Proceedings of the 2nd International Conference on Modern Medicine and Global Health
DOI: 10.54254/2753-8818/45/20240617

Multifunctional GelMA platforms with Hybrid hydrogel

Jingye Zhou[1,4,5,†], Jinmoqian Wang[2,6,†], Minghao Liu[3,7,†]

[1]Department of Veterinary Medicine, Yangzhou University, Yangzhou, 225009, China,
[2]Huaai Preparatory Academy, Cheng Du,610000, China,
[3]Department of Forensic Medicine, Wannan Medical College, Wuhu,241002, China,
[4]Corresponding author

[5]18952592583@163.com
[6]Mark_wjmq@163.com
[7]18905540623@163.com
[†]All the authors contributed equally to this work and should be considered as co-first author.

Abstract. Polymer hydrogels, 3D scaffolds for tissue adjustment and delivery systems for therapeutic molecules and cells, are a fascinating platform. For example, GelMA methacrylate has become a representative hydrogel preparation and has wide-ranging applications in biomedicine. Recent research on GelMA-based hydrogels has focused on combining them with other things is a helpful way to make GelMA better for some uses, such as carbon nanotubes (CNT), graphene oxide (GO), tiny particles from minerals, other natural materials, and man-made materials, aiming to provide GelMA with enhanced biological properties and physicochemical. The benefits of this approach are manifold: i) change the electrical conductivity and simulate the propagation of electrical signals, present electrical/magnetic properties to generate for repair of specific tissues, ii) promote bone cell growth without specific growth factors, and iii) can closely mimic the natural tissue environment. iv) Facilitates tissue culture and drug delivery. The method utilized in GelMA can also be applied to other existing polymer hydrogel systems.

Keywords: Hybrid hydrogel, Multifunctional, Therapeutics, GelMA platforms, Tissue repair

1. Introduction

Multifunctional hydrogels are a significant component of engineered damaged tissues because they closely resemble the cellular microenvironment. So far, the particular materials have been actively studied to progress a variety of biological alternatives and stimulate predictable biological responses [1-5]. Despite these remarkable properties, multifunctional hydrogels still have varies limitations, such as little mechanical stiffness, little thermal stability, and rapid degradation rates, which limit their effective use in a variety of applications [6-9]. This is the significance of hybrid hydrogel system. These hydrated polymer networks with covalent or non-covalent deep-rooted hydrogel systems can serve as multi-response platforms, mimic natural cell matrices, and have regenerative properties of engineered damaged tissues. These mixed nanomaterials also showed improved biocompatibility, acceptable cell viability, proliferation and differentiation response to cells.

Hybrid hydrogels are made by mixing different things together. These things are chosen because they have specific properties. GelMA is a material that cells respond to, and it can be changed in many ways. Making hybrid materials by mixing GelMA with other things is a helpful way to make GelMA better for some uses. In this passage, we will talk about hybrid hydrogels that are made using GelMA and other things like carbon nanotubes (CNT), graphene oxide (GO), tiny particles from minerals, other natural materials, and man-made materials

2. Carbon nanotubes (CNTS), graphene oxide (GO), and graphene incorporated GelMA

In certain cases of building body tissues, we need materials that are stronger than what GelMA hydrogels usually provide (about 30 kPa in compression strength). To make GelMA hydrogels stronger, we can mix them with higher amounts of the GelMA material before they're fully formed (called prepolymer solutions). However, doing this can affect other important qualities like how fast they break down, how many tiny holes they have (porosity), and how well cells spread and grow in a 3D environment.

So, scientists have looked into creating hybrid hydrogels by combining GelMA with other materials. This is a way to make GelMA hydrogels stronger while still being good for cells to grow on. For instance, researchers called Shin and his team mixed Carbon Nanotubes (CNTs) into GelMA hydrogels. This made the hydrogels stiffer, but it didn't stop cells from growing in them. They tried different amounts of CNTs and found that having 0.5 mg/mL of CNTs in 5% GelMA made the hydrogels much stronger (31 kPa) compared to just GelMA hydrogels (10 kPa). Cells also grew well on these hydrogels [10-12].

The researchers also found that adding CNTs changed how well electricity could move through the hydrogels. This is important for building tissues like the heart or nerves. By arranging the CNTs in a certain way using electricity, they could control how cells grow. Another team, led by Ramón-Azcón, used electricity to line up the CNTs within the GelMA hydrogels. This made the hydrogels even better at conducting electricity, which helps with cell growth and protein production [11]. When they tried this with muscle cells, the cells grew better and showed more signs of functioning normally.

Another material called Graphene Oxide (GO) was used to make GelMA hydrogels stronger too. Researchers like Shin found that when they mixed GO with GelMA, the hydrogels became better at supporting cells' growth and alignment [11]. This was useful for making complex artificial tissues that are stable and safe for cells. The GO mixed in GelMA could also be changed to make the hydrogels either stronger or more conductive without making them hard to work with [11].

Some scientists mixed GelMA with a special form of GO called Methacrylated Graphene Oxide (MeGO). This made the hydrogels much tougher and resistant to breaking [13]. Cells also grew better on these hydrogels compared to just GelMA ones. Moreover, GelMA with a kind of GO that helps with gene delivery was used to help injured heart tissues in rats. By using this special GelMA, researchers could deliver growth factors directly to the damaged heart area and help it heal better.

One more team led by Ahadian came up with a simple and environmentally friendly way to mix Graphene Oxide into GelMA. This made the hydrogels both stronger and better at conducting electricity [14]. By adding materials like Carbon Nanotubes and Graphene Oxide to GelMA hydrogels, scientists can create materials that are stronger and can conduct electricity. These materials can be used to make scaffolds for growing cells in the lab, especially for tissues like the heart and muscles, where electrical signals are important.

3. GelMA hydrogel containing inorganic nanoparticles

The enhancement of GelMA hydrogels has been achieved by adding substances like gold [15,16] and other particles made from non-living materials. This addition has led to improvements in the strength of the hydrogels, their ability to conduct electricity, respond to heat or magnets, and their usefulness for biological purposes. For instance, researchers Heo et al. included gold nanoparticles (GNP) in GelMA hydrogels for creating materials for bone tissue engineering (shown in Figure 3C). Their study showed that these GelMA-GNP hydrogels helped human adipose-derived stem cells (ADSCs) to grow and change into bone-building cells. This was proven by seeing higher levels of alkaline phosphatase (ALP) activity (up to 85% increase at day 14) and an increase in the expression of three genes linked to bone formation: bone sialoprotein (BSP), osteocalcin (OCN), and runt-related transcription factor 2 (RUNX2) [16].

Another idea was to make GelMA hydrogels mixed with a substance called hydroxyapatite. This mix was applied as a coating for titanium (Ti) surfaces, which are used in bone implants. They made the titanium surfaces more reactive by treating them with alkali and then coating them with a GelMA film. When these GelMA-coated titanium materials were put in a solution that imitates concentrated human plasma for three days, a layer of hydroxyapatite-GelMA formed, helping the implant integrate better with the bone [17].

Researchers also recently made hybrid GelMA hydrogels with something called 2D nanosilicates. These are tiny particles that don't come from living things, and they have special properties like being anisotropic (having different properties along different directions) and having a large surface area. These nanosilicates improved how the GelMA hydrogel interacted with cells enclosed within it. The hybrid hydrogels became about four times stronger when compressed and had larger holes compared to regular GelMA hydrogels. They tested these hybrid hydrogels with preosteoblast NIH MC3T3 cells in the lab, and the results were impressive. The hybrid hydrogels promoted bone cell growth even without the presence of specific growth factors in the cell culture medium. This was confirmed by a three-fold increase in ALP activity and a four-fold increase in the formation of a mineralized matrix. These findings showed a promising way to grow bone tissue without needing certain growth factors [18-19].

4. Hybrid hydrogels based on GelMA and other biopolymers

Researchers have delved into the development and application of hybrid hydrogels formed by combining GelMA with various other biopolymers, with diverse objectives in mind. These objectives encompass enhancing their structural integrity, tailoring properties such as porosity, swelling capacity, and degradation rates, and conferring specific functionalities, like electrical conductivity [20,21].

One particularly notable pairing receiving significant attention involves hyaluronic acid methacrylate (HAMA) and GelMA, as depicted in Figure 3D. Given that hyaluronic acid (HA) and collagen are predominant constituents in numerous tissues, including the heart, cartilage, and nervous tissues, the synergy of GelMA and HAMA hydrogels appears promising for mimicking natural tissue environments. Recent investigations into the physical characteristics of GelMA-HAMA hybrids, encompassing swelling, degradation, strength, and cellular responses in lab settings, have unveiled remarkable improvements in strength compared to single-material hydrogels. For instance, the incorporation of 2% HAMA elevated the strength of 5% GelMA hydrogels from 4 to 36 KPa. Similarly, the inclusion of 2% HAMA increased the strength of 10% GelMA hydrogels from 32 to 72 KPa [22].

Levett and colleagues also conducted research on hybrid hydrogels fabricated from GelMA and HAMA, as well as from chondroitin sulfate methacrylate (CSMA) and GelMA, for the purpose of engineering cartilage tissue. In their experiments, human chondrocytes (cells found in cartilage) were embedded in GelMA-HAMA and GelMA-CSMA hydrogels, resulting in enhanced cartilage formation. This was assessed through the analysis of gene activity and specialized imaging techniques. The introduction of HAMA to GelMA structures induced cell rounding and increased the production and dispersion of new material within the structure. Consequently, this improved cartilage formation process led to a 114 kPa strength increase for GelMA-HAMA and GelMA-CSMA structures over an 8-week lab growth period, while GelMA-only structures only experienced a 26 kPa increase over the same duration [23,24].

Recent studies have proposed the combination of GelMA and HAMA as a method for investigating changes and developments in tissue cells, particularly valvular tissue interstitial cells (VICs) found in heart valves. This approach offers valuable insights into diseases affecting heart valves. Additionally, GelMA has been successfully combined with silk fibroin (SF) to establish a polymer network. Silk alone is an excellent material for tissue growth due to its manageability and strength. Researchers like Xiao and his team fabricated and analyzed GelMA-SF polymer networks through a straightforward process: first, they employed light to bond GelMA and silk fibroin, followed by a special solution treatment to reinforce the silk fibroin. The assessment encompassed the strength, swelling capacity, degradation, and suitability for creating 3D scaffolds (structures for cell cultivation in the lab). The exploration of dual-polymer networks involving GelMA and other materials has also yielded promising results [25].

Shin and collaborators managed to create robust hydrogels capable of accommodating cells, specifically NIH-3T3 fibroblasts, using a two-step process. They combined modified biomolecules known as gellan gum methacrylate (GGMA) with GelMA and utilized light to fuse them together. Testing the hydrogels with cells demonstrated their suitability for cell growth and their potential to emulate the strength of tissues like cartilage or tendons. The integration of a second polymer into GelMA offers expanded possibilities by enhancing the strength of GelMA structures and granting them new functionalities [25].

Bae and his team observed that cells within pullulan methacrylate (PulMA) hydrogels aggregated into clusters instead of elongating. The size of these clusters could be controlled by adjusting the mixing ratios of the two materials. This suggests that GelMA-PulMA hydrogels may be valuable for creating controlled micro-tissues. Other combinations involving GelMA and a second biopolymer include GelMA with dextran glycidyl methacrylate (DexMA). When the right proportions of DexMA are added to GelMA, the resulting hydrogels exhibit reduced swelling and significantly enhanced strength compared to GelMA alone. Moreover, Liu and his team demonstrated that hydrogels produced by blending DexMA (modified with lysine) and GelMA could be utilized for cultivating blood vessels in the lab. The strength of these hydrogels could be modulated by varying the concentrations of different molecules. Cells from artery smooth muscles introduced into these hydrogels exhibited growth, spreading, and network formation, mimicking the development of blood vessels. Another innovative application of GelMA involves creating patterns within hydrogels [26].

Li and his colleagues harnessed a specialized molecule known as a collagen mimetic peptide (GMP) to generate patterns within GelMA hydrogels. This process involved binding GMP to GelMA and subsequently using light to facilitate their connection. This method holds promise for shaping specific structures within GelMA hydrogels [27].

In a recent breakthrough, Visser and his team incorporated fragments of horse cartilage matrix into GelMA hydrogels. They observed that these additions promoted cell growth in a manner reminiscent of natural cartilage formation. Considering that the extracellular matrix (ECM), the natural environment of cells, comprises diverse components, the creation of hydrogels incorporating multiple biopolymers provides a means to further tailor the behavior of hybrid hydrogels, aligning them more closely with natural tissues. Future research endeavors may center on optimizing the blending of GelMA with other materials to craft hydrogels ideally suited for specific tissue engineering applications [27]

5. Hybrid hydrogels based on GelMA and synthetic polymers

Numerous studies have explored the development and assessment of hybrid hydrogels formed by the combination of GelMA and synthetic polymers. One such study conducted by Hutson and his colleagues delved into the creation of hybrid hydrogels utilizing GelMA (up to 15% w/v) and PEG (up to 10% w/v). This fusion of GelMA and PEG derivatives, crosslinked through light exposure, resulted in hybrid hydrogels that exhibited versatile physical and biological characteristics. Interestingly, the incorporation of GelMA (5–15% w/v) into PEG (5 and 10% w/v) significantly enhanced the attachment and spreading of fibroblast cells on the hydrogel surfaces. To illustrate, on PEG hydrogel surfaces, less than 1% of the surface area was covered by fibroblasts after six hours of cultivation. In contrast, when using a 10% GelMA-10% PEG hybrid, over 10% of the surface area was occupied by fibroblasts within the same time frame. Moreover, fibroblast cells encapsulated within GelMA-PEG hybrid hydrogels managed to form intricate three-dimensional cell networks after seven days of culture, a phenomenon not observed in pure PEG hydrogels [28-30].

In another investigation, Qi and his team harnessed the distinct properties of GelMA and PEG to control the environments conducive to cell growth. They employed micromolding and photomasking techniques to meticulously arrange 3D hybrid hydrogel arrays based on GelMA and PEG derivatives, as depicted in Figure 3F. Remarkably, embryoid bodies derived from mouse embryonic stem cells (ESCs) displayed directed growth with structured vasculogenic differentiation within these tailored bipolar extracellular matrices. Significantly, the oriented differentiation of each embryoid body within the customized matrix was linked to potential cell interactions among different sections of the embryoid bodies exposed to varying microenvironments [31].

Nanopatterned hybrid frameworks, composed of GelMA, HA, and PEG dimethacrylate, underwent examination by Nemeth et al. with the goal of inducing chondrogenesis from dental pulp stem cells (DPSCs). Their observations, coupled with gene and protein expression analysis, revealed that both nanopatterning and the inclusion of HA prompted DPSCs to undergo chondrogenic differentiation. Similarly, Pedrón et al. employed PEG-GelMA-HAMA hydrogels as a simulated matrix to mimic gliomas for the study of human glioblastoma multiforme (hGBM), an aggressive form of brain cancer [32].

In a separate study, Serafim and his team characterized a range of hybrid hydrogels produced from GelMA and polyacrylamide (PAA) using a straightforward "one-pot" synthesis involving photopolymerization. They demonstrated a direct relationship between the composition and relevant properties of the hybrid PAA-GelMA, which has applications in tissue culture and drug delivery. These properties included swelling, mechanical strength, porosity of the resulting covalent network, and degradability. Boere and colleagues outlined a relatively complex process for creating novel hybrid materials by grafting poly-methacrylated-poly(hydroxymethylglycolide-co-ε-caprolactone)-poly(ε-caprolactone) (pMHMGCL-PCL) onto GelMA hydrogel surfaces. This covalent linkage between modified PCL and GelMA yielded increased resistance to recurring axial and rotational forces at the interface. Remarkably, human chondrocytes embedded in these constructs successfully generated cartilage-specific matrix in vitro, as evidenced by immunostaining outcomes, after six weeks of cultivation. Moreover, introducing these constructs into rats led to extensive collagen II deposition, including deposition at the interface between the implant and native tissues.

In certain advanced applications, materials with specific attributes are essential. For instance, in the field of heart valve engineering, materials must endure the dynamic stresses within the heart valve environment. To achieve hybrid scaffolds with improved mechanical properties, electrospun microfiber scaffolds made of poly(glycerol sebacate) (PGS) and poly(ε-caprolactone) (PCL) were incorporated into a hybrid hydrogel composed of HAMA-GelMA. This hybrid hydrogel provided sheep mitral valve interstitial cells with a more uniform spatial arrangement in three dimensions compared to unmodified GelMA hydrogels or microfiber scaffolds lacking GelMA. The hybrid system, as opposed to just electrospun fibers or hydrogel scaffolds, offered an ideal three-dimensional framework for heart valve tissue engineering [33,34].

With contemporary methods of controlled polymerization and a wide array of available synthetic polymers, there are boundless opportunities to tailor hybrid hydrogels by combining GelMA with other synthetic polymers. This combination can occur at the molecular level by directly blending them as the initial polymer solution or at the supramolecular level by introducing engineered assemblies of synthetic polymers into the GelMA hydrogel matrix, such as microfibers embedded in the GelMA hydrogel as reinforcing additives. These possibilities open up new avenues for potential applications of hybrid GelMA hydrogels within the biomedical field.

6. Conclusion and prospect

GelMA is prepared by one-step chemical modification using natural polymer gelatin as raw material. The introduction of photocrosslinked methacryloyl substituents makes it convenient and rapid to gelate under light irradiation in the presence of photoinitiators. Nowadays, methacrylic acid gelatin (GelMA) has become into a representative hydrogel preparation, which has wide-ranging applications in biomedicine. Modern research areas focus on the combination of GelMA-based hydrogels with bioactive and functional nanomaterials in order to afford enhanced physical, chemical and biological properties for GelMA. Its achievements have a great impact on the application of modern science and technology, as well as biology and medicine.

In various other biomedical applications to be searched, biomaterials based on GelMA will still become high candidate materials in the area of biomaterials research because of their high stability and

DOI: 10.54254/2753-8818/45/20240617

wide range of adhibitions. In the near future, the application of GelMA will become wider and wider with the emergence of new micro-manufacturing technology. GelMA-based smart hydrogels are designed to seal or even heal surgical injuries, release oxygen and other nutrients, or capture and remove by-products that inhibit cells. This is also the future research direction of GelMA.

Acknowledgement

Jingye Zhou, Jinmoqian Wang, and Minghao Liu contributed equally to this work and should be considered co-first authors.

References

[1] A.C. Daly, L. Riley, T. Segura, J.A. Burdick, Hydrogel microparticles for biomedical applications, Nat. Rev. Mater. 5 (1) (2020) 20–43.
[2] S. Hinderer, S.L. Layland, K. Schenke-Layland, ECM and ECM-like materials—biomaterials for applications in regenerative medicine and cancer therapy, Adv. Drug Deliv. Rev. 97 (2016) 260–269.
[3] J. Li, D.J. Mooney, Designing hydrogels for controlled drug delivery, Nat. Rev. Mater. 1 (12) (2016) 1–17.
[4] L. Li, J.M. Scheiger, P.A. Levkin, Design and applications of photoresponsive hydrogels, Adv. Mater. 31 (26) (2019), 1807333.
[5] R.V. Ulijn, N. Bibi, V. Jayawarna, P.D. Thornton, S.J. Todd, R.J. Mart, A. M. Smith, J.E. Gough, Bioresponsive hydrogels, Mater. Today 10 (4) (2007) 40–48.
[6] A.S. Hoffman, Hydrogels for biomedical applications, Adv. Drug Deliv. Rev. 64 (2012) 18–23.
[7] N.A. Peppas, J.Z. Hilt, A. Khademhosseini, R. Langer, Hydrogels in biology and medicine: from molecular principles to bionanotechnology, Adv. Mater. 18 (11) (2006) 1345–1360.
[8] T. Billiet, M. Vandenhaute, J. Schelfhout, S. Van Vlierberghe, P. Dubruel, A review of trends and limitations in hydrogel-rapid prototyping for tissue engineering, Biomaterials 33 (26) (2012) 6020–6041.
[9] T.R. Hoare, D.S. Kohane, Hydrogels in drug delivery: progress and challenges, Polymer 49 (8) (2008) 1993–2007.
[10] Shin SR, Bae H, Cha JM, Mun JY, Chen Y-C, Tekin H, et al. Carbon Nanotube Reinforced Hybrid Microgels as Scaffold Materials for Cell Encapsulation. ACS Nano. 2012;6:362-72.
[11] Shin SR, Aghaei-Ghareh-Bolagh B, Dang TT, Topkaya SN, Gao X, Yang SY, et al. Cell-laden Microengineered and Mechanically Tunable Hybrid Hydrogels of Gelatin and Graphene Oxide. Advanced Materials. 2013;25:6385-91.
[12] Ramon-Azcon J, Ahadian S, Estili M, Liang X, Ostrovidov S, Kaji H, et al. Dielectrophoretically Aligned Carbon Nanotubes to Control Electrical and Mechanical Properties of Hydrogels to Fabricate Contractile Muscle Myofibers. Advanced Materials. 2013;25:4028-34.
[13] Cha C, Shin SR, Gao X, Annabi N, Dokmeci MR, Tang X, et al. Controlling Mechanical Properties of Cell-Laden Hydrogels by Covalent Incorporation of Graphene Oxide. Small. 2014;10:514-23.
[14] Ahadian S, Estili M, Surya VJ, Ramon-Azcon J, Liang X, Shiku H, et al. Facile and green production of aqueous graphene dispersions for biomedical applications. Nanoscale. 2015;7:6436-43.
[15] Ahadian S, Ramon-Azcon J, Estili M, Obregon R, Shiku H, Matsue T. Facile and rapid generation of 3D chemical gradients within hydrogels for high-throughput drug screening applications. Biosensors & Bioelectronics. 2014;59:166-73.
[16] Heo DN, Ko W-K, Bae MS, Lee JB, Lee D-W, Byun W, et al. Enhanced bone regeneration with a gold nanoparticle-hydrogel complex. Journal of Materials Chemistry B. 2014;2:1584-93.
[17] Tan G, Zhou L, Ning C, Tan Y, Ni G, Liao J, et al. Biomimetically-mineralized composite coatings on titanium functionalized with gelatin methacrylate hydrogels. Applied Surface Science. 2013;279:293-9.

[18] Xavier JR, Thakur T, Desai P, Jaiswal MK, Sears N, Cosgriff-Hernandez E, et al. Bioactive Nanoengineered Hydrogels for Bone Tissue Engineering: A Growth-Factor-Free Approach. ACS Nano. 2015;9:3109-18.

[19] Gaharwar AK, Mihaila SM, Swami A, Patel A, Sant S, Reis RL, et al. Bioactive Silicate Nanoplatelets for Osteogenic Differentiation of Human Mesenchymal Stem Cells. Advanced Materials. 2013;25:3329-36.

[20] Levett PA, Melchels FPW, Schrobback K, Hutmacher DW, Malda J, Klein TJ. A biomimetic extracellular matrix for cartilage tissue engineering centered on photocurable gelatin, hyaluronic acid and chondroitin sulfate. Acta Biomaterialia. 2014;10:214-23.

[21] Wang H, Zhou L, Liao J, Tan Y, Ouyang K, Ning C, et al. Cell-laden photocrosslinked GelMA-DexMA copolymer hydrogels with tunable mechanical properties for tissue engineering. Journal of Materials Science-Materials in Medicine. 2014;25:2173-83.

[22] Camci-Unal G, Cuttica D, Annabi N, Demarchi D, Khademhosseini A. Synthesis and Characterization of Hybrid Hyaluronic Acid-Gelatin Hydrogels. Biomacromolecules.2013;14:1085-92.

[23] Levett PA, Melchels FPW, Schrobback K, Hutmacher DW, Malda J, Klein TJ. A biomimetic extracellular matrix for cartilage tissue engineering centered on photocurable gelatin, hyaluronic acid and chondroitin sulfate. Acta Biomaterialia. 2014;10:214-23.

[24] Hjortnaes J, Camci-Unal G, Hutcheson JD, Jung SM, Schoen FJ, Kluin J, et al. Directing Valvular Interstitial Cell Myofibroblast-Like Differentiation in a Hybrid Hydrogel Platform. Advanced Healthcare Materials. 2015;4:121-30.

[25] Shin H, Olsen BD, Khademhosseini A. The mechanical properties and cytotoxicity of cell-laden double-network hydrogels based on photocrosslinkable gelatin and gellan gum biomacromolecules. Biomaterials. 2012;33:3143-52.

[26] Bae H, Ahari AF, Shin H, Nichol JW, Hutson CB, Masaeli M, et al. Cell-laden microengineered pullulan methacrylate hydrogels promote cell proliferation and 3D cluster formation. Soft Matter. 2011;7:1903-11.

[27] Li Y, San BH, Kessler JL, Kim JH, Xu Q, Hanes J, et al. Non-Covalent Photo-Patterning of Gelatin Matrices Using Caged Collagen Mimetic Peptides. Macromolecular Bioscience. 2015;15:52-62.

[28] Daniele MA, Adams AA, Naciri J, North SH, Ligler FS. Interpenetrating networks based on gelatin methacrylamide and PEG formed using concurrent thiol click chemistries for hydrogel tissue engineering scaffolds. Biomaterials. 2014;35:1845-56.

[29] Fu Y, Xu K, Zheng X, Giacomin AJ, Mix AW, Kao WJ. 3D cell entrapment in crosslinked thiolated gelatin-poly(ethylene glycol) diacrylate hydrogels. Biomaterials. 2012;33:48-58.

[30] Fathi A, Lee S, Breen A, Shirazi AN, Valtchev P, Dehghani F. Enhancing the mechanical properties and physical stability of biomimetic polymer hydrogels for micro-patterning and tissue engineering applications. European Polymer Journal. 2014;59:161-70.

[31] Qi H, Du Y, Wang L, Kaji H, Bae H, Khademhosseini A. Patterned Differentiation of Individual Embryoid Bodies in Spatially Organized 3D Hybrid Microgels. Advanced Materials. 2010;22:5276-81.

[32] Nemeth CL, Janebodin K, Yuan AE, Dennis JE, Reyes M, Kim D-H. Enhanced Chondrogenic Differentiation of Dental Pulp Stem Cells Using Nanopatterned PEG-GelMA-HA Hydrogels. Tissue Engineering Part A. 2014;20:2817-29.

[33] Boere KWM, Visser J, Seyednejad H, Rahimian S, Gawlitta D, van Steenbergen MJ, et al. Covalent attachment of a three-dimensionally printed thermoplast to a gelatin hydrogel for mechanically enhanced cartilage constructs. Acta Biomaterialia. 2014;10:2602-11.

[34] Eslami M, Vrana NE, Zorlutuna P, Sant S, Jung S, Masoumi N, et al. Fiber-reinforced hydrogel scaffolds for heart valve tissue engineering. Journal of Biomaterials Applications. 2014;29:399-410.