Progress in Polymer Science 39 (2014) 1973–1986

Contents lists available at ScienceDirect

Progress in Polymer Science

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

Editorial/Preface

In situ-forming injectable hydrogels for regenerative medicine

Jeong-A. Yang[a,1], Junseok Yeom[a,1], Byung Woo Hwang[a], Allan S. Hoffman[b], Sei Kwang Hahn[a,c,*]

[a] *Department of Materials Science and Engineering, Pohang University of Science and Technology (POSTECH), San 31 Hyoja-dong, Nam-gu, Pohang, Kyungbuk 790-784, Republic of Korea*
[b] *Department of Bioengineering, University of Washington, Box 355061, Seattle, WA 98195, USA*
[c] *Wellman Center for Photomedicine, Harvard Medical School and Massachusetts General Hospital, 65 Landsdowne St. UP-5, Cambridge, MA 02139, USA*

ARTICLE INFO

Article history:
Received 3 January 2014
Received in revised form 25 May 2014
Accepted 9 July 2014
Available online 19 July 2014

Keywords:
Artificial extracellular matrix
Cell therapy
Injectable hydrogel
Regenerative medicine
Tissue regeneration

ABSTRACT

Regenerative medicine involves interdisciplinary biomimetic approaches for cell therapy and tissue regeneration, employing the triad of cells, signals, and/or scaffolds. Remarkably, the field of therapeutic cells has evolved from the use of embryonic and adult stem cells to the use of induced pluripotent stem cells. For application of these cells in regenerative medicine, cell fate needs to be carefully controlled *via* external signals, such as the physical properties of an artificial extracellular matrix (ECM) and biologically active molecules in the form of small molecules, peptides, and proteins. It is therefore crucial to develop biomimetic scaffolds, reflecting the nanoenvironment of three-dimensional (3D) ECM in the body. Here, we describe *in situ*-forming injectable hydrogel systems, prepared using a variety of chemical crosslinkers and/or physical interactions, for application in regenerative medicine. Selective and fast chemical reactions under physiological conditions are prerequisites for *in situ* formation of injectable hydrogels. These hydrogels are attractive for regenerative medicine because of their ease of administration, facile encapsulation of cells and biomolecules without severe toxic effects, minimally invasive treatment, and possibly enhanced patient compliance. Recently, the Michael addition reaction between thiol and vinyl groups, the click reaction between bis(yne) molecules and multiarm azides, and the Schiff base reaction have been investigated for generation of injectable hydrogels, due to the high selectivity and biocompatibility of these reactions. Noncovalent physical interactions have also been proposed as crosslinking mechanisms for *in situ* forming injectable hydrogels. Hydrophobic interactions, ionic interactions, stereocomplex formation, complementary pair formation, and host–guest interactions drive the formation of 3D polymeric networks. In particular, supramolecular hydrogels have been developed using the host–guest chemistry of cyclodextrin (CD) and cucurbituril (CB), which allows highly selective, simple, and biocompatible crosslinking. Molecular recognition and complex formation of supramolecules, without the need for additional additives, have been successfully applied to the 3D network formation of polymer chains. Finally, we review the current state of the art of injectable hydrogel systems for application in regenerative medicine, including cell therapy and tissue regeneration.

* Corresponding author at: Department of Materials Science and Engineering, Pohang University of Science and Technology (POSTECH), San 31 Hyoja-dong, Nam-gu, Pohang, Kyungbuk 790-784, Republic of Korea. Tel.: +82 54 279 2159; fax: +82 54 279 2399.
E-mail address: skhanb@postech.ac.kr (S.K. Hahn).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.progpolymsci.2014.07.006
0079-6700/

Contents

1. Introduction

Regenerative medicine is the term for biomedical approaches to improve, restore, or replace biological functions of damaged tissues and organs, using cells, physico-chemical factors, and/or engineered scaffolds [1]. These translational approaches include cell therapies, immunomodulation therapy *via* infused cells, and tissue regeneration or engineering using artificial organs and tissues [1,2]. In recent years, engineered scaffolds have been investigated widely as an artificial extracellular matrix (ECM) to provide structural support and growth factors for the spatiotemporal control of encapsulated cells [3]. The ECM naturally consists of an interlocking mesh of fibrous proteins and glycosaminoglycans (GAGs). GAGs are carbohydrate polymers, such as heparan sulfate, chondroitin sulfate, and keratan sulfate, which are usually attached to ECM proteins to form proteoglycans. Hyaluronic acid (HA) is an exceptional non-proteoglycan polysaccharide. Collagens are the most abundant protein in the ECM, while elastin, laminin, and fibronectin are also important components of the ECM. Cell-to-ECM adhesion is regulated by cellular adhesion molecules (CAMs) of integrins that bind cells to fibronectin and laminin in the ECM [4,5]. The cell fate is regulated by external signals, such as cell–cell interactions, the physical properties of the ECM, and growth factors [6,7].

As an artificial ECM, synthetic scaffolds should possess appropriate mechanical properties, porous structures that allow free diffusion of nutrients and wastes, and degradability that matches the rate of cellular growth, enabling spatiotemporal control of encapsulated cells. They should be easily fabricated in a way that minimizes cell damage and cytotoxic byproducts. A variety of synthetic scaffolds have been developed for tissue engineering, including hydrogels [8,9], porous nanostructures [10–12], and nanofibers [13,14]. In particular, hydrogels, three-dimensional (3D) networks of crosslinked polymer chains, have been widely investigated as promising artificial ECMs for *in vitro* and *in vivo* tissue engineering applications [15–17]. As schematically shown in Fig. 1, *in situ*-forming cytocompatible hydrogels can be prepared using non-toxic chemical crosslinkers, enzymes for biological crosslinking, physical interactions such as hydrophobic interaction and ionic interaction, and supramolecular chemistry. These injectable hydrogels have many advantages for various biomedical applications, including their ease of administration, simple cell encapsulation, the minimally invasive treatment, and the possibly enhanced patient compliance [18–22]. In addition, injectable hydrogels can easily take on a complex shape and can adhere to the surrounding tissues during hydrogel formation. However, to our knowledge, there are few *in situ*-forming hydrogels available for long-term cell encapsulation, due to the absence of chemical crosslinking systems that are completely biocompatible, the narrow range of physiologically acceptable triggering stimuli for physical crosslinking, and the low stability of physically crosslinked hydrogels in the body [15,23]. Furthermore, it is not easy to provide adequate growth factors to cells encapsulated in hydrogels for their proliferation and differentiation. To achieve this, cellular adhesion moieties and/or growth factors should be introduced into the hydrogel in a spatiotemporally controlled manner (Fig. 1). The systematic investigation on the fate of cells encapsulated in the hydrogels over time is one of the most important but currently unmet biomedical requirements for the successful application of hydrogels to regenerative medicine.

In this review, we describe various injectable hydrogel systems used in regenerative medicine, including cell

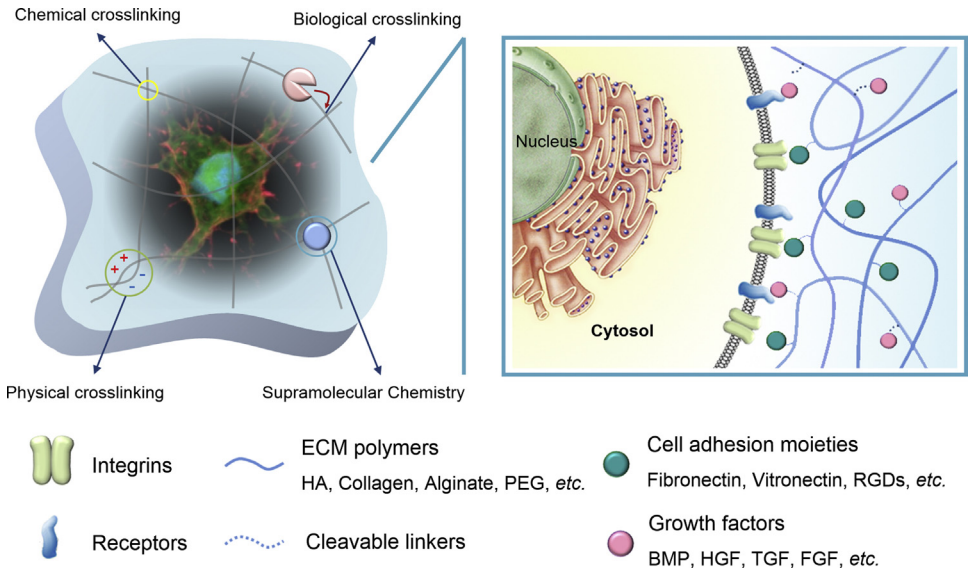

Fig. 1. Schematic representation of crosslinking mechanisms. Mechanisms used for *in situ* forming injectable hydrogels (left) and the interaction between cell integrins or receptors and biomolecules conjugated to extracellular matrix (ECM) polymers (right).

therapy and tissue regeneration. First, we review injectable hydrogels prepared using non-toxic chemical crosslinkers, employing the Michael addition reaction between thiol and vinyl groups, the click reaction between bis(yne) molecules and multiarm azides, and the Schiff base reaction. Second, we review injectable hydrogels prepared using enzymes for biological crosslinking, such as hydrogen peroxidase for the crosslinking of an HA–tyramine conjugate. Third, we review injectable hydrogels prepared using physical interactions, such as hydrophobic interactions and ionic interactions, and external stimuli-responsive hydrogel systems, of which the formation is triggered by temperature and/or pH changes. Fourth, we discuss supramolecular hydrogels prepared using host–guest chemistry involving cyclodextrin (CD) and cucurbituril (CB). Finally, we describe several examples of *in situ*-forming injectable hydrogels applied in cell therapy and tissue regeneration, with a view to further clinical development.

2. *In situ*-forming injectable hydrogels

2.1. Injectable hydrogels prepared by chemical crosslinking

2.1.1. Michael addition for formation of injectable hydrogels

Michael addition is the nucleophilic addition of a carbanion or a nucleophile, such as thiols and amines, to an α,β-unsaturated carbonyl compound [24]. The reaction is highly selective under physiological conditions, without involving toxic reagents and side products. Accordingly, this reaction has been widely exploited for the preparation of injectable hydrogels for biomedical applications (Fig. 2). For example, the Michael addition reaction can occur between thiol and vinyl sulfone (VS) or aminoethyl methacrylate (AEMA). As a polymer backbone, synthetic and natural polymers have been used for the preparation

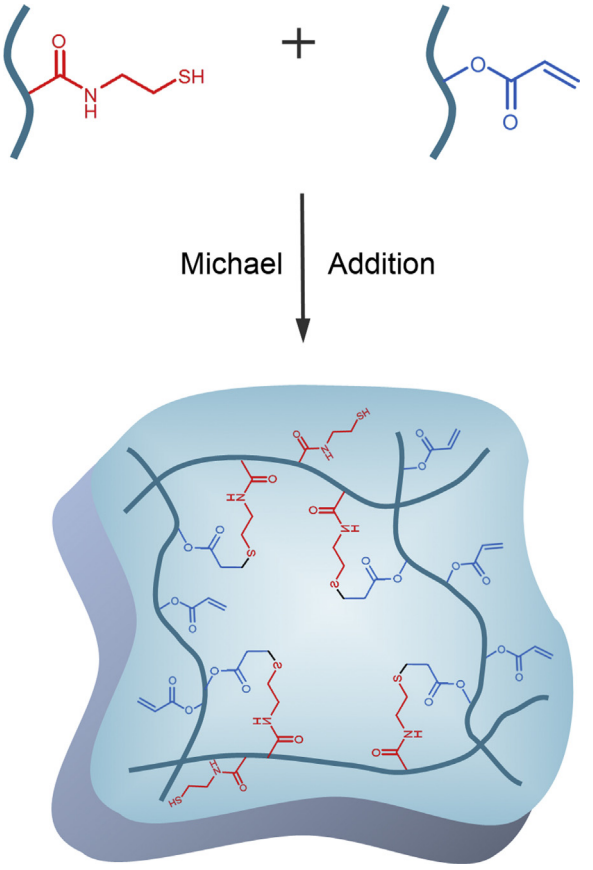

Fig. 2. Schematic illustration of *in situ* hydrogel formation using Michael addition [28], Copyright 2010.
Reproduced with permission from Elsevier Ltd.

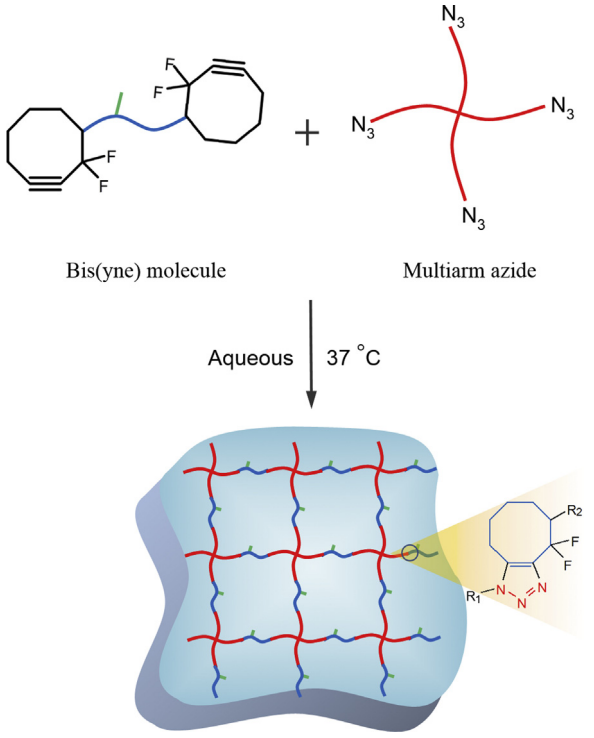

Fig. 3. Schematic illustration of *in situ* hydrogel formation using click chemistry [40], Copyright 2009.
Reproduced with permission from the Nature Publishing Group.

of hydrogels, such as poly(ethylene glycol) (PEG), collagen, HA, and heparin [25–29]. Cells and biopharmaceuticals can be encapsulated within such a hydrogel by simple mixing with the polymer precursor solutions. Extracel™ is one of the typical injectable hydrogels created by Michael addition between a thiol-modified carboxymethyl HA and gelatin modified with diacrylated PEG [25,30–32]. The gelation time can be controlled by changing the concentration and the pH of the polymer precursor solutions. Extracel™ has been applied to drug delivery [30], post-surgical adhesion barriers [31], and tissue engineering [25,32].

2.1.2. Click reaction for formation of injectable hydrogels

Click chemistry is a Cu(I)-catalyzed reaction between azide and terminal acetylene groups, forming 1,2,3-triazoles [33,34]. This reaction is widely employed for biomedical applications, because of the high yield, regiospecificity, absence of toxic byproducts, and rapid reactivity under physiological conditions. Using click chemistry, various hydrogels have been developed for drug delivery and tissue engineering applications [35–38]. A polymeric 3D network has been fabricated using a dipolar cycloaddition reaction between the two types of derivatives in the presence of a catalytic amount of Cu(I), at room temperature. Recently, copper-free click chemistry has also been developed using azide–alkyne cycloaddition between difluorinated cyclooctyne ($DIFO_3$) and azide, and applied to *in situ* hydrogel formation (Fig. 3) [39,40]. Simple mixing of the polymer precursor solutions with a cell suspension resulted in hydrogel formation *via* the highly specific click reaction of azide with acetylene, encapsulating the cells. In addition, post-modification of the hydrogel, on demand, was performed by subsequent functionalization of the remaining azide or acetylene group.

2.1.3. Schiff base reaction for formation of injectable hydrogels

Injectable hydrogels can be prepared by a Schiff base reaction between an amine group and an aldehyde group, without additional chemical crosslinking reagents. The residual functional groups within the hydrogel can be used for covalent conjugation of therapeutic molecules or additional crosslinking. HA, chitosan, dextran, chondroitin sulfate, and poly(vinyl alcohol) have been used for the preparation of hydrogels *via* the Schiff base reaction [41–45]. The gelation time and physical properties of these hydrogels are dependent on the ratio of the amine and aldehyde groups. Although the cells entrapped in these hydrogels have been reported to maintain their normal morphology, aldehyde groups can react with other amine groups in biomolecules of cells in the body during the crosslinking reaction.

2.1.4. Enzyme-mediated injectable hydrogels

Tyramine-conjugated polymers have been used for *in situ* hydrogel formation in the presence of H_2O_2 and horseradish peroxidase (Fig. 4) [46–49]. The enzymatically crosslinked hydrogels can be prepared within 10 min, depending on the polymer concentration and the enzyme/tyramine ratio. Polymer–tyramine hydrogels with high elasticity have been used as drug delivery depot systems [46] and tissue engineering scaffolds [47–49].

2.1.5. Photo-crosslinked injectable hydrogels

Methacrylated polymers have been used for *in situ* hydrogel formation by photo-crosslinking with a photoinitiator (Fig. 5) [50]. The hydrogel precursor solution is injected into the body and is then exposed to visible or ultraviolet (UV) light. Photo-crosslinking has also been used to improve the mechanical properties and stability of physically crosslinked hydrogels [51,52]. For example, methacrylic acid was introduced into thermosensitive polymers or electrostatically crosslinked hydrogels. The thermosensitive photopolymerized hydrogels demonstrated improved mechanical properties. Nevertheless, the practical applications of photo-crosslinked hydrogels are limited due to the possible toxicity of photoinitiators, the long exposure time, and the short penetration depth of light sources [53,54].

2.2. Injectable hydrogels prepared by electrostatic interaction

2.2.1. Alginate-based injectable hydrogels

Alginate is an anionic polysaccharide derived from brown algae. This biopolymer is a common excipient in the pharmaceutical industry. Alginate hydrogels are formed by simple mixing of alginate solutions with divalent cations, such as Ca^{2+}, Mg^{2+}, and Ba^{2+}. Alginate-based hydrogels have been widely used for drug delivery [61], cell therapy [62,63], and tissue engineering [55–57]. They have

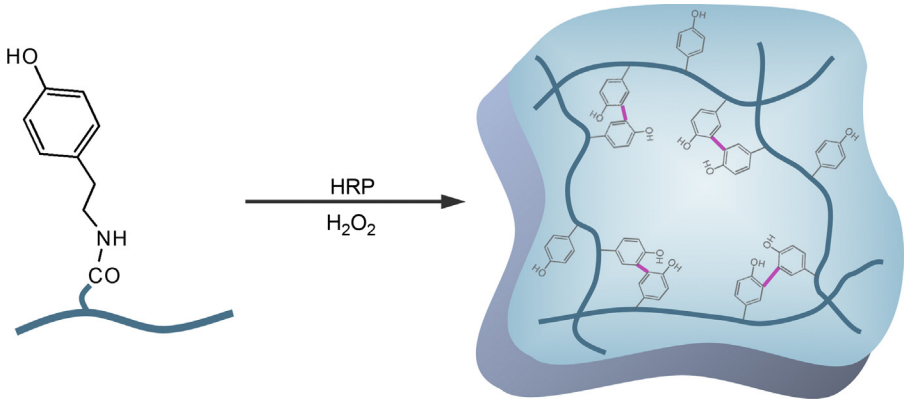

Fig. 4. Schematic illustration of *in situ* hydrogel formation using an enzymatic crosslinking reaction with horseradish peroxidase (HRP) and H_2O_2 [46], Copyright 2011.
Reproduced with permission from Elsevier Ltd.

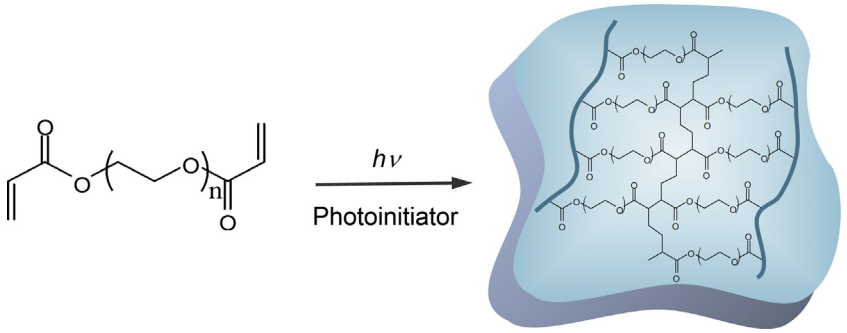

Fig. 5. Schematic illustration of *in situ* hydrogel formation using photo-crosslinking [50], Copyright 1999.
Reproduced with permission from the United States National Academy of Sciences.

been used in clinical trials and are a component of FDA approved medical products [63]. The formation and the mechanical strength of alginate-based hydrogels can be controlled by changing the concentration and the type of cation added. For example, the rate of alginate hydrogel formation increased with increasing total calcium content in the case of $CaCO_3$/D-glucono-δ-lactone (GDL) and $CaSO_4$/$CaCO_3$/GDL systems [58]. The mechanical properties of the alginate hydrogels were improved with increasing alginate concentration, total calcium content, molecular weight, and glucuronic acid content of the alginate. However, alginate hydrogels formed by ionic interaction are not stable in the body, because ionic molecules diffuse out from the hydrogels into the body fluid [59,60]. In addition, the formation of alginate hydrogel is difficult to control, and the hydrogel has poor cell adhesion [61]. To improve the stability and mechanical properties of this type of hydrogel, highly stretchable and tough alginate hydrogels have been prepared by additional covalent crosslinking (Fig. 6) [62,63]. Furthermore, alginate hydrogel was prepared by photo-crosslinking of methacrylated alginate, which maintained enhanced mechanical properties for 4 weeks in mice [51]. An issue with alginate hydrogel is that this polymer is naturally derived, and thus if it is not adequately purified, contaminants can lead to inflammation or immune responses. However, appropriately purified materials can be successfully applied to biomedical applications [64].

2.2.2. Chitosan-based injectable hydrogels

Chitosan is an alternating copolymer of 2-acetamido-2-deoxy-β-D-glucopyranose and 2-amino-2-deoxy-D-glucopyranose derived from naturally occurring chitin. Chitosan can form a hydrogel complex with polyanionic molecules *via* electrostatic interaction [65]. Temperature- and pH-responsive chitosan-based hydrogels have been prepared with polyol-salts possessing a single anionic head, such as glycerol-, sorbitol-, fructose-, or glucose-phosphate salts (polyol- or sugar-phosphate) [66–69]. The driving force behind hydrogel formation includes hydrogen bonding, electrostatic interaction, and hydrophobic interaction between chitosan and polyol-phosphate salts. The chitosan solution remains liquid at physiological pH and turns into a hydrogel at body temperature. Drugs and cells can be easily entrapped within the hydrogel by mixing them with the precursor solution at low temperature prior to injection.

2.3. Stimuli-responsive injectable hydrogels

2.3.1. Temperature-responsive injectable hydrogels

Some polymers undergo solubility changes and phase transitions in response to environmental temperature [70,71]. This threshold is referred to as the lower critical solution temperature (LCST) [72]. For example, poly(*N*-isopropylacrylamide) (PNIPAAm) undergoes phase

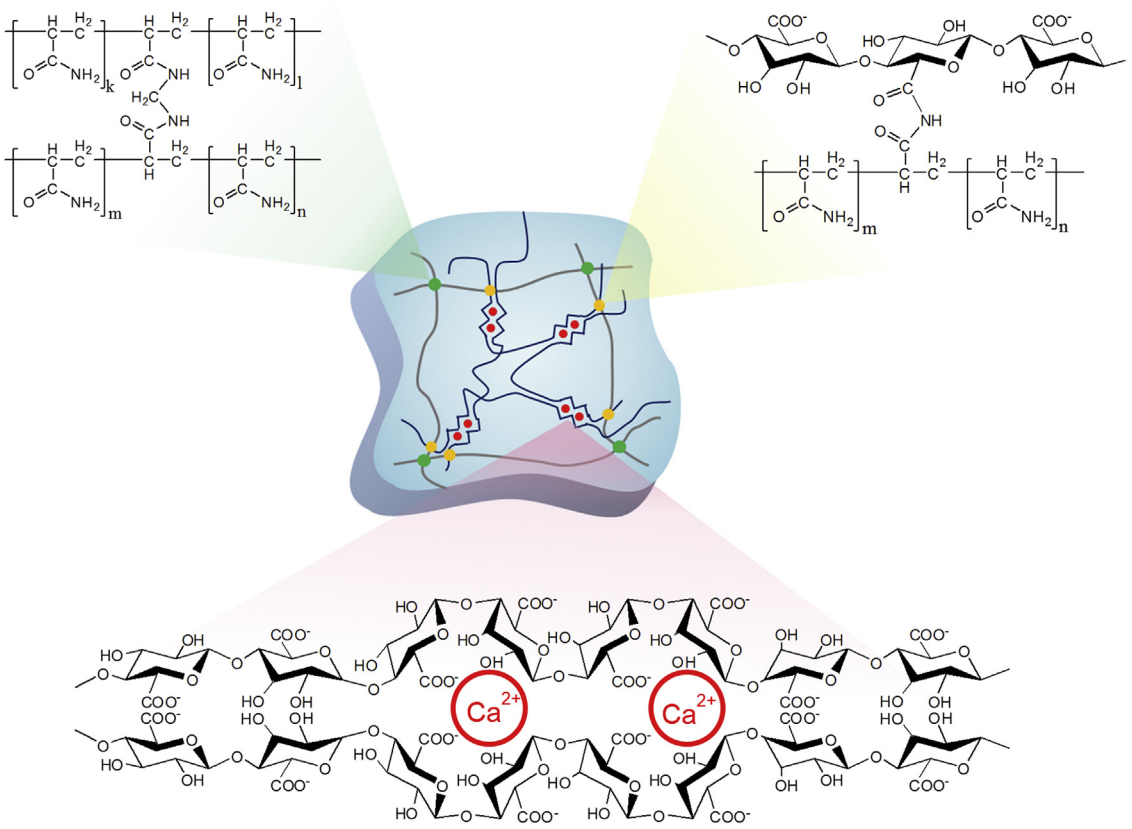

Fig. 6. Schematic illustration of *in situ* hydrogel formation using chemical crosslinking and ionic interaction between alginate and calcium ions [62], Copyright 2012.
Reproduced with permission from the Nature Publishing Group.

transition at temperatures above 32 °C in an aqueous solution. Because the LCST of PNIPAAm is increased by copolymerization with a hydrophilic polymer, *in situ* gelation temperature can be adjusted to body temperature [70]. In addition, some amphiphilic polymers were used for hydrogel formation, by micellar packing, in response to temperature changes [73]. Linear and star-shaped block copolymers, composed of central hydrophilic polyethylene oxide (PEO) and terminal PNIPAAm, showed a temperature-responsive behavior, forming relatively strong injectable hydrogels [72]. Recently, biodegradable temperature-responsive hydrogels were developed for biomedical applications by combining non-biodegradable PNIPAAm and biodegradable polymers [74–76]. The HA-*g*-PNIPAAm conjugate forms a hydrogel network, exhibiting reversible temperature-responsive solubility [75]. The degradation rate, swelling ratio, and cytocompatibility of the hydrogels can be controlled by changing the weight ratio of PNIPAAm to HA for tissue engineering applications.

A PEO–PPO–PEO triblock copolymer, under the trade name of Pluronic®, is one of the most commonly used thermosensitive hydrogels for biomedical applications. Dehydration and increasing hydrophobicity of the PPO block with increasing temperature results in micelle formation, which is the driving force for *in situ* hydrogel formation. This hydrogel formation is dependent on the concentration and temperature of the polymer precursor solution. Pluronic® with a different composition and molecular weight of copolymer has been used for applications in drug delivery, gene delivery, tissue adhesion prevention, and tissue engineering [77]. However, Pluronic® systems have the disadvantages of having weak mechanical strength and being non-biodegradable. Biodegradable PEG–PLGA–PEG also forms a thermoresponsive hydrogel similar to the PEO–PPO–PEO triblock copolymer systems [78]. Despite the wide clinical exploitation of PLGA based copolymers with FDA approval, they are known to cause harmful side-effects to biomolecules, cells, and tissues in some cases after they are degraded to acidic monomers [79,80]. To alleviate these issues, porous devices, microparticles, and hydrogels have been developed using this type of polymers [80].

2.3.2. *Dual-responsive injectable hydrogels*

The main disadvantage of physically crosslinked thermosensitive hydrogels is their weak stability and mechanical properties in the body. Accordingly, dual-responsive hydrogels have been developed to alleviate these problems. For example, temperature- and pH-responsive hydrogels have been developed using PNIPAAm-based copolymers. PNIPAAm is copolymerized with pH-responsive segments, such as poly(propylacrylic

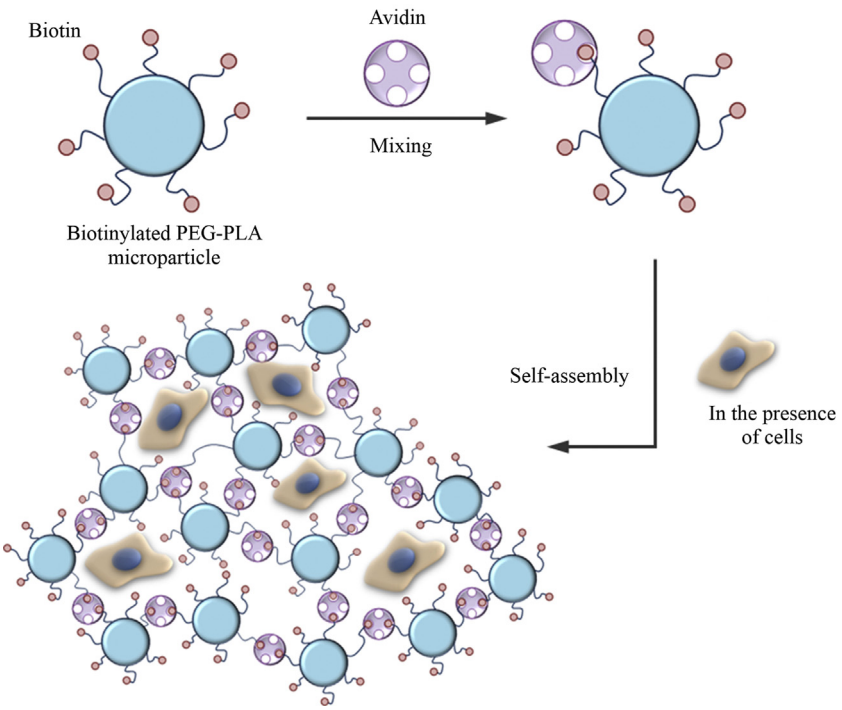

Fig. 7. Schematic illustration of *in situ* hydrogel formation in the presence of cells using ligand–receptor interaction [85], Copyright 2003. Reproduced with permission from John Wiley & Sons Inc.

acid) (PPAA), poly(*N*-isopropylmaleamic acid) (PNIPMAA), and poly(methacrylic acid) (PMAA) [81]. These synthetic polymers are not only temperature responsive but also significantly pH responsive due to the presence of carboxyl groups. In addition, temperature- and pH-responsive hydrogels have been prepared with multiblock copolymers [82]. The pH-responsive sulfamethazine oligomers (SMO) have been conjugated to both ends of thermoresponsive poly(ε-caprolactone-*co*-lactide)–PEG–poly(ε-caprolactone-*co*-lactide). The resulting SMO–PCLA–PEG–PCLA–SMO multiblock copolymer solution shows a reversible sol–gel transition at pH 7.2 and body temperature. The mechanism of hydrogel formation is the hydrophobic interaction between SMO and PCLA blocks. These temperature- and pH- (dual) responsive hydrogels have enhanced mechanical strength and prevent gelation of the precursor solution in the needle during injection into the body. Other dual-responsive hydrogel systems that enhance the mechanical properties of physically crosslinked hydrogels have also been developed using photo- and temperature-responsive hydrogel systems [83,84].

2.4. Supramolecular injectable hydrogels prepared by self-assembly

2.4.1. Self-assembling injectable hydrogels by complementary binding

Self-assembling hydrogels have been developed using various complementary bindings, such as ligand–receptor pairs [85–87], antigen–antibody pairs [88–90], and base-pairing interactions [91–93]. Because ligand–receptor pairs have an extremely high binding affinity, the formation of a complex between a receptor and a ligand can be used to drive formation of injectable hydrogels. For example, a streptavidin–biotin pair has been used for the preparation of injectable hydrogels (Fig. 7) [85]. PLA–PEG–biotin microparticles have been crosslinked with avidin to generate 3D porous matrices that self-assemble at the injection site. In addition, multiple repeats of tryptophan-rich domains and proline-rich peptide domains have been used for hydrogel formation [86]. The amount of crosslinking protein affected the hydrogel formation rate, as well as the physical strength of the hydrogels. Moreover, growth factors could be added to the hydrogel precursor solution to promote cellular functions within the hydrogel. Antibody–antigen interaction has also been used for the formation of an injectable 3D network [88–90]. Simple mixing of antibody-conjugated polymer and antigen-grafted polymer solutions can result in hydrogel formation. Treatment with free antigen or free antibody also affects the physical properties of these hydrogels. In addition, self-assembling hydrogels have been prepared using three complementary branched DNA sequences [91–93]. However, the relative difficulties in mass production and chemical modification of biomolecules, as well as the potential safety issues involved, should be addressed to expand the applications of these hydrogels to regenerative medicine.

2.4.2. Self-assembling injectable hydrogels by host–guest interaction of cyclodextrin

As an alternative to biological complementary binding pairs, self-assembling hydrogels have been developed

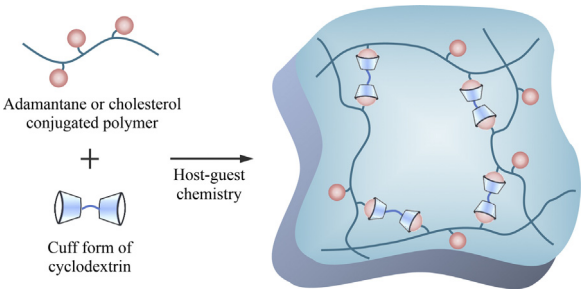

Fig. 8. Schematic illustration of *in situ* hydrogel formation using host–guest interaction of cyclodextrin [96], Copyright 2006. Reproduced with permission from John Wiley & Sons Inc.

using host–guest interaction of the cyclodextrin (CD) family (Fig. 8). CDs are series of natural cyclic oligosaccharides composed of six, seven, or eight D-glucopyranoside units (α, β, and γ-CD). They have a hydrophobic inner cavity, by which they can generate an inclusion complex with other guest molecules, such as PEG, adamantane, and cholesterol [94]. Recently, injectable hydrogels that make use of an inclusion complex of CD have emerged as another series of promising physical hydrogels that can be used in various biomedical applications [95–97]. PEG can penetrate the inner cavity of α-CD to generate an inclusion complex. Injectable hydrogels have been created by mixing high molecular weight PEG and α-CD in aqueous solution [95]. This type of hydrogel is reversibly thixotropic and non-degradable high molecular weight PEG is not ideal for *in vivo* applications. To improve the stability of the hydrogel, PEO–PPO–PEO was used to make a complex with α-CD. β-CD and adamantane, and β-CD and cholesterol pairs have also been investigated for the preparation of injectable hydrogels [96,97]. However, CD-based hydrogels have an intrinsic limitation in *in vivo* applications, due to the low binding affinity of CD to guest molecules (*e.g.* $K \sim 10^2\ M^{-1}$ for α-CD-PEG) and the low stability of the resulting hydrogels in the body [98].

2.4.3. *Self-assembling injectable hydrogels by host–guest interaction of cucurbituril*

Cucurbit[*n*]uril (*n* = 5–8, 10; CB[*n*]), which is a hollowed pumpkin-like symmetrical macromolecule, can form a complex with acyl ammonium ion with exceptionally high binding affinity and selectivity in aqueous solution (Fig. 9). Accordingly, the CB–guest interaction has been exploited as a useful tool for noncovalent conjugation, with negligible cytotoxicity, for biomedical applications [99]. The cavity size of CB[*n*] and the length of the guest molecule for hydrophobic interaction and hydrogen bonding are important factors for the formation of a CB–guest complex. For example, CB[8] forms a complex with two guest molecules, an electron-deficient first guest, such as viologen, and an electron-rich second guest, such as 2-naphthol [100]. Also, CB[6] binds tightly to the protonated forms of diaminohexane (DAH) and spermine (SPM) in order to make ultrastable host–guest complexes [101], with a high binding constant of 10^{10}–$10^{12}\ M^{-1}$, which is almost comparable to that of streptavidin and biotin. In our previous study, the host–guest interaction between CB[6] and DAH was used as a selective crosslinking system for the preparation of injectable hydrogels, without requiring additional reagents and stimuli (Fig. 9) [101]. Supramolecular HA hydrogels encapsulating cells can be prepared easily within 2 min by simple mixing of CB[6]–HA and DAH–HA solutions in the presence of cells. Interestingly, modular post-modification of the hydrogel can be achieved using various "tags" attached to CB[6], allowing control of proliferation and morphology of the encapsulated cells [101]. We have been able to confirm the feasibility of CB[6]/DAH–HA hydrogels with good mechanical stability, enzymatic degradability, and negligible toxicity that are applicable to cell therapy and tissue regeneration. For further *in vivo* applications, the long-term safety of CB[6]/DAH–HA hydrogels should be investigated carefully with the optimization of complicated synthetic processes.

3. *In situ*-forming injectable hydrogels for regenerative medicine

The field of regenerative medicine includes cell therapy and biomedical approaches to tissue regeneration. Cell therapy involves delivery of therapeutic cells to appropriate tissues for the treatment of diseases. Autologous cell therapy is performed using cells from a patient, while allogeneic cell therapy uses cells from donors. Tissue regeneration or engineering is performed using a triad of cells, signaling molecules, and scaffolds for the repair and regeneration of damaged body parts. In addition to external signals derived from the microenvironment, cell–cell interaction *via* transmembrane proteins is also important for determining the cell fate [102]. 3D hydrogels have been investigated extensively as a cell carrier and as an artificial ECM for the spatiotemporal control of cells by means of an adequate supply of growth factors. Below follows a detailed description of injectable hydrogels for use in cell therapy and tissue regeneration.

3.1. *Injectable hydrogels for cell therapy*

Red blood cell injection, platelet transfusion, and bone marrow transplantation are typical examples of cell-based therapies. In general, the number of cells (from several millions to hundreds of millions of cells) should be injected to achieve effective cell therapy [103–105]. The delivery of cells with adequate carriers can extend the duration of the therapeutic effect, which holds a great advantage, particularly for patients suffering from chronic diseases such as diabetes. Islet transplantation is a promising therapy for the treatment of diabetes. Loss and poor engraftment of islets are major obstacles for islet therapy. P(NIPAAm-*co*-AA) has been investigated as a thermoresponsive injectable cellular scaffold for islet encapsulation. The viability of islets entrapped within a P(NIPAAm-*co*-AA) hydrogel and the subsequent secretion of insulin were improved markedly in comparison to those of free islets [106]. In addition, insulin secretion was further enhanced by the addition of glucagon-like peptide 1 (GLP-1) to the hydrogel [107]. Another injectable hydrogel system for islet encapsulation was developed using Michael addition between PEG-maleimides and dithiol

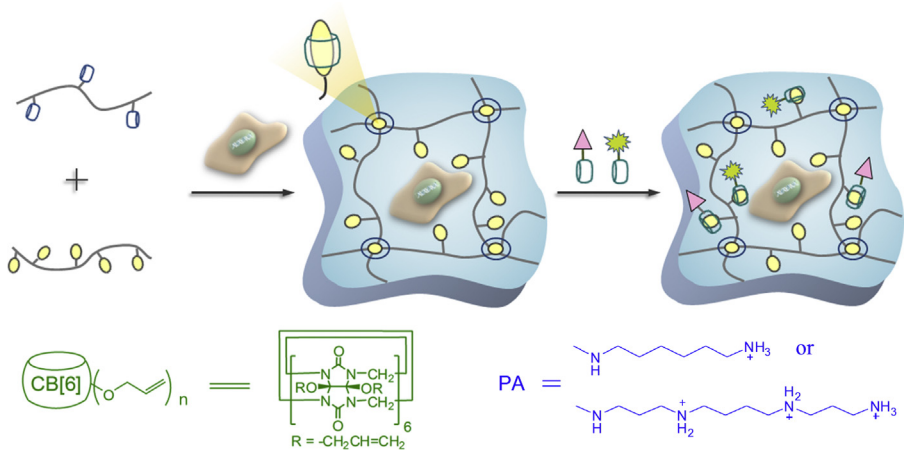

Fig. 9. Schematic illustration of *in situ* CB[6]/DAH–HA hydrogel formation in the presence of genetically engineered mesenchymal stem cells. Also shown is its modular modification, using highly selective and strong host–guest interactions [101], Copyright 2012. Reproduced with permission from the American Chemical Society.

crosslinkers containing protease-cleavable peptides. The hydrogel was functionalized with an RGD peptide and/or VEGF to improve viability and engraftment of cells [108]. Islet transplantation using VEGF-releasing PEG hydrogels resulted in reversal of hyperglycemia in diabetic model mice. Glucose tolerance tests confirmed the normoglycemia in the diabetic mice that had been treated with islets encapsulated in the VEGF-releasing PEG hydrogels. Alginate micro-hydrogels loaded with bioactive glass and fibroblasts were also developed to deliver VEGF for long-term treatment of ischemia [109]. The bioactive glass enhanced the endothelial cell proliferation and VEGF secretion from fibroblasts in the alginate beads.

As an alternative to drug delivery, cells have been genetically modified to produce and secrete therapeutic biomolecules, such as cytokines, hormones, and proteins. RGD-conjugated alginate was used to encapsulate genetically engineered C_2C_{12} myoblasts to secrete murine erythropoietin (EPO) [110]. *In vitro* EPO secretion from cells loaded in alginate microcapsules using RGD was higher than that of the control group. In addition, subcutaneous injections of cell-loaded hydrogels resulted in a relatively high EPO release, maintaining a high hematocrit level, with minimal inflammatory reaction. Photo-crosslinked PEG hydrogel has also been developed and used to encapsulate genetically engineered fibroblasts expressing BMP-2 for bone regeneration in bone-defect animal models [111]. Recently, we reported hydrogel optical waveguides for *in vivo* cell-based sensing and therapy (Fig. 10). PEG-diacrylate was photo-crosslinked encapsulating optogenetic cells, which can be triggered by optical signals, to assess the nanotoxicity of quantum dots and to produce GLP-1 for the treatment of diabetes as model diagnostic and therapeutic systems [112,113]. Photolabile hydrogels that contain a cysteine compound with a sulfhydryl protecting group was used to guide 3D cell growth and migration for cell therapy [114].

Remarkably, gene expression was reported to be significantly higher in mesenchymal stem cells (MSCs) than in normal cells [115]. Transplanted allogeneic or xenogeneic stem cells have shown prolonged survival and even engraftment in various tissues of host organisms, due to the non-immunogenicity of MSCs [116]. Accordingly, genetically modified MSCs have been extensively investigated for the treatment of intractable diseases [117–119]. The therapeutic gene was packaged into a delivery vehicle, such as an adenovirus, which was then transduced into MSCs isolated from the patient. In this approach, adequate cell carriers can greatly reduce the number of cells needed for therapeutic applications. We have previously reported *in situ* formation and modular modification of CB[6]/DAH–HA hydrogels [101]. For tissue engineering applications, the supramolecular CB[6]/DHA–HA hydrogels were modularly modified with a cyclic RGDyK peptide. After 2 weeks, the number of NHDF human fibroblast cells in the hydrogel increased 5-fold, consistent with the characteristic cell adhesion and proliferation behaviors in the context of a RGD environment. We have also carried out *in situ* formation and modular modification of hydrogels under the skin of mice by sequential subcutaneous injections of CB[6]–HA, DAH–HA, and FITC–CB[6] [101]. We confirmed the feasibility of supramolecular hydrogels as a 3D artificial ECM for various genetically modified stem cell therapies.

3.2. Injectable hydrogels for tissue regeneration

The importance of tissue regeneration has increased significantly with prolonged life expectancy [120]. Regenerated skin, cartilage, and bone are already clinically available, and meaningful progress has already been achieved for regeneration of bladder, liver, and heart tissue, as well as spinal cord nerves [121–123]. Injectable hydrogels have been investigated widely as an artificial scaffold for regenerative medicine. For example, *in situ*-forming HA hydrogels were developed using the Michael addition reaction between thiol-group substituted HA (HA–SH) and VS-modified PEG (PEG–VS) for cartilage repair [26]. Chondrocytes were homogeneously encapsulated within the hydrogel by mixing the polymer precursor solutions. The cells survived and proliferated for 3 weeks.

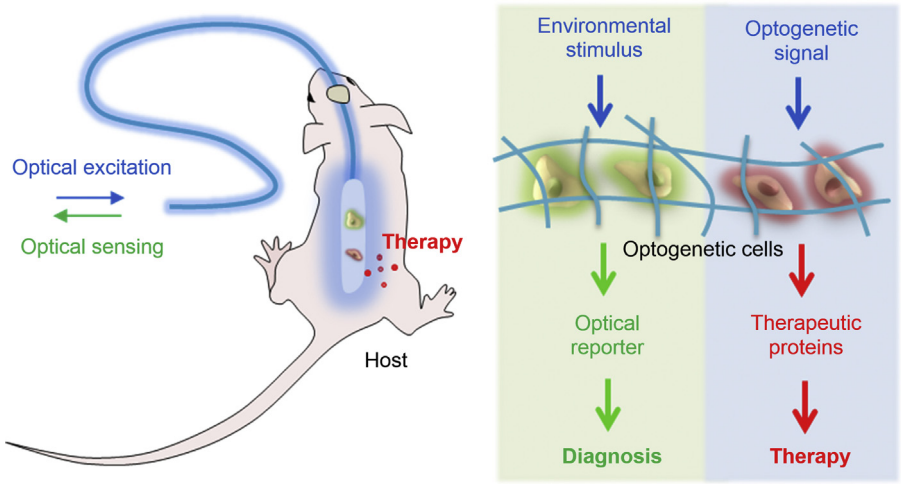

Fig. 10. Schematic illustration of the hydrogel optical waveguide for *in vivo* optogenetic cell-based sensing and therapy [112]. Copyright 2013. Reproduced with permission from the Nature Publishing Group.

Collagen type II and GAG accumulated in the hydrogels, reflecting the feasibility of using these hydrogels for cartilage regeneration. Other injectable hydrogels prepared by the Michael addition reaction were developed using heparin–SH and PEG–diacrylate for primary hepatocyte culture [28]. The encapsulated primary hepatocytes showed enhanced hepatic function in rats. The expression of albumin-, urea-, and liver-specific products were maintained at high levels for 3 weeks after cell encapsulation. In addition, hepatocytes cultured within heparin-based hydrogels containing hepatocyte growth factors exhibited higher levels of albumin and urea production than the cells entrapped within the hydrogel alone. Moreover, dextran–tyramine conjugate and heparin–tyramine conjugate were co-crosslinked for application in chondrogenesis [46]. Heparin interacts with ECM components and modulates cell signaling by cell–matrix interaction. Heparin can promote cell adhesion, proliferation, and differentiation. Peptide-based hydrogels have also been developed using click chemistry for cell delivery [124]. An RGD peptide was functionalized using azide and four-arm PEG was modified with acetylene, to fabricate injectable hydrogels by click chemistry. Primary human dermal fibroblasts were then encapsulated within the PEG–peptide hydrogels to assess the feasibility of using this type of hydrogel for wound healing. The attachment and proliferation of the encapsulated cells were markedly improved by increasing the RGD peptide content in the hydrogels.

The precursor solution of the SMO–PCLA–PEG–PCLA–SMO multiblock copolymer showed a reversible sol–gel transition at pH 7.2 and body temperature [82]. Under physiological conditions, human MSCs and recombinant human BMP-2 protein were easily encapsulated within the hydrogels. Subcutaneous injection of the precursor polymer solution containing human MSCs and BMP-2 resulted in formation of a hydrogel encapsulating stem cells and bioactive molecules on the back of mice, within 10 min. The hydrogel maintained its shape, and the encapsulated human MSCs survived and proliferated for up to 7 weeks. These pH- and temperature-responsive hydrogels were able to enhance the mechanical strength of the hydrogels and prevented the formation of the hydrogel from the precursor solution during injection into the body. Injectable polyurethane acrylate systems were also developed using poly(propylene glycol) or biodegradable poly(caprolactone diol) and hydroxyethyl methacrylate for biomedical applications [52]. The mechanical properties of the cured polymers were comparable to that of soft tissues, exhibiting good biocompatibility in the dorsum of rats for more than 4 weeks. Moreover, a two-component protein-engineered physical hydrogel system was reported to encapsulate various cell types, including PC-12 neuronal-like cells, human umbilical vein endothelial cells, and murine adult neural stem cells [86]. Multiple repeating units of tryptophan- and proline-rich peptide domains caused a sol–gel phase transition by hetero-assembly of the peptide domains. The encapsulated neural stem cells were induced to undergo self-renewal, differentiation, and neurite extension within the hydrogels, forming 3D stable networks.

Supramolecular hydrogels using host–guest interaction of CD have also been developed for stem cell encapsulation [125]. MPEG–PCL–MPEG triblock copolymers could generate a complex with α-CD for tissue engineering applications. ECV304 cells and bone marrow-derived MSCs were encapsulated in the hydrogels with ease, and they maintained their morphologies within the hydrogels, demonstrating the feasibility of using these hydrogels for further applications. On the basis of our previous work [101], we developed more advanced supramolecular HA hydrogels as artificial scaffolds for controlled chondrogenesis of human MSCs, using mono-allyloxyCB[6]-conjugated HA (monoCB[6]–HA), DAH–HA, and drug–CB[6] (Fig. 9) [126]. Dexamethasone (Dexa) could be controlled-released by the hydrolysis of ester-linkages in Dexa–CB[6] which was modularly introduced to CB[6]/DAH–HA hydrogels

for effective chondrogenesis in the presence of TGF-β. Taken together, we confirmed the feasibility of using supramolecular hydrogels as a 3D artificial ECM for cell therapies and tissue engineering applications.

4. Conclusion and perspectives

In this review, we summarized a wide range of injectable hydrogels that have been applied to regenerative medicine, including cell therapy and tissue regeneration. Each hydrogel system has its own advantages and disadvantages in terms of applications in regenerative medicine. Injectable hydrogels prepared by chemical crosslinking demonstrate good mechanical properties, but *in vivo* applications have been limited due to the possible cytotoxicity of the reactive chemical crosslinkers. In contrast, injectable hydrogels prepared by physical crosslinking can be formed easily without reactive chemical reagents, but the hydrogels have poor stability and mechanical properties in the body. Supramolecular injectable hydrogels are fabricated by self-assembly of receptor–ligand pairs, complementary pairs, and host–guest pairs. We have developed injectable supramolecular HA hydrogels using strong and selective host–guest interaction between CB[6]–HA and DAH–HA, which demonstrated the feasibility of using these hydrogels as tissue-engineered scaffolds for cell therapy and tissue regeneration.

The major challenges related to using *in situ* forming hydrogels for regenerative medicine are the attainment of 3D microenvironment of cells in the ECM with biocompatibility, biodegradability, connected pores for active nutrient transport, and spatiotemporal control of cells with adequate growth factors. Engineering tissue functions requires in-depth understanding of stem cell biology, cell–cell communication, tissue dynamics, biomaterials, physicochemical processes, and bioengineering designs. Achieving all these requirements and integrating them into novel designs represent the main task confronting multidisciplinary tissue engineers.

As a next-generation regenerative medicine, organoids have been extensively investigated for new drug screening and therapeutic applications since 2010 [127]. For example, cerebral organoids have been generated successfully in a culture dish to model microcephaly [128,129]. This emerging field of organoids, developed *via* spatiotemporal control of stem cells by means of hydrogels, will open the door to futuristic regenerative medicine.

Acknowledgements

This research was supported by the Bio & Medical Technology Development Program of the National Research Foundation (NRF), funded by the Korean government (MEST) (No. 2012M3A9C6049791). This study was also supported by a Mid-career Researcher Program through NRF grant funded by the MEST (No. 2012R1A2A2A06045773). This work was supported by the IT Consilience Creative Program of MKE and NIPA (C1515-1121-0003).

References

[1] Petit-Zeman S. Regenerative medicine. Nat Biotechnol 2001;19:201–6.
[2] Hunt JA. Regenerative medicine: materials in a cellular world. Nat Mater 2008;7:617–8.
[3] Kim BS, Park IK, Hoshiba T, Jiang HL, Choi YJ, Akaike T, Cho CS. Design of artificial extracellular matrices for tissue engineering. Prog Polym Sci 2011;36:238–68.
[4] Gonzalez AM, Gonzales M, Herron GS, Nagavarapu U, Hopkinson SB, Tsuruta D, Jones JCR. Complex interactions between the laminin alpha 4 subunit and integrins regulate endothelial cell behavior in vitro and angiogenesis in vivo. Proc Natl Acad Sci USA 2002;99:16075–80.
[5] Bast Jr RC, Hennessy B, Mills GB. The biology of ovarian cancer: new opportunities for translation. Nat Rev Cancer 2009;9:415–28.
[6] Lutolf MP, Gilbert PM, Blau HM. Designing materials to direct stem-cell fate. Nature 2009;462:433–41.
[7] Kong HJ, Mooney DJ. Microenvironmental regulation of biomacromolecular therapies. Nat Rev Drug Discov 2007;6:455–63.
[8] Dash M, Chiellini F, Ottenbrite RM, Chiellini E. Chitosan-A versatile semi-synthetic polymer in biomedical applications. Prog Polym Sci 2011;36:981–1014.
[9] Deming TJ. Synthetic polypeptides for biomedical applications. Prog Polym Sci 2007;32:858–75.
[10] Puppi D, Chiellini F, Piras AM, Chiellini E. Polymeric materials for bone and cartilage repair. Prog Polym Sci 2010;35:403–40.
[11] Vert M. Polymeric biomaterials: strategies of the past vs. strategies of the future. Prog Polym Sci 2007;32:755–61.
[12] Ma PX. Biomimetic materials for tissue engineering. Adv Drug Deliv Rev 2008;60:184–98.
[13] Patel S, Kurpinski K, Quigley R, Gao HF, Hsiao BS, Poo MM, Li S. Bioactive nanofibers: synergistic effects of nanotopography and chemical signaling on cell guidance. Nano Lett 2007;7:2122–8.
[14] Agarwal S, Greiner A, Wendorff JH. Electrospinning of manmade and biopolymer nanofibers-progress in techniques, materials, and applications. Adv Funct Mater 2009;19:2863–79.
[15] Seliktar D. Designing cell-compatible hydrogels for biomedical applications. Science 2012;336:1124–8.
[16] Hoffman AS. Hydrogels for biomedical applications. Adv Drug Deliv Rev 2002;54:3–12.
[17] Lutolf MP, Hubbell JA. Synthetic biomaterials as instructive extracellular microenvironments for morphogenesis in tissue engineering. Nat Biotechnol 2005;23:47–55.
[18] Li YL, Rodrigues J, Tomas H. Injectable and biodegradable hydrogels: gelation, biodegradation and biomedical applications. Chem Soc Rev 2012;41:2193–221.
[19] Kretlow JD, Klouda L, Mikos AG. Injectable matrices and scaffolds for drug delivery in tissue engineering. Adv Drug Deliv Rev 2007;59:263–73.
[20] Park H, Temenoff JS, Tabata Y, Caplan AI, Mikos AG. Injectable biodegradable hydrogel composites for rabbit marrow mesenchymal stem cell and growth factor delivery for cartilage tissue engineering. Biomaterials 2007;28:3217–27.
[21] Burdick JA, Prestwich GD. Hyaluronic acid hydrogels for biomedical applications. Adv Mater 2011;23:H41–56.
[22] Ko DY, Shinde UP, Yeon B, Jeong B. Recent progress of in situ formed gels for biomedical applications. Prog Polym Sci 2013;38:672–701.
[23] Yu L, Ding J. Injectable hydrogels as unique biomedical materials. Chem Soc Rev 2008;37:1473–81.
[24] Mather BD, Viswanathan K, Miller KM, Long TE. Michael addition reactions in macromolecular design for emerging technologies. Prog Polym Sci 2006;31:487–531.
[25] Zheng Shu X, Liu Y, Palumbo FS, Luo Y, Prestwich GD. In situ crosslinkable hyaluronan hydrogels for tissue engineering. Biomaterials 2004;25:1339–48.
[26] Jin R, Moreira Teixeira LS, Krouwels A, Dijkstra PJ, van Blitterswijk CA, Karperien M, Feijen J. Synthesis and characterization of hyaluronic acid-poly(ethylene glycol) hydrogels via Michael addition: an injectable biomaterial for cartilage repair. Acta Biomater 2010;6:1968–77.
[27] Tae G, Kim YJ, Choi WI, Kim M, Stayton PS, Hoffman AS. Formation of a novel heparin-based hydrogel in the presence of heparin-binding biomolecules. Biomacromolecules 2007;8:1979–86.
[28] Kim M, Lee JY, Jones CN, Revzin A, Tae G. Heparin-based hydrogel as a matrix for encapsulation and cultivation of primary hepatocytes. Biomaterials 2010;31:3596–603.

[29] Yang JA, Kim H, Park K, Hahn SK. Molecular design of hyaluronic acid hydrogel networks for long-term controlled delivery of human growth hormone. Soft Matter 2011;7:868–70.
[30] Cai S, Liu Y, Zheng SX, Prestwich GD. Injectable glycosaminoglycan hydrogels for controlled release of human basic fibroblast growth factor. Biomaterials 2005;26:6054–67.
[31] Liu Y, Shu XZ, Prestwich GD. Reduced postoperative intra-abdominal adhesions using Carbylan-SX, a semisynthetic glycosaminoglycan hydrogel. Fertil Steril 2007;87:940–8.
[32] Shu XZ, Liu Y, Palumbo F, Prestwich GD. Disulfide-crosslinked hyaluronan–gelatin hydrogel films: a covalent mimic of the extracellular matrix for in vitro cell growth. Biomaterials 2003;24:3825–34.
[33] Binder WH, Sachsenhofer R. 'Click' chemistry in polymer and materials science. Macromol Rapid Commun 2007;28:15–54.
[34] Moses JE, Moorhouse AD. The growing applications of click chemistry. Chem Soc Rev 2007;36:1249–62.
[35] Nimmo CM, Shoichet MS. Regenerative biomaterials that "click": simple, aqueous-based protocols for hydrogel synthesis, surface immobilization, and 3D patterning. Bioconjugate Chem 2011;22:2199–209.
[36] van Dijk M, van Nostrum CF, Hennink WE, Rijkers DT, Liskamp RM. Synthesis and characterization of enzymatically biodegradable PEG and peptide-based hydrogels prepared by click chemistry. Biomacromolecules 2010;11:1608–14.
[37] Malkoch M, Vestberg R, Gupta N, Mespouille L, Dubois P, Mason AF, Hedrick JL, Liao Q, Frank CW, Kingsbury K, Hawker CJ. Synthesis of well-defined hydrogel networks using click chemistry. Chem Commun 2006:2774–6.
[38] Johnson JA, Lewis DR, Diaz DD, Finn MG, Koberstein JT, Turro NJ. Synthesis of degradable model networks via ATRP and click chemistry. J Am Chem Soc 2006;128:6564–5.
[39] DeForest CA, Anseth KS. Cytocompatible click-based hydrogels with dynamically tunable properties through orthogonal photoconjugation and photocleavage reactions. Nat Chem 2011;3:925–31.
[40] DeForest CA, Polizzotti BD, Anseth KS. Sequential click reactions for synthesizing and patterning three-dimensional cell microenvironments. Nat Mater 2009;8:659–64.
[41] Ossipov DA, Brannvall K, Forsberg-Nilsson K, Hilborn J. Formation of the first injectable poly(vinyl alcohol) hydrogel by mixing of functional PVA precursors. J Appl Polym Sci 2007;106:60–70.
[42] Tan H, Chu CR, Payne KA, Marra KG. Injectable in situ forming biodegradable chitosan-hyaluronic acid based hydrogels for cartilage tissue engineering. Biomaterials 2009;30:2499–506.
[43] Wang DA, Varghese S, Sharma B, Strehin I, Fermanian S, Gorham J, Fairbrother DH, Cascio B, Elisseeff JH. Multifunctional chondroitin sulphate for cartilage tissue–biomaterial integration. Nat Mater 2007;6:385–92.
[44] Jia X, Yeo Y, Clifton RJ, Jiao T, Kohane DS, Kobler JB, Zeitels SM, Langer R. Hyaluronic acid-based microgels and microgel networks for vocal fold regeneration. Biomacromolecules 2006;7:3336–44.
[45] Maia J, Ferreira L, Carvalho R, Ramos MA, Gil MH. Synthesis and characterization of new injectable and degradable dextran-based hydrogels. Polymer 2005;46:9604–14.
[46] Kim KS, Park SJ, Yang JA, Jeon JH, Bhang SH, Kim BS, Hahn SK. Injectable hyaluronic acid–tyramine hydrogels for the treatment of rheumatoid arthritis. Acta Biomater 2011;7:666–74.
[47] Jin R, Moreira Teixeira LS, Dijkstra PJ, van Blitterswijk CA, Karperien M, Feijen J. Chondrogenesis in injectable enzymatically crosslinked heparin/dextran hydrogels. J Control Release 2011;152:186–95.
[48] Jin R, Moreira Teixeira LS, Dijkstra PJ, Karperien M, van Blitterswijk CA, Zhong ZY, Feijen J. Injectable chitosan-based hydrogels for cartilage tissue engineering. Biomaterials 2009;30:2544–51.
[49] Jin R, Hiemstra C, Zhong Z, Feijen J. Enzyme-mediated fast in situ formation of hydrogels from dextran–tyramine conjugates. Biomaterials 2007;28:2791–800.
[50] Elisseeff J, Anseth K, Sims D, McIntosh W, Randolph M, Langer R. Transdermal photopolymerization for minimally invasive implantation. Proc Natl Acad Sci USA 1999;96:3104–7.
[51] Chou AI, Akintoye SO, Nicoll SB. Photo-crosslinked alginate hydrogels support enhanced matrix accumulation by nucleus pulposus cells in vivo. Osteoarthr Cartil 2009;17:1377–84.
[52] Pereira IHL, Ayres E, Patricio PS, Goes AM, Gomide VS, Junior EP, Orefice RL. Photopolymerizable and injectable polyurethanes for biomedical applications: synthesis and biocompatibility. Acta Biomater 2010;6:3056–66.
[53] Ovsianikov A, Malinauskas M, Schlie S, Chichkov B, Gittard S, Narayan R, Lobler M, Sternberg K, Schmitz KP, Haverich A. Three-dimensional laser micro- and nano-structuring of acrylated poly(ethylene glycol) materials and evaluation of their cytoxicity for tissue engineering applications. Acta Biomater 2011;7:967–74.
[54] Williams CG, Malik AN, Kim TK, Manson PN, Elisseeff JH. Variable cytocompatibility of six cell lines with photoinitiators used for polymerizing hydrogels and cell encapsulation. Biomaterials 2005;26:1211–8.
[55] Stevens MM, Qanadilo HF, Langer R, Shastri VP. A rapid-curing alginate gel system: utility in periosteum-derived cartilage tissue engineering. Biomaterials 2004;25:887–94.
[56] Ashton RS, Banerjee A, Punyani S, Schaffer DV, Kane RS. Scaffolds based on degradable alginate hydrogels and poly(lactide-co-glycolide) microspheres for stem cell culture. Biomaterials 2007;28:5518–25.
[57] Zhao LA, Weir MD, Xu HHK. An injectable calcium phosphate–alginate hydrogel–umbilical cord mesenchymal stem cell paste for bone tissue engineering. Biomaterials 2010;31:6502–10.
[58] Kuo CK, Ma PX. Ionically crosslinked alginate hydrogels as scaffolds for tissue engineering: part 1. Structure, gelation rate and mechanical properties. Biomaterials 2001;22:511–21.
[59] Ikada Y. Challenges in tissue engineering. J R Soc Interface 2006;3:589–601.
[60] Donati I, Asaro F, Paoletti S. Experimental evidence of counterion affinity in alginates: the case of nongelling ion Mg^{2+}. J Phys Chem B 2009;113:12877–86.
[61] Nair LS, Laurencin CT. Biodegradable polymers as biomaterials. Prog Polym Sci 2007;32:762–98.
[62] Sun JY, Zhao X, Illeperuma WR, Chaudhuri O, Oh KH, Mooney DJ, Vlassak JJ, Suo Z. Highly stretchable and tough hydrogels. Nature 2012;489:133–6.
[63] Lee KY, Mooney DJ, Alginate:. properties and biomedical applications. Prog Polym Sci 2012;37:106–26.
[64] Igarashi T, Iwasaki N, Kawamura D, Tsukuda Y, Kasahara Y, Todoh M, Tadano S, Minami A. Therapeutic effects of intra-articular ultrapurified low endotoxin alginate administration on experimental osteoarthritis in rabbits. Cartilage 2012;3:70–8.
[65] Berger J, Reist M, Mayer JM, Felt O, Gurny R. Structure and interactions in chitosan hydrogels formed by complexation or aggregation for biomedical applications. Eur J Pharm Biopharm 2004;57:35–52.
[66] Molinaro G, Leroux JC, Damas J, Adam A. Biocompatibility of thermosensitive chitosan-based hydrogels: an in vivo experimental approach to injectable biomaterials. Biomaterials 2002;23:2717–22.
[67] Chenite A, Chaput C, Wang D, Combes C, Buschmann MD, Hoemann CD, Leroux JC, Atkinson BL, Binette F, Selmani A. Novel injectable neutral solutions of chitosan form biodegradable gels in situ. Biomaterials 2000;21:2155–61.
[68] Tan HP, Marra KG. Injectable biodegradable hydrogels for tissue engineering applications. Materials 2010;3:1746–67.
[69] Yan JH, Yang L, Wang GR, Xiao Y, Zhang BH, Qi NM. Biocompatibility evaluation of chitosan-based injectable hydrogels for the culturing mice mesenchymal stem cells in vitro. J Biomater Appl 2010;24:625–37.
[70] Klouda L, Mikos AG. Thermoresponsive hydrogels in biomedical applications. Eur J Pharm Biopharm 2008;68:34–45.
[71] Stile RA, Healy KE. Thermo-responsive peptide-modified hydrogels for tissue regeneration. Biomacromolecules 2001;2:185–94.
[72] Ruel-Gariepy E, Leroux JC. In situ-forming hydrogels – review of temperature-sensitive systems. Eur J Pharm Biopharm 2004;58:409–26.
[73] Alexandridis P, Zhou DL, Khan A. Lyotropic liquid crystallinity in amphiphilic block copolymers: temperature effects on phase behavior and structure for poly(ethylene oxide)-b-poly(propylene oxide)-b-poly(ethylene oxide) copolymers of different composition. Langmuir 1996;12:2690–700.
[74] Zhang XZ, Wu DQ, Sun GM, Chu CC. Novel biodegradable and thermosensitive Dex-AI/PNIPA. Am hydrogel Macromol Biosci 2003;3:87–91.
[75] Ha DI, Lee SB, Chong MS, Lee YM, Kim SY, Park YH. Preparation of thermo-responsive and injectable hydrogels based on hyaluronic acid and poly(*N*-isopropylacrylamide) and their drug release behaviors. Macromol Res 2006;14:87–93.
[76] Feng L, Hao JY, Xiong CD, Deng XM. A novel biodegradable and thermosensitive polymer with PEG-analogue macromolecular structure. Chem Commun 2009:4411–3.
[77] Fusco S, Borzacchiello A, Netti PA. Perspectives on: PEO–PPO–PEO triblock copolymers and their biomedical applications. J Bioact Compat Polym 2006;21:149–64.

[78] Jeong B, Bae YH, Kim SW. In situ gelation of PEG–PLGA–PEG triblock copolymer aqueous solutions and degradation thereof. J Biomed Mater Res 2000;50:171–7.
[79] Disthabanchong S, Radinahamed P, Stitchantrakul W, Hongeng S, Rajatanavin R. Chronic metabolic acidosis alters osteoblast differentiation from human mesenchymal stem cells. Kidney Int 2007;71:201–9.
[80] Liu H, Slamovich EB, Webster TJ. Less harmful acidic degradation of poly(lacticco-glycolic acid) bone tissue engineering scaffolds through titania nanoparticle addition. Int J Nanomed 2006;1:541–5.
[81] Xu XD, Zhang XZ, Cheng SX, Zhuo RX, Kennedy JF. A strategy to introduce the pH sensitivity to temperature sensitive PNIPAAm hydrogels without weakening the thermosensitivity. Carbohydr Polym 2007;68:416–23.
[82] Kim HK, Shim WS, Kim SE, Lee KH, Kang E, Kim JH, Kwon IC, Lee DS. Injectable in situ-forming pH/thermo-sensitive hydrogel for bone tissue engineering. Tissue Eng 2009;15A::923–33.
[83] Potta T, Chun C, Song SC. Dual cross-linking systems of functionally photo-cross-linkable and thermoresponsive polyphosphazene hydrogels for biomedical applications. Biomacromolecules 2010;11:1741–53.
[84] Tai H, Howard D, Takae S, Wang W, Vermonden T, Hennink WE, Stayton PS, Hoffman AS, Endruweit A, Alexander C, Howdle SM, Shakesheff KM. Photo-cross-linked hydrogels from thermoresponsive PEGMEMA–PPGMA–EGDMA copolymers containing multiple methacrylate groups: mechanical property, swelling, protein release, and cytotoxicity. Biomacromolecules 2009;10:2895–903.
[85] Salem AK, Rose FRAJ, Oreffo ROC, Yang XB, Davies MC, Mitchell JR, Roberts CJ, Stolnik-Trenkic S, Tendler SJB, Williams PM, Shakesheff KM. Porous polymer and cell composites that self-assemble in situ. Adv Mater 2003;15:210–3.
[86] 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.
[87] Kiick KL. Peptide- and protein-mediated assembly of heparinized hydrogels. Soft Matter 2008;4:29–37.
[88] Miyata T, Asami N, Uragami T. Structural design of stimuli-responsive bioconjugated hydrogels that respond to a target antigen. J Polym Sci Pol Phys 2009;47:2144–57.
[89] Miyata T, Asami N, Uragami T. Preparation of an antigen-sensitive hydrogel using antigen–antibody bindings. Macromolecules 1999;32:2082–4.
[90] Miyata T, Asami N, Uragami T. A reversibly antigen-responsive hydrogel. Nature 1999;399:766–9.
[91] Um SH, Lee JB, Park N, Kwon SY, Umbach CC, Luo D. Enzyme-catalysed assembly of DNA hydrogel. Nat Mater 2006;5:797–801.
[92] Xing YZ, Cheng EJ, Yang Y, Chen P, Zhang T, Sun YW, Yang ZQ, Liu DS. Self-assembled DNA hydrogels with designable thermal and enzymatic responsiveness. Adv Mater 2011;23:1117–21.
[93] Wei B, Cheng I, Luo KQ, Mi YL. Capture and release of protein by a reversible DNA-induced sol–gel transition system. Angew Chem Int Ed 2008;47:331–3.
[94] Davis ME, Brewster ME. Cyclodextrin-based pharmaceutics: past, present and future. Nat Rev Drug Discov 2004;3:1023–35.
[95] Li J. Self-assembled supramolecular hydrogels based on polymer–cyclodextrin inclusion complexes for drug delivery. NPG Asia Mater 2010;2:112–8.
[96] Kretschmann O, Choi SW, Miyauchi M, Tomatsu I, Harada A, Ritter H. Switchable hydrogels obtained by supramolecular cross-linking of adamantyl-containing LCST copolymers with cyclodextrin dimers. Angew Chem Int Ed 2006;45:4361–5.
[97] van de Manakker F, van der Pot M, Vermonden T, van Nostrum CF, Hennink WE. Self-assembling hydrogels based on beta-cyclodextrin/cholesterol inclusion complexes. Macromolecules 2008;41:1766–73.
[98] Lehn J-M. From molecular to supramolecular chemistry. In: Supramolecular chemistry. Weinheim: Wiley-VCH; 1995. p. 1–9.
[99] Lee JW, Samal S, Selvapalam N, Kim HJ, Kim K. Cucurbituril homologues and derivatives: new opportunities in supramolecular chemistry. Acc Chem Res 2003;36:621–30.
[100] Appel EA, Biedermann F, Rauwald U, Jones ST, Zayed JM, Scherman OA. Supramolecular cross-linked networks via host–guest complexation with cucurbit[8]uril. J Am Chem Soc 2010;132:14251–60.
[101] Park KM, Yang JA, Jung H, Yeom J, Park JS, Park KH, Hoffman AS, Hahn SK, Kim K. In situ supramolecular assembly and modular modification of hyaluronic acid hydrogels for 3D cellular engineering. ACS Nano 2012;6:2960–8.
[102] Kirouac DC, Madlambayan GJ, Yu M, Sykes EA, Ito C, Zandstra PW. Cell–cell interaction networks regulate blood stem and progenitor cell fate. Mol Syst Biol 2009;5, 293 (1–20).
[103] Stamm C, Westphal B, Kleine HD, Petzsch M, Kittner C, Klinge H, Schumichen C, Nienaber CA, Freund M, Steinhoff G. Autologous bone-marrow stem-cell transplantation for myocardial regeneration. Lancet 2003;361:45–6.
[104] García-Olmo D, García-Arranz M, Herreros D, Pascual I, Peiro C, Rodríguez-Montes JA. A phase I clinical trial of the treatment of Crohn's fistula by adipose mesenchymal stem cell transplantation. Dis Colon Rectum 2005;48:1416–23.
[105] Le Blanc K, Frassoni F, Ball L, Locatelli F, Roelofs H, Lewis I, Lanino E, Sundberg B, Bernardo ME, Remberger M, Dini G, Egeler RM, Bacigalupo A, Fibbe W, Ringden O. Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study. Lancet 2008;371:1579–86.
[106] Vernon B, Kim SW, Bae YH. Insulin release from islets of Langerhans entrapped in a poly(*N*-isopropylacrylamide-co-acrylic acid) polymer gel. J Biomater Sci Polym Ed 1999;10:183–98.
[107] Gappa H, Baudys M, Koh JJ, Kim SW, Bae YH. The effect of zinc-crystallized glucagon-like peptide-1 on insulin secretion of macroencapsulated pancreatic islets. Tissue Eng 2001;7A:: 35–44.
[108] Phelps EA, Headen DM, Taylor WR, Thule PM, Garcia AJ. Vasculogenic bio-synthetic hydrogel for enhancement of pancreatic islet engraftment and function in type 1 diabetes. Biomaterials 2013;34:4602–11.
[109] Keshaw H, Forbes A, Day RM. Release of angiogenic growth factors from cells encapsulated in alginate beads with bioactive glass. Biomaterials 2005;26:4171–9.
[110] Orive G, De Castro M, Kong HJ, Hernandez RM, Ponce S, Mooney DJ, Pedraz JL. Bioactive cell-hydrogel microcapsules for cell-based drug delivery. J Contr Release 2009;135:203–10.
[111] Bikram M, Fouletier-Dilling C, Hipp JA, Gannon F, Davis AR, Olmsted-Davis EA, West JL. Endochondral bone formation from hydrogel carriers loaded with BMP2-transduced cells. Ann Biomed Eng 2007;35:796–807.
[112] Choi M, Choi JW, Kim S, Nizamoglu S, Hahn SK, Yun SH. Light-guiding hydrogels for cell-based sensing and therapy. Nat Photonics 2013;7:987–94.
[113] Koch L. Technology: let there be light – new implantable hydrogels for in vivo optical sensing and therapy. Nat Rev Endocrinol 2013;10:2.
[114] Luo Y, Shoichet MS. A photolabile hydrogel for guided three-dimensional cell growth and migration. Nat Mater 2004;3: 249–53.
[115] Oh HJ, Park EJ, Lee SY, Soh JW, Kong IS, Choi SW, Ra JC, Kang SK, Lee BC. Comparison of cell proliferation and epigenetic modification of gene expression patterns in canine foetal fibroblasts and adipose tissue-derived mesenchymal stem cells. Cell Prolif 2012;45:438–44.
[116] Wojciechowski AB, Englund U, Lundberg C, Warfvinge K. Survival and long distance migration of brain-derived precursor cells transplanted to adult rat retina. Stem Cells 2004;22:27–38.
[117] Lindvall O, Kokaia Z. Stem cells for the treatment of neurological disorders. Nature 2006;441:1094–6.
[118] Cheng ZK, Ou LL, Zhou X, Li F, Jia XH, Zhang YG, Liu XL, Li YM, Ward CA, Melo LG, Kong DL. Targeted migration of mesenchymal stem cells modified with CXCR4 gene to infarcted myocardium improves cardiac performance. Mol Ther 2008;16:571–9.
[119] van der Flier LG, Clevers H. Stem cells, self-renewal, and differentiation in the intestinal epithelium. Annu Rev Physiol 2009;71:241–60.
[120] Badylak SF, Nerem RM. Progress in tissue engineering and regenerative medicine. Proc Natl Acad Sci USA 2010;107:3285–6.
[121] Horner PJ, Gage FH. Regenerating the damaged central nervous system. Nature 2000;407:963–70.
[122] Laflamme MA, Murry CE. Heart regeneration. Nature 2011;473:326–35.
[123] Michalopoulos GK, DeFrances MC. Liver regeneration. Science 1997;276:60–6.
[124] Liu SQ, Ee PLR, Ke CY, Hedrick JL, Yang YY. Biodegradable poly(ethylene glycol)-peptide hydrogels with well-defined structure and properties for cell delivery. Biomaterials 2009;30:1453–61.
[125] Wu DQ, Wang T, Lu B, Xu XD, Cheng SX, Jiang XJ, Zhang XZ, Zhuo RX. Fabrication of supramolecular hydrogels for drug delivery and stem cell encapsulation. Langmuir 2008;24: 10306–12.

[126] Jung H, Park JS, Yeom J, Selvapalam N, Park KM, Oh K, Yang JA, Park KH, Hahn SK, Kim K. 3D tissue engineered supramolecular hydrogels for the controlled chondrogenesis of human mesenchymal stem cells. Biomacromolecules 2014;15:707–14.
[127] Sasai Y. Next-generation regenerative medicine: organogenesis from stem cells in 3D culture. Cell Stem Cell 2013;12:520–30.
[128] Lancaster MA, Renner M, Martin CA, Wenzel D, Bicknell LS, Hurles ME, Homfray T, Penninger JM, Jackson AP, Knoblich JA. Cerebral organoids model human brain development and microcephaly. Nature 2013;501:373–9.
[129] Bershteyn M, Kriegstein AR. Cerebral organoids in a dish: progress and prospects. Cell 2013;155:19–20.