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Review

Synthesis, properties, and biomedical applications of gelatin methacryloyl (GelMA) hydrogels

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ABSTRACT

Gelatin methacryloyl (GelMA) hydrogels have been widely used for various biomedical applications due to their suitable biological properties and tunable physical characteristics. GelMA hydrogels closely resemble some essential properties of native extracellular matrix (ECM) due to the presence of cell-attaching and matrix metalloproteinase responsive peptide motifs, which allow cells to proliferate and spread in GelMA-based scaffolds. GelMA is also versatile from a processing perspective. It crosslinks when exposed to light irradiation to form hydrogels with tunable mechanical properties. It can also be microfabricated using different methodologies including micromolding, photomasking, bioprinting, self-assembly, and microfluidic techniques to generate constructs with controlled architectures. Hybrid hydrogel systems can also be formed by mixing GelMA with nanoparticles such as carbon nanotubes and graphene oxide, and other polymers to form networks with desired combined properties and characteristics for specific biological applications. Recent research has demonstrated the proficiency of GelMA-based hydrogels in a wide range of tissue engineering applications including engineering of bone, cartilage, cardiac, and vascular tissues, among others. Other applications of GelMA hydrogels, besides tissue engineering, include fundamental cell research, cell signaling, drug and gene delivery, and biosensing.

1. Introduction

Hydrogels are crosslinked network of hydrophilic polymers that can swell in water to capture many times their original mass. Physical and biochemical properties of hydrogels largely depend on their compositions, methods used for their polymerization, and their crosslinking density. Hydrogels provide a versatile platform to include desired combinations of properties for designed applications [1]. Numerous hydrogels have been developed based on natural and/or synthetic polymers using various kinds of crosslinking chemistry towards different biomedical applications, such as regenerative medicine, drug delivery, and tissue adhesives [2]. In particular, hydrogels for biomedical applications are designed to resemble the characteristics of native extracellular matrix (ECM) and to provide three-dimensional (3D) supports for cellular growth and tissue formation [3]. Hydrogels have been also widely used in 3D culturing to study cell-matrix and cell–cell interactions, and cellular proliferation, migration [4], and differentiation [5]. To this

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aim, hydrogels based on naturally occurring biopolymers have potential advantages over synthetic polymers, such as excellent biocompatibility, low immunoresponse, and possible bioactive motifs encoded in their chemical structures.

In this contribution, we review recent research on the synthesis, characterizations, and biomedical applications of gelatin methacryloyl (GelMA), which is also frequently referred as gelatin methacrylate [6–9], methacrylated gelatin [10–13], methacrylamide modified gelatin [14], or gelatin methacrylamide [15–18] in literature by different authors. Based on the fact that GelMA is a gelatin derivative containing a majority of methacrylamide groups and a minority of methacrylate groups, we suggest that "gelatin methacryloyl" is a more suitable name, which also matches the widely accepted abbreviation GelMA.

GelMA undergoes photoinitiated radical polymerization (i.e. under UV light exposure with the presence of a photoinitiator) to form covalently crosslinked hydrogels. As the hydrolysis product of collagen, the major component of ECM in most tissues, gelatin contains many arginine-glycine-aspartic acid (RGD) sequences that promote cell attachment [19], as well as the target sequences of matrix metalloproteinase (MMP) that are suitable for cell remodeling [20]. When compared to collagen, the advantages of gelatin include better solubility and less antigenicity [21,22]. The hydrolysis process also denatures the tertiary structure of collagen, reducing its structural variations due to different sources. A gelatin solution has, on its own, the unique property of gelation at low temperatures to form physically crosslinked hydrogels [14,23]. In addition, several chemical reactions have been applied to covalently crosslink gelatin [24–27]. Conveniently, introduction of methacryloyl substituent groups confers to gelatin the property of photocrosslinking with the assistance of a photoinitiator and exposure to light, due to the photopolymerization of the methacryloyl substituents [14]. This polymerization can take place at mild conditions (room temperature, neutral pH, in aqueous environments, etc.), and allows for temporal and spatial control of the reaction [6]. This enables microfabrication of the hydrogels to create unique patterns, morphologies, and 3D structures, providing ideal platforms to control cellular behaviors, to study cell–biomaterial interactions, and to engineer tissues [6,28].

It is worth mentioning that the chemical modification of gelatin by methacrylic anhydride (MA) generally only involves less than 5% of the amino acid residues in molar ratio [14], which implies that most of the functional amino acid motifs (such as the RGD motifs and MMP-degradable motifs) will not be significantly influenced. Specifically, the RGD motifs do not contain groups that will react with MA, which ensures the retention of good cell adhesive properties of GelMA [6,19,29]. Furthermore, the *in vitro* enzymatic degradation of GelMA hydrogels by type I and type II collagenases (also known as MMP-1 and MMP-8, respectively) proceeds at accelerated rates, indicating the existence of MMP-sensitive motifs in GelMA [30,31].

Since its first synthesis report [14], GelMA hydrogels have been thoroughly studied in terms of physical and biochemical properties for many different applications ranging from tissue engineering, to drug and gene delivery. In this review, we will focus on studies related to GelMA hydrogel synthesis and characterization as well as its composites. We will also summarize the reported methods for microfabrication of GelMA hydrogels, and the applications of resulting GelMA-based biomaterials.

2. Synthesis and characterization of GelMA hydrogels

Different protocols have been reported for the preparation of GelMA, but they are all essentially minor variations of a general method first reported by Van Den Bulcke et al. [14]. Briefly, GelMA is synthesized by the direct reaction of gelatin with MA in phosphate buffer (pH = 7.4) at 50 °C. This reaction introduces methacryloyl substitution groups on the reactive amine and hydroxyl groups of the amino acid residues [14] (Fig. 1A). Different degrees of methacryloyl substitution can be achieved in GelMA by tunning the amount of MA added to the reaction mixture, which produces GelMA with different physical properties. Maintaining a higher pH during the reaction enhances the reactivity of amine and hydroxyl groups, thereby leading to a higher degree of substitution [32]. Once the substitution reaction is stopped by diluting the reaction mixture (typically 5X) with phosphate buffer, the resulting solution should then be dialyzed against deionized water through a dialysis tubing (12–14 kDa molecular weight cutoff) for 5–7 days to allow complete removal of the low-molecular-weight impurities (including unreacted MA and methacrylic acid byproducts, etc.), which are potentially cytotoxic. Finally, the dialyzed solution can be freeze dried and stored, preferably under refrigeration, until use. Note that the reaction of gelatin and MA is a two-phase reaction, where an organic compound is added and dispersed into an aqueous phase. As a result, the rate of MA addition and the conditions of mixing might have specific effects on the quality of the dispersion, and consequently, on the degree of methacryloyl substitution in the final product. To our knowledge, the effect of different mixing conditions on the properties of the resulting GelMA has not been studied in detail and remains a topic for further optimization studies.

Photocrosslinking of the synthesized GelMA can be conducted using a water-soluble initiator under UV light. Common choices for photoinitiators include 2-hydroxy-1-[4-(2-hydroxyethoxy) phenyl]-2-methyl-1-propanone (Irgacure 2959) [6,29] and lithium acylphosphinate salt (LAP) [33]. Irgacure 2959, a commercially available photoinitiator, has a solubility in water of at least 5 mg/mL [34], which is sufficiently high for most photopolymerization conducted in aqueous environments. LAP, a recently developed alternative water-soluble photoinitiator, has a higher solubility in water (up to 8.5 wt%) and a higher molar extinction coefficient at 365 nm than Irgacure 2959 [33]. The degree of substitution, GelMA concentration, initiator concentration, and UV exposure time are the major parameters that allow tuning of the physical properties of the resulting GelMA hydrogels [14].

Characterization of the physical properties (i.e. porosity, elastic modulus, degradation, and water swelling) and cell response parameters (i.e. cell viability, proliferation, differentiation and spreading) on GelMA hydrogels is key to determining the suitability of these polymers for different tissue engineering applications. GelMA offers high versatility regarding the tuning of its characteristics by manipulating its synthesis and processing (i.e. conditions of crosslinking). For example, the compressive modulus of GelMA can be fine-tuned by varying the degree of methacryloyl substitution or by adding inorganic or organic components to GelMA (Fig. 1B), making it suitable for a wide range of biomedical applications. GelMA hydrogels can also be subjected to cryogenic treatments (i.e. freeze drying) to generate porous scaffolds with controlled pore sizes and porosity [35]. Van Vlierberghe et al. reported the preparation of porous GelMA hydrogels upon cryogenic treatments of the chemically crosslinked hydrogels [36]. They identified that the average pore sizes were inversely related to the concentration of GelMA solution and the cooling rate, and they successfully prepared GelMA hydrogels with gradient pore sizes using a gradient cooling rate strategy [36,37]. We and others have also shown that the pore sizes in GelMA hydrogel can be tuned by changing the degree of methacryloyl substitution (Fig. 1C) [9,38]. For example, Chen et al. [9] synthetized GelMA hydrogels with different substitution degrees (49.8, 63.8, and 73.2%) using 1, 5, and 10 M MA solutions, respectively. In these experiments, the degree