Vol. 29, No. 2, 2025, Pages 36-43

Thi-Qar Medical Journal (TQMJ)

Thi-Qar Medical Journal was established in 2006 and is published by the College of Medicine, University of Thi-Qar, Iraq

ISSN (PRINT): 1992-9218 | ISSN (ONLINE): 3006-4791

www.jmed.utq.edu.iq utjmed@utq.edu.iq

Characterization of Gelatin Methacrylate (GelMA) Hydrogels for Cartilage Tissue Engineering Applications

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Received: 25.8.2025
Revised:20.10.2025
Accepted: 30.10.2025
DOI:
10.32792/jmed.2025.29.21

Keywords:
GelMA
Hydrogel
Characterization
Cartilage tissue applications

How to cite
Hussein Mishbak, Mohammed A. Albadri, Mohammedsadiq A. Hasan. Characterization of Gelatin Methacrylate (GelMA) Hydrogels for Cartilage Tissue Engineering Applications. *Thi-Qar Medical Journal (TQMJ)*. 2025;Volume(29):36-43

Abstract

Articular cartilage, a tissue that is particularly known for its self-repairing deficiency, is a major challenge in regenerative medicine. Gelatin Methacrylate (GelMA) is a multifunctional and biocompatible hydrogel, which has attracted much attention as a potential scaffold material for cartilage tissue engineering. This paper gives a detailed characterization of GelMA hydrogels with regard to the effect of polymer concentration on their physicochemical and biological properties. We performed an in-depth analysis of GelMA at concentrations of 5%, 10% and 15% (w/v) and their mechanical strength, swelling kinetics, degradation rates and porous architecture. Additionally, we have synthesized and critically reviewed optimal parameters for 3D bio-printing of constructs based on GelMA for cartilage regeneration. Our results show that the mechanical properties are significantly improved with an increase in the concentration of GelMA from 5% to 15%, and the compressive modulus has been increased from 3.3 +- 0.5 kPa to 30.0 +- 4.0 kPa. This enhancement, however, comes with a decrease in porosity, swelling ratio and degradation rate. While no one GelMA formulation reproduced the mechanical properties of native articular cartilage, a 10% (w/v) concentration of GelMA, with optimized 3D bioprinting parameters, appears to be a well-balanced formulation. It provides a good compromise between mechanical stability and microenvironment that allows the viability, proliferation, and chondrogenesis of cells. This critical review highlights the opportunities of the GelMA as a highly tunable and promising platform in which effective and clinically relevant cartilage repair and regeneration strategies can be developed.

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1. Introduction

Articular cartilage is a special type of connective tissue which covers the ends of bones in synovial joints and provide a smooth, low-friction surface for the movement of joints. However, it is a highly avascular, alymphatic and aneural tissue, which has a very limited intrinsic healing ability after injury or in degenerative disorders such as osteoarthritis (1–4). As a result, cartilage defects frequently cause chronic pain, loss of the joint's function, and a significant decrease in the quality of life of millions of people around the world (5). Current clinical procedures such as microfracture, autologous chondrocyte implantation (ACI) and osteochondral grafting have been less successful, with the formation of mechanically inferior fibrocartilage and with a lack of long term and durable repair (6,7).

Tissue engineering has become a promising alternative with the aim of regenerative functional cartilage tissue generation by using a combination of cells, bioactive molecules and biomaterial scaffolds (8). An optimal scaffold for cartilage tissue engineering should be biocompatible and biodegradable and should have mechanical properties that can withstand the challenging biomechanical

environment of an articulating joint. Moreover, it should be able to support chondrocyte viability, proliferation and deposition of a new extracellular matrix (ECM) (9–11)

Among the different biomaterials under investigation, Gelatin Methacrylate (GelMA) has been of great interest because of its excellent biocompatibility, tunable physicochemical properties and resemblance to the native ECM (12–15). GelMA is a photocurable hydrogel prepared by the functionalization of gelatin with methacrylic anhydride which adds photoreactive methacrylate groups. This can be used to form covalently crosslinked hydrogels upon exposure to ultraviolet (UV) light in the presence of a photoinitiator (16,17). The parent material, gelatin is derived from collagen which is the main protein component of ECM, native of the body but retains important cell binding motifs, such as arginine-glycine-aspartic acid (RGD) sequences, which promote cells adhesion, migration and proliferation (18–20) .

The tunability of the properties of GelMA is one of its main strengths. By adjusting the GelMA concentration, the level of methacrylation and the crosslinking conditions, the mechanical stiffness, swelling behavior, degradation rate and porosity of the hydrogel can be tailored to meet the specific needs of cartilage tissue engineering(21–23). Furthermore, the emergence of 3D bioprinting has allowed the creation of complex architectures of scaffolds bearing a patient specific design with fine control over the distribution of cells and bioactive factors in space, further promoting the potential of GelMA in this regard(24,25).

The objective of this study is to investigate the characterization of GelMA hydrogels for cartilage tissue engineering purposes. By systematically exploring the mechanical properties, swelling and degradation kinetics, porosity and 3D bioprinting parameters of GelMA at various concentrations, we hope to build a basic data set for guiding the rational design and fabrication of constructs from GelMA for effective cartilage regeneration.

2. 2. Materials and Methods

2.1 Synthesis and Hydrogel Formulation of GelMA

GelMA was produced according to a previously established protocol (26). Briefly, gelatin (Type A, from porcine skin, 300 bloom, Sigma-Aldrich, UK) was dissolved in phosphate buffered saline PBS (Sigma-Aldrich, UK) at 50° C to prepare a 10% (w/v) solution. Methacrylic anhydride (Sigma-Aldrich) was then added dropwise to the gelatin solution at a rate of 0.5 mL/min and reacted for 3 hours at 50°C under continuous stirring, PH was kept at 7-8 during the reaction time by adding 5M NaOH. The reaction then was terminated by diluting the mixture with warm PBS and the resulting solution was dialyzed against distilled water for 7 days at 40°C using a dialysis membrane (12-14 kDa MWCO) to remove the unreacted methacrylic anhydride and other by-products. The purified GelMA solution was subsequently lyophilized to get a white porous foam which was kept at -80° C dialysis membrane (12-14 kDa MWCO) until further use.

GelMA hydrogels discs were produced by dissolving the lyophilized GelMA in PBS at 60°C to obtain final concentrations of 5%, 10% and 15% (w/v). For photopolymerization, a photoinitiator, lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP, Sigma-Aldrich) was added to the GelMA solution at a concentration of 0.05-0.1% (w/v). The hydrogel precursor solution was then poured into cylindrical molds (hole diameter: 8mm) and exposed to UV light (365 nm, 10-13 mW/cm2) (Model Dymax 2000-EC, Dymax Europe GmbH, Wiesbaden, Germany) for almost 45 seconds for the crosslinking reaction. As depicted in Fig**. 1.**

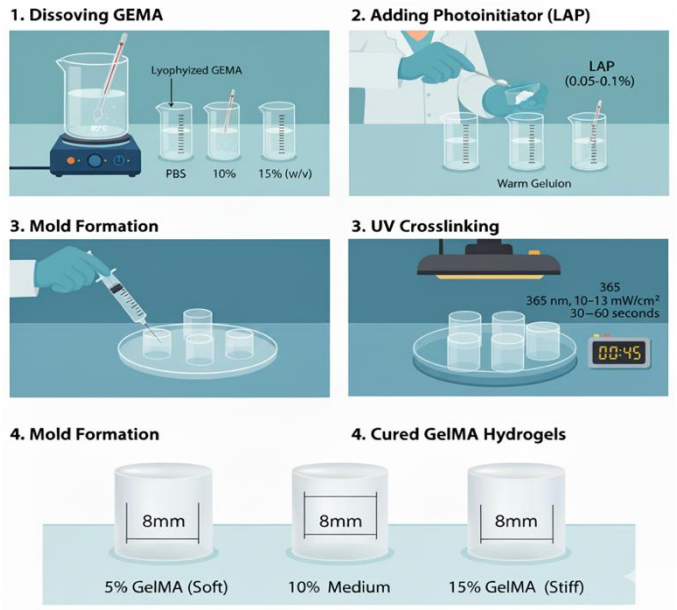

Fig. 1: (1) Dissolving GelMA: Dissolution of lyophilized powder of GelMA in PBS at 60 degrees Celsius. (2) Introduction of Photoinitiator (LAP): LAP (0.05-0.1% w/v) is added to the GelMA solution. (3) Mold Formation & UV Crosslinking: The GelMA-LAP solution is prepared and poured into 8mm cylindrical molds and exposed to UV light (with 365 nm wavelength, 10 - 13 mW/cm2) for 15 - 30 seconds for crosslinking. (4) Cured GelMA Hydrogels: Resulting hydrogels exhibit different stiffness depending on the concentration of GelMA (e.g. 5% soft, 10% medium, 15% stiff).

2.2 GelMA Hydrogels Characterizations

The physicochemical properties of the GelMA hydrogels were tested in conditions that simulate a physiological environment (37°C).

2.2.1 Mechanical Testing

The mechanical properties of the hydrogels were tested with the help of a universal testing machine. Unconfined compression tests were conducted at a rate of 30%/min to measure the compressive modulus. Tensile tests were carried out at a strain rate of 1 mm/min to determine the Young's modulus and ultimate tensile strength.

2.2.2 Swelling Behavior

The swelling ratio of the hydrogels was determined by immersing the lyophilized hydrogels in PBS at 37C and measuring the weight at different time intervals over 72 hours. The formula used to calculate the swelling ratio was (Wet Weight - Dry Weight) / Dry Weight.

2.2.3 Degradation Profile

The in vitro degradation of the hydrogels was evaluated by incubating them in a PBS solution with a concentration of 2U/mL of collagenase type II (Sigma Aldrich) at 37°C. The percent of residual weight was determined at various time intervals for 14 days.

2.3 Statistical Analysis

The analysis of variance (ANOVA) was applied to question the data one-way, and post-hoc analysis was conducted using Tukey. Differences were considered statistically significant at * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$. GraphPad Prism software (GraphPad Software Inc., San Diego, CA, USA) was used in this research.

3. Results and Discussion

3.1 GelMA Hydrogels Mechanical Properties

The mechanical properties of GelMA hydrogels are important for their use in cartilage tissue engineering where they have to be able to withstand the complex loading environment of the joint. Our results show that the mechanical properties of GelMA hydrogels are very sensitive to the polymer concentration as indicated in fig. 2.

The stress-strain curves of 5%, 10% and 15% GelMA hydrogels show a clear stiffening response with concentration. All three formulations behave almost linearly at low strain (less than 10 percent) and low stress (less than 1 kPa) with the 5 percent hydrogel generating negligible stress (less than 1 kPa) and the 15 percent hydrogel generating only around 3 kPa. Beyond 10% it is no longer linear, 10% GelMA displays a moderate strain-stiffening behavior, with values of 10-12 kPa at 40% strain, whereas 15% formulation demonstrates a stiffer behavior with values of 10-22 kPa at the same strain. In contrast, the 5% GelMA is very compliant throughout, with stress values below 2kPa already at 40% strain. The results show that with higher GelMA concentration, both initial stiffness and strain-dependent reinforcement will increase, thus higher concentration gels are a more suitable choice for load-bearing applications.

The compressive modulus was significantly increased with increasing GelMA concentration from 3.3 +- 0.5 kPa for 5% GelMA to 30.0 +- 4.0 kPa for 15% GelMA. The Young's modulus also increased from 2.0 +- 0.3 kPa for 5% GelMA to 22.0 +- 3.2 kPa for 15% GelMA.

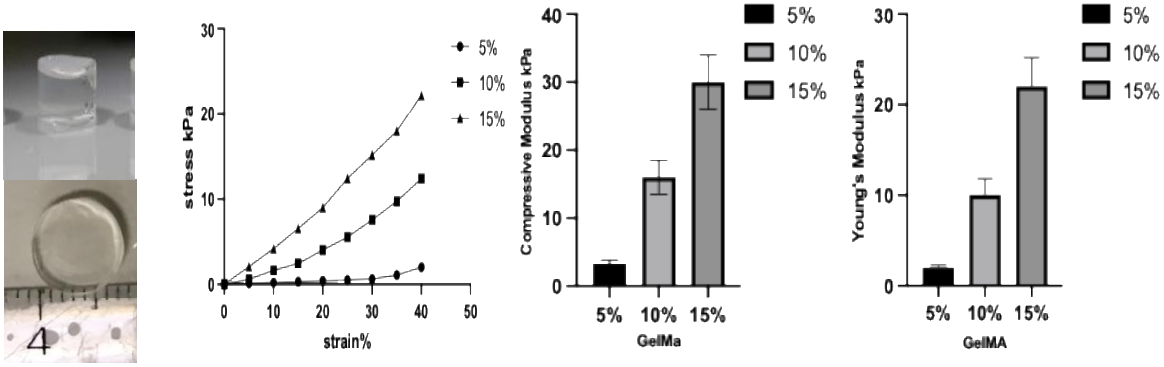

Fig. 2: A- Stress vs Strain curve B- Compressive modulus (kPa) C- Young's modulus (kPa)

These results are in agreement with other studies that have also demonstrated that an increase in the polymer concentration results in a denser polymer network and increased crosslinking density, which in turn produces a stiffer and stronger hydrogel (27). However, it should be mentioned that even in the highest concentration of 15%, the compressive modulus of the GelMA hydrogel remains an order of magnitude lower than the value of a native articular cartilage, which is in the range of 250-3000 kPa (28,29). This mechanical mismatch is still a major issue in the field and several approaches to improve the mechanical properties of GelMA hydrogels are under investigation including the use of reinforcing agents such as nanoparticles, nanofibers, or other polymers (30,31).

3.2. Swelling and degradation

The swelling and degradation behavior of GelMA hydrogels are important parameters that affect the transport of nutrients and wastes as well as long-term stability of the scaffold fig. 3. GelMA swelling and degradation indicate that the swelling ratio and the degradation rate are inversely proportional to the GelMA concentration.

The swelling ratio of 5% GelMA hydrogel was the greatest after 72 hours with a swelling ratio of 9.2, whereas the swelling ratio of 15% GelMA hydrogel was the lowest with a swelling ratio of 6.2. This is due to the fact that the higher concentration hydrogels have a denser polymer network which limits the influx of water molecules [14]. Similarly, the 5% GelMA hydrogel degraded completely within 14 days in the presence of collagenase whereas the 10% and 15% formulations retained 4% and 8% of their weight, respectively. This is because higher concentration hydrogels have higher crosslinking density and therefore are more resistant to enzymatic degradation [15].

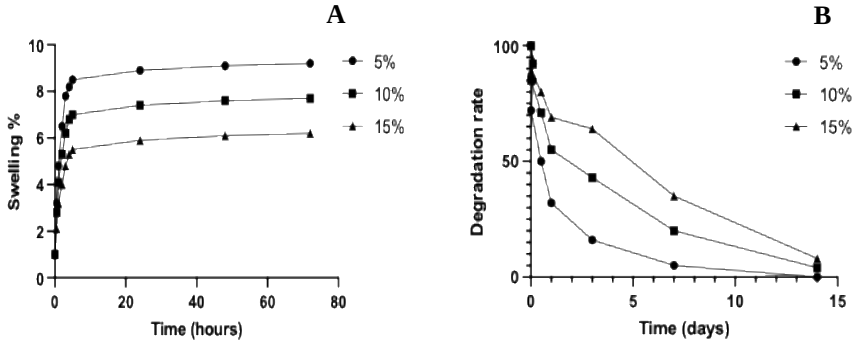

Fig. 3: GelMA hydrogel (5%, 10% and 15%) A- Swelling rate B- degradation rate

It is an ongoing research question what the optimal swelling and degradation profile of a scaffold for tissue engineering of cartilage is. High swelling ratio is good for nutrient diffusion, but it may result in the reduction of mechanical properties. Similarly, a fast rate of degradation may not be enough to provide long-term support to the regenerating tissue, whereas a slow rate of degradation may inhibit tissue integration and remodeling [16]. Therefore, the ideal swelling and degradation behavior will probably be application dependent and dependent on the tissue regeneration time point.

4. Discussion

The overall aim of this study was to characterize the Gelatin Methacrylate (GelMA) hydrogels at different polymer concentrations (5%, 10% and 15% w/v) to establish their suitability for cartilage tissue engineering applications. The results show clearly that GelMA is a tunable biomaterial, where a slight variation of polymer concentration results in significant and predictable variations in mechanical properties, swelling behavior, and degradation rates. These results are important for the rational design of GelMA-based scaffolds for articular cartilage regeneration.

A significant result of this research is the direct and significant correlation of GelMA concentration with the mechanical stiffness of the resulting hydrogels. As the concentration was increased from 5% to 15% both the compressive and Young's moduli increased by almost an order of magnitude. This is in accordance with the known literature, which explains this stiffening by a denser polymer network and a higher crosslinking density. For cartilage tissue engineering, which requires scaffolds to be able to withstand large biomechanical loads, this tunability is a major advantage. The best mechanical profile of all groups tested is the 15% GelMA formulation with compressive modulus of 30.0 +- 4.0 kPa.

Also, one of the major points discussed is the continuous mechanical mismatch between these GelMA hydrogels and native articular cartilage. Even at the highest concentration, the compressive modulus of our GelMA hydrogels is still an order of magnitude lower than native cartilage (250-3000 kPa). This mechanical mismatch has been one of the major issues in the field, and a major roadblock for the direct translation of pure GelMA scaffolds to the clinic for load-bearing purposes.

Furthermore, the study also emphasizes on the complex interaction among the physicochemical properties of GelMA hydrogels. As the concentration of polymer was increased, we noticed that the swelling ratio, the rate of and degradation of the scaffolds decrease. This is a logical consequence of a higher density of the polymer network which limits the entry of water and is a more formidable barrier to enzymatic degradation by collagenase.

The 5% GelMA hydrogels, due to their high swelling ratio and fast degradation, provide a microenvironment that is very conducive to nutrient and waste transport, which is necessary for cell viability. However, this comes at the expense of poor mechanical integrity and in vivo life. On the other hand, hydrogels made of 15% GelMA have better mechanical stability but block cellular infiltration and tissue integration with a reduced porosity and slower degradation.

These considerations are the key to the design of any tissue engineering scaffold. An ideal scaffold should be able to provide adequate mechanical support in the early stages of the healing process and also slowly degrade to permit the formation of new extracellular matrix by the encapsulated cells. Our results imply that the 10% GelMA formulation is a good compromise. It has medium mechanical properties, a satisfactory degradation pattern and a porous structure that can provide support for the viability and proliferation of cells,

so that it is a suitable candidate for cartilage regeneration.

5. Conclusion

Gelatin Methacrylate (GelMA) is a highly versatile and promising biomaterial for cartilage tissue engineering as its properties can be highly tuned by modulating its concentration, degree of methacrylation and crosslinking conditions. This study has given a complete characterization of GelMA hydrogels, showing the exchange that exist between mechanical strength and biological favorability. While no single GelMA formulation is a perfect match to the properties of native articular cartilage, a concentration of 10% (w/v) GelMA, in combination with optimized 3D bioprinting parameters, showed to be a good compromise for cartilage applications. Future studies should be aimed at creating composite GelMA hydrogels with improved mechanical properties that better match those of native cartilage, without compromising their excellent biological properties. The incorporation of reinforcing materials, such as nanoparticles or nanofibers or other polymers, as well as more complex and biomimetic scaffold architectures using advanced bioprinting techniques will be important for advancing the field of GelMA-based cartilage tissue engineering and the translation of these promising technologies for clinical applications.

Acknowledgments:

The authors express their sincere gratitude to the University of Thi-Qar, Iraq for their continuous support and facilitation in the completion of this research project.

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