Published in final edited form as:
Expert Rev Med Devices. 2011 September ; 8(5): 607–626. doi:10.1586/erd.11.27.

Design properties of hydrogel tissue-engineering scaffolds

Junmin Zhu[1,†] and **Roger E Marchant**[1]

[1]Department of Biomedical Engineering, Case Western Reserve University, 10900 Euclid Avenue, Cleveland, OH 44106, USA

Abstract

This article summarizes the recent progress in the design and synthesis of hydrogels as tissue-engineering scaffolds. Hydrogels are attractive scaffolding materials owing to their highly swollen network structure, ability to encapsulate cells and bioactive molecules, and efficient mass transfer. Various polymers, including natural, synthetic and natural/synthetic hybrid polymers, have been used to make hydrogels via chemical or physical crosslinking. Recently, bioactive synthetic hydrogels have emerged as promising scaffolds because they can provide molecularly tailored biofunctions and adjustable mechanical properties, as well as an extracellular matrix-like microenvironment for cell growth and tissue formation. This article addresses various strategies that have been explored to design synthetic hydrogels with extracellular matrix-mimetic bioactive properties, such as cell adhesion, proteolytic degradation and growth factor-binding.

Keywords

bioactive scaffold; extracellular matrix; hydrogel; polymer; tissue engineering

Hydrogels are water-swollen polymeric networks, usually consisting of crosslinked hydrophilic polymers that can swell but do not dissolve in water. This ability to swell under biological conditions makes them an ideal class of materials for biomedical applications, such as drug delivery and tissue engineering [1–14]. Hydrogels possess a 3D network structure, crosslinked together either physically or chemically. This insoluble cross-linked structure allows effective immobilization and release of active agents and biomolecules. Owing to their high water content, hydrogels resemble natural soft tissue more than any other type of polymeric biomaterials. Hydrogel materials generally exhibit good biocompatibility and high permeability for oxygen, nutrients and other water-soluble metabolites, making them attractive scaffolds for use in cell encapsulation [6–17]. Most hydrogel materials are injectable [18,19] and can be formed via photopolymerization [20,21], which can be carried out under mild conditions in the presence of living cells. This allows homogeneous seeding of cells throughout the scaffold materials and formation of hydrogels *in situ*.

[†]Author for correspondence: Tel.: +1 216 368 0270, Fax: +1 216 368 4969, junmin.zhu@case.edu.

Financial & competing interests disclosure

This work was supported in part by the National Institutes of Health (Grants 1RC1EB010795 and 1R01HL087843). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Hydrogels can be classified into physical and chemical hydrogels based on their cross-linking mechanism [3,12]. Physical crosslinks include entangled chains, hydrogen bonding, hydrophobic interaction and crystallite formation. While these physical crosslinks may not be permanent junctions, they are sufficient to keep the hydrogel from dissolving in an aqueous media. Chemical (or covalent) crosslinks, on the other hand, are permanent junctions formed by covalent bonds. One common way to create a covalently crosslinked network is to polymerize end-functionalized macromers [7,11,21]. Hydrogel networks may include both permanent junctions and semipermanent junctions like chain entanglements. The type and degree of crosslinking influences many of the network properties, like swelling properties, elastic modulus and transport of molecules [22]. Hydrogels can further be classified by their ionic charge (neutral, cationic, anionic and ampholytic), structure (amorphous, semicrystalline and hydrogen-bond) and preparation methods (homopolymer, copolymer, multipolymer and interpenetrating polymer network) [12,22].

The control of the hydrogel network structure allows for the proper design and characterization of the degradation of hydrogel scaffolds, diffusion of bioactive molecules and migration of cells through the network [12,22]. Four important swelling parameters have been used to define the network structure of hydrogels, including:

- The swelling ratio (Q), including the mass swelling ratio (Q_m) and the volume swelling ratio (Q_v)
- The polymer volume fraction in the swollen state ($\upsilon_{2,s}$)
- The number average molecular weight between cross-links (M_c)
- The network mesh size (ξ) (Figure 1).

They can be defined by the following equations [23,24]:

$$Q_m=(W_g - W_p)/W_p \quad \text{(Equation 1)}$$

$$Q_v=V_g/V_p=(Q_m+1)\rho_2/\rho_1 \quad \text{(Equation 2)}$$

$$\upsilon_{2,s}=V_p/V_g=Q_v^{-1} \quad \text{(Equation 3)}$$

$$M_c=M_0/2X \quad \text{(Equation 4)}$$

$$\xi=\upsilon_{2,s}^{-\frac{1}{3}}\left(\gamma_0^2\right)^{\frac{1}{2}}=Q_v^{\frac{1}{3}}\left(\gamma_0^2\right)^{\frac{1}{2}} \quad \text{(Equation 5)}$$

where W_g is the weight of the equilibrium swollen gel, W_p is the weight of the polymer, V_p is the volume of the polymer, V_g is the volume of the equilibrium swollen gel, ρ_1 is the solvent density, ρ_2 is the M_0 is the polymer density, molecular weight of the polymer repeating unit, X is the degree of crosslinking, and $\left(\gamma_0^2\right)^{\frac{1}{2}}$ is the root-mean-square end-to-end distance of network chains between two adjacent crosslinks in the equilibrium state. Q and

$\upsilon_{2,s}$ can be measured from swelling experiments (Equations 1–3), while M_c and ξ can be calculated by the equilibrium swelling or rubber elasticity theories (Equations 4–5) [25–27].

Hydrogels have been used as an important class of tissue-engineering scaffolds because they can provide a soft tissue-like environment for cell growth and allow diffusion of nutrients and cellular waste through the elastic hydrogel network. They have advantages over other types of polymeric scaffolds, such as easy control of structural parameters (e.g., Q, $\upsilon_{2,s}$, M_c, ξ), high water content, promising biocompatibility and adjustable scaffold architecture. This article summarizes the recent progress in the design and synthesis of hydrogel scaffolds for tissue engineering. It begins with an overview of the properties of polymers used for designing and fabricating hydrogel scaffolds, and then briefly describes the use of the natural extracellular matrix (ECM) as a design model for engineering bioactive hydrogels, followed by highlighting three types of ECM-mimetic hydrogels, including cell-adhesive, enzyme-sensitive and growth factor (GF)-bearing hydrogels. Finally, five-year perspective and some key issues are provided regarding the applications of hydrogel tissue-engineering scaffolds, and the challenges in the design and synthesis of bioactive or biomimetic hydrogels.

Polymers used for fabricating hydrogel scaffolds

Hydrogel networks can be created by natural, synthetic or their hybrid polymers. Based on the polymer origin, hydrogels can be classified into three major types: natural, synthetic and synthetic/natural hybrid hydrogels. This section describes the properties of polymers that have been used for designing and fabricating hydrogel scaffolds.

Natural polymers

Natural polymers have been used to make natural hydrogels as scaffolds for tissue engineering owing to their biocompatibility, inherent biodegradability and critical biological functions. There are four major types of natural polymers (Table 1), including:

- Proteins [28–39], such as collagen, gelatin, fibrin, silk, lysozyme, Matrigel™, and genetically engineered proteins [40–49], such as calmodulin (a calcium-binding protein), elastin-like polypeptides and leucine zipper;
- Polysaccharides [50–55], such as hyaluronic acid (HA), agarose, dextran and chitosan;
- Protein/polysaccharide hybrid polymers [56–63], such as collagen/HA, laminin/cellulose, gelatin/chitosan and fibrin/alginate;
- DNA [64–68].

However, the use of natural hydrogels is often restricted because of concerns regarding potential immunogenic reactions and relatively poor mechanical properties [7,15–17].

Various proteins have been used to make natural-hydrogel tissue-engineering scaffolds. Among them, collagen, the most abundant protein in mammals, is a representative natural polymer to fabricate natural hydrogels. Collagen can be degraded naturally by metallo-matrix proteinases (MMPs) – specifically, collagenase – allowing for local degradation controlled by cells present in the engineered tissue. Gelatin is a derivative of collagen, formed by breaking the natural triple-helix structure of collagen into single-strand molecules by hydrolysis. Gelatin is less immunogenic compared with its precursor and presumably retains informational signals like the Arg–Gly–Asp (RGD) sequence, thus promoting cell adhesion, migration, differentiation and proliferation [18,29]. Matrigel is a gelatinous protein mixture secreted by Engelbreth–Holm–Swarm (EHS) mouse sarcoma cells, mainly

consisting of laminin, collagen type IV, enlactin and various GFs [37]. This mixture resembles the complex extracellular environment found in many tissues, and has been used widely as scaffolds for cell differentiation, tissue vascularization and angiogenesis [38,39]. Protein-based hydrogels can be formed by thermal gelation and their mechanical properties can be enhanced using chemical crosslinkers such as glutaraldehyde.

Polysaccharides are another major type of natural polymer used to make hydrogels for tissue engineering. The ECM component hyaluronic acid (HA) possesses a non-sulfated glycosaminoglycan (GAG) structure and is widely distributed throughout the ECM of all connective tissues. HA plays an essential role in many biological processes such as tissue hydration, nutrient diffusion, proteoglycan organization and cell differentiation. Polysaccharides can be modified with the attachment of various functional groups such as acrylate, thiol and amine for hydrogel formation [50,51]. A variety of polysaccharides like HA, heparin, chitosan, dextran and alginate have been explored as hydrogels for tissue engineering owing to their good biocompatibility, biodegradability, as well as excellent gel-forming properties [51–55]. Polysaccharide hydrogels can be formed by covalent crosslinking, chemical conjugation, esterification and polymerization. In addition, polysaccharides have been combined with proteins such as collagen, gelatin, laminin and fibrin to form an interpenetrating network or composite hydrogels [56–64].

DNA is a polynucleotide with deoxyribose sugars joined at both the 3′-hydroxyl and 5′-hydroxyl groups to phosphate groups through ester links. Two polynucleotide chains, held together by weak intermolecular forces, form a DNA molecule. DNA has received considerable attention as a promising building material for fabricating hydrogels owing to its ability to form predictable hydrogel networks through self-assembly, electrostatic interaction, chemical crosslinking or enzymatic ligation [65,66]. The distinct difference between DNA hydrogels and other natural hydrogels is that the crosslinking of DNA can be realized very efficiently using self-assembly or ligase-mediated reactions carried out under physiological conditions. Fine-tuning of these hydrogels is easily accomplished by adjusting the initial concentrations and types of DNA monomers. DNA molecules can be designed and synthesized with multiple arms and complementary sticky ends. These branched DNA monomers include X-, Y- and T-shaped DNA [67–69], which can be tailored to form DNA hydrogel networks for specific biomedical applications, such as 3D cell culture, cell transplant therapy, controlled drug delivery and cell-free protein production. DNA hydrogels are biodegradable, and their biodegradability is dependent on the branched structure and concentration of DNA molecules, loaded drugs and the environment (e.g., in the absence or presence of nucleases) [65].

Synthetic polymers

Compared with natural polymers, synthetic polymers possess more reproducible physical and chemical properties, which is critical for the fabrication of tissue-engineering scaffolds. Currently, synthetic polymers have emerged as an important alternative choice for fabricating hydrogel tissue-engineering scaffolds because they can be molecularly tailored with block structures, molecular weights, mechanical strength and biodegradability [7–17]. Synthetic polymers used for preparing synthetic hydrogels can be classified into three major types, including nonbiodegradable [70–81], biodegradable [82–106] and bioactive polymers [107–119].

Nonbiodegradable synthetic polymers—For nonbiodegradable applications in tissue engineering, it is essential for the hydrogels to maintain physical and mechanical integrity. Mechanical stability of the gel is an important consideration when designing a scaffold. The strength of hydrogels can be increased by incorporating crosslinking agents, comonomers,

and increasing the degree of crosslinking [21–23]. There is an optimal degree of crosslinking since a higher degree of crosslinking also leads to brittleness and less elasticity. Elasticity of the gel is important to give flexibility to the crosslinked chains, and to facilitate the movement or diffusion of the incorporated bioactive agents. Thus, a compromise between mechanical strength and flexibility is necessary for the appropriate use of the nonbiodegradable hydrogels as tissue-engineering scaffolds.

Nonbiodegradable synthetic hydrogels can be prepared from the copolymerization of various vinylated monomers or macromers [70–78], such as 2-hydroxyethyl methacrylate (HEMA), 2-hydroxypropyl methacrylate (HPMA), acrylamide (AAm), acrylic acid (AAc), *N*-isopropylacrylamide (NIPAm), and methoxyl poly(ethylene glycol) (PEG) monoacrylate (mPEGMA or PEGMA), with crosslinkers, such as *N*,*N*′-methylenebis(acrylamide) (MBA), ethylene glycol diacrylate (EGDA) and PEG diacrylate (PEGDA), as shown in Figure 2. Another method to form nonbiodegradable hydrogels is to use nonbiodegradable polymers [79–81], such as self-assembly of Pluronic® polymers with a structure of poly(ethylene oxide) (PEO)-poly(propylene oxide) (PPO)-PEO, chemical cross-linking of modified poly(vinyl alcohol) (PVA), and radiation cross-linking of linear or branched PEG. Nonbiodegradable hydrogels have been used for engineering bone and cartilage [76,78], but are limited in engineering vascular constructs or other soft tissues owing to their nonbiodegradability.

Poly(*N*-isopropylacrylamide) (PNIPAm) has been investigated extensively as a thermo-sensitive polymer, which can form thermosensitive hydrogels from free radical copolymerizing of NIPAm with crosslinkers like MBA [73,74]. PNIPAm hydrogels swell in water at temperatures less than the lower critical solution temperature (~32°C). The formation of hydrogen bonds between water molecules and the amide groups of PNIPAm plays a dominant role in the intermolecular association. However, when the temperature is higher than the lower critical solution temperature, hydrophobic interaction between the isopropyl groups of PNIPAm side chains plays a more dominant role, which results in phase separation and deswelling of the hydrogels. Pluronic is another polymer that can form thermoreversible hydrogels [75]. This unique property of temperature-responsive swelling/deswelling can be used to detach cell layers for engineering special tissues like cornea or cell sheets [73,79].

PEG is the most widely investigated polymer used to make hydrogels due to its unique properties, such as solubility in water and in organic solvents, nontoxicity, low protein adhesion and nonimmunogenicity [76–78]. Furthermore, the end hydroxyl groups of PEG molecules can be easily modified with various functional groups, such as carboxyl, thiol and acrylate, or attached to other molecules or bioactive agents [7]. PEG-based hydrogels can be prepared by radiation crosslinking of PEG or free radical polymerization of PEG macromers. PVA is another synthetic hydrophilic polymer that has been explored as hydrogels for tissue-engineering applications [80,81]. PVA hydrogels can be formed by physically crosslinking through repeated freezing/thawing methods, or chemically crosslinked with glutaraldehyde or epichlorohydrin. PVA can also be modified with acryloyl chloride or glycidyl methacrylate to generate reactive acrylate groups through the pendant hydroxyl groups, followed by crosslinking polymerization to form hydrogels. In addition, PVA can blend with other water-soluble polymers to form hydrogels.

Biodegradable synthetic polymers—Biodegradability is one of the most important considerations of scaffolds for tissue engineering. It is highly desirable to ensure that the biodegradation rate coincides with new tissue regeneration at the defect site [2,6,8]. Many polymers created in nature are biodegradable, such as proteins, cellulose, starch and chitin, but they are limited in making hydrogel scaffolds with tailored biodegradability and

mechanical properties. Synthetic biodegradable polymers have been extensively studied throughout the last decades. Polyesters are the most widely used biodegradable polymer for scaffold fabrication, including poly(lactic acid) (PLA), poly(glycolic acid) (PGA), poly(ε-caprolactone) (PCL) and their copolymers [6,17]. They can be used to modify hydrophilic polymers like PEG to form acrylated macromers or amphiphilic polymers for fabricating biodegradable hydrogels via chemical or physical crosslinking [82–93]. For example, as shown in Figure 3, triblock copolymers, PLA–PEG–PLA and PEG–PLA–PEG have been synthesized and end capped with acrylate groups to generate PLA-modified PEG diacrylates [82,84]. These polyester-containing macromers can be photopolymerized to form hydrolytically degradable hydrogels. In addition, some crosslinkers containing functional groups, such as acetal, ketal, disulfide and poly(propylene fumarate) (PPF), have been used to make biodegradable PEG hydrogels [94–96].

Michael addition has been used to form PEG hydrogels with enhanced biodegradation. For example, PEGDA or multi-arm PEG-acrylated macromers can react with thiol-containing molecules like dithiothreitol or cysteine-containing peptides via Michael addition to create a hydrogel network with a thioether bond proximal to the acrylate ester bond [86,87]. The presence of the thioether bond establishes a positive charge on the carbonyl carbon of the acrylate ester group, thereby enhancing its reactivity toward nucleophilic hydroxyl anions in the primary step of ester hydrolysis. Another strategy to make biodegradable PEG hydrogels is to incorporate disulfide linkage into PEGDA structure to generate disulfide-containing PEG diacrylate, PEG(SS) DA (Figure 3) [89]. The disulfide linkages can be cleaved reductively by thiol-containing molecules, such as cysteine and glutathione. Thus, the resulting hydrogels can be degraded by cysteine- containing peptides or proteins, which offers a convenient pathway to control the scaffold biodegradability.

Synthetic self-assembling peptides have attracted much attention for use in peptide hydrogels because of their excellent bio-compatibility and biodegradability, adaptable structure that allows for specific interaction, and nanofibrous network formation that mimics the natural ECM fibrillar structure [97–99]. There are two major types of these peptides, including self-complementary peptides (SCPs) and peptide amphiphiles (PAs). SCPs, such as Arg–Ala–Asp (RAD)-16, consist of short oligomers of alternating hydrophilic and hydrophobic amino acid residues that trigger self-assembly into well-ordered nanofibers and then further into hydrogel scaffolds upon exposure to physiological pH and ionic strength [97]. There are two distinctive sides for SCPs: one hydrophobic and the other hydrophilic. The hydrophobic side forms a double sheet inside of a fiber. The hydrophilic side is ionic self-complementary owing to the presence of both positive and negative side chains on one side of the β-sheet, which forms the outside of the nanofibers that interact with water molecules, forming an extremely high water content hydrogel. PAs are a class of molecules that combine the structural features of amphiphilic surfactants with the peptides as the hydrophilic block and alkyl or fluorenylmethyloxycarbonyl (Fmoc) groups as the hydrophobic block [100,101]. The most important design element of PAs is the amphiphilic nature of the molecules. The amphiphilicity that results from the incorporation of the hydrophobic alkyl or Fmoc group allows self-assembly of PAs into nanofibers, followed by entangling to form a hydrogel network. Research results have shown that self-assembling peptide nanofibrous hydrogels have the capacity to form stable hydrogels for encapsulating cells for tissue engineering [102–106].

Bioactive synthetic polymers—The major limitation of the above synthetic hydrogels as tissue-engineering scaffolds is lack of cell-specific bioactivities, such as cell adhesion, migration and cell-mediated biodegradation. To overcome this limitation, bioactive molecules have been incorporated into synthetic hydrogels to mediate specific cell functions [2,3,26,27]. The principle is to attach those bioactive elements (e.g., peptides and GFs) to

the hydrogel network during or after hydrogel formation [7,11], as shown in Figure 4. A variety of ECM component-derived peptides or bioactive molecules have been used to modify synthetic polymers for fabricating bioactive hydrogels, including cell-adhesive [107,108], enzyme-sensitive [104,105], GF-binding [110,111] and other bioactive hydrogels [112–119], such as matrix protein-binding, immune-isolating and nitric oxide (NO)-bearing. Their physical properties (e.g., network parameters, mechanical strength and diffusive profile) and bioactivities (e.g., cell adhesion, migration and scaffold biodegradation) can be tailored by molecular design. Compared with natural hydrogels, bioactive synthetic hydrogels offer an improved control of the matrix architecture and chemical composition, and also provide a biomimetic environment for cell growth and tissue formation.

Synthetic/natural hybrid polymers

Synthetic polymers can be easily synthesized on a large scale and manipulated at a molecular level by polymerization, crosslinking and functionalization; however, most synthetic hydrogels alone usually only function as passive scaffolds for cells and do not foster active cellular interactions [17,18]. As mentioned previously, natural polymers like proteins exhibit distinct tertiary structures, and regulate active cellular response, biological recognition and cell-triggered remodeling. Thus, the combination of the characteristics of synthetic and natural polymers to make hybrid hydrogels has become a direct approach to create bioactive hydrogel scaffolds for tissue engineering. These hybrid hydrogel polymers include:

- PEG-modified natural polymers [120–126], such as heparin, dextran, HA, fibrinogen and albumin;
- PNIPAm-modified natural polymers, such as collagen, chitosan and alginate [127–131];
- Synthetic peptide-modified proteins or polysaccharides [132–136];
- PVA and other synthetic polymer (e.g., Pluronic)-modified natural polymers [137–140].

Compared with using bioactive synthetic polymers, this method is advantageous in creating bioactive hydrogels without complicated synthesis for bioconjugation; however, it still has concerns in immunogenic reactions and infection when using animal-derived natural polymers.

Synthetic/natural hybrid hydrogels can be made by covalent bonding of synthetic and natural polymer blocks via chemical conjugation or polymerization. The synthetic block provides tunable physical properties, while the natural block provides specific biological functions. Many naturally occurring biopolymers, such as collagen, fibrinogen, hyaluronic acid, chitosan and heparin, have been used to make hybrid hydrogels with synthetic polymers, such as PEG, PNIPAm and PVA [121,128,138]. The hybridization can occur at a molecular level depending on the size and nature of building blocks. This hybrid method considerably expands the design and application of hydrogels, which offers the flexibility in engineering hydrogel scaffolds with desirable molecular architectures, chemical compositions and mechanical properties. Much research has been carried out to maintain the structure and function of natural polymers upon chemical modification in order to design well-integrated hybrid materials with structurally and biologically active components.

Design & synthesis of ECM-mimetic hydrogel scaffolds

The extracellular matrix (ECM) is a complex network structure that surrounds and supports cells. It is filled with ECM molecules like proteins and proteoglycans, which are secreted by

the cells (Figure 5). Cell receptors bind both soluble and tethered signaling cues from the ECM environment, while simultaneously, cells send out signals to actively construct and degrade their microenvironment for remodeling. Thus, the ECM acts not only as a mechanical scaffold for the cells, but also a bioactive and dynamic environment that mediates cellular functions [141,142]. It is highly desirable to synthesize scaffolds to mimic the structure and biofunctions of the natural ECM [143–145]. To date, numerous bioactive peptide sequences derived from ECM proteins such as fibronectin, laminin and collagen, have been incorporated into synthetic hydrogels. To tether ECM-derived biomolecules to the hydrogel networks, reactive groups, such as acrylate, amine, thiol, azide, maleimide and biotin/strepavidin, have been used to functionalize peptides and polymers for hydrogel formation. Bioactive molecules, such as cell-adhesive peptides (CAPs), enzyme-sensitive peptides (ESPs), GFs and other specially functionalized molecules have been used to modify synthetic hydrogels to mimic one or more ECM biofunctions, such as cell adhesion [146–181], proteolytic degradation [182–208], GF-binding [209–214], matrix protein-binding [112–114], immune-isolating [115–117] and nitric oxide (NO)-binding [118,119]. This section mainly focuses on the design and fabrication of three major type bioactive hydrogels with ECM-mimetic properties, including cell-specific adhesion, enzyme-sensitive biodegradation and GF binding.

Cell-adhesive hydrogels

Cell attachment to the ECM is an obvious prerequisite for a number of important cell-function processes, such as cell proliferation and cell migration [141,142]. The ECM provides cell-adhesive domains for binding cell surface receptors, such as integrins, selectins, CD44 and syndecan. These interactions between cell-binding domains and cell receptors play central roles in the tissue development, organization and maintenance, by providing anchorage and triggering signals that direct cell function, cell-cycle progression and expression of differentiated phenotypes. To mimic these specific cell/matrix interactions, a variety of ECM protein-derived CAPs have been used for cell-adhesive modification of synthetic hydrogels (Figure 6A). Unlike the entire protein structure, which is subject to denaturation and degradation, short peptide sequences have the advantage of being relatively stable for modification, tunable for cell binding, and are easy to synthesize on a large scale. However, this approach assumes that the selected short peptide sequence retains its biological functional specificity when isolated from its native protein structure. A good example is the RGD sequence, which retains its integrin-binding specificity even though there is some decrease in affinity relative to the native ECM protein such as fibronectin. On the other hand, if the sequence is part of an ordered secondary structure (e.g., β-sheet and α-helix) in the native protein, it is unlikely that specificity will be retained.

The general method for fabricating cell-adhesive hydrogels is to chemically conjugate CAPs on the hydrogel network or copolymerize CAP-modified monomers with other macromers. Bioadhesive peptides are mainly derived from six ECM proteins, including fibronectin [146–164], vitronectin [165], bone sialoprotein [165], laminin [166–175], collagen [176–178] and elastin [179–181]. The most commonly used CAP for cell-adhesive modification is RGD, which is derived from the integrin-binding domain of fibronectin, laminin and collagen. Other peptide sequences include fibronectin-derived KQAGDV, REDV and PHSRN, laminin-derived YIGSR, LGTIPG, IKVAV, PDGSR, LRE, LRGDN and IKLLI, collagen-derived DGEA and GFOGER, and elastin-derived VAPG (Table 2).

Various reactive groups, such as amine, carboxyl, thiol, azide and vinyl, have been used to functionalize peptides for incorporation into hydrogels [7,11,13]. Among them, acrylation is the most widely applied method to modify the peptide N-terminus to generate peptide monoacrylate [107], such as RGD-monoacrylate (RGD-MA) and RGD-PEG monoacrylate (RGD-PEGMA) with a PEG spacer (Figure 6B). Both RGD-MA and RGD-PEGMA can

copolymerize with PEGDA or other macromers to create cell-adhesive hydrogels. For example, human umbilical vein endothelial cells (HUVECs) possessed a rounded morphology with no evidence of spreading 4 h after seeding on PEGDA hydrogels (Figure 7A), and had a decreased cell density after 24 h (Figure 7B), suggesting that HUVECs have only weak, nonspecific interactions with this material. However, the PEGDA hydrogels with incorporation of 1% (w/v) of RGD-PEGMA showed higher initial cell attachment and some cell spreading 4 h after seeding of HUVECs (Figure 7C) and extensive spreading after 24 h (Figure 7D). The enhanced attachment and spreading on RGD-modified PEGDA hydrogels were attributed to the specific binding of HUVECs to the RGD ligands present on the hydrogel surface.

To control the peptide spatial organization, RGD peptides can be attached in the middle of the PEGDA chain to create RGD-PEGDA (Figure 6B) [147]. RGD-PEGDA has a similar structure to PEGDA with two acrylate groups on both ends, which has the advantage to be incorporated into hydrogels with higher peptide density without significantly affecting the scaffold mechanical properties, compared with RGD-PEGMA. In addition, the RGD sequence in the cell-binding domain of fibronectin is exposed at the tip of a random coil loop with a spatial constraint that results in increased affinity for cell binding. To enhance the cell binding, a PEG macromer with cyclic RGD (cRGD) attached in the middle of PEG chain, cRGD-PEGDA, has been synthesized [148]. Results show that the incorporation of cRGD peptides into the PEGDA hydrogels can better mimic the native RGD loop structure and benefits the cell-binding affinity in the cell-specific adhesion.

Enzyme-sensitive hydrogels

Desirable tissue formation requires the cells to express signals to control the biodegradation of synthetic scaffolds like the natural remodeling of the ECM [2,6–8,14]. If the biodegradation is more rapid than the tissue regeneration, the scaffolds will lose their carrier function for cell growth; on the other hand, if the biodegradation is too slow compared with tissue regeneration, the scaffolds will impede tissue regeneration. As well-known, the proteolytic degradation of the natural ECM is an essential feature of a variety of biological processes, such as cell migration, tissue repair and remolding [108,109]. Most ECM proteins, such as collagen [182–184], laminin [185–187] and fibrin [188–190], have specific cleavage sites for degradation by enzymes, such as matrix metalloproteinases (MMPs), plasmin and elastase (Table 3). Among them, MMPs play a crucial role in defining the cellular environment through regulated degradation and processing of ECM proteins [182,183]. The incorporation of polyester segments (e.g., PLA and PGA) into synthetic hydrogels has been used to enhance the scaffold biodegradation, but this hydrolytic degradation process is not responsive to cellular signals or cell-secreted enzymes. The best way to impart biodegradability is to exploit the proteolytic degradation mechanisms presented in the ECM with the incorporation of ESP sequences.

Various ESPs have been used to prepare enzyme-sensitive synthetic hydrogels [182–208], as listed in Table 3. To incorporate ESPs into hydrogels, two major methods have been explored, including:

- Free radical polymerization of ESP diacrylates, such as ESP-PEGDA macromers prepared from acrylation of ESP diamine (containing two amine groups on both peptide ends) with a PEG spacer (Figure 8A) [197–201];
- Michael addition of ESP dithiol (containing two cysteine residues on both peptide ends) with multi-arm PEG vinyl sulfone or acrylate (Figure 8B) [189,190,193,194].

Peptides like collagen-derived GPQGIAGQ and peptide library-derived GPQGIWGQ, APGL and LGPA have been used to make MMP-sensitive hydrogels [184,191–194,197–

200], while fibrin-derived YKNRD and VRN have been used to make plasmin-sensitive hydrogels [189,190]. Elastase-sensitive peptides (e.g., AAAAAAA, AAPV and AAPVRGGG) [201–204] and chymotrypsin-sensitive peptides (e.g., GGYRG) [205] have been used for proteolytic modification of PEG hydrogels. Short peptide sequences, such as GL, GFL and GFGL, have also been functionalized with dimethacrylate for crosslinking HEMA or HEMA/PEGMA to make papain-sensitive hydrogels [206]. In addition, ESP trithiols (with three cysteine residues) like GCYKNRGCYKNRCG have been developed to make plasmin-sensitive hydrogels by Michael addition with 4-arm PEG acrylate or sulfone [188]. Compared with ESP dithiols, this kind of design of trifunctional crosslinking peptides has the advantage of preventing nonfunctional dangling ends during Michael addition and enhance the number of elastically active crosslinks in the hydrogel networks.

The enzyme-sensitive designs can also be used to modulate cell adhesion to synthetic hydrogels. The incorporation of enzyme-cleavable CAPs is expected to mimic the natural ECM that provides temporary cues for the regulation of cellular responses and tissue development. PENFF is one of the major peptide sequences at the MMP-13 cleavage site of aggrecan, a cartilage ECM component [207]. A cysteine-containing bifunctional peptide, CPENFFRGD has been incorporated into PEG hydrogels by thiol–acrylate photopolymerization [208]. This peptide has the sequence of PENFF for MMP-13-sensitive cleavage and the RGD motif for cell adhesion. The resulting hydrogels provide a platform that mimics the native upregulation and downregulation of cell-adhesive proteins by the cell-secreted enzymes in the ECM to mediate cell differentiation.

GF-bearing hydrogels

Growth factors are a class of proteins or polypeptides that play a key role in modulating cell functions, such as differentiation, migration, proliferation and gene expression [209,210]. The dosage response of GFs like VEGF is highly sensitive for tissue formation [211,212]. ECM components like proteins and glycans have functional domains for binding GFs and modulating their release [213,214]. To mimic the function of the ECM as the reservoir of GFs, researchers have incorporated GFs into hydrogels during or after the hydrogel fabrication by covalent and noncovalent means. Specifically, there are four major strategies for incorporating GFs into synthetic hydrogels, including direct loading [215–218], carrier-encapsulating [219–226], covalent bonding [227–233] and reverse binding [234–243], as shown in Figure 9.

Direct loading—Hydrogels have unique characteristics, such as the ability to act as carriers for controlling the release of bioactive molecules and as scaffolds for encapsulating cells [2–12]. It is highly desirable to combine tissue engineering with controlled drug delivery in the same system to regulate cell response and tissue formation. Both nonbiodegradable and biodegradable hydrogels have been used for encapsulating GFs for controlled release owing to their highly swollen crosslinked network structure [215–218]. The easiest way is to load GFs into hydrogels directly during hydrogel formation (Figure 9A). Various models have been developed to predict the release of active agents from hydrogels as a function of time. The release rate-limiting step is dependent on different mechanisms, including diffusion-, swelling- and chemically-controlled release. Diffusion is the most widely applicable mechanism to describe drug release from hydrogels [23]. Swelling-controlled release occurs when diffusion of drug is faster than hydrogel swelling. Chemically-controlled release is determined by reactions occurring within a hydrogel scaffolds. The most common reactions are the cleavage of polymer chains via hydrolytic or enzymatic degradation. The direct loading of GFs into hydrogels typically shows a rapid burst release during the initial phase, since the rate of protein release is generally diffusion-controlled through aqueous channels within the hydrogels [109,110]. Thus, it is a great

challenge for the direct loading method to control the GF release over a long time without burst release.

Carrier encapsulating—To retard the release of GFs from hydrogels and achieve a sustained release over extended periods, GF-loaded carrier systems, such as microparticles and nanoparticles, have been incorporated into hydrogels [109,212], as shown in Figure 9B. A variety of polymers have been used to fabricate micro-/nano-particles as carrier systems for encapsulating and releasing GFs, including synthetic polymers [212,219,220], such as PVA, PEO-PPO-PEO, PLA, PLGA and PCL, and natural polymers [110,221–226], such as gelatin, alginate, chitosan and dextran sulfate. Compared with the direct loading method, the strategy of using delivery systems has several advantages, such as protecting GFs from inactivation occurring in biological environments, and supplying adequate local GF concentration in the form of temporal and spatial gradients. Sustained release from encapsulated carrier systems in hydrogels can provide an optimal level of GFs over extended periods, which is required for the formation of stable tissues. However, the carrier encapsulating method may still have the initial burst release, and the use of hydrophobic polyesters may result in the denaturation of GFs.

Covalent bonding—As an alternative to the previously described two methods, GFs can also be covalently attached to the hydrogel network (Figure 9C). For example, recombinant VEGF has been engineered with cysteine for tethering to PEG networks by Michael-type addition with multi-arm PEG vinyl sulfone [227–229]. In addition, GFs, such as bFGF, EGF and TGFβ can also be acrylated with acryloyl-PEG-carboxy succinimidyl ester (Acr-PEG-NHS) and copolymerize with PEG macromers to generate GF-tethered PEG hydrogels [230–232]. Results show the covalently tethered GFs maintaining mitogenic activity, and enhancing fibroblast proliferation and migration. In addition, GF-derived peptides like bone morphogenetic protein (BMP)-derived KIPKASSVPTELSAISTLYL have been incorporated into PEG hydrogels by Click chemistry [233] in order to enhance the osteogenic differentiation of bone marrow stromal cells. The covalent bonding method is effective in eliminating the burst release of GFs; however, this method needs the chemical functionalization of GFs, which may result in structural damage to the GFs.

Reverse binding—Naturally, GFs associate with the ECM components, especially glycosaminoglycans (GAGs) such as heparin, chondroitin sulfate and HA [213,214]. This association is important to stabilize the GF's active conformation and protect it from immediate clearance. Those GAGs play an important role in modulating the stability, activity, release and spatial localization of GFs. To mimic the GF binding mechanism of GAGs in the natural ECM, a variety of methods have been developed to chemically functionalize heparin, chondroitin sulfate and HA for making GAG-bearing hydrogels by thiol-acrylate or thiol-maleimide Michael addition, specific binding, amine-carboxyl conjugation and copolymerization [234–243]. Heparin is a linear, unbranched, highly sulfated GAG, and it has been used to mediate a wide range of biological activities such as cell adhesion, cell mobility, cell proliferation and tissue morphogenesis via binding to various cell regulatory proteins [234,235]. The polysaccharide backbone of heparin has hydroxyl and carboxyl groups, which are versatile for chemical modification and bioconjugation. The carboxyl groups on heparin can react directly with the amine groups on multi-arm PEG or its derivatives, and the hydroxyl groups can be acrylated to form heparin macromers for copolymerization with other macromers [234–242]. Another method to make GF-binding hydrogels is to develop affinity hydrogels [242,243], for example, using biotin-containing PEG hydrogels for specific interaction with strepavidin-modified GFs like bFGF, or making hydrogels with incorporated GF-binding peptide, KRTGQYK, for binding of bFGF [243].

The development of GF-associating or binding hydrogels has emerged as an important strategy to mimic the ECM biofunction to deliver GFs (Figure 9D). This method has the advantage of maintaining the biological bioactivity of GFs upon release and overcoming the potential damage to GFs that may result from the covalent bonding method. However, it needs to attach GF-binding components like GAGs to the hydrogel network, and it still remains a challenge in controlling the loading and release of GFs since these processes are dependent on the affinity of GFs with GAGs.

Expert commentary

Hydrogels are promising scaffolds for tissue-engineering applications due to their high swollen 3D structure, ability to encapsulate cells and bioactive molecules, efficient mass transfer, and easily manipulated physical properties. Highly hydrated hydrogels provide ideally cellular microenvironments for cell proliferation and differentiation. Natural polymers have frequently been used to make hydrogel scaffolds for tissue-engineering applications owing to their biocompatibility, inherent biodegradability and critical biological functions. Compared with natural polymers, synthetic polymers possess more reproducible chemical and physical properties, which is critical for the fabrication of tissue-engineering scaffolds. Bioactive synthetic hydrogels have emerged as promising hydrogel scaffolds because they can be molecularly tailored with block structures, molecular weights, mechanical strength and biodegradability, and also they can mimic the natural ECM to provide a desirable cellular environment for supporting cell growth.

To develop suitable hydrogel scaffolds, the biodegradation rate and mechanical strength of hydrogels must match the tissue growth and the new ECM production. In general, these properties can be fine-tuned through variations in the chemical structure, crosslinking density and peptide incorporation in hydrogels. For a given hydrogel system, activities of seeded cells can be regulated by attaching specific bioactive moieties to the hydrogel network. The attachment of ECM-derived peptides to synthetic polymers has emerged as an important strategy for fabricating bioactive hydrogels. Much effort has been devoted to the control of ligand density and spatial distribution in synthetic hydrogels to modulate specific cellular responses for tissue formation. A number of cell lines, including fibroblasts, chondrocytes, vascular smooth muscle cells and endothelial cells, osteoblasts, neural cells and stem cells have been immobilized on bioactive hydrogels to provide fundamental knowledge of cell/scaffold interactions.

Five-year view

Cells and bioactive molecules can be readily integrated into the soft tissue-like hydrogel scaffolds. Although many efforts have been made to improve hydrogels for the development of functional engineered tissues, the future success in engineering of large tissues or organs is highly dependent on the design of bioactive hydrogel scaffolds with controlled physical, chemical and biological properties. A desirable bioactive hydrogel scaffold property is to mimic the structural and biological properties of the natural ECM found in tissue. Current bioactive synthetic polymers are still limited in mimicking multiple biofunctions of the ECM. An important future work is to mimic the ECM as closely as possible, in order to design synthetic hydrogels that will form an ideal microenvironment to support cell growth and tissue regeneration. There is a continuing need to develop novel strategies to control the incorporation and release of cellular biofactors like GFs so that specific signals can be delivered in an appropriate spatial and temporal manner.

References

1. Kopecek J. Hydrogel biomaterials: a smart future? Biomaterials. 2007; 28(34):5185–5192. [PubMed: 17697712]
2. Lutolf MP. Biomaterials: Spotlight on hydrogels. Nat Mater. 2009; 8(6):451–453. [PubMed: 19458644]
3. Chung HK, Park TG. Self-assembled and nanostructured hydrogels for drug delivery and tissue engineering. Nano Today. 2009; 4(5):429–437.
4. Oh JK. Engineering of nanometer-sized cross-linked hydrogels for biomedical applications. Can J Chem. 2010; 88(3):173–184.
5. Ulijn RV, Bibi N, Jayawarna V, et al. Bioresponsive hydrogels. Mater Today. 2007; 10(4):40–48.
6. Lee J, Cuddihy MJ, Kotov NA. Three-dimensional cell culture matrices: state of the art. Tissue Eng Part B. 2008; 14(1):61–86.
7. Zhu J. Bioactive modification of poly(ethylene glycol) hydrogels for tissue engineering. Biomaterials. 2010; 31(17):4639–4656. [PubMed: 20303169]
8. Geckil H, Xu F, Zhang XH, Moon S, Demirici U. Engineering hydrogels as extracellular matrix mimics. Nanomedicine. 2010; 5(3):469–484. [PubMed: 20394538]
9. Hunt NC, Grover LM. Cell encapsulation using biopolymer gels for regenerative medicine. Biotechnol Lett. 2010; 32(6):733–742. [PubMed: 20155383]
10. Drury JL, Mooney DJ. Hydrogels for tissue engineering: scaffold design variables and applications. Biomaterials. 2003; 24(24):4337–4351. [PubMed: 12922147]
11. Liu SQ, Tay R, Khan M, Ee PLR, Hedrick JL, Yang YY. Synthetic hydrogels for controlled stem cell differentiation. Soft Matter. 2010; 6(1):67–81.
12. Slaughter BV, Khurshid SS, Fisher OZ, Khademhosseini A, Peppas NA. Hydrogels in regenerative medicine. Adv Mater. 2009; 21(32–33):3307–3329. [PubMed: 20882499]
13. Nicodemus GD, Bryant SJ. Cell encapsulation in biodegradable hydrogels for tissue engineering applications. Tissue Eng Part B Rev. 2008; 14(2):149–165. [PubMed: 18498217]
14. Cushing MC, Anseth KS. Hydrogel cell culture. Science. 2007; 316(5828):1133–1134. [PubMed: 17525324]
15. Nuttelman CR, Rice MA, Rydholm AE, Salinas CN, Shah DN, Anseth KS. Macromolecular monomers for the synthesis of hydrogel niches and their application in cell encapsulation and tissue engineering. Prog Polym Sci. 2008; 33(2):167–170. [PubMed: 19461945]
16. Brandl F, Sommer F, Goepferich A. Rational design of hydrogels for tissue engineering: Impact of physical factors on cell behavior. Biomaterials. 2007; 28(2):134–146. [PubMed: 17011028]
17. Varghese S, Elisseeff JH. Hydrogels for musculoskeletal tissue engineering. Adv Polym Sci. 2006; 203:95–144.
18. Tan H, Marra KG. Injectable, biodegradable hydrogels for tissue engineering applications. Materials. 2010; 3:1746–1767.
19. Kretlow JD, Klouda L, Mikos AG. Injectable matrices and scaffolds for drug delivery in tissue engineering. Adv Drug Deliver Rev. 2007; 59 (4–5):263–273.
20. Ifkovits JL, Burdick JA. Review: photopolymerizable and degradable biomaterials for tissue engineering applications. Tissue Eng. 2007; 13(10):2369–2385. [PubMed: 17658993]
21. Nguyen TK, West JL. Photopolymerizable hydrogels for tissue engineering applications. Biomaterials. 2002; 23(22):4307–4314. [PubMed: 12219820]
22. Hoffman AS. Hydrogels for biomedical applications. Adv Drug Deliver Rev. 2002; 43(1):3–12.
23. Lin CC, Metters AT. Hydrogels in controlled release formulations: network design and mathematical modeling. Adv Drug Deliver Rev. 2006; 58(12–13):1379–1408.
24. Peppas NA, Huang Y, Torres-Lugo M, Ward JH, Zhang J. Physicochemical foundations and structural design of hydrogels in medicine and biology. Annu Rev Biomed Eng. 2000; 2:9–29. [PubMed: 11701505]
25. Peppas NA, Hilt JZ, Khademhosseini A, Langer R. Hydrogels in biology and medicine: From Molecular principles to bionanotechnology. Adv Mater. 2006; 18(11):1345–1360.

26. Deiber JA, Ottone ML, Piaggio MV, Peirotti MB. Characterization of cross-linked polyampholytic gelatin hydrogels through the rubber elasticity and thermodynamic theories. Polymer. 2009; 50(25):6065–6075.
27. Freudenberg U, Herman A, Welzel PB, et al. A star-PEG-heaprin hydrogel platform to aid cell replacement therapies for neurodegeneration diseases. Biomaterials. 2009; 30(28):5049–5060. [PubMed: 19560816]
28. Glowacki J, Mizuno S. Collagen scaffolds for tissue engineering. Biopolymers. 2007; 89(5):338–344. [PubMed: 17941007]
29. Sakai S, Hirose K, Taguchi K, Ogushi Y, Kawakami K. An injectable, *in situ* enzymatically gellable, gelatin derivative for drug delivery and tissue engineering. Biomaterials. 2009; 30(20): 3371–3377. [PubMed: 19345991]
30. Kimelman-Bleich N, Pelled G, Sheyn D, et al. The use of a synthetic oxygen carrier-enriched hydrogel to enhance mesenchymal stem cell-based bone formation *in vivo*. Biomaterials. 2009; 30(27):4639–4648. [PubMed: 19540585]
31. Wang Y, Kim HJ, Vunjak-Novakovic G, Kaplan DL. Stem cell-based tissue engineering with silk biomaterials. Biomaterials. 2006; 27(36):6064–6082. [PubMed: 16890988]
32. Daamen WF, Veerkamp JH, van Hest JCM, van Kuppevelt TH. Elastin as a biomaterial for tissue engineering. Biomaterials. 2007; 28(30):4378–4398. [PubMed: 17631957]
33. Mol A, van Lieshout MI, Dam-de Veen CG, et al. Fibrin as a cell carrier in cardiovascular tissue engineering applications. Biomaterials. 2005; 26(16):3113–3121. [PubMed: 15603806]
34. Osathanon T, Linnes ML, Rajachar RM, Ratner BD, Somerman MJ, Giachelli CM. Microporous nanofibrous fibrin-based scaffolds for bone tissue engineering. Biomaterials. 2008; 29(30):4091–4099. [PubMed: 18640716]
35. Yan H, Saiani A, Gough JE, Miller AF. Thermoreversible protein hydrogel as cell scaffold. Biomacromolecules. 2006; 7(10):2776–2782. [PubMed: 17025352]
36. Yan H, Frielinghaus H, Nykanen A, Ruokolainen J, Saiani A, Miller AF. Thermoreversible lysozyme hydrogels: properties and an insight into the gelation pathway. Soft Matter. 2008; 4(6): 1313–1325.
37. Kleinman HK, Martin GR. Matrigel: basement membrane matrix with biological activity. Semin Cancer Biol. 2005; 15(5):378–386. [PubMed: 15975825]
38. Morritt AN, Bortolotto SK, Dilley RJ, et al. Cardiac tissue engineering in an *in vivo* vascularized chamber. Circulation. 2007; 115(3):353–360. [PubMed: 17200440]
39. Ponce ML. Tube formation: an *in vitro* matrigel angiogenesis assay. Methods Mol Biol. 2009; 467:183–188. [PubMed: 19301671]
40. Ehrick JD, Deo SK, Browning TW, Bachas LG, Madou MJ, Daunert S. Genetically engineered protein in hydrogels tailors stimuli-responsive characteristic. Nat Mater. 2005; 4(4):298–302. [PubMed: 15765106]
41. Sengupta D, Heilshorn S. Protein-engineered biomaterials: Highly tunable tissue engineering scaffolds. Tissue Eng Part B. 2010; 16(3):285–293.
42. Wong Po Foo CTS, Lee JS, Mulyasasmita W, Parisi-Amon A, Heilshorn SC. Two-component protein-engineered physical hydrogels for cell encapsulation. Proc Natl Acad Sci USA. 2009; 105(52):22067–22072. [PubMed: 20007785]
43. MacEwan SR, Chilkoti A. Elastin-like polypeptides: biomedical applications of tunable biopolymers. Pept Sci. 2010; 94 (1):60–77.
44. Romano NH, Sengupta D, Chung C, Heilshorn SC. Protein-engineering biomaterials: nanoscale mimcs of the extracellular matrix. Bioichim Biophy Acta. 2011; 1810(3):339–349.
45. Dinerman AA, Cappello J, Ghandehari H, Hoag SW. Swelling behavior of a genetically engineered silk-elastin like protein polymer hydrogel. Biomaterials. 2002; 23(21):4203–4210. [PubMed: 12194523]
46. Greish K, Araki K, Li D, et al. Silk-elastin like protein polymer hydrogels for localized adenoviral gene therapy of head and neck tumors. Biomacromolecules. 2009; 10(10):2183–2188. [PubMed: 19722557]

47. Davis NE, Ding S, Forster RE, Pinkas DM, Barron AE. Modular enzymatically crosslinked protein polymer hydrogels for *in situ* gelation. Biomaterials. 2010; 31(28):7288–7297. [PubMed: 20609472]
48. Banta S, Wheeldon IR, Blenner M. Protein engineering in the development of functional hydrogels. Annu Rev Biomed Eng. 2010; 12:167–186. [PubMed: 20420519]
49. Kaufmann D, Fiedler A, Junger A, Auernheimer J, Kessler H, Weberskirch R. Chemical conjugation of linear and cyclic RGD moieties to a recombinant elastin-mimetic polypeptide – a versatile approach towards bioactive protein hydrogels. Macromol Biosci. 2008; 8(6):577–588. [PubMed: 18350537]
50. Leach JB, Bivens KA, Patrick CW, Schmidt CE. Photocrosslinked hyaluronic acid hydrogels: Natural, biodegradable tissue engineering scaffolds. Biotechnol Bioeng. 2003; 82(5):578–589. [PubMed: 12652481]
51. Ramamurthi A, Vesely I. Ultraviolet light-induced modification of crosslinked hyaluronan gels. J Biomed Mater Res A. 2003; 66(2):317–329. [PubMed: 12889002]
52. Denizli BK, Can HK, Rzaev ZMO, Guner A. Preparation conditions and swelling equilibria of dextran hydrogels prepared by some crosslinked agents. Polymer. 2004; 45(19):6431–6435.
53. Kuo CK, Ma PX. Ionically crosslinked alginate hydrogels as scaffolds for tissue engineering: part I. Structure, gelation rate and mechanical properties. Biomaterials. 2001; 22(6):511–521. [PubMed: 11219714]
54. Kim IY, Seo SJ, Moon HS, et al. Chitosan and its derivatives for tissue engineering applications. Biotechnol Adv. 2008; 26(1):1–21. [PubMed: 17884325]
55. Liang Y, Liu W, Han B, et al. An *in situ* formed biodegradable hydrogel for reconstruction of the corneal endothelium. Coll Surf B. 2011; 82(1):1–7.
56. Davidenko N, Campbell JJ, Thian ES, Watson CJ, Cameron RE. Collagen-hyaluronic acid scaffolds for adipose tissue engineering. Acta Biomater. 2010; 6(10):3957–3968. [PubMed: 20466086]
57. Stabenfeldt SE, Garcia AJ, LaPlaca MC. Thermoreversible laminin-functionalized hydrogel for neural tissue engineering. J Biomed Mater Res A. 2006; 77(4):718–725. [PubMed: 16555267]
58. Shikanov A, Xu M, Woodruff TK, Shea LD. Interpenetrating fibrin-alginate matrices for in vitro ovarian follicle development. Biomaterials. 2009; 30(29):5476–5485. [PubMed: 19616843]
59. Sakai S, Hashimoto I, Kawakami K. Synthesis of an agarose-gelatin conjugate for use as a tissue engineering scaffold. J Biosci Bioeng. 2007; 103(10):22–26. [PubMed: 17298896]
60. Huang Y, Onyeri S, Siewe M, Moshfeghian A, Madihally SV. *In vitro* characterization of chitosan–gelatin scaffolds for tissue engineering. Biomaterials. 2005; 26(36):7616–7627. [PubMed: 16005510]
61. Dhandayuthapani B, Krishnan UM, Sethuraman S. Fabrication and characterization of chitosan-gelatin blend nanofiber for skin tissue engineering. J Biomed Mater Res B Appl Biomater. 2010; 94(1):264–272. [PubMed: 20524203]
62. Tan H, Wu J, Lao L, Gao C. Gelatin/chitosan/hyaluronan scaffold intergrated with PLGA microspheres for cartilage tissue engineering. Acta Biomater. 2009; 5(1):328–337. [PubMed: 18723417]
63. Rosellini E, Cristallini C, Barbani N, Vozzi G, Giusti P. Preparation and characterization of alginate/gelatin blend films for cardiac tissue engineering. J Biomed Mater Res A. 2009; 91(2): 447–453. [PubMed: 18985761]
64. Liu Y, Chan-Park MB. Hydrogel based on interpenetrating polymer networks of dextran and gelatin for vascular tissue engineering. Biomaterials. 2009; 30(2):196–207. [PubMed: 18922573]
65. Um SH, Lee JB, Park N, Kwon SY, Umbach CC, Luo D. Enzyme-catalysed assembly of DNA hydrogel. Nat Mater. 2006; 5(10):797–801. [PubMed: 16998469]
66. Xing Y, Cheng E, Yang Y, et al. Self-assembled DNA hydrogels with designable thermal and enzymatic responsiveness. Adv Mater. 2011; 23(9):1117–1121. [PubMed: 21181766]
67. Lee CK, Shin SR, Lee SH, et al. DNA hydrogel fiber with self-entanglement prepared by using an ionic liquid. Angew Chem Int Ed. 2008; 47(13):2470–2474.
68. Park N, Kahn J, Rice EJ, et al. High-yield cell-free protein production from P-gel. Nat Proto. 2009; 4 (12):1759–1770.

69. Park N, Um SH, Funabashi H, Xu J, Luo D. A cell-free protein-production gel. Nat Mater. 2009; 8(5):432–437. [PubMed: 19329993]
70. Schneider GB, English A, Abraham M, Zaharias R, Stanford C, Keller J. The effect of hydrogel charge density on cell attachment. Biomaterials. 2004; 25(15):3023–3028. [PubMed: 14967535]
71. Hejcl A, Sedy J, Kapcalova M, et al. HPMA-RGD hydrogels seeded with mesenchymal stem cells improve functional outcome in chronic spinal cord injury. Stem Cells Delvelop. 2010; 19(10): 1535–1546.
72. Woerly S, Pinet E, de Robertis P, Van Diep D, Bousmina M. Spinal cord repair with PHPMA hydrogel containing RGD peptide (NeuroGel™). Biomaterials. 2001; 22(10):1095–1111. [PubMed: 11352090]
73. Takezawa T, Mori Y, Yoshizato K. Cell culture on a thermo-responsive polymer surface. Nat Biotechnol. 1990; 8(9):854–856.
74. Vihola H, Laukkanen A, Valtola L, Tenhu H, Hirvonen J. Cytotoxicity of thermosensitive polymers poly(*N*-isopropylacrylamide), poly(*N*-vinylcaprolactam) and amphiphilically modified poly(*N*-vinylcaprolactam). Biomaterials. 2005; 26(16):3055–3064. [PubMed: 15603800]
75. Park KH. Improved long-term culture of hepatocytes in a hydrogel containing Arg-Gly-Asp (RGD). Biotechnol Lett. 2002; 24(14):1131–1135.
76. Buxton AN, Zhu J, Marchant RE, West JL, Yoo JU, Johnstone B. Design and characterization of poly(ethylene glycol) photopolymerizable semi-interpenetrating networks for chondrogenesis of human mesenchymal stem cells. Tissue Eng. 2007; 13(10):2549–2560. [PubMed: 17655489]
77. Beamish JA, Zhu J, Kottke-Marchant K, Marchant RE. The effects of monoacrylate poly(ethylene glycol) on the properties of poly(ethylene glycol) diacrylate hydrogels used for tissue engineering. J Biomed Mater Res A. 2010; 92(2):441–450. [PubMed: 19191313]
78. Yang F, Williams CG, Wang D, Lee H, Manson PN, Elisseeff J. The effect of incorporating RGD adhesive peptide in polyethylene glycol diacrylate hydrogel on osteogenesis of bone marrow stromal cells. Biomaterials. 2005; 26(30):5991–5998. [PubMed: 15878198]
79. Higuchi A, Aoki N, Yamamoto T, et al. Temperature-induced cell deattachment on immobilized pluronic surface. J Biomed Mater Res A. 2006; 79(2):380–392. [PubMed: 16883586]
80. Schmedlen RH, Masters KS, West JL. Photocrosslinked polyvinyl alcohol hydrogels that can be modified with cell adhesion peptides for use in tissue engineering. Biomaterials. 2002; 23(22): 4325–4332. [PubMed: 12219822]
81. Ossipov DA, Brannvall K, Forsberg-Nilsson K, Hilborn J. Formation of the first injectable poly(vinyl alcohol) hydrogel by mixing of functional PVA precursors. J Appl Polym Sci. 2007; 106(1):60–70.
82. Sawhney AS, Pathak CP, Hubbell JA. Bioerodible hydrogels based on photopolymerized poly(ethylene glycol)-co-poly(α-hydroxy acid) diacrylate macromers. Macromolecules. 1993; 26(4):581–587.
83. Jiang Z, Hao J, You Y, Liu Y, Wang Z, Deng X. Biodegradable and thermosensitive hydrogels of poly(ethylene glycol)-poly(ε-caprolactone-co-glycolide)-poly(ethylene glycol) aqueous solutions. J Biomed Mater Res A. 2008; 87(1):45–51. [PubMed: 18080306]
84. Clapper JD, Skeie JM, Mullins RF, Guymon A. Development and characterization of photopolymerizable biodegradable materials from PEG–PLA–PEG block macromonomers. Polymer. 2007; 48(22):6554–6564.
85. Sanabria-DeLong N, Agrawal SK, Bhatia SR, Tew GN. Impact of synthetic technique on PLA–PEO–PLA physical hydrogel properties. Macromolecules. 2007; 40(22):7864–7873.
86. Hudalla GA, Eng TS, Murphy WL. An approach to modulate degradation and mesenchymal stem cell behavior in poly(ethylene glycol) networks. Biomacromolecules. 2008; 9(3):842–849. [PubMed: 18288800]
87. Rydholm AE, Anseth KS, Bowman CN. Effects of neighboring sulfides and pH on ester hydrolysis in thiol-acrylate photopolymers. Acta Biomater. 2007; 3(4):449–455. [PubMed: 17276150]
88. Zhang J, Skardal A, Prestwich GD. Engineered extracellular matrices with cleavable crosslinkers for cell expansion and easy cell recovery. Biomaterials. 2008; 29(34):4521–4531. [PubMed: 18768219]

89. Deshmukh M, Singh Y, Gunaseelan S, Gao D, Stein S, Sinko PJ. Biodegradable poly(ethylene glycol) hydrogels based on a self-elimination degradation mechanism. Biomaterials. 2010; 31(26): 6675–6684. [PubMed: 20561680]

90. Zustiak SP, Leach JB. Hydrolytically degradable poly(ethylene glycol) hydrogel scaffolds with tunable degradation and mechanical properties. Biomacromolecules. 2010; 11(5):1348–1357. [PubMed: 20355705]

91. Li Q, Wang J, Shahani S, et al. Biodegradable and photocrosslinkable poly(phophoester hydrogel. Biomaterials. 2006; 27(7):1027–1034. [PubMed: 16125222]

92. Haihara S, Matsumura S, Fisher JP. Synthesis and characterization of cyclic acetal based degradable hydrogels. Eur J Pharm Biopharm. 2008; 68(1):67–73. [PubMed: 17888640]

93. Malkoch M, Vestberg R, Gupta N, et al. Synthesis of well-defined hydrogel networks using Click chemistry. Chem Commun. 2006; 26:2774–2776.

94. Jo S, Engel PS, Mikos AG. Synthesis of poly(ethylene glycol)-tethered poly(propylene fumarate) and its modification with GRGD peptide. Polymer. 2000; 41(21):7595–7604.

95. He X, Ma J, Jabbari E. Effect of grafting RGD and BMP-2 protein-derived peptides to a hydrogel substrate on osteogenic diffierentiation of marrow stromal cells. Langmuir. 2008; 24(21):12508–12516. [PubMed: 18837524]

96. Atzet S, Curtin S, Trinh P, Bryant S, Ratner B. Degradation poly(2-hydroxyl methacrylate)-co-polycaprolactone hydrogels for tissue engineering scaffolds. Biomacromolecules. 2008; 9(12): 3370–3377. [PubMed: 19061434]

97. Hauser CAE, Zhang S. Designer self-assembling peptides nanofiber biological materials. Chem Soc Rev. 2010; 39:2780–2790. [PubMed: 20520907]

98. Zhao X, Zhang S. Designer self-assembling peptide materials. Macromol Biosci. 2007; 7(1):13–22. [PubMed: 17225214]

99. Stupp SI. Self-assembly and biomaterials. Nano Lett. 2010; 10(12):4783–4786.

100. Capito RM, Azevedo HS, Velichko YS, Mata A, Stupp SI. Self-assembly of large and small molecules into hierarchically ordered sacs and membranes. Science. 2008; 319(5871):1812–1816. [PubMed: 18369143]

101. Ulijin RV, Smith AM. Designing peptide based nanomaterials. Chem Soc Rev. 2008; 37:664–675. [PubMed: 18362975]

102. Jayawarna V, Richardson SM, Hirst AR, et al. Introducing chemical functionality into Fmoc-peptide gels for cell culture. Acta Biomater. 2009; 5(3):934–943. [PubMed: 19249724]

103. Galler KM, Aulisa L, Regan KR, D'Souza RN, Hartgerink JD. Self-assembling multidomain peptide hydrogels: designed susceptibility to enzymatic cleavage allows enhanced cell migration and spreading. J Am Chem Soc. 2010; 132(9):3217–3223. [PubMed: 20158218]

104. Kopesky PW, Vanderploeg EJ, Sandy JS, Kurz B, Grodzinsky AJ. Self-assembling peptide hydrogels modulate *in vitro* chondrogenesis of bovine bone marrow stromal cells. Tissue Eng A. 2010; 16(2):465–477.

105. Anderson JM, Andukuri A, Lim DJ, Jun HW. Modulating the gelation properties of self-assembling peptide amphiphiles. ACS Nano. 2009; 3(10):3447–3454. [PubMed: 19791757]

106. Liu J, Song H, Zhang L, Xu H, Zhao X. Self-assembly-peptide hydrogels as tissue engineering scaffolds for three-dimensional culture of chondrocytes *in vitro*. Macromol Biosci. 2010; 10(10): 1164–1170. [PubMed: 20552605]

107. Hern DL, Hubbell JA. Incorporation of adhesion peptides into nonadhesive hydrogels useful for tissue resurfacing. J Biomed Mater Res. 1998; 39(2):266–276. [PubMed: 9457557]

108. Shin H, Jo S, Mikos AG. Biomimetic materials for tissue engineering. Biomaterials. 2003; 24(24):4353–4364. [PubMed: 12922148]

109. Lutolf MP, Hubbell JA. Synthetic biomaterials as instructive extracellular microenvironments for morphogenesis in tissue engineering. Nat Biotechnol. 2005; 23(1):47–55. [PubMed: 15637621]

110. Silva AKA, Richard C, Bessodes M, Scherman D, Merten OW. Growth factor delivery approaches in hydrogels. Biomacromolecules. 2009; 10(1):9–18. [PubMed: 19032110]

111. Zisch AH, Lutolf MP, Hubbell JA. Biopolymeric delivery matrices for angiogenic growth factors. Cardiovasc Pathol. 2003; 12(6):295–310. [PubMed: 14630296]

112. Lee HJ, Lee JS, Chansakul T, Yu C, Elisseef JH, Yu SM. Collagen mimetic peptide-conjugated photopolymerizable PEG hydrogel. Biomaterials. 2006; 27(30):5268–5276. [PubMed: 16797067]
113. Jing P, Rudra JS, Herr AB, Collier JH. Self-assembling peptide-polymer hydrogels designed from the coiled coil region of fibrin. Biomacromolecules. 2008; 9(9):2438–2446. [PubMed: 18712921]
114. Salinas CN, Anseth KS. Decorin moieties tethered into PEG networks induce chondrogenesis of human mesenchymal stem cells. J Biomed Mater Res A. 2009; 90(2):456–464. [PubMed: 18546186]
115. Cheung CY, McCartney SJ, Anseth KS. Synthesis of polymerizable superoxide dismutase mimetics to reduce reactive oxygen species damage in transplanted biomedical devices. Adv Funct Mater. 2008; 18(20):3119–3126.
116. Cheung CY, Anseth KS. Synthesis of immunoisolation barriers that provide localized immunosuppression for encapsulated pancreatic islets. Bioconjug Chem. 2006; 17(4):1036–1042. [PubMed: 16848413]
117. Lin CC, Metters AT, Anseth KS. Functional PEG-peptide hydrogels to modulate local inflammation induced by the pro-inflammatory cytokine TNFα. Biomaterials. 2009; 30(28): 4907–4914. [PubMed: 19560813]
118. Su J, Hu BH, Lowe WL, Kaufman DB, Messersmith PB. Anti-inflammatory peptide-functionalized hydrogels for insulin-secreting cell encapsulation. Biomaterials. 2010; 31(2):308–314. [PubMed: 19782393]
119. Lipke EA, West JL. Localized delivery of nitric oxide from hydrogels inhibits neointima formation in a rat carotid balloon injury model. Acta Biomater. 2005; 1(6):597–606. [PubMed: 16701840]
120. Jia X, Kiick KL. Hybrid multicomponent hydrogels for tissue engineering. Macromol Biosci. 2009; 9(2):140–156. [PubMed: 19107720]
121. Hiemstra C, van der Aa LJ, Zhong Z, Kijkstra PJ, Jan Feijen. Rapidly *in situ*-forming degradable hydrogels form dextran thiols through Michael addition. Biomacromolecules. 2007; 8(5):1548–1556. [PubMed: 17425366]
122. Zieris A, Prokoph S, Levental KR, et al. FGF-2 and VEGF functionalization of starPEG-heparin hydrogels to modulate biomolecular and physical cues of angiogenesis. Biomaterials. 2010; 31(31):7985–7994. [PubMed: 20674970]
123. Jin R, Teixeira LSM, Krouwels A, et al. Synthesis and characterization of hyaluronic acid-poly(ethylene glycol) hydrogels via Michael addition: an injectable biomaterial for cartilage repair. Acta Biomater. 2010; 6(6):1968–1977. [PubMed: 20025999]
124. Strehin I, Nahas Z, Arora K, Nguyen T, Elisseeff J. A versatile pH sensitive chondroitin sulphate–PEG tissue adhesive and hydrogel. Biomaterials. 2010; 31(10):2788–2797. [PubMed: 20047758]
125. Rizzi SC, Hubbell JA. Recombinant protein-co-PEG networks as cell-adhesive and proteolytically degradable hydrogel matrixes. Part I: development and physiochemical characteristics. Biomacromolecules. 2005; 6(3):1226–1238. [PubMed: 15877337]
126. Appelman TP, Mizrahi J, Elisseeff JH, Seliktar D. The influence of biological motifs and dynamic mechanical stiulation in hydrogel scaffold systems on the phenotype of chondrocytes. Biomaterials. 2011; 32(6):1508–1516. [PubMed: 21093907]
127. Li F, Griffith M, Li Z, et al. Recruitment of multiple cell lines by collagen-synthetic copolymer matrices in corneal regeneration. Biomaterials. 2005; 26(16):3093–3104. [PubMed: 15603804]
128. Wang C, Stewart RJ, Kopecek J. Hybrid hydrogels assembled from synthetic polymers and coiled-coil protein domains. Nature. 1999; 397(6718):417–420. [PubMed: 9989405]
129. Chen JP, Cheng TH. Thermo-responsive chitosan-graft-poly(*N*-isopropylacrylamide) injectable hydrogel for cultivation of chondrocytes and meniscus cells. Macromol Biosci. 2006; 6(12): 1026–1039. [PubMed: 17128421]
130. Tan H, Ramirez CM, Miljkovic N, Li H, Rubin JP, Marra KG. Thermosensitive injectable hyaluronic acid hydrogel for adipose tissue engineering. Biomaterials. 2009; 30(36):6844–6853. [PubMed: 19783043]
131. Prabaharan M, Mano JF. Stimuli-responsive hydrogels based on polysaccharides incorporated with thermo-resoponsive polymers as novel biomaterials. Macromol Biosci. 2006; 6(12):991–1008. [PubMed: 17128423]

132. Myles JL, Burgess BT, Dickinson RB. Modification of the adhesive properties of collagen by covalent grafting with RGD peptides. J Biomater Sci Polym Ed. 2000; 11(1):69–86. [PubMed: 10680609]
133. Shachar M, Tsur-Gang O, Dvir T, Leor J, Cohen S. The effect of immobilized RGD peptide in alginate scaffolds on cardiac tissue engineering. Acta Biomater. 2011; 7(1):152–162. [PubMed: 20688198]
134. Connelly JT, Garcia AJ, Levenston ME. Inhibition of *in vitro* chondrogenesis in RGD-modified three-dimensional alginate gels. Biomaterials. 2007; 28(6):1071–1083. [PubMed: 17123602]
135. Khetan S, Katz JS, Burdick JA. Sequential crosslinking to control cellular spreading in 3-dimensional hydrogels. Soft Matter. 2009; 5(8):1601–1606.
136. Shu XZ, Ghosh K, Liu Y, Palumbo FS, Clark RA, Prestwich GD. Attachment and spreading of fibroblasts on an RGD peptide-modified hyaluronan hydrogel. J Biomed Mater Res A. 2004; 68(2):365–375. [PubMed: 14704979]
137. Kimura T, Nam K, Mutsuo S, et al. Preparation of poly(vinyl alcohol)/DNA hydrogels via hydrogen bonds formed on ultra-high pressurization and controlled release of DNA from the hydrogels for gene delivery. J Artif Organs. 2007; 10(2):104–108. [PubMed: 17574513]
138. Bryant SJ, Davis-Arehart KA, Luo N, Shoemaker RK, Arthur JA, Anseth KS. Synthesis and characterization of photopolymerized multifunctional hydrogels: water-soluble poly(vinyl alcohol) and chondroitin sulfate macromers for chondrocyte encapsulation. Macromolecules. 2004; 37(18):6726–6733.
139. Lin C, Zhao P, Li F, Guo F, Li Z, Wen X. Thermosensitive *in situ*-forming dextranpluronic hydrogels through Michael addition. Mater Sci Eng C. 2010; 30(8):1236–1244.
140. Wang C, Kopecek J, Stewart RJ. Hybrid hydrogels cross-linked by genetically engineered coiled-coil block proteins. Biomacromolecules. 2001; 2(3):912–920. [PubMed: 11710049]
141. Scott JE. Extracellular matrix, supramolecular organization and shape. J Anat. 1995; 187:259–269. [PubMed: 7591990]
142. Rhodes JM, Simons M. The extracellular matrix and blood vessel formation; not just a scaffold. J Cell Mol Med. 2007; 11(2):176–205. [PubMed: 17488472]
143. Ma PX. Biomimetic materials for tissue engineering. Adv Drug Deliver Rev. 2008; 60(2):184–198.
144. Chen R, Hunt JA. Biomimetic materials processing for tissue-engineering processes. J Mater Chem. 2007; 17(38):3974–3979.
145. Tibbitt MW, Anseth KS. Hydrogels as extracellular matrix mimics for 3D cell culture. Biotechnol Bioeng. 2009; 103(4):655–663. [PubMed: 19472329]
146. Zhu J, Beamish JA, Tang C, Kottke-Marchant K, Marchant RE. Extracellular matrix-like cell-adhesive hydrogels form RGD-containing poly(ethylene glycol) diacrylate. Macromolecules. 2006; 39(4):1305–1307.
147. Zhu J, Tang C, Kottke-Marchant K, Marchant RE. Design and synthesis of biomimetic hydrogel scaffolds with controlled organization of cyclic RGD peptides. Bioconjug Chem. 2009; 20(2): 333–339. [PubMed: 19191566]
148. Zhu J, Marchant RE. Solid-phase synthesis of tailed cyclic RGD peptides using glutamic acid: unexpected glutarimide formation. J Pept Sci. 2008; 14:690–696. [PubMed: 18085515]
149. Liu SQ, Ee PLR, Ke CY, Hedrick JL, Yang YY. Biodegradable poly(ethylene glycol)-peptide hydrogels with well-defined structure and properties for cell delivery. Biomaterials. 2009; 30(8): 1453–1461. [PubMed: 19097642]
150. Polizzotti BD, Fairbanks BD, Anseth KS. Three-dimensional biochemical patterning of Click-based composite hydrogels via thiolene photopolymerization. Biomacromolecules. 2008; 9(4): 1084–1087. [PubMed: 18351741]
151. Liu SQ, Tian Q, Wang L, et al. Injectable biodegradable poly(ethylene glycol)/RGD peptide hybrid hydrogels for *in vitro* chondrogenesis of human mesenchymal stem cells. Macromol Rapid Commun. 2010; 31(13):1148–1154. [PubMed: 21590868]
152. Zhou M, Smith AM, Das AK, et al. Self-assembled peptide-based hydrogels as scaffolds for anchorage-dependent cells. Biomaterials. 2009; 30(13):2523–2530. [PubMed: 19201459]

153. Comisar WA, Hsiong SX, Kong HJ, Mooney MJ, Linderman JJ. Multi-scale modeling to predict ligand presentation within RGD nanopatterned hydrogels. Biomaterials. 2006; 27(10):2322–2329. [PubMed: 16316682]

154. Studenovska H, Vodicka P, Proks V, Hlucilova J, Motlik J, Rypacek F. Synthetic poly(amino acid) hydrogels with incorporated cell-adhesive peptide for tissue engineering. J Tissue Eng Regen Med. 2010; 4(6):454–463. [PubMed: 20084624]

155. Herten M, Jung TE, Rothamel D, et al. Biodegradation of different synthetic hydrogels made of polyethylene glycol hydrogel/RGD-peptide modificaitons: an immunohistochemical study in rats. Clin Oral Impl Res. 2009; 20(2):116–125.

156. Schmidt DR, Kao WJ. Monocyte activation in response to polyethylene glycol hydrogels grafted with RGD and PHSRN separated by interpositional spacers of various length. J Biomed Mater Res A. 2007; 83(3):617–625. [PubMed: 17503491]

157. Benoit DSW, Anseth KS. The effect on osteoblast function of coloclized RGD and PHSRN epitopes on PEG surfaces. Biomaterials. 2005; 26(25):5209–5220. [PubMed: 15792548]

158. Masters KS, Shah DN, Walker G, Leinwand LA, Anseth KS. Designing scaffolds for valvular interstitial cells: cell adhesion and function on naturally derived materials. J Biomed Mater Res A. 2004; 71(1):172–180. [PubMed: 15368267]

159. Peyton SR, Raub CB, Keschrumrus VP, Putnam AJ. The use of poly(ethylene glycol) hydrogels to investigate the impact of ECM chemistry and mechanics on smooth muscle cells. Biomaterials. 2006; 27(28):4881–4893. [PubMed: 16762407]

160. Park CH, Hong YJ, Park K, Han DK. Peptide-grafted lactide-based poly(ethylene glycol) porous scaffolds for specific cell adhesion. Macromol Res. 2010; 18(5):526–532.

161. Massia SP, Hubbell JA. Vascular endothelial cell adhesion and spreading promoted by the peptide REDV of the IIICS region of plasma fibronectin is mediated by integrin α4 β1. J Biol Chem. 1992; 267(20):14019–14036. [PubMed: 1629200]

162. Drake SL, Varnum J, Mayo KH, Letourneau PC, Furcht LT, McCarthy JB. Structure features of fibronectin synthetic peptide FN-C/H II, responsive for cell adhesion, neurite extension, and heparin. J Biol Chem. 1993; 268(21):15859–15867. [PubMed: 8340411]

163. Hansen LK, O'Leary JJ, Skubitz APN, Furcht LT, McCarthy JB. Identification of a homologous heparin binding peptide sequence present in fibronectin and the 70 kDa family of heat-shock proteins. Biochim Biophys Acta. 1995; 1252(1):135–145. [PubMed: 7548155]

164. Woods A, McCarthy JB, Furcht LT, Couchman JR. A synthetic peptide from the COOH-terminal heparin-binding domain of fibronectin promotes focal adhesion formation. Mol Biol Cell. 1993; 4(6):605–613. [PubMed: 8374170]

165. Klim JR, Li L, Wrighton PJ, Piekarczyk MS, Kiessling LL. A defined glycosaminoglycan-binding substratum for human pluripotent stem cells. Nat Methods. 2010; 7(12):989–994. [PubMed: 21076418]

166. Nomizu M, Weeks BS, Weston CA, Kim WH, Kleinman HK, Yamada Y. Structure–activity study of a laminin α1 chain active peptide segment Ile–Lys–Val–Ala–Val (IKVAV). FEBS Lett. 1995; 365(2–3):227–231. [PubMed: 7781784]

167. Hynd MR, Frampton JP, Dowell-Mesfin N, Turner JN, Shain W. Directed cell growth on protein-functionalized hydrogel surfaces. J Neurosci Meth. 2007; 162(1–2):255–263.

168. Saha K, Irwin EF, Kozhukh J, Schaffer DV, Healy KE. Biomimetic interfacial interpenetrating polymer networks control neural stem cell behavior. J Biomed Mater Res A. 2007; 81(1):240–249. [PubMed: 17236216]

169. Santiago LY, Nowak RW, Rubin JP, Marra KG. Peptide-surface modification of poly(caprolactone) with laminin-derived sequences for adipose-derived stem cell applications. Biomaterials. 2006; 27(15):2962–2969. [PubMed: 16445976]

170. Zustiak SP, Durbal R, Leach JB. Influence of cell-adhesive peptide ligands on poly(ethylene glycol) hydrogel physical, mechanical and transport properties. Acta Biomater. 2010; 6(9):3404–3414. [PubMed: 20385260]

171. Tsur-Gang O, Ruvinov E, Landa N, et al. The effects of peptide-based modification of alginate on left ventricular remodeling and function after myocardial infarction. Biomaterials. 2009; 30(2):189–195. [PubMed: 18849071]

172. Xu J, Zhou X, Ge H, et al. Endothelial cells anchoring by functionalized yeast polypeptide. J Biomed Mater Res A. 2008; 87(3):819–821. [PubMed: 18228253]

173. Fittkau MH, Zilla P, Bezuidenhout D, et al. The selective modulating of endothelial cell mobility on RGD peptide containing surfaces by YIGSR peptides. Biomaterials. 2005; 26(2):167–174. [PubMed: 15207463]

174. Webber LM, Anseth KS. Hydrogel encapsulation environments functionalized with extracellular matrix interactions increase islet insulin secretion. Matrix Biol. 2008; 27(8):667–673. [PubMed: 18773957]

175. Webber LM, Haydam KN, Haskins K, Anseth KS. The effects of cell-matrix interactions on encapsulated β-cell function within hydrogels functionalized with matrix-derived adhesive peptides. Biomaterials. 2007; 28(19):3004–3011. [PubMed: 17391752]

176. Luzak B, Golanski J, Rozalski M, Boncler MA, Watala C. Inhibition of collagen-induced platelet reactivity by DGEA peptide. Acta Biochim Pol. 2003; 50(4):1119–1128. [PubMed: 14739999]

177. Mineur P, Guignandon A, Lambert CA, Lapiere CM, Nusgens BV. RGDS and DGEA-induced $[Ca^{2+}]_i$ signaling in human dermal fibroblast. Biochim Biophys Acta. 2005; 1746(1):28–37. [PubMed: 16199103]

178. Renner C, Sacca B, Moroder L. Synthetic heterotrimeric collagen peptides as mimics of cell adhesion sites of the basement membrane. Biopolymers. 2004; 76(1):34–47. [PubMed: 14997473]

179. Mann BK, West JL. Cell adhesion peptides alter smooth muscle cell adhesion, proliferation, migration, and matrix protein synthesis on modified surfaces and in polymer scaffolds. J Biomed Mater Res. 2002; 60(1):86–93. [PubMed: 11835163]

180. Mann BK, Schmedlen RH, West JL. Tethered-TGF-β increases extracellular matrix production of vascular smooth muscle cells. Biomaterials. 2001; 22(5):439–444. [PubMed: 11214754]

181. Mann BK, Tsa AT, Scott-Burden T, West JL. Modification of surfaces with cell adhesion peptides alters extracellular matrix deposition. Biomaterials. 1999; 20(23–24):2281–2286. [PubMed: 10614934]

182. Nagase H, Fields GB. Human matrix metalloproteinase specificity studies using collagen sequence-based synthetic peptides. Biopolymers. 1996; 40(4):399–416. [PubMed: 8765610]

183. Turk BE, Huang LL, Piro ET, Cantley LC. Determination of protease cleavage site motifs using mixture-based oriented peptide libraries. Nat Biotechnol. 2001; 19(7):661–667. [PubMed: 11433279]

184. Miller JS, Shen CJ, Legant WR, Baranski JD, Blakely BL, Chen CS. Bioactive hydrogels made from step-growth derived PEG-peptide macromers. Biomaterials. 2010; 31(13):3736–3743. [PubMed: 20138664]

185. Tsubota Y, Mizushima H, Hirosaki T, Higashi S, Yasumitsu H, Miyazaki K. Isolation and activity of proteolytic fragment of laminin-5 α3 chain. Biochem Biophys Res Commun. 2000; 278(3): 614–620. [PubMed: 11095958]

186. Ogawa T, Tsubota Y, Maeda M, Kariya Y, Miyazaki K. Regulation of biological activity of laminin-5 by proteolytic processing of γ2 chain. J Cell Biochem. 2004; 92(4):701–714. [PubMed: 15211568]

187. Pirila E, Sharabi A, Salo T, et al. Matrix metalloproteinases process the laminin-5 γ2 chain and regulate epithelial cell migration. Biochem Biophys Res Commun. 2003; 303(4):1012–1017. [PubMed: 12684035]

188. Shikanov A, Smith RM, Xu M, Woodruff TK, Shea LD. Hydrogel network design using multifunctional macromers to coordinate tissue maturation in ovarian follicle culture. Biomaterials. 2011; 32(10):2524–2531. [PubMed: 21247629]

189. Jo YS, Rizzi SC, Ehrbar M, Weber FZ, Hubbell JA, Lutolf MP. Biomimetic PEG hydrogels crosslinked with minimal plasmin-sensitive tri-amino acid peptides. J Biomed Mater Res A. 2010; 93(3):870–877. [PubMed: 19701911]

190. Halstenberg S, Panitch A, Rizzi S, Hall H, Hubbell JA. Biologically engineered protein-graft-poly(ethylene glycol) hydrogels: a cell adhesive and plasmin-degradable biosynthetic material for tissue repair. Biomacromolecules. 2002; 3(4):710–723. [PubMed: 12099815]

191. Fosang AJ, Last K, Knauper V, Murphy G, Neame PJ. Degradation of cartilage aggrecan by collagenase-3 (MMP-13). FEBS Lett. 1996; 380(1–2):17–20. [PubMed: 8603731]
192. Salinas CN, Anseth KS. The enhancement of chondrogenic differentiation of human mesenchymal stem cells by enzymatically regulated RGD functionalities. Biomaterials. 2008; 29(15):2370–2377. [PubMed: 18295878]
193. Bott K, Upton Z, Schrobback K, et al. The effect of matrix characteristics on fibroblast proliferation. Biomaterials. 2010; 31(32):8454–8464. [PubMed: 20684983]
194. Lutolf MP, Weber FE, Schmoekel HG, et al. Repair of bone defects using synthetic mimetics of collagenous extracellular matrices. Nat Biotechnol. 2003; 21(5):513–518. [PubMed: 12704396]
195. Raeber GP, Lutolf MP, Hubbell JA. Mechanisms of 3-D migration and matrix remodeling of fibroblasts within artificial ECMs. Acta Biomater. 2007; 3(5):615–629. [PubMed: 17572164]
196. Phelps EA, Landazuri N, Thule PM, Taylor WB, Garcia AJ. Bioartificial matrices for therapeutic vascularization. Proc Natl Acad Sci USA. 2010; 107(8):3323–3328. [PubMed: 20080569]
197. DeForest CA, Polizzotti BD, Anseth KS. Sequential click reactions for synthesizing and patterning three-dimensional cell microenvironments. Nat Mater. 2009; 8(8):659–664. [PubMed: 19543279]
198. He X, Jabbari E. Material properties and cytocompatibility of injectable MMP degradable poly(lactide ethylene oxide fumarate) hydrogel as a carrier for marrow stromal cells. Biomacromolecules. 2007; 8(3):780–792. [PubMed: 17295540]
199. Lee SH, Moon JJ, Miller JS, West JL. Poly(ethylene glycol) hydrogels conjugated with a collagenase-sensitive fluorogenic substrate to visualized collagenase activity during three-dimensional cell migration. Biomaterials. 2007; 28(20):3163–3170. [PubMed: 17395258]
200. Keen I, Lambert L, Chirila TV, Paterson SM, Whittaker AK. Degradable hydrogels for tissue engineering – part I: synthesis by RAFT polymerization and characterization of PHEMA containing enzymatically degradable crosslinks. J Biomim Biomater Tissue Eng. 2010; 6:67–85.
201. Patel PN, Gobin AS, West JL, Patrick CW. Poly(ethylene glycol) hydrogel system supports preadipocyte viability, adhesion, and proliferation. Tissue Eng. 2005; 11(9–10):1498–1505. [PubMed: 16259604]
202. West JL, Hubbell JA. Polymeric biomaterials with degradation sites for proteases involved in cell migration. Macromolecules. 1999; 32 (1):241–244.
203. Mann BK, Gobin AS, Tsai AT, Schmedlen RH, West JL. Smooth muscle cell growth in photopolymerized hydrogels with cell adhesion and proteolytically degradable domains: synthetic ECM analogs for tissue engineering. Biomaterials. 2001; 22(22):3045–3051. [PubMed: 11575479]
204. Patrick AG, Ulijin RV. Hydrogels for the detection and management of protease levels. Macromol Biosci. 2010; 10(10):1184–1193. [PubMed: 20593363]
205. Pak CC, Erukulla RK, Ahl PL, Janoff AD, Meers P. Elastase activated liposomal delivery to nucleated cells. Biochim Biophys Acta. 1999; 1419(2):111–126. [PubMed: 10407064]
206. Aimetti AA, Tibbitt MW, Anseth KS. Human neutrophil elastase responsive delivery from poly(ethylene glycol) hydrogels. Biomacromolecules. 2009; 10(6):1484–1489. [PubMed: 19408953]
207. Casadio YS, Brown DH, Chirila TV, Kraatz HB, Baker MV. Biodegradable poly(2-hydroxyethyl methacrylate) (PHEMA) and poly{(2-hydroxyl methacrylate)-co-[poly(ethylene glycol) methyl ether methacrylate]} hydrogels containing peptide-based cross-linking agents. Biomacromolecules. 2010; 11(11):2949–2959.
208. Fairbanks BD, Schwartz MP, Halevi AE, Nuttelman CR, Bowman CN, Anseth KS. A versatile synthetic extracellular matrix mimic via thioi-norbornene photopolymerization. Adv Mater. 2009; 21(48):5005–5010.
209. Ahrendt G, Chickering DE, Ranieri JP. Angiogenic growth factor: A review for tissue engineering. Tissue Eng. 1998; 4(2):117–130.
210. Chen RR, Mooney DJ. Polymeric growth factor delivery strategies for tissue engineering. Pharm Res. 2003; 20(80):1103–1112. [PubMed: 12948005]
211. Phelps EA, Garcia AJ. Update on therapeutic vascularization strategies. Regen Med. 2009; 4(1): 65–80. [PubMed: 19105617]

212. Zhang S, Uludag H. Nanoparticle systems for growth factor delivery. Pharm Res. 2009; 26(7): 1561–1579. [PubMed: 19415467]

213. Taipale J, Keski-Oja J. Growth factors in the extracellular matrix. FASEB J. 1997; 11(1):51–59. [PubMed: 9034166]

214. Whitelock JM, Murdoch AD, Iozzo RV, Underwoods PA. The degradation of human endothelial cell-derived perlecan and release bound basic fibroblast growth factor by stromelysin, collagenase, plasmin, and heparanases. J Biol Chem. 1996; 271(17):10079–10086. [PubMed: 8626565]

215. Hiemstra C, Zhong Z, van Steenbergen MJ, Hennink WE, Feijen J. Release of model proteins and basic fibroblast growth factor from in situ forming degradable dextran hydrogels. J Control Release. 2007; 122(1):71–78. [PubMed: 17658651]

216. Andreopoulos FM, Persaud I. Delivery of basic fibroblast growth factor (bFGF) from photoresponsive hydrogel scaffolds. Biomaterials. 2006; 27(11):2468–2476. [PubMed: 16321436]

217. Burdick JA, Mason MN, Hinman AD, Thorne K, Anseth KS. Delivery of osteoinductive growth factors from degradable PEG hydrogels influences osteoblast differentiation and mineralization. J Control Release. 2002; 83(1):53–63. [PubMed: 12220838]

218. van de Wetering P, Metters AT, Schoenmakers RG, Hubbell JA. Poly(ethylene glycol) hydrogels formed by conjugate addition with controllable swelling, degradation, and release of pharmaceutically active proteins. J Control Release. 2005; 102(3):619–627. [PubMed: 15681084]

219. Lim SM, Oh SH, Lee HH, Yuk SH, Im GI, Lee JH. Dual growth factor-releasing nanoparticle/hydrogel system for cartilage tissue engineering. J Mater Sci Mater Med. 2010; 21(9):2593–2600. [PubMed: 20577785]

220. Ferreira LS, Gerecht S, Fuller J, Shieh HF, Vunjak-Novakovic G, Langer R. Bioactive hydrogel scaffolds for controllable vascular differentiation of human embryonic stem cells. Biomaterials. 2007; 28(17):2706–2717. [PubMed: 17346788]

221. Moya ML, Cheng MH, Huang JJ, et al. The effect of FGF-1 loaded alginate microbeads on neovascularization and adipogenesis in a vascular pedicle model of adipose tissue engineering. Biomaterials. 2010; 31(10):2816–2826. [PubMed: 20080298]

222. Chen RR, Silve EA, Yuen WW, et al. Integrated approach to designing growth factor delivery systems. FASEB J. 2007; 21(14):3896–3903. [PubMed: 17644610]

223. Park H, Temenoff J, Tabata Y, Caplan AI, Mikos AG. Injectable biodegradable hydrogel composites for rabbit marrow mesenchymal stem cell and growth factor delivery for cartilage tissue engineering. Biomaterials. 2007; 28(21):3217–3227. [PubMed: 17445882]

224. Jay SM, Stepherd BR, Bertram JP, Pober JS, Saltzman WM. Engineering of multifunctional gels integrating highly efficient growth factor delivery with endothelial cell transplantation. FASEB J. 2008; 22(8):2919–2956.

225. Jay SM, Saltzman WM. Controlled delivery of VEGF via modulation of alginate microparticle ionic crosslinking. J Control Release. 2009; 134(1):26–34. [PubMed: 19027807]

226. Ho YC, Wu SJ, Mi FL, et al. Thiol-modified chitosan sulfate nanoparticles for protection and release of basic fibroblast growth factor. Bioconjug Chem. 2010; 21(1):28–38. [PubMed: 20000719]

227. Ehrbar M, Rizzi SC, Hlushchuk R, et al. Enzymatic formation of modular cell-instructive fibrin analogs for tissue engineering. Biomaterials. 2007; 28(26):3856–3866. [PubMed: 17568666]

228. Zisch AH, Schenk U, Schense JC, Sakiyama-Elbert SE, Hubbell JA. Covalently conjugated VEGF–fibrin matrices for endothelialization. J Control Release. 2001; 72(1–3):101–103. [PubMed: 11389989]

229. Seliktar D, Zisch AH, Lutolf MP, Wrana JL, Hubbell JA. MMP-2 sensitive, VEGF-bearing bioactive hydrogels for promotion of vascular healing. J Biomed Mater Res A. 2004; 68(4):704–716. [PubMed: 14986325]

230. Gobin AS, West JL. Effects of epidermal growth factor on fibroblast migration through biomimetic hydrogels. Biotechnol Prog. 2003; 19(6):1781–1785. [PubMed: 14656156]

231. Mann BK, Schmedlen RH, West JL. Tethered-TGF-β increases extracellular matrix production of vascular smooth muscle cells. Biomaterials. 2001; 22(5):439–444. [PubMed: 11214754]

232. DeLong SA, Moon JJ, West JL. Covalently immobilized gradients of bFGF on hydrogel scaffolds for directed cell migration. Biomaterials. 2005; 26(16):3227–3234. [PubMed: 15603817]

233. He X, Ma J, Jabbari E. Effect of grafting RGD and BMP-2 protein-derived peptides to a hydrogel substrate on osteogenic diffierentiation of marrow stromal cells. Langmuir. 2008; 24(21):12508–12516. [PubMed: 18837524]

234. Benoit DSW, Durney AR, Anseth KS. The effect of heparin-functionalized PEG hydrogels on three-dimensional human mesenchymal stem cell osteogenic differentiation. Biomaterials. 2007; 28(1):66–77. [PubMed: 16963119]

235. Tae G, Kim YJ, Choi WI, Kim M, Stayton PS, Hoffman AS. Formation of a novel heparin-based hydroel in the presence of heparin-binding biomolecules. Biomacromolecules. 2007; 8(6):1979–1986. [PubMed: 17511500]

236. Zhang L, Furst EC, Kiick KL. Manipulation of hydrogel assembly and growth factor delivery via the use of peptide-polysaccharide interactions. J Control Release. 2006; 114(2):130–142. [PubMed: 16890321]

237. Nie T, Akins RE, Kiick KL. Production of heparin-containing hydrogels for modulating cell responses. Acta Biomater. 2009; 5(3):865–875. [PubMed: 19167277]

238. Yamaguchi N, Zhang L, Chae BS, Palla CS, Furst EM, Kiick KL. Growth factor mediated assembly of cell receptor-responsive hydrogels. J Am Chem Soc. 2007; 129(11):3040–3041. [PubMed: 17315874]

239. Nakamura S, Ishihara M, Obara K, et al. Controlled release of fibroblast growth factor-2 from an injectable 6-O-desulfated heparin hydrogel and subsequent effect on *in vivo* vascularization. J Biomed Mater Res A. 2006; 78(2):364–371. [PubMed: 16673389]

240. Fujita M, Ishihara M, Simizu M, et al. Vascularization *in vivo* caused by the controlled release of fibroblast growth factor-2 from an injectable chitosan/non-anticoagulant heparin hydrogels. Biomaterials. 2004; 25(4):699–706. [PubMed: 14607508]

241. Cai S, Liu Y, Zheng X, Prestwich GD. Injectable glycosaminoglyosaminoglycan hydrogels for controlled release of human basic fibroblast growth factor. Biomaterials. 2005; 26(30):6054–6067. [PubMed: 15958243]

242. Rajangam K, Arnold MS, Rocco MA, Stupp SI. Peptide amphiphile nanostructure-heparin interactions and their relationship to bioactivity. Biomaterials. 2008; 29(23):3298–3305. [PubMed: 18468676]

243. Lin CC, Anseth KS. Controlling affinity binding with peptide-functionalized poly(ethylene glycol) hydrogels. Adv Funct Mater. 2009; 19(14):2325–2331. [PubMed: 20148198]

Key issues

- Hydrogels are a class of water-swollen polymers with physical or chemical crosslinks, high water content and physical properties similar to soft tissues.
- Hydrogels can be prepared from natural, synthetic or synthetic/natural hybrid polymers, and can encapsulate both cells and bioactive molecules for regulating cellular response and guiding tissue formation.
- The equilibrium swelling capacity of hydrogels is a balance between swelling and elastic forces, and the proper design of swelling parameters allows for hydrogel scaffolds to control the diffusion of bioactive molecules and migration of cells through the complex network structure.
- Natural hydrogels possess inherent biocompatibility, biodegradability and biologically recognizable moieties that support cellular activities; however, they may not provide sufficient mechanical properties and evoke immune/inflammatory responses.
- Synthetic hydrogels can be tailored with structures, biodegradability and functionality. The emerging biomimetic strategy has attracted much attention to design and synthesize extracellular matrix (ECM)-like bioactive hydrogels for tissue engineering.
- Short peptide sequences derived from the bioactive domains of ECM components have been used to design bioactive synthetic hydrogels as tissue-engineering scaffolds with ECM-mimetic biofunctions, such as cell-specific adhesion, enzyme-sensitive degradation and growth factor-binding.
- There is a continuing need for highly efficient methods for fabricating bioactive hydrogels for tissue engineering, in order to mimic the ECM structure and function with conjugating a broad class of bioactive molecules to regulate cellular response.

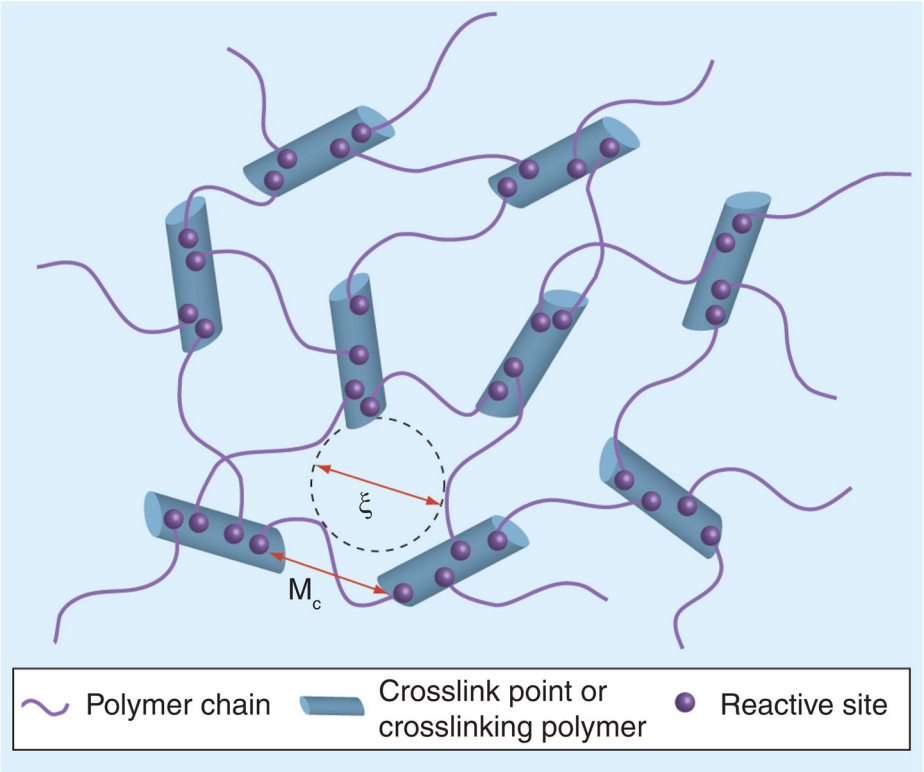

Figure 1. Schematic of hydrogel structure with hydrophilic polymer chains connected through crosslink points or crosslinking polymers

M_c represents the number average molecular weight between two adjacent crosslinks, which is related to the degree of crosslinking. ξ represents the network mesh size and is indicative of the distance between consecutive crosslinking points.

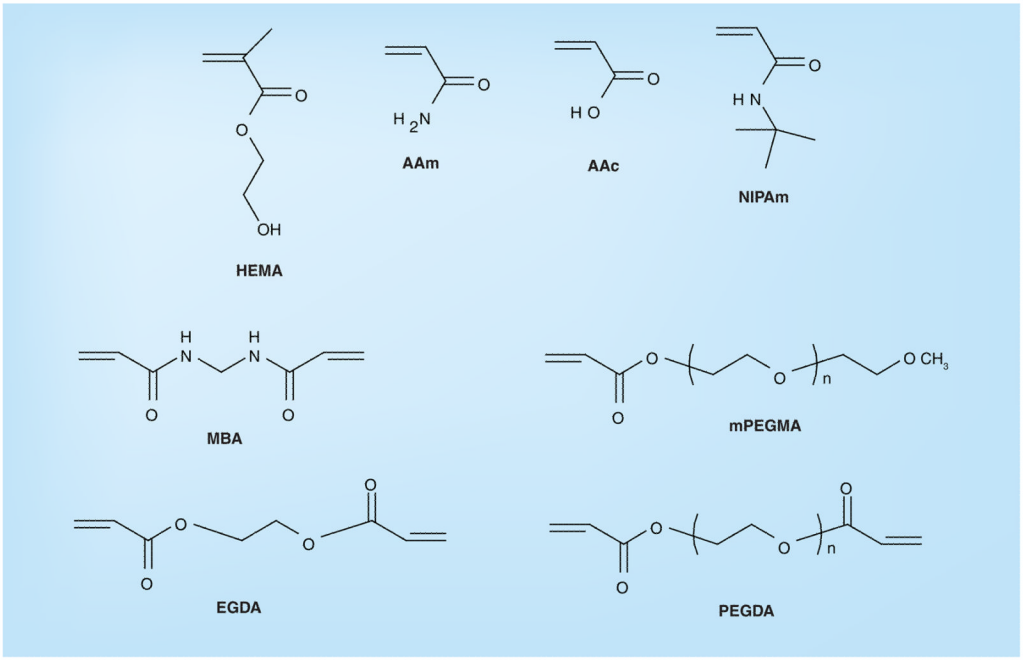

Figure 2. Structures of monomers or macromers (HEMA, AAm, AAc, NIPAm and mPEGMA), and crosslinkers (MBA, EGDA and PEGDA) for preparing nondegradable synthetic hydrogels

AAc: Acrylic acid; AAm: Acrylamide; EGDA: Ethylene glycol diacrylate; HEMA: 2-hydroxyethyl methacrylate; MBA: *N*,*N*′-methylenebis(acrylamide); mPEGMA: Methoxyl poly(ethylene glycol) monoacrylate; NIPAm: *N*-Isopropylacrylamide; PEGDA: Poly(ethylene glycol) diacrylate.

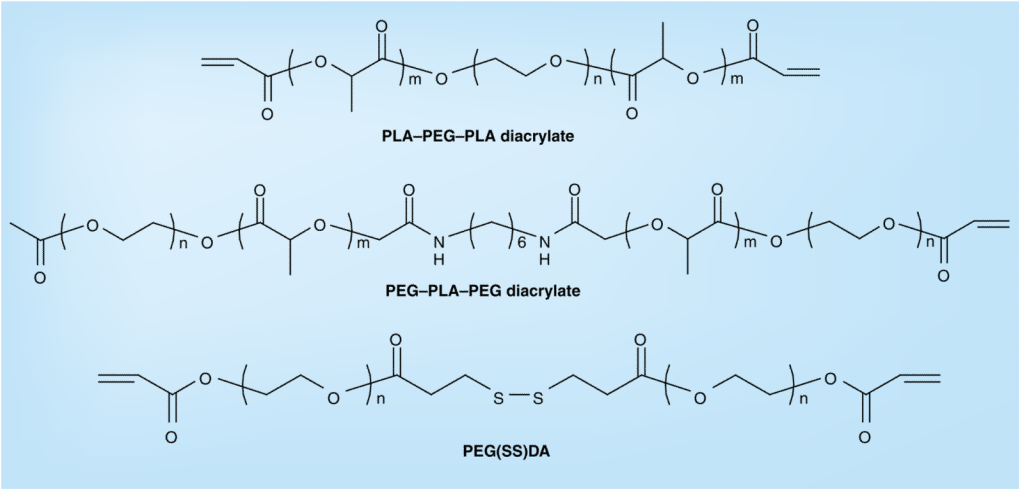

Figure 3. Structures of macromers, PLA–PEG–PLA and PEG–PLA–PEG diacrylates, and PEG(SS)DA for preparing degradable synthetic hydrogels.<
br>PEG: Poly(ethylene glycol); PEG(SS)DA: Disulfide-containing PEG diacrylate; PLA: Poly(lactic acid).

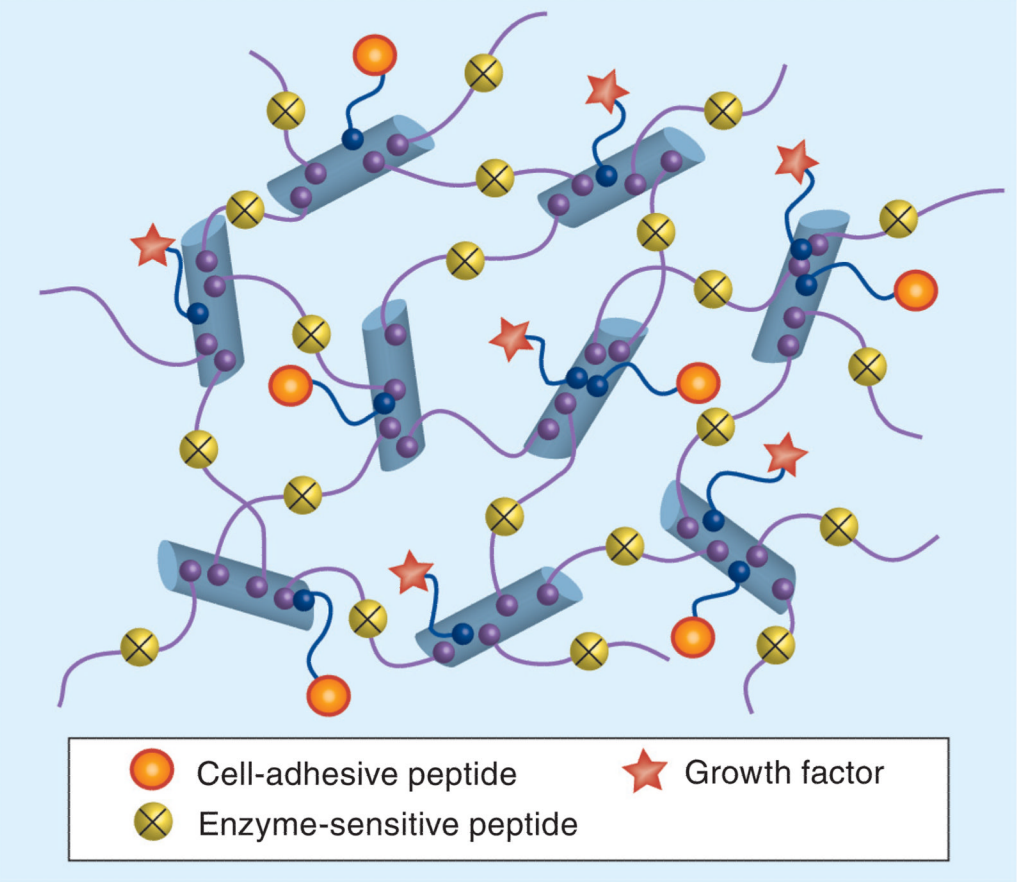

Figure 4. Model of bioactive synthetic hydrogels

Cell-adhesive and enzyme-sensitive peptides can be incorporated into hydrogels to make hydrogels as cell-adhesive and biodegradable scaffolds. Growth factors can also be covalently attached on, or reversely bind with, the hydrogel network to mediate cellular response and regulate tissue formation.

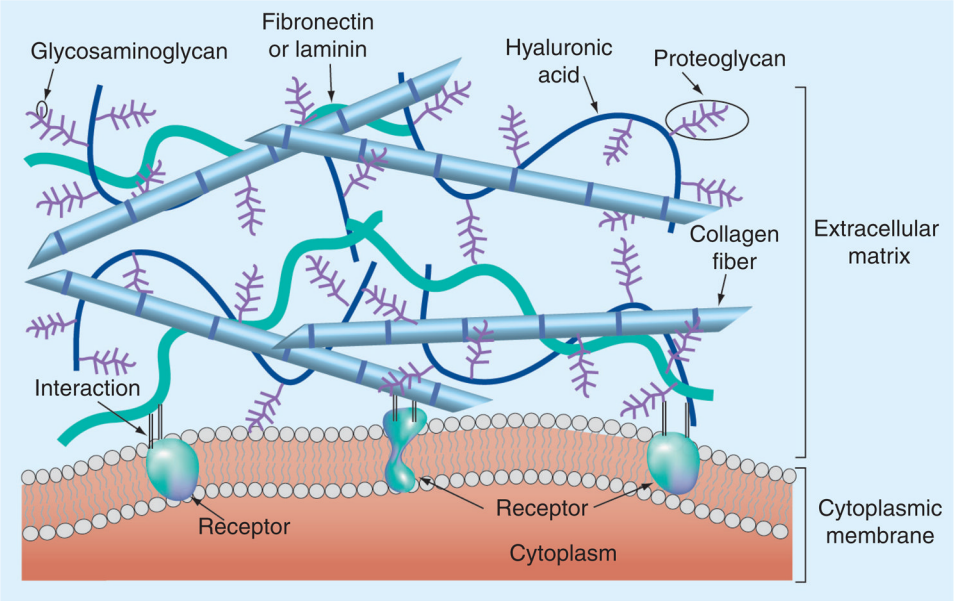

Figure 5. Model of complex 3D structure of the natural extracellular matrix and the interactions between cells and the extracellular matrix components

Extracellular matrix proteins such as collagen, laminin and fibronectin are embedded in highly negatively charged polysaccharide-rich glycans, including glycosaminoglycans and proteoglycans. The extracellular matrix components provide cell-adhesive domains for binding cell-surface receptors, such as intergrins, selectins, CD44 and syndecan.

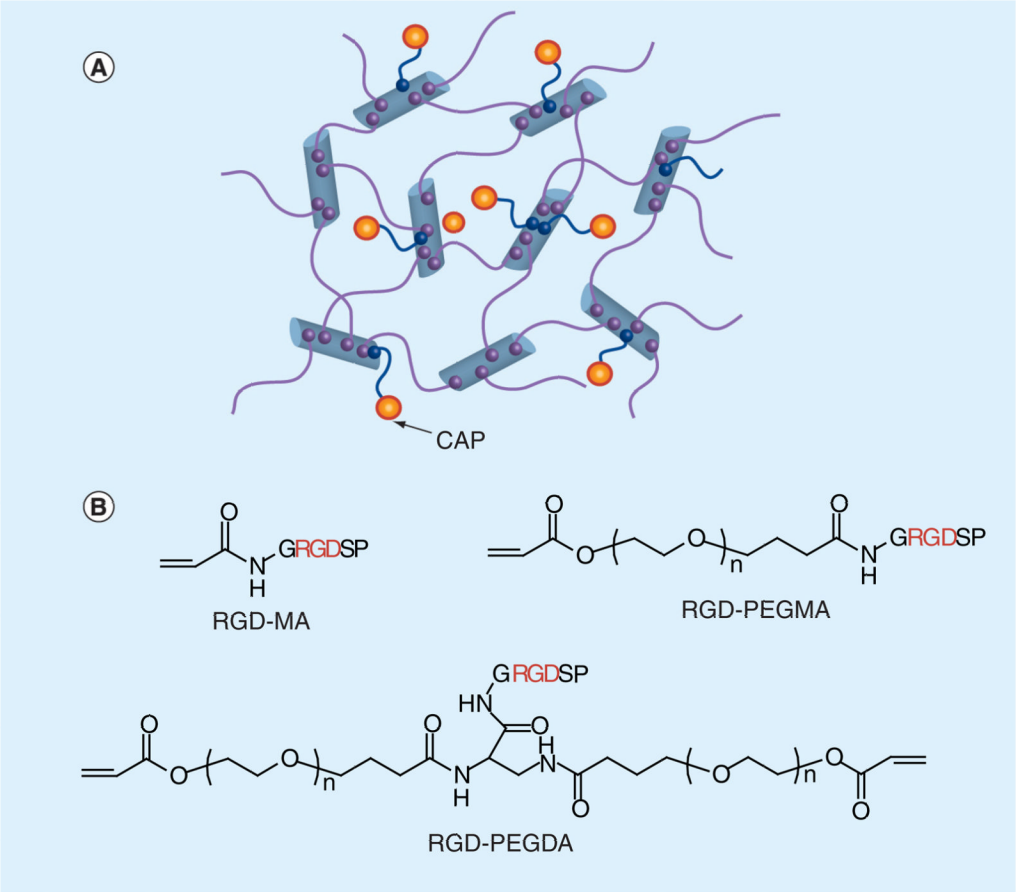

Figure 6. Preparation of cell-adhesive hydrogels

(A) Model of cell-adhesive hydrogels. Cell-adhesive peptides can be incorporated into the hydrogel network by various methods, such as free radical copolymerization, Michael addition and Click chemistry. **(B)** Structures of RGD-modified poly(ethylene glycol) macromers: RGD-MA, RGD-PEGMA and RGD-PEGDA.

CAP: Cell-adhesive peptide; MA: Monoacrylate; PEGDA: Poly(ethylene glycol) diacrylate; PEGMA: Poly(ethylene glycol) monoacrylate; RGD: Arg–Gly–Asp.

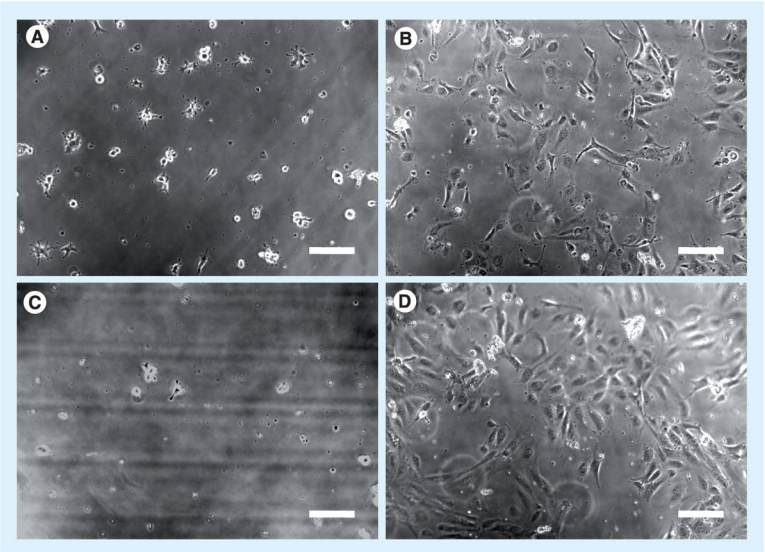

Figure 7. Phase contrast images of 2D seeding and culturing of human umbilical vein endothelial cells on hydrogels

(A & B) 2 and 24 h after seeding human umbilical vein endothelial cells (HUVECs) on 10% (w/v) poly(ethylene glycol) diacrylate (PEGDA) hydrogels, respectively; **(C & D)** 2 and 24 h after seeding HUVECs on Arg–Gly–Asp (RGD)-PEGDA hydrogels made by copolymerization of RGD-poly(ethylene glycol) monoacrylate (PEGMA; 1%, w/v) and PEGDA (9%, w/v), respectively. The images show that HUVECs seeded on RGD-PEGDA hydrogels exhibited higher initial cell attachment, greater cell spreading, and higher cell density than on PEGDA hydrogels. (Scale bar: 100 μm).

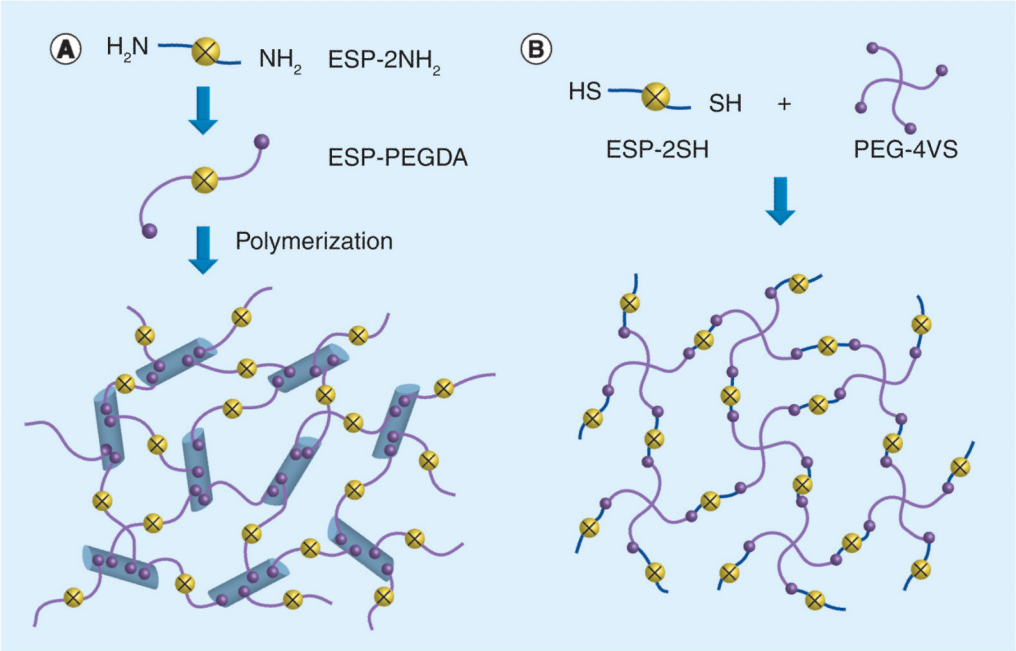

Figure 8. Schematic of the methods for the preparation of enzyme-sensitive hydrogels

(A) Free radical polymerization of ESP-containing PEGDA (ESP-EGDA). ESP-PEGDA can be synthesized by the conjugation of ESP-$2NH_2$ with acrylate-PEG-NHS. **(B)** Michael addition of ESP-2SH and multiarm PEG sulfone, such as PEG-4VS.

ESP: Enzyme-sensitive peptide; ESP-$2NH_2$: ESP diamine; ESP-2SH: ESP-dithiol; PEG-4VS: 4-arm poly(ethylene glycol) vinyl sulfone; PEGDA: Poly(ethylene glycol) diacrylate.

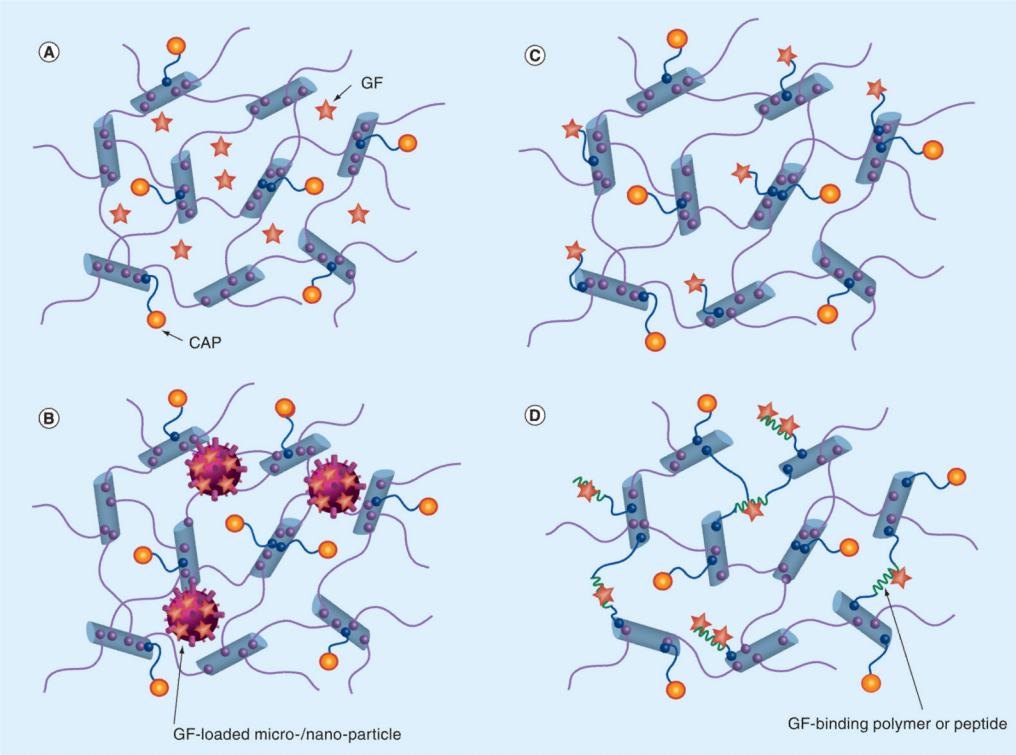

Figure 9. Schematic of growth factor-bearing hydrogels

(A) Direct loading: GFs are encapsulated into hydrogels directly during hydrogel preparation. **(B)** Carrier systems: carrier systems like micro- or nano-particles are used to encapsulate GFs first, which are subsequently embedded in hydrogels during hydrogel preparation. **(C)** Covalent bonding: GFs are covalently attached on the hydrogel network through chemical conjugation or copolymerization. **(D)** Reverse binding: GF-binding polymers or short peptides are incorporated into hydrogels by various reactions, such as free radical copolymerization, Michael addition and chemical conjugation. The resulting hydrogels can control the delivery of GFs through the reverse binding between GFs and the incorporated GF-binding polymers or peptides.

CAP: Cell-adhesive peptide; GF: Growth factor.

Table 1

Polymers used for fabricating hydrogel scaffolds for tissue engineering.

Hydrogel type	Polymer	Ref.
Natural hydrogel		
Protein	Collagen, elastin, fibrin, silk, lysozyme, Matrigel™	[28–39]
	Genetically engineered proteins	[40–49]
Polysaccharide	HA, alginate, chitosan, dextran	[50–55]
Protein/polysaccharide	Collagen/HA, laminin/cellulose, fibrin/alginate	[56–58]
	Gelatin/agarose, chitosan, alginate, dextran	[59–64]
DNA	X-, Y-, T-DNA, linear plasmid DNA	[65–69]
Synthetic hydrogel		
Nonbiodegradable	PHEMA, PHPMA, PNIPAm, Pluronic®	[70–76]
	PEGDA, PVA	[77–81]
Biodegradable	Degradable PEG	[82–93]
	PPF-PEG, PHEMA-PCL	[94–96]
	Synthetic peptides	[97–106]
Bioactive	Cell-adhesive hydrogels	[107,108]
	Enzyme-sensitive hydrogels	[109,110]
	Growth factor-bearing hydrogels	[110,111]
	Other bioactive hydrogels	[112–119]
Synthetic/natural hybrid hydrogel		
	PEG/dextran, heparin, HA, CS, protein	[120–126]
	PNIPAm/proteins, chitosan, HA, alginate	[127–131]
	Synthetic peptides/proteins, polysaccharides	[132–136]
	PVA/DNA, CS; Pluronic/dextran; PHPMA/protein	[137–140]

CS: Chondroitin sulfate; HA: Hyaluronic acid; PCL: Poly(ε-caprolactone); PEG: Poly(ethylene glycol); PEGDA: Poly(ethylene glycol) diacrylate; PHEMA: Poly(2-hydroxyethyl methacrylate); PHPMA: Poly(2-hydroxypropyl methacrylate); PNIPAm: Poly(N-isopropylacrylamide); PPF: Poly(propylene fumarate); PVA: Poly(vinyl alcohol).

Table 2

Origins of cell-adhesive peptides and their cell receptors.

Origin	Cell-adhesive peptides	Cell receptor	Ref.
Fibronectin	RGD[†]	Integrin	[146–155]
	PHSRN	Integrin $\alpha_5\beta_1$	[156,157]
	EILDV	Integrin $\alpha_4\beta_1$	[158]
	KQAGDV	Integrin	[159]
	REDV	Integrin $\alpha_4\beta_1$	[160,161]
	LIGRKK	Heparin	[162,163]
	SPPRRARV	Heparin	[164]
	WQPPRARI	Heparin	[164,165]
Vitronectin	GKKQRFRHRNRKG	Heparin	[165]
Bone sialoprotein	FHRRIKA	Heparin	[165]
Laminin	IKVAV	110-kDa protein	[166–168]
	YIGSR	67-kDa protein	[169–173]
	PDGSR	Integrin	[174,175]
	LRGDN	Integrin	[174]
	LRE	Integrin	[175]
	IKLLI	Heparin	[175]
Collagen	DGEA	Integrin $\alpha_2\beta_1$	[176,177]
	GFOGER	Integrin	[177]
	GDR, GRD	Integrin $\alpha_2\beta_1$	[178]
Elastin	VAPG	67-kDa protein	[179–181]

[†]RGD is also derived from laminin and collagen.

Table 3

Origins of enzyme-sensitive peptides and their sensitive enzymes.

Origin	Enzyme-sensitive peptide	Sensitive enzyme	Ref.
Collagen-I	GPQGIAGQ	MMP-1	[182–184]
Laminin	QLLADTPV	MMP	[185]
	YSGDENP	MMP	[186]
	DENPDIE	MMP-12	[187]
Fibrinogen	YKNR, YKNRD	Plasmin	[188,189]
	YKNS, YKND	Plasmin	[188]
	NRV, NRD	Plasmin	[190]
Peptide library	GPQGIWGQ	MMP-1, MMP-12	[191–194]
	GPQGILGQ	MMP-1	[195]
	GPQGLA	MMP-13	[196]
	LGPA	MMP-1	[197–199]
	APGL	MMP-1	[200]
	AAAAAAAAA	Elastase	[201]
	AAPV	Elastase	[202,203]
	AAPVRGMG	Elastase	[204]
	GGYRG	Chymotrypsin	[204]
	GL, GFL, GFGL	Papain	[206]
Aggrecan	PENFF	MMP-13	[207,208]

MMP: Matrix metalloproteinase.