Bioconjugate Chemistry

Review

pubs.acs.org/bc

Bioconjugated Hydrogels for Tissue Engineering and Regenerative Medicine

This paper was originally submitted for the "Biofunctional Biomaterials: The Third Generation of Medical Devices", published as the July 15, 2015, issue of Bioconjugate Chemistry (Vol. 26, No. 7).

Samad Ahadian,[†] Ramin Banan Sadeghian,[†,#] Sahar Salehi,[†,#] Serge Ostrovidov,[†,#] Hojae Bae,[‡] Murugan Ramalingam,[†,§] and Ali Khademhosseini*[,†,‡,∥,⊥,¶,●]

[†]WPI-Advanced Institute for Materials Research, Tohoku University, Sendai 980-8577, Japan

[‡]College of Animal Bioscience and Technology, Department of Bioindustrial Technologies, Konkuk University, Hwayang-dong, Kwangjin-gu, Seoul 143-701, Republic of Korea

[§]Centre for Stem Cell Research, Institute for Stem Cell Biology and Regenerative Medicine, Christian Medical College Campus, Vellore 632002, India

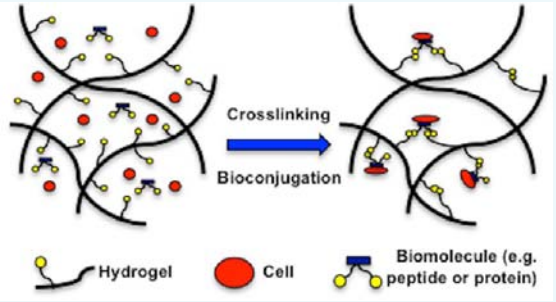
[∥]Department of Medicine, Center for Biomedical Engineering, Brigham and Women's Hospital, Harvard Medical School, Cambridge, Massachusetts 02139, United States

[⊥]Harvard-MIT Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, United States

[¶]Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, Massachusetts 02115, United States

[●]Department of Physics, Faculty of Science, King Abdulaziz University, Jeddah 21569, Saudi Arabia

ABSTRACT: Hydrogels are hydrophilic polymer networks with high water content, which have played an important role as scaffolds for cells, as carriers for various biomolecules (e.g., drugs, genes, and soluble factors), and as injectable biomaterials in tissue engineering (TE) and regenerative medicine. Bioconjugation is an approach for improving the performance of hydrogels using cell-responsive components, such as proteins and peptides, which have high affinity to regulate cellular behaviors and tissue morphogenesis. However, the current knowledge on the role of those bioconjugated moieties in controlling cellular functions and tissue morphogenesis and bioconjugation methods are limited in the context of TE and organogenesis. Moreover, micro- and nanofabrication techniques have been used to manipulate bioconjugated hydrogels for regulating cell behaviors and function. This Review therefore describes synthesis, characteristics, and manipulation of various bioconjugated hydrogels and their potential in TE applications with special emphasis on preclinical/clinical translation.



1. INTRODUCTION

Bioconjugation techniques, a covalent coupling of two or more distinct molecules to achieve a specific functionality, have been utilized extensively to obtain reliable, controllable, and functional combinations of various biomolecules for wide biomedical applications. Bioconjugation has been used as a tool for the discovery and elucidation of cellular and biological interactions,[1] the development of biological assays[2] and diagnostic tools,[3] bioimaging,[4] and the fabrication of novel and functional biomaterials.[5] Various bioconjugation reactions and pathways have been reported in which functional and conjugated biomolecules are created. For example, fluorescent probes can be generated through the conjugation of organic fluorescent markers with biomolecules of interest (e.g., DNA and amino acids), allowing for the investigation of biological processes involving the target molecule.[6] The efficiency of bioconjugation can be increased using novel bioconjugation reactions and approaches involving chemoselectivity, high-throughput screening, and high sensitivity and reproducibility.[7]

The extracellular matrix (ECM) is composed of a mixture of several biomolecules, including collagens and glycoproteins arranged in distinct structures that are essentially unique to a specific tissue. It is also a rich repository of various soluble factors. All of these ECM biomolecules are needed for growth, development, and maintenance of cells and tissue organization in the body. In addition, the ECM regulates other biological activities, such as wound healing and antimicrobial activity in developed tissue constructs.[8] Due to the structural and functional complexity of the ECM, the design and fabrication of artificial and biomimetic ECM in vitro remains a major

Received: June 30, 2015
Revised: August 13, 2015
Published: August 17, 2015

DOI: 10.1021/acs.bioconjchem.5b00360
Bioconjugate Chem. 2015, 26, 1984−2001

challenge in tissue regeneration.[8] Bioconjugation approaches play a crucial role in synthesizing novel biomaterials as the artificial ECM or in improving the performance of existing biomaterials in tissue engineering (TE) and regenerative medicine.[9] A variety of synthesis reactions and chemical modification techniques are available to fabricate a wide range of biomimetic materials for different TE applications.

In this Review, we focus on the impact of bioconjugated hydrogels in TE and regenerative medicine. We first introduce the concept of hydrogel-based TE followed by various methods and strategies for the bioconjugation with different biomolecules. Next, we provide an overview of different fabrication technologies used in engineering tissue constructs using bioconjugated hydrogels. Preclinical/clinical trials of bioconjugated hydrogels are then discussed. Finally, commonly used conjugation techniques for bioconjugated hydrogels are discussed with future research directions.

2. CONCEPT OF TE

Although some tissues in the body are intrinsically capable of regeneration, they are not able to regenerate large volumes to replace diseased, lost, or damaged tissue. Moreover, the regenerative ability of tissues is substantially decreased with aging.[10] Treating tissue defects with injection of cells has been attempted. However, this approach is not highly efficient for tissue regeneration due to immunorejection and poor cell survival and retention following injection.[11] In addition, surgical reconstruction is unable to fully repair lost tissues and organs and often causes the morbidity of the donor site.[12] In this regard, TE has evolved as an exciting and multidisciplinary field of research aiming to recreate native tissues, with the goal of restoring diseased or damaged tissues in the body.[13,14]

Engineered tissues have been used for additional important applications in vitro, such as drug screening.[15] They may replace the corresponding animal models for testing novel pharmacoactive compounds, reducing the time and effort involved in the screening process. More importantly, engineered human tissues more closely resemble the physiological activity and response of native human tissues than animal models.[15] In general, cells, scaffolds, and soluble factors are key components for regenerating and engineering tissues.[16] To fabricate tissues in vitro, cells are often seeded onto scaffolds and cultured using an appropriate medium containing necessary soluble factors. The scaffold mediates different signaling pathways from the soluble factors and other sources to regulate different cellular behaviors, such as migration, adhesion, proliferation, and differentiation,[17] thereby providing a suitable artificial ECM microenvironment for the cells. In particular, the scaffold provides mechanical integrity and stability and adhesive sites for the cells during tissue morphogenesis and function.[18]

3. HYDROGELS IN TE

Hydrogels are high-water-content crosslinked macromolecules with physicochemical properties that have a certain similarity to the ECM.[19] These materials have been used as scaffolds for cells, as carriers of various biomolecules, and as injectable biomaterials in TE and regenerative medicine applications, mainly due to their ease of fabrication and biological and physicochemical characteristics.[20]

Hydrogels can be categorized into two major groups according to their origin: naturally derived and synthetically derived.[21] Naturally derived hydrogels are made from natural sources and are composed of either polysaccharides or fibrous structural proteins, such as collagen, gelatin, elastin, and fibrin. Naturally derived hydrogels often interact with cells through existing cell-binding motifs within the hydrogel and thereby regulate various cellular behaviors. However, these materials may induce an immune response in the body, likely due to high interactions with biological moieties. In addition, they often exhibit poor mechanical properties, stability, and reproducibility.[22] In contrast, synthetic hydrogels [e.g., poly(vinyl alcohol) (PVA), poly(ethylene glycol) (PEG), and poly(2-hydroxyethyl methacrylate)] consist of synthetic macromolecules and do not generally result in an immunogenic response. Furthermore, synthetic hydrogels can be produced in a large-scale manner with tunable mechanical and chemical properties. However, synthetic hydrogels do not often provide effective interactions with cells and may give rise to an inflammatory response after implantation.[23]

4. CONJUGATED HYDROGELS AND THEIR IMPORTANCE IN TE

There is no hydrogel that fulfills all of the requirements of an ideal TE scaffold. Bioconjugation can be used to improve properties and performance of hydrogels used in TE. Various biomolecules and bioconjugation approaches have been used to create biomimetic and functional hydrogels for use in TE and regenerative medicine. For example, a versatile and biofriendly strategy for creating a stable, three-dimensional (3D), and tunable structure of cell-laden hydrogels in aqueous environments is by using cross-linkable moieties in the structure of hydrogels. Some hydrogels (e.g., gelatin methacryloyl (GelMA)[24] and tropoelastin methacryloyl[25]) include such cross-linkable moieties. These hydrogels can be conjugated with other hydrogels to increase processability and facile cross-linking of composite gel compared with single gel counterparts. More importantly, 3D cell-laden structures of the composite hydrogels can be fabricated in mild conditions without a significant loss of the cell viability.[26] Therefore, it would be possible to fabricate any desired hydrogel or cell-laden microstructure and, by extension, any tissue organization using this bioconjugation approach. The precise control of tissue organization and structure is important for many TE applications. For example, skeletal muscle is a tightly packed and organized tissue in which muscle myofibers are anisotropically aligned in one direction. This particular structure of muscle myofiber is crucial in generating contractile forces within the tissue for movement and other physiological activities.[27] High processability and facile fabrication of bioconjugated hydrogels are useful to recreate such cellular structures.

Other biomolecules, including proteins and peptides, have also been conjugated to hydrogels. These biomolecules often tune or improve the cell-responsive and physical properties of the obtained bioconjugated hydrogels.[28] These bioconjugated hydrogels can be designed to mediate different cellular behaviors, such as adhesion, migration, proliferation, and differentiation, which directly affect tissue formation and functionality. One of the earliest synthesized bioconjugated hydrogels was a protein-conjugated hydrogel in which a triblock artificial protein containing leucine domains was conjugated to PEG hydrogel to accurately control the pH and temperature of its gelation,[29] which is important for cell-encapsulation studies and the release of biomolecules entrapped in the gel. Following

DOI: 10.1021/acs.bioconjchem.5b00360
Bioconjugate Chem. 2015, 26, 1984−2001

the latter study, many bioconjugated hydrogels have been synthesized and reported for different tissue regeneration applications,[28] which are discussed in the following sections. In particular, great advances in click chemistry allow the use of facile, highly selective, and efficient methods for the synthesis of multicomponent conjugated hydrogels. Moreover, this approach offers mild and nontoxic chemistries for cell encapsulation and hydrogel formation both in vitro and in vivo.[29]

5. CLASSIFICATION OF BIOCONJUGATED HYDROGELS

5.1. Peptide-Conjugated Hydrogels. A peptide is a sequence of few amino acids (by convention less than 50 amino acids) condensed to each other through amide bonds. Some peptides contain bioactive domains that are responsible for cell binding and function, as well as matrix degradation, and they can be conjugated to a variety of hydrogels to improve their biofunctionality.[30] This section describes the classification of a variety of peptide-conjugated hydrogels.

PEG hydrogels are favorable for TE applications due to their porosity, mechanical properties, and in vivo biocompatibility.[31] However, PEG hydrogels fail to provide cell attachment sites, and they are not biodegradable. The modification of PEG hydrogels with cell-adhesive and enzyme-sensitive peptides can improve their biofunctionality and degradation, making them more suitable substitutes for the ECM.[32] Recently, important works have been reported in the synthesis of photopatternable peptide-conjugated hydrogels with physicochemical characteristics that can locally be tailored for cell culture and tissue fabrication. For example, Mosiewicz et al. demonstrated the controlled spatiotemporal attachment of primary human MSCs by photopatterning peptide-conjugated PEG.[33] First, they prepared a peptide substrate of activated transglutaminase factor XIII, and then they covalently incorporated it into the PEG hydrogels. A confocal laser beam was used to locally activate the substrate. Generally, diacrylated PEG hydrogels are cross-linked either by chemical activation or through UV exposure. However, free-radical cross-linking methods often suffer from reduced viability of encapsulated cells and therefore are not optimal for in vitro and in vivo delivery of cell-laden hydrogels. Interestingly, hydrogels cross-linked by Michael-type addition do not employ UV light and toxic free radicals. Using this strategy, Phelps et al. used maleimide as a cross-linker moiety to create a bioactive PEG hydrogel and then conjugated it with a thiol-containing adhesive peptide for cell encapsulation studies.[34]

Alginate (Alg) is a linear polysaccharide and is naturally extracted from brown seaweed.[35] As an injectable and biodegradable hydrogel, it can be used to deliver bioactive proteins and peptides, as well as anti-inflammatory and anticancer drugs.[36] However, Alg does not efficiently interact with cells. To overcome this drawback, Bubenikova et al. immobilized cell-adhesive thiol-terminated Arg-Gly-Asp (RGD) peptides onto an Alg hydrogel.[37] The RGD peptide was linked to Alg through a disulfide exchange between the 2-pyridyldithio sequence of the Alg and the thiol-terminated peptide. In a recent study, Madl et al. conjugated two different peptides associated with bone morphogenetic protein-2 (BMP-2; a potent inducer of osteogenesis) with Alg through an orthogonal coupling strategy.[38] The bioconjugated gels improved the osteogenesis of osteoblasts and mesenchymal stem cells (MSCs). Jeon and Alsberg conjugated a cell adhesive ligand Gly-Arg-Gly-Asp-Ser-Pro (GRGDSP) with Alg to develop a photo-cross-linkable hydrogel that resulted in enhanced cell attachment, spreading, proliferation, and glycosaminoglycan production by the embedded chondrocytes.[39] The acrylated-peptide was used to couple to the hydrogel backbone during the photopolymerization. This work was an improvement over their earlier attempt, in which the RGD peptide was directly coupled to Alg macromer, resulting in controlled adhesiveness, but the product lacked the other advantages.[40] In the former work, adjusting the concentration of GRGDSP in the Alg precursor solution allowed for the precise control over the adhesion, spreading, and proliferation of human MSCs in the gels. Interestingly, the inclusion of GRGDSP in the Alg hydrogel did not affect its mechanical properties or degradation rate. Dhoot et al. modified the surface of Alg through the covalent attachment of a Tyr-Ile-Gly-Ser (TIGS) peptide to the carboxylic acid groups of Alg.[41] These authors observed enhanced adhesion and neurite outgrowth of NB2a neuroblastoma cells onto the peptide-conjugated Alg hydrogels in contrast with the pure Alg gels.

Chitosan can be obtained through deacetylation of chitin, and it acts as a biomimetic biomaterial because of its similar structure to the glycosaminoglycans found in native tissues, such as cornea and cartilage tissues. Chitosan and its derivatives have various applications in TE.[42] Overproduction of reactive oxygen species is closely associated with myocardial infarction.[43] Chitosan is known for its ability to scavenge reactive oxygen species, making it a suitable host for cardiomyocytes grown for myocardial repair in ischemic conditions. A high molecular weight or concentration of chitosan is required to shape it into a hydrogel form. However, the antioxidant activity of chitosan declines with increasing molecular weight, necessitating an auxiliary means to retain its antioxidant capacity. To this end, Li et al. introduced glutathione into chitosan chloride via the formation of amide bonds.[43] This peptide bioconjugation increased the oxygen scavenging capability of chitosan gels.[43] Miklas et al. reported Gln-His-Arg-Glu-Asp-Gly-Ser (QHREDGS) peptide sequence that can be used as a small and inexpensive cardio-protective and vasculogenic molecule for incorporation into collagen-chitosan hydrogels.[44] The peptide promoted encapsulated endothelial cells to form tube-like structure in more rapid and robust manner in comparison to the control hydrogel without the QHREDGS peptide.

Collagen, the main component of the ECM, has numerous applications in tissue scaffolding and repair.[45] Because naturally derived collagens have limitations, such as thermal instability and pathogenic contamination, peptide-conjugated collagens have been developed to compensate the physical properties and function of pure collagens.[46] Reis et al. conjugated a peptide with a collagen type I-chitosan hydrogel to form a thermoresponsive hydrogel.[47] Cardiomyocytes encapsulated in these bioconjugated gels showed improved morphology, viability, metabolism, and beating activity. Through a recombinant DNA technology, Hiraoka et al. modified collagen type I with a laminin-derived small peptide by fusing the peptide to the N- or C-terminus of collagen.[48] This peptide improved the attachment and viability of neurosphere-forming cells through the integrin pathway.

Gellan gum (GG) is an anionic and linear polysaccharide with repeating molecular units of D-glucuronic acid, D-glucose, L-rhamnose, and D-glucose. It is a suitable scaffolding material for engineering cartilage tissue with low toxicity.[49] However, it

DOI: 10.1021/acs.bioconjchem.5b00360
Bioconjugate Chem. 2015, 26, 1984−2001

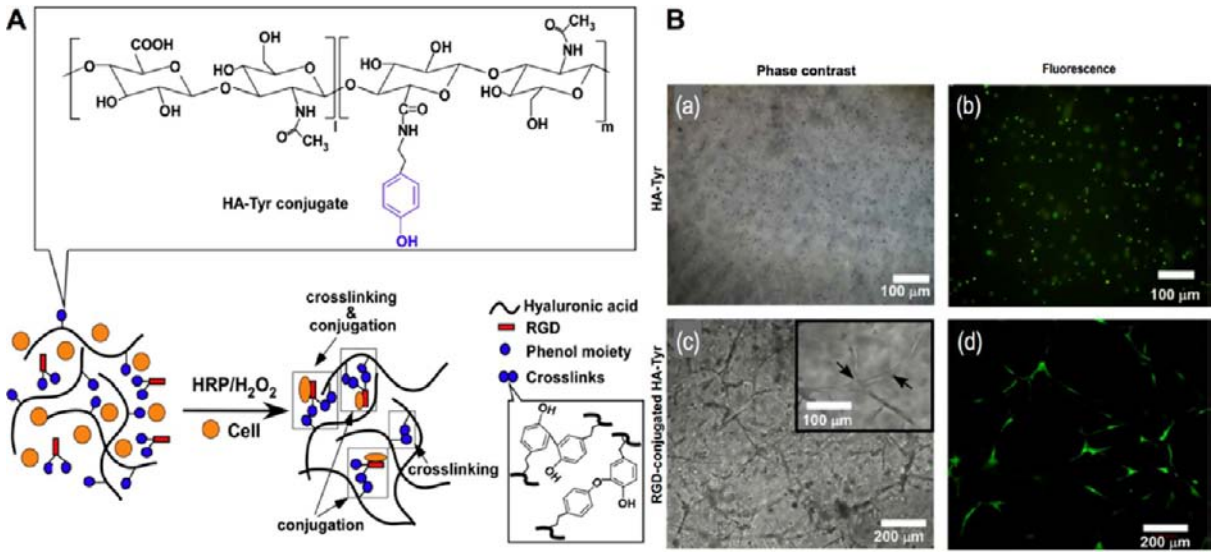


Figure 1. Synthesis and TE applications of select peptide-conjugated hydrogels. (A) RGD conjugation to HA-tyramine during gel formation via an enzymatic oxidation reaction for cell encapsulation studies. (B) Phase contrast and fluorescence images of fibroblasts and HUVECs cocultured in HA-tyramine (a, b) and HA-tyramine-RGD (c, d) hydrogels. The inset of (c) shows the formation of capillary-like structures in the RGD-modified hydrogel. Fluorescence images were taken after CD31 staining of HUVECs. Reproduced with permission, copyright 2014, Elsevier B.V. B.[30]

can only host attachment-independent cell lines, such as blood cells due to the lack of cell attachment sites in its structure. To overcome this limitation, Ferris et al. conjugated GG with a RGD peptide to induce the attachment required for the normal growth and function of anchored cells, such as fibroblasts and oligodendrocytes.[50] The authors assessed the cellular phenotypes of C2C12 and PC12 cell lines in response to the peptide-conjugated GG and observed improved adhesion and proliferation in both cell lines.

Hyaluronic acid (HA) is a polysaccharide and can ubiquitously be found in the human body. HA has been used in the clinic for over 30 years and is capable of regulating many cellular behaviors and function.[51] Wang et al. developed an RGD-conjugated HA as an injectable hydrogel in which H_2O_2 and horseradish peroxidase (HRP) were simultaneously used as cross-linkers during the gel formation process (Figure 1A).[30] The conjugated gels enhanced the vascularization of cocultured human umbilical vein endothelial cells (HUVECs) and human fibroblasts. The bioconjugated hydrogels were highly efficient at forming the capillary-like structure of these cells (Figure 1B). Interestingly, the enzymatic reaction induced by the HRP and hydrogen peroxide enables the incorporation of other bioactive molecules during the hydrogel cross-linking reaction. HA hydrogels have extensively been used for vascularization of engineered tissues.[52] The facile incorporation of appropriate soluble factors (e.g., vascular endothelial growth factor (VEGF)) in the peptide-conjugated gel and a precise control on their release can significantly promote vascular formation within the hydrogels. Yamada et al. conjugated laminin-derived cell-adhesive peptides with HA.[53] The PC12 cells cultured on the 2D conjugated hydrogels exhibited improvements in cell spreading and adhesion as well as neurite outgrowth. In addition, the 3D peptide-conjugated hydrogels showed improved cell viability and gene expression similar to that observed on conventional 3D scaffolds.

PVA can be synthesized from poly(vinyl acetate) through hydrolysis, alcoholysis, or aminolysis. The hydrophilicity of PVA can be changed by controlling its molecular weight and degree of hydrolysis.[54] PVA has been widely used in biomedical applications. However, the stabilization of PVA hydrogels and the incorporation of bioactive cargos onto the hydrogel usually require the use of toxic chemicals, such as glutaraldehyde. As an alternative, Chong et al. developed a thiol-terminated microstructured PVA and conjugated it with a natural thiol-containing peptide.[55] They succeeded in conjugating the peptide in the gel phase to increase the cell-adhesive nature of PVA. The authors evaluated the bioconjugated hydrogel using hepatocellular carcinoma cells and demonstrated a considerable improvement in the cell adhesion to the microstructured peptide-conjugated PVA hydrogel.

5.2. Protein-Conjugated Hydrogels. Proteins are natural macromolecules consisting of one or more than one type of amino acid residues. Proteins play a crucial role in the body and are important ingredients for tissue regeneration.[56,57] These biomolecules act as biological cues for cells. However, their sensitive chemical structures are a major limitation for their direct use in regenerative medicine.[58] The poor chemical stability of proteins under certain conditions and their tendency to aggregate lead to an immune response and the loss of their bioactivity.[59] Therefore, finding new ways to preserve protein function during their processing or delivery has been a major challenge in TE and regenerative medicine.[60] A useful approach for protecting proteins from degradation and preventing an immunogenic reaction is to bind them with other macromolecules, such as heparin.[61] Various protein carriers, including particles and cross-linked polymer networks, have been developed to facilitate their controlled release in medium.[62]

Hydrogels are often prepared under mild conditions that allow proteins to retain stability and function.[63] In addition, they can be fabricated in different shapes and physical structures with the ability to release proteins in a controlled manner.[64] Various approaches have been employed to control the incorporation of proteins into hydrogels and their delivery to target sites.[65] Proteins can be loaded into hydrogels either by physical entrapment in the gels or by chemical conjugation to the hydrogel backbone. They can be released from the hydrogels via swelling, degradation, or erosion of the gels.[63] Because certain proteins (e.g., growth factors) act locally and

DOI: 10.1021/acs.bioconjchem.5b00360
Bioconjugate Chem. 2015, 26, 1984−2001

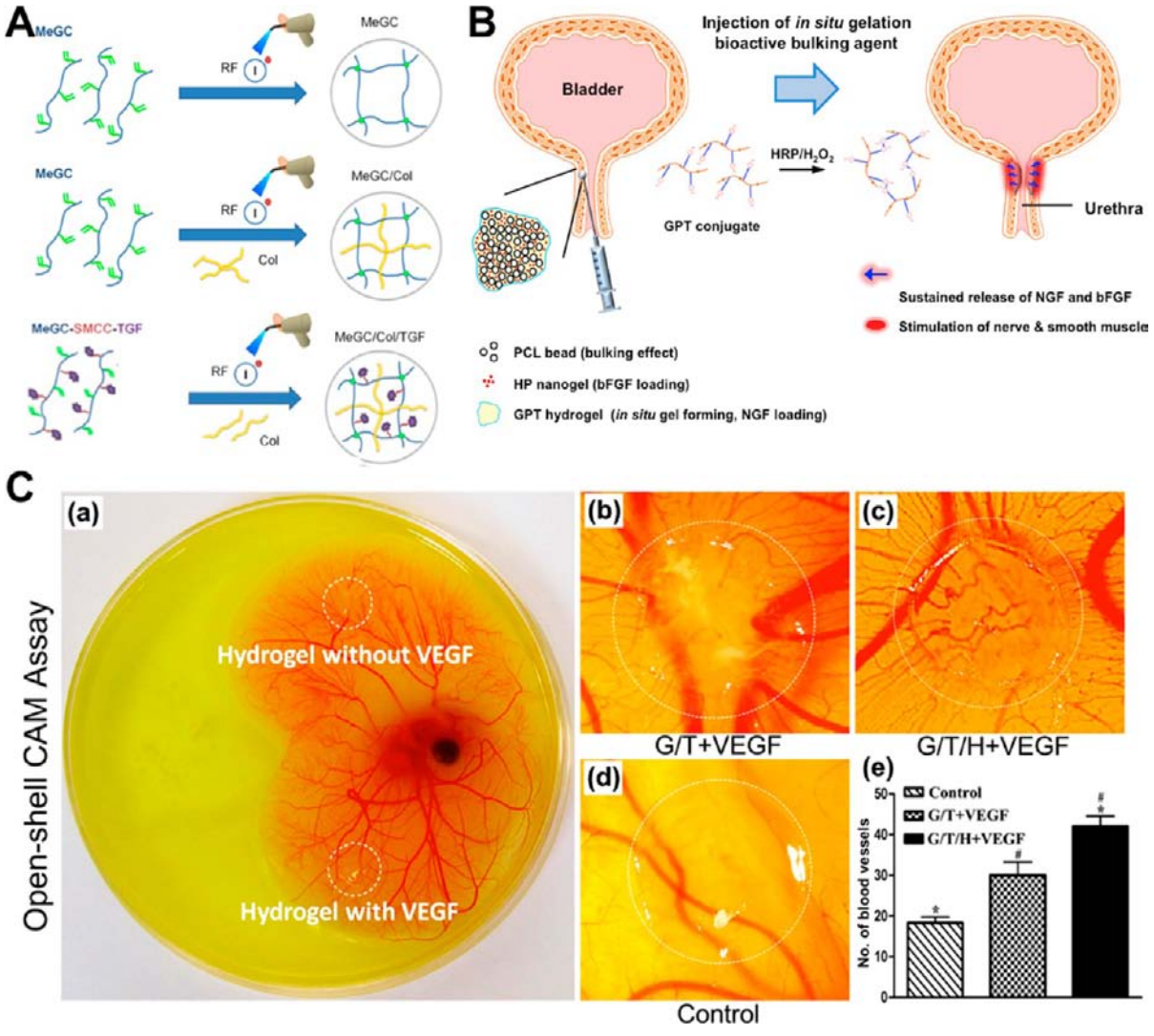


Figure 2. Synthesis and TE applications of select protein-conjugated hydrogels. (A) Schematic diagram showing the synthesis of functionalized MeGC hydrogels. The MeGC hydrogels were formed by irradiation by visible blue light in the presence of riboflavin as a photoinitiator. The MeGC-collagen type II hydrogels were prepared by mixing the MeGC solution with collagen type II, followed by photopolymerization. TGF-β1 was bioconjugated to the hydrogel via a linker. Reproduced with permission, copyright 2015, BioMed Central.[68] (B) Schematic of the two growth factors (NGF and bFGF) loaded in the gels for nerve and smooth muscle TE. Reproduced with permission, copyright 2015, Springer.[82] (C) Effect of released VEGF from VEGF-loaded gelatin-tyramine-heparin (G/T/H) hydrogels using a CAM assay. (a) Hydrogels loaded with 1 μg of VEGF or without VEGF were placed on a chicken embryo CAM. (b) 2.5 wt % G/T hydrogel loaded with 1 μg VEGF after 5 days of incubation. (c) 2.5 wt % G/T/H hydrogel loaded with 1 μg VEGF after 5 days of incubation. (d) Control: 2.5 wt % G/T hydrogel without VEGF after 5 days of incubation. (e) Quantification of the blood vessels surrounding the gel. Values are represented as the mean ± SEM (*p < 0.01, #p < 0.05). Reproduced with permission, copyright 2015, Elsevier B.V.B.[85]

over short diffusion distances, their bioconjugation to hydrogels is preferred to physical entrapment in gels to effectively preserve their function.[65] In this section, we review the progress in developing protein-conjugated hydrogels for the regeneration of different tissues.

Transforming growth factor-β1 (TGF-β1) was used as a chondrogenesis inducer[66] in photopolymerizable methacrylated chitosan (MeGC) hydrogels.[67] The MeGC was synthesized via free radical polymerization under visible blue light in the presence of a photoinitiator. Following synthesis, TGF-β1 protein was chemically attached to the MeGC prior to the hydrogel formation using a succinimidyl-4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (SMCC) linker (Figure 2A). Kim et al. obtained a prolonged release (12% up to 21 days) of TGF-β1 and collagen type II proteins using this hydrogel.[68] The presence of collagen type II enhanced TGF-β1-mediated chondrogenesis, while the bioconjugated hydrogel increased the differentiation of human MSCs, as well as cellular condensation and the deposition of cartilaginous ECM, compared with the pure MeGC hydrogels. Jha et al. also demonstrated the bioconjugation of TGF-β1 to HA hydrogels using heparin.[69] HA is a suitable hydrogel for bioconjugation to a variety of proteins.[70] The latter bioconjugated hydrogel was synthesized by in situ cross-linking of acrylated HA and growth factor via a Michael-type addition reaction. The resulting hydrogel enhanced the cell survival and engraftment of encapsulated murine cardiac progenitors to the host tissue after transplantation. In addition, the vascularization and tube formation of the grafted tissue was observed, as confirmed by the expression of angiogenic factors.

DOI: 10.1021/acs.bioconjchem.5b00360
Bioconjugate Chem. 2015, 26, 1984−2001

Duan et al. engineered heart valve tissue using MSCs derived from bone marrow or adipose tissue.[71] They studied the effect of the prolonged and controlled release of basic fibroblast growth factor (bFGF) covalently bound to PEG on the fibroblast differentiation of the stem cells compared with bFGF freshly added to medium. The conjugated bFGF-PEG hydrogels promoted MSC fibroblast differentiation to a greater extent than soluble bFGF in the medium. Another protein involved in cardiac tissue regeneration is stromal derived factor-1α (SDF-1α). This protein is a chemokine from a family of pro-inflammatory mediators that induces a chemotactic effect to guide stem cell migration from the bone marrow to ischemic tissue.[72] Prokoph et al. demonstrated that functionalized star PEG-heparin hydrogels containing SDF-1α significantly improved the migration of early endothelial progenitor cells in contrast with gels without SDF-1α.[73] A histological analysis of the SDF-1α-loaded hydrogels subcutaneously implanted in nude mice showed a significantly greater number of endothelial cells and the initiation of angiogenesis in the hydrogels after 7 days. This recruitment of progenitor cells in the ischemic tissue is important for the treatment of myocardial tissue. Rabbany et al. used Alg hydrogel to deliver SDF-1α in order to accelerate the rate of tissue healing and to reduce the formation of scar tissue.[74] The role of SDF-1α in decreasing wound size is attributed to the recruitment of progenitor cells to the site of injury, thereby inducing re-epithelialization and revascularization. One of the challenges in cardiac tissue regeneration following myocardial infarction is the delivery of multiple proteins.[75] In a relevant study, Ruvinov et al. evaluated the effect of the dual delivery of hepatocyte growth factor (HGF) and insulin-like growth factor-1 (IGF-1) conjugated to an injectable Alg hydrogel for myocardial regeneration.[76] The bioconjugation of these growth factors protected them from proteolysis and therefore maximized their therapeutic effect during tissue reconstruction. Myocardial injection of IGF-1/HGF-conjugated hydrogels in a rat model of myocardial infarction confirmed the significant effect of these proteins on cardiac tissue regeneration and the reduction of apoptosis. The conjugated hydrogels also decreased scar fibrosis formation after 4 weeks of hydrogel injection.

Siddiqui and Pramanik recently showed an improvement in cell-binding of chitosan/nano beta tricalcium phosphate (β-TCP) composite hydrogels by the bioconjugation of fibrin to the gels using carbodimide cross-linking.[77] The high porosity and compressive strength of chitosan/nano β-TCP hydrogels were not affected by the fibrin conjugation. Attachment, proliferation, osteogenic differentiation, and mineralization of human MSCs were significantly enhanced on the fibrin-conjugated hydrogels compared with the nonconjugated hydrogels. In another study, Noh et al. introduced heparin-conjugated fibrin gels for a dual delivery of a short peptide and BMP-2 to human MSCs.[78] The ex vivo implantation of differentiated cells for bone regeneration was not efficient due to a reduction in cell viability after the implantation. However, the bioconjugated hydrogel increased the cell survival due to effective and controllable release of the conjugated proteins in situ. The sustained release of proteins from the gels was based on an interaction between the heparin-binding motif of the proteins and the heparin conjugated to the fibrin gel. The in vivo study also demonstrated high efficacy of the protein-conjugated hydrogels for bone regeneration.

Protein-conjugated hydrogels have been used in the field of neural TE.[79] Semaphorin-3A (a soluble protein that induces axonal growth in dopaminergic neurons[80]) was conjugated to PEG-silica nanocomposite gels to enhance axonal outgrowth of neurons.[81] Conjugated hydrogels containing either 2 or 5 μg/mL of semaphorin 3A increased the axon growth by up to 53−68% compared with controls. Oh et al. studied the simultaneous regeneration of neural and smooth muscle tissues using protein-conjugated hydrogels.[82] The material consisted of three components: bFGF-loaded heparin-pluronic nanogels, nerve growth factor (NGF)-loaded PEG-gelatin-tyramine nanogels, and polycaprolactone (PCL) beads for regenerating weak tissues around the urethra. Weakness in these tissues causes stress urinary incontinency, a major medical problem in adult females. The continuous and dual release of growth factors (bFGF for more than 4 weeks and NGF for approximately 1 week) from hydrogels resulted in the regeneration of damaged nerve and smooth muscle tissues and, by extension, the recovery of urethra function and contractibility. Figure 2B shows a schematic of the regeneration process. Enzymatically cross-linked Alg-dopamine hydrogels have also been proposed for neural regeneration and repair.[83] This conjugated hydrogel was synthesized by the in situ coupling of Alg with dopamine, catalyzed by 1-ethyl-3-(3-(dimethylamino)propyl) carbodiimide hydrochloride and *N*-hydroxysulfosuccinimide in the presence of HRP and H_2O_2. Compared with control Alg hydrogel, the bioconjugated hydrogel demonstrated higher cell adhesion and elasticity.

Protein-conjugated hydrogels have widely been used for vascular TE.[84] For instance, Li et al. synthesized an injectable VEGF-conjugated gelatin hydrogel to induce angiogenesis.[85] These authors introduced tyramine and heparin into gelatin chains to provide both enzymatic cross-linking points in the gel and binding domains for VEGF conjugation. VEGF is a well-known soluble factor that induces the vascularization of different tissues.[86] The gelatin concentration can be altered to tailor the mechanical properties and degradation rate of the bioconjugated hydrogels. The sustained release of VEGF in vitro for over 3 weeks was observed, and in vivo angiogenesis assay of chicken chorioallantoic membrane (CAM) revealed the formation of blood vessels after 5 days of incubation. Figure 2C shows the resulting in vivo vascularization by the VEGF-conjugated and control hydrogels. In another study, Drinnan et al. conjugated injectable PEGylated fibrin gels with platelet-derived growth factor and TGF-β1 through amine groups of the PEGylated fibrin.[87] These authors compared the induction of neovascularization in ischemic tissue by the release of these factors with induction by soluble factors physically entrapped in the gels. A controlled release of bioconjugated growth factors maintained their bioactivity and improved the vascularization of tissues compared with the nonconjugated growth factors. It was also found that the release of growth factors was directly related to the degradation rate of fibrin gels, and by adjusting the ratio of PEG to fibrinogen the release rate of growth factors could be modulated and controlled.

5.3. Hydrogel−Hydrogel Conjugates. Hydrogel−hydrogel conjugates are often composites of chemically bonded natural and/or synthetic hydrogels that are randomly or selectively distributed within the hydrogel network. These hydrogels exhibit tunable structural, mechanical, and biological properties. The synthetic hydrogels control the mechanical and structural properties of hydrogel−hydrogel conjugates, while natural hydrogels are mainly responsible for regulating cellular properties of gels, such as adhesion, proliferation, matrix production, and enzyme activity.[88]

DOI: 10.1021/acs.bioconjchem.5b00360
Bioconjugate Chem. 2015, 26, 1984−2001

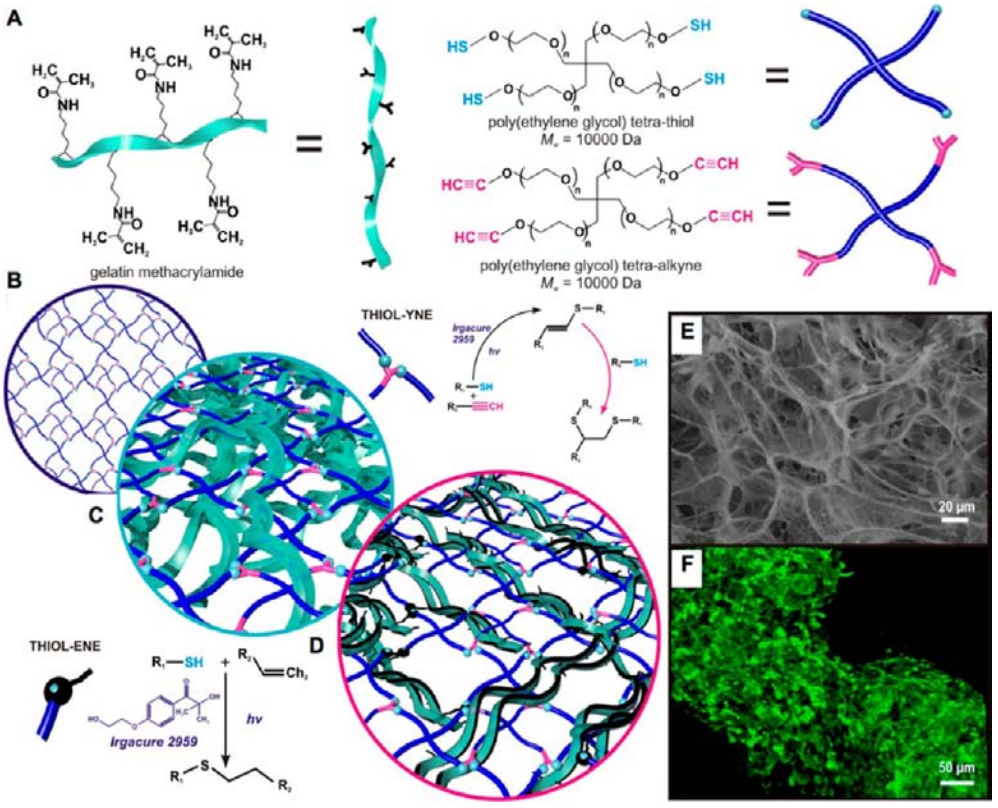


Figure 3. Fabrication and cell encapsulation of a bioconjugated GelMA-PEG hydrogel. (A) Chemical structure of GelMA and PEG precursors. (B) Well-defined network architecture of conjugated hydrogels intertwined with either (C) physically incorporated GelMA and PEG or (D) covalently cross-linked hydrogels, in which GelMA reacts with both itself and the PEG network. (E) Architectural heterogeneity emulated native ECM. (F) Encapsulated fibroblasts in conjugated GelMA-PEG hydrogels, where cytoskeletal F-actin fiber staining illustrates the density and interaction of the encapsulated cells. Reproduced with permission, copyright 2014, Elsevier B.V. B.[99]

PEG hydrogel has widely been used for the formation of hydrogel−hydrogel conjugates.[89,90] PEG can be functionalized to provide suitable sites for the chemical binding to other hydrogels. Following the pioneering work of Sawhney et al.,[91] researchers have exploited the copolymer structures of PEG hydrogels to control their physicochemical properties. In particular, glycolide, lactide, and caprolactone blocks have been grafted to PEG hydrogels to tune their chemical properties and alter their degradation.[92] Seidlits et al. conjugated fibronectin-functionalized PEG (PEG-FN) to HA to induce endothelial cell adhesion and angiogenesis.[93] Both HA and PEG-FN hydrogels were acrylated to induce photosensitivity. Then, the mixture of hydrogels was cross-linked by applying UV light. The chemical attachment of FN to the conjugated hydrogels increased cell viability and attachment compared with the adsorbed FN on the gels (control sample) due to its higher retention and uniform distribution within the conjugated hydrogel. Therefore, HA-PEG-FN conjugated hydrogels are valuable for the study of angiogenesis in vitro. A similar approach was used to acrylate HA and PEG hydrogels and then conjugate them for potential protein delivery and TE applications.[94] Interestingly, the photopolymerization of the hydrogels did not affect the stability and function of proteins. In addition, the authors found that the release of bovine serum albumin protein from the gels could be controlled by changing the PEG or HA concentration. The precise control of protein release from hydrogels is desired for various regenerative purposes, such as vaccination,[95] therapeutic application,[96] and TE and regenerative medicine.[97] We fabricated composite hydrogels of PEG and GelMA to improve cell adhesion and proliferation compared with the pristine PEG gel.[98] These composite hydrogels offered tunable stiffness, degradation, and cell binding properties, indicating their potential for application in 3D cell culture and regenerative medicine. In a similar study, PEG hydrogel was conjugated to GelMA using a click chemistry approach, forming an interpenetrating gel network for use in cell culture studies[99] (Figure 3). These hybrid hydrogels showed a higher stiffness and lower gelation dissolution compared with the pristine gels, which are favorable characteristics for long-term cell adhesion and encapsulation in hybrid hydrogels. Other conjugated hydrogels to PEG include ECM-based hydrogels,[100] collagen,[101] chitosan,[102] chitosan−collagen,[103] and Alg,[104] which provide a range of biological, chemical, and physical properties for different TE and regenerative medicine applications.

Gelatin is a natural thermoresponsive, cell-adhesive, and inexpensive hydrogel derived from the alkaline or acidic hydrolysis of collagen.[105] There are two types of gelatin: A and B. Type A gelatin is obtained through the acid treatment of collagen, while the type B gelatin is produced using an alkaline process.[106] Gelatin contains reactive amino groups in its side chain, making it a suitable hydrogel for the fabrication of gelatin-hydrogel conjugates.[107] Sakai et al. synthesized gelatin−agarose conjugates by reacting functional amino groups of gelatin with 1,1-carbonyldiimidazole-activated agarose.[108,109] Agarose is a naturally derived polysaccharide that forms a gel when its solution is cooled to ∼35 °C.[110] Agarose has been used as a delivery vehicle for drugs[111] and as a TE scaffold for

DOI: 10.1021/acs.bioconjchem.5b00360
Bioconjugate Chem. 2015, 26, 1984−2001

neural[112] and cartilage[113] tissues. However, it exhibits a low adhesiveness and a poor ability to promote cell proliferation because it does not contain any cell-adhesive moieties. The conjugation of agarose to gelatin provides a facile and inexpensive approach for enhancing its cell adhesiveness. In another study, HA was conjugated to gelatin through a click chemistry reaction at a mild temperature.[114] The click chemistry employed resulted in an interpenetrable conjugated hydrogel with significantly higher mechanical and degradation properties compared with the bioconjugated gel components. The properties of the conjugated hydrogel could be controlled simply by changing the cross-linking density of the gel. Interestingly, the compressive stress of the fabricated conjugated hydrogels was comparable to that observed for native cartilage tissue.

GG has a low mechanical strength and high gelation temperature.[115] To overcome these limitations, Tang et al. prepared oxidized GG conjugated with carboxymethyl chitosan to obtain a composite hydrogel with improved mechanical properties and a low gelation temperature[116] due to a strong network of hydrogen bonds and high molecular weight of chitosan.[117,118] The chondrocytes cultured in these gels exhibited high cellular viability. It is important to note that it is not easy to mix chitosan with GG due to the formation of an insoluble polyelectrolyte complex. The bioconjugation of these hydrogels is a practical solution to avoid this problem. In another study, Shin et al. reported that conjugated GelMA and methacrylated GG formed an interpenetrable network of gels that could be used in cell encapsulation studies.[119] Human fibroblasts seeded within the conjugated gel remained viable during 3 days of culture. However, the cell viability and function was lower than in pure GelMA gels. Therefore, the same group proposed a microgel-reinforced hydrogel consisting of GelMA and GG, which demonstrated improved biological and mechanical properties compared with the pristine gel network.[120] Sponge-like GG-HA hydrogels have also been synthesized with high stability for use in skin regeneration.[121] In mouse models, the hydrogels were degraded upon implantation, leading to the formation of dense and thick skin tissue.

Chitosan can easily be grafted to catechol in an aqueous medium under ambient conditions using a reductive amination reaction.[122] The obtained hydrogels were soft with excellent load bearing properties. In addition, due to the reversible nature of the chitosan−catechol interaction at certain pH values, the hydrogels were self-healing. The catechol-functionalized chitosan was conjugated with thiolated Pluronic F-127 copolymers to produce an adhesive and temperature-sensitive hydrogel.[123] The conjugated hydrogel was highly viscous at room temperature, but its viscosity was reduced at body temperature. Moreover, it showed superior hemostatic properties for antibleeding and regenerative applications.

5.4. Other Bioconjugated Hydrogels. In the following section, we present select examples of bioconjugated hydrogels that have not been conjugated with proteins, peptides, or other hydrogels. Hydrogels conjugated with heparin allow the retention of soluble factors and are highly adhesive. Thus, Foster et al. conjugated a thiolated heparin with a diacrylated PEG hydrogel via thiol−ene coupling for a long-term maintenance of primary hepatocytes.[124] After 3 weeks of culture, the authors observed higher albumin secretion and cytochrome P450 activity in cells seeded in the bioconjugated gels compared with the control gels. Leach et al. conjugated glycidyl methacrylated-HA with acrylated PEG-hexaglycine to obtain a photo-cross-linkable hydrogel for TE applications.[125] Kim et al. conjugated a glucagon-like biomolecule to poly(*N*-vinyl-2-pyrroridone-*co*-acrylic acid) via a PEG spacer and used it to culture islet cells for insulin production.[126] Biphosphonates (BPs) have an anti-osteoporotic effect due to their high affinity for calcium ions.[127] Hulsart-Billström et al. reported that HA hydrogels functionalized with BP ligands by reacting aldehyde-modified HA with BP and hydrazide-modified HA.[128] The resulting BP-HA hydrogel was then used to study the controlled release of BMP-2. The conjugated hydrogel enhanced the retention time and bioactivity of BMP-2 in the polymer network.

Dynamic materials containing reversible covalent or non-covalent bonds have numerous biomedical applications.[129] Among these materials, complexes of boronic acid and 1,2- or 1,3-diols allow the formation of dynamic bonds with boronate ester.[130] Deng et al. used this property to fabricate two different hydrogels by reacting 2-acrylamidophenylboronic acid with PVA or with a catechol-functionalized copolymer, resulting in a hydrogel that is self-healing at neutral and acidic pH.[131]

Some studies have focused on the fabrication of bioconjugated hydrogels capable of in situ polymerization. Among them, Sakloetsakun et al. made a chitosan-thioglycolic acid hydrogel by reacting the amino groups of chitosan with the carboxyl group of thioglycolic acid.[132] To obtain a 3D gel network in a short time period, Zhang et al. fabricated an oxidized dextran-thiolated chitosan hydrogel using polymeric network interpenetration via Schiff-base formation and disulfide bond inter-cross-linking.[133] The in vitro biocompatibility studies of these bioconjugated gels demonstrated no cytotoxicity, and in vivo implantation of the gels resulted in a mild tissue response in mouse models. Raja et al. fabricated a 3-(3,4-dihydroxyphenyl)-2-propenoic acid bioconjugated gelatin-based injectable hydrogel (dubbed caffeic acid-bioconjugated gel) for wound healing.[134] These authors observed that the bioconjugated gels promoted the migration of fibroblasts in vitro and played a free radical scavenger role. In vivo studies of the gels revealed their biocompatibility, biodegradability, and enhanced healing properties. Koeheler et al. used a Diels−Alder reaction to fabricate maleimide-containing hydrogels to drive the osteogenic differentiation of encapsulated human MSCs.[135] The Fejen group prepared a thiol-conjugated HA for which the gelation and degradation rates were tunable.[136] In addition, high cell viability and functionality were observed when chondrocytes were cultured in the bioconjugated gels. Desai et al. fabricated a click Alg hydrogel using carbodiimide chemistry.[137] High gel stability in culture and good cell viability were observed when the fibroblasts were encapsulated in the hydrogels. When injected subcutaneously into mice, the hydrogel maintained its stability over the course of several months and resulted in a minimal inflammatory response. Wu et al. conjugated polyphosphate hydroxyl groups to hydrazide-modified HA,[138] resulting in an injectable gel with osteoconductive properties.

6. FABRICATION TECHNIQUES OF BIOCONJUGATED HYDROGELS

6.1. Electrospinning. Electrospinning uses an electric field to extract micro- and nanofibers from a polymer solution and to deposit them on a collector after ejection. This fabrication method has been extensively used to make fibrous structures from various polymers in a versatile, easy, and reproducible

DOI: 10.1021/acs.bioconjchem.5b00360
Bioconjugate Chem. 2015, 26, 1984−2001

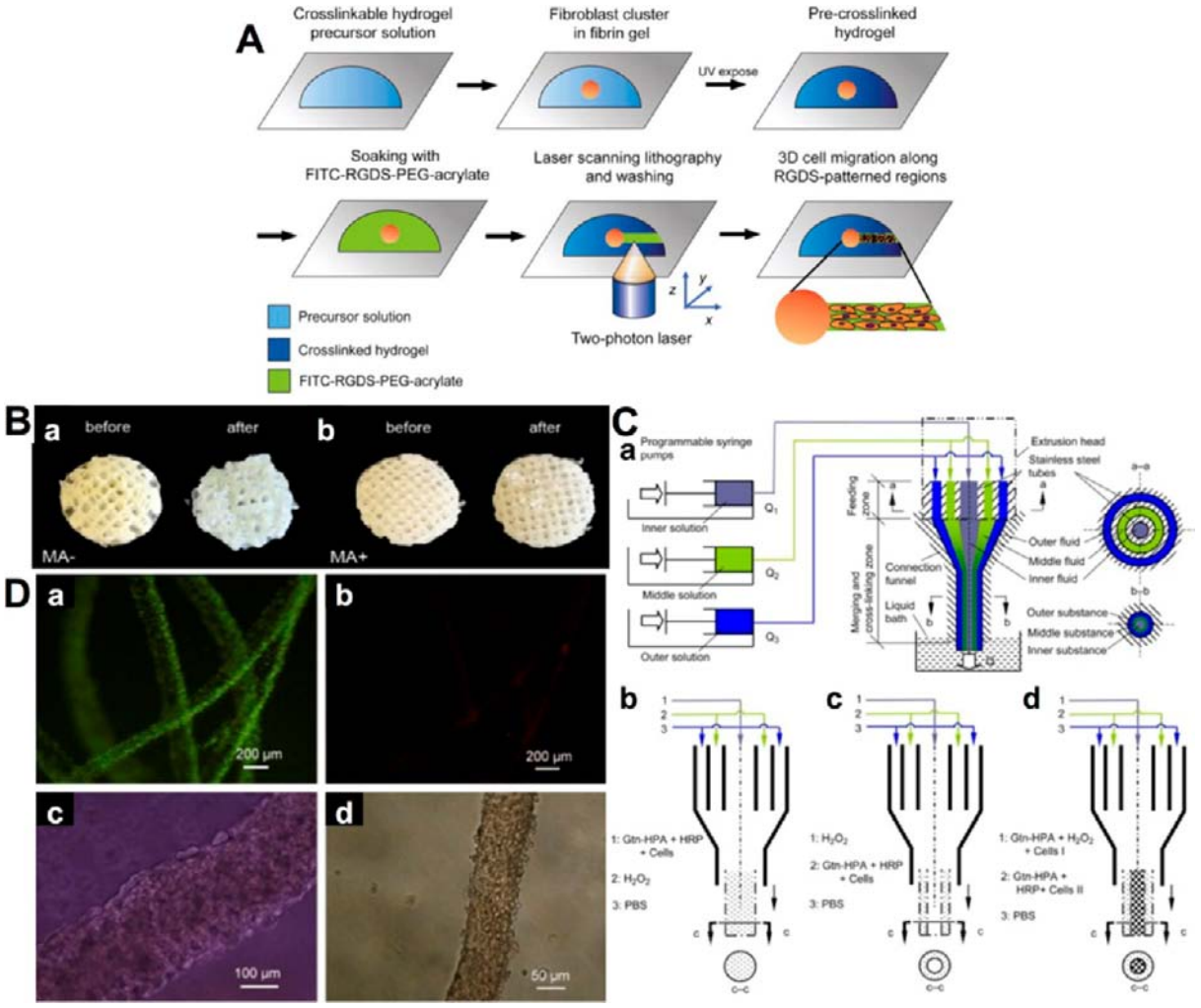


Figure 4. Microfabrication techniques used to engineer bioconjugated hydrogels for TE. (A) Photolithography technique for 3D RGDS patterning in the PEG hydrogel. First, the fibroblasts in fibrin clusters were photopolymerized into the collagenase-sensitive PEG hydrogels upon exposure to UV light. The hydrogels were soaked in PEG-RGDS solution, allowing its diffusion into the bulk of the material. Reproduced with permission, copyright 2008, Elsevier B.V.B.[155] (B) Photographs of printed poly(hydroxymethylglycolide-*co*-ε-caprolactone)/PCL functionalized with methacrylate (MA+) and without functionalization (MA−) before and after the application of force. Reproduced with permission, copyright 2014, Elsevier B.V. B.[164] (C) Multiphase laminar flow and its application in forming cell-seeded gelatin-HPA hydrogel fibers. (a) Multiphase laminar flow from a triple-orifice extruder. (b) Cell-seeded single-layered gelatin-HPA solid fibers can be formed by pumping cell-mixed hydrogel precursors through the inner orifice and H_2O_2 solution through the middle orifice. (c) Cell-seeded gelatin-HPA hollow fibers can be obtained by switching the solutions in the inner and middle orifices of b. (d) Cell-seeded dual-layered gelatin-HPA solid fibers can be synthesized by mixing gelatin-HPA and another type of cell with H_2O_2 and pumping the mixture through the inner orifice of c. In b−d, phosphate buffered saline (PBS) is pumped through the outer orifice as a sheath fluid. Reproduced with permission, copyright 2009, Elsevier B.V. B.[171] (D) Live−dead assay of the immobilized MDCK cells in gelatin-HPA fibers. Live cells were labeled green, and dead cells are shown in red. (c) Solid fiber embedded with the MDCK cells after 4 days in culture medium. (d) NIH/3T3 cells in a solid fiber after 10 days of culture. Reproduced with permission, copyright 2009, Elsevier B.V. B.[171]

procedure.[139] Electrospun fibers made from hydrogels or other biomacromolecules have been used for different biomedical applications, such as wound dressing,[140] enzyme immobilization,[141] production of artificial blood vessels,[142] and as drug or gene carriers[143] and TE scaffolds.[144] It is also possible to prepare aligned electrospun fibers by including a rotating mandrel or disk into the electrospinning setup.[145] Aligned fibers can be used to mimic the anisotropic structure of native ECM fibers.

The use of electrospinning technology to manipulate bioconjugated hydrogels is an asset for the development of biomimetic scaffold structures for different tissue regeneration applications. For example, Lee et al. reported the use of electrospun PCL-collagen fibers to control the release kinetics of heparin conjugated on hydrogel fibers.[146] Fiber morphology was shown to be an important parameter affecting heparin release and cell affinity of gels. In another study, chitosan−agarose fibers were fabricated using an electrospinning technique.[147] Of particular note is the important role played by chitosan in adjusting the viscosity of agarose for electrospinning. In its absence, pure agarose could not be processed due to its high viscosity. Block copolymer fibers of PEG and poly(D,L-lactide) conjugated with the RGD peptide were also prepared using an electrospinning approach.[148] The fiber shape and its large surface/volume ratio helped to control the immobilization of the peptide on the gel surface, and therefore determined its bioreactivity. Human fibroblasts successfully adhered to and proliferated on the fibers conjugated with the RGD peptide. In a recent study, Wade et al. prepared electrospun nanofibers of nondegradable methacrylated peptide

DOI: 10.1021/acs.bioconjchem.5b00360
Bioconjugate Chem. 2015, 26, 1984−2001

HA (MePHA) and protease-degradable MePHA hydrogels.[149] The biodegradation of the electrospun fibers was achieved proteolytically both in vitro and in vivo similarly to the ECM degradation.

6.2. Photopatterning. Photopatterning or photolithography is a popular approach for fabrication of hydrogel structures. In this approach, a photomask with a desired pattern is prepared. Some areas of the photomask are opaque to block light exposure, and other areas are transparent to allow passage of light through the mask. Upon exposure of a photomasked hydrogel to light, the areas under the opaque regions remain un-cross-linked and washed out, while the other areas are cross-linked and form the negative of the mask pattern.[150]

In one study, Wong et al. synthesized a photosensitive hydrogel through the conjugation of ortho-nitrobenzyl groups to PEG.[151] These authors were able to locally control swelling properties of the hydrogels using a micropatterning technique. In another work, a layer of photo-cross-linkable PEG-*co*-(L-lactide) diacrylate was confined between PEG-diacrylate layers,[152] and a micropattern within the PEG-*co*-(L-lactide) diacrylate layer was created upon UV irradiation. The micropatterned areas were then dissolved in a high pH medium to form 3D microchannels. However, cells could not be encapsulated in the fabricated hydrogels because of harsh basic conditions. PEG hydrogels conjugated with RGDS and VEGF ligands were also spatially patterned using photolithography technique[153,154] at micropattern widths varying from 10 to 200 μm. The formation of tubular structures of HUVECs on the PEG micropatterns was increased when smaller patterns were used compared with wider ones. In another study, peptide-conjugated PEG hydrogels were micropatterned using two-photon laser scanning photolithography and were used to assess the cell migration of human fibroblasts.[155] Unlike conventional micropatterning approaches, the newly developed technique was able to generate 3D-patterned gels that allow the creation of complex tissue constructs in vitro (Figure 4A). Au et al. micropatterned an agarose gel and then conjugated it with collagen.[156] The adhesion and proliferation of hepatocytes on the fabricated micropatterns were improved compared with nonpatterned agarose samples. A proper attachment of hepatocytes to culture substrates is crucial for maintaining their cellular phenotype and function in vitro.[157] In addition, the micropatterned structures provided anisotropic alignment for hepatocytes, mimicking their native ECM organization.[158]

6.3. Bioprinting. Despite major advances in the fabrication of tissue constructs, there is still a huge gap between fabricated tissues and cinically relevant ones.[159] Bioprinting has recently emerged as a powerful technique capable of filling this gap by producing large-scale and complicated tissue structures.[160] In particular, bioprinting hydrogels and cell-laden hydrogels help to precisely reproduce the 3D and hierarchical architecture of native tissues by sequentially depositing hydrogel layers. Novel bioprinting approaches are capable of creating custom-made cell-laden architectures with high cell viability.[161]

Gao et al. prepared acrylated peptides conjugated with a PEG hydrogel and coprinted them with human MSCs using an inkjet bioprinter followed by photopolymerization.[162] This fabrication process was surprisingly efficient and preserved cell viability and differentiation, and the low viscosity of the conjugated PEG hydrogel minimized printhead clogging. In addition, the printed hydrogel was highly efficient at inhibiting stem cell hypertrophy during chondrogenic differentiation. In another study, Levato et al. used bioprinting technology to manipulate cell-laden microcarriers.[163] The microcarriers of MSCs in polylactic acid were first obtained using a static culture or a bioreactor. The cell-laden microcarriers were then encapsulated in GelMA-GG bioinks and used for the fabrication of bilayered osteochondral constructs. The MSCs were able to differentiate into bone cells within the microcarriers. In another study, Boere et al. synthesized a thermoplastic polymer consisting of poly(hydroxymethylglycolide-*co*-ε-caprolactone)/PCL (pHMGCL/PCL) functionalized with methacrylate groups and conjugated it with GelMA hydrogel using a photopolymerization step[164] (Figure 4B). The 3D printed and scaffolds of (pHMGCL/PCL) were mechanically reinforced with GelMA hydrogel and used as a scaffold to repair a focal articular cartilage defect. The chondrocytes cultured within the scaffolds produced functional cartilage tissues in both in vitro and in vivo conditions.

6.4. Microfluidics. Microfluidics is a multidisciplinary field of research in which small volumes of fluid are manipulated at the microscale level. The field emerged in the early 1980s and now has wide applications in different disciplines of science and technology.[165] The applications of microfluidics in the field of biomedicine are numerous,[166] ranging from fabrication of tissue structures[167] and biological assays[168] to single-cell analysis.[169] In particular, microfluidic technologies offer an interesting approach for creating functional hydrogels with 3D morphologies and tunable chemical structures.[170]

Various microfluidic systems have been employed to fabricate bioconjugated hydrogels in TE. For instance, Hu et al. developed a microfluidic platform to fabricate cell-laden and hollow microfibers of gelatin conjugated with hydroxyphenylpropionic acid (HPA)[171] (Figure 4C). Fibers as small as 20 μm were formed by enzymatically cross-linking the gelatin with the HPA moving through a capillary tube. The experimental conditions were sufficiently mild to allow high viability of cells encapsulated in the fibers (Figure 4D). The same research group also used a triple-orifice spinneret to produce solid and hollow fibers of gelatin-HPA hydrogels.[172] The flow rate determined the diameter of the fibers. In the latter study, the porous fibers were generated upon the selective removal of fiber sections by sodium citrate or collagenase. A gradient of gelatin-HPA hydrogels was also fabricated in a microfluidic channel.[173] This gradient was used to record the chemotactic response of human cancerous cells in a high-throughput and cost-effective manner. In general, gradient systems aim to recapitulate the anisotropic structural and chemical properties of the native ECM. Chitosan−agarose microgels with a controllable diameter were also prepared with a microfluidic technique.[174] In another study, Jun et al. fabricated collagen-Alg fibers to enhance the immunoprotection of pancreatic islet cells.[175] The technique enabled a uniform mass production of islets encapsulated in the fibers. Collagen-Alg hydrogels closely mimicked the native ECM of the pancreatic islets. The pancreatic islets cultured in collagen-Alg fibers showed a higher viability and insulin secretion compared with free islets and those cultured in Alg fibers alone.

7. PRECLINICAL/CLINICAL TRIALS OF BIOCONJUGATED HYDROGELS

TE has made great progress in fabricating simple and planar tissues and organs for clinical applications, such as skin,[176] cornea,[177] and bladder.[178] However, other tissues still require complex and sophisticated tissue replacements in the clinic. In

DOI: 10.1021/acs.bioconjchem.5b00360
Bioconjugate Chem. 2015, 26, 1984−2001

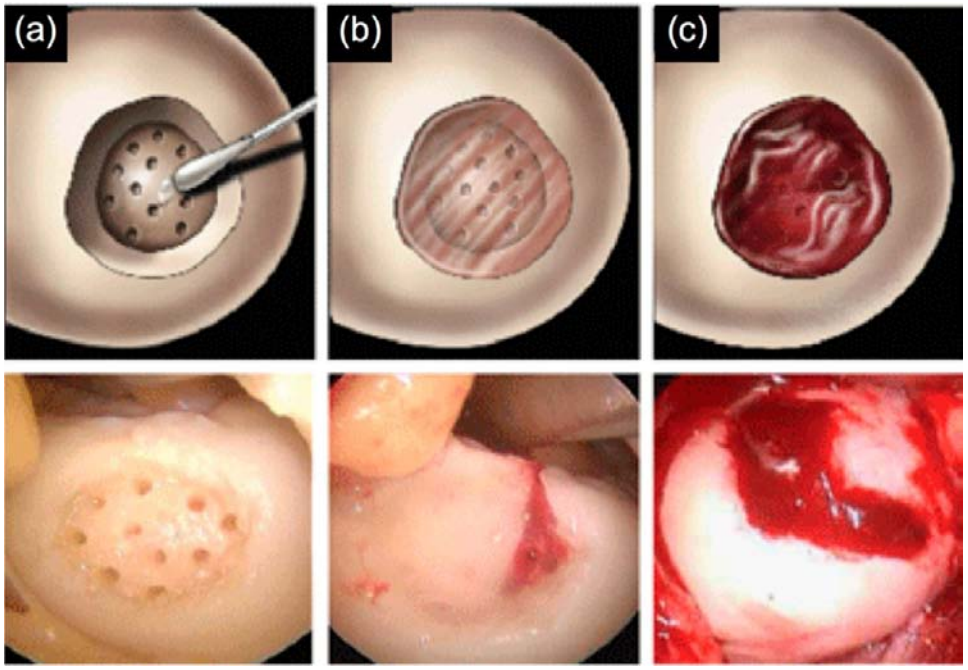


Figure 5. Bioconjugated hydrogels in preclinical/clinical studies. Hydrogel-adhesive implantation into a cartilage defect. (a) The adhesive was applied to the base and walls of the defect followed by surgical microfracture. (b) The hydrogel solution was injected into the defect and photopolymerized in situ with light. (c) Bleeding from the microfracture holes was trapped in and around the hydrogel-adhesive conjugate. Reproduced with permission, copyright 2013, American Association for the Advancement of Science.[185]

particular, the creation of multiple tissue layers and spatial control over them and vascularization within the tissues are major hurdles in fabricating complicated tissues and organs.[179] In this section, we focus on application of bioconjugated hydrogels as smart and functional scaffolds in preclinical and clinical studies.

Tang et al. prepared a thermoresponsive and composite hydrogel of chitosan-PVA.[180] Qi et al. used this hydrogel combined with Ad-hTGF-β1 transfected MSCs for repairing articular cartilage defects in a rabbit model.[181] Twenty-four rabbits were used in the study. The performance of the hydrogel was evaluated using naked-eye observation, immunohistological staining of collagen type II, and Masson's trichrome staining. After 16 weeks of implantation, the cell-laden hydrogel completely healed the defects, while control animal models still suffered from the defects. There are four main strategies for cartilage regeneration in preclinical/clinical trials: cells encapsulated in or cultured on a scaffold, a gene activated matrix, genetically modified cells, and a combination of these methods.[182] Hydrogels have been used as matrices for supporting chondrocyte implantation and repairing cartilage defects.[183] Conjugated hydrogels may play a significant role in cartilage repair in the clinic by providing suitable physical or biological cues and functionality for implants. For example, Wang et al. conjugated PEG hydrogel with a chondroitin sulfate adhesive to enhance hydrogel adhesion to cartilage and bone tissues in articular defects.[184] The bioconjugation of the hydrogel helped its integration with slippery surface of cartilage surroundings in vivo. More importantly, applying light caused the formation of the hydrogel-adhesive conjugate in situ within minutes. Therefore, the hydrogel was able to conform to any defect shape. A preclinical evaluation of the hydrogel using large animals (caprine model) demonstrated its safety and high performance for treating cartilage defects.[184] Moreover, clinical studies of the hydrogel using 15 human patients showed higher regeneration of cartilage defects compared with control species with negligible side effects[185] (Figure 5).

OctoPlus Company developed a hydrogel named PolyActive that is a biodegradable copolymer composed of PEG and poly(butylene terephtalate) (PBT). The PEG segment provides the material elasticity, while the PBT segment cross-links the gel through physical interactions. The PolyActive is a thermoresponsive gel, which has been used in preclinical studies for repairing bone and cartilage defects.[186] Since the PolyActive supports calcium phosphate formation, it was also commercialized as bone cement restrictor (SynPlug) by IsoTis Company in 2001. Despite commercialization of some bioconjugated hydrogels and their successful preclinical and clinical applications in tissue regeneration, there is still a huge gap between most bioconjugated hydrogels and their clinical applications for humans.

8. CONJUGATION TECHNIQUES FOR HYDROGELS

A variety of conjugation techniques have been used to prepare bioconjugated hydrogels from the hydrogels and conjugated moieties. Michael addition, click, Schiff base, and enzyme-mediated reactions are among the most widely used conjugation techniques for hydrogels because of high selectivity, efficiency, and biocompatibility[187] as described below.

The Michael addition reaction results in the nucleophilic addition of a nucleophile or a carbanion (e.g., amines and thiols) to an α,β-unsaturated carbonyl compound. This reaction has high selectivity for efficient coupling of compounds under physiological conditions, without producing side products or toxic reagents.[188] Different natural and synthetic hydrogels have been involved in the Michael addition reaction to synthesize the bioconjugated hydrogels, such as PEG-HA,[136] PEG-chitosan,[189] and heparin-PEG.[190] Soluble factors and cells can

DOI: 10.1021/acs.bioconjchem.5b00360
Bioconjugate Chem. 2015, 26, 1984−2001

also be encapsulated in such bioconjugated hydrogels by mixing them with the hydrogel precursors.[191] A notable bioconjugated hydrogel is Extracel, which is an injectable hydrogel synthesized using the Michael addition reaction between gelatin modified with diacrylated PEG and a thiol-modified carboxymethyl HA.[192]

Click chemistry often refers to a Cu(I)-catalyzed reaction between acetylene and azide groups, forming 1,2,3-triazoles.[193] The click chemistry has wide biomedical applications because its starting materials (i.e., azides and terminal alkynes) are stable in biological media, leading to facile introduction of these chemical groups into a variety of biomolecules. In addition, click chemistry reactions provide rapid reactivity with no toxic byproducts and have high yield, selectivity, and control on the reactions.[194] Ossipov et al. first introduced the click reaction as a selective and efficient technique for hydrogel synthesis. They functionalized PVA with either azide or acetylene groups, and cross-linked it by mixing its aqueous solution with copper sulfate and sodium ascorbate as the catalyst.[195] The click chemistry has also been used to synthesize bioconjugated hydrogels. For instance, Liu et al. cross-linked tetraacetylene PEG with diazide-functionalized RGD peptide in an aqueous medium with sodium ascorbate and copper sulfate as the catalyst.[196] The gelation time was varied between 2 and 30 min as a function of temperature, precursor concentrations, and catalyst. An increase in the amount of copper sulfate or temperature resulted in a decrease in the gelation time. Similarly, van Dijk et al. reported the reaction of alkyne-functionalized star-shaped PEG molecules with a protease-sensitive bis-azido peptide in an aqueous solution in the presence of sodium ascorbate and copper sulfate.[197] Interestingly, the conformation of protein or peptide component in bioconjugated gels can be controlled using click reactions providing a well-defined system to study cellular behavior. The main shortcoming of click chemistry in biomedical applications is the use of copper-based catalysts.[194] Copper is a cytotoxic element and may cause Alzheimer's disease and hepatitis.[198] However, the use of copper-free click chemistry (e.g., strain-promoted azide−alkyne coupling reaction) is an alternative approach to copper-based catalysts in click reactions.[199]

Schiff base reaction can be used to conjugate a hydrogel with other biological moieties. This reaction requires an aldehyde group and an amine group in reactive compounds and does not use any additional chemical cross-linking reagent.[200] Gelatin,[134] PEG,[201] and chitosan[202] have been used for the synthesis of bioconjugated hydrogels using the Schiff base reaction.

Enzyme-mediated reaction can also be used to prepare bioconjugated hydrogels in the presence of horseradish peroxidase and H_2O_2 in a rapid manner.[203] The time for hydrogel formation depends on the concentration of hydrogel precursor and enzyme/hydrogel ratio. Gelatin-PEG hydrogel was prepared using the enzyme-mediated technique and proposed as an injectable hydrogel for tissue regeneration in vivo.[204] The gelation time varied from a few seconds to a few minutes by changing the concentration of horseradish peroxidase. Enzyme-mediated reactions are efficient and highly selective and occur in mild conditions. Interestingly, these reactions are also naturally involved in metabolic and biological processes in living organisms.[205] Therefore, they can further broaden the design and synthesis of functional and biomimetic bioconjugated hydrogels.

9. CONCLUSIONS AND FUTURE DIRECTIONS

TE and regenerative medicine have recently emerged as exciting research fields with numerous preclinical and clinical trials and products using hydrogels. However, there is still a need to design and fabricate highly complex and sophisticated hydrogel materials mimicking the complex structure and function of the native ECM.[206] Unfortunately, there is no hydrogel that possesses all of the characteristics of the native ECM for a given TE application. Therefore, a wide variety of synthetic, natural, and synthetic−natural composite hydrogels have been developed to facilitate specific interactions with cells through their well-defined chemical (e.g., cell-adhesion and biofunctionality) and physical properties (e.g., mechanics, structure, porosity, and interconnectivity).[207] Thus, bioconjugation of hydrogels has become a useful approach for synthesis or modification of hydrogels for tissue regeneration. Here, we reviewed and discussed representative bioconjugated materials composed of different biomolecules, such as proteins, peptides, and hydrogels, which help to regulate the function and morphogenesis of different cell and tissue types. Micro- and nanofabrication techniques for engineering bioconjugated hydrogels for regenerative purposes were also described.

Rapid advances in life sciences and technologies have greatly aided the process of hydrogel design and fabrication for tissue regeneration. In particular, novel discoveries in biology, chemical synthesis and production, and chemical reaction mechanisms, as well as close collaborations between biologists, chemists, material scientists, and engineers, are key factors for the development of novel and functional bioconjugated hydrogels.

Controlling biological and physicochemical properties of hydrogels is critical for manipulating different cell behaviors and therefore for fabricating functional tissue constructs. In particular, the dynamic nature of the native ECM should be considered in the design and fabrication of functional hydrogels. Dynamic hydrogels aim to provide a dynamic scaffold for cells to receive and respond to biological signals over time with appropriate spatial resolution. Conventional methods for fabricating hydrogels have relied on static and well-defined design, which considerably limits their dynamic properties.[208,209] Bioconjugation approaches possess great potential for developing hydrogels that mimic the spatiotemporal and dynamic properties of the ECM microenvironment. Some dynamic hydrogels have already been synthesized. For example, Murphy et al. reported the synthesis of a dynamic PEG hydrogel conjugated with the calmodulin protein.[210] The conformational changes of calmodulin due to ligand binding caused a change in the hydrogel volume. However, there is ample opportunity to develop dynamic hydrogels for TE applications that would be responsive to biological signals during tissue morphogenesis both in vitro and in vivo.

An increasing number of bioconjugated hydrogels with multiple chemical, biological, and physical functionalities closely mimicking the ECM have been developed. Taking cues from the native ECM to design and fabricate hydrogels is a good way to create a suitable hydrogel for tissue regeneration.[211] However, considerable time and effort are required to make the large numbers of developed bioconjugated hydrogels suitable for specific TE applications. In this regard, high-throughput screening is a useful approach to synthesize bioconjugated hydrogels and optimize their properties. In particular, high-throughput screening approaches could im-

DOI: 10.1021/acs.bioconjchem.5b00360
Bioconjugate Chem. 2015, 26, 1984−2001

prove our understanding of the chemical interactions between hydrogels and their conjugated biomaterials, as well as cell−hydrogel interactions.

Micro- and nanotechnology approaches are extremely powerful tools for the construction of bioconjugated hydrogels to control the intra- and extracellular signaling pathways and cell functions of particular cell types.[212] In the future, bioconjugated hydrogels must be further integrated with novel and advanced technologies (e.g., 3D printing[213] and textile technology[214]) to fabricate biomimetic and hierarchical architectures for tissue regeneration. Due to the high sensitivity of conjugated moieties (e.g., soluble factors) to processing conditions, special care may be required to preserve hydrogel properties and functions.

Ultimately, a major step toward wide and practical application of the proposed bioconjugated hydrogels is successful clinical translation to humans in a safe and cost-effective manner. While a large number of excellent bioconjugated hydrogels have been developed and tested for their ability to regulate cell behaviors and tissue fabrication in vitro, in vivo analysis of these biomaterials is limited. Therefore, it is necessary to accelerate the translation process of developed bioconjugated hydrogels into the clinic using standard protocols. Despite these challenges, bioconjugated hydrogels are likely to prove invaluable in the future development of tissue engineering, with great challenges and also great expectations ahead.

AUTHOR INFORMATION

Corresponding Author

*Fax: +1 (617) 768-8477. E-mail: alik@rics.bwh.harvard.edu.

Author Contributions

#Ramin Banan Sadeghian, Sahar Salehi, and Serge Ostrovidov contributed equally to this work.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by the World Premier International Research Center Initiative (WPI), MEXT, Japan.

REFERENCES

(1) Jaiswal, J. K., Mattoussi, H., Mauro, M., and Simon, S. M. (2002) Long-term multiple color imaging of live cells using quantum dot bioconjugates. *Nat. Biotechnol. 21*, 47−51.

(2) Huo, Q. (2007) A perspective on bioconjugated nanoparticles and quantum dots. *Colloids Surf., B 59*, 1−10.

(3) Liong, M., Fernandez-Suarez, M., Issadore, D., Min, C., Tassa, C., Reiner, T., Fortune, M. S., Toner, M., Lee, H., and Weissleder, R. (2011) Specific pathogen detection using bioorthogonal chemistry and diagnostic magnetic resonance. *Bioconjugate Chem. 22*, 2390−2394.

(4) Fichna, J., and Janecka, A. (2003) Synthesis of target-specific radiolabeled peptides for diagnostic imaging. *Bioconjugate Chem. 14*, 3−17.

(5) Zalipsky, S. (1995) Functionalized poly (ethylene glycols) for preparation of biologically relevant conjugates. *Bioconjugate Chem. 6*, 150−165.

(6) Gonçalves, M. S. T. (2009) Fluorescent labeling of biomolecules with organic probes. *Chem. Rev. 109*, 190−212.

(7) da Costa, J. P., Oliveira-Silva, R., Daniel-da-Silva, A., and Vitorino, R. (2014) Bionanoconjugation for proteomics applications-An overview. *Biotechnol. Adv. 32*, 952−970.

(8) Brown, B. N., and Badylak, S. F. (2014) Extracellular matrix as an inductive scaffold for functional tissue reconstruction. *Translational Res. 163*, 268−285.

(9) Schense, J. C., and Hubbell, J. A. (1999) Cross-linking exogenous bifunctional peptides into fibrin gels with factor XIIIa. *Bioconjugate Chem. 10*, 75−81.

(10) Shin, H., Jo, S., and Mikos, A. G. (2003) Biomimetic materials for tissue engineering. *Biomaterials 24*, 4353−4363.

(11) Zouraq, F. A., Stölting, M., and Eberli, D. (2013) Skeletal muscle regeneration for clinical application, In *Regenerative Medicine and Tissue Engineering* (Andrades, J. A., Ed.) INTECH.

(12) Cezar, C. A., and Mooney, D. J. (2015) Biomaterial-based delivery for skeletal muscle repair. *Adv. Drug Delivery Rev. 84*, 188−197.

(13) Khademhosseini, A., Vacanti, J. P., and Langer, R. (2009) Progress in tissue engineering. *Sci. Am. 300*, 64−71.

(14) Lin, X., Huang, J., Shi, Y., and Liu, W. (2015) Tissue engineering and regenerative medicine in applied research: a year in review of 2014. *Tissue Eng., Part B 21*, 177−186.

(15) Madden, L., Juhas, M.,. Kraus, W. E., Truskey, G. A., and Bursac, N. (2015) Bioengineered human myobundles mimic clinical responses of skeletal muscle to drugs. *eLife*, 10.7554/eLife.04885

(16) Griffith, L. G., and Naughton, G. (2002) Tissue engineering-current challenges and expanding opportunities. *Science 295*, 1009−1014.

(17) Rosso, F., Giordano, A., Barbarisi, M., and Barbarisi, A. (2004) From cell-ECM interactions to tissue engineering. *J. Cell. Physiol. 199*, 174−180.

(18) Khademhosseini, A., and Langer, R. (2007) Microengineered hydrogels for tissue engineering. *Biomaterials 28*, 5087−5092.

(19) Annabi, N., Tamayol, A., Uquillas, J. A., Akbari, M., Bertassoni, L. E., Cha, C., Camci-Unal, G., Dokmeci, M. R., Peppas, N. A., and Khademhosseini, A. (2014) 25th Anniversary article: rational design and applications of hydrogels in regenerative medicine. *Adv. Mater. 26*, 85−124.

(20) Slaughter, B. V., Khurshid, S. S., Fisher, O. Z., Khademhosseini, A., and Peppas, N. A. (2009) Hydrogels in regenerative medicine. *Adv. Mater. 21*, 3307−3329.

(21) Peppas, N. A., Hilt, J. Z., Khademhosseini, A., and Langer, R. (2006) Hydrogels in biology and medicine: From molecular principles to bionanotechnology. *Adv. Mater. 18*, 1345−1360.

(22) Holzapfel, B. M., Reichert, J. C., Schantz, J. T., Gbureck, U., Rackwitz, L., Nöth, U., Jakob, F., Rudert, M., Groll, J., and Hutmacher, D. W. (2013) How smart do biomaterials need to be? A translational science and clinical point of view. *Adv. Drug Delivery Rev. 65*, 581−603.

(23) Rosso, F., Marino, G., Giordano, A., Barbarisi, M., Parmeggiani, D., and Barbarisi, A. (2005) Smart materials as scaffolds for tissue engineering. *J. Cell. Physiol. 203*, 465−470.

(24) Nichol, J. W., Koshy, S. T., Bae, H., Hwang, C. M., Yamanlar, S., and Khademhosseini, A. (2010) Cell-laden microengineered gelatin methacrylate hydrogels. *Biomaterials 31*, 5536−5544.

(25) Annabi, N., Mithieux, S. M., Zorlutuna, P., Camci-Unal, G., Weiss, A. S., and Khademhosseini, A. (2013) Engineered cell-laden human protein-based elastomer. *Biomaterials 34*, 5496−5505.

(26) Du, Y., Ghodousi, M., Qi, H., Haas, N., Xiao, W., and Khademhosseini, A. (2011) Sequential assembly of cell-laden hydrogel constructs to engineer vascular-like microchannels. *Biotechnol. Bioeng. 108*, 1693−1703.

(27) Ostrovidov, S., Hosseini, V., Ahadian, S., Fujie, T., Parthiban, S. P., Ramalingam, M., Bae, H., Kaji, H., and Khademhosseini, A. (2014) Skeletal muscle tissue engineering: Methods to form skeletal myotubes and their applications. *Tissue Eng., Part B 20*, 403−436.

(28) Jabbari, E. (2011) Bioconjugation of hydrogels for tissue engineering. *Curr. Opin. Biotechnol. 22*, 655−660.

(29) Petka, W. A., Harden, J. L., McGrath, K. P., Wirtz, D., and Tirrell, D. A. (1998) Reversible hydrogels from self-assembling artificial proteins. *Science 281*, 389−392.

(30) Wang, L. S., Lee, F., Lim, J., Du, C., Wan, A. C. A., Lee, S. S., and Kurisawa, M. (2014) Enzymatic conjugation of a bioactive peptide

DOI: 10.1021/acs.bioconjchem.5b00360
Bioconjugate Chem. 2015, 26, 1984−2001

into an injectable hyaluronic acid-tyramine hydrogel system to promote the formation of functional vasculature. *Acta Biomater. 10*, 2539−2550.
(31) Mahoney, M. J., and Anseth, K. S. (2006) Three-dimensional growth and function of neural tissue in degradable polyethylene glycol hydrogels. *Biomaterials 27*, 2265−2274.
(32) Zhu, J. (2010) Bioactive modification of poly(ethylene glycol) hydrogels for tissue engineering. *Biomaterials 31*, 4639−4656.
(33) Mosiewicz, K. A., Kolb, L., van der Vlies, A. J., Martino, M. M., Lienemann, P. S., Hubbell, J. A., Ehrbar, M., and Lutolf, M. P. (2013) In situ cell manipulation through enzymatic hydrogel photopatterning. *Nat. Mater. 12*, 1072−1078.
(34) Phelps, E. A., Enemchukwu, N. O., Fiore, V. F., Sy, J. C., Murthy, N., Sulchek, T. A., Barker, T. H., and García, A. J. (2012) Maleimide cross-linked bioactive PEG hydrogel exhibits improved reaction kinetics and cross-linking for cell encapsulation and in situ delivery. *Adv. Mater. 24*, 64−70.
(35) Rowley, J. A., Madlambayan, G., and Mooney, D. J. (1999) Alginate hydrogels as synthetic extracellular matrix materials. *Biomaterials 20*, 45−53.
(36) Li, Y., Rodrigues, J., and Tomas, H. (2012) Injectable and biodegradable hydrogels: gelation, biodegradation and biomedical applications. *Chem. Soc. Rev. 41*, 2193−2221.
(37) Bubenikova, S., Stancu, I. C., Kalinovska, L., Schacht, E., Lippens, E., Declercq, H., Cornelissen, M., Santin, M., Amblard, M., and Martinez, J. (2012) Chemoselective cross-linking of alginate with thiol-terminated peptides for tissue engineering applications. *Carbohydr. Polym. 88*, 1239−1250.
(38) Madl, C. M., Mehta, M., Duda, G. N., Heilshorn, S. C., and Mooney, D. J. (2014) Presentation of BMP-2 mimicking peptides in 3D hydrogels directs cell fate commitment in osteoblasts and mesenchymal stem cells. *Biomacromolecules 15*, 445−455.
(39) Jeon, O., and Alsberg, E. (2013) Photofunctionalization of alginate hydrogels to promote adhesion and proliferation of human mesenchymal stem cells. *Tissue Eng., Part A 19*, 1424−1432.
(40) Jeon, O., Powell, C., Ahmed, S. M., and Alsberg, E. (2010) Biodegradable, photocrosslinked alginate hydrogels with independently tailorable physical properties and cell adhesivity. *Tissue Eng., Part A 16*, 2915−2925.
(41) Dhoot, N. O., Tobias, C. A., Fischer, I., and Wheatley, M. A. (2004) Peptide-modified alginate surfaces as a growth permissive substrate for neurite outgrowth. *J. Biomed. Mater. Res. 71A*, 191−200.
(42) Kim, I. Y., Seo, S. J., Moon, H. S., Yoo, M. K., Park, I. Y., Kim, B. C., and Cho, C. S. (2008) Chitosan and its derivatives for tissue engineering applications. *Biotechnol. Adv. 26*, 1−21.
(43) Li, J., Shu, Y., Hao, T., Wang, Y., Qian, Y., Duan, C., Sun, H., Lin, Q., and Wang, C. (2013) A chitosan-glutathione based injectable hydrogel for suppression of oxidative stress damage in cardiomyocytes. *Biomaterials 34*, 9071−9081.
(44) Miklas, J. W., Dallabrida, S. M., Reis, L. A., Ismail, N., Rupnick, M., and Radisic, M. (2013) QHREDGS enhances tube formation, metabolism and survival of endothelial cells in collagen-chitosan hydrogels. *PLoS One 8*, e72956.
(45) Ferreira, A. M., Gentile, P., Chiono, V., and Ciardelli, G. (2012) Collagen for bone tissue regeneration. *Acta Biomater. 8*, 3191−3200.
(46) Luo, T., and Kiick, K. L. (2013) Collagen-like peptides and peptide-polymer conjugates in the design of assembled materials. *Eur. Polym. J. 49*, 2998−3009.
(47) Reis, L. A., Chiu, L. L. Y., Liang, Y., Hyunh, K., Momen, A., and Radisic, M. (2012) A peptide-modified chitosan-collagen hydrogel for cardiac cell culture and delivery. *Acta Biomater. 8*, 1022−1036.
(48) Hiraoka, M., Kato, K., Nakaji-Hirabayashi, T., and Iwata, H. (2009) Enhanced survival of neural cells embedded in hydrogels composed of collagen and laminin-derived cell adhesive peptide. *Bioconjugate Chem. 20*, 976−983.
(49) Yu, F., Cao, X., Zeng, L., Zhang, Q., and Chen, X. (2013) An interpenetrating HA/G/CS biomimic hydrogel via Diels-Alder click chemistry for cartilage tissue engineering. *Carbohydr. Polym. 97*, 188−195.
(50) Ferris, C. J., Stevens, L. R., Gilmore, K. J., Mume, E., Greguric, I., Kirchmajer, D. M., Wallace, G. G., and in het Panhuis, M. (2015) Peptide modification of purified gellan gum. *J. Mater. Chem. B 3*, 1106−1115.
(51) Burdick, J. A., and Prestwich, G. D. (2011) Hyaluronic acid hydrogels for biomedical applications. *Adv. Mater. 23*, H41−H56.
(52) Pardue, E. L., Ibrahim, S., and Ramamurthi, A. (2008) Role of hyaluronan in angiogenesis and its utility to angiogenic tissue engineering. *Organogenesis 4*, 203−214.
(53) Yamada, Y., Hozumi, K., Katagiri, F., Kikkawa, Y., and Nomizu, M. (2013) Laminin-111-derived peptide-hyaluronate hydrogels as a synthetic basement membrane. *Biomaterials 34*, 6539−6547.
(54) Wong, W. H. and Mooney, D. J. (1997) Synthesis and properties of biodegradable polymers used as synthetic matrices for tissue engineering, in *Synthetic biodegradable polymer scaffolds*, pp 51−82, Springer.
(55) Chong, S.-F., Smith, A. A. A., and Zelikin, A. N. (2013) Microstructured, functional PVA hydrogels through bioconjugation with oligopeptides under physiological conditions. *Small 9*, 942−950.
(56) Lahiri, D. K., Farlow, M. R., Greig, N. H., and Sambamurti, K. (2002) Current drug targets for Alzheimer's disease treatment. *Drug Dev. Res. 56*, 267−281.
(57) Reddi, A. R. (1998) Role of morphogenetic proteins in skeletal tissue engineering and regeneration. *Nat. Biotechnol. 16*, 247−252.
(58) Sheridan, M. H., Shea, L. D., Peters, M. C., and Mooney, D. J. (2000) Bioadsorbable polymer scaffolds for tissue engineering capable of sustained growth factor delivery. *J. Controlled Release 64*, 91−102.
(59) Vermonden, T., Censi, R., and Hennink, W. E. (2012) Hydrogels for protein delivery. *Chem. Rev. 112*, 2853−2888.
(60) Mikos, A. G., Herring, S. W., Ochareon, P., Elisseeff, J., Lu, H. H., Kandel, R., Schoen, F. J., Toner, M., Mooney, D., Atala, A., et al. (2006) Engineering complex tissues. *Tissue Eng. 12*, 3307−3339.
(61) Freeman, I., Kedem, A., and Cohen, S. (2008) The effect of sulfation of alginate hydrogels on the specific binding and controlled release of heparin-binding proteins. *Biomaterials 29*, 3260−3268.
(62) Szlachcic, A., Zakrzewska, M., and Otlewski, J. (2011) Longer action means better drug: Tuning up protein therapeutics. *Biotechnol. Adv. 29*, 436−441.
(63) van Vlierberghe, S., Dubruel, P., and Schacht, E. (2011) Biopolymer-based hydrogels as scaffolds for tissue engineering applications: a review. *Biomacromolecules 12*, 1387−1408.
(64) Seliktar, D. (2012) Designing cell-compatible hydrogels for biomedical applications. *Science 336*, 1124−1128.
(65) Censi, R., Di Martino, P., Vermonden, T., and Hennink, D. W. (2012) Hydrogels for protein delivery in tissue engineering. *J. Controlled Release 161*, 680−692.
(66) Park, H., Temenoff, J. S., Holland, T. A., Tabata, Y., and Mikos, A. G. (2005) Delivery of TGF-b1 and chondrocytes via injectable, biodegradable hydrogels for cartilage tissue engineering applications. *Biomaterials 26*, 7095−7103.
(67) Choi, B., Kim, S., Lin, B., Li, K., Bezouglaia, O., Kim, J., Evseenko, D., Aghaloo, T., and Lee, M. (2015) Visible-light-initiated hydrogels preserving cartilage extracellular signaling for inducing chondrogenesis of mesenchymal stem cells. *Acta Biomater. 12*, 30−41.
(68) Kim, J., Lin, B., Kim, S., Choi, B., Evseenko, D., and Lee, M. (2015) TGF-β1 conjugated chitosan collagen hydrogels induce chondrogenic differentiation of human synovium-derived stem cells. *J. Biol. Eng. 9*, 1.
(69) Jha, A. K., Tharp, K. M., Ye, J., Santiago-Ortiz, J. L., Jackson, W. M., Stahl, A., Schaffer, D. V., Yeghiazarians, Y., and Healy, K. E. (2015) Enhanced survival and engraftment of transplanted stem cells using growth factor sequestering hydrogels. *Biomaterials 47*, 1−12.
(70) Oh, E. J., Park, K., Kim, K. S., Kim, J., Yang, J. A., Kong, J. H., Lee, M. Y., Hoffman, A. S., and Hahn, S. K. (2010) Target specific and long-acting delivery of protein, peptide, and nucleotide therapeutics using hyaluronic acid derivatives. *J. Controlled Release 141*, 2−12.
(71) Duan, B., Hockaday, L. A., Das, S., Xu, C., and Butcher, J. T. (2015) Comparison of mesenchymal stem cell source differentiation

DOI: 10.1021/acs.bioconjchem.5b00360
Bioconjugate Chem. 2015, 26, 1984−2001

toward human pediatric aortic valve interstitial cells within 3D engineered matrices. *Tissue Eng., Part C 21*, 795−807.
(72) Wen, J., Zhang, J. Q., Huang, W., and Wang, Y. (2012) SDF-1α and CXCR4 as therapeutic targets in cardiovascular disease. *Am. J. Cardiovasc. Dis 2*, 20−28.
(73) Prokoph, S., Chavakis, E., Levental, K. R., Zieris, A., Freudenberg, U., Dimmeler, S., and Werner, C. (2012) Sustained delivery of SDF-1a from heparin-based hydrogels to attract circulating pro-angiogenic cells. *Biomaterials 33*, 4792−4800.
(74) Rabbany, S. Y., Pastore, J., Yamamoto, M., Miller, T., Rafii, S., Aras, R., and Penn, M. (2010) Continuous delivery of stromal cell-derived factor-1 from alginate scaffolds accelerates wound healing. *Cell Transplantation 19*, 399−408.
(75) Vunjak-Novakovic, G., Tandon, N., Godier, A., Maidhof, R., Marsano, A., Martens, T. P., and Radisic, M. (2010) Challenges in cardiac tissue engineering. *Tissue Eng., Part B 16*, 169−187.
(76) Ruvinov, E., Leor, J., and Cohen, S. (2011) The promotion of myocardial repair by the sequential delivery of IGF-1 and HGF from an injectable alginate biomaterial in a model of acute myocardial infarction. *Biomaterials 32*, 565−578.
(77) Siddiqui, N., and Pramanik, K. (2015) Development of fibrin conjugated chitosan/cano b-TCP composite scaffolds with improved cell supportive property for bone tissue regeneration. *J. Appl. Polym. Sci. 132*, 41534.
(78) Noh, S. S., Bhang, S. H., La, W. G., Lee, S., Shin, J. Y., Ma, Y. J., Jang, H. K., Kang, S., Jin, M., Park, J., et al. (2015) A dual delivery of substance P and bone morphogenetic protein-2 for mesenchymal stem cell recruitment and bone regeneration. *Tissue Eng., Part A 21*, 1275−1287.
(79) Carballo-Molina, O. A., and Velasco, I. (2015) Hydrogels as scaffolds and delivery systems to enhance axonal regeneration after injuries. *Front. Cell. Neurosci. 9*, 13.
(80) Hernandez-Montiel, H. L., Tamariz, E., Sandoval-Minero, M. T., and Varela-Echavarria, A. (2008) Semaphorins 3A, 3C, and 3F in mesencephalic dopaminergic axon pathfinding. *J. Comp. Neurol. 506*, 387−397.
(81) Tamariz, E., Wan, A. C. A., Pek, Y. S., Giordano, M., Hernandez-Padron, G., Varela-Echavarrıa, A., Velasco, I., and Castano, V. M. (2011) Delivery of chemotropic proteins and improvement of dopaminergic neuron outgrowth through a thixotropic hybrid nanogel. *J. Mater. Sci.: Mater. Med. 22*, 2097−2109.
(82) Oh, S. H., Bae, J. W., Kang, J. G., Kim, I. G., Son, J. Y., Lee, J. Y., Park, K. D., and Lee, J. H. (2015) Dual growth factor-loaded in situ gel-forming bulking agent: passive and bioactive effects for the treatment of urinary incontinence. *J. Mater. Sci.: Mater. Med. 26*, 33.
(83) Hou, J., Li, C., Guan, Y., Zhang, Y., and Zhu, X. X. (2015) Enzymatically crosslinked alginate hydrogels with improved adhesion properties. *Polym. Chem. 6*, 2204−2213.
(84) Stegemann, J. P., Kaszuba, S. N., and Rowe, S. L. (2007) Review: advances in vascular tissue engineering using protein-based biomaterials. *Tissue Eng. 13*, 2601−2613.
(85) Li, Z., Qu, T., Ding, C., Ma, C., Sun, H., Li, S., and Liu, X. (2015) Injectable gelatin derivative hydrogels with sustained vascular endothelial growth factor release for induced angiogenesis. *Acta Biomater. 13*, 88−100.
(86) Simón-Yarza, T., Formiga, F. R., Tamayo, E., Pelacho, B., Prosper, F., and Blanco-Prieto, M. J. (2012) Vascular endothelial growth factor-delivery systems for cardiac repair: An Overview. *Theranostics 2*, 541−552.
(87) Drinnan, C. T., Zhang, G., Alexander, M. A., Pulido, A. S., and Suggs, L. J. (2010) Multimodal release of transforming growth factor-β1 and the BB isoform of platelet derived growth factor from PEGylated fibrin gels. *J. Controlled Release 147*, 180−186.
(88) Lau, H. K., and Kiick, K. L. (2015) Opportunities for multicomponent hybrid hydrogels in biomedical applications. *Biomacromolecules 16*, 28−42.
(89) Hubbell, J. A. (2003) Materials as morphogenetic guides in tissue engineering. *Curr. Opin. Biotechnol. 14*, 551−558.
(90) Harris, J. M. (1992) *Introduction to Biotechnical and Biomedical Applications of Poly(ethylene glycol)*, Springer.
(91) Sawhney, A. S., and Hubbell, J. A. (1992) Poly (ethylene oxide)-graft-poly (L-lysine) copolymers to enhance the biocompatibility of poly (L-lysine)-alginate microcapsule membranes. *Biomaterials 13*, 863−870.
(92) Nuttelman, C. R., Rice, M. A., Rydholm, A. E., Salinas, C. N., Shah, D. N., and Anseth, K. S. (2008) Macromolecular monomers for the synthesis of hydrogel niches and their application in cell encapsulation and tissue engineering. *Prog. Polym. Sci. 33*, 167−179.
(93) Seidlits, S. K., Drinnan, C. T., Petersen, R. R., Shear, J. B., Suggs, L. J., and Schmidt, C. E. (2011) Fibronectin-hyaluronic acid composite hydrogels for three-dimensional endothelial cell culture. *Acta Biomater. 7*, 2401−2409.
(94) Leach, J. B., and Schmidt, C. E. (2005) Characterization of protein release from photocrosslinkable hyaluronic acid-polyethylene glycol hydrogel tissue engineering scaffolds. *Biomaterials 26*, 125−135.
(95) Andrianov, A. K., Chen, J., and Payne, L. G. (1998) Preparation of hydrogel microspheres by coacervation of aqueous polyphosphazene solutions. *Biomaterials 19*, 109−115.
(96) Ichikawa, H., and Peppas, N. A. (2003) Novel complexation hydrogels for oral peptide delivery: In vitro evaluation of their cytocompatibility and insulin-transport enhancing effects using Caco-2 cell monolayers. *J. Biomed. Mater. Res. 67*, 609−617.
(97) Lee, K. Y., Peters, M. C., Anderson, K. W., and Mooney, D. J. (2000) Controlled growth factor release from synthetic extracellular matrices. *Nature 408*, 998−1000.
(98) Hutson, C. B., Nichol, J. W., Aubin, H., Bae, H., Yamanlar, S., Al-Haque, S., Koshy, S. T., and Khademhosseini, A. (2011) Synthesis and characterization of tunable poly(ethylene glycol): Gelatin methacrylate composite hydrogels. *Tissue Eng., Part A 17*, 1713−1723.
(99) Daniele, M. A., Adams, A. A., Naciri, J., North, S. H., and Ligler, F. S. (2014) Interpenetrating networks based on gelatin methacrylamide and PEG formed using concurrent thiol click chemistries for hydrogel tissue engineering scaffolds. *Biomaterials 35*, 1845−1856.
(100) Grover, G. N., Rao, N., and Christman, K. L. (2014) Myocardial matrix-polyethylene glycol hybrid hydrogels for tissue engineering. *Nanotechnology 25*, 014011.
(101) Chan, B. K., Wippich, C. C., Wu, C. J., Sivasankar, P. M., and Schmidt, G. (2012) Robust and semi-interpenetrating hydrogels from poly(ethylene glycol) and collagen for elastomeric tissue scaffolds. *Macromol. Biosci. 12*, 1490−1501.
(102) Cao, L., Cao, B., Lu, C., Wang, G., Yu, L., and Ding, J. (2015) An injectable hydrogel formed by in situ cross-linking of glycol chitosan and multi-benzaldehyde functionalized PEG analogues for cartilage tissue engineering. *J. Mater. Chem. B 3*, 1268.
(103) Rafat, M., Li, F., Fagerholm, P., Lagali, N. S., Watsky, M. A., Munger, R., Matsuura, T., and Griffith, M. (2008) PEG-stabilized carbodiimide crosslinked collagen-chitosan hydrogels for corneal tissue engineering. *Biomaterials 29*, 3960−3972.
(104) Mahou, R., Tran, N. M., Dufresne, M., Lagallais, C., and Wandrey, C. (2012) Encapsulation of Huh-7 cells within alginate-poly (ethylene glycol) hybrid microspheres. *J. Mater. Sci.: Mater. Med. 23*, 171−179.
(105) Ibusuki, S., Fujii, Y., Iwamoto, Y., and Matsuda, T. (2003) Tissue-engineered cartilage using an injectable and in situ gelable thermoresponsive gelatin: fabrication and in vitro performance. *Tissue Eng. 9*, 371−384.
(106) Hosseini, V., Ahadian, S., Ostrovidov, S., Camci-Unal, G., Chen, S., Kaji, H., Ramalingam, M., and Khademhosseini, A. (2012) Engineered contractile skeletal muscle tissue on a microgrooved methacrylated gelatin substrate. *Tissue Eng., Part A 18*, 2453−2465.
(107) Dreesmann, L., Ahlers, M., and Schlosshauer, B. (2007) The pro-angiogenic characteristics of a cross-linked gelatin matrix. *Biomaterials 28*, 5536−5543.
(108) Sakai, S., Hashimoto, I., and Kawakami, K. (2007) Synthesis of an agarose-gelatin conjugate for use as a tissue engineering scaffold. *J. Biosci. Bioeng. 103*, 22−26.

DOI: 10.1021/acs.bioconjchem.5b00360
Bioconjugate Chem. 2015, 26, 1984−2001

(109) Sakai, S., Hashimoto, I., and Kawakami, K. (2007) Agarose-gelatin conjugate for adherent cell-enclosing capsules. *Biotechnol. Lett. 29*, 731−735.
(110) Normand, V., Lootens, D. L., Amici, E., Plucknett, K. P., and Aymard, P. (2000) New insight into agarose gel mechanical properties. *Biomacromolecules 1*, 730−738.
(111) Liu, J., and Li, L. (2005) SDS-aided immobilization and controlled release of camptothecin from agarose hydrogel. *Eur. J. Pharm. Sci. 25*, 237−244.
(112) Jain, A., Kim, Y. T., McKeon, R. J., and Ballamkonda, R. V. (2006) In situ gelling hydrogels for conformal repair of spinal cord defects, and local delivery of BDNF after spinal cord injury. *Biomaterials 27*, 497−504.
(113) Gruber, H. E., Hoelscher, G. L., Leslie, K., Ingram, J. A., and Hanley, E. N., Jr. (2006) Three-dimensional culture of human disc cells within agarose or a collagen sponge: assessment of proteoglycan production. *Biomaterials 27*, 371−376.
(114) Yu, F., Cao, X., Zeng, L., Zhang, Q., and Chen, X. (2013) An interpenetrating HA/G/CS biomimic hydrogel via Diels-Alder click chemistry for cartilage tissue engineering. *Carbohydr. Polym. 97*, 188−195.
(115) Coutinho, D. F., Sant, S. V., Shin, H., Oliveira, J. T., Gomes, M. E., Neves, N. M., Khademhosseini, A., and Reis, R. L. (2010) Modified gellan gum hydrogels with tunable physical and mechanical properties. *Biomaterials 31*, 7494−7502.
(116) Tang, Y., Sun, J., Fan, H., and Zhang, X. (2012) An improved complex gel of modified gellan gum and carboxymethyl chitosan for chondrocytes encapsulation. *Carbohydr. Polym. 88*, 46−53.
(117) Di Martino, A., Sittinger, M., and Risbud, M. V. (2005) Chitosan: a versatile biopolymer for orthopaedic tissue-engineering. *Biomaterials 26*, 5983−5990.
(118) Muzzarelli, R. A. A. (1988) Carboxymethylated chitins and chitosans. *Carbohydr. Polym. 8*, 1−21.
(119) Shin, H., Olsen, B. D., and Khademhosseini, A. (2012) The mechanical properties and cytotoxicity of cell-laden double-network hydrogels based on photocrosslinkable gelatin and gellan gum biomacromolecules. *Biomaterials 33*, 3143−3152.
(120) Shin, H., Olsen, B. D., and Khademhosseini, A. (2014) Gellan gum microgel-reinforced cell-laden gelatin hydrogels. *J. Mater. Chem. B 2*, 2508−2516.
(121) Cerqueira, M. T., da Silva, L. P., Santos, T. C., Pirraco, R. P., Correlo, V. M., Reis, R. L., and Marques, A. P. (2014) Gellan gum-hyaluronic acid spongy-like hydrogels and cells from adipose tissue synergize promoting neoskin vascularization. *ACS Appl. Mater. Interfaces 6*, 19668−19679.
(122) Yavvari, P. S., and Srivastava, A. (2015) Robust, self-healing hydrogels synthesized from catechol rich polymers. *J. Mater. Chem. B 3*, 899−910.
(123) Ryu, J. H., Lee, Y., Kong, W. H., Kim, T. G., Park, T. G., and Lee, H. (2011) Catechol-functionalized chitosan/pluronic hydrogels for tissue adhesives and hemostatic materials. *Biomacromolecules 12*, 2653−2659.
(124) Foster, E., You, J., Siltanen, C., Patel, D., Haque, A., Anderson, L., and Revzin, A. (2015) Heparin hydrogel sandwich cultures of primary hepatocytes. *Eur. Polym. J.*, DOI: 10.1016/j.eurpolymj.2014.12.033.
(125) Leach, J. B., Bivens, K. A., Collins, C. N., and Schmidt, C. E. (2004) Development of photocrosslinkable hyaluronic acid-polyethylene glycol-peptide composite hydrogels for soft tissue engineering. *J. Biomed. Mater. Res. 70A*, 74−82.
(126) Kim, S., Kim, S. W., and Bae, Y. H. (2005) Synthesis, bioactivity and specificity of glucagon-like peptide-1 (7−37)/polymer conjugate to isolated rat islets. *Biomaterials 26*, 3597−3606.
(127) Russell, R. G. G., Watts, N. B., Ebetino, F. H., and Rogers, M. J. (2008) Mechanism of action of bisphosphonates: similarities and differences and their potential influence on clinical efficacy. *Osteoporosis Int. 19*, 733−759.
(128) Hulsart-Billström, G., Yuen, P. K., Marsell, R., Hilborn, J., Larsson, S., and Ossipov, D. (2013) Bisphosphonate-linked hyaluronic acid hydrogel sequesters and enzymatically releases active bone morphogenetic protein-2 for induction of osteogenic differentiation. *Biomacromolecules 14*, 3055−3063.
(129) Wojtecki, R. J., Meador, M. A., and Rowan, S. J. (2011) Using the dynamic bond to access macroscopically responsive structurally dynamic polymers. *Nat. Mater. 10*, 14−27.
(130) Cheng, F., and Jäkle, F. (2011) Boron-containing polymers as versatile building blocks for functional nanostructured materials. *Polym. Chem. 2*, 2122−2132.
(131) Deng, C. C., Brooks, W. A., Abboud, K. A., and Sumerlin, B. S. (2015) Boronic acid-based hydrogels undergo self-healing at neutral and acidic pH. *ACS Macro Lett. 4*, 220−224.
(132) Sakloetsakun, D., Hombach, J. M. R., and Bernkop-Schnürch, A. (2009) In situ gelling properties of chitosan-thioglycolic acid conjugate in the presence of oxidizing agents. *Biomaterials 30*, 6151−6157.
(133) Zhang, H., Qadeer, A., and Chen, W. (2011) In situ gelable interpenetrating double network hydrogel formulated from binary components: thiolated chitosan and oxidized dextran. *Biomacromolecules 12*, 1428−1437.
(134) Thirupathi Kumara Raja, S., Thiruselvi, T., Aravindhan, R., Baran Mandal, A., and Gnanamani, A. (2015) *In vitro* and *in vivo* assessments of a 3-(3,4-dihydroxyphenyl)-2-propenoic acid bioconjugated gelatin-based injectable hydrogel for biomedical applications. *J. Mater. Chem. B 3*, 1230−1244.
(135) Koehler, K. C., Alge, D. L., Anseth, K. S., and Bowman, C. N. (2013) A Diels-Alder modulated approach to control and sustain the release of dexamethasone and induce osteogenic differentiation of human mesenchymal stem cells. *Biomaterials 34*, 4150−4158.
(136) Jin, R., Moreira Teixeira, L. S., Krouwels, A., Dijkstra, P. J., van Blitterswijk, C. A., Karperien, M., and Feijen, J. (2010) Synthesis and characterization of hyaluronic acid-poly(ethylene glycol) hydrogels via Michael addition: An injectable biomaterial for cartilage repair. *Acta Biomater. 6*, 1968−1977.
(137) Desai, R. M., Koshy, S. T., Hilderbrand, S. A., Mooney, D. J., and Joshi, N. S. (2015) Versatile click alginate hydrogels crosslinked via tetrazine-norbornene chemistry. *Biomaterials 50*, 30−37.
(138) Wu, A. T. H., Aoki, T., Sakoda, M., Ohta, S., Ichimura, S., Ito, T., Ushida, T., and Furukawa, K. S. (2015) Enhancing osteogenic differentiation of MC3T3-E1 cells by immobilizing inorganic polyphosphate onto hyaluronic acid hydrogel. *Biomacromolecules 16*, 166−173.
(139) Greiner, A., and Wendorff, J. H. (2007) Electrospinning: a fascinating method for the preparation of ultrathin fibers. *Angew. Chem., Int. Ed. 46*, 5670−5703.
(140) Zahedi, P., Rezaeian, I., Ranaei-Siadat, S. O., Jafari, S. H., and Supaphol, P. (2009) A review on wound dressings with an emphasis on electrospun nanofibrous polymeric bandages. *Polym. Adv. Technol. 21*, 77−95.
(141) Wu, L., Yuan, X., and Sheng, J. (2005) Immobilization of cellulase in nanofibrous PVA membranes by electrospinning. *J. Membr. Sci. 250*, 167−173.
(142) Buttafoco, L., Kolkman, N. G., Poot, A. A., Dijkstra, P. J., Vermes, I., and Feijen, J. (2005) Electrospinning collagen and elastin for tissue engineering small diameter blood vessels. *J. Controlled Release 101*, 322.
(143) Katti, D. S., Robinson, K. W., Ko, F. K., and Laurencin, C. T. (2004) Bioresorbable nanofiber-based systems for wound healing and drug delivery: Optimization of fabrication parameters. *J. Biomed. Mater. Res. 708*, 286−296.
(144) Ostrovidov, S., Shi, X., Zhang, L., Liang, X., Kim, S. B., Fujie, T., Ramalingam, M., Chen, M., Nakajima, K., Al-Hazmi, F., et al. (2014) Myotube formation on gelatin nanofibers-multi-walled carbon nanotubes hybrid scaffolds. *Biomaterials 35*, 6268−6277.
(145) Bellan, L. M., Pearsall, M., Cropek, D. M., and Langer, R. (2012) A 3D interconnected microchannel network formed in gelatin by sacrificial shellac microfibers. *Adv. Mater. 24*, 5187−5191.
(146) Lee, J., Yoo, J. J., Atala, A., and Lee, S. J. (2012) Controlled heparin conjugation on electrospun poly(ε-caprolactone)/gelatin

DOI: 10.1021/acs.bioconjchem.5b00360
Bioconjugate Chem. 2015, 26, 1984−2001

fibers for morphology-dependent protein delivery and enhanced cellular affinity. *Acta Biomater. 8*, 2549−2558.
(147) Teng, S. H., Wang, P., and Kim, H. E. (2009) Blend fibers of chitosan-agarose by electrospinning. *Mater. Lett. 63*, 2510−2512.
(148) Lösel, R., Grafahrend, D., Möller, M., and Klee, D. (2010) Bioresorbable electrospun fibers for immobilization of thiol-containing compounds. *Macromol. Biosci. 10*, 1177−1183.
(149) Wade, R. J., Bassin, E. J., Rodell, C. B., and Burdick, J. A. (2015) Protease-degradable electrospun fibrous hydrogels. *Nat. Commun. 6*, 6639.
(150) Selimović, Š., Oh, J., Bae, H., Dokmeci, M., and Khademhosseini, A. (2012) Microscale strategies for generating cell-encapsulating hydrogels. *Polymers 4*, 1554−1579.
(151) Wong, D. Y., Griffin, D. R., Reed, J., and Kasko, A. M. (2010) Photodegradable hydrogels to generate positive and negative features over multiple length scales. *Macromolecules 43*, 2824−2831.
(152) Chiu, Y. C., Larson, J. C., Perez-Luna, V. H., and Brey, E. M. (2009) Formation of microchannels in poly (ethylene glycol) hydrogels by selective degradation of patterned microstructures. *Chem. Mater. 21*, 1677−1682.
(153) Leslie-Barbick, J. E., Shen, C., Chen, C., and West, J. L. (2011) Micron-scale spatially patterned, covalently immobilized vascular endothelial growth factor on hydrogels accelerates endothelial tubulogenesis and increases cellular angiogenic responses. *Tissue Eng., Part A 17*, 221−229.
(154) Moon, J. J., Hahn, M. S., Nsiah, B. A., and West, J. L. (2009) Micropatterning of poly (ethylene glycol) diacrylate hydrogels with biomolecules to regulate and guide endothelial morphogenesis. *Tissue Eng., Part A 15*, 579−585.
(155) Lee, S. H., Moon, J. J., and West, J. L. (2008) Three-dimensional micropatterning of bioactive hydrogels via two-photon laser scanning photolithography for guided 3D cell migration. *Biomaterials 29*, 2962−2968.
(156) Au, A. Y., Hasenwinkel, J. M., and Frondoza, C. G. (2012) Micropatterned agarose scaffolds covalently modified with collagen for culture of normal and neoplastic hepatocytes. *J. Biomed. Mater. Res., Part A 100*, 342−352.
(157) Tanimizu, N., and Miyajima, A. (2007) Molecular mechanism of liver development and regeneration. *Int. Rev. Cytology 259*, 1−48.
(158) Mooney, D. J., Langer, R., and Ingber, D. E. (1995) Cytoskeletal filament assembly and the control of cell spreading and function by extracellular matrix. *J. Cell Sci. 108*, 2311−2320.
(159) Sadri-Ardekani, H., and Atala, A. (2015) Regenerative medicine for the treatment of reproductive system disorders: current and potential options. *Adv. Drug Delivery Rev. 82−83*, 145−152.
(160) Kolesky, D. B., Truby, R. L., Gladman, A. S., Busbee, T. A., Homan, K. A., and Lewis, J. A. (2014) 3D bioprinting of vascularized, heterogeneous cell-laden tissue constructs. *Adv. Mater. 26*, 3124−3130.
(161) Derby, B. (2012) Printing and prototyping of tissues and scaffolds. *Science 338*, 921−926.
(162) Gao, G., Yonezawa, T., Hubbell, K., Dai, G., and Cui, X. (2015) Inkjet-bioprinted acrylated peptides and PEG hydrogel with human mesenchymal stem cells promote robust bone and cartilage formation with minimal printhead clogging. *Biotechnol. J.*, DOI: 10.1002/biot.201400635.
(163) Levato, R., Visser, J., Planell, J. A., Engel, E., Malda, J., and Mateos-Timoneda, M. A. (2014) Biofabrication of tissue constructs by 3D bioprinting of cell-laden microcarries. *Biofabrication 6*, 1−12.
(164) Boere, K. W. M., Visser, J., Seyednejad, H., Rahimian, S., Gawlitta, D., van Steenbergen, M. J., Dhert, W. J. A., Hennink, W. E., Vermonden, T., and Malda, J. (2014) Covalent attachment of a three-dimensionally printed thermoplast to a gelatin hydrogel for mechanically enhanced cartilage constructs. *Acta Biomater. 10*, 2602−2611.
(165) Tabeling, P. (2010) *Introduction to Microfluidics*, Oxford University Press.
(166) Sackmann, E. K., Fulton, A. L., and Beebe, D. J. (2014) The present and future role of microfluidics in biomedical research. *Nature 507*, 181−189.
(167) Huh, D., Matthews, B. D., Mammoto, A., Montoya-Zavala, M., Hsin, H. Y., and Ingber, D. E. (2010) Reconstituting organ-level lung functions on a chip. *Science 328*, 1662−1668.
(168) Ahadian, S., Ramón-Azcón, J., Estili, M., Obregón, R., Shiku, H., and Matsue, T. (2014) Facile and rapid generation of 3D chemical gradients within hydrogels for high-throughput drug screening applications. *Biosens. Bioelectron. 59*, 166−173.
(169) Qasaimeh, M. A., Gervais, T., and Juncker, D. (2011) Microfluidic quadrupole and floating concentration gradient. *Nat. Commun. 2*, 464.
(170) Nichol, J. W., and Khademhosseini, A. (2009) Modular tissue engineering: engineering biological tissues from the bottom up. *Soft Matter 5*, 1312−1319.
(171) Hu, M., Kurisawa, M., Deng, R., Teo, C. M., Schumacher, A., Thong, Y. X., Schumacher, K. M., and Yimg, J. Y. (2009) Cell immobilization in gelatin-hydroxyphenylpropionic acid hydrogel fibers. *Biomaterials 30*, 3523−3531.
(172) Hu, M., Deng, R., Schumacher, K. M., Kurisawa, M., Ye, H., Purnamawati, K., and Ying, J. Y. (2010) Hydrodynamic spinning of hydrogel fibers. *Biomaterials 31*, 863−869.
(173) Al-Abboodi, A., Tjeung, R., Doran, P. M., Yeo, L. Y., Friend, J., and Chan, P. P. Y. (2014) In situ generation of tunable porosity gradients in hydrogel-based scaffolds for microfluidic cell culture. *Adv. Healthcare Mater. 3*, 1655−1670.
(174) Zamora-Mora, V., Velasco, D., Hernández, R., Mijangos, C., and Kumacheva, E. (2014) Chitosan/agarose hydrogels: Cooperative properties and microfluidic preparation. *Carbohydr. Polym. 111*, 348−355.
(175) Jun, Y., Kim, M. J., Hwang, Y. H., Jeon, E. A., Kang, A. R., Lee, S.-H., and Lee, D. Y. (2013) Microfluidics-generated pancreatic islet microfibers for enhanced immunoprotection. *Biomaterials 34*, 8122−8130.
(176) MacNeil, S. (2008) Biomaterials for tissue engineering of skin. *Mater. Today 11*, 26−35.
(177) Tsai, R. J. -F., Li, L.-M., and Chen, J.-K. (2000) Reconstruction of damaged corneas by transplantation of autologous limbal epithelial cells. *N. Engl. J. Med. 343*, 86−93.
(178) Atala, A., Bauer, S. B., Soker, S., Yoo, J. J., and Retik, A. B. (2006) Tissue-engineered autologous bladders for patients needing cystoplasty. *Lancet 367*, 1241−1246.
(179) Hollister, S. J. (2009) Scaffold design and manufacturing: From concept to clinic. *Adv. Mater. 21*, 3330−3342.
(180) Tang, Y., Du, Y., Li, Y., Wang, X., and Hu, X. (2009) A thermosensitive chitosan/poly(vinyl alcohol) hydrogel containing hydroxyapatite for protein delivery. *J. Biomed. Mater. Res., Part A 91A*, 953−963.
(181) Qi, B., Yu, A., Zhu, S., Zhou, M., and Wu, G. (2013) Chitosan/poly(vinyl alcohol) hydrogel combined with Ad-hTGF-β1 transfected mesenchymal stem cells to repair rabbit articular cartilage defects. *Exp. Biol. Med. 238*, 23−30.
(182) Madeira, C., Santhagunam, A., Salgueiro, J. B., and Cabral, J. M. S. (2015) Advanced cell therapies for articular cartilage regeneration. *Trends Biotechnol. 33*, 35−42.
(183) Spiller, K. L., Maher, S. A., and Lowman, A. M. (2011) Hydrogels for the repair of articular cartilage defects. *Tissue Eng., Part B 17*, 281−299.
(184) Wang, D. A., Varghese, S., Sharma, B., Strehin, I., Fermanian, S., Gorham, J., Fairbrother, D. H., Cascio, B., and Elisseeff, J. H. (2007) Multifunctional chondroitin sulphate for cartilage tissue-biomaterial integration. *Nat. Mater. 6*, 385−392.
(185) Sharma, B., Fermanian, S., Gibson, M., Unterman, S., Herzka, D. A., Cascio, B., Coburn, J., Hui, A. Y., Marcus, N., Gold, G. E., et al. (2013) Human cartilage repair with a photoreactive adhesive-hydrogel composite. *Sci. Transl. Med. 5*, 167ra6.
(186) Waris, E., Ashammakhi, N., Lehtimäki, M., Tulamo, R.-M., Törmälä, P., Kellomäki, M., and Konttinen, Y. T. (2008) Long-term bone tissue reaction to polyethylene oxide/polybutylene terephthalate copolymer (PolyActive®) in metacarpophalangeal joint reconstruction. *Biomaterials 29*, 2509−2515.

DOI: 10.1021/acs.bioconjchem.5b00360
Bioconjugate Chem. 2015, 26, 1984−2001

(187) Yang, J.-A., Yeom, J., Hwang, B. W., Hoffman, A. S., and Hahn, S. K. (2014) In situ-forming injectable hydrogels for regenerative medicine. *Prog. Polym. Sci. 39*, 1973−1986.
(188) Mather, B. D., Viswanathan, K., Miller, K. M., and Long, T. E. (2006) Michael addition reactions in macromolecular design for emerging technologies. *Prog. Polym. Sci. 31*, 487−531.
(189) Han, J., Zhang, J., Yin, R., Ma, G., Yang, D., and Nie, J. (2011) Electrospinning of methoxy poly(ethylene glycol)-grafted chitosan and poly(ethylene oxide) blend aqueous solution. *Carbohydr. Polym. 83*, 270−276.
(190) Shah, S. S., Kim, M., Cahill-Thompson, K., Tae, G., and Revzin, A. (2011) Micropatterning of bioactive heparin-based hydrogels. *Soft Matter 7*, 3133−3140.
(191) Hahn, S. K., Oh, E. J., Miyamoto, H., and Shimobouji, T. (2006) Sustained release formulation of erythropoietin using hyaluronic acid hydrogels crosslinked by Michael addition. *Int. J. Pharm. 322*, 44−51.
(192) Shu, X. Z., Liu, Y., Palumbo, F., and Prestwich, G. D. (2003) Disulfide-crosslinked hyaluronan-gelatin hydrogel films: a covalent mimic of the extracellular matrix for in vitro cell growth. *Biomaterials 24*, 3825−3834.
(193) Rostovtsev, V. V., Green, L. G., Fokin, V. V., and Sharpless, K. B. (2002) A stepwise Huisgen cycloaddition process: Copper(I)-catalyzed regioselective "ligation" of azides and terminal alkynes. *Angew. Chem., Int. Ed. 41*, 2596−2599.
(194) Nimmo, C. M., and Shoichet, M. S. (2011) Regenerative biomaterials that "Click": simple, aqueous-based protocols for hydrogel synthesis, surface immobilization, and 3D patterning. *Bioconjugate Chem. 22*, 2199−2209.
(195) Ossipov, D. A., and Hilborn, J. (2006) Poly(vinyl alcohol)-based hydrogels formed by "click chemistry. *Macromolecules 39*, 1709−1718.
(196) Liu, S. Q., Ee, P. L. R., Ke, C. Y., Hedrick, J. L., and Yang, Y. Y. (2009) Biodegradable poly(ethylene glycol)-peptide hydrogels with well-defined structure and properties for cell delivery. *Biomaterials 30*, 1453−1461.
(197) Van Dijk, M., van Nostrum, C. F., Hennink, W. E., Rijkers, D. T. S., and Liskamp, R. M. J. (2010) Synthesis and characterization of enzymatically biodegradable PEG and peptide-based hydrogels prepared by click chemistry. *Biomacromolecules 11*, 1608−1614.
(198) Wang, T., and Guo, Z. J. (2006) Copper in medicine: homeostasis, chelation therapy and antitumor drug design. *Curr. Med. Chem. 13*, 525−537.
(199) Baskin, J. M., Prescher, J. A., Laughlin, S. T., Agard, N. J., Chang, P. V., Miller, I. A., Lo, A., Codelli, J. A., and Bertozzi, C. R. (2007) Copper-free click chemistry for dynamic in vivo imaging. *Proc. Natl. Acad. Sci. U. S. A. 104*, 16793−16797.
(200) Li, Y., Liu, C., Tan, Y., Xu, K., Lu, C., and Wang, P. (2014) In situ hydrogel constructed by starch-based nanoparticles via a Schiff base reaction. *Carbohydr. Polym. 110*, 87−94.
(201) Saito, H., Hoffman, A. S., and Ogawa, H. I. (2007) Delivery of doxorubicin from biodegradable PEG hydrogels having Schiff base linkages. *J. Bioact. Compat. Polym. 22*, 589−601.
(202) Lü, S., Gao, C., Xu, X., Bai, X., Duan, H., Gao, N., Feng, C., Xiong, Y., and Liu, M. (2015) Injectable and self-healing carbohydrate-based hydrogel for cell encapsulation. *ACS Appl. Mater. Interfaces 7*, 13029−13037.
(203) Jin, R., Hiemstra, C., Zhong, Z., and Feijen, J. (2007) Enzyme-mediated fast in situ formation of hydrogels from dextran-tyramine conjugates. *Biomaterials 28*, 2791−2800.
(204) Park, K. M., Ko, K. S., Joung, Y. K., Shin, H., and Park, K. D. (2011) In situ cross-linkable gelatin-poly(ethylene glycol)-tyramine hydrogel via enzyme-mediated reaction for tissue regenerative medicine. *J. Mater. Chem. 21*, 13180−13187.
(205) Hu, J., Zhang, G., and Liu, S. (2012) Enzyme-responsive polymeric assemblies, nanoparticles and hydrogels. *Chem. Soc. Rev. 41*, 5933−5949.
(206) Dvir, T., Timko, B. P., Kohane, D. S., and Langer, R. (2011) Nanotechnological strategies for engineering complex tissues. *Nat. Nanotechnol. 6*, 13−22.
(207) Rice, J. J., Martino, M. M., De Laporte, L., Tortelli, F., Briquez, P. S., and Hubbell, J. A. (2013) Engineering the regenerative microenvironment with biomaterials. *Adv. Healthcare Mater. 2*, 57−71.
(208) Burdick, J. A., and Murphy, W. L. (2012) Moving from static to dynamic complexity in hydrogel design. *Nat. Commun. 3*, 1269.
(209) Lim, H. L., Hwang, Y., Kar, M., and Varghese, S. (2014) Smart hydrogels as functional biomimetic systems. *Biomater. Sci. 2*, 603−618.
(210) Murphy, W. L., Dillmore, W. S., Modica, J., and Mrksich, M. (2007) Dynamic hydrogels: Translating a protein conformational change into macroscopic motion. *Angew. Chem., Int. Ed. 46*, 3066−3069.
(211) Custódio, C. A., Reis, R. L., and Mano, J. F. (2014) Engineering biomolecular microenvironments for cell instructive biomaterials. *Adv. Healthcare Mater. 3*, 797−810.
(212) Obregón, R., Ramón-Azcón, J., Ahadian, S., Shiku, H., Bae, H., Ramalingam, M., and Matsue, T. (2014) The use of microtechnology and nanotechnology in fabricating vascularized tissues. *J. Nanosci. Nanotechnol. 14*, 487−500.
(213) Bertassoni, L. E., Cardoso, J. C., Manoharan, V., Cristino, A. L., Bhise, N. S., Araujo, W. A., Zorlutuna, P., Vrana, N. E., Ghaemmaghami, A. M., Dokmeci, M. R., and Khademhosseini, A. (2014) Direct-write bioprinting of cell-laden methacrylated gelatin hydrogels. *Biofabrication 6*, 024105.
(214) Hosseini, V., Kollmannsberger, P., Ahadian, S., Ostrovidov, S., Kaji, H., Vogel, V., and Khademhosseini, A. (2014) Fiber-assisted molding (FAM) of surfaces with tunable curvature to guide cell alignment and complex tissue architecture. *Small 10*, 4851−4857.

DOI: 10.1021/acs.bioconjchem.5b00360
Bioconjugate Chem. 2015, 26, 1984−2001