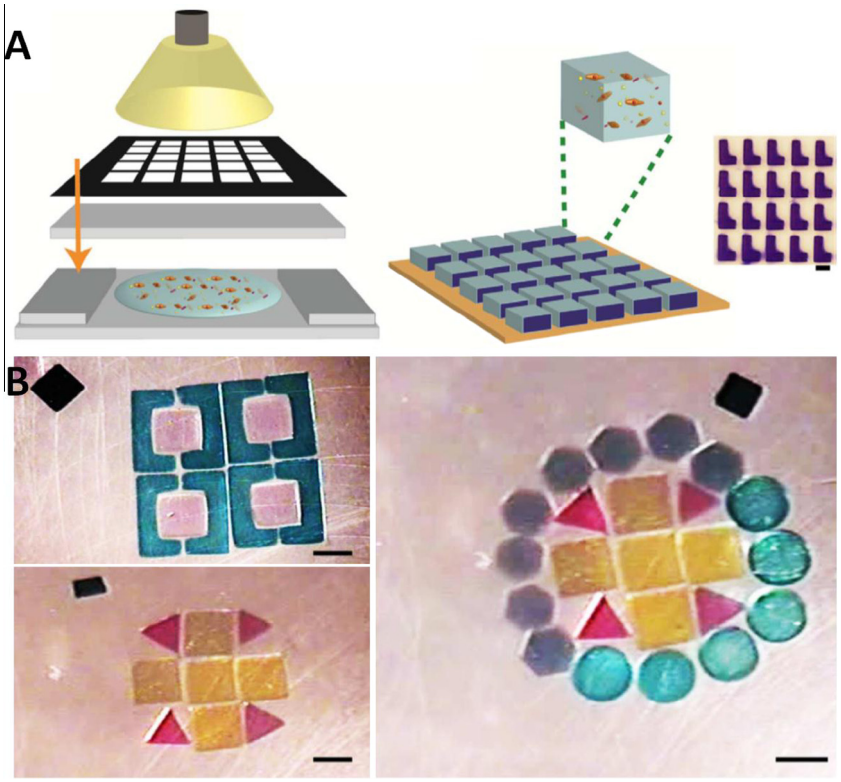

Fig. 3. Coding 2D complex functional materials using untethered magnetic micro-robots. (A) Fabrication of cell-encapsulating hydrogels via UV photocrosslinking; (B) micro-robotic coding and reconfiguration of poly(ethylene glycol) dimethacrylate hydrogels with various shapes into complex planar constructs. Scale bar, 1 mm [18].

viability and proliferation. Through regulating the developed magnetic field, its facile and easy to construct complex structural cellular microenvironment using cell-laden hydrogels in both 2D and 3D. In addition, it hold great potential in assembling hydrogels encapsulated with different types of cells, in which cell behavior can be regulated by growth factors secreted by others (smooth muscle cells and endothelial cells). Therefore, these magnetic micro-robots could provide capability for engineering cell microenvironment with multiple structures using individual cell-encapsulating hydrogel units, especially for tissue remodeling and mechanobiology in a 3D setting.

Additional efforts have also been made to mimic the cell mechanical microenvironment by using magnetic hydrogels. A typical example is the development of integrated M-gel systems, where the cell-encapsulating part and magnetic actuation part are connected to engineer the cell mechanical microenvironment *in vitro* [26]. For example, the fabrication of polydimethylsiloxane (PDMS) microcantilevers and the generation of M-gels on such cantilevers have been used to mimic cell mechanical microenvironment (Fig. 4). In this system, the cells are encapsulated in the M-gels in the PDMS arrays, containing thousands of microwells that each is composed of two microcantilevers separated by 500 μm. A 100 μm diameter nickel sphere was encapsulated into one side of the M-gel that was adhered to cantilever in each well to generate a magnetic pillar used for both static and dynamic actuation as well as loading to the cell-encapsulating M-gels. An external MF is also applied to cellular constructs to enable simultaneous quantification of both tissue stiffness and contraction force, which are two of the most critical mechanical parameters for guiding cellular biological behaviors [5]. The mechanical properties of the micro-tissues attributed from the cells and the collagen matrix were decoupled in both static and dynamic loading conditions, shedding new light on the mechanics of engineered tissues. Such a system holds great potential to investigate biomechanics of multiple types of tissues, and can also serve as a platform for high-throughput drug screening.

2.2. Photoresponsive hydrogels for engineering cell microenvironment

Among various stimuli-responsive hydrogels, photoresponsive hydrogels have drawn much attention owing to their unique and controllable property in response to photo stimulation, which makes it useful in a wide range of biomedical applications. Typically, photoresponsive hydrogels include photochromic chromophores as the photoreactive group within the 3D hydrogel network. Upon excitation, the photochromic moiety first captures the optical signal [28], which is then converted by the

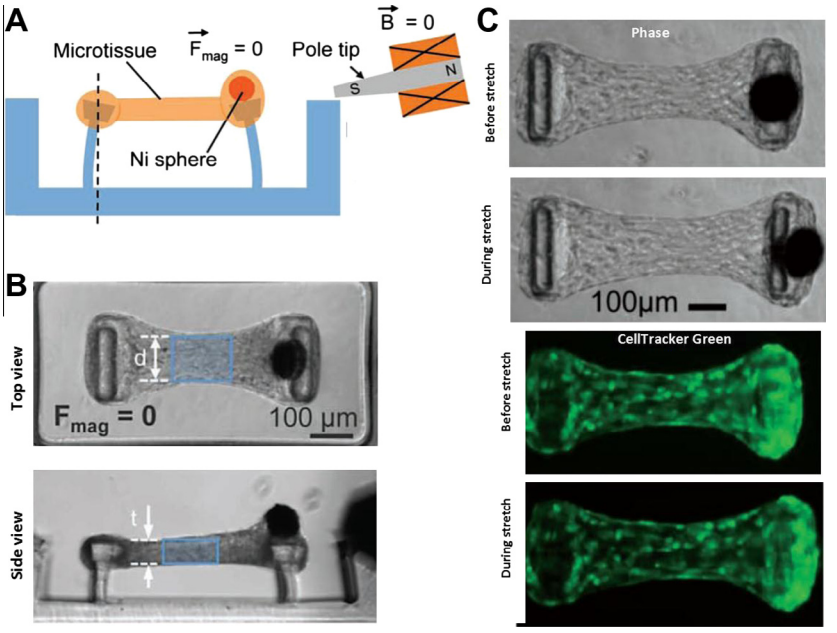

Fig. 4. M-gels composed of cell-encapsulating part and magnetic actuation part developed for mimicking cellular mechanical microenvironment. (A) Fabrication process for magnetic actuation hydrogels integrated with a PDMS mold; (B) a microscopy image of M-gels before and after magnetic stretch loading; (C) phase contrast and live cell labeled (CellTracker Green) images of microtissues before and during stretch [20].

chromophores in the photoreceptor to a chemical signal through photoreaction processes such as isomerization, cleavage and dimerization [29]. One excitation could induce multiple photoreaction processes depending on the types of the photoreactive groups that are sensitive to a certain irradiation wavelength (e.g., 4-methacryloyloxyazobenzene for photoisomerization at 366 nm [30], o-nitrobenzyl acrylate for photocleavage at 365 nm [31], and 4-methyl-(7-(methacryloyl)-oxyethyloxy) coumarin for photodimerization at >310 nm [32]). In photoresponsive hydrogels, diverse photoreactions have been used to tune the properties and functions of hydrogels, including degradability [33], reversibility [34], hydrophilia [35], adhesion [36] and polarity [37], which have made it applicable to a variety of biomedical applications [38–41].

Because of the sensitive response to an excitation at a specific light wavelength, photoresponsive hydrogels have been utilized to manipulate the cell microenvironment through either photodegradation or bulked. Upon a specific irradiation, the degradation process starts and results in the collapse of hydrogels and the breakage of hydrogel network at both macro- and micro-levels. For instance, Kloxin et al. synthesized PEG-based photodegradable hydrogels, which firstly went through photopolymerization processes in the presence of cells and then completely degraded in 10 min. The degradation rate can be precisely controlled by the irradiation wavelength and intensity. In addition, the human mesenchymal stem cells (hMSCs) encapsulated in the hydrogels changed to a more spread morphology from an original rounded shape with maintained cell viability through photodegradation during culture [33], which could be attributed to the reduced crosslinking density (Fig. 5A). Taking advantage of the photodegradable hydrogels, the authors further fabricated cell-culture substrates with tunable elasticity to investigate and identify the critical substrate elasticity that promotes or suppresses the activation of valvular interstitial cells (VIC) to myofibroblasts [42]. The elasticity of the hydrogel can be tuned by adjusting the irradiation time. Therefore, the photodegradable hydrogels can be utilized to dynamically engineer scaffolds to mimic the mechanical microenvironment of native extracellular matrix (ECM), therefore to direct cell migration, proliferation, and fate.

In addition, the photocrosslinkable hydrogels have also been utilized to manipulate the cell microenvironment. The crosslinking or polymerization process under photo irradiation can enhance the mechanical strength and stabilizes the hydrogel network for the cells encapsulated in the hydrogels. For instance, photocrosslinked hydrogels incorporating poly(NIPAAm-co-AAc) and sensitive peptide (QPQGLAK-NH_2) can act as an intelligent matrices for human embryonic stem cell (hESCs) to support short-term self-renewal and maintenance [43]. These hydrogels provide favorable matrix stiffness by adjusting the degree of crosslinking (Fig. 5B). Recently, a novel dual-photocrosslinked hydrogel system has been developed to study the proliferation and differentiation of human adipose-derived stem cells. In this study, micropatterning was utilized to create hydrogel regions crosslinked by different mechanisms and to manipulate the properties of hydrogels spatially by changing the ratio of crosslinked regions using different micropattern sizes. The results showed that micropattern sizes affect the growth and differentiation of the stem cells encapsulated within the

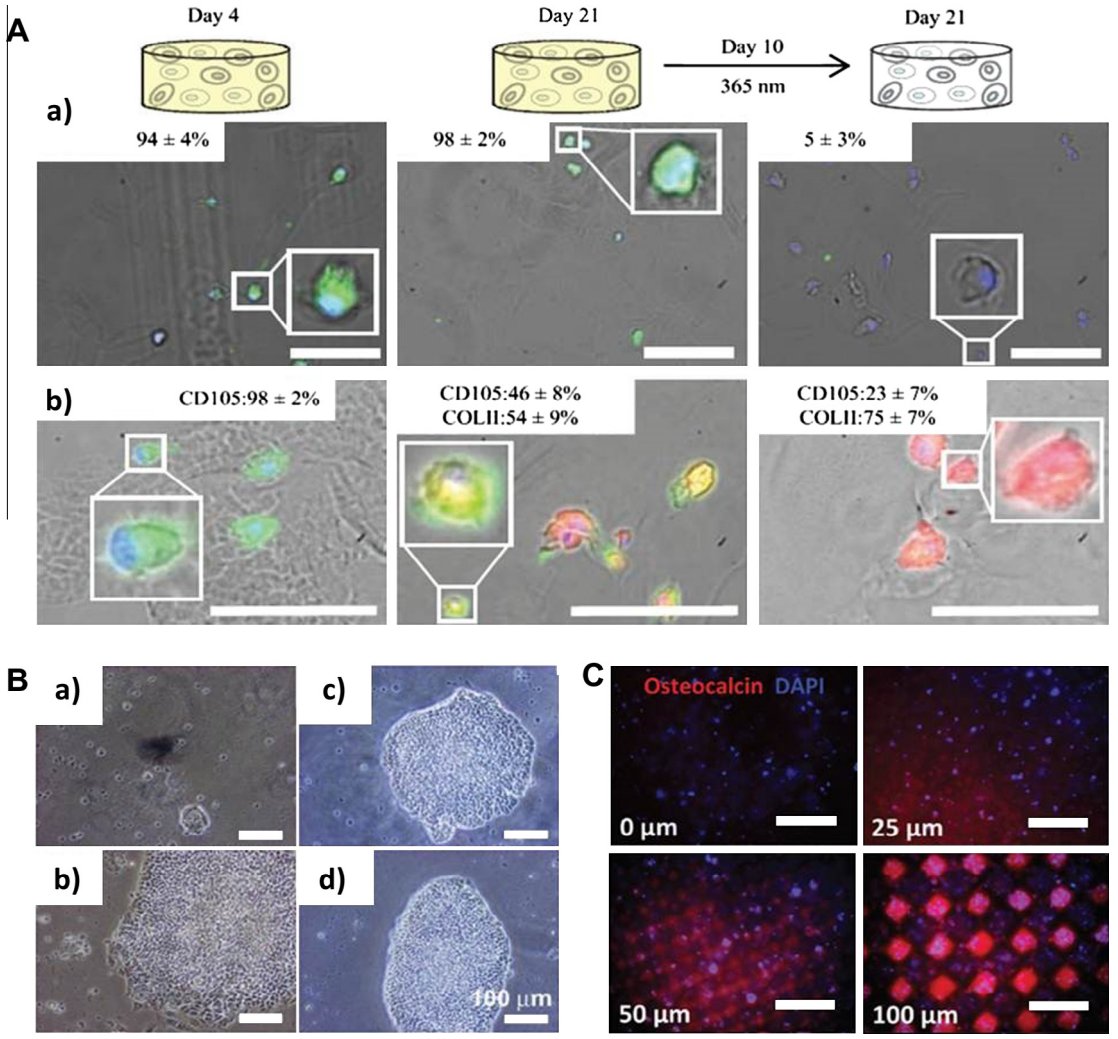

Fig. 5. Engineering cell microenvironment using photoresponsive hydrogels. (A) Chondrogenic differentiation of hMSCs on photodegradable hydrogel substrates for (a) 4 days; (b) 21 days; (c) 21 days with photo-stimulation on day 10, and the photodegradable process promotes the chondrogenic differentiation of hMSCs; (B) the hESCs cultured on photocrosslinkable hydrogels with adhesion ligand concentrations of (a) 0 μM; (b) 45 μM; (c) 105 μM; (d) 150 μM, and the hESCs exhibited tight borders and morphologies more similar to the undifferentiated hESCs with increased degree of crosslinking; (C) fluorescence micrographs of hASCs encapsulated in the micropatterned hydrogels with varied sizes through photocrosslinking (osteocalcin is shown in red and nuclei in blue) after 4 weeks of culture in the osteogenic differentiation media [33,42–44]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

hydrogels, which would be due to the varied hydrogel stiffness and transport properties [44] (Fig. 5C).

2.3. Conductive hydrogels for engineering cell microenvironment

Conductive hydrogels are another type of functional hydrogels, which can actively respond to an external electrical stimulation, and have attracted great attention for the applications of tissue engineering, regenerative medicine and drug delivery [16,45]. The conductivity of the hydrogels is usually realized by using either conductive polymers (i.e., conductive polymer-based hydrogels) or incorporating conductive composites into hydrogels (i.e., acid-doped or inorganics-added conductive hydrogels). Inorganic substances including graphite [35,36], carbon fibers, carbon nanotubes [39,40], and metal particles [37,38] have been extensively investigated for the development of conductive hydrogels. The conductive polymer-based conductive hydrogels are commonly constructed by combining the conductive polymers with other highly hydrated hydrogels through a crosslinking process.

The application of conductive hydrogel for engineering cell microenvironment has been motivated by the cellular response to the electrical stimulation observed both *in vivo* and *in vitro*. A variety of cells have been observed to possess the ability to transfer electrical signal and actively respond to external electrical stimulation both *in vivo* and *in vitro*, such as stem cells [46], neurons [47] and cardiomyocytes [48]. For instance, the heart muscles possess a

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DC conductivity of 0.1 S/m and *in vivo* the beating frequency of cardiomyocytes can be changed by an external electrical stimulation [49]. The signal conduction of the nerve tissue can also been affected by the electrical stimulation [50]. The electrical stimulation has also been found to affect a variety of cellular behaviors *in vivo*, such as the extension of the motile process, division, migration and differentiation [51]. In addition, the electrical stimulation has been found to affect the extension and the direction of the neurite outgrowth from the neurons and promoted nerve regeneration *in vitro* [52]. Electrical stimulation could also influence the differentiation of embryonic stem cells (ESCs) [53]. Various mechanisms have been proposed to explain the cellular response to electrical stimulation. It is believed that by changing the action potential of the cellular membrane, the electrical stimulation induces multiple intra-cellular activities, such as the redistribution of cytoplasmic materials, the activation of biomolecule transportation across the plasma membrane, the opening and closing of the voltage-dependent ion channels, and protein synthesis [54]. Therefore, engineering the cell electrical microenvironment *in vitro* is also important for tissue regeneration and maturation.

As the bioelectric phenomena are most significant for the heart and nerve system, conductive hydrogels have been widely explored to reconstruct the 3D cell microenvironment for the regeneration of cardiac and nerve tissues. Shin et al. developed a conductive hydrogel by incorporating carbon nanotubes (CNT) into a gelatin derivative hydrogel (GelMA) for cardiac tissue regeneration (Fig. 6) [49]. The adhesion, alignment and the maturation of the neonatal rat cardiomyocytes cultured on the CNT–GelMA hydrogel film were significantly improved compared to those on the non-conductive GelMA hydrogels. The incorporation of CNTs also promoted the cell–cell coupling and enhanced the overall contractive function of the cardiac tissue, as confirmed by the significantly higher expression of sarcomeric α-actin, connexin 43 (Cx-43) for cell–cell metabolic and electrical coupling. Upon electrical stimulation, the beating behavior of the cardiac tissues cultured on the CNT–GelMA hydrogels is more stable and the beating rate is 3 times of those on the GelMA ones. More

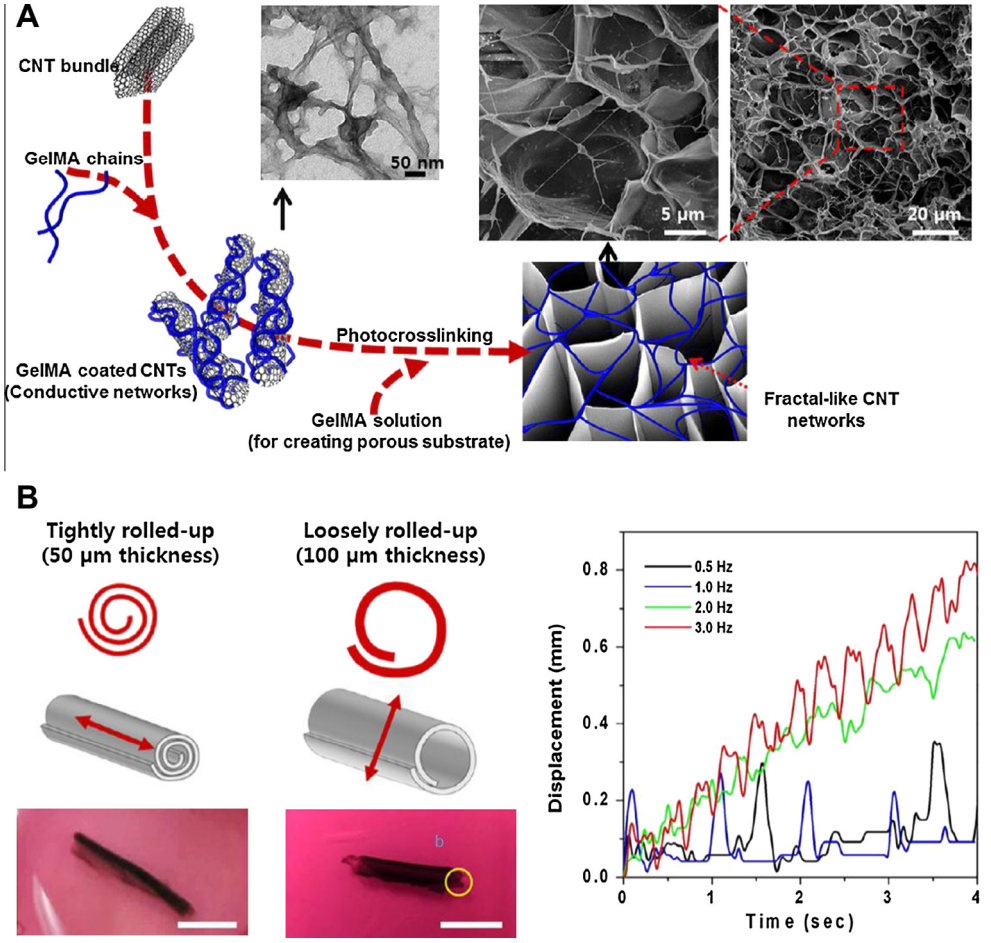

Fig. 6. CNT/GelMA for engineering cardiac tissues and bioactuators. (A) Preparation procedure of CNT/GelMA hydrogels; (B) two engineered cardiac patches form different shaped tubular actuators showing different corresponding beating directions and different pumping frequencies under electrical stimulation [47].

importantly, the electrical stimulation can dramatically reduce the excitation threshold (85% lower) of the cardiac cells cultured on the CNT–GelMA. This could be attributed to the improved cell–cell electrical coupling facilitated by the conductive CNT meshwork. In another example, single walled carbon nanotubes (SWNTs) were combined with gelatin to fabricate conductive hydrogels for the construction of cardiac tissues. After electrical stimulation for 5 days, the cells encapsulated in the SWNT/gelatin scaffolds form compact multi-cellular aggregates, while small sporadic aggregates were observed in the gelatin scaffolds [54]. Similarly, human cardiomyocytes cultured on a carbon nanofiber/poly-2-hydroxyethyl methacrylate (pHEMA) composite substrate showed improved cell adhesion and proliferation compared to those cultured on the pure pHEMA substrate [55].

Conductive hydrogels have also been investigated for engineering cell microenvironment to repair and regenerate the peripheral nerve or spinal cord [52]. Hydrogels incorporating electrically conductive materials have demonstrated great potentials to improve the nerve regeneration especially under an external electrical stimulation [56]. For instance, a novel electrically conductive hydrogel combining oligo (polyethylene glycol) fumarate (OPF) with polypyrrole (PPy) has been utilized to encapsulate PC12 cells for nerve regeneration, and exhibited improved cell attachment and neurite extension compared to the pure OPF hydrogels. More importantly, the quantification of neurite extensions suggested that the OPF–PPy hydrogel promoted more neurites per cell, longer neurites and higher amounts of cells bearing neurite extension. These results indicate the importance of the cell electrical microenvironment for enhancing cellular attachment and differentiation during nerve regeneration [57]. In another example, multi-walled carbon nanotubes were combined with poly(2-hydroxyethyl methacrylate)(pHEMA) for peripheral nerve repair. Human neuroblastoma SH-SY5Y cells seeded on the conductive hydrogel and pure pHEMA hydrogels were subject to an electrical stimulation with a potential of 1 V or 2 V, and only the cells on the mwCNT loaded membrane survived, indicating the protection function of the encapsulated mwCNT to the cells (Fig. 7A) [58]. In addition to cardiac and nerve tissue engineering, conductive hydrogels have been reported to engineer cell microenvironment for artificial muscle regeneration. A composite hydrogel composed of poly (ε-caprolactone), polyacrylic acid/polyvinyl alcohol and mwCNTs was developed for muscle tissue construction. The artificial muscle tissue was activated with motion when exposed to the electrical stimulation, and exhibited improved cell proliferation compared to non-conductive control (Fig. 7B) [59].

2.4. Other functional hydrogels for engineering cell microenvironment

Besides the functional hydrogels discussed above, other hydrogels with properties such as temperature response, molecule response and high stretchability have also been utilized to engineer cell microenvironment.

2.4.1. Thermoresponsive hydrogels

Temperature sensitive hydrogels can sense the external temperature changes and undergo a swelling/shrinkage transition. These hydrogels usually shrink when the temperature is above the Lower Critical Solution Temperature (LCST) and swell when the temperature drops below the LCST. The shrinkage behavior is attributed to the enhanced hydrophobic interactions among the inter-polymer chains resulted from the decreased water solubility of these hydrogels with increasing temperature [60,61]. Due to these unique properties, many attempts have been made to engineer 3D cell microenvironment using thermoresponsive hydrogels. One approach is to manipulate the cell mechanical microenvironment through the deformation of hydrogels induced by temperature changes. For instance, the 3T3 mouse fibroblasts encapsulated within poly (Nisopropyl acrylamide) (PNIPAAm) hydrogels underwent a stretching process due to the hydrogel being swollen by the decrease of external temperature [62]. The mechanical stimulation as induced by temperature change has been demonstrated to regulate the cell morphology, such as the formation of a paxillin-containing fibrous structure in the cell cytoplasm and filopodia-like structure at the peripheral regions [62]. Another strategy is to modulate the hydrophobicity of the thermoresponsive hydrogels by temperature changes, which could change the absorption of cell-adhesive proteins. For example, the pNIPAM hydrogel-coated substrates have been demonstrated with the capability to support the attachment and spreading of cells when the temperature is increased above the LSCT, while they lose the ability leading to the cell detachment from the substrate when the temperature drops below the LSCT (Fig. 8). This is due to the fact that the increased hydrophobicity with temperature increase can provoke the absorption of proteins to the coating surface, thus regulating the cell biological microenvironment to facilitate cell attachment and spreading. However, the thermoresponsive coating starts to swell and hydrate when the temperature drops, thus resulting in weak binding of the cells to the hydrogel coating [63].

2.4.2. Molecule-responsive hydrogels

Molecule sensitive hydrogels usually undergo a swelling process in response to certain specific molecules, such as glucose [64], enzyme [65] and antigen [66], which can be utilized to manipulate cell microenvironment for various applications. Specifically, enzyme-sensitive hydrogels are designed based on the enzyme-digesting process of certain biodegradable polymers, which has been used to modulate cell mechanical microenvironment through enzyme-sensitive degradation of hydrogels [60]. For instance, a matrix metalloproteinase 7 (MMP7)-sensitive hydrogel has been developed by incorporating MMP7 in the backbone of poly (ethylene glycol) diacrylate (PEGDA) hydrogels to encapsulate hMSCs [65]. Thus the expression of MMP7 within the hydrogels has a temporal pattern corresponding to the cartilage development. The encapsulated hMSCs within the enzyme-sensitive degradable hydrogels formed neocartilage constructs with more

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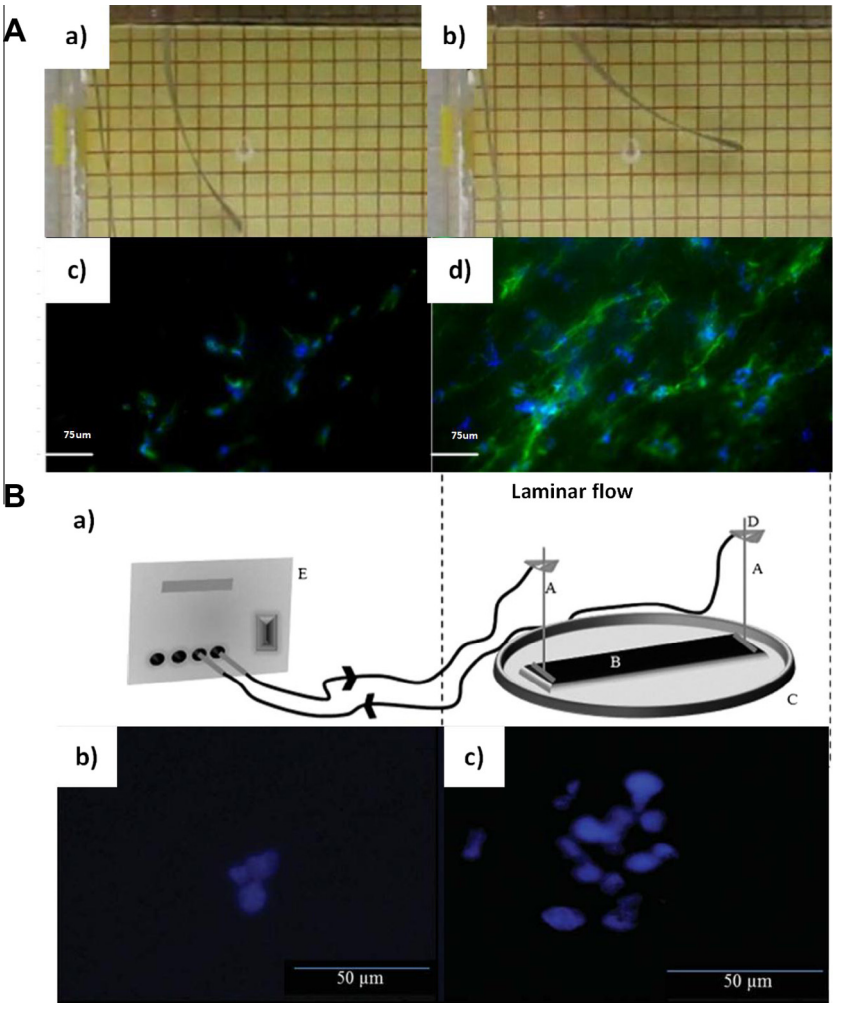

Fig. 7. Conductive hydrogels for skeletal muscle regeneration and peripheral nerve repair. (A) Multi-component scaffolds for skeletal muscle regeneration (a and b) artificial muscle strips exposed to electrical stimulation (a) initially; (b) first bend; (c and d) fluorescent staining images (cytoskeletal actin is shown in green and nuclei in blue) revealing better proliferation on the PCL–MWCNT-H scaffolds (d) than the PCL (c). (B) Multi-walled CNT–pHEMA composite conduits for peripheral nerve repair (a) the setup for electrical stimulation to the cells seeded on the mwCNT–pHEMA membranes; (b and c) Fluorescence micrographs of 24 h culture of cells on the membranes; (b) pHEMA membrane with 1 V electrical potential application; (c) mwCNT–pHEMA membrane with 1 V of electrical potential [56,57]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

extensive regeneration of collagenous matrix compared to the non-degradable scaffold.

2.4.3. Tough and stretchable hydrogels for engineering cell microenvironment

Despite many superior properties of hydrogels, most hydrogels cannot stand an extensive mechanical loading such as stretching. Therefore, the practical applications of conventional hydrogels are limited by the low mechanical strength, especially as structural materials [67]. The poor mechanical properties are mainly due to the inhomogeneity of polymeric structures, which can be categorized into motility, spatial, topological and connectivity inhomogeneity. The networks may break from the weakest link if the networks fail to behave cooperatively, leading to reduced mechanical strength [68]. Great efforts have been made to improve the mechanical properties of hydrogels by designing unique network structures, including topological hydrogels with sliding linkers [69], hydrogels with double-networks [70], and homogeneous-structural hydrogels fabricated from tetrahedron-like macro monomers (Fig. 9A). These mechanically enhanced hydrogels have been explored to engineer cell mechanical microenvironment through tuning the stiffness and the stress that cells experience to modulate cell behaviors. For instance, Annabi et al. developed a highly elastic methacrylated tropoelastin (MeTro) hydrogel, which exhibits remarkable mechanical property such as reversible deformation with a low energy loss and high resilience on stretching with extensibility up to 400% (Fig. 9C). By adjusting the methacrylation degree and the concentration of MeTro, hydrogels with elastic moduli and ultimate strengths can be tuned.

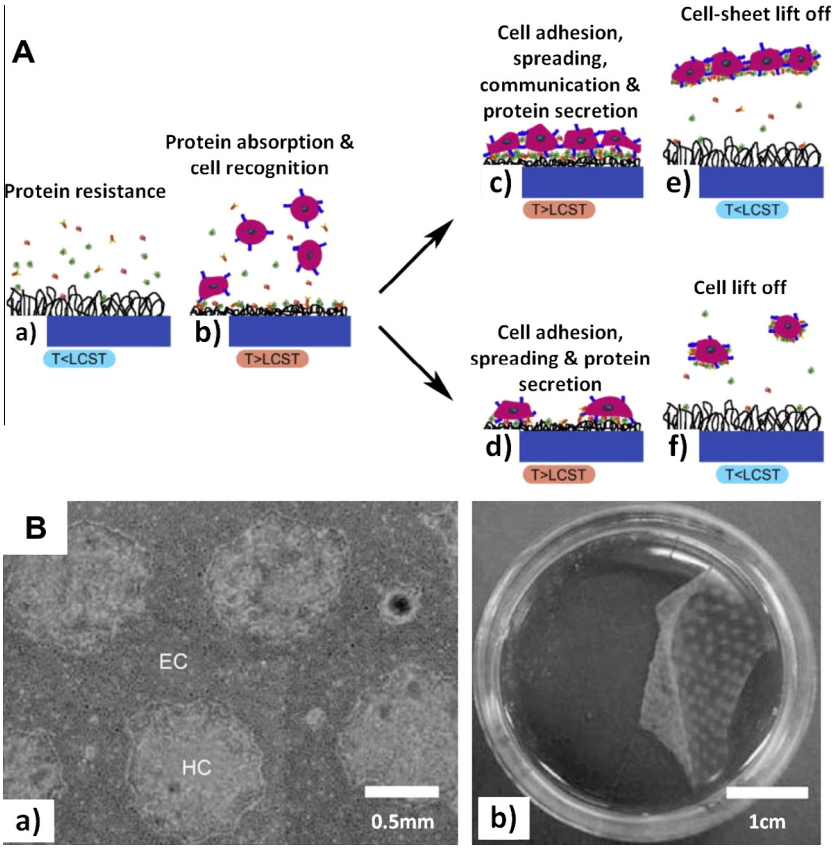

Fig. 8. Thermoreponsive cell sheet attachment and release. (A) A model for the cellular attachment and release; (B) endothelial cells (ECs) adhere to pNIPAM at 37 °C and detaches as a monolayer when temperature drops [61].

The neonatal rat cardiomyocytes exhibited preference to a MeTro hydrogel with a tensile modulus of 15 kPa than a GelMA hydrogel with better cell adherence and spreading, and the formation of a layer of elongated cells compared to scattered cell clusters on a GelMA hydrogel. This could be attributed to the matching mechanical property that MeTro hydrogels provided to the native myocardium [71].

3. Conclusions and future perspectives

Cells *in vivo* reside in a dynamic and complex microenvironment, which provides the cells with mechanical integrity, physical structure and biochemical components. Hydrogels have become an attractive model to mimic the cell microenvironment *in vitro*. The complex and dynamic properties of cell microenvironment require developing hydrogels with more functions, such as magnetic response, electrical conductivity and photo response. Magnetic hydrogels can be applied to engineering 3D cellular microenvironments owing to the sensitive and the controllable properties to an external MF. Photoresponsive hydrogels can be employed to spatially control the bioactivity and mechanical properties as well as degradability on the microscale by methods such as photopatterning, UV triggered cleavage, polymerization and isomerization. The electrical stimulation can influence the contraction of cardiomyocytes and nerve regeneration, which makes the conductive hydrogels especially important.

Although functional hydrogels have demonstrated great capability in manipulating dynamic and complex cell microenvironment *in vitro*, these are still several challenges. For instance, the distribution of the added functional molecules and nanomaterials within the hydrogels may vary due to their aggregation or precipitation in hydrogel precursors, which would lead to difficulties in precise spatial control of the cell microenvironment through external stimulation. Another challenge is to achieve a temporal control of cell microenvironment along tissue development and maturation, as the biological and mechanical microenvironment changes at different cell differentiation and tissue developmental stages. Moreover, the presence of the functional molecules and nanomaterials within the hydrogels may create other concerns for the applications. For example, the heat generated from the MPs exposed to the alternating current (AC) based MF may have a negative effect on the cells. Therefore, hydrogels endowed with multiple functionalities and the ability for precise control of the cell microenvironment spatially and temporally are greatly desired for future studies in the engineering of cell microenvironment.

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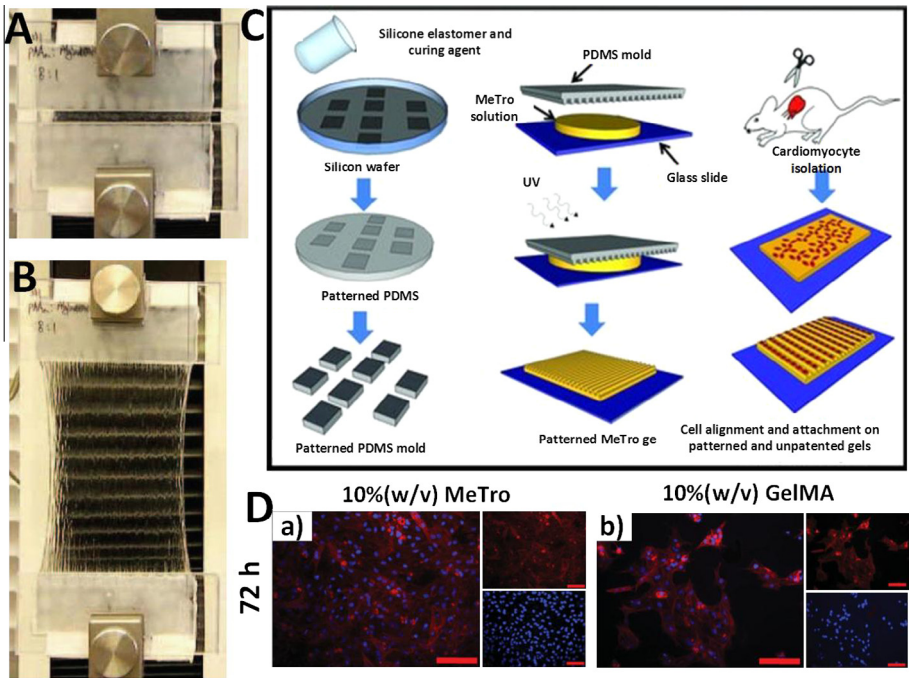

Fig. 9. Tough hydrogels with high stretchability show influence on cell attachment and spreading. (A–B) images revealing high stretchability of hydrogels; (C) fabrication of the highly elastic micropatterned hydrogels; (D) cardiomyocytes cultured on 10% (w/v) MeTro hydrogels demonstrate better cell attachment and spreading than on GelMA at different culture times (smaller panels show F-actin (top) and cell nuclei (bottom) stained samples) (scale bar, 100 μm) [65,69].

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