Progress in Polymer Science 37 (2012) 645–658

Contents lists available at SciVerse ScienceDirect

Progress in Polymer Science

journal homepage: www.elsevier.com/locate/ppolysci

Trends in polymer science

Polymers used to influence cell fate in 3D geometry: New trends

Yukie Aizawa[a,1], Shawn C. Owen[a,b,1], Molly S. Shoichet[a,b,c,*]

[a] Department of Chemical Engineering & Applied Chemistry, University of Toronto, Ontario M5S 3E5, Canada
[b] Institute of Biomaterials & Biomedical Engineering, University of Toronto, Ontario M5S 3G9, Canada
[c] Department of Chemistry, University of Toronto, Ontario M5S 3H6, Canada

ARTICLE INFO

Article history:
Received 7 June 2011
Received in revised form 8 November 2011
Accepted 14 November 2011
Available online 19 November 2011

Keywords:
Polymer scaffold
Synthetic extracellular matrix
Tissue engineering
Biological models

ABSTRACT

The extracellular matrix (ECM) is a hydrogel-like structure comprised of several different biopolymers, encompassing a wide range of biological, chemical, and mechanical properties. The composition, organization, and assembly of the ECM play a critical role in cell function. Cellular behavior is guided by interactions that occur between cells and their local microenvironment, and this interrelationship plays a significant role in determining physiological functions. Bioengineering approaches have been developed to mimic native tissue microenvironments by fabricating novel bioactive hydrogel scaffolds. This review explores material designs and fabrication approaches that are guiding the design of hydrogels as tissue engineered scaffolds. As the fundamental biology of the cellular microenvironment is often the inspiration for material design, the review focuses on modifications to control bioactive cues such as adhesion molecules and growth factors, and summarizes the current applications of biomimetic scaffolds that have been used *in vitro* as well as *in vivo*.

1. Introduction

Tissue engineering has been traditionally described as the process of creating functional three-dimensional (3D) tissues using scaffolds or devices that facilitate cell growth, organization, and differentiation [1]. In particular, scaffolds made from natural and synthetic polymers have been used to drive the formation and maintenance of 3D tissue structures that can be tailored to specific applications for the repair or regeneration of tissues and organs [2]. The interactions of cells and biomaterials comprise a dynamic regulatory system responsible for tissue regeneration, thus 3D biomimetic scaffolds with spatially controlled features are essential in tissue development for ultimate applications in regenerative medicine [3]. Over the last decade, bioengineering approaches have been developed to mimic the cellular microenvironment, thereby providing insight into the natural interactions between cells and their environment [4–8]. In particular, hydrogels have been studied intensively and used as tissue engineering scaffolds because they can provide a hydrated, three-dimensional environment similar to soft tissues that allow the diffusion of nutrients and cellular waste through elastic networks [9,10]. There is a growing, insightful body of literature demonstrating the significant differences in cell phenotype that arise when cells are cultured on traditional two-dimensional (2D) surfaces as compared to their native 3D microenvironment [11–19]. We do not emphasize this transition in cell culture strategies, but instead focus on the 3D geometry.

This review explores some of the material designs and fabrication approaches that are leading the development of 3D bioactive hydrogels as tissue engineering scaffolds. As the fundamental biology of the cellular microenvironment is often the inspiration for material design, the review focuses on hydrogel syntheses and modifications that mimic the extracellular matrix (ECM), with a

* Corresponding author at: Donnelly Centre, University of Toronto, 160 College Street, Room 514, Toronto, ON, M5S 3E1, Canada.
Tel.: +1 416 978 1460; fax: +1 416 978 4317.
E-mail address: molly.shoichet@utoronto.ca (M.S. Shoichet).
[1] Equal contribution.

0079-6700/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.progpolymsci.2011.11.004

particular focus on controlling bioactive cues such as adhesion molecules and growth factors. The review also summarizes current biomimetic scaffolds that have been used *in vitro* as well as *in vivo*.

2. The extracellular matrix

The environment surrounding cells in tissue is a complex network composed of proteins, carbohydrates and growth factors called the ECM. The dynamic, heterogeneous composition of the ECM provides a wide range of biological, chemical, and mechanical properties [20–22]. The structure of all organs is comprised of specific cells and ECM components organized to facilitate its physiological functions [23]. The specific composition and architecture unique to each tissue is beyond the scope of this review. Instead, we focus on several ubiquitous ECM properties central to tissue engineering.

The ECM generally includes two classes of proteins: insoluble and soluble [24]. Collagen is the major insoluble fibrous protein in the ECM and accounts for nearly 30% of all proteins found in the body [25]. Of the 28 different types of collagen, 80–90% consist of types I, II, III, and IV. Collagens are distinguished by their ability to form fibers with high tensile strength and to organize into networks, providing structural and connective support for tissue [26,27]. The ECM contains two major soluble proteins: multiadhesive matrix proteins and proteoglycans [28]. Multiadhesive matrix proteins have multiple domains that interact with various types of collagen and cell surface adhesion molecules, and are responsible for attaching cells to the ECM and initiating various cellular responses [21,24,28,29]. Laminin and fibronectin are two important multiadhesive proteins in the ECM [21,30–32]. With 15 types identified, laminin is the most abundant constituent of basal lamina after type IV collagen [20]. Fibronectin is primarily involved in attaching cells to all matrices that contain fibrous collagen [20]. Importantly, the most common protein mimetic peptide, RGD, is used for cell adhesion in tissue engineering and is based on the cell recognition site of fibronectin [33,34].

Proteoglycans are another major constituent of the ECM. The basic proteoglycan contains a core protein with one or more covalently bound polysaccharide chains [21]. Many ECM and cell surface proteoglycans facilitate cell–matrix interactions and help present certain growth factors to their cell-surface receptors [21]. Hyaluronan (HA) is an extremely long (5–20,000 kDa), negatively charged polysaccharide which forms highly hydrated gels [35].

Together, these major ECM components play a critical instructive role in mediating key cell functions such as: cell adhesion, growth factor binding, proteolytic degradation, and mechanical support [21,24,28,36]. Overall, the composition, organization, and assembly of these constituents at the molecular level give the ECM its properties, which is unique for each tissue type [36]. Cellular behavior is guided by interactions that occur between cells and their local microenvironment, and this interrelationship plays a significant role in determining physiological functions [20,21]. Therefore, the natural ECM is an attractive model for design and fabrication of bioactive scaffolds for tissue engineering.

3. Mimicking the extracellular matrix

3.1. Scaffold fabrication

This section addresses the recent progress in material design and fabrication approaches that have led to the development of bioactive three-dimensional hydrogels as tissue engineering scaffolds.

Poly(ethylene glycol) (PEG) is the most widely investigated synthetic polymer used for scaffold fabrication [2,11,37–40]. Current PEG hydrogels utilize both linear and branched forms of the polymer and take advantage of the plethora of functional groups that have been incorporated onto PEG terminal end groups, including: acetylene, acrylate, amine, azide, carboxyl, and thiol. For example, the Marra lab investigated 4- and 8-arm PEG hydrogels as injectable scaffolds [41]. Amine-terminated PEG was crosslinked with genipin and tested for gelation time, swelling, and the level of cell adhesion. Results show that the degree of cell adhesion on 4-arm PEG hydrogels was significantly greater than that on 8-arm gels.

Sulfated glycosaminoglycans (GAGs) are among the main biopolymers found in the ECM [42,43]. Hyaluronan is a ubiquitous non-sulfated GAG, and present in all connective tissue as a major constituent of the ECM [43,44]. HA has a key role in morphogenesis and is therefore an important factor in tissue engineering [44]. Recently, Shoichet and co-workers [45] designed covalently crosslinked HA gels by taking advantage of Diels–Alder click cycloaddition chemistry. HA was modified with furan functional groups and reacted with difunctional maleimide-PEG furan to yield HA crosslinked gels where the mechanical and swelling properties were tuned by the amount of crosslinker as depicted in Fig. 1. Previously, Prestwich and co-workers [46] developed a covalently crosslinked, synthetic ECM using HA. In this approach, the disulfide hydrazide 3,3′-di(thiopropionyl) bishydrazide (DTPH) first modifies the carboxyl groups of HA, chondroitin, or heparin. Second, the disulfide bonds are reduced with dithiothreitol (DTT) to give the thiol-modified macromonomers such as HA-DTPH, chondroitin-DTPH, and heparin-DTPH. Third, the monomers are crosslinked by the electrophilic addition of thiol–ene to form hydrogels [42]. Fig. 2 illustrates the basic chemistry of the three-step process. Alternatively, crosslinking with difunctional electrophiles can be accomplished in the presence or absence of cells, to give biocompatible hydrogels. Using this strategy, Prestwich and co-workers [46] further demonstrated the synthesis of cell-adhesive hydrogels by crosslinking thiol-modified gelatin (gelatin-DTPH) with HA-DTPH to support cell attachment, growth and proliferation in 3D culture. Their work has expanded to include the development of matrices composed of co-crosslinked HA-DTPH, chondroitin-DTPH and heparin-DTPH, and more recently formulations with thiol-modified HA, gelatin, and heparin in order to control the release rate of growth factors [47].

Alginate is a natural polymer, with a composition similar to glycosaminoglycan, the main component of

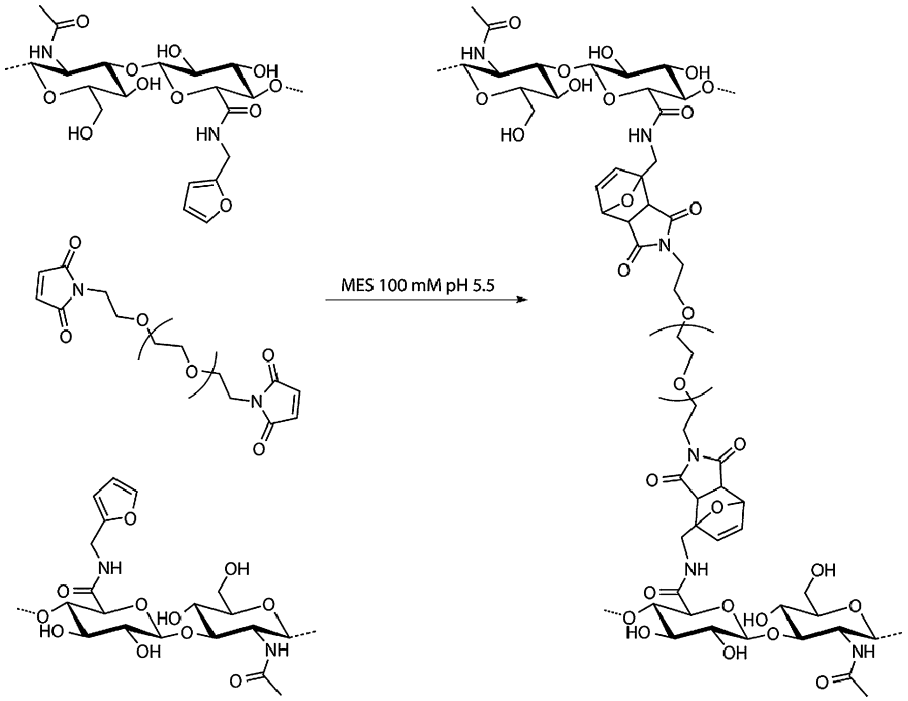

Fig. 1. Schematic representation of the formation of the Diels–Alder HA-PEG hydrogels by crosslinking HA-furan with $(maleimide)_2$-PEG [45].

native ECMs in tissue [48]. Alginate is a naturally derived polysaccharide composed of linearly assembled (1–4) linked β-mannuronic acid (M) and α-L-guluronic acid (G) monomers [48]. Alginate gels are formed when blocks of G-monomers and divalent cation (e.g., Ca^{2+}) interact to form ionic bridges between different polymer chains [48]. Alginate gels are considered biocompatible and have been used to transplant cells in a variety of applications, yet the degradation of typical alginate hydrogels is very slow and poorly controlled [49–51]. Chitosan, a deacetylated form of chitin derived from the shells of crustaceans and exoskeletons of some arthropods, has been extensively studied in the past decade to form hydrogels, scaffolds, and fibers for tissue engineering applications [52,53]. Chitosan is an attractive material because of its biodegradability, biocompatibility, antibacterial, and wound-healing properties. By varying the degree of acetylation, the degradation rate and the level of cell adhesion can be modified [52,53]. Li et al. recently designed 3D scaffolds formed from chitosan–alginate complexes by ionically bonding the amine group of chitosan with the carboxylate group of alginate and then further crosslinking the gel with divalent calcium ions, Ca^{2+}, thereby forming ionic bridges. The physically crosslinked gel shows improved mechanical strength compared to gels of chitosan or aliginate alone [54]. This highly porous chitosan–alginate scaffold supported sustained self-renewal of human embryonic stem cells without feeder cells, demonstrating the potential for engineered scaffolds to be used for both defined *in vitro* cultures and implantation of stem cell populated scaffolds in tissue engineering [54].

Polypeptide-based ECM hydrogels have generated significant attention recently as they can be specifically engineered. For example, Heilshorn and co-workers [55] reported a genetic strategy to prepare polypeptide-based ECM hydrogels. In this study, multiple repeats of tryptophan-rich and proline-rich peptide domains were encoded in a modular genetic construct using recombinant protein technology. The two domains associate into anti-parallel β-sheet structures to form a physically crosslinked hydrogel. This strategy allows simple and gentle cell encapsulation without compromising cell viability and without the use of any crosslinking agents or environmental triggers. Similar results have been shown in other studies in the development of well-defined, three-dimensional structures based on molecular interactions (hydrogen bonds, disulfide bonds, electrostatic and ionic interactions, etc.) [56]. In another example, Stupp and co-workers [57–59] developed peptide amphiphilic assemblies, which formed long cylindrical nanofibers that crosslinked in the presence of salts. Importantly, the nanofibers formed highly oriented hydrogels via a liquid-crystalline phase, resulting in guided cell growth *in vivo* [58]. Due to strong inter-fibril interactions, the hydrogels also exhibited high stability [58]. This injectable material supported the growth of blood vessels along the fiber axis as post-infarct therapies and healing of critical wounds [60], bone and cartilage regeneration [61], and axon regeneration in spinal cord injury [62].

3.2. Controlling mechanical properties

The ECM acts as a mechanical support to organize cells into specific tissues and control cell behavior.

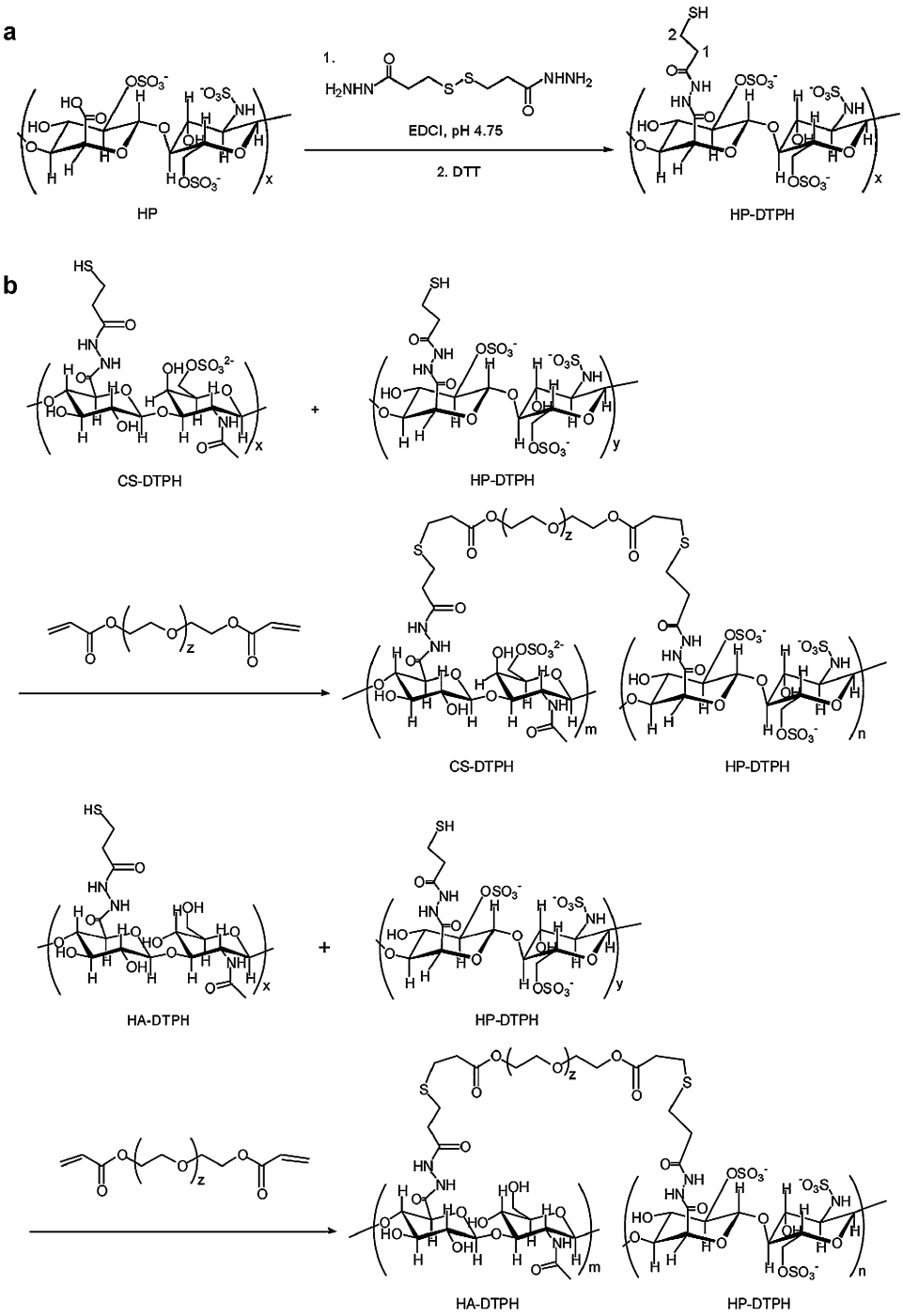

Fig. 2. (a) Chemical modification of heparin to give HP-DTPH. (b) Crosslinking of GAG-DTPH by addition of PEGDA [46].

Tissues are exquisitely sensitive to mechanical forces (e.g., hemodynamic forces in blood vessels, and tension in skin and muscle), which are transmitted through the ECM to individual cells [36]. Many studies have investigated the influence of mechanical stimuli on cell shape over the past years, demonstrating that cell shape is intimately related to gene expression [3,10,15,63,64]. The molecular mechanisms by which cell shape change is translated into biochemical signals are starting to be understood and several studies suggest that cells can be switched between entirely different gene programs through alterations of ECM structure or mechanics, independent of growth factor or integrin binding [15,63]. In order to mimic the mechanical aspects of natural tissue, collagen (the most

abundant protein in mammals) has been used to enhance the functionality of engineered tissues. Comprised of triple α-helices, collagen self-assembles to form a fibrillar structure [65], which has been taken advantage of in the design of engineered tissue scaffolds. However, collagen-based scaffolds are weak in nature and contract extensively during gelation and cell encapsulation [26,27]. One group, led by Khademhosseini, has sought to address both of these drawbacks by mixing collagen with photo-crosslinkable methacrylated hyaluronic acid (MeHA) [64]. By changing the methacrylation and concentration of the HA, the moduli of the hydrogels can be varied over three orders of magnitude and the authors demonstrated that these composite hydrogels achieve an increase in strength, failure stress, and stiffness and thus provide better mechanical control than collagen alone. Enhanced mechanical properties also resulted in higher levels of NIH-3T3 cell viability (>75–85%) upon cell encapsulation.

Important work by Discher and co-workers [66] demonstrated that the mechanical properties of the matrix impact the differentiation profile of mesenchymal stem cells (MSCs) in two-dimensional culture. By preparing collagen-coated polyacrylamide gels that mimicked the elasticity of various tissues, the MSCs differentiated into lineages that corresponded to the stiffness of the native environment. Recent studies by Schaffer and co-workers [15] also demonstrate the dependence on matrix rigidity for adult neural progenitor cells derived from the hippocampus. The authors synthesized interpenetrating polymer networks (IPNs) through the sequential, free-radical polymerization of poly(acrylamide) using TEMED and ammonium persulfate as catalysts. From this approach, the authors were able to vary the modulus within a range of 10–10,000 Pa, while allowing for surface modification of the gel networks to modulate cell adhesion (Fig. 3). These results suggest that neural progenitors are directed to neurons on softer gels (100–500 Pa) and glial cells on harder gels (1000–10,000 Pa).

Interestingly, using neural stem/progenitor cells derived from the subventricular zone of adult rats, Shoichet's lab found similar results using methacrylamide-modified chitosan where rigidity was controlled using a photolabile crosslinker [67]. In order to gain insight into the mechanism of response to cells in 3D culture, the Shoichet lab modified methacrylamide chitosan scaffolds with cell adhesive RGD peptides and interferon-gamma [68], which had been shown to promote differentiation of neural stem/progenitor cells to neurons [69]. Scaffolds with encapsulated cells were photo-crosslinked using the cytocompatible photo-initiator 2,2-dimethoxy-2-phenylacetophenone. The NSPCs within modified gels differentiated preferentially to neurons.

To gain further perspective on the role of mechanical properties in 3D, the Anseth group [70–72] has been exploring chemical modifications of poly(ethylene glycol)-based hydrogel materials. Several crosslinking methods have been used to synthesize PEG hydrogels, including Michael-type addition and click chemistry [45,73]. In the work by Anseth and co-workers [71], a photocleavable crosslinking diacrylate macromer was first synthesized by attaching a photodegradable, nitrobenzyl ether-derived moiety, to PEG-*bis*-amine. Photodegradable PEG hydrogels were then synthesized by redox-initiated, free radical polymerization with PEG monoacrylate in phosphate-buffered saline (PBS) (Fig. 4). Upon UV irradiation, the PEG is released. As the storage modulus is proportional to the hydrogel crosslinking density, the degradation rate and resulting material properties, such as stiffness, were predictably manipulated with light intensity and wavelength. The authors demonstrated that the morphology of encapsulated human mesenchymal stem cells (hMSCs) was regulated in real-time using these photodegradable PEG hydrogels. Anseth et al. recently suggested a sophisticated alternative strategy to synthesize PEG hydrogels with tunable moduli using copper-free, azide–alkyne click chemistry. In this approach, a 4-arm PEG tetraazide is reacted with a bis(cyclooctyne)-peptide to form multifunctional hydrogels [72]. By using the biocompatible cyclooctyne molecule, the cycloaddition of alkyne–azide occurs in the absence of a catalyst. Furthermore, crosslinking can be controlled spatially and temporally with

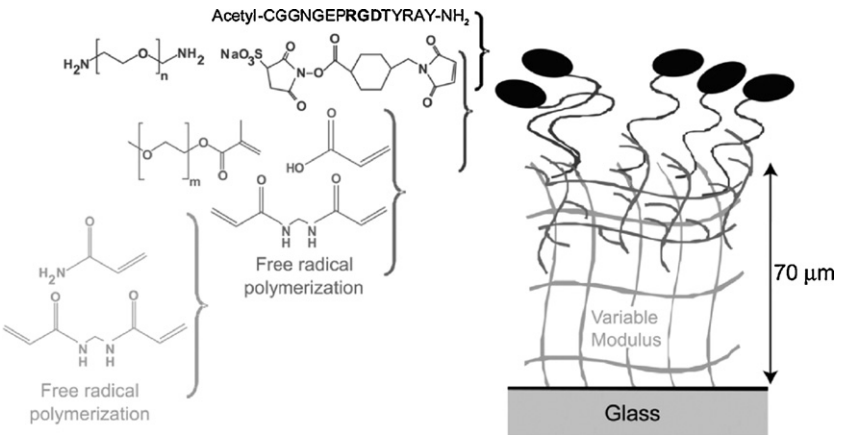

Fig. 3. Schematic of sequential polymerizations and grafting chemistry used to synthesize vmIPN [15].

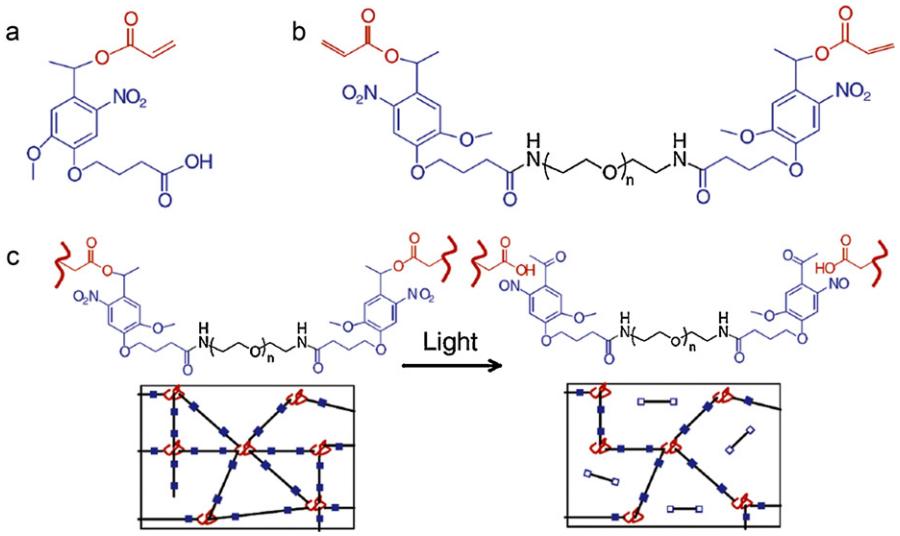

Fig. 4. Synthesis of photodegradable hydrogel for tuning gel mechanical properties. (a) The base photodegradable acrylic monomer. (b) The photodegradable crosslinking macromer composed of PEG (black), photolabile moieties (blue), and acrylic end groups (red). (c) Macromer was polymerized with PEGA creating gels connected by PEG with photolabile groups (blue boxes) [71]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

exposure to light. The mechanical properties of the gel can be tuned by altering the length of the PEG arms as well as the stoichiometric ratio of azide:alkyne on the PEG and peptide crosslinker, respectively. For example, the gels containing the lowest molecular weight PEG showed the highest moduli and lowest swelling whereas those with the highest molecular weight PEG provided the lowest moduli and highest swelling. Through the bioorthogonal thiol–ene click reaction, biomolecules were also conjugated to the hydrogel backbone without changing the network structure.

3.3. Regulating matrix degradation

In addition to hormonal cues and biochemical signaling, the proteolytic degradation of the natural ECM is an essential feature of a variety of biological processes, such as cell migration, tissue repair and remodeling. Most ECM proteins, including collagen, fibrin, fibronectin, and laminin have specific cleavage sites for degradation by enzymes, such as matrix metalloproteinases (MMPs), plasmin, and elastase. In a specific example, proteolytic degradation of the ECM is one of the key processes in angiogenesis where extensive endothelial cell proliferation requires degradation of the extracellular matrix to permit migration and tube formation [74]. Several enzymes, including MMPs, degrade the proteins that keep the vessel walls solid, which allows the endothelial cells to escape into the interstitial matrix. This course of ECM degradation is highly controlled and coordinated because unguarded dissolution of ECM would result in a loss of the integrity, and thereby function, of the microvasculature [74].

In recreating suitable matrices to recapitulate these processes, it is critical to include components that allow for the natural remodeling of the ECM as seen in the cells' native environment. Recent work by Burdick and Khetan [75] demonstrated three-dimensional, spatially controlled remodeling in patterned hydrogels using HA and enzyme-sensitive peptides. Unlike the entire protein structure, which is subject to denaturation and degradation, short peptide sequences have the advantage of being relatively stable for modification, tunable for cell binding, and easy to be synthesized on a large scale. In Burdick's study, a two-step protocol was used to develop crosslinked hydrogels. In the first step, crosslinked hydrogels were synthesized via reactions between multi-acrylate HA macromers and bifunctional MMP-degradable peptides and cell-adhesive peptides [75]. A photo-initiator was mixed together in this first step. In the second step, the mixtures were exposed to UV light to initiate free radical photopolymerization of the remaining acrylate groups. The resulting hydrogels were expected to prevent cellular remodeling in the presence of non-degradable covalent crosslinks versus allowing cellular remodeling in the presence of degradable crosslinks. As the secondary crosslinking is initiated by light, spatially distinct zones of remodeling were created as seen in Fig. 5. When human mesenchymal stem cells or chick aortic arches were encapsulated in patterned hydrogels, outgrowth of cells, as a result of gel remodeling, was observed in biodegradable versus restrictive regions of the gels. The results suggest that proteolytic degradation is necessary to support cellular spreading and differentiation. Similar studies have been investigated for the development of bioactive hydrogels incorporating proteolytic peptide sequences using poly(ethylene glycol) as a backbone. Although PEG is neither cell adhesive nor biodegradable, PEG hydrogels have been modified with bioactive molecules, such as cell adhesive and enzyme-sensitive peptides. Anseth and Salinas [76] reported incorporation of a cysteine-containing, bifunctional

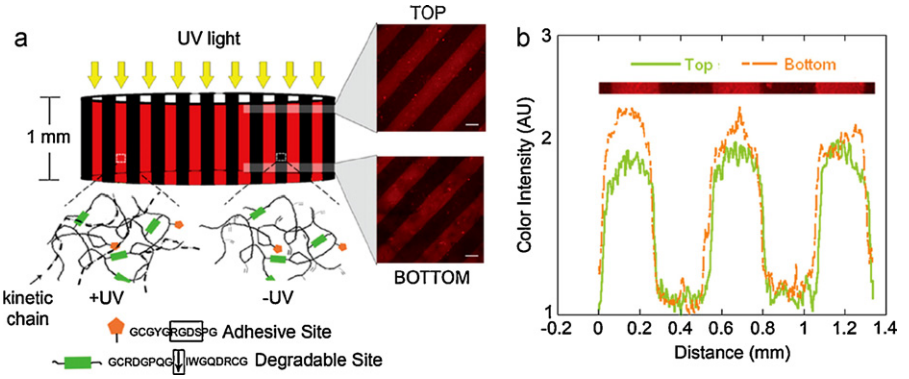

Fig. 5. Photopatterning of AHA hydrogels. (a) Sequentially crosslinked hydrogel photopatterned using a high resolution photomask. Inset images show the top and bottom surfaces of hydrogels patterned with 250 μm stripes. (b) Quantification of photopattern fidelity at the top and bottom gel surfaces [75].

peptide, CPENFFRGD into PEG hydrogels by thiol–acrylate photopolymerization. This peptide has the RGD motif for cell adhesion and the sequence of PENFF for MMP-13-sensitive cleavage, both of which are important for the differentiation of human mesenchymal stem cells. Peptides like collagen-derived GPQGIAGQ [77] and peptide library-derived GPQGIWGQ [78] have also been used to make MMP-sensitive PEG hydrogels, while fibrin-derived YKNRD and VRN have been used to make plasmin-sensitive PEG hydrogels.

For cell transplantation studies in tissue engineering, scaffolds are often designed to degrade over time thereby allowing cell integration with the host and new tissue formation. The degradation rate of scaffolds should be designed to match that of new tissue regeneration at the defect site. If degradation rate is more rapid than that of tissue regeneration, the mechanical integrity at the implant site may be sub-optimal. Conversely, if the degradation is too slow, the scaffolds may impede tissue regeneration. Efforts by Mooney and co-workers [50] have been made to achieve this balance by synthesizing cell-adhesive alginate hydrogels with tunable degradation rates for use as artificial constructs in muscle replacement strategies. Mooney et al. utilized alginates with bimodal molecular weight distributions, one of which has undergone partial oxidation of the polymer to facilitate subsequent hydrolytic breakdown. When alginate is oxidized by reacting with sodium periodate, the carbon–carbon bond of the cis-diol group in the uronate residue is cleaved, which creates hydrolytically labile bonds in the polysaccharide (Fig. 6). This approach provides control over the degradation rate by varying the degree of oxidation where increased degrees of oxidation result in accelerated rates of degradation [50]. Primary skeletal muscle cells encapsulated in this gel demonstrated higher proliferation in degradable than in non-degradable gels, indicating that the degradability of the gels influences cell fate in 3D culture [79].

3.4. Incorporating biochemical signals

In the ECM, some growth factors are active in the bound state, while others are active only after being released by enzymatic cleavage of the matrix. In either case, growth factors may be synthesized and sequestered in the extracellular matrix for immediate activity, or for liberation and activity at a much later time as regulated by the enzymatic demand of cells in the environment.

In early work, Hubbell and Schense [80] attempted to exploit and mimic growth factor–matrix interactions using fibrin hydrogels and peptides derived from transglutaminase enzyme factor XIIIa. In a recent work, the Hubbell lab has explored the use of a fibrin-binding variant of VEGF, with an intervening plasmin-sensitive linker [81]. Whereas $VEGF_{121}$ simply mixed within fibrin diffused out within a few hours, the engineered variant form of $VEGF_{121}$ was quantitatively bound within fibrin and remained there until liberated by active plasmin as depicted in Fig. 7. This demonstrated that bound $VEGF_{121}$ induces endothelial cell (EC) proliferation, as well as endothelial progenitor cell maturation into endothelial cells. Indeed, the matrix-bound forms are seen to be more effective than native $VEGF_{121}$ at promoting maturation.

Cellular migration and architectural assembly are driven by extracellular spatial and temporal biomolecular cues [35]. In biological tissues, two important classes of spatial molecular mechanisms are responsible for guiding cell motility and organization: chemotaxis and haptotaxis [35]. By mimicking these microenvironments in ECM, the Shoichet group has advanced cell guidance strategies using growth factor bound photoactive hydrogels

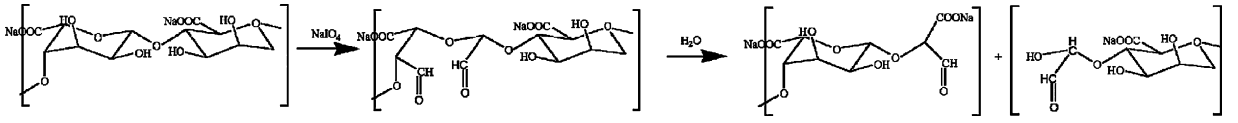

Fig. 6. Periodate oxidation of alginate creates open chain adducts that are susceptible to hydrolytic scission [50].

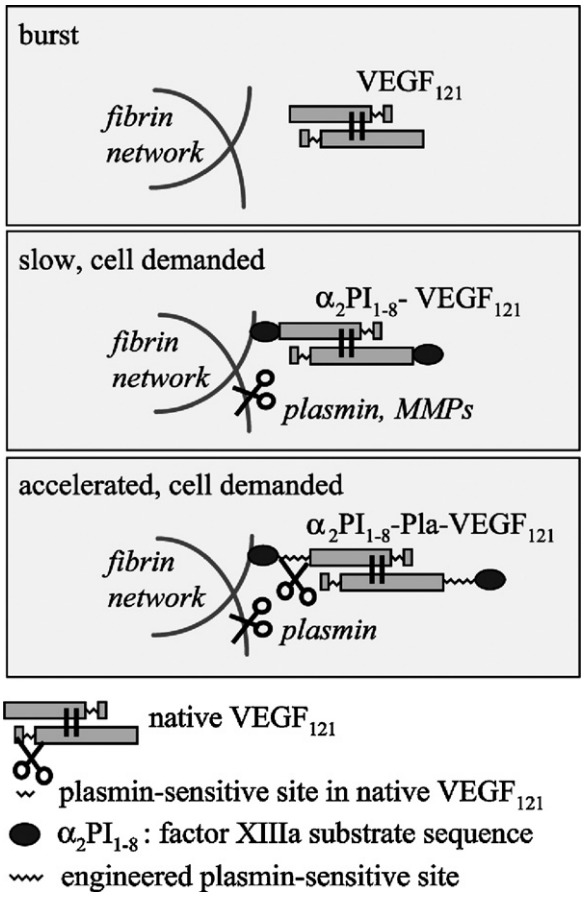

Fig. 7. Native $VEGF_{121}$ is freely diffusible in the aqueous milieu of the fibrin matrix and is released by passive, diffusive burst [81].
© 2005 with permission from Elsevier.

formed from agarose—a naturally derived polysaccharide derived from agar which is extracted from agarophyte seaweeds [82]. Using nitrobenzyl-protected thiols, patterns are produced within agarose hydrogels by uncaging sulfhydryl groups upon exposure to conventional He/Ne 325 nm laser source. These exposed thiols react readily within maleimide-terminated peptides and proteins, yielding peptide-/protein-modified agarose gels localized throughout specific volumes for the study of neurite outgrowth. Shoichet et al. later expanded this approach to three-dimensional patterns using two-photon patterning techniques by chemically modifying agarose with thiol-protected 6-bromo-7-hydroxycoumarin Fig. 8. This approach allows the creation of more complex patterned gels, including the production of islands (<20 μm^3) at defined depths that can be linked to create a variety of geometries (Fig. 9) [83]. Recent studies by the Shoichet lab demonstrated that a concentration gradient of $VEGF_{165}$ was immobilized within defined volumes of the agarose hydrogel, by increased exposure to multiphoton light, thereby creating a concentration gradient of coumarin–deprotected agarose–thiol groups which were available to react with maleimide-modified $VEGF_{165}$ [84]. By mimicking the cues that guide ECs during vascular development, ECs were shown to follow an immobilized $VEGF_{165}$ concentration gradient in a 3D hydrogel, with tip and stalk cells identified and tubular-like structures formed. Furthermore, by taking advantage of orthogonal physical binding pairs, barnase–barstar and streptavidin–biotin, multiple growth factors were simultaneously immobilized in distinct volumes in 3D hydrogels to guide stem cell differentiation [85].

One important aspect of current research is consideration of cell–cell interactions. In addition to chemical and physical properties of the surrounding ECM as described above, cellular functions *in vivo* are influenced by interactions with nearby cells. Previous studies by Bhatia and co-workers [86] reported that hepatocytes, found in liver tissue, were stabilized by co-culturing with fibroblasts, inducing the liver-specific functions and preserving the maximal levels of functional integrin expression. These co-cultured hepatocytes preferentially adhered to poly(ethylene glycol)-based hydrogels photo-patterned with cell adhesive peptides, resulting in higher levels of albumin and urea, indicative of hepatocyte functionality.

Interestingly, recent work has implicated a strong functional interaction between neural progenitor cells (NPCs) and endothelial cells within the stem cell niche and has shown spatial proximity between established neural and vascular networks [87]. Lavik and co-workers [88] demonstrated that hydrogels formed by crosslinking PEG with polylysine around salt-leached polylactic-co-glycolic acid (PLGA) supports the co-culture of ECs and NPCs *in vivo* by

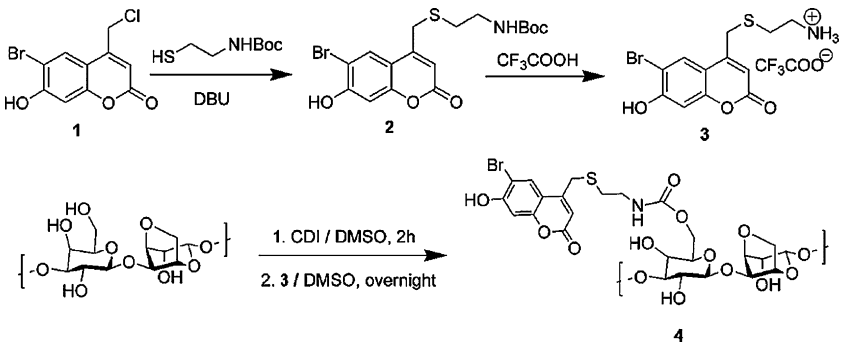

Fig. 8. Conjugation of photochemically masked thiol to polysaccharide backbones [83].
© 2008 American Chemical Society.

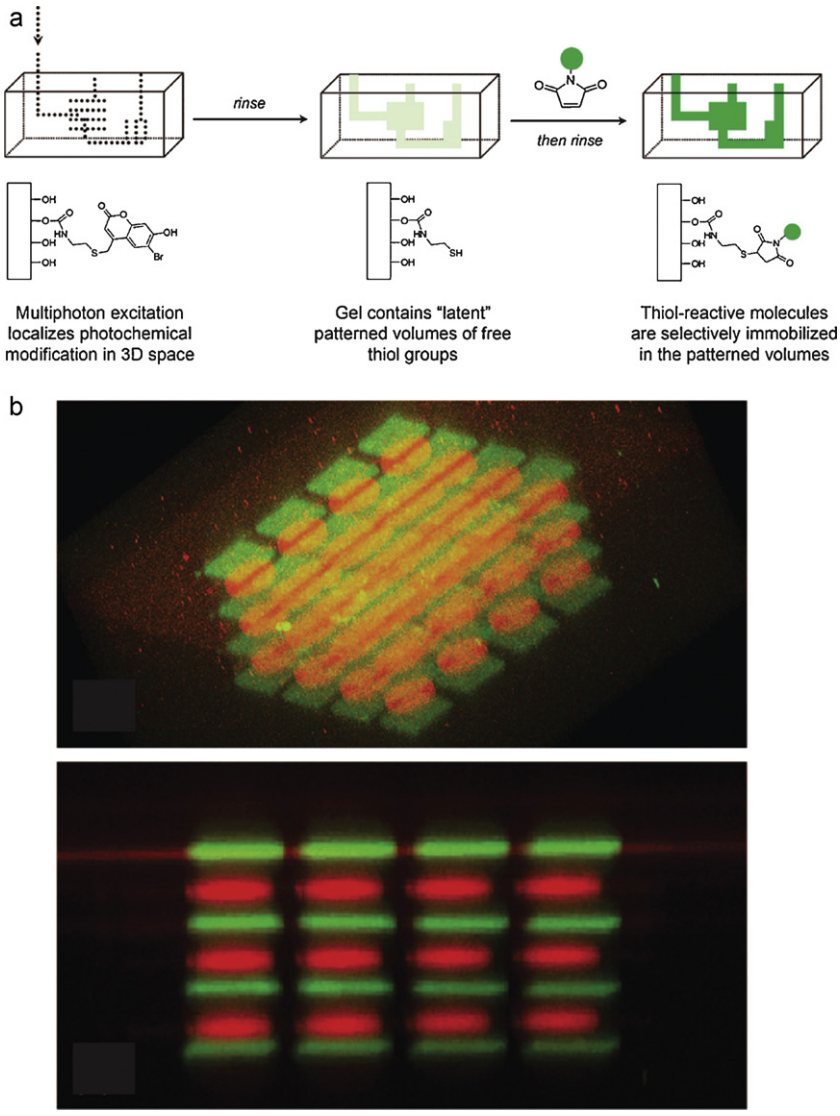

Fig. 9. (a) Schematic representation of multiphoton chemical patterning in hydrogels. (b) Oblique and side views of an array of 3D patterned squares (green), over-patterned with a second array of circles (red) [83]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

promoting the stabilization of microvascular networks. The authors suggested that the mechanism involved in these interactions can be investigated further using 3D hydrogel scaffolds; therefore, synthetic scaffolds are promising platforms for stem cell culture *in vitro*, providing the ability to elucidate complex biological mechanisms of the stem cell niche.

4. Current applications

4.1. In vitro applications

Studies using natural or synthetic materials have begun to elucidate the role of tissue structure and 3D organization of niche cues on differentiation, proliferation, migration, matrix deposition, development and pathogenesis [2]. By designing polymeric biomaterials with the appropriate properties and providing a controlled microenvironment for cells, 3D scaffolds hold great promise for decoupling comprehensive microenvironment variables and effectively understanding the physiological systems *in vivo*. Moreover, these 3D engineered scaffolds may be used for *in vitro* screening applications, and some have already been shown to provide an excellent model where pharmaceuticals can be tested prior to animal studies [3].

The Bhatia group has created a 3D photo-crosslinked PEG hydrogel platform as a high-throughput assay using dielectrophoretic forces (DEP) as shown in Fig. 10 [89]. The DEP system allows precise control over single cells to investigate cell shape, organization and interactions at micron-scale resolution. In this system, living cells are

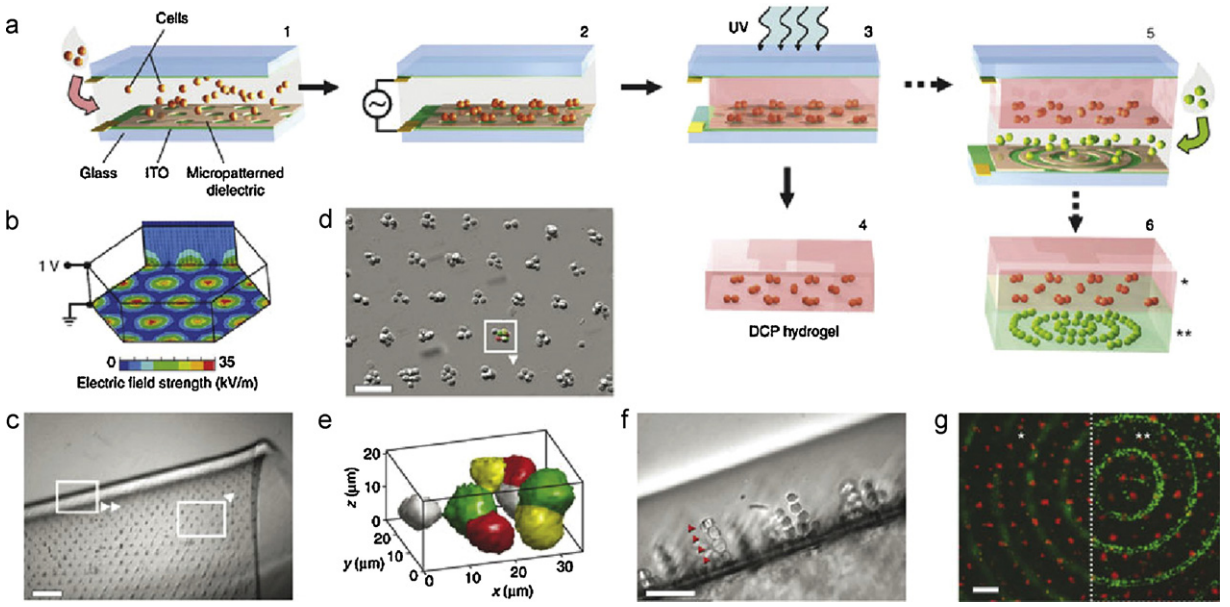

Fig. 10. Fabrication method and examples of DCP hydrogels. (a) Cells in prepolymer solution are introduced into transparent chambers and localized to micropatterned gaps. UV light is used to polymerize the hydrogel. (b) Electrical field strength model. (c–f) Embedded fibroblast clusters shown in hydrogels. (g) A bilayered hydrogel containing distinct layers of fibroblasts (rings and clusters) [5].

arrayed under DEP within the uncrosslinked PEG solution. Single cells are then encapsulated by photo-crosslinking PEG, forming clusters with precise size and shape within the hydrogels, and maintaining 3D microstructures with high cell viability during the cell culture period. This 3D cellular microarray system allows for the investigation of cell–cell interactions that resemble *in vivo* behavior. Bhatia and co-workers [5] later demonstrated that 3D structures of bovine articular chondrocytes exhibit altered matrix biosynthesis compared with those in 2D culture; cumulative matrix proteins synthesized by chondrocytes were decreased in a dose-dependent manner with increasing cluster size.

The Shoichet and Zandstra groups have collaborated to demonstrate that encapsulating embryonic stem cells in VEGF-modified agarose hydrogels can drive their hematopoietic differentiation under defined conditions in bioreactors [90]. This *in vitro* model has been shown to be predictive of the temporal and microenvironmental events that occur *in vivo* during embryonic development.

In the past few years, 3D scaffolds have been designed for applications in cancer where the goal is to better understand tumor progression, metastasis and provide a tool for screening therapeutics *in vitro*. 3D culture systems are designed to bridge the gap between *in vitro* and *in vivo* cancer models by retaining the *in vivo* phenotype through mimicking the structure of the tumor microenvironment. Although commercially available Matrigel® matrix has been used in 3D tumor studies, Matrigel® is ill-defined and inconsistent in composition, making results difficult to interpret. The Mooney group has created a simple 3D human tumor model using poly(lactide-co-glycolide) (PLG) [91]. The PLG scaffolds provide a biocompatible porous culture system which the authors used to investigate the micro-environmental conditions representative of tumors *in vivo*. Using this 3D model, they showed aspects of cancer progression, demonstrating the relevance of this culture system to *in vivo* tumor characteristics [91]. Fischbach and co-workers [92] have continued this work and expanded the tumor model to include mineralized scaffolds to study breast cancer bone metastasis as well as collagen I-based scaffolds to study tumor angiogenesis and vasculogenesis [93].

4.2. *In vivo* applications

In vivo, the ultimate function of the scaffold is to facilitate functional tissue repair. Therefore, the tissue engineered construct must be designed to foster local tissue growth as well as to promote integration with the host tissue. Integration with the host tissue requires the use of biocompatible material and connectivity between implanted constructs/cells and host tissue. One of the limitations of cell-scaffolds is that the cells that are more than 100–200 μm away from the vascular network die due to lack of oxygen and nutrients. Recent studies by the Langer group demonstrated that pre-vascularized scaffolds supported survival of transplanted cells [94]. In this study, endothelial cells were seeded together with fibroblasts and myoblasts (muscle cells) into a scaffold comprised of 50% poly(L-lactic acid) (PLLA) and 50% poly(lactic-co-glycolic acid). The PLGA was selected for its rapid degradation profile, allowing for cellular ingrowth, whereas the PLLA was selected to provide mechanical support to 3D structures [95]. In addition, the blended polymers were fabricated using a salt-leaching process, exhibiting highly porous network that allowed endothelial cells to form vascular networks within the scaffolds. Results show that after

implantation into nude mice, the pre-vascularized scaffold successfully integrated with host microvessels and, importantly, promoted viability of the implanted muscle cells [94].

Recent studies by Song and co-workers [96] have investigated injectable self-crosslinkable polyphosphazene hydrogels. In this study, thiol- and acrylate-based polyphosphazenes were prepared as shown in Fig. 11. These blended polymers exhibited a solution state at low temperature and a transparent gel state at physiological temperature due to physical entanglements and chemical crosslinking via Michael addition of thiols across acrylate double bonds, providing control over mechanical properties. Physical crosslinking brought the reactive double bond of acrylate together with thiol groups in the polymer network, and facilitated a fast gelling transition through chemical crosslinking. As expected, the rate of gel degradation depended on the degree of crosslinking.

Scaffolds are currently used to retain cells at a desired location, serving as a template for 3D cell assembly, survival and engraftment *in vivo*. Several 3D scaffolds have been used for this purpose including PEG-based hydrogels [97,98], and Matrigel. PEG-based hydrogels have great promise as in situ forming scaffolds due to the facile control of mechanical, chemical and architectural properties [97,98]. In the Shoichet lab, an injectable hydrogel of hyaluronan and methylcellulose (HAMC) has been used for *in vivo* applications [99,100]. HAMC has been shown to be biocompatible, biodegradable, inverse thermal gelling and easily injectable through fine 30–34-gauge needles, resulting in minimally invasive surgery. Studies have shown that HAMC is a promising gel for localized delivery of therapeutic agents to the spinal cord and brain, and stem cells to the retina [101–103]. Importantly, HAMC has demonstrated some therapeutic benefit on its own where it has promoted healing and attenuated the inflammatory response in the CNS [100].

Chen et al. [104] developed in situ gelling hydrogels composed of thiolated chitosan and oxidized dextran. In this investigation, the structure presents interpenetrating double-network hydrogels via Schiff base chemistry and disulfide bond crosslinking. Importantly, *in vivo* results

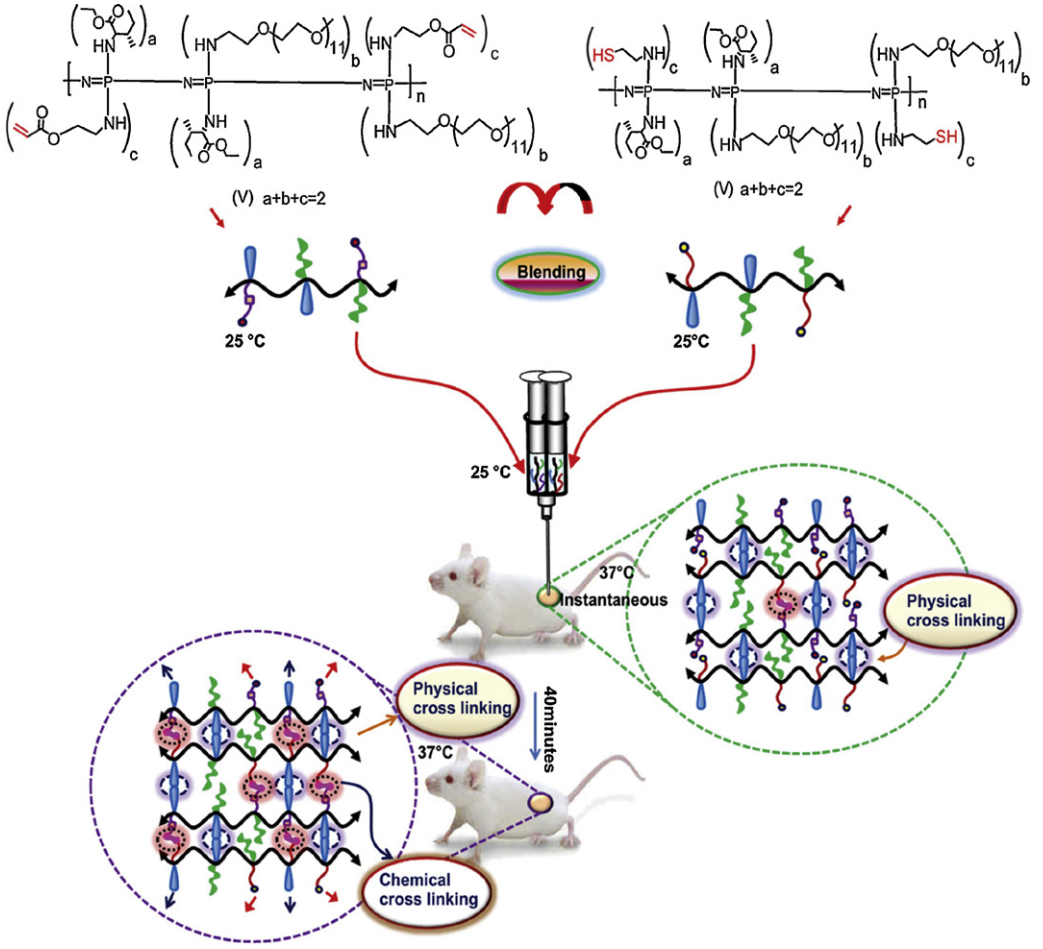

Fig. 11. Schematic illustrations depicting the proposed physical and chemical crosslinking mechanism of the injectable, self-assembling and dual crosslinkable polymer blend hydrogel system [96].

from subdermal implantation in mice models demonstrate that this hydrogel is not only highly resistant to degradation but also induces a very mild tissue response.

Recently, there has been great interest in injectable materials composed of peptide amphiphiles that self-assemble into nanofibers by the addition of electrolyte solutions or changes in pH [57–59]. By conjugating a bioactive sequence into the epitope segment, peptide amphiphiles function as nanofibers for stimulating biological activity. In particular, Stupp and co-workers [62] reported that an IKVAV-bearing peptide amphiphiles promote axonal regeneration in a mouse model of spinal cord injury.

5. Conclusions and future perspectives

Tissue engineering presents the possibility of creating or regenerating various organs or organ-like structures for potential therapeutic intervention. Since the concept was first proposed by Langer and Vacanti [1], the use of living cells in combination with biodegradable scaffold materials has yielded several clinical successes in the recreation of a wide variety of tissues, including cartilage, bone, skin and blood vessels. In addition, engineered tissues have been created as *in vitro* 3D physiological models, providing more biologically relevant complexity than traditional 2D cultures. In these strategies, scaffolds have been designed to incorporate both biochemical and mechanical cues in an attempt to reconstruct tissues that resemble the native structures, whether the application is *in vitro* or *in vivo*.

Despite recent advances in the development of bioactive hydrogels, several challenges still remain including recapitulating the dynamic cellular microenvironment in the design of scaffolds. New scaffolds that provide both spatial and temporal resolution and that are responsive to cells will be particularly attractive in the future. Stem cells are compelling in the design of engineered tissues where one can imagine designing scaffolds with factors that will specifically and preferentially promote the differentiation of stem cells to the several different phenotypes within that engineered tissue scaffold. By this strategy, multiple cell types can grow together in one scaffold, with each influencing the other, and thereby providing a more biomimetic environment with which to interrogate cells.

Extending *in vitro* stem cell-niche engineering to *in vivo* cellular strategies poses additional challenges. Stem-cell transplantation requires a scaffold that regulates the presentation of ligands, is sensitive to stimuli, provides structural support, and has the ability to induce cell migration or invasion into the scaffold. The promise of such scaffolds *in vivo* is just beginning to be explored as a means to deliver stem cells; however, the main challenges to the field remain as cell survival and integration into the host tissue.

Innovative biomaterial strategies are required to overcome the current challenges of cell survival and integration after transplantation. By creating biomimetic, cell-responsive tissue scaffolds that include ECM components and multiple cell types, the field will advance in terms of understanding cell function and fate in response to, for example, therapeutics used in screening applications while at the same time providing insight into the microenvironment required for greater transplantation success. Engineered polymers can be used as a platform to better mimic the stem cell niche, allowing for multiple stimuli and many cell types to be explored individually or in combination. Control over the ECM will facilitate investigation of cell biology, and allow the complexity of the system to be evaluated in terms of individual components, including soluble signals, cell–substrate interactions, and cell–cell contacts. The 3D bioengineered matrices will be advanced for continued use both *in vitro* as a model of disease progression and *in vivo* for tissue regeneration.

Acknowledgments

The authors are grateful to the Natural Sciences and Engineering Research Council of Canada (NSERC), the Killam Research Fellowship (to MSS) and the Japanese Long-Term Study Abroad Scholarship (to YA) for funding.

References

[1] Langer R, Vacanti JP. Tissue engineering. Science 1993;260:920–6.
[2] Shoichet MS. Polymer scaffolds for biomaterials applications. Macromolecules 2010;43:581–91.
[3] Griffith LG, Swartz MA. Capturing complex 3D tissue physiology in vitro. Nat Rev Mol Cell Biol 2006;7:211–24.
[4] Cushing MC, Anseth KS. Hydrogel cell cultures. Science 2007;316:1133–4.
[5] Albrecht DR, Underhill GH, Wassermann TB, Sah RL, Bhatia SN. Probing the role of multicellular organization in three-dimensional microenvironments. Nat Methods 2006;3:369–75.
[6] Fisher OZ, Khademhosseini A, Langer R, Peppas NA. Bioinspired materials for controlling stem cell fate. Acc Chem Res 2009;43:419–28.
[7] Owen SC, Shoichet MS. Design of three-dimensional biomimetic scaffolds. J Biomed Mater Res A 2010;94:1321–31.
[8] Marklein RA, Burdick JA. Controlling stem cell fate with material design. Adv Mater 2010;22:175–89.
[9] Johnson JA, Turro NJ, Koberstein JT, Mark JE. Some hydrogels having novel molecular structures. Prog Polym Sci 2010;35:332–7.
[10] Kloxin AM, Kloxin CJ, Bowman CN, Anseth KS. Mechanical properties of cellularly responsive hydrogels and their experimental determination. Adv Mater 2010;22:3484–94.
[11] Slaughter BV, Khurshid SS, Fisher OZ, Khademhosseini A, Peppas NA. Hydrogels in regenerative medicine. Adv Mater 2009;21:3307–29.
[12] Madurantakam PA, Cost CP, Simpson DG, Bowlin GL. Science of nanofibrous scaffold fabrication: strategies for next generation tissue-engineering scaffolds. Nanomedicine 2009;4:193–206.
[13] Lutolf MP, Gilbert PM, Blau HM. Designing materials to direct stem-cell fate. Nature 2009;462:433–41.
[14] Lund AW, Yener B, Stegemann JP, Plopper GE. The natural and engineered 3D microenvironment as a regulatory cue during stem cell fate determination. Tissue Eng Part B 2009;15:371–80.
[15] Saha K, Keung AJ, Irwin EF, Li Y, Little L, Schaffer DV, Healy KE. Substrate modulus directs neural stem cell behavior. Biophys J 2008;95:4426–38.
[16] Daley WP, Peters SB, Larsen M. Extracellular matrix dynamics in development and regenerative medicine. J Cell Sci 2008;121:255–64.
[17] Tsang VL, Bhatia SN. Fabrication of three-dimensional tissues. Adv Biochem Eng Biotechnol 2007;103:189–205.
[18] Tsang VL, Bhatia SN. Three-dimensional tissue fabrication. Adv Drug Deliv Rev 2004;56:1635–47.
[19] Stelzer EHK, Pampaloni F, Reynaud EG. The third dimension bridges the gap between cell culture and live tissue. Nat Rev Mol Cell Biol 2007;8:839–45.
[20] Unsicker K, Bruckner-Tuderman L, von der Mark K, Pihlajaniemi T. Cell interactions with the extracellular matrix. Cell Tissue Res 2010;339:1–5.

[21] Baudino TA, Bowers SLK, Banerjee I. The extracellular matrix: at the center of it all. J Mol Cell Cardiol 2010;48:474–82.
[22] Hynes RO. The extracellular matrix: not just pretty fibrils. Science 2009;326:1216–9.
[23] Nelson CM, Bissell MJ. Of extracellular matrix, scaffolds and signaling: tissue architecture regulates development, homeostasis and cancer. Annu Rev Cell Dev Biol 2006;22:287–309.
[24] Birkedalhansen H. Proteolytic remodeling of extracellular-matrix. Curr Opin Cell Biol 1995;7:728–35.
[25] Ricard-Blum S. The collagen family. Cold Spring Harbor Perspect Biol 2011;3:a004978/1–9/19.
[26] Knott L, Bailey AJ. Collagen cross-links in mineralizing tissues: a review of their chemistry, function and clinical relevance. Bone 1998;22:181–7.
[27] Ashwin PT, McDonnell PJ. Collagen cross-linkage: a comprehensive review and directions for future research. Br J Ophthalmol 2010;94:965–70.
[28] Badylak SF, Freytes DO, Gilbert TW. Extracellular matrix as a biological scaffold material: structure and function. Acta Biomater 2009;5:1–13.
[29] Heino J, Kapyla J. Cellular receptors of extracellular matrix molecules. Curr Pharm Des 2009;15:1309–17.
[30] Malinda KM, Kleinman HK. The laminins. Int J Biochem Cell B 1996;28:957–9.
[31] Timpl R, Brown JC. The laminins. Matrix Biol 1994;14:275–81.
[32] Burgeson RE, Chiquet M, Deutzmann R, Ekblom P, Engel J, Kleinman H, Martin GR, Meneguzzi G, Paulsson M, Sanes J, Timpl R, Tryggvason K, Yamada Y, Yurchenco PD. A new nomenclature for the laminins. Matrix Biol 1994;14:209–11.
[33] Buck CA, Horwitz AF. Cell-surface receptors for extracellular-matrix molecules. Annu Rev Cell Biol 1987;3:179–205.
[34] Ruoslahti E, Pierschbacher MD. New perspectives in cell-adhesion—RGD and integrins. Science 1987;238:491–7.
[35] Louderbough JMV, Lopez JI, Schroeder JA. Matrix hyaluronan alters epidermal growth factor receptor-dependent cell morphology. Cell Adhes Migr 2010;4:26–31.
[36] Bissell MJ, Hall HG, Parry G. How does the extracellular-matrix direct gene-expression. J Theor Biol 1982;99:31–68.
[37] Zhu J. Bioactive modification of poly(ethylene glycol) hydrogels for tissue engineering. Biomaterials 2010;31:4639–56.
[38] Tessmar JK, Goepferich AM. Customized peg-derived copolymers for tissue-engineering applications. Macromol Biosci 2007;7:23–39.
[39] Tessmar JK, Goepferich AM. Matrices and scaffolds for protein delivery in tissue engineering. Adv Drug Deliv Rev 2007;59:274–91.
[40] Loh XJ, Li J. Biodegradable thermosensitive copolymer hydrogels for drug delivery. Expert Opin Ther Pat 2007;17:965–77.
[41] Tan H, DeFail AJ, Rubin JP, Chu CR, Marra KG. Novel multiarm peg-based hydrogels for tissue engineering. J Biomed Mater Res A 2010;92:979–87.
[42] Prestwich GD, Shu XZ, Liu Y, Cai S, Walsh JF, Hughes CW, Ahmad S, Kirker KR, Yu B, Orlandi RR, Park AH, Thibeault SL, Duflo S, Smith ME. Injectable synthetic extracellular matrices for tissue engineering and repair. Adv Exp Med Biol 2007;585:125–33.
[43] Hargittai I, Hargittai M. Molecular structure of hyaluronan: an introduction. Struct Chem 2008;19:697–717.
[44] Balazs EA. The role of hyaluronan in the structure and function of the biomatrix of connective tissues. Struct Chem 2009;20:233–43.
[45] Nimmo CM, Owen SC, Shoichet MS. Diels–Alder click cross-linked hyaluronic acid hydrogels for tissue engineering. Biomacromolecules 2011;12:824–30.
[46] Cai SS, Liu YC, Shu XZ, Prestwich GD. Injectable glycosaminoglycan hydrogels for controlled release of human basic fibroblast growth factor. Biomaterials 2005;26:6054–67.
[47] Liu Y, Cai S, Shu XZ, Shelby J, Prestwich GD. Release of basic fibroblast growth factor from a crosslinked glycosaminoglycan hydrogel promotes wound healing. Wound Repair Regen 2007;15:245–51.
[48] Yang JS, Xie YJ, He W. Research progress on chemical modification of alginate: a review. Carbohydr Polym 2011;84:33–9.
[49] Wei HL, Yang Z, Chen Y, Chu HJ, Zhu J, Li ZC. Characterisation of n-vinyl-2-pyrrolidone-based hydrogels prepared by a Diels–Alder click reaction in water. Eur Polym J 2010;46:1032–9.
[50] Boontheekul T, Kong HJ, Mooney DJ. Controlling alginate gel degradation utilizing partial oxidation and bimodal molecular weight distribution. Biomaterials 2005;26:2455–65.
[51] Liu HC, Wang CC, Yang KC, Lin KH, Lin FH. A highly organized three-dimensional alginate scaffold for cartilage tissue engineering prepared by microfluidic technology. Biomaterials 2011;32:7118–26.
[52] Cho CS, Kim IY, Seo SJ, Moon HS, Yoo MK, Park IY, Kim BC. Chitosan and its derivatives for tissue engineering applications. Biotechnol Adv 2008;26:1–21.
[53] Kim H, Cooke MJ, Shoichet MS. Creating permissive microenvironments for stem cell transplantation into the central nervous system. Trends Biotechnol 2011, in press, doi:10.1016/j.tibtech.2011.07.002.
[54] Zhang MQ, Li ZS, Leung M, Hopper R, Ellenbogen R. Feeder-free self-renewal of human embryonic stem cells in 3D porous natural polymer scaffolds. Biomaterials 2010;31:404–12.
[55] Foo CTSWP, Lee JS, Mulyasasmita W, Parisi-Amon A, Heilshorn SC. Two-component protein-engineered physical hydrogels for cell encapsulation. Proc Natl Acad Sci USA 2009;106:22067–72.
[56] Liu B, Liu Y, Lewis AK, Shen W. Modularly assembled porous cell-laden hydrogels. Biomaterials 2010;31:4918–25.
[57] Zhang SM, Greenfield MA, Mata A, Palmer LC, Bitton R, Mantei JR, Aparicio C, de la Cruz MO, Stupp SI. A self-assembly pathway to aligned monodomain gels. Nat Mater 2010;9:594–601.
[58] Stupp SI. Self-assembly biomaterials. Nano Lett 2010;10:4783–6.
[59] Stupp SI, Hartgerink JD, Beniash E. Self-assembly and mineralization of peptide-amphiphile nanofibers. Science 2001;294:1684–8.
[60] Stupp SI, Rajangam K, Behanna HA, Hui MJ, Han XQ, Hulvat JF, Lomasney JW. Heparin binding nanostructures to promote growth of blood vessels. Nano Lett 2006;6:2086–90.
[61] Stupp SI, Palmer LC, Newcomb CJ, Kaltz SR, Spoerke ED. Biomimetic systems for hydroxyapatite mineralization inspired by bone and enamel. Chem Rev 2008;108:4754–83.
[62] Tysseling-Mattiace VM, Sahni V, Niece KL, Birch D, Czeisler C, Fehlings MG, Stupp SI, Kessler JA. Self-assembling nanofibers inhibit glial scar formation and promote axon elongation after spinal cord injury. J Neurosci 2008;28:3814–23.
[63] Ingber DE. Mechanical control of tissue morphogenesis during embryological development. Int J Dev Biol 2006;50:255–66.
[64] Brigham MD, Bick A, Lo E, Bendali A, Burdick JA, Khademhosseini A. Mechanically robust and bioadhesive collagen and photocrosslinkable hyaluronic acid semi-interpenetrating networks. Tissue Eng Part A 2009;15:1645–53.
[65] Pachence JM. Collagen-based devices for soft tissue repair. J Biomed Mater Res 1996;33:35–40.
[66] Engler AJ, Sen S, Sweeney HL, Discher DE. Matrix elasticity directs stem cell lineage specification. Cell 2006;126:677–89.
[67] Leipzig ND, Shoichet MS. The effect of substrate stiffness on adult neural stem cell behavior. Biomaterials 2009;30:6867–78.
[68] Leipzig ND, Wylie RG, Kim H, Shoichet MS. Differentiation of neural stem cells in three-dimensional growth factor-immobilized chitosan hydrogel scaffolds. Biomaterials 2011;32:57–64.
[69] Leipzig ND, Xu C, Zahir T, Shoichet MS. Functional immobilization of interferon-gamma induces neuronal differentiation of neural stem cells. J Biomed Mater Res A 2010;93:625–33.
[70] DeForest CA, Sims EA, Anseth KS. Peptide-functionalized click hydrogels with independently tunable mechanics and chemical functionality for 3D cell culture. Chem Mater 2010;22:4783–90.
[71] Kloxin AM, Kasko AM, Salinas CN, Anseth KS. Photodegradable hydrogels for dynamic tuning of physical and chemical properties. Science 2009;324:59–63.
[72] Kloxin AM, Tibbitt MW, Kasko AM, Fairbairn JA, Anseth KS. Tunable hydrogels for external manipulation of cellular microenvironments through controlled photodegradation. Adv Mater 2010;22:61–6.
[73] Lutolf MP, Hubbell JA. Synthetic biomaterials as instructive extracellular microenvironments for morphogenesis in tissue engineering. Nat Biotechnol 2005;23:47–55.
[74] Prior BM, Yang HT, Terjung RL. What makes vessels grow with exercise training? J Appl Physiol 2004;97:1119–28.
[75] Khetan S, Burdick JA. Patterning network structure to spatially control cellular remodeling and stem cell fate within 3-dimensional hydrogels. Biomaterials 2010;31:8228–34.
[76] Salinas CN, Anseth KS. The influence of the rgd peptide motif and its contextual presentation in peg gels on human mesenchymal stem cell viability. J Tissue Eng Regen Med 2008;2:296–304.
[77] Lutolf MP, Lauer-Fields JL, Schmoekel HG, Metters AT, Weber FE, Fields GB, Hubbell JA. Synthetic matrix metalloproteinase-sensitive hydrogels for the conduction of tissue regeneration: engineering cell-invasion characteristics. Proc Natl Acad Sci USA 2003;100:5413–8.
[78] Lee SH, Miller JS, Moon JJ, West JL. Proteolytically degradable hydrogels with a fluorogenic substrate for studies of cellular proteolytic activity and migration. Biotechnol Prog 2005;21:1736–41.

[79] Boontheekul T, Hill EE, Kong HJ, Mooney DJ. Regulating myoblast phenotype through controlled gel stiffness and degradation. Tissue Eng 2007;13:1431–42.
[80] Schense JC, Hubbell JA. Cross-linking exogenous bifunctional peptides into fibrin gels with factor XIIIa. Bioconjug Chem 1999;10:75–81.
[81] Ehrbar M, Metters A, Zammaretti P, Hubbell JA, Zisch AH. Endothelial cell proliferation and progenitor maturation by fibrin-bound vegf variants with differential susceptibilities to local cellular activity. J Control Release 2005;101:93–109.
[82] Luo Y, Shoichet MS. A photolabile hydrogel for guided three-dimensional cell growth and migration. Nat Mater 2004;3: 249–53.
[83] Wosnick JH, Shoichet MS. Three-dimensional chemical patterning of transparent hydrogels. Chem Mater 2008;20:55–60.
[84] Aizawa Y, Wylie R, Shoichet M. Endothelial cell guidance in 3D patterned scaffolds. Adv Mater 2010;22:4831–5.
[85] Wylie RG, Ahsan S, Aizawa Y, Maxwell KL, Morshead CM, Shoichet MS. Spatially controlled simultaneous patterning of multiple growth factors in three-dimensional hydrogels. Nat Mater 2011;10:799–806.
[86] Tsang VL, Chen AA, Cho LM, Jadin KD, Sah RL, DeLong S, West JL, Bhatia SN. Fabrication of 3D hepatic tissues by additive photopatterning of cellular hydrogels. FASEB J 2007;21:790–801.
[87] Shen Q, Goderie SK, Jin L, Karanth N, Sun Y, Abramova N, Vincent P, Pumiglia K, Temple S. Endothelial cells stimulate self-renewal and expand neurogenesis of neural stem cells. Science 2004;304:1338–40.
[88] Ford MC, Bertram JP, Hynes SR, Michaud M, Li Q, Young M, Segal SS, Madri JA, Lavik EB. A macroporous hydrogel for the coculture of neural progenitor and endothelial cells to form functional vascular networks in vivo. Proc Natl Acad Sci USA 2006;103: 2512–7.
[89] Albrecht DR, Tsang VL, Sah RL, Bhatia SN. Photo- and electropatterning of hydrogel-encapsulated living cell arrays. Lab Chip 2005;5:111–8.
[90] Rahman N, Purpura KA, Wylie RG, Zandstra PW, Shoichet MS. The use of vascular endothelial growth factor functionalized agarose to guide pluripotent stem cell aggregates toward blood progenitor cells. Biomaterials 2010;31:8262–70.
[91] Fischbach C, Chen R, Matsumoto T, Schmelzle T, Brugge JS, Polverini PJ, Mooney DJ. Engineering tumors with 3D scaffolds. Nat Methods 2007;4:855–60.
[92] Pathi SP, Kowalczewski C, Tadipatri R, Fischbach C. A novel 3-D mineralized tumor model to study breast cancer bone metastasis. PLoS ONE 2010;5:e8849/1–10.
[93] Cross VL, Zheng Y, Won Choi N, Verbridge SS, Sutermaster BA, Bonassar LJ, Fischbach C, Stroock AD. Dense type I collagen matrices that support cellular remodeling and microfabrication for studies of tumor angiogenesis and vasculogenesis in vitro. Biomaterials 2010;31:8596–607.
[94] Levenberg S, Rouwkema J, Macdonald M, Garfein ES, Kohane DS, Darland DC, Marini R, van Blitterswijk CA, Mulligan RC, D'Amore PA, Langer R. Engineering vascularized skeletal muscle tissue. Nat Biotechnol 2005;23:879–84.
[95] Mooney DJ, Park S, Kaufmann PM, Sano K, Mcnamara K, Vacanti JP, Langer R. Biodegradable sponges for hepatocyte transplantation. J Biomed Mater Res 1995;29:959–65.
[96] Potta T, Chun C, Song SC. Injectable dual cross-linkable polyphosphazene blend hydrogels. Biomaterials 2010;31:8107–20.
[97] Ding JD, Yu L. Injectable hydrogels as unique biomedical materials. Chem Soc Rev 2008;37:1473–81.
[98] Weber FE, Wechsler, Fehr D, Molenberg A, Raeber G, Schense JC. A novel tissue occlusive poly(ethylene glycol) hydrogel material. J Biomed Mater Res A 2008;85:285–92.
[99] Kang CE, Poon PC, Tator CH, Shoichet MS. A new paradigm for local and sustained release of therapeutic molecules to the injured spinal cord for neuroprotection and tissue repair. Tissue Eng Part A 2009;15:595–604.
[100] Gupta D, Tator CH, Shoichet MS. Fast-gelling injectable blend of hyaluronan and methylcellulose for intrathecal, localized delivery to the injured spinal cord. Biomaterials 2006;27:2370–9.
[101] Cooke MJ, Wang YF, Shoichet MS. Controlled epi-cortical delivery of epidermal growth factor for the stimulation of endogenous neural stem cell proliferation in stroke-injured brain. Biomaterials 2011;32:5688–97.
[102] Ballios BG, Cooke MJ, van der Kooy D, Shoichet MS. A hydrogel-based stem cell delivery system to treat retinal degenerative diseases. Biomaterials 2010;31:2555–64.
[103] Wang YF, Lapitsky Y, Kang CE, Shoichet MS. Accelerated release of a sparingly soluble drug from an injectable hyaluronan-methylcellulose hydrogel. J Control Release 2009;140:218–23.
[104] Zhang H, Qadeer A, Chen W. In situ gelable interpenetrating double network hydrogel formulated from binary components: thiolated chitosan and oxidized dextran. Biomacromolecules 2011;12:1428–37.