Recent advances in high-strength and elastic hydrogels for 3D printing in biomedical applications

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Abstract

Three-dimensional (3D) printing enables the production of personalized tissue-engineered products with high tunability and complexity. It is thus an attractive and promising technology in the pharmaceutical and medical fields. Printable and biocompatible hydrogels are attractive materials for 3D printing applications because they offer favorable biomimetic environments for live cells, such as high water content, porous structure, bioactive molecule incorporation, and tunable mechanical properties and degradation rates. However, most conventional hydrogel materials are brittle and mechanically weak and hence cannot meet the mechanical needs for handling and soft and elastic tissue use. Thus, the development of printable, high strength, and elastic hydrogel materials for 3D printing in tissue repair and regeneration is critical and interesting. In this review, we summarized the recent reports on high strength and elastic hydrogels for printing use and categorized them into three groups, namely double-network hydrogels, nanocomposite hydrogels, and single-network hydrogels. The reinforcing mechanisms of these high strength hydrogels and the strategies to improve their printability and biocompatibility were further discussed. These high-strength and elastic hydrogels may offer opportunities to accelerate the development of 3D printing technology and provide new insights for 3D-printed product design in biomedicine.

Keywords

hydrogel; high strength; elasticity; 3D printing; tissue repair

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Conflicts of interest

There are no conflicts of interest to declare.

Introduction

Three-dimensional (3D) printing is an advanced manufacturing technique in drug formulation, tissue repair and regeneration, and medical devices and disease models because of the demand for customized medical and pharmaceutical products [1]. Currently, 3D printing technologies can be divided into four major categories, namely vat photopolymerization-based printing, powder-based printing, droplet-based printing, and extrusion-based printing [2]. Vat photopolymerization-based printing technologies such as stereolithography (SLA), direct light processing (DLP), and continuous liquid interface production (CLIP) use photosensitive polymers exposed to light with a specific wavelength for solidification [3, 4]. The powder-based printing technologies, such as selective laser sintering (SLS), direct metal laser sintering (DMLS), selective laser melting (SLM), and electron beam melting (EBM), utilize localized heating to fuse the materials [5]. In droplet-based printing technologies such as multijet modeling (MJM), laser-induced forward transfer (LIFT), and wax deposition modeling (WDM) liquid droplets are ejected onto a substrate to form a layer-by-layer construct [6–8]. Compared to the above three printing technologies, the extrusion-based printing is the most popular method in biomedicine for 3D-printed scaffolds, especially for cell-laden 3D constructs, because a wide range of printable materials are available and easy to use [9]. The extrusion-based printing technologies including fused deposition modeling (FDM) and direct ink writing (WID) can extrude printable materials with/without cells from a nozzle and deposit filaments layer-by-layer on a platform to form a 3D construct [10, 11].

The printable materials for tissue repair and regeneration are required to have adequate printability, sufficient mechanical strength, strong interfacial strength, and desirable biocompatibility. However, it remains challenging to obtain ideal printable biomaterials. Thus, it is very important to develop new printable biomaterials for 3D-printed constructs in tissue repair and regeneration. The biocompatible and biodegradable hydrogel inks are generally flowable liquids that can be easily extruded and rapidly solidified, using chemical or physical stimulations to maintain their shapes. They can also provide favorable environments for various cells that mimic the extracellular matrix (ECM), such as high water content, porous structure, and tunable mechanical properties and degradation. Most importantly, these hydrogel inks are capable of directly loading living cells and bioactive molecules [12–14]. Thus, they are highly attractive and promising biomaterials for 3D printing, especially for extrusion-based printing with/without cell loading. Most hydrogels used in 3D printing are natural or synthetic polymer-based, such as gelatin [15], fibrin [16], hyaluronic acid (HA) [17, 18], alginate [19], poly(ethylene glycol) (PEG) [20, 21], and methacrylated gelatin (GelMA) [22, 23]. However, these conventional hydrogels are weak and brittle, which makes it difficult for them to mimic the biomechanics of soft and elastic tissues such as skin, tendon, cartilage, skeletal muscle, and blood vessels. For example, the cardiac muscle is highly elastic and can contract and relax to pump blood approximately 2.5 billion times in a 70-year lifetime [24, 25]. Cartilage exhibits high toughness and low sliding friction and is shock-absorbent [26]. The elasticity of blood vessels is critical for the vessels to withstand the pulsatile pressure and propagation of pulse waves. It is difficult to mimic these properties using these weak and brittle hydrogels. Additionally, these fragile hydrogels

are not good for handling and cannot retain shape well during and after 3D printing. Hence, it is desirable to develop high-strength, elastic, and biomimetic hydrogels to mimic those soft and resilient tissues. Recently, several hydrogels with high strength and elasticity for extrusion printing have been developed. In this review, we summarize three main categories of high-strength elastic hydrogels (Fig. 1), including double-network (DN) hydrogels, nanocomposite hydrogels, and single-network (SN) hydrogels, with their applications to extrusion-based 3D printing in the biomedical field. Other strengthened hydrogels like slide ring gels [27] are not reviewed because they are not popular. Additionally, the approaches to improve the printability and biocompatibility of these high-strength elastic hydrogels are also discussed.

Double-network hydrogels

DN hydrogels possess interpenetrating polymer network (IPN) structures, which exhibit a significantly higher mechanical strength than the conventional single-network (SN) hydrogel from each component. They have been extensively investigated for biomedical applications (Fig. 1A) [28, 29]. Generally, one network is stiff and brittle, and hence it can be fractured to dissipate energy, and the other network is soft and ductile, and hence it can sustain large deformation. This combination endows the DN hydrogel with a high toughness [26, 30]. For example, the typical DN hydrogels synthesized from poly(2-acrylamido-2-methylpropanesulfonic acid) (PAMPS; first network) and polyacrylamide (PAAm; second network) can reach a compressive fracture stress rate at 17.2 MPa, which is more than 20 times higher than those of SN counterparts (PAMPS or PAAm SN hydrogels) [28]. However, this type of DN hydrogel lacks elasticity because of the permanent, irreversible rupture of the covalent bond in the first network [31, 32]. Various physical networks with reversible noncovalent associations (such as ionic crosslinking, hydrogen bonding, and hydrophobic interaction) have been introduced into DN hydrogels for energy dissipation to obtain highly elastic and high strength hydrogels [33–36].

The metal ions can coordinate with corresponding ligands to form reversible metal ion-ligand interactions, which have been used as physical crosslinkers to prepare high strength and elastic DN hydrogels [33, 37–40]. Calcium ion (Ca^{2+}) crosslinked alginate/PAAm DN hydrogels can be stretched more than 20 times their initial length and reach a fracture energy of up to 9,000 J/m^2 [33]. Inspired by the Ca-alginate/PAAm strategy, tough aluminum ion (Al^{3+}) crosslinked alginate/poly(*N*-isopropylacrylamide) (PNIPAM) hydrogels and lanthanide ion (Ln^{3+}) crosslinked alginate/PAAm hydrogels were also developed [35, 36]. Additionally, a DN protein hydrogel composed of a covalently cross-linkable protein (CCP)-based elastic network and a zinc ion (Zn^{2+}) crosslinked protein-based dissipating network possessed high strength, toughness, and stretchability [39]. The addition of a Zn^{2+} crosslinked protein resulted in maximal increase in tensile stress of approximately two orders of magnitude and a 14-fold increase in breaking strain compared to the CCP alone.

The hydrogen bonding interaction is also commonly involved in strengthening hydrogels because it is relatively stable in an aqueous environment, and its dissociation energy is low. But the association strength of multiple hydrogen bonds in the microdomains between polymer chains may be equivalent to a covalent bond [41]. A polyvinyl alcohol (PVA)/

PAAm DN hydrogel was strong and tough because one of its two networks is from PVA crystallites induced by hydrogen bonds, which can reversibly undergo association/ dissociation and dissipate energy. This reversible network enhanced the toughness and self-recovery property of the hydrogel [42]. Hydrogen bonds are also widespread in natural macromolecules, which have been used to prepare tough and elastic DN hydrogels [34, 43, 44]. An agar/PAAm DN hydrogel was synthesized through a healing-cooling-photopolymerization process. The agar with a thermoreversible sol-gel transition induced by hydrogen bonding served as the first network, and a photopolymerized PAAm was the second network [34]. The resulting agar/PAAm hydrogel had a high mechanical strength and an excellent shape-recovery property. The compression strength of the agar/PAAm DN hydrogel (38 MPa) was 10 times and 633 times higher than that of the PAAm hydrogel (3.8 MPa) and the agar hydrogel (0.06 MPa), respectively. Furthermore, the tensile strength (1.0 MPa) and toughness (9 MJ/m^3) of the DN hydrogel were approximately 3.3 times and 4.7 times higher than those of the PAAm hydrogel (0.3 MPa andl.9 MJ/m^3), respectively.

Incorporating hydrophobic interactions can also increase the mechanical strength of the hydrogel [31, 45, 46]. A series of poly(dodecyl glyceryl itaconate) (PDGI)/PAAm hydrogels exhibited a markedly enhanced mechanical strength from 38 kPa (pure PAAm hydrogel) to 600 kPa (PDGI/PAAm bilayer hydrogel) because of the lipid-like PDGI mobile bilayers and their reversible hydrophobic interactions [31, 46]. The supramolecular guest-host (GH) assembly between a host cavity (e.g., cyclodextrin) and a guest molecule that is predominantly formed by hydrophobic interactions has also been utilized to develop a self-recoverable network for energy dissipation in the DN hydrogel system [35, 47].

Dynamic covalent bonds, such as imine, hydrazone, and the Diels–Alder click reaction, are significantly stronger than the physical networks described above, and they are also reversible [36, 48–52]. Hydrazine-modified elastin-like protein and aldehyde-modified HA were combined to form a DN hydrogel through the thermal assembly of the elastin-like protein and dynamic covalent hydrazone bonds between hydrazine and aldehyde [36]. This dual-crosslinked hydrogel demonstrated apparent shear-thinning and self-recovery properties. Its mechanical stiffness was approximately 1000 times higher than that of a pure HA hydrogel. Another DN hydrogel was designed by combining a Diels–Alder cycloaddition with a hydrophobic/hydrophilic interaction of Pluronic F127 [48]. which also showed improved mechanical and inherent self-healing properties.

The DN hydrogels with a high elasticity and mechanical strength have demonstrated their ability to be printed into complex 3D constructs (Table 1) [40, 53–57]. For example, a Fe^{3+} crosslinked sodium alginate/poly(acrylamide-co-acrylic acid) DN hydrogel with high toughness and strength and self-recovery was effectively 3D printed by optimizing the viscosity of the ink [40]. The combination increased the tensile strength and toughness of the hydrogel from 0.1 MPa and 1.05 MJ/m^3 to 3.24 MPa and 25.10 MJ/m^3, respectively. A hydrogel based on double networks of a dynamic hydrazone bonding interaction and a covalent photo-crosslinking (thiol and norbornene) network formed a 3D construct through extrusion printing. It had self-recovery and enhanced mechanical properties [54]. To further improve the rheological properties of the hydrogel inks for extrusion printing, some nanoscale materials such as nanoclay, cellulose nanocrystals, and graphene oxide were

incorporated [55–57]. A highly stretchable and tough PEGDA/alginate hydrogel was prepared by both photo-crosslinking and Ca^{2+} crosslinking. It had a fracture toughness of approximately 1500 J/m^2, which was more than 7 times higher than that of a pure PEGDA hydrogel [55]. The nanoclay was then added as a rheology modifier for 3D printing. The PEGDA/alginate/nanoclay hydrogel ink was printed into various complex 3D constructs, which could be cellularized by infiltrating cells/collagen solution into the pores of the 3D printed PEGDA/alginate/nanoclay mesh with cell viability at 95% during 7 days of culture (Fig. 2). A sodium alginate/PAAm DN hydrogel filled with amino-graphene oxide (aGO) showed high mechanical strength, favorable toughness, thixotropic property, and 3D printing processability, where the aGO acted not only as a rheology modifier but also as a physical co-crosslinker to strengthen the alginate/PAAm DN hydrogel [57]. The aGO/alginate/PAAm hydrogel achieved a compressive stress and tensile strength of 33.2 MPa and 862.7 kPa, which were 47.4 and 35.2 times higher than those of the PAAm SN hydrogel, respectively, and 2.7 and 4.0 times higher than those of the alginate/PAAm DN hydrogel, respectively.

Nanocomposite hydrogels

In the past decades, nanocomposite hydrogels have become known as a new class of hydrogels that incorporates nanoscale materials into hydrated polymeric hydrogel s to improve their mechanical performance (Fig. 1B). A series of nanomaterials such as inorganic, polymeric, and metal/metal oxide nanomaterials have been physically blended or covalently conjugated with the polymeric network to generate nanocomposite hydrogels [58].

Inorganic nanomaterials include carbon-based nanomaterials (e.g., carbon nanotubes (CNT), graphene, graphene oxide (GO), C60, and nanodiamonds), and ceramic nanoparticles (e.g., hydroxyapatite, silica, silicate, calcium phosphate, and bioactive glass) [59–61]. Carbon-based nanomaterials can not only strengthen the mechanical properties of the hydrogel but also offer it additional electroactive and optical functions. A family of CNT-GelMA hybrid hydrogels were designed for cardiac tissue engineering and bioactuators [62]. The addition of 0 to 5 mg/mL of CNT in GelMA led to an increase in electrical conductivity and increased the compressive modulus from 10 to 32 kPa. This CNT-GelMA nanocomposite hydrogel seeded with rat cardiomyocytes showed an increased spontaneous and synchronous beating rate (3 times higher than that of the cardiomyocytes-seeded GelMA hydrogel), a partial cell alignment, and well-developed F-actin cross-striations. Although physically blending the nanoparticles into a hydrogel is straightforward for processability and manipulation [63], the covalent bindings between polymer chains and nanoparticles can further transfer the applied force within the entire network, thus largely enhancing the mechanical strength and toughness of the hydrogel [58, 64]. "Grafting-to" approaches (reactions between the functional groups of polymers and GO) and "grafting-from" methods (such as atom transfer radical polymerization (ATRP), and free radical polymerization (FRP), and *in situ* Ziegler-Natta polymerization) have been used to graft polymer chains onto GO to achieve GO nanocomposite hydrogels with enhanced mechanical properties [65–68]. On the other hand, most inorganic ceramic nanoparticles contain some minerals that naturally exist in the human body and can provide biofunctions to promote *in vivo* tissue growth. Hydroxyapatite nanoparticles were incorporated into a PEG hydrogel to form a

tough and elastomeric nanocomposite hydrogel, which supported osteoblast cell adhesion [69]. The addition of the hydroxyapatite nanoparticles to the PEG hydrogel resulted in a maximal 10-fold increase in toughness, an 8-fold increase in fracture strength, and a 3-fold increase in tensile modulus compared to the PEG hydrogel alone. Nanoclay such as Laponite (a synthetic silicate) has been used to improve the mechanical and rheological properties of a hydrogel due to the strong surface interactions between polymers and silicate nanoparticles. These interactions result in a physical crosslinking of polymer chains by silicate sheets, which allows the polymer chains to reversibly absorb and desorb on the nanosilicate surfaces and also makes the nanocomposite hydrogel become stronger and markedly shear-thinned [58, 70–72]. The ultimate stresses of Laponite/poly(ethylene oxide) (PEO) nanocomposite hydrogels increased from ~25 to ~40 MPa when the Laponite concentration was varied from 40% to 70%, and the nanocomposite hydrogel supported the growth and osteogenic differentiation of human mesenchymal stem cells [73].

Polymeric nanomaterials, especially dendritic polymers (dendrimers and hyperbranched polymers), were also used to reinforce polymeric hydrogels. Polyamidoamine (PAMAM) dendritic nanoparticles were incorporated into a collagen hydrogel to improve its mechanical stiffness and cell proliferation [74]. Additionally, these dendritic polymer-based composite hydrogels could be combined with controlled drug release functions [75, 76].

Metallic or metal oxide nanomaterials such as gold (Au), silver (Ag), iron oxide, titanium, and alumina can be combined with hydrogels to obtain nanocomposite hydrogels [77]. These metal/metal oxide nanocomposite hydrogels can not only possess enhanced mechanical properties but also exhibit additional functions such as conductivity, magnetic properties, or antimicrobial properties. A golden nanowire/alginate nanocomposite hydrogel demonstrated improved mechanical properties. Its electroactivity enhanced the electrical communications between adjacent cardiac cells and promoted the synchronous contraction of seeded cardiomyocytes with electrical stimulation [78]. The addition of Ag nanoparticles into an alginate/poly(vinyl alcohol) (PVA)/poly(N-vinyl-2-pyrrolidone) (PVP) hydrogel increased its compressive stiffness up to 115.2 kPa compared to PVA hydrogels (64.9 kPa) and Ag/alginate/PVA hydrogels (79.4 kPa), with an antimicrobial function against *Escherichia coli* [79].

Some nanomaterial-reinforced hydrogels were employed to be 3D printed (Table 1) [80–86]. A GelMA/nanosilicate hydrogel exhibited a 4-fold increase in compressive modulus and a 10-fold increase in compressive stress when the nanosilicate content levels reached 2%, compared to the GelMA hydrogel alone (Fig. 3) [84]. This GelMA/nanosilicate hydrogel supported the viability of encapsulated preosteoblasts with 4 days of culture and were 3D printed into precisely designed scaffolds. In addition, a porous osteochondral scaffold was 3D printed from a combination of poly(ethylene glycol) diacrylate (PEGDA), nanocrystalline hydroxyapatite, and PLGA nanospheres encapsulated with chondrogenic transforming growth factor-β1 (TGF-β1). The hydroxyapatite nanocrystals provided mechanical reinforcement, nanotexturization, and osteoconductivity [85]. This 3D-printed nanocomposite scaffold also supported human bone marrow-derived mesenchymal stem cell adhesion and growth, as well as osteochondral differentiation *in vitro*. A PEGDA/cellulose nanocrystal (CNC) hydrogel was also 3D printed into complex architectures, in which the

CNC from abaca plants provided the desirable strength and toughness for 3D-printable hydrogels [86]. The addition of 0.3 wt% CNC to the PEG hydrogel led to a two-fold increase in tensile strength (from 0.6±0.2 (PEG hydrogel alone) to 1.2±0.3 MPa) and a four-fold increase in fracture energy (from 6±3 (PEG hydrogel alone) to 25±14 mJ) compared to the PEG hydrogel alone.

Single-network hydrogels

Compared with the DN and nanocomposite hydrogels, the SN hydrogel is more easily handled during printing because only a single polymer and a single stimulation for crosslinking are involved, which greatly simplifies the hydrogel-precursor preparation and gelation process. Most of the current SN hydrogels still possess two crosslinking network mechanisms although they use a single polymer component (Fig. 1C). One crosslinking network is an irreversible covalent crosslinking formed by the main polymer chains to provide a mechanical stable structure. The other network is a reversible covalent/ noncovalent interaction formed by segments or side groups/branches of the main polymer chains for energy dissipation to obtain elastic and high strength hydrogels. These various dynamic covalent/noncovalent associations include hydrophobic interactions, hydrogen bonding, ionic crosslinking, and hydrazone bonds [54, 87–90]. A high-strength and elastic SN hydrogel was prepared from the photopolymerization of oligo(trimethylene carbonate)-poly(ethylene glycol)-oligo(trimethylene carbonate) (OTMC-PEG-OTMC) diacrylate, in which the OTMC blocks enabled the fracture stress and toughness to increase from 3.3±1.0 MPa and 130.2±45.4 kJ/m^3 (PEG hydrogel alone) to 5.2±1.3 MPa and 215.3±46.4 kJ/m^3, respectively [87]. Its mechanical strength and elasticity were attributed to the hydrophobic interactions between OTMC moieties in the polymer chains, which could dissipate energy when force/stress was applied to the network. Similarly, our group designed a highly elastic, photo-crosslinked polycaprolactone-poly(ethylene glycol)-polycaprolactone (PCL-PEG-PCL) diacrylate (PEG-PCL-DA) hydrogel [88], where the hydrophobic interactions between the PCL segments could dissipate energy, thus endowing the hydrogel with elasticity. The PEG-PCL-DA (PEG-PCL(24k)-DA) at a concentration of 40% had a tensile strength of 34.5±2.5 kPa, which was 8.2 times higher than that of PEG-DA at the same concentration (4.2±1.2 kPa). The irreversible deformation of PEG-PCL(24K)-DA (~20%) was only half that of PEG-DA (~45%), indicating the greatly enhanced elasticity of the PEG-PCL-DA hydrogel compared to the PEG-DA. A dual-amide structure in each monomer can further amplify the hydrogen bonding between polymer chains, thus greatly enhancing the mechanical properties of the hydrogel. A photo-crosslinked, high-strength, and self-healable SN hydrogel synthesized from a monomer of *N*-acryloyl glycinamide (NAGA) with two amides showed a tensile strength of 160 kPa-1.1 MPa, an elongation at break of 600–1400%, and a Young's modulus of 50–150 kPa[89]. A dual-crosslinked SN hydrogel, poly(acrylamide-co-acrylic acid) (poly(AAm-co-AAc)) with an ion Fe^{3+} addition exhibited high strength, toughness, and good self-recovery. In this hydrogel, the polymerization between AAc and AAm formed a covalent crosslinking network, and the interaction between Fe^{3+} and the AAc carboxyl groups produced the other ion-crosslinking network [90]. The Fe^{3+} crosslinking network resulted in a dramatic increase in tensile strength and toughness from 100 kPa and 0.4±0.06 MJ/m^3 (poly(AAm-co-AAc) hydrogel alone) to 5.9

MPa and 27.8±1.01 MJ/m^3 (poly(AAm-co-AAc) hydrogel/Fe^{3+}), respectively. In some cases, a high-strength SN hydrogel can even be obtained using only a single dynamic crosslinking network. For example, high shear-thinning and self-recovery properties of the hydrogel were realized only through dynamic covalent bonds (hydrazone bonds) between hydrazide-modified HA and aldehyde-modified HA [54].

The SN hydrogels have been proved to be 3D printable (Table 1). The PEG-PCL-DA hydrogel was printed with various human cells with a cell viability of more than 83% after 7 days of culture and was also able to be printed into complex patterns (Fig. 4) [88]. A series of methacrylated HA (MeHA)-based SN hydrogels with supramolecular interactions were prepared using a single stimulation of photo-crosslinking with dual networks, including one photo-crosslinking network from MeHA, and one network from a GH assembly between β-cyclodextrin (β-CD) and adamantane (Ad) moieties on the MeHA polymer chains [91]. These SN hydrogels were printed into multilayer constructs and maintained stable for a month of incubation in PBS at 37 °C without a significant reduction in compressive modulus (from 20.8±2.0 to 16.7±1.9 kPa after 30 days, when the concentrations of MeHA-Ad and MeHA-β-CD were both 7.5%). Notably, some HA-based SN hydrogels with single dynamic crosslinking could also be printed due to their good shear-thinning properties [54, 92].

Methods to improve the elastic hydrogel printability and biocompatibility

Ideal hydrogel inks for extrusion-based 3D printing should meet the following requirements: (i) the inks should have good printability to enable a 3D structure with shape integrity and fidelity; (ii) the hydrogels can provide sufficient mechanical strength to support the printed structure after deposition; (iii) the interfacial strength between the printed layers should be high enough to prevent delamination; and (iv) the hydrogel components, reactions, and printing parameters should be safe for loaded cells and bioactive molecules [54, 93, 94].

Many studies have investigated how altering the components, concentrations, and printing parameters of hydrogel inks affects their printability [95–97]. For example, the printability of twelve commonly used hydrogels, such as collagen, chitosan, and alginate, were systematically evaluated [96]. It was found that the hydrogel inks with a high viscosity (e.g., chitosan, chitosan-collagen, and methylcellulose-hyaluronan) or inks with fast gelling properties (e.g., Extracel™ hydrogel, Extracel™ UV, and PEGDA) showed relatively high printing accuracy. A gelatin-based hydrogel was 3D printed with embryonic stem cells (ESCs) [95], and it was demonstrated that a higher gelatin concentration and a lower printing temperature resulted in a gelatin solution with a higher viscosity and better printability. Hence, increasing the viscosity and gelation rate of the hydrogel ink can improve its printability. A high viscosity can prevent the hydrogel inks from spreading out after deposition, and a fast gelling property can solidify the extruded hydrogel filaments quickly and preserve the printed shape [98, 99]. However, during printing, an increase in the viscosity of the ink causes increase in shear stress, the major cause of cell damage in extrusion-based cell printing [15, 100]. Additionally, highly viscous hydrogel ink may also cause handling and extrusion difficulties and fractured printing filaments, consequently compromising the printability of the ink [95, 96]. Thus, optimizing the viscosity of the hydrogel ink is one of the most crucial factors to be considered for the inks used for

extrusion-based 3D printing applications, especially for cell-laden extrusion-based 3D bioprinting. High shear-thinning hydrogel inks are appropriate candidates. Their viscosities decrease with increase in shear stress when pressure is applied to a nozzle, thereby facilitating their deposition. After the inks go through the nozzle, the shear stress is removed, and the viscosity of the ink increases rapidly to retain its printed shape [101, 102]. Reversible interactions between polymer chains in the hydrogels can not only provide desirable mechanical properties but also enhance shear-thinning to improve gel printability, as discussed above. For example, GH-assembled hydrogels have desirable shear-thinning properties because the GH interaction can be disrupted by shear stress during 3D printing and recover immediately after extrusion, which has been proved to limit ink collapse upon printing and result in thinner printed filament [91, 92, 103]. Nanoclays incorporated into hydrogel inks also improve their shear-thinning properties, as these polymer chains can reversibly absorb and desorb on the nanoclay surfaces under shear stress [55, 83]. Additionally, the dynamic hydrazone bonds can also enable ink shear-thinning during extrusion and maintain a high shape fidelity [54]. Thus, introduction of multiple reversible interactions into the hydrogel ink system is a promising way to improve the printability of the hydrogel ink and increase its mechanical strength and elasticity.

3D bioprinting advances tissue-engineered product manufacturing by depositing live cells, inks, and bioactive molecules layer-by-layer to form the desired cellularized constructs with specific structures [15]. However, it is challenging to maintain cells with high viability and functionality inside hydrogel inks during/after printing [104]. The biological requirements for hydrogel inks mainly include cytocompatibility and bioactivity [105]. For cytocompatibility, the ink and its degradation products should be nontoxic to cells *in vitro* and not cause any immunological response to the host *in vivo.* Most high-strength elastic hydrogels based on natural/synthetic polymers (such as PEG, PEO, HA, and GelMA) possess good cytocompatibility [36, 55, 62, 73]. Shear stress might be one of the main factors for cellular damage induced during the bioprinting process [15]. Altering the properties of the hydrogel inks or printing parameters, such as by increasing ink viscosity and extrusion speed or decreasing the nozzle diameter, can result in a shear stress increase, which would cause cell damage. However, the high viscosity of the ink is beneficial for shape fidelity as discussed above, and a nozzle with a small diameter can enhance the printing resolution. Both factors contribute to a high printing quality. Thus, there exists a balance between ink printability and cell viability. Some studies have evaluated ink properties and optimized printing parameters to achieve both good printability and high cell viability [95, 100, 106–109]. These studies demonstrated that the cell survival rate after printing is closely tied to the viscosity of the hydrogel ink and the shear stress produced during printing. Thus, improving the ink shear-thinning behavior and cell survival rate must be simultaneously considered. Additionally, the crosslinking methods are another important factor that cannot be neglected for cell survival. For photo-crosslinking, photo-initiators and light irradiation (such as UV) can induce toxicity to kill and damage cells during the printing process. Thus, selecting biocompatible chemicals and a safe light wavelength (such as visible light) and reducing the irradiation time would improve cell survival. Furthermore, incorporating bioactive molecules (e.g., hydroxyapatite and bioactive peptide) into the hydrogel is a feasible and simple way to greatly enhance the bioactivity of 3D-printed

hydrogels [82, 85, 91, 110, 111]. An alginate-bone formation peptide-1 (BFP-1) SN hydrogel was prepared by covalently conjugating the BFP-1, a 15-amino acid peptide derived from bone morphogenetic protein-7 (BMP-7), to alginate and then processed into a porous scaffold under Ca^{2+} crosslinking using printing [111]. This 3D-printed alignate-BFP-1 scaffold supported the growth of human adipose-derived stem cells and promoted bone regeneration in a rabbit calvarial defect model. Because of the friendly environment of the hydrogel, the incorporated bioactive molecules could maximally maintain their bioactivity, which was not necessarily a significant concern. However, such molecule introduction may change the mechanical properties and shear-thinning behavior of the hydrogel, which must be optimized before printing.

Some of the high-strength elastic hydrogel inks have been printed into 3D constructs and then cultured with cells *in vitro* [55, 85, 91]. However, only a few were directly printed with live cells in the inks [88, 93]. A PEG-PCL-DA hydrogel was directly printed with various human cells and showed more than 80% cell survival after printing and 7 days of cell culture [88]. An alginate/methylcellulose DN hydrogel was printed with mouse fibroblast L929 cells and exhibited high cell viability (more than 95%) after 5 days of culture [93]. Some 3D-printed high strength and elastic hydrogels, such as PNAGA/nanoclay, collagen/hydroxyapatite, and alignate-BFP-1 hydrogels, have been used for bone regeneration in rat and rabbit bone defect models [83, 110, 111]. However, it is rarely reported that the 3D-printed hydrogels were evaluated for mechanically active soft tissue repair in animal models, which may be attributed to the low mechanical strength and brittleness of the hydrogels. The cell-laden printed hydrogels and their *in vivo* functional evaluations were rarely seen compared to the solid polymer-printed scaffolds. It is expected that the development of high strength and elastic hydrogels will provide new opportunities to bridge such gaps.

Summary and future perspectives

Many studies aim to improve the strength and elasticity of the hydrogel materials to broaden their applications in 3D printing and tissue engineering. Compared to the natural or synthetic hydrogel systems that are fragile and weak, these new high strength and elastic hydrogels should have tremendous potential for biomedical applications, especially in constructing soft and elastic tissues such as skin, skeletal muscle, and blood vessels. The strategies for designing high strength and elastic hydrogels are to introduce multiple covalent/noncovalent interactions not limited to two networks into the hydrogel system. These general principles of hydrogel development also need to be further investigated.

To improve the printability of these high strength hydrogels, various nanoscale additives can be incorporated into the hydrogel to enable the shear-thinning behaviors suitable for extrusion-based 3D printing. These interactions can also increase the mechanical properties of the hydrogels and maintain the desired 3D shapes. Another consideration is the biocompatibility and bioactivity of the hydrogels. Selecting biocompatible material-based hydrogel systems (such as gelatin and PEG) and incorporating bioactive molecules into the hydrogel are reasonable and feasible solutions because these materials and methodologies have been extensively investigated and often used in research and clinics. However, improving the printability and printing parameters (such as speed, nozzle size, and pressure)

of the inks to maximally maintain cell survival remains a challenge for cell printing, which requires further optimization of these factors and development of new printing techniques to solve such problems.

Despite substantial progress, the availability of new types of high strength and elastic hydrogels remains limited. In addition, these elastic hydrogels need to be further evaluated for 3D printing and tissue repair use, and there are few *in vivo* studies evaluating their performance. In the future, through a deeper understanding of the general principles of elasticity and strengthening of the hydrogels, new chemical and physical structures are expected to be designed to produce a new generation of high strength and elastic hydrogels for 3D printing, including cell printing. The current elastic hydrogel systems can also be further optimized and modified to improve their bioprintability and performance *in vitro* and *in vivo* and broaden their biomedical applications in tissue repair and regeneration.

Acknowledgment

Abbreviations

AAc	Acrylic acid
AAm	Acrylamide
Ad	Adamantane
AMPS	2-Acrylamido-2-methylpropanesulfonic acid
ATRP	Atom transfer radical polymerization
BFP-1	Bone formation peptide-1
BMP-1	Bone morphogenetic protein-7
CNT	Carbon nanotubes
CNC	Cellulose nanocrystal
CD	Cyclodextrin
CLIP	Continuous liquid interface production
DGI	Dodecyl glyceryl itaconate
DN	Double network
DLP	Direct light processing
DMLS	Direct metal laser sintering
EBM	Electron beam melting

ELP	Elastin-like protein
ECM	Extracellular matrix
FRP	Free radical polymerization
FDM	Fused deposition modeling
GH	Guest-host
GO	Graphene oxide
GelMA	Methacrylated gelatin
HA	Hyaluronic acid
IPN	Interpenetrating polymer network
LIFT	Laser-induced forward transfer
MeHA	Methacrylated hyaluronic acid
MJM	Multijet modeling
NAGA	*N*-Acryloyl glycinamide
NIPAM	*N*-Isopropylacrylamide
PAMAM	-Polyamidoamine
PCL	Polycaprolactone
PVA	Poly(vinyl alcohol)
PVP	Poly(*N*-vinyl-2-pyrrolidone)
PEG	Poly(ethylene glycol)
PEO	Poly(ethylene oxide)
PEGDA	Poly(ethylene glycol) diacrylate
SLA	Stereolithography
SLS	Selective laser sintering
SLM	Selective laser melting
SN	Single network
TMC	Trimethylene carbonate
WDW	Wax deposition modeling
WID	Direct ink writing

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Statement of significance

Biocompatible and biodegradable hydrogels are highly attractive in 3D printing because of their desirable printability and friendly environment for loading bioactive molecules and living cells. The development of high strength and elastic hydrogels changes the conventional impression of weak and brittle hydrogels and provides new opportunities and inspirations for 3D printing and biomedical applications. In this review, we analyzed the hydrogel reinforcement mechanisms, summarized recent progresses in developing high strength and elastic hydrogels for 3D printing, and discussed the strategies to improve the printability and biocompatibility of the hydrogel inks.
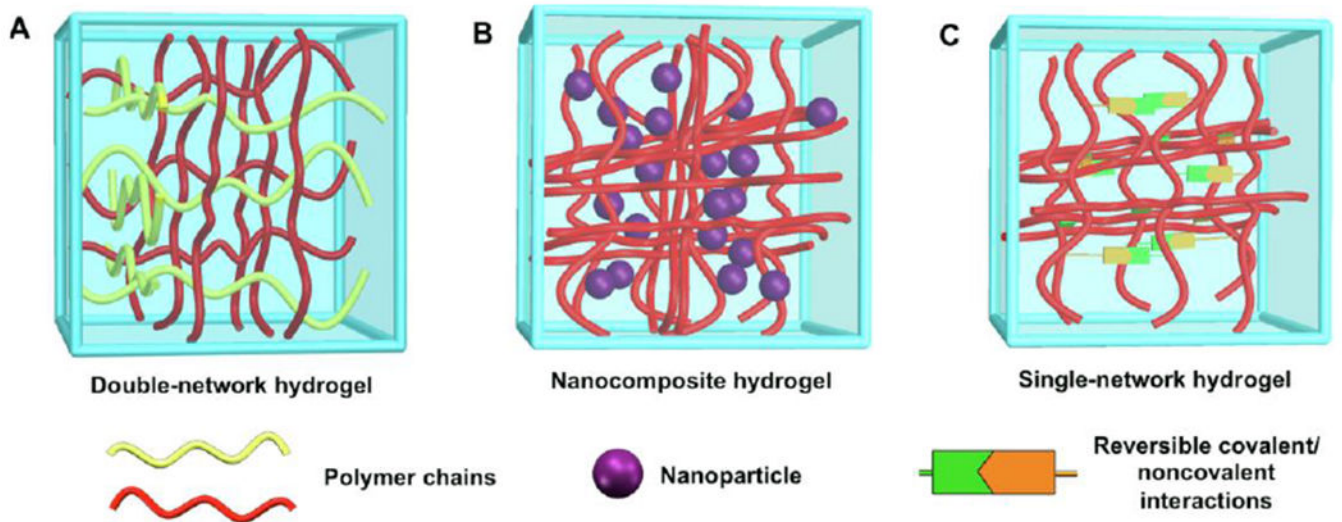
Fig. 1.
A schematic illustration of high-strength and elastic hydrogels with three major reinforcing mechanisms: (A) a double-network hydrogel consisting of two polymer components-based networks, (B) a nanocomposite hydrogel consisting of nanomaterials and one polymer network, and (C) a single-network hydrogel consisting of a single polymer component network with additional reversible interactions.
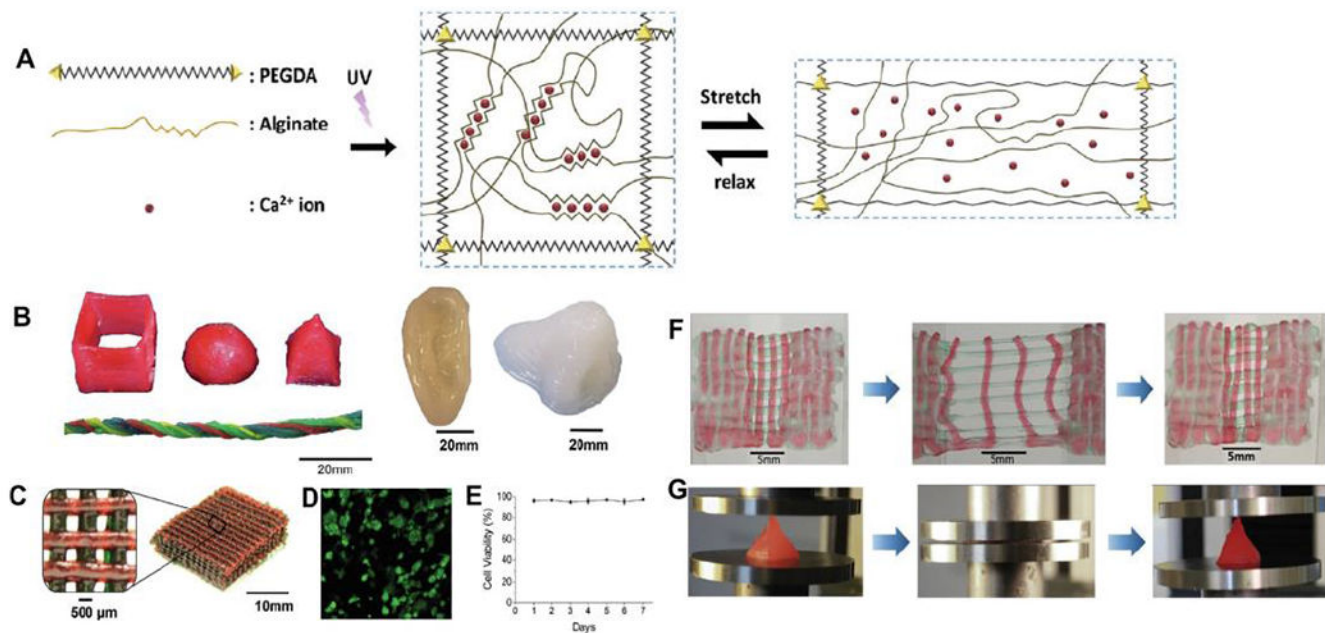
Fig. 2.
A double-network hydrogel from PEGDA and alginate. (A) Illustration of the PEGDA/
alginate DN hydrogel with covalently (UV irradiation) and ionically (Ca^{2+}) crosslinked
networks. (B) 3D-printed samples with PEGDA/alginate/nanoclay hydrogel. The nanoclay
addition is to improve the shear-thinning behavior. (C) A mesh printed with the PEGDA/
alginate/nanoclay hydrogel. (D) Live-dead staining and (E) viability of host human
embryonic kidney (HEK) cells in a collagen hydrogel infused into the 3D-printed mesh. (F)
Stretchability of a printed bilayer mesh from PEGDA/alginate/nanoclay hydrogel. (G)
Compression and recovery of a printed pyramid. Reprinted with permission from [55].
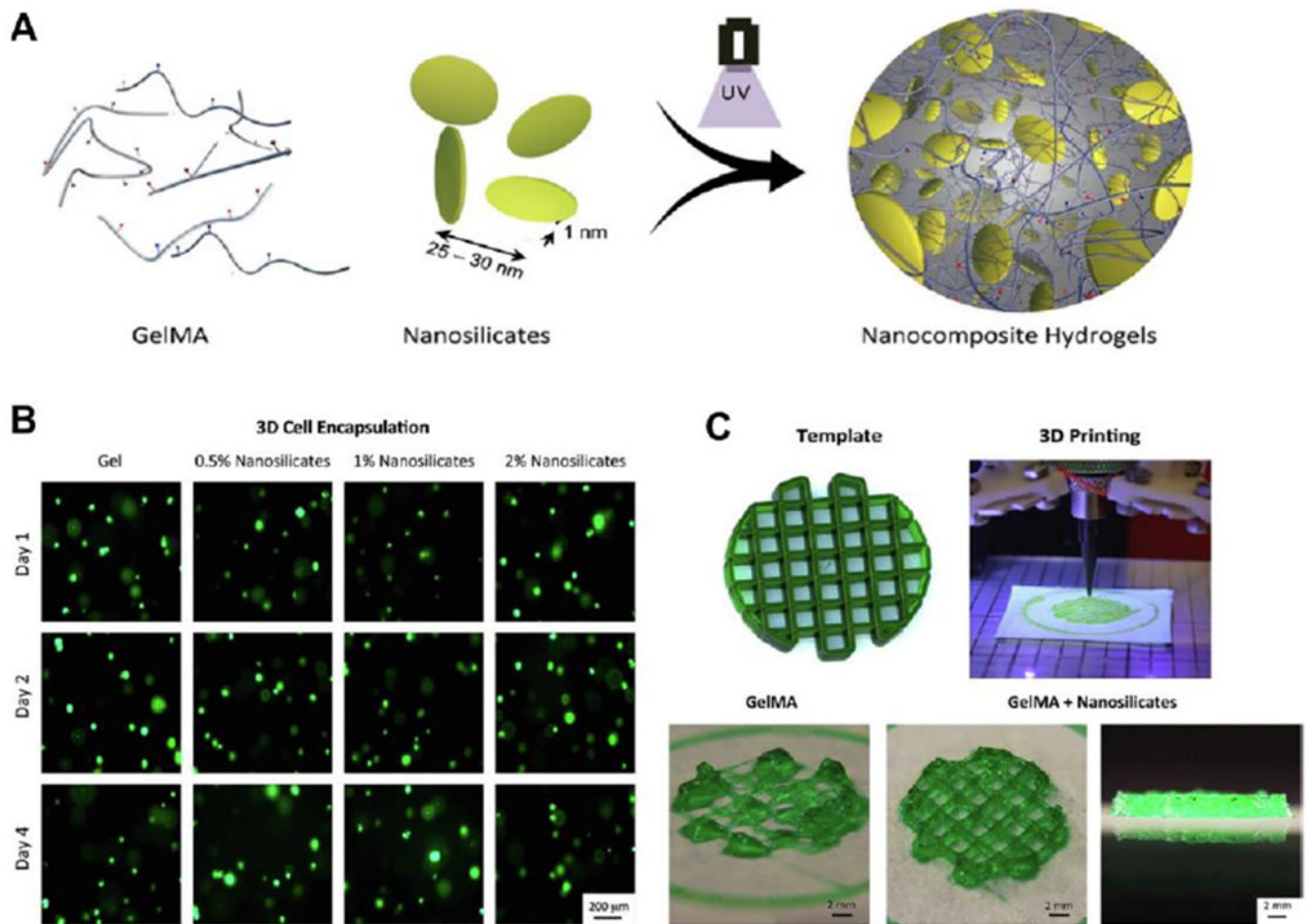Copyright 2015 Wiley-VCH.

Fig. 3.
A nanocomposite hydrogel from GelMA and nanosilicate. (A) Schematic preparation of the GelMA/nanosilicate hydrogels through covalently crosslinking with UV exposure. (B) Preosteoblast survival after encapsulation in the nanocomposite hydrogels was shown by live/dead staining. (C) 3D printed constructs from the GelMA/nanosilicate hydrogel showed desirable size and shape stability. Adapted with permission from [84]. Copyright 2015 American Chemical Society.
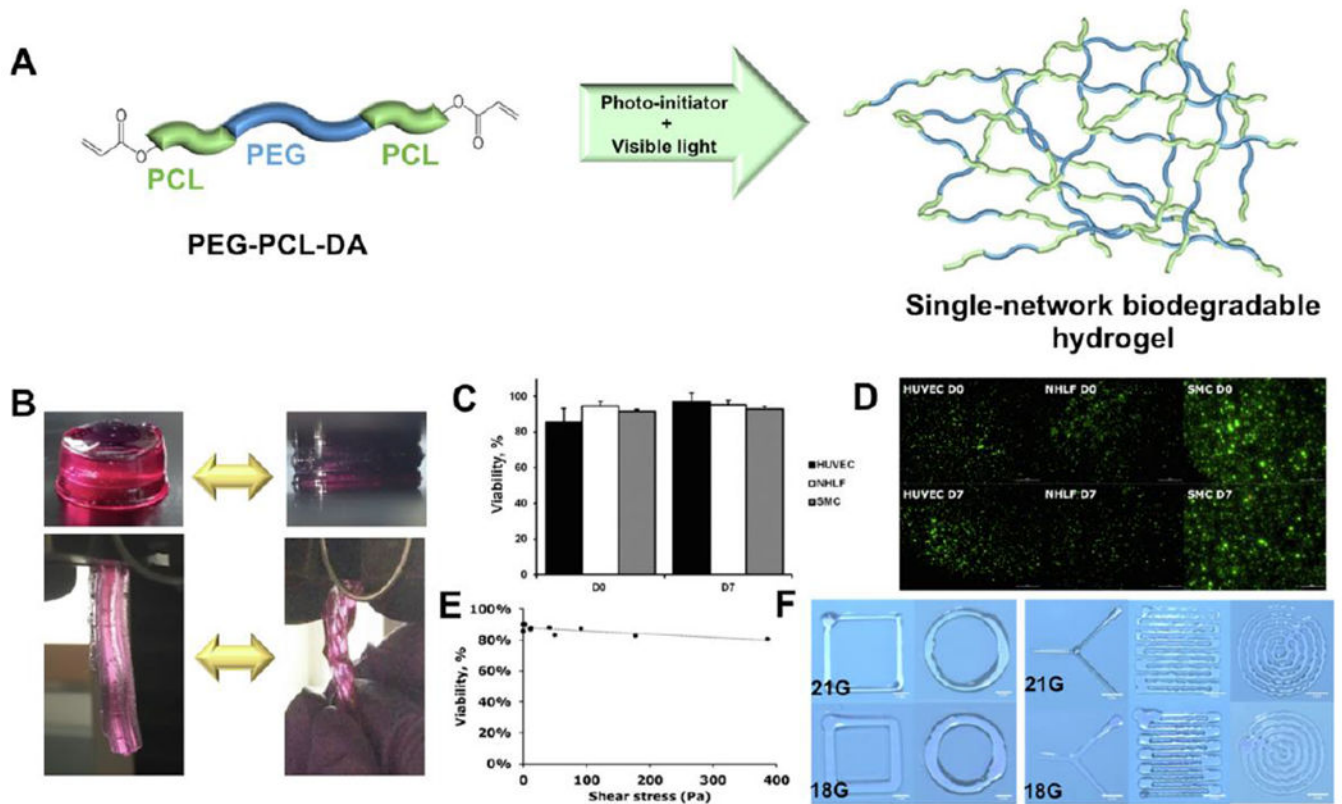
Fig. 4.
A single-network elastic hydrogel from a triblock copolymer of PEG-PCL-DA. (A) The preparation of elastic PEG-PCL-DA SN hydrogel by covalently crosslinking under visible light. (B) The hydrogel can be compressed and twisted, and then recovered. (C-D) Cell viability of 3 different human cells in 10% elastic PEG-PCL(24K)-DA hydrogel through 7 days of culture. (Scale bars = 500 μm). (E) Effect of shear stress on cell viability evaluated immediately after printing. (F) Complex patterns were printed using different needle sizes. Reproduced with permission from [88]. Copyright 2018 American Chemical Society.

Table 1.

Summary of high-strength and elastic hydrogels for 3D printing

	Hydrogel components	Reinforcing factors #	Application	References
Double-network hydrogel	Alginate/Poly(acrylamide-co-acrylic acid)	Ionic crosslinking	- *	[40]
	κ-Carrageenan/PAAm	Ionic crosslinking	Strain sensor	[53]
	HA-Aldehyde/HA-Hydrazide/ Norbornene-HA	Hydrazone bond	-	[54]
	Alginate/PEGDA/Nanoclay	Ionic crosslinking	-	[55]
	Alginate/Gelatin/Cellulose nanocrystals	Ionic crosslinking	-	[56]
	Alginate/PAAm/amino-GO	Ionic crosslinking	-	[57]
Nanocomposite hydrogel	PNIPAM/GO/Laponite	Laponite/GO	-	[80]
	HA/Gelatin/Au nanoparticles/	Au nanoparticles	-	[81]
	Alginate/nanocellulose	Nanocellulose	Wound dressing	[82]
	PNAGA/Nanoclay	Nanoclay	Bone regeneration	[83]
	GelMA/Nanosilicate	Nanosilicate	Bone tissue engineering	[84]
	PEGDA/PLGA nanosphere/ Nanocrystalline hydroxyapatite	Nanocrystalline hydroxyapatite	Osteochondral tissue repair	[85]
	PEGDA/Cellulose nanocrystal	Cellulose nanocrystal	-	[86]
Single-network hydrogel	PEG-PCL-DA	Hydrophobic interactions	-	[88]
	β-CD-MeHA/Ad-MeHA	Guest-host interaction	-	[91]

*No specific application is mentioned in the reference.