Biomedical Materials & Devices (2023) 1:191–201
https://doi.org/10.1007/s44174-022-00023-2

REVIEW

Hybrid Hydrogels Based on Methacrylate-Functionalized Gelatin (GelMA) and Synthetic Polymers

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Received: 15 June 2022 / Accepted: 11 August 2022 / Published online: 8 September 2022

Abstract

This review on hybrid hydrogels prepared from methacrylated gelatin (GelMA) and synthetic polymers presents their advantageous properties and potential applications in biomedical engineering. It describes the preparation of the different types of frequently used hybrid hydrogel networks: co-networks, interpenetrating networks (IPNs), and semi-interpenetrating networks (semi-IPNs) and gives an overview of the different methods of preparing biomedical devices from these materials.

Keywords Methacrylate-functionalized gelatin · Methacrylated gelatin · Gelatin methacrylamide · GelMA · Hybrid hydrogels · Natural and synthetic networks

Introduction

Hydrogels are three-dimensional (3D) networks prepared from hydrophilic polymers. The high water content, adjustable chemical and physical properties, and the ability to encapsulate cells, biological macromolecules (such as peptides/proteins, nucleotides and antibodies), and therapeutic agents, open up a variety of potential applications in the biomedical field, such as tissue engineering, wound healing, and drug delivery [1–3]. Hydrogels made of natural or synthetic polymers have been widely reported and have their respective advantages and disadvantages. For example, natural hydrogels made of hyaluronic acid, chitosan, alginate, and chondroitin sulfate are widely used due to their good biocompatibility and biodegradability, but their application is limited due to poor mechanical properties and the difficulty to adjust degradation rates and biological function [3, 4]. On the other hand, hydrogels made of synthetic polymers offer a wider range of chemical, physical and mechanical properties. Researchers have investigated the properties of synthetic hydrogel biomaterials in detail and have successfully developed hydrogels showing high-strength, self-healing, stimulus-responsive, adhesive, and antibacterial properties. However, the biological performance of such high-performance synthetic polymer hydrogels often needs to be further improved. Therefore, to obtain hydrogels with good biocompatibility and mechanical properties at the same time, hybrid hydrogels made of synthetic and natural polymers have appeared [5–7]. The hybridization of synthetic and natural polymers in a single hydrogel network can form a new class of material with the beneficial properties of both types of materials without their respective disadvantages. Compared to single-component hydrogels, hybrid hydrogels exhibit an improvement of mechanical properties and biocompatibility that closer meets the properties required in the biomedical field [8–12].

Although the benefits are attractive, current research on synthetic-natural hybrid hydrogels is very limited [12]. The hydrophilic synthetic polymers that are used to form hydrogels with natural polymers also have the disadvantage of having inferior mechanical properties. Although network structures such as interpenetrating network (IPN) structures have been used in hybrid hydrogel systems to enhance their mechanical and biological properties, the improvements have been limited [13–15].

Hydrophobic polymers maintain their advantageous mechanical properties in an aqueous environment and could be used to enhance the mechanical properties of hydrophilic

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networks. However, it is non-trivial to combine hydrophobic polymers with hydrophilic natural polymers in a homogenous hybrid hydrogel network. Unlike conventional composite materials, the constituents of these natural and synthetic hybrid materials need to be mixed at the nano or molecular level. Mixing at this submicroscopic scale leads to homogeneous materials with characteristics in between those of the two original components and in some cases to materials with new properties [16].

Methylacrylate-functionalized gelatin (GelMA) is a photosensitive biological material that can rapidly be cured by photo-initiated radical polymerization upon exposure to UV or blue light [17]. Hereby, a crosslinked 3D gelatin-based network is formed that still has cellular adhesion sites and can support cell proliferation and migration. The gelatin network has good biocompatibility and is mainly used for tissue engineering and 2D and 3D cell culture [18]. In addition, aqueous GelMA solutions can form physical gels upon cooling, making them very useful in bioprinting *(i.e.*, in the extrusion-based additive manufacturing of biomaterial solutions containing cells). A variety of constructs have been manufactured, such as cell-containing scaffolds for the tissue engineering of cartilage, skin, blood vessels, and heart patches [19, 20]. However, like most hydrogel materials, GelMA hydrogels are also brittle and lack good mechanical properties.

The preparation of hybrid GelMA and synthetic polymer hydrogels can be an approach to fabricate gelatin-based material with enhanced properties, and some research on hybrid GelMA and synthetic functionalized polymers has been done. In this review, we summarize the research on hybrid natural and synthetic hydrogels prepared from GelMA and synthetic polymers, and give an overview of the different methods of preparing biomedical devices from these materials.

GelMA-Based Hydrogels

Gelatin is a high-molecular weight polypeptide obtained by partial hydrolysis of the collagen that is present in the connective or epidermal tissue of animals. Gelatin has many interesting physical and chemical properties, such as its hydrophilicity, its biological activity allowing interaction with cells and tissues, and its ability to reversibly form a physical gel at relatively low temperatures. Hydrogels prepared from unmodified gelatin often cannot meet the requirements of medical materials as their thermal stability, degradation characteristics, and mechanical properties are sub-optimal [18, 21]. In practice, gelatin is often adapted. The gelatin molecule contains many reactive groups, such as –COOH, $–NH_2$, and –OH groups, which allow modification and functionalization of the molecule to adjust its gelation behavior and mechanical properties [22, 23]. Lopez-Cebral reacted the carboxylic acid groups of gelatin with 1-ethyl-3-[3-dimethlyaminopropyl] carbodiimide (EDC), allowing the subsequent coupling of ethylenediamine and spermine to the gelatin via amide bonds [24]. The resulting cationization of gelatin at physiological pH, especially with the endogenous spermine polyamine, is of great interest in the controlled release of plasmid DNA.

Hydrophobic compounds such as dodecanal were reacted with the amino groups of gelatin in 30% solutions of ethanol in water. In experiments determining the adhesion strength to tissues under wet conditions, it was shown that the hydrophobically modified gelatin had higher interfacial bonding strengths to soft tissues than non-modified gelatin [25, 26].

To prepare stable covalently crosslinked gelatin hydrogels, methacrylic anhydride (MA) has been used to functionalize gelatin and obtain photo-crosslinkable gelatin methacrylamide (GelMA). In Fig. 1A, first reported by Van den Bulcke [27], upon reaction of (part of) the amino groups of gelatin with methacrylic anhydride, a crosslinked GelMA hydrogel network could be prepared by photo-initiated radical polymerization of the methacrylamide groups in the presence of a UV light-sensitive photo-initiator. In Fig. 1B, since then, GelMA hydrogels have been widely studied. They have been used in the regeneration of soft tissues like cartilage [28], heart valves [29], tendons [30], and muscles [31]. Many studies have shown that GelMA hydrogels are also suitable substrates for two-dimensional cell seeding and three-dimensional cell encapsulation, and can be processed by manufacturing methods like micro-molding, self-assembling systems, microfluidics, and bioprinting [32–36]. In these networks, the amino acid sequence of gelatin itself is not affected, and functional peptide sequences such as cell-binding RGD and sequences susceptible to enzymatic degradation remain present. Therefore, crosslinked GelMA hydrogels remain biologically active and promote cell adhesion. They can also be degraded by proteases in vitro and in vivo [18, 37].

By controlling the degree of functionalization and the crosslinking density, the water uptake, degradation rate, and mechanical properties of GelMA hydrogels can be varied. For use as a scaffolding material in tissue engineering, hydrogels must have adequate mechanical characteristics and appropriate degradation times to maintain space and allow for tissue reconstruction [38–40]. Also, when the hydrogel is formed in situ, short gelation times are desired to reduce operation times. In some applications, such as in guided bone regeneration, the properties of GelMA hydrogels that can be reached are still inadequate and restrict their use [41].

To further tailor and improve the properties of GelMA hydrogels, GelMA has been compounded with synthetic polymers [32]. Hydrophilic water-soluble functionalized polymers (macromers) such as functionalized polyethylene

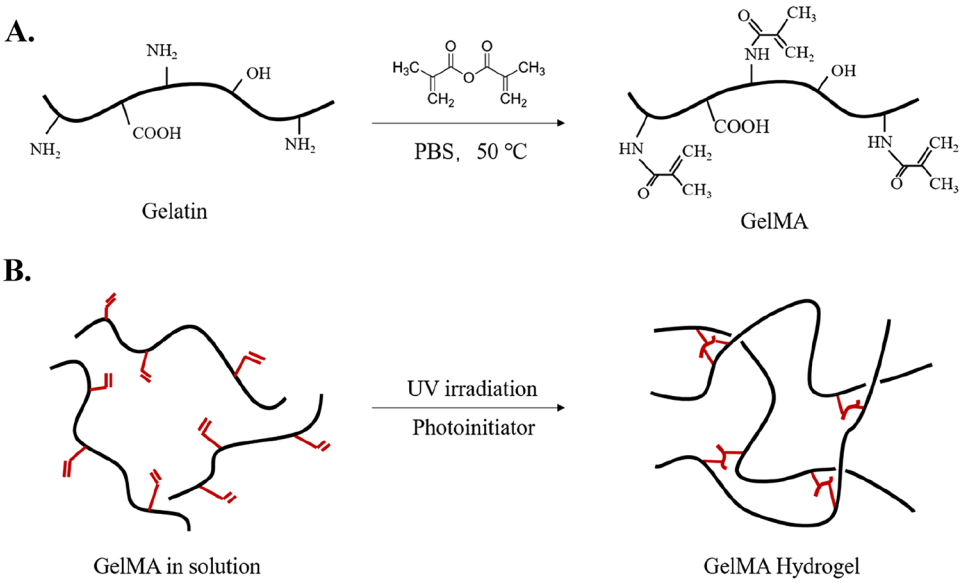

Fig. 1 Scheme illustrating the preparation of A. GelMA and B. GelMA hydrogels

glycol (PEG) [42] and poly(vinyl alcohol) (PVA) [43] have been used to prepare natural-synthetic hybrid hydrogels with GelMA. The process is facile, as the hydrophilic macromers and GelMA can be homogeneously mixed in a common aqueous solution and crosslinked to form hybrid hydrogel networks. Polyesters like poly(lactide) (PLA) and poly(ε-caprolactone) (PCL) [44] are very well known and the most often employed hydrophobic polymers in tissue engineering due to their biocompatibility, tunable degradability, and excellent mechanical properties. The preparation of hybrid GelMA hydrogels with hydrophobic macromers is more difficult, as a common solvent needs to be found.

Preparation of GelMA-Based Hybrid Hydrogels

Hydrogels are physically or chemically crosslinked hydrophilic polymers. These networks can be formed from hydrophilic polymer chains by physical entanglement, electrostatic interactions, covalent bonding, etc. [45, 46]. GelMA can form networks in a multitude of ways: aqueous GelMA solutions can form physically crosslinked hydrogels by variation of environmental stimuli such as temperature, pH, and the addition of multivalent cations [18]. Covalently crosslinked GelMA hydrogels can be prepared by photo-initiated radical polymerization, whereby the polymerized methacrylamide groups form multivalent cross-linkages between the gelatin chains.

In combination with synthetic polymers, different kinds of hybrid networks with GelMA can be prepared. Frequently used strategies for forming GelMA-based natural-synthetic hybrid hydrogels involve the preparation of (a) Co-networks (b) Interpenetrating networks (IPNs), and (c) Semi-interpenetrating networks (Semi-IPNs), see Fig. 2.

GelMA Co-networks

Copolymerization of methacrylate-functionalized synthetic polymers (macromers) and GelMA is the most commonly used method to prepare GelMA-based hybrid hydrogels. In the presence of a photo-initiator, mixtures of methacrylate-functionalized synthetic polymers and GelMA copolymerize in solution to form hybrid networks upon exposure to visible

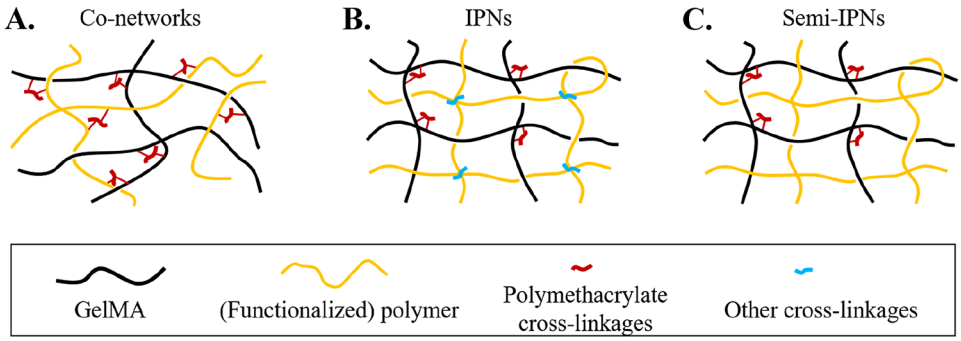

Fig. 2 Schematic representation of different possible hybrid hydrogel network structures. **A** Co-networks, **B** Interpenetrating networks (IPNs), and **C** Semi-interpenetrating networks (Semi-IPNs)

or UV light [47]. Kuo et al. prepared natural-synthetic hybrid networks by photo-crosslinking methacrylated poly(vinyl alcohol) (PVAMA), methacrylated alginate (AlgMA), and GelMA [43]. PVAMA and AlgMA assisted in increasing the water content of the formed hydrogels, which led to high porosity structures that allowed the migration of cells, while GelMA enhanced the cell entrapment efficiency.

Double-bond-modified hyaluronic acid (HAMA) and gelatin (GelMA) were used to form stable hybrid gel microspheres [48] with good affinity for tumor cells. This 3D cell-culturing model simulates the tumor-extracellular matrix microenvironment and has been applied in different drug screening models.

As illustrated in Fig. 3A, we copolymerized PEG-dimethacrylate (PEG-dMA) and GelMA in 0.5% acetic acid solution to prepare hybrid hydrogels. While human mesenchymal stem cells did not proliferate on PEG-dMA hydrogels, proliferation of the cells on the prepared natural-synthetic hybrid hydrogels was similar to that on GelMA hydrogels. Furthermore, the toughness of 50% PEG-dMA: 50% GelMA hybrid hydrogels prepared in this manner was 2.5 times higher than that of hydrogels prepared from either PEG-dMA or GelMA [49].

Besides copolymerization with methacrylate-functionalized polymers, GelMA can be copolymerized by photo-initiated radical copolymerization with monomers such as acrylamide (AM), see Fig. 3B. Compared with single poly(acrylamide) (PAM) and GelMA hydrogels, (PAM)-GelMA hybrid hydrogels showed enhanced compression strengths (up to 0.38 MPa) and higher elasticity (storage modulus of up to 1000 Pa) [47]. Moreover, the addition of PAM sequences in the co-network influenced the network density and water affinity which allowed control of degradability as well [50].

While hybrid GelMA hydrogels can easily be obtained by copolymerization with functionalized hydrophilic macromers or monomers in common aqueous solutions, it is more difficult to do so with hydrophobic macromers like those based on PLA or PCL. Such networks, consisting of a hydrophilic as well as a hydrophobic phase, can have even better mechanical properties, especially with regard to their toughness and resilience [51]. We have prepared hybrid hydrogel co-networks with very high toughness by photo-crosslinking methacrylate-functionalized poly (trimethylene carbonate) (PTMC) and GelMA in a DMSO/formic acid mixture. A water-swollen hybrid hydrogel consisting of 75% methacrylated PTMC and 25% GelMA was found to be extremely tough (with a work to fracture in tensile testing of 386 N/mm^2), which is two orders of magnitude higher than that of the corresponding single GelMA hydrogel. Moreover, this hybrid hydrogel also showed good cell adhesion [52].

IPN GelMA Networks

Another way to fabricate hybrid gelatin-based networks is by preparing interpenetrating networks (IPNs). IPN structures are composed of two or more separate polymer networks, in which the elastically active macromolecular chains of the networks permeate each other without being covalently bound to each other [53]. These distinct networks, with different properties or functions, can be combined in a stable structure that possesses the properties of the constituent networks. In some cases, specific properties can be greatly enhanced in interpenetrating gelatin hydrogel networks. By constructing multi-component interpenetrating network structures, the mechanical properties of GelMA-based hydrogels can be enhanced and the swelling/de-swelling response rate can be accelerated [54–57].

The preparation of GelMA-based IPN hydrogels can be done in a synchronal or in a sequential manner. In the synchronal manner, the two networks are formed simultaneously by different polymerization or crosslinking mechanisms

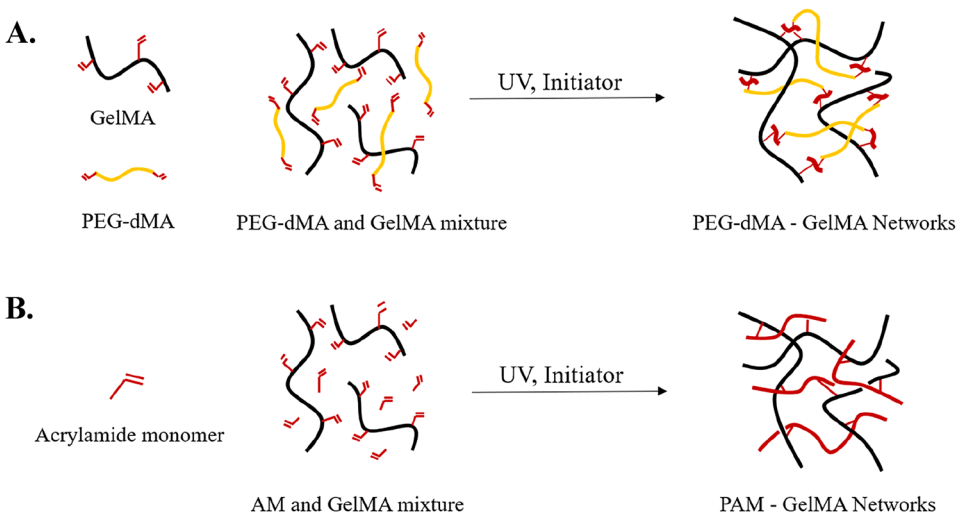

Fig. 3 **A** Preparation of PEG-dMA and GelMA hybrid hydrogels, **B** Preparation of PAM and GelMA hybrid hydrogels

[58]. For example, in preparing GelMA and alginate IPN hydrogels, a GelMA network is formed by photo-crosslinking while at the same time, alginate is physically crosslinked with Ca^{2+} ions [59]. Most IPN GelMA and synthetic polymer hydrogels are prepared sequentially, where first network I is formed after which network II is formed using the same or different crosslinking mechanisms [60].

GelMA and poly(2-hydroxyethyl methacrylate) (pHEMA) IPN hydrogels were prepared in a sequential manner [58]. A GelMA solution (15 w/v % GelMA in phosphate-buffered saline (PBS), 0.5 w/v % I2959 as photo-initiator) and a HEMA solution (97.75% HEMA, 1.25% N,N,N',N'-tetramethyl-ethylenediamine (TEMED) and 1% ethylene glycol dimethacrylate (EGDMA)) were prepared separately. The GelMA and HEMA solutions (8:2, v:v) were mixed with a freshly prepared 10% solution of ammonium persulfate (APS) in PBS. In the presence of TEMED and APS, the HEMA monomer quickly polymerizes to form a crosslinked pHEMA network with EGDMA. Although GelMA can also polymerize in the presence of TEMED and APS, the reaction is much slower under the above conditions, and in this first stage, a pHEMA network is predominantly formed. After exposing the mixture to UV light, the GelMA component also polymerizes, thereby leading to the formation of a GelMA-HEMA IPN hydrogel network. Compressive testing showed that the modulus of the GelMA hydrogel significantly increased with the incorporation of crosslinked pHEMA in the structure (6.53 *vs.* 155.49 kPa, respectively). Cell-culturing experiments showed that human corneal keratocytes can proliferate within the GelMA-HEMA IPN hydrogel, while the single pHEMA hydrogel was not suitable for cell growth.

Also, sequentially, IPN hydrogels were formed in an aqueous environment by first crosslinking pectin-grafted poly(ε-caprolactone) (pectin-g-PCL) with calcium ions (Ca^{2+}), and then photo-crosslinking the GelMA component. The obtained IPN hydrogels had comparatively much higher compressive modulus values, which increased from 39 to 5029 kPa [61].

A composite-biodegradable network IPN hydrogel that promotes in situ bone regeneration was prepared in a two-step crosslinking process as well [62]. First inorganic polyhedral oligomeric silsesquioxane (POSS) was suspended in a methacrylated chitosan (CSMA) solution in dimethyl sulfoxide (DMSO). After photo-crosslinking the CSMA, a well-defined nanocomposite CS-POSS hydrogel was formed. Then, after removing DMSO, the composite CS-POSS network was soaked in an aqueous GelMA solution and the GelMA was photo-crosslinked to form the second network. This composite IPN GelMA hydrogel exhibits enhanced stiffness and toughness, resulting from the rigid POSS units and their unique properties of energy dissipation. The biological components of the IPN hydrogel framework (chitosan and gelatin) are biodegradable and can be replaced by newly formed tissue. When loaded with mesenchymal stem cells (MSCs), accelerated in situ bone regeneration was observed upon implanting these nanocomposite IPN hydrogels into skull defect of rats.

Semi-IPN GelMA Networks

Semi-interpenetrating networks (semi-IPNs) are combinations of a crosslinked polymer and a non-crosslinked (linear) polymer. The presence of non-crosslinked polymers in the structure reduces the friction between the molecular chains, increases the free volume between the polymer chains, and enables the polymer chains to adjust their conformation and arrangement to external forces. Through the effective arrangement and slippage of polymer chains, the elongation to break and toughness of semi-IPN hydrogels are improved [63].

A semi-IPN hydrogel made from GelMA and hydrophilic polyvinylpyrrolidone (PVP) with potential application in the treatment of wounds has been described [64]. In the semi-IPN network, GelMA forms a network upon photo-crosslinking and PVP is entangled with the network. The elasticity modulus of the hydrogels, as determined by tensile testing, could be increased from 46 to 190 kPa by adjusting the GelMA to PVP ratio in the networks.

Although GelMA hydrogels with relatively good mechanical properties can be obtained by incorporating hydrophilic polymer in the networks, some biomedical applications require hydrogels with even better mechanical properties. In the preparation of blood vessel grafts or blood vessel tissue engineering scaffolds, implants with high burst resistance and suture retention strength are required, while load-bearing bone tissue engineering scaffolds and other orthopedic applications require high compressive strengths and elasticity moduli. For this, GelMA networks have also been compounded with hydrophobic polymers. But, as these polymers are insoluble in water, it is challenging to prepare homogenous combinations with GelMA and GelMA networks.

To homogeneously mix GelMA with hydrophobic polymers in solution, several methods have been explored. By grafting the hydrophobic poly(ε-caprolactone) (PCL) to pectin, the pectin-g-PCL graft copolymer can dissolve in PBS and form a stable mixture with dissolved GelMA. Upon photo-crosslinking, a semi-INP hydrogel of GelMA and pectin-g-PCL with compressive moduli ranging from 3.1 to 10.4 kPa were obtained [61].

As is the case for the GelMA co-networks which we discussed previously, use of an organic solvent that allows the dissolution of the hydrophobic polymer as well as GelMA can be used to prepare homogeneous semi-IPNs upon crosslinking. Not many such solvents are available, as gelatin and GelMA are insoluble in most common organic solvents.

However, it has been shown that GelMA is soluble in dimethyl sulfoxide (DMSO), trifluoroethanol (TFE), hexafluoroisopropanol (HFIP), formic acid, acetic acid, and mixtures of these solvents [2].

A series of fibrous semi-IPN hydrogels based on GelMA and PCL for application in vascular engineering were fabricated in HFIP [65]. In these hydrogels, the GelMA network ensured biomimetic biological activity and enhanced vascular endothelial cell adhesion and remodeling, while the presence of PCL resulted in favorable mechanical properties. Variation of the composition allowed tuning of the properties of the resulting semi-IPNs. Tensile testing showed that semi-IPN GelMA/PCL hybrid hydrogels can be elongated up to 300%, with elasticity modulus and tensile strength values of up to 6.5 and 3.0 MPa, respectively.

In other work using HFIP as a common solvent, polyethylene glycol (PEG) was included in the hybrid semi-IPN as well [66]. This PCL/PEG/GelMA semi-INP was found to have significantly reduced bacterial attachment to the surface of the material. Compared to PCL, this reduction in bacterial adhesion was more than 90%.

Manufacturing of GelMA-Based Hybrid Hydrogel Tissue Engineering Scaffolds

Electrospinning

Fibrous scaffolds are some of the earliest extracellular matrix substitutes used in tissue engineering. In recent years, electrospinning has attracted widespread attention as a method for preparing tissue engineering scaffolds with ultrafine fibers [67]. In the process, a polymer solution or melt is ejected from a nozzle under the action of a strong electric field to yield very thin fibers [68, 69]. In principle, any polymeric material that can be dissolved or melted can be processed by electrospinning, this includes natural as well as synthetic polymers and macromers and their mixtures. When solvents are used, the solvent volatilizes upon ejection of the polymer solution and a fibrous porous scaffold free of solvent is obtained [70]. This is important when mixtures of GelMA and hydrophobic polymers are electrospun using solvents like HFIP, TFE, etc. [71, 72].

Porous hybrid hydrogel scaffolds with bactericidal activity were made by electrospinning mixtures of PCL, PEG, and GelMA using HFIP as a solvent [66]. In the prepared fibrous scaffold, the PCL component ensures suitable mechanical properties, while the PEG and GelMA provide water absorption and the desired biological properties. In serum-free medium, the fibrous hydrogel scaffold provided an optimal environment for the maintenance of the keratocyte phenotype of cultured cells and regeneration of damaged corneal stroma.

To obtain porous scaffolds, PCL/GelMA hybrid hydrogels were also electrospun from a mixture of TFE and acetic acid (4:1, v/v). The blood compatibility of the photo-crosslinked porous membranes was evaluated, and it was shown that the membranes had low values of thrombogenicity and did not trigger any hemolytic effects [72].

3D Printing and Bio-printing

3D printing refers to a digital manufacturing technology that produces 3-dimensional objects by the precise computer-controlled deposition of materials in sequential layers. The necessary data can be obtained from computed tomography (CT) or computer-aided design (CAD) models [73, 74]. 3D-printed tissue engineering scaffolds can improve and enhance cell-culturing models and provide a rapid and robust approach to assemble functional tissues in vitro [75].

The ideal network structure, rheological behavior, biomechanical, and biochemical characteristics of a 3D-printing material for use in tissue engineering have recently been discussed [76]. Due to its excellent biocompatibility and rapid photo-crosslinking characteristics, GelMA has become one of the most often used materials in 3D printing and has been widely used to print scaffold constructs for the engineering of skin, nerve, and other soft tissues [77, 78]. Most human tissues and organs are complex combinations of extracellular matrix components with specific biological or mechanical effects. A single material ink will likely not create a microenvironment for cells that mimics the circumstances in the body. This makes that multi-material bio-printing is becoming increasingly important. Therefore, GelMA hybrids with other biomedical polymeric materials are being used as inks for 3D (bio-)printing [79, 80].

Extrusion-based 3D printing is the most often used additive manufacturing in biomedical engineering. Here, a polymer melt or a viscous polymer solution (ink) is pressurized and extruded through a nozzle. The extruded filament is laid down in a specific pattern, after which it solidifies. Three-dimensional constructs are formed by extruding and patterning the filament in a layer-by-layer manner. A main advantage of this technique is the availability of a wide range of biocompatible materials. [81, 82]. As GelMA itself can form stable non-crosslinked physical gels at somewhat reduced temperatures, GelMA solutions have frequently been used as 3D-printing inks [19]. However, extrusion-based printing also has some drawbacks. Its printing accuracy is relatively low compared with other printing methods, generally in the order of 100 µm. Moreover, the gelation, curing, shear thinning, and other properties of inks need to be taken into account during the printing [83].

Stereolithography (SLA) uses a laser beam to draw a shape on the surface of a light-curing liquid resin, allowing the sequential layer-by-layer manufacturing of detailed

hydrogel structures. Hydrogel tissue engineering scaffolds of varying shapes and sizes have been prepared from common PEGDA and GelMA solutions; for example, 3D vascular structures were printed in this way. By adjusting the concentration of the inks, structures with layer thicknesses and resolutions varying between 42 and 83 μm have been printed [84].

Digital light processing (DLP) is another 3D-printing platform that has attracted much interest in tissue engineering. In DLP, use is made of digital masks to photo-cure the single layer of a light-curing resin at once, thereby significantly increasing the printing speed. Using DLP, high-resolution nerve guidance conduits have been made using aqueous GelMA and PEGDA solutions [85]. Using a 32.5% solution of GelMA and PEGDA (GelMA: PEGDA = 7.5: 25) in DPBS, a printed nerve guidance conduit with a Young's modulus of approximately 4.5 MPa was prepared.

Unlike conventional 3D printing, bio-printing uses live cells and other biological materials together with natural or synthetic polymers as inks in extrusion-based 3D printing to create organ-like structures in which the cells can proliferate [86]. Bio-printing has many uses in the biomedical field, allowing, *e.g.*, the regeneration and repair of tissues, the study of organ development, the evaluation of drugs, and pathological mechanisms [87, 88]. Due to the presence of cells, rigorous demands regarding cell compatibility are put on the inks used and on the applied 3D-printing processing conditions [89].

Using extrusion-based 3D printers equipped with a UV light source, the cell-containing GelMA solutions can be photo-crosslinked quickly, making the final cell-containing construct stable at physiological temperatures [19, 90]. Human mesenchymal stem cells (hMSCs) have been mixed into combined PEG-dMA and GelMA solutions, to fabricate cell-laden scaffolds by 3D bio-printing. The PEG-dMA-GelMA scaffolds embedded with hMSCs showed improved mechanical properties with evenly distributed cells that developed into homogeneous tissues. Over 80% of the cells survived the printing process and showed excellent osteogenic and chondrogenic differentiation capacity [91].

Microfluidics, Cell Encapsulation, and Bio-printing

Microfluidic technologies make use of microchannels (tens to hundreds of microns in width) to process or manipulate minute amounts of liquids (nanoliter volumes) [92, 93]. In recent years, microfluidic techniques have been used to fabricate microgels for application in tissue engineering. Using microfluidics, particles of different sizes and shapes can be obtained by variation of the size of the microchannels, adjusting the fluid velocity and adapting the droplet shape [91, 94–96]. GelMA hydrogels for biomedical use have often been prepared using microfluidic systems [97]. When combined with 3D bio-printing methods, the cell-containing microspheres or microwires fabricated using microfluidics can directly be used to prepare functional tissue-like structures[98].

Wang et al. prepared GelMA microgels with a core–shell structure using a droplet microfluidic system [99]. The microfluidic device consisted of a core–shell droplet generation unit and a microgel photo-polymerization unit. A methyl cellulose solution, a GelMA solution, and mineral oil were respectively injected into the core channel, the shell channel, and the continuous flow channel of the droplet generation unit. The GelMA shell of the generated particles was photo-crosslinked upon exposure to UV light as the particles flowed through a serpentine channel in the photo-crosslinking unit. By adjusting the flow rates, microgels with sizes ranging from 44.9 to 366.6 μm could be obtained. Using this method, microgels encapsulating hepatocytes (HepG2) and human umbilical vein endothelial cells (HUVECs) were successfully prepared. These cell-loaded microgels have potential application in the construction of microtissues or the examination of cell–cell interactions [100, 101].

Overview of Applications of GelMA and Synthetic Polymer Hybrid Hydrogels

As described in the foregoing sections, hybrid hydrogels based on GelMA and synthetic polymers have been used in a variety of applications. Table 1 gives an overview of representative materials and applications in the biomedical field.

Conclusions

GelMA can be combined with functionalized and non-functionalized synthetic polymers to form different kinds of hydrogels, and hybrid co-networks, and interpenetrating networks and semi-interpenetrating networks have been prepared. The mechanical and biological properties of GelMA-based hybrid hydrogels are very good, making these materials highly applicable in biomedical engineering. These materials have been processed into medical implants and tissue engineering scaffolds in several ways including electrospinning, 3D printing, bio-printing, and by use of microfluidics. It should be noted, however that there are still some important limitations in preparing hybrid GelMA hydrogels comprising cells. When it concerns hydrophobic synthetic polymers, the organic solvents required to prepare the mixtures are usually detrimental to cells, and in many cases, it will be required to modify the polymer with hydrophilic moieties.

Although the application of GelMA-based hybrid hydrogels in tissue engineering and other application areas of

Table 1 Overview of applications of hybrid hydrogels based on GelMA and synthetic polymers

Type of network	Components	Application	Particulars	[References]
Co-networks	GelMA/ PVAMA/ Alginate-MA	Cell entrapment	Highly porous hydrogels with high water content	[43]
	GelMA/ HAMA	Drug screening	Cell-loaded gel microspheres simulating the tumor-extra cellular matrix	[48]
	GelMA/ PEG-dMA	Tissue engineering	Tough hybrid hydrogels	[49]
	GelMA/ PAA	Drug delivery	Network design and composition controls hydrogel characteristics	[50]
	GelMA/ PTMC-dMA	Tissue engineering	Hybrid hydrogels with extremely high toughness	[52]
	GelMA/ PEG-dMA	Bio-printing	Hydrogels with improved mechanical properties showing osteogenic and chondrogenic differentiation	[91]
IPNs	GelMA/ Chitosan-MA/ POSS	Tissue engineering	Tough composites with high modulus for bone regeneration	[3]
	GelMA/ polyHEMA	Tissue engineering	Hybrid hydrogel with low modulus for cornea regeneration	[58]
	GelMA/ pectin-grafted PCL	Tissue engineering	Hybrid hydrogels with relatively high modulus	[61]
	GelMA/ Methyl cellulose	Microfluidics	Core–shell microgels encapsulating cells for use as organoids or microtissues	[99]
Semi-IPNs	GelMA/PVP	Wound treatment	Hybrid materials with high water affinity and adequate mechanical properties	[5]
	GelMA/ PCL	Tissue engineering	Hybrid materials with mechanical and biological properties suited for vascular engineering	[65]
	GelMA/ PCL/PEG	Antibacterial materials	Materials showing highly reduced bacterial adhesion	[66]
	GelMA/ PCL	Tissue engineering	Electrospun biodegradable meshes with good haemocompatibility for use as vascular grafts	[72]

biomedicine is still in its initial stages, more and more researchers realize their potential and continuously improve the performance of these hybrid materials. It can be expected that in the near future, more advanced GelMA-based hydrogels with multiple functions and sensitivities to a variety of stimuli will become available.

Funding Funding was provided by China Scholarship Council (Grant No. 201608440247).

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