ISSN 2616-5880 Vol. 6, Issue 3: 79-84, DOI: 10.25236/AJMC.2025.060311

A Generalized Fabrication Strategy towards the Mechanical Double-Network Hydrogel Scaffold for Potential Tissue Engineering

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Abstract: *Tissue engineering provides a prominent solution for repairing damaged tissue and helping the regeneration of the peripheral cells. The artificial filler, hydrogel, for example, can provide an ideal microenvironment for the settlement of body cells and the diffusion of the medicine. To provide its normal function, sufficient mechanical strength, compatibility with the peripheral tissue, low operation requirement, and the ability to facilitate the generation of peripheral tissue are required. One kind of double-network (DN) hydrogel (GEL-PAA) will be made and tested in this study, which can be made by exposing the solution of gelatin (GEL) and polyacrylic acid (PAA) to ultraviolet radiation. Through the ultraviolet radiation, the GEL forms a chain-entanglement network with carboxy groups in PAA. This physically strong network enables the hydrogel (GEL) to have excellent physical properties, such as high tensile strength, high compression resistance, and high ductility. In addition, in vitro biological studies reveal that this hydrogel (GEL-PAA) is capable of activating the bone mesenchymal stem cells (BMSCs)and facilitating the growth and proliferation of the cells, providing a prerequisite for repairing the damaged tissue. Overall, this method demonstrated here is capable and universal for constructing mechanically bioactive scaffolds, demonstrating the spread of their bio-applicability as well as long-lasting biological function and mechanical support, promoting the advancement of tissue engineering and raising tissue engineering to a higher level.*

Keywords: *Hydrogel, Tissue Engineering, Rigidity PAA Gel*

1. Introduction

Articular tissue injuries represent a common health problem associated with pain, joint deterioration, and possible dysfunction. Although surgical methods and clinical approaches—including autologous implantation—have been used for many years, limitations in available implants and suboptimal long-term outcomes of recovery remain contentious, highlighting a demand for novel designs and treatments [1-5]. In this context, tissue engineering has arisen as a viable option for cartilage therapy [6-10]. Among possible scaffold materials, hydrogels are particularly appealing due to their highly hydrous, crosslinked three-dimensional construction and advantageous biocompatibility. Particularly, in articular cartilage restructuring, their mechanical performance is crucial, as they have to offer structural support while also furnishing a microenvironment conducive to sustaining the chondrogenic phenotype. Nevertheless, many hydrogels suffer from softness or brittleness, rendering them unsuitable for load-bearing applications.

Double-network (DN) hydrogels are elastic materials composed of two interpenetrating polymer networks with distinct characteristics: one is stiff and brittle, which enables it to dissipate energy through fracture, while the other is soft and stretchable, allowing it to remain intact under deformation. The synergy of these networks gives DN hydrogels a balanced mechanical property, combining stiffness with toughness, which makes them attractive for tissue engineering scaffolds. Nonetheless, conventional DN hydrogel synthesis often involves complex procedures, toxic initiators, and additional chemical agents, limiting their broader adoption in biological contexts. Thus, there is an urgent need to develop hydrogel scaffolds that integrate suitable mechanical properties, biological compatibility, and application-specific traits—such as high strength, biocompatibility, and biomimetic microenvironments resembling natural tissues.

A protein derivative produced through partial hydrolysis of collagen, gelatin displays a variety of

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physicochemical attributes that support its use across food, pharmaceutical, and biomedical sectors. Its properties are strongly affected by molecular composition and external criteria. Moreover, gelatin's mechanical, rheological, thermal, and swelling behaviors are not fixed but can be modulated through factors such as molecular weight (Bloom index), pH, temperature, crosslinking methods (physical or chemical), and incorporation of modifiers like plasticizers or fillers. This adaptability allows gelatin to be customized for uses from food texture enhancement to sophisticated biomedical scaffolds. However, for use in scaffold design, key requirements include not only biocompatibility and porous structure but also adequate mechanical strength and the ability to promote chondrogenic differentiation of stem cells.

Polyacrylic acid (PAA) is a synthetic, water-soluble polymer formed from polymerized acrylic acid monomers. It contains a backbone of carbon-carbon chains with pendant carboxyl groups (–COOH), which govern its reactivity and functional characteristics. Owing to its polyelectrolyte behavior, responsive solution properties, strong chelating ability, and adjustable rheology, PAA serves as a highly adaptable functional polymer with uses in industrial, environmental, and biomedical fields—including roles as an anti-inflammatory agent, disinfectant, wound-healing promoter, and moisturizer.

Guided by this background, the present study aims to fabricate a high-performance hybrid dual-network hydrogel composed of gelatin and polyacrylic acid (GEL–PAA), with tunable structure and mechanics achieved via modulation of the gelatin network. Initially, gelatin was incorporated into a covalently crosslinked PAA network to produce a highly ductile GEL–PAA hydrogel (Figure 1). The resulting DN hydrogels exhibit advantageous mechanical properties due to dynamic coordination effects, demonstrating high tensile strength, substantial elastic modulus, exceptional fracture toughness, and strong fatigue resistance. Furthermore, the hydrogel showed good cytocompatibility, supporting cell viability and scaffold integration. Thus, this work establishes a proof of concept for the GEL–PAA DN hydrogel as a mechanobiologically active scaffold capable of facilitating tissue regeneration, with promising potential for various biomedical applications.

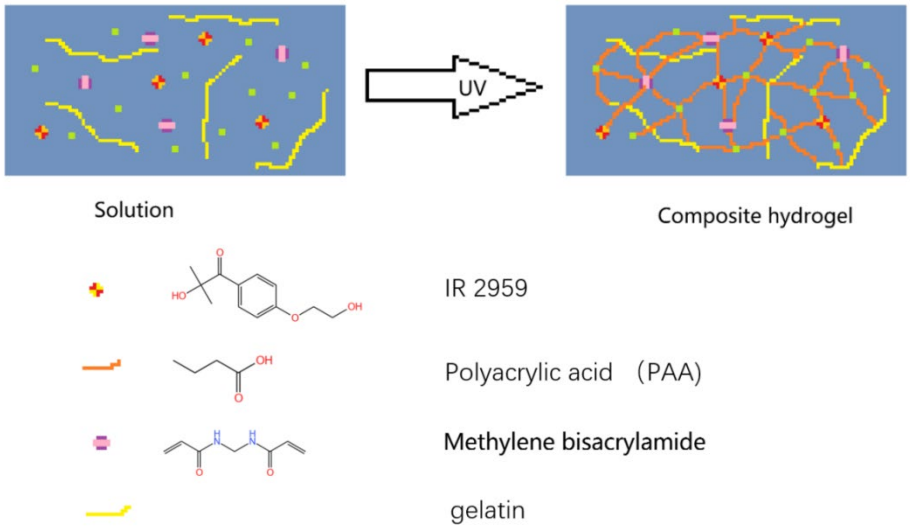

Figure 1 Schematic illustrations of fabricated GEL-PAA DN hydrogel.

2. Experimental section

2.1 Materials

Acrylic acid (98%, Energy Chemical Reagent Co., Ltd.), N, N'-methylene-bis-acrylamide (MBA, 98%, Energy Chemical Reagent CO., Ltd.), 2-hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiopheno (Irgacure 2959, Alfa Aesar), and gelatin(Sigma-Aldrich). The chemicals listed are the fundamental compositions of the GEL-PAA DN hydrogel, whereas in the follow-up experiments evaluating the hydrogel, other related reagents are used. All of these other reagents are purchased from Concord Reagent Co., Ltd. and applied to the sample subsequently, except for the bone marrow mesenchymal stem cells (BMSCs), which are provided by China's infrastructure of cell line resources.

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2.2 Preparation of GEL-PAA DN hydrogel

With photoinitiated radical polymerization, the solutions including AA(1.8g), GEL(1.0g), MBA solution (118ul), 0.02mol% of AA monomer, Cmba=10mg ml^-1), and IR 2959 (57.6mg, 1 mol% of AA monomer) were dissolved in 10 ml of deionized water and mixed together sufficiently. Then, ultraviolet radiation was applied to the intended molds containing the mixture for 4 hours, producing the GEL-PAA DN hydrogel.

2.3 characterization

A microscopic image generation and two mechanical tests were applied to the GEL-PAA DN hydrogel in this section. The mechanical test involved the compressive tests done on hydrogels solidified within glass sample vials with a diameter of 9 mm and the tensile tests done on hydrogels solidified within plastic tubes with a diameter of 6mm. All tests were operated on an Instron 3365 apparatus at an ambient temperature, using samples that were precisely measured based on diameter. The tensile tests were run at a 50 mm min^-1 velocity, while the compression tests were run at a 5mm min^-1 velocity. The electron microscopy (SEM) images were obtained by a JSM-6700F microscope, using free-dried samples covered with a Pt layer using sputter-coating technology. The sputter-coating technology was adopted in 90s in order to ensure the samples have high conductivity.

2.4 Cytotoxicity and cell proliferation assay

The cell counting Kit-8 (CCK-8) assay was used to measure the cytotoxicity of the hydrogel. Technically, bone marrow mesenchymal stem cells (BMSCs) were exposed to hydrogel extracts, and the absorbance of the CCK-8 solution was measured to calculate cell viability. First, the cells were placed in a culture dish at 37 degrees Celsius for 24 hours in a 5% CO2 environment and allowed to reproduce. At the same time, control cells were grown in DMEM media with 10% fetal bovine serum(FBS). Then, the hydrogel extracts were incubated in the cell solution and cultured for 1, 3, and 7 days. After the cells were combined thoroughly with the extract, 10 μL of CCK-8 solution can be substituted into the medium, replacing the cells. At last, the absorbance of the solution was tested by a microplate reader at a wavelength of 450nm, reflecting the current cell number. To convert absorbance to cell viability, we use the equation:

$$\text{Cell viability (\%)} = (A_{sample}-A_{blank})/ (A_{control}-A_{blank}) \times 100\% \quad (1)$$

2.5 Live/dead straining assay

After calculating cell viability, live/dead staining can be performed to clarify the result. The cells were combined with red fluorescent propidium iodide(PI) stain and green fluorescence (AM) stain at 37 degrees Celsius for 1 h. Then, by applying confocal laser scanning microscopy(CLSM), the fluorescence emission of the GMSCs was analyzed at excitation wavelengths of 568nm and 488nm. Specifically, the fluorophores are detected to derive the actual live-cell ratio compared to that of dead cells. (Figure 2)

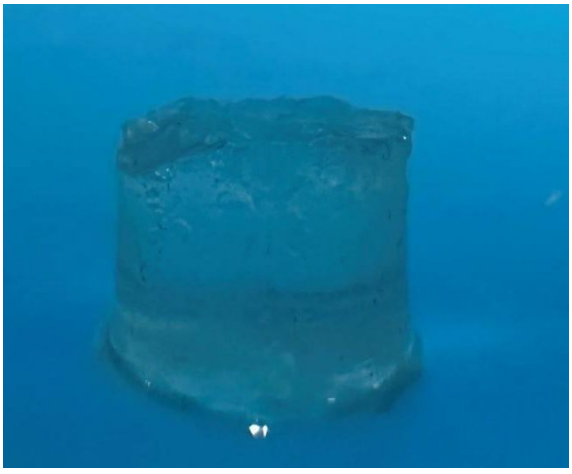

Figure 2 General observation image of GEL-PAA DN hydrogel

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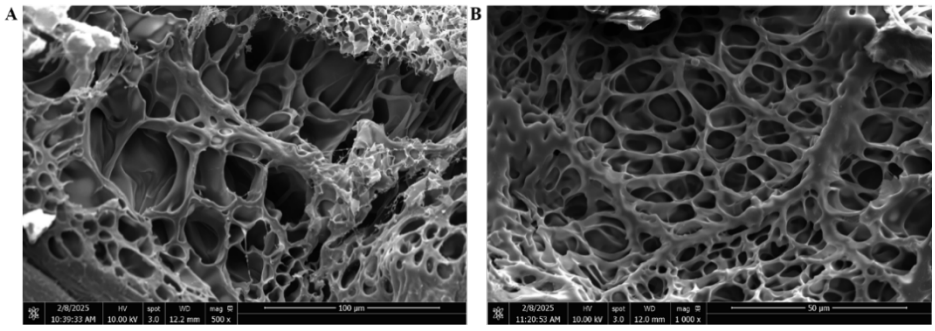

Figure 3 (A) PAA hydrogel and (B) GEL-PAA DN hydrogel: Scanning Electron Microscopy images.

Figure 3 demonstrated that the PAA hydrogel exhibited a uniform honeycomb structure, whereas the GEL-PAA DN hydrogel presented a dense clustered morphology accompanied by porous features. This observation indicates the aggregation state of the DN hydrogels as well as their superior stability.

2.6 Mechanics evaluation of PAA hydrogel and GEL-PAA DN hydrogel

It was obvious that the unique network structure gave the GEL-PAA DN hydrogel excellent mechanical properties. As shown in Figure 4 and Figure 5, the GEL-PAA DN hydrogel was transparent and highly resistant to different kinds of deformation—like stretching, compressing, knotting, and crossing—without getting serious damage. Once the pressure was taken away, the hydrogel went back to its original shape quickly. This shows it has great elasticity and toughness.

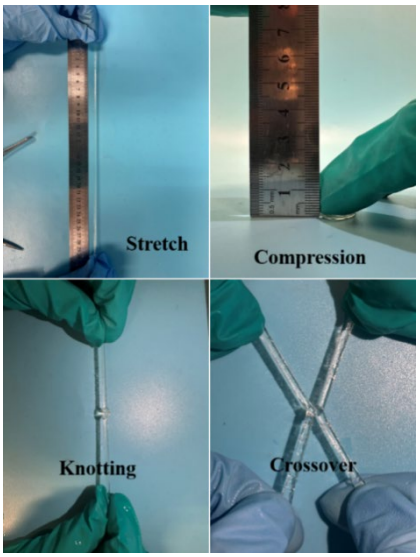

Figure 4 General measurements for the GEL-PAA DN hydrogel's outstanding mechanical properties involve stretching, compression, knotting, and crossover stretching.

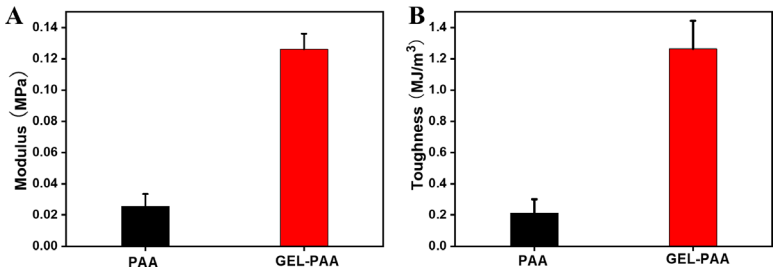

Figure 5 (A) Compressive modulus of PAA hydrogel vs. GEL-PAA DN hydrogel. (B) Tensile toughness of PAA hydrogel vs. GEL-PAA DN hydrogel.

2.7 Cell viability and proliferation

Given the crucial role of biocompatibility in tissue engineering implants, the CCK-8 assay was

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employed in the study to evaluate the cell growth and proliferation capabilities of PAA hydrogel and GEL-PAA hydrogel. After culturing bone marrow mesenchymal stem cells (BMSCs) in the medium containing the hydrogel extract for 24 hours, the results shown in Figure 6 indicates a slight decrease in cell activity. This decrease in activity was mainly due to the acidic environment of the medium caused by the PAA structural units. However, when the culture time was extended to 3 days, the cell proliferation rate gradually increased. This phenomenon suggests that both hydrogels mentioned above can provide favorable conditions for cell attachment and growth.

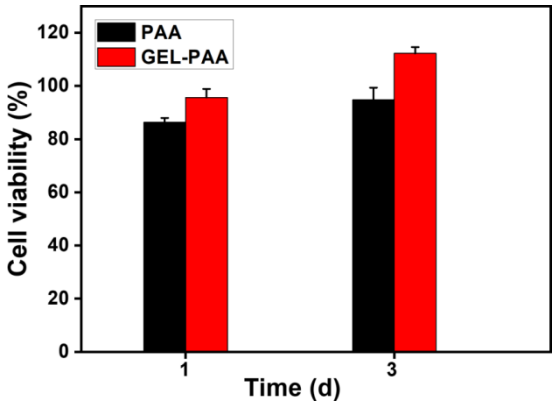

Figure 6 Cell viability and proliferation upon cultivation with PAA and GEL-PAA hydrogel extracts at various time points.

Live/dead staining analysis presented in Figure 7 further corroborates the favorable outcomes observed from the green fluorescent signals emitted by these two hydrogels, explicitly indicating that the vast majority of cells remained viable and intact following their culture with the respective hydrogel extracts. Beyond merely confirming cell survival, the distinct luminous green cell populations also serve to reflect the crucial role of these hydrogels in mediating biological regulation—an attribute that is particularly pivotal for supporting cellular functions in tissue engineering applications.

Therefore, due to the comprehensive set of advantageous properties exhibited by the GEL-PAA double-network (DN) hydrogel(its adaptable gel-forming behavior that enables easy integration with different biological microenvironments, its excellent mechanical performance provide stable structural support for cell growth and tissue development, and its good biocompatibility validated by the live/dead staining results) the GEL-PAA DN hydrogel emerges as a highly promising and readily applicable candidate for use as suitable scaffolds in cartilage tissue engineering. In addition to these properties, this hydrogel can be conveniently fabricated via a simple one-step soaking method that avoids complex processing procedures. This combination of desirable characteristics not only addresses key requirements for cartilage scaffold design but also lays a solid foundation for its potential usage into practical tissue engineering strategies.

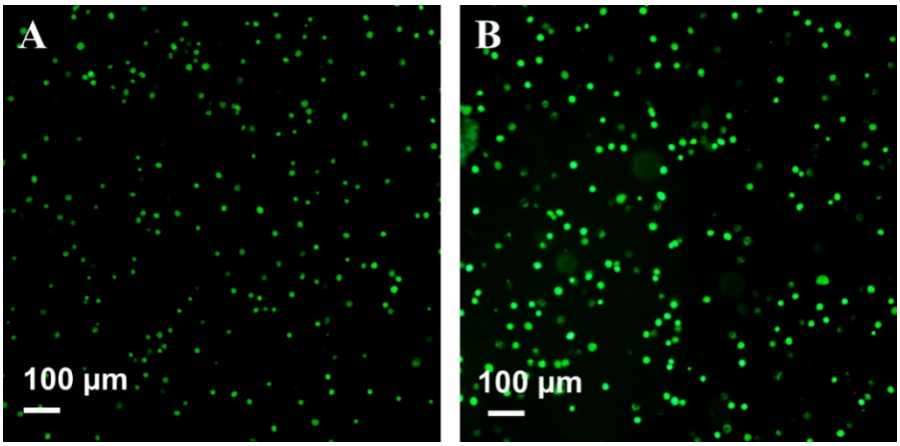

Figure 7 Day 1 live/dead staining of BMSCs treated with (A) PAA and (B) GEL-PAA hydrogel extracts. Green cells represent live ones, and red cells represent dead ones.

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3. Conclusions

In conclusion, this study preliminarily explored the soaking strategy to fabricate a high-strength, mechanically biological hydrogel. Utilizing various desired physical and chemical properties of the chain entanglement between PACG and/or GEL, hydrogen bond interactions, and attraction between charged particles, the final hydrogel product exhibits a porous network and excellent mechanical properties, including strength and resilience. Those properties provide an ideal microenvironment for cells to maintain their viability and to grow. It also stimulates the cell to proliferate and differentiate stably, making the hydrogel capable of facilitating tissue regeneration. Thus, this finding provides a universal strategy to craft various bioactive DN hydrogels for implantation. In addition, it also has great implications for understanding hydrogel scaffolds' long-term biological effects and for assisting tissue regeneration in the future.

References

[1] B.B. Xu, J. Ye, B.S. Fan, X.J. Wang, J.Y. Zhang, S.T. Song, Y.F. Song, W.B. Jiang, X. Wang, J.K. Yu, Protein-spatiotemporal partition releasing gradient porous scaffolds and anti-inflammatory and antioxidant regulation remodel tissue engineered anisotropic meniscus, Bioact. Mater. 20 (2023), 194–207.

[2] J.S. Zhang, S.Y. Lv, X.D. Zhao, Y. Sun, S.H. Ma, F. Zhou, Biobased, self-healing, and recyclable polyurethane derived hydrogel-elastomer hybrids for efficient lubrication. Prog. Org. Coat. 188 (2024), 108212.

[3] T.J. Zhu, H.F. Wang, Z.H. Jing, D.Y. Fan, Z.J. Liu, X. Wang, Y. Tian, High efficacy of tetra-PEG hydrogel sealants for sutureless dural closure, Bioact. Mater. 8 (2022), 12.

[4] B. Sun, H.F. Wang, B. Xiao, H.C. Yan, H.Q. Wu, R.C. Zhang, Y. Zhang, W. Yuan, X. Wang, C.G. Shi, Bioactive composite hydrogel with effects of robust promoting osteogenesis and immunomodulation for osteoporotic bone regeneration, Chem. Eng. J. 476 (2023), 146743.

[5] X.Y. Huang, Y.X. Lou, Y.H. Duan, H. Liu, J. Tian, Y. Shen, X. Wei, Biomaterial scaffolds in maxillofacial bone tissue engineering: a review of recent advances, Bioact. Mater. 33 (2024), 129–156.

[6] J.A. Burdick, G.D. Prestwish, Hyaluronic acid hydrogels for biomedical applications, Adv. Mater. 23 (2011), 41.

[7] X.Y. Dou, H.F. Wang, F. Yang, H. Shen, X. Wang, D.C. Wu, One-step soaking strategy toward anti-swelling hydrogels with a stiff "armor", Adv. Sci. 10 (2023), 2206242.

[8] Y.J. Hua, H.T. Xia, L.T. Jia, J.Z. Zhao, D.D. Zhao, X.Y. Yan, Y.Q. Zhang, S.J. Tang, G.D. Zhou, L.Y. Zhu, Q.N. Lin, Ultrafast, tough, and adhesive hydrogel based on hybrid photocrosslinking for articular cartilage repair in water-filled arthroscopy, Sci. Adv. 7 (2021), eabg0628.

[9] Y.Y. Yang, X. Wang, F. Yang, L.N. Wang, D.C. Wu, Highly elastic and ultratough hybrid ionic-covalent hydrogels with tunable structures and mechanics, Adv. Mater. 30 (2018), 1707071.

[10] X.Y. Wang, H.J. Kim, Ultra-stretchable dual-network ionic hydrogel strain sensor with moistening and anti-freezing ability, Prog. Org. Coat. 166 (2022), 106784.