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REVIEW

3D Printing of Hybrid-Hydrogel Materials for Tissue Engineering: a Critical Review

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Abstract

Purpose Key natural polymers, known as hydrogels, are an important group of materials in the design of tissue-engineered constructs that can provide a suitable habitat for cell attachment and proliferation. However, in comparison to tissues within the body, these hydrogels display poor mechanical properties. Such properties cause challenges in 3D printing of hydrogel scaffolds as well as their surgical handling after fabrication. For this reason, the purpose of this study is to critically review the 3D printing processes of hydrogels and their characteristics for tissue engineering application.

Methods A search of Google Scholar and PubMed has been performed from 2003 to February 2022 using a combination of keywords. A review of the types of 3D printing is presented. Additionally, different types of hydrogels and nano-biocomposite materials for 3D printing application are critically reviewed. The rheological properties and crosslinking mechanisms for the hydrogels are assessed.

Results Extrusion-based 3D printing is the most common practice for constructing hydrogel-based scaffolds, and it allows for the use of varying types of polymers to enhance the properties and printability of the hydrogel-based scaffolds. Rheology has been found to be exceedingly important in the 3D printing process; however, shear-thinning and thixotropic characteristics should also be present in the hydrogel. Despite these features of extrusion-based 3D printing, there are limitations to its printing resolution and scale.

Conclusion Combining natural and synthetic polymers and a variety of nanomaterials, such as metal, metal oxide, non-metal, and polymeric, can enhance the properties of hydrogel and provide additional functionality to their 3D-printed constructs.

Lay Summary Essential natural materials, known as hydrogels, are important for the design of tissue-engineered constructs that allow for cells to attach and grow while receiving the nutrients necessary for growth as well. However, in comparison to the cells in the body, the hydrogels have poor functional properties. Because of this, the purpose of this study is to assess the 3D printing processes of the hydrogels, and the appropriate characteristics of the hydrogels that allow this material to be useful in tissue engineering processes. A review of the types of 3D printing is presented, and the different types of materials used for 3D printing are also assessed. Lastly, the properties of hydrogels that make them ideal for 3D printing are reviewed. The most common type of 3D printing is extrusion-based 3D printing. It allows for the use of different types of natural materials to enhance the properties and printing ability for the hydrogel-based cellular environment. The flow of a material is found to be important in 3D printing as well as the consistency and capability of a material. However, there are challenges to the 3D printing quality and size for extrusion-based 3D printing. Overall, different 3D printing techniques should be created to enhance the construction capabilities of 3D printing to meet the most favorable environment for cellular growth and attachment.

Keywords 3D printing · Hydrogel scaffold · Tissue engineering · Hybrid-hydrogel materials · Hydrogel 3D printing

Introduction

Tissue engineering aims to develop three-dimensional (3D) functional tissue and organ using combinations of biomaterials, cells, and/or bioactive molecules [1–3]. 3D printing, or additive manufacturing, is an advanced process for the

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generation of 3D structures [4]. 3D printing technology compared to the traditional processing methods can manufacture very complex structures with computer-aided design and manufacturing technologies [5]. The 3D printing processes of polymers [6], ceramics, and metals [7, 8] have achieved great success. Recently, 3D printing has also shown great advantages in the preparation of tissue-engineering scaffolds. Some scaffolds with complex structures can be designed and customized, for example, based on the medical images obtained through computed tomography. Hydrogels are composed of a polymeric structure that is highly hydrated and can be modified to respond to different external stimuli, including temperature, light, and biological signals [9]. These distinguishing characteristics make hydrogels ideal habitats for cell attachment and proliferation within their hydrated hydrogel networks.

3D-printed hydrogels have many diverse uses from robotic to medical applications [10–13]. Since hydrogels provide ample space for cell growth while facilitating the delivery of necessary metabolites and nutrients to the encapsulated cells, 3D-printed hydrogels play an important role in scaffold fabrication for tissue engineering applications [14]. 3D hydrogel and cell printing, known as biofabrication, can be utilized to produce living organisms. However, in comparison with tissues that are found naturally such as ligaments and cartilage, most hydrogels exhibit poor mechanical strength. Improvements in hydrogel's mechanical properties and bioactivity are therefore a challenge for material scientists. One of the most efficient ways of integrating and combining various mechanical properties and functions that cannot be achieved by a single hydrogel is to use a hydrogel composite system. Physical interactions with the hydrogel matrix are the basis for traditional inorganic reinforcements. These physical interactions create strong adhesion between the matrix of reinforcements and the hydrogel, while the quantity, size, and shape of reinforcements increase hydrogel properties. Chemical groups and the formation of covalent bonds at the interface result in superior interfacial bonding strength (40,400 kJ/mol), which is commonly greater than the physical interaction (816 kJ/mol) [15]. This results in improved strength of the hydrogel composite system, and an enhanced printability of hydrogel composites, extending the use of 3D printing in hydrogels.

In general, the main advantage of using hydrogels is the significant biocompatibility of this class of material [16, 17]. Because of their perfect cell-attractant property, some widely used materials for biomedical applications are hydrogels, such as gelatin and collagen, which explains the necessity of considering the 3D printing of hydrogels as an important subject. However, the main challenge related to their printing procedure are their rheological characteristics [16]. For instance, challenging printing process of gelatin-based ink because of its premature gelation can result in constructs with low quality [18], although some good 3D-printed gelatin constructs have also been reported recently [19–21].

In this article, additive manufacturing processes of hydrogel and hydrogel composites for tissue engineering application will be reviewed first. Following that, the structure–property relationships of different hydrogels will be discussed in-depth (based on natural polymers and synthetic polymers), and a variety of nanomaterials such as metal, metal oxide, and polymeric nanomaterials incorporated into hydrogel matrix to improve their properties. Furthermore, the role of hydrogel properties (viscosity, shear thinning, and yield stress) in 3D printing control will be addressed. Additionally, this article is concluded by discussing some challenges of 3D printing hybrid-hydrogel materials.

Additive Manufacturing Processes of Hydrogels

3D printing is a novel technology for custom 3D structures through the layer-by-layer deposition of materials. In 1981, stereolithography was introduced as the original 3D printing technology, and then in 1992, fused deposition modeling (FDM), a type of extrusion-based 3D printing, was invented [22]. 3D printing of hydrogel can be classified into three different categories: 3D printing based on extrusion, 3D printing based on inkjet printers, and 3D printing based on lasers. The different methods of 3D printing have both advantages and disadvantages. Detailed information for each technology is listed below.

Extrusion-Based 3D Printing

The most common method for fabricating hydrogel-based scaffolds is extrusion-based 3D printing. Sol or viscous liquids are extruded from a nozzle using force and solidified into a stage. 3D structures are built layer by layer using sequential extrusion of materials following predesigned computer modeling. Interlayer adhesion plays a vital role in successful 3D printed structures. As a result, different hydrogel parameters like solidification temperature, shear thinning, thixotropy, and the gelling mechanism are fundamental.

Inkjet 3D Printing

Thermal inkjet printing and piezoelectric inkjet printing could be classified as 3D inkjet printing. A thermally induced inkjet printer makes use of a heater to heat the surrounding ink and generate rapidly expanding vapor

bubbles, resulting in expulsion of ink droplets from the print head. Then, piezoelectric actuators apply pulses to extrude ink out of the chamber. Multilayered droplets and tiny 3D structures have been created using inkjet 3D printing. While inkjet 3D printing possesses numerous benefits, such as high spatial resolution (40–400 µm) and fast print speeds (5000 drops/s), it is not appropriate to print complex and large biofabrication constructs because this technology could only produce small droplets.

Laser-Based 3D Printing

Laser-based 3D printing creates 3D structures under laser energy deposition in a tank of photocurable hydrogels, in precisely built patterns [23]. In this process, UV radiation induces radical polymerization or crosslinking of a polymer within a monomer reservoir. A thin single layer of gel forms on the surface of photocurable liquid after UV exposure, which is progressively pushed upward or downward with the sample stage to allow the next layer to develop on top of previous layers. As a result, designed 3D structures are materialized directly in the liquid vat. The laser-based 3D printing offers many advantages. First, it is simple to produce high-resolution droplets (50 ~ 60 µm). In particular, two-photon polymerization will create constructs with a resolution of about 100 nm. Second, due to the lack of mechanical forces during printing, high cell viability (about 95%) can be achieved. Lastly, due to the lack of direct contact with the inks and the dispenser, there is a reduced risk of contamination.

Hydrogels for 3D Printing Application

Biocompatibility and degradability make physical hydrogels based on natural polymers widely used for tissue engineering [24–26]. Physical hydrogels, however, are generally weak by nature; therefore, the printed filaments easily spread. Alternatively, synthetic hydrogels often show poor biocompatibility and non-natural degradation but can be prepared with enhanced mechanical properties. Hence, producing biocompatible hydrogels with enhanced mechanical properties and degradability by a 3D printing technique is challenging. Many researchers have employed hydrogel composites to overcome this limitation. In the following sections, the 3D printability of different hydrogels is primarily reviewed and the latest advances in the design of hydrogels and hydrogel composites for complex 3D structures with high fidelity through a 3D print strategy are presented.

Natural Polymers

Hydrogels made from natural polymers, including proteins and polysaccharides (Fig. 1), are the most attractive biofabrication inks as the swollen networks allow oxygen permeability to provide a highly hydrated environment of cell proliferation, nutrients, and water-soluble metabolites. The various gelation mechanisms derived from various natural polymer molecular structures create specific 3D network structures and gel properties that determine the 3D printability of the resulting hydrogels. As a result, the primary focus will be on natural polymer gelation mechanisms in aqueous solution, as well as the

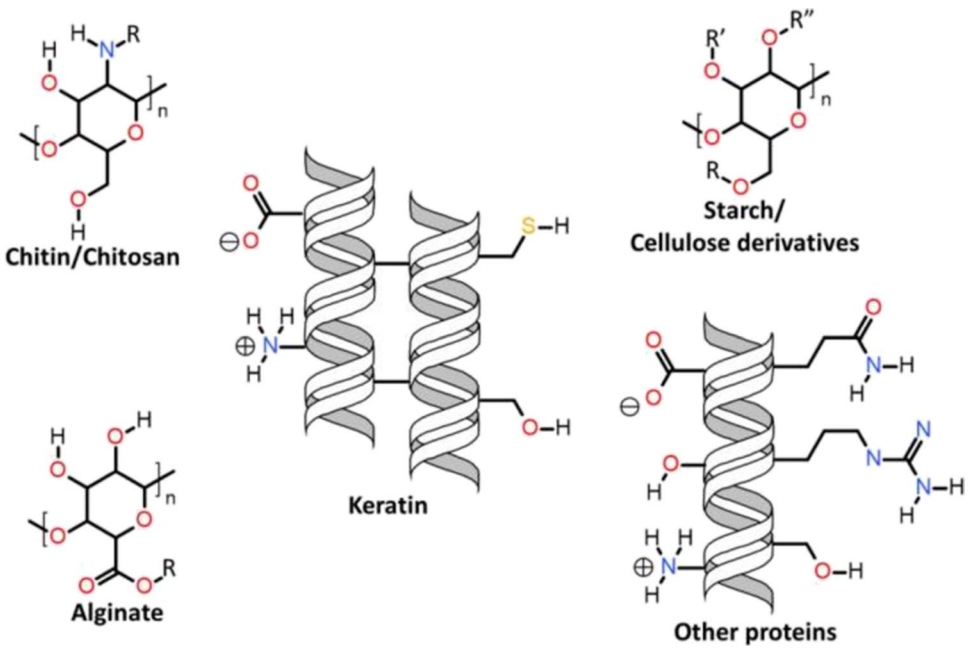

Fig. 1 Schematic representation of the natural polymers. Obtained from reference [27] distributed under the Creative Commons Attribution

structure-property relationship of natural polymer hydrogels and their impact on 3D printing.

Collagen

Collagen is a key component of the extracellular matrix; thus, collagen has been commonly utilized in the past decade for bio-application. There are several collagen types, but the most common is Type I with triple-helical structures that can bundle into fibrils. Through its self-assembling behavior, Type I collagen may be used as a hydrogel for tissue engineering. It is agreed that the gelation of collagen involves three steps: (1) fibril formation from triple helices; (2) linear fibril growth; (3) linear fibril network structure formation [28]. Yang and his colleagues developed 3D-printed scaffolds by mixing Type I collagen with sodium alginate to act as 3D printing inks, and the resulting alginate/collagen significantly increased gene expression, facilitated cell adhesion, and increased cell proliferation [29]. Recently, the printability of collagen hydrogels at various temperatures and the gelation behavior of collagen during heating have been thoroughly studied. As illustrated in Fig. 2, a structurally stable, highly porous, and biocompatible block was created by adjusting processing parameters, concentration, and crosslinking [30].

Gelatin

As a derivative of collagen, gelatin maintains the collagen structure and facilitates cell adhesion and proliferation. Gelatin can dissolve at high temperatures in an aqueous solution to form a sol. It forms a gel with decreasing temperature, as shown in Fig. 3 [31].

Alginate

Alginate aqueous solution becomes solid-like gel in the presence of divalent cations [32]. Due to the coordination interaction between G blocks and divalent cations, the association of the G blocks of different polymer chains with divalent cations results in the creation of a 3D gel network. Because of its high biocompatibility and ease of processing in 3D printing, alginate is the most widely utilized hydrogel in biofabrication [33]. The alginate hydrogel, however, is often mechanically weak, leading to the collapse of patterns built in 3D. To produce greater quality 3D printings, many approaches have been used to increase the 3D printability of alginate hydrogel. Li and colleagues discovered that increasing the amount of the ionic crosslinker (Ca^{2+}) and graphene oxide in hydrogel alginate can improve pattern fidelity. However, the divalent cations in the junctions might progressively dissolve into the cell culture medium, resulting in poor stability of the printed alginate hydrogel

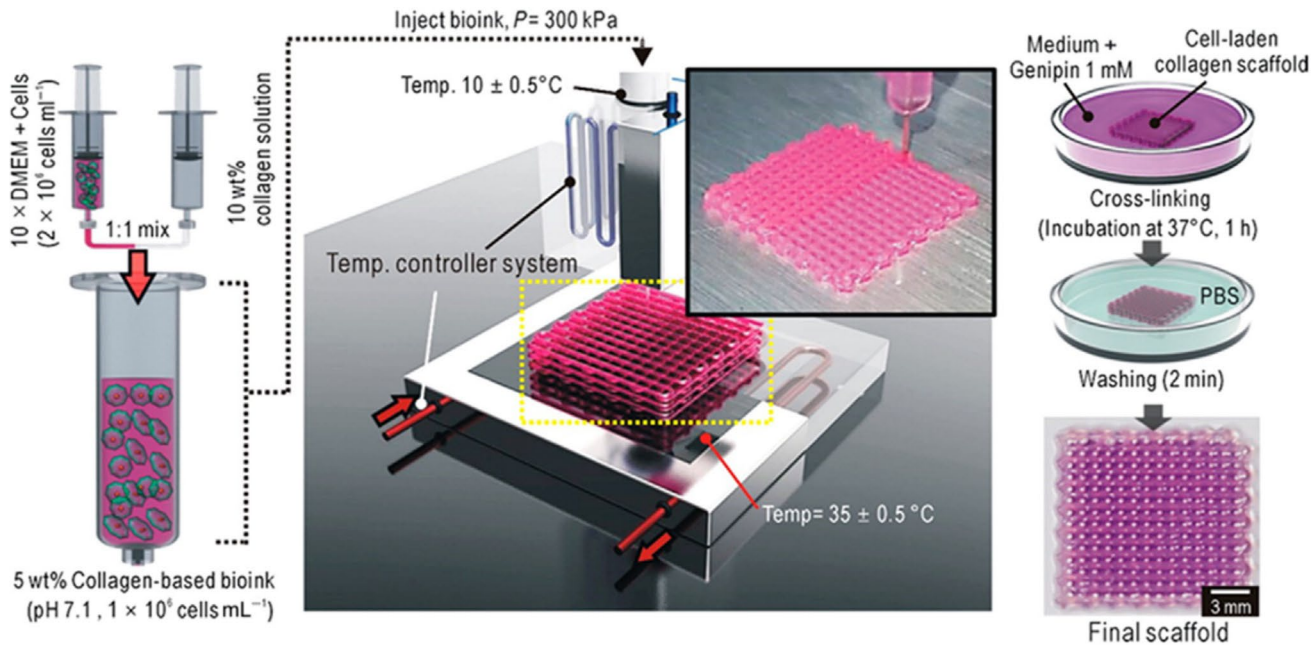

Fig. 2 3D fabrication of a collagen hydrogel mesh incorporating porous cells and temperature controls. Obtained from reference [30] with permission

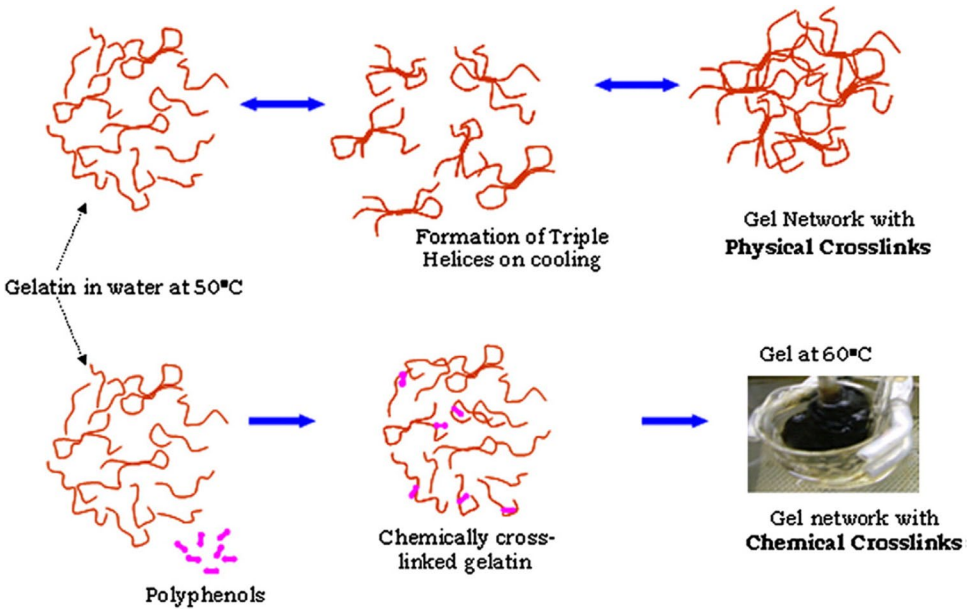

Fig. 3 Schematic representation of physical gelation and gelation after phenolic crosslinking. Obtained from reference [31] with permission

scaffolds. Jia and colleagues also discovered that in vitro growth medium quickly reduced the mechanical power of alginate hydrogels [34]. Gao and colleagues recently developed a coaxial bioprinting strategy to create the hollow alginate filament (Figs. 4 and 5) [35].

This strategy is based on the hollow calcium alginate filaments by employing a coaxial nozzle. More specifically, 3D structures of high-strength cell-laden hydrogel with built-in microchannels were made through fusion of neighboring hollow filaments by regulating the crosslinking time sequence [35].

Synthetic Polymers

Synthetic polymer hydrogels have a wide range of properties due to differences in chemical structures, production processes, and water content or crosslinking. New smart biomaterials can be created by changing the chemical composition

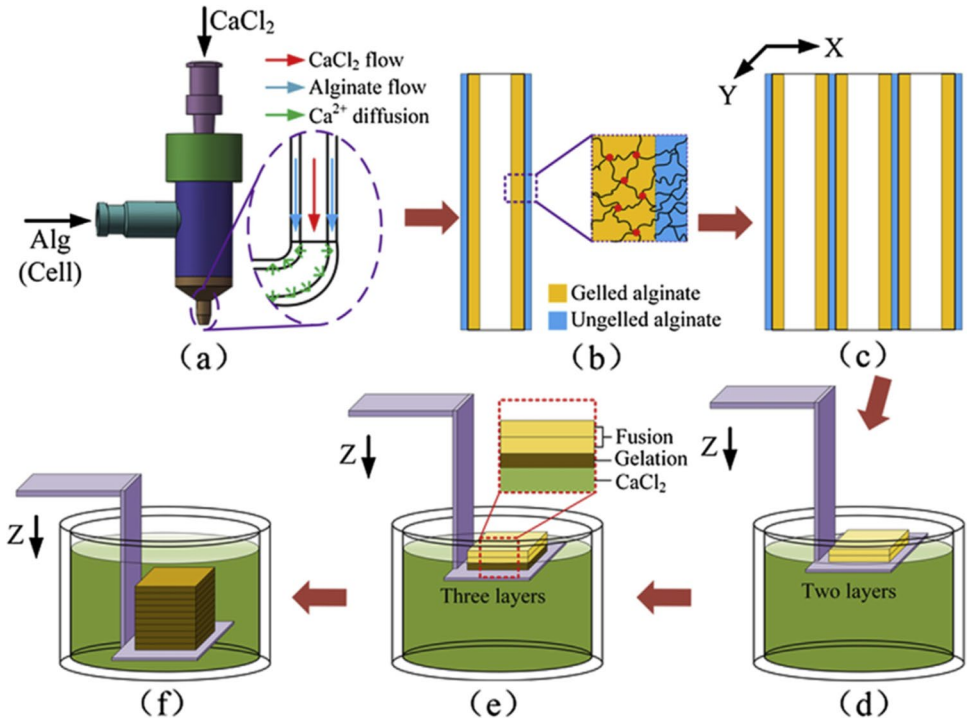

Fig. 4 Fabrication process of a 3D alginate structure with built-in microchannels. Obtained from reference [35] with permission

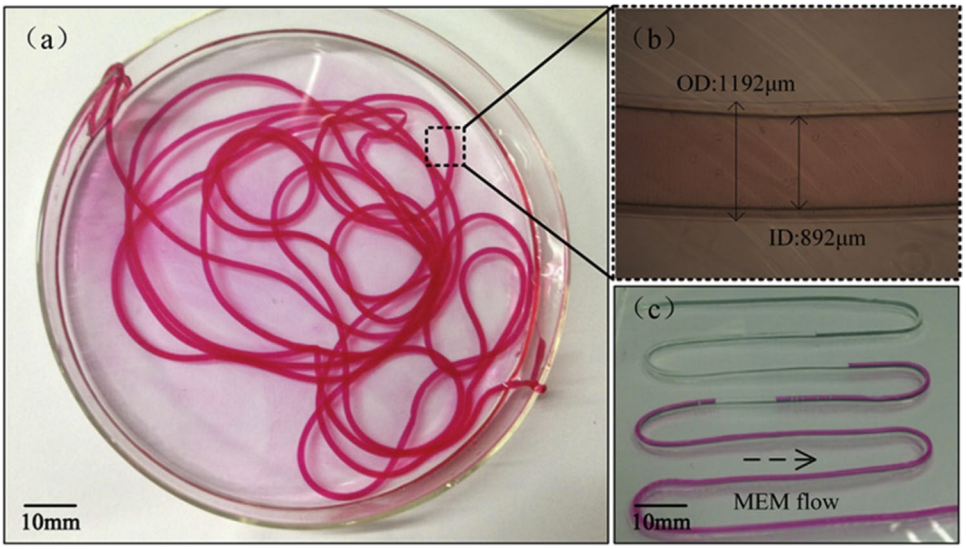

Fig. 5 (a) Printed alginate hollow filaments. (b) The filament under an inverted microscope. (c) Perfusion of cell culture media (MEM). Obtained from reference [35] with permission

or simply one of the synthesis components. Polyethylene glycol (PEG) is one type of hydrophilic macromolecule, and typically, PEG hydrogel is difficult to use as 3D printer ink. However, hydrogels formed using PEG derivatives as crosslinkers in various compositions can be used for 3D printing.

Nano-biocomposite for 3D Printing Application

Nano-biocomposites obtained by applying nanofillers to biopolymers can result in promising materials because they show improved properties without eco-toxicity with the protection of the biodegradability of the material [25]. Such materials are primarily intended for biomedical applications and various short-term applications, such as packaging, farming, and hygiene equipment. In the following sections, the application of these novel materials in 3D printing will be reviewed.

Nanoparticle-Hydrogel Composite

Nanocomposite hydrogels have become known in the past decades as a new class of hydrogels, incorporating nanoscale materials into hydrated polymeric hydrogels to improve their mechanical performance. A variety of nanomaterials, such as metal, metal oxide, non-metal, and polymeric, were physically mixed or covalently coupled with the hydrogel network to create nanoparticle-hydrogel composites.

Metal and Metal Oxide Nanoparticle-Hydrogel Composite

To produce nanocomposite hydrogels, metallic and metal oxide nanomaterials such as gold (Au), silver (Ag), iron oxide (Fe_3O_4, Fe_2O_3), titania (TiO_2), alumina (Al_2O_3), and zirconia (ZrO_2) can be mixed with hydrogels [36]. These metal nanoparticle-hydrogel composites may not only have enhanced mechanical properties but also have additional functions such as conductivity, magnetic properties, or antimicrobial properties. Improved mechanical property has been demonstrated by adding a golden nanowire in alginate hydrogel. Its electroactivity enhanced electrical communication between adjacent cardiac cells and encouraged the synchronous contraction of seeded cardiomyocytes with electrical stimulation [37].

Silver (Ag) nanoparticles added to an alginate/poly (vinyl alcohol) (PVA)/poly (N-vinyl-2-pyrrolidone) (PVP) hydrogel resulted in an increased compressive stiffness from 64.9 kPa (PVA hydrogel) and 79.4 kPa (Ag/alginate/PVA) to 115.2 kPa.

Some hydrogels reinforced with nanomaterials were utilized for 3D printing. Gaharwar and his coworker incorporated nanosilicates in GelMA hydrogel. The results demonstrated a fourfold increase in compressive modulus of GelMA/nanosilicate compared to hydrogel alone. Moreover, when the concentration of silicate reached 2%, the compressive stress showed a fourfold increase in comparison to GelMA hydrogel. This GelMA/nanosilicate was 3D printed into the designed scaffold.

Another common approach in applying nanoparticles for the preparation of nano-biocomposite is using the metal nanoparticles as crosslinkers. Skardal et al., for example, utilized Au nanoparticles to crosslink a printable semi-synthetic extracellular matrix hydrogel made up of thiol-modified bio-macromonomers produced from hyaluronic acid and gelatin for tissue engineering applications [38]. Electrostatic interaction between positively charged collagen strands and [$AuCl_4$] ions was used by Xing et al. to create a collagen-Au composite hydrogel. This interaction

resulted in the reduction of $[AuCl_4]^-$ ions and forming Au nanoparticles that act as the crosslinker for collagen chains [39].

Zhu and his colleagues created a novel gold nanocomposite bio-ink for printing three-dimensional cardiac tissue constructs that may repair damaged tissue and serve as drug screening platforms. When compared to constructions lacking gold nanorods, the cardiac cells in the printed gold nanorod constructs had better cell adhesion and organization [40].

Tognato et al. reported a novel oxide nanocomposite hydrogel ink that was created using GelMa and iron oxide to fabricate the 3D magnetic starfish soft robot [41]. This 3D structure showed magnetic remote control and cell guidance effect where C_2C_{12} skeletal myoblasts can differentiate into myotubes without inducing a medium.

In addition, the non-toxic photo cross-linkable hydrogel gelatin methacryloyl (GelMA) was used to fabricate biodegradable soft helical microswimmers in three dimensions using two-photon polymerization (Fig. 6) [42].

Polymeric Nanoparticle-Hydrogel Composite

Natural hydrogels are usually very soft/flexible and weak/brittle, and most synthetic hydrogels also have low toughness and limited recoverability disadvantages. The fabrication of high-strength hydrogels for tissue engineering remains very difficult. One of the most effective techniques for integrating and combining diverse hydrogel functions and qualities that cannot be achieved by a single hydrogel is to use a hydrogel composite system. During the last two decades, one of the proposed composite designs is a double network. The interpenetration of two polymer networks in double-network hydrogel composite systems forms a unique microstructure, which strongly affects the mechanical performances of the resulting hydrogel. Double-network hydrogels consist of two polymers with different physical characteristics [43]. Due to its exceptional strength and toughness, double-network hydrogels were introduced to 3D printing [44, 45]. For example, Liu et al. reported the fabrication of κ-carrageenan/PAAm double-network hydrogel by combining an ionically crosslinked κ-carrageenan network with a covalently crosslinked polyacrylamide (PAAm) network. κ-carrageenan/PAAm double-network hydrogels demonstrated both excellent recoverability and self-healing capabilities.

κ-carrageenan/PAAm could be used as printing ink to construct complicated 3D structures, such as cone and dumbbell-shaped patterns. As shown in Fig. 7, 3D structures also demonstrated excellent mechanical strength after UV curing [44].

Gellan gum (GG)/poly(ethylene glycol) diacrylate (PEGDA) double-network hydrogel was developed for

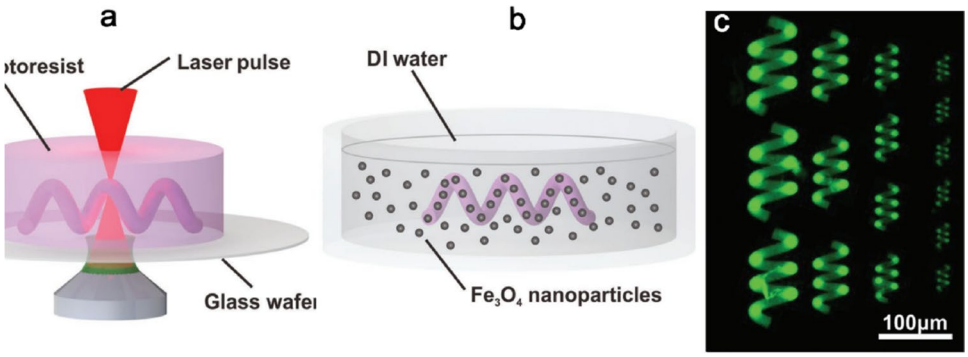

Fig. 6 3D printing for soft robots. (A) Creation of GelMA hydrogel helical microswimmers. (a) 2 P P printing. (b) Decoration with Fe3O4 nanoparticles. (c) Image of helical microstructures. Obtained from reference [42] with permission

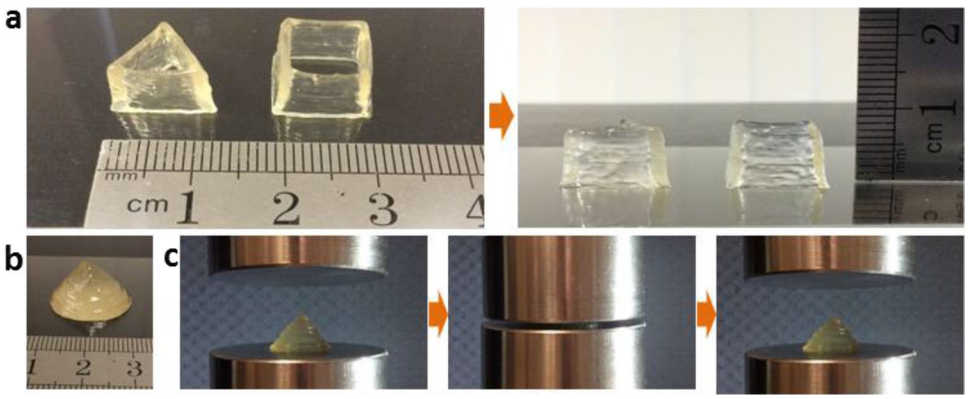

Fig. 7 3D-printed κ-carrageenan/PAAm DN hydrogel, (a) hollow triangular prism and cube 3D patterns, and (b) a cone pattern. (c) The printed cone sample could return to its original shape after undergoing a compressive strain of 90% and relaxation. Obtained from reference [44] with permission

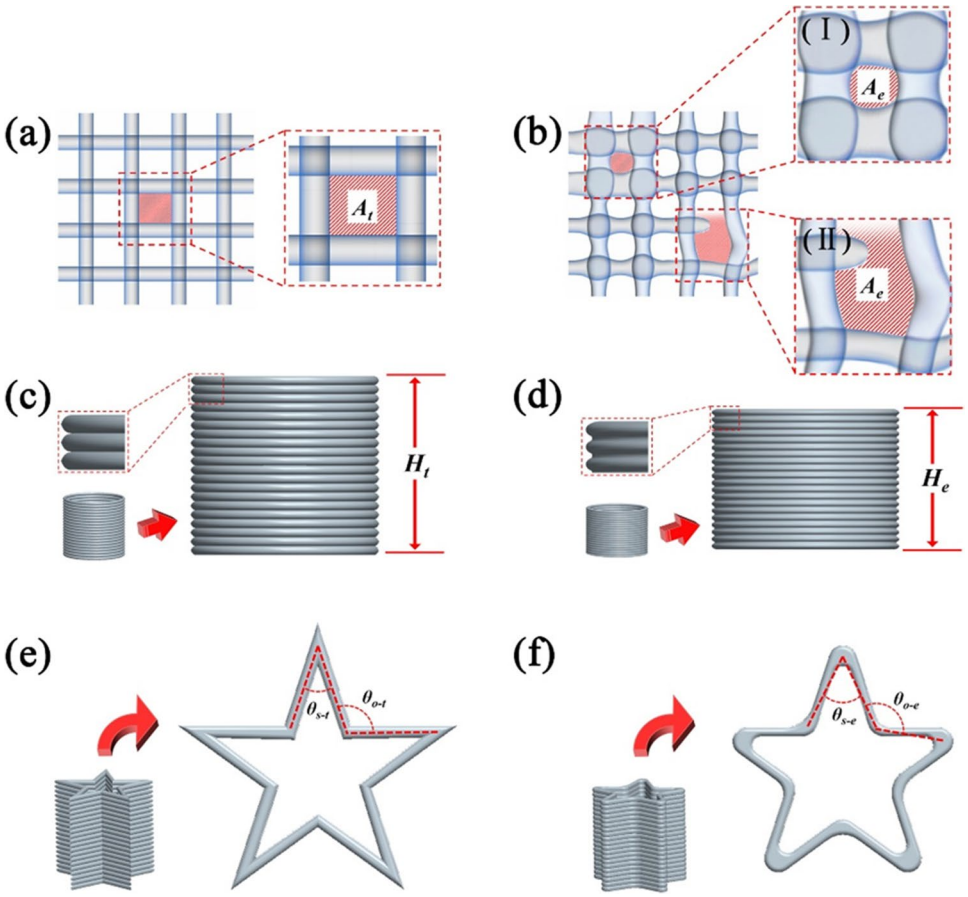

Fig. 8 A schematic of the evaluation of hydrogel printability. This image shows the lattice matrix, thin-walled tube, and pentagram tube-like structure in its model (a, c, e) and practical (b, d, f) form. (b) (I) The liquid-like bio-ink represented here. (b) (II) A representation of a bio-ink with too high viscosity. Obtained from reference [46] with permission

extrusion-based 3D bioprinting [46]. The models and practical shapes of the 3D structures are schematically shown in Fig. 8. The printed cylindrical structure can also sustain a certain amount of pressure in the lateral direction, as illustrated in Fig. 9c. The pressure deformation can be recovered once the stress is released. Furthermore, after pressing repeatedly, the thin-walled tube construction may swiftly return to its original shape.

Characteristics of Hydrogels for 3D Printing

The suitability of a hydrogel to a particular method of biofabrication depends primarily on its physicochemical properties under the conditions imparted by the instrument of biofabrication [47]. The rheological properties of hydrogels and mechanisms for crosslinking are key parameters that determine hydrogel printability [16, 47, 48]. In the following

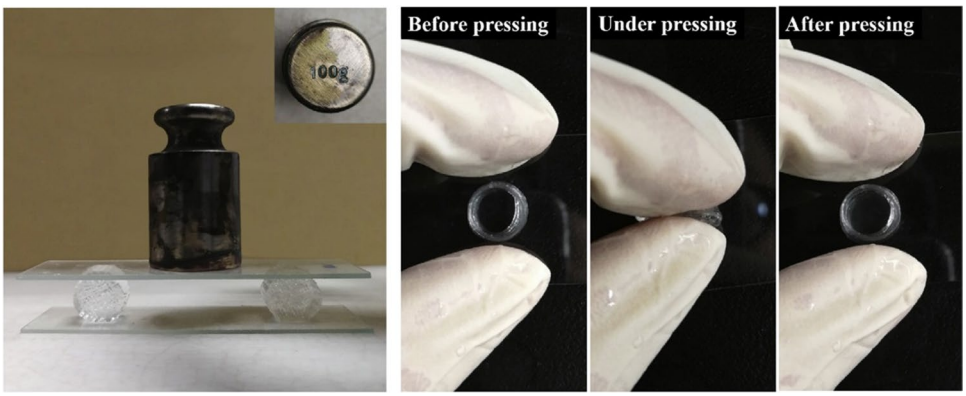

Fig. 9 An optical image of a printed cylindrical construct under compression. After pressing, the thin-walled tube structure retook its original shape. Obtained from reference [46] with permission

sections, these two characteristics of hydrogels for 3D printing will be reviewed.

Rheological Properties

Rheology studies the flow of matter in response to an external force. It is known that the rheological properties of hydrogels play a vital role in controlling their printability and 3D-printed structures [49]. However, the evaluation of rheology in the additive manufacturing process has not been thoroughly studied. Here, some rheological properties of hydrogels such as viscosity, shear thinning, and yield stress which define the printability of hydrogel ink are discussed.

Viscosity

Viscosity measures the resistance of a fluid to deformation at a given rate. The viscosity of hydrogels, as a non-Newtonian fluid, is dependent on shear rate. Therefore, the viscosity of hydrogels increases or decreases as the shear rate increases. During the 3D printing process, the printable hydrogel should be in filament form instead of drop form. Hence, the viscosity of materials is a critical factor in the additive manufacturing process.

Jia et al. discovered a direct link between printability and viscosity by evaluating hydrogel printability using a point-to-point method. The findings revealed that for a piston-driven system, the optimal kinematic viscosity range is between 400 and 3000 mm^2/s [34].

Moreover, the viscosity profile of different hydrogel systems has been studied to improve rheological properties for ideal 3D printing [50, 51]. Murphy et al. looked at a wide range of viscosities for several 3D printing methods, including inkjet, extrusion, and laser-assisted. According to their research, a sample with a viscosity range of 30 to > 6 107 mPa·s is appropriate for an extrusion-based 3D printer. [52]. In a study by Blaeser et al., the effect of hydrogel viscosity and nozzle size on shear stress during 3D printing was investigated [53]. They used three different concentrations of alginate (0.5, 1.0, 1.5 wt/v%) and nozzle diameters of 150 and 300 µm. Their results demonstrated that by increasing the hydrogel viscosity, while the concentration of alginate was 0.5 wt/v%, the shear stress difference between the two nozzles diminished.

There have been numerous studies to investigate how the alteration of hydrogel inks' properties impacts their printability [54, 55]. It was reported that the highly viscous hydrogel inks such as chitosan, chitosan-collagen, and methylcellulose-hyaluronan showed relatively high printing accuracy. The high viscosity of hydrogel inks can keep them from spreading after deposition during the 3D printing process [52, 56]. However, extremely viscous hydrogel ink might cause handling and extrusion problems, and broken printing filaments, consequently, affecting the printability of hydrogel inks. Therefore, the viscosity of the hydrogel inks should be optimized as an important factor for the inks used for extrusion-based 3D printing.

The effect of nanoparticles on hydrogel viscosity Recently, the combination of nanomaterials with polymers has attracted the attention of researchers due to enhanced thermal, mechanical, and electrical properties of polymer nanocomposites [57–61]. Furthermore, incorporating nanoparticles into a hydrogel matrix alters the rheology of the hydrogel, affecting its printability. As previously said, viscosity is the most researched material quality for 3D printing, and it varies depending on the printing process. Extrusion-based 3D printing, for example, needs inks with a greater viscosity to offer mechanical strength and form fidelity. Viscous inks, on the other hand, can clog nozzles. The most common route to alter the ink's viscosity is adding materials that modify rheology. Several studies have reported the influence of adding the nanoparticles on polymer matrix and observing their printability. On the other hand, nanoparticles have been introduced to improve bio-ink viscosity to retain shapes after depositing the ink onto the substrate. However, the viscosity should not be too high to reach the pressure limit of the instrument.

Some nanoscale materials such as nanoclay, cellulose nanocrystals, graphene oxide, carbon nanotube, and silica nanoparticles have been introduced to improve the rheological properties of hydrogel inks for the 3D printing process [62–64]. By adding nanoclay to a polyethylene glycol (PEG)/alginate matrix, Zhao and colleagues studied its rheological properties. The nanoclay particles are physically crosslinked with one another and with polymer networks to enhance the viscosity of the pre-gel solution. They validated the rise in the viscosity of the pre-gel solution as a function of nanoclay concentration using a cone and plate rheometer. They developed hydrogel ink with exceptional printability [62]. In another report, two-dimensional nanosilica was used to optimize the bio-inks for 3D printing [65]. In this study, Gaharwar et al. examined the effect of nanosilica on the rheological behavior of κ-carrageenan (κCA), a linear sulfated polysaccharide. The connection between κCA and nanosilica is strong, resulting in improved physical interactions and excellent form fidelity and structural integrity of the printed structure. The effect of silica nanoparticle concentration on the printability of gelatin and alginates hydrogels was studied by Roopavath et al. [66]. In this study, the viscosity of hydrogels was investigated by gradual addition of silica nanoparticles. The results showed that the viscosity of hydrogels increased, as the concentration of silica nanoparticles increased up to 2.5 wt%. However, after addition of 2.5 to 5.0 wt%, the viscosity remained nearly the same, indicating the saturation and phase separation, resulting in

reduced resistance. Agglomeration of silica nanoparticles occurred after a certain addition of them to the hydrogels; therefore, the incorporation of 2.5 wt% and 5.0 wt% of silica into hydrogels was selected as suitable inks for 3D printing.

The incorporation of nanoparticles into the polymeric matrix is a wide approach to enhance the thermal, mechanical, or electrical properties of polymeric materials. However, in terms of 3D printing, integration of nanoparticles with polymer matrix limits processability due to increases in the viscosity of the overall matrix. This is the main reason that limits the loading concentration of nanoparticles in 3D printable hydrogels. Hence, ongoing research and development are being carried out on novel 3D printing technologies to print a wider range of composites with various rheological properties [67].

Shear Thinning

A practical factor for 3D printing is the "shear-thinning" type of non-Newtonian fluid. In rheology, shear thinning is a phenomenon in which the viscosity of fluid decreases under shear strain. Shear thinning can be time-dependent; this behavior is known as thixotropic [68]. The advantage of this property in inkjet printing not only prevents clogging nozzle but also improves solidification after deposition. Generally, the hydrogels for 3D printing should possess shear thinning and thixotropic characteristics. The shear-thinning behavior of hydrogel ink guarantees the extrusion of hydrogel out of the nozzle, and the thixotropic property ensures the maintenance of the extruded filament shape after printing.

Taking advantage of the shear-thinning property, Markstedt and coworkers reported the printability of alginate that incorporated nanofibrillated cellulose (NFC). In this study, they confirmed the poor shape fidelity of pure alginate when printing. However, using NFC in the alginate matrix improved the shape fidelity of this hydrogel. In addition, the flow curve of 2.5% NFC dispersion showed the shear-thinning property of this nanofibrillated that led to excellent printability.

Wu et al. [38] created a 3D-printed bio-ink made of gellan gum (GG) and poly(ethylene glycol) diacrylate (PEGDA). Using different concentrations of GG/PEGDA, they tested the rheology, printability, fidelity, mechanical characteristics, and cytocompatibility of a double-network hydrogel. The results showed the concentration of GG has the greatest effect on the rheology of the system and makes the system shear thinning; however, the presence of PEGDA promotes the network reassembly during the recovery process. The optimal concentrations for 3D printing were 1.5 wt.% GG and 10 wt.% PEGDA.

Yield Stress

The yield stress is linked to suspension stability and will reveal the suspension's long-term stability as well as the particles' inclination to settle. Because particle sedimentation causes inhomogeneous printing, it is undesirable. Other rheological experiments used to determine the degree of sedimentation in suspensions include oscillatory tan determination, creep recovery, and stress relaxation tests [49].

Flow initiation plays an essential role during the 3D printing process. It is defined by yield stress; a printable material should behave under the yield point as solid-like and above it as liquid-like. Concentrated samples of polymers usually experience yield stress due to interactions between their chains. The chains tend to align when a shear charge is applied, and the material flows. Contrary to this, when the shear load is removed the material experiences a recovery [69].

To describe the yield stress, routine rheology measurements were taken. For determining material yield stress, a shear stress ramp is a helpful tool. It is feasible to evaluate the viscosity at which the material begins to flow first and so approximate its "at-rest" viscosity by graphing the viscosity over the shear stress.

Paxton and his colleagues, for example, calculated the yield stress for commercially available crème, poloxamer 407, alginate-based inks, and an alginate-gelatine composite material [70]. The results are shown in Fig. 10.

The yield stress was calculated by intersecting two tangents for the materials that showed evident yield stress, one in the plateau area of viscosity where the material is elastically deformed and the other in the region where viscosity decreases and the material flows. The shear stress ramp for the alginate-gelatin sample was given at 37 °C, as shown in Fig. 10, implying very low viscosity and yield stress when compared to the other samples. The thermoresponsive rise in viscosity induced by the cooled collection plate is critical for alginate-gelatin printability.

Conclusion

Specific materials, techniques, and applications are at the center of the hydrogel 3D printing difficulties. The use of various network architectures to create new hydrogels with great 3D printability and mechanical properties has shown to be a viable technique. Due to its versatility under various printing conditions, nozzle-based 3D printing is the main technique to produce hydrogel-based scaffolds or devices. Rheology is highly important to an extrusion-based 3D printing operation, and priority is given to the rheological property of hydrogel used as the 3D printer's ink. Shear-thinning and thixotropic characteristics should be present in the

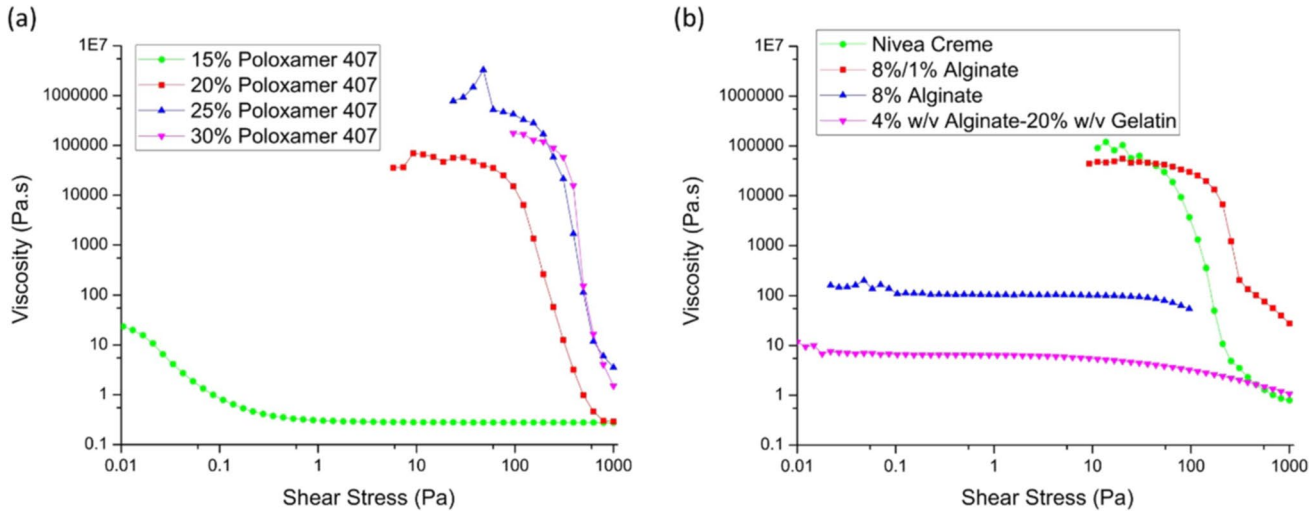

Fig. 10 For all samples, shear stress ramp measurements were conducted at 24 °C except for the alginate-gelatin (4%w/v alginate hydrogel, 20%w/v gelatin) sample which was measured at 37 °C. Obtained from reference [70] distributed under the Creative Commons Attribution

proposed hydrogels. This is because the hydrogel ink can be swiftly extruded from a small nozzle due to the shear-thinning behavior, and the thixotropic characteristic allows the extruded filament to keep its shape and sustain self-weight. There are some challenges of extrusion-based 3D printing, including resolution limitations and scale limitations. The resolution of nozzle-based 3D printing is relatively low when compared to laser-based 3D printing and inkjet 3D printing technologies. In addition, the resolution depends on the solid loading or particle size of hydrogel composites. On the other hand, several sensitive biocomponents such as cells and growth factors in hydrogel or hydrogel composite inks, high temperature, and speed of printing or external pressure can result in loss of function or damage to sensitive biocomponents. To address these drawbacks, certain innovative techniques should be considered to improve manufacturing productivity by enhancing deposition capability in achieving optimum scaffolding requirements.

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