Preparation, Characterization and Application of Injectable Tissue Bioadhesives

Pengcheng Zhao *

Wisdom Lake Academy of Pharmacy, Xi'an Jiaotong-Liverpool University, Suzhou, 215123, China

* Corresponding Author Email: 2978931346@qq.com

Abstract. Hydrogel are medical wound dressings for accelerating wound healing, because it can maintain a moist environment, promote tissue regeneration, and absorb wound exudates, which provide a favorable microenvironment for tissue repair while reducing the risk of complications. In this work, aminated gelatin and four-arm polyethylene glycol activated ester were employed to construct an injectable and mechanically robust bioadhesive. After optimization, the bioadhesive exhibits good handling properties, rapid gelation, and excellent biocompatibility. It can firmly adhere to skin tissue surfaces and effectively promote wound healing, demonstrating that this material is a convenient, effective, and biosafe tissue adhesive. Notably, the bioadhesive's overall performance is significantly superior to that of commercially available fibrin glue. This study provides a new strategy for development of next-generation tissue bioadhesives.

Keywords: hydrogel, bioadhesives, injeactable.

1. Introduction

The skin serves as the primary mechanical and biological barrier between the human body and the external environment, and disruption of its structural integrity and function can result in different types of wounds. Causes of skin tissue injury include burn trauma, internal medical diseases, and iatrogenic injury. Skin wound healing generally is composed of four sequential and potentially overlapping phases: hemostasis, inflammation, proliferation, and remodeling. These processes are precisely regulated by a complex network composed of multiple cell types and biological mediators. However, when the local wound microenvironment is unfavorable for repair, such as in the presence of uncontrolled inflammatory responses, infection, cellular dysfunction, malnutrition, cellular senescence, or imbalanced proteolysis, the normal wound healing process may be impaired or interrupted, leading to delayed healing or progression to chronic wounds, thereby severely affecting patient health and even quality of life.

Since Winter proposed the theory of moist wound healing in 1962, fundamental, translational, and clinical studies focusing on the wound repair microenvironment have been continuously advanced and refined [1]. Medical wound dressings can cover wounds and act as temporary barriers. They function as effective temporary barriers by preventing microbial invasion, preserving normal cellular activity, and coordinating the processes of wound repair and tissue regeneration, thereby serving as an important strategy for enhancing clinical wound healing. An ideal medical wound dressing is expected to exhibit the following features: (1) adequate safety and tissue compatibility; (2) the capability to maintain a suitable moist wound environment, allow gas exchange, and absorb wound exudates to a certain extent; (3) sufficient physical and mechanical strength to preserve structural integrity while preventing microbial penetration; (4) appropriate microstructural and biochemical characteristics that support cell proliferation and migration; and (5) minimal secondary damage to the wound site [2,3].

Hydrogels possess unique advantages in wound repair management due to their excellent hydrophilicity, biocompatibility, and extracellular matrix (ECM)-like three-dimensional porous structure. In addition to optimizing hydrogel properties by modifying the polymer backbone, concentration ratios, and crosslinking methods, hydrogels can also be combined with bioactive molecules, drugs, or cells to construct delivery systems, thereby enabling the customization of locally applied medical wound dressings for different types of wounds. With interdisciplinary integration

and development, hydrogels have evolved from simple physical coverage or single-function materials into multifunctional composites, showing broad application prospects in the field of wound repair. Different hydrogel matrix materials exert distinct biological effects on various cell types during the wound repair process, and consequently have different impacts on the progression of wound healing.

Tissue bioadhesives represent a typical class of such materials, among which fibrin glue is a sealant and adhesive, which is widely used in surgical procedures and is critically involved in the physiological process of wound healing [4]. Fibrin glue is generally extracted from animal or human blood and can serve as an autologous or allogeneic scaffold in tissue engineering [5]. To date, fibrin glue remains a commonly used biomedical hydrogel in clinical practice, and no toxic degradation products or immune reactions have been observed during its use. Fibrin gel can be formed through the crosslinking of fibrinogen in the presence of thrombin during the coagulation process [6], and its degradation rate can be regulated by aprotinin. Moreover, skeletal muscle cells, smooth muscle cells, and chondrocytes can be loaded by fibrin glue for applications in tissue engineering. However, the drawbacks of fibrin glue include poor toughness, insufficient adhesive strength, and high cost, which significantly limit its clinical application [7].

Collagen is the main component of the extracellular matrix in mammalian tissues such as skin, bone, cartilage, tendons, and ligaments. Since collagen contains specific protein sequences that can be recognized and enzymatically degraded by cells, it exhibits excellent biocompatibility and is therefore a widely used material that can satisfy multiple biological requirements [8]. Collagen has been used as tissue engineering scaffolds, artificial skin, cancer therapy materials, and drug carriers. Through chemical modification, including the incorporation of fibronectin, chondroitin sulfate, or low-molecular-weight hyaluronic acid into the collagen matrix, the cell adhesion properties of the resulting gels can be further optimized. Many tissue adhesives use collagen as a raw material. However, collagen has poor mechanical strength and cannot meet the requirements of certain specialized applications, such as load-bearing tissues including cartilage and muscle. In addition, collagen is expensive, shows poor batch-to-batch reproducibility during production, and presents certain immunogenicity and potential risks of disease transmission [9].

Therefore, in response to the characteristics of skin wound injuries under emergency conditions, such as continuous bleeding and irregular wound morphology, this study developed an injectable, tough tissue-sealing hemostatic hydrogel (AG-PEG gel) by in situ chemical crosslinking of aminated gelatin (AG) with four-arm polyethylene glycol succinimidyl succinate (Tetra-PEG-NHS). The resulting AG-PEG gel exhibits ultrahigh toughness, tunable gelation time, and biodegradability (Figure 1). After amidation treatment of gelatin, reactive amino groups on AG readily undergo coupling with the activated ester groups of Tetra-PEG-NHS, forming an AG-PEG gel with robust adhesion to diverse wet tissues, such as bleeding organs and smooth blood vessel surfaces. In vitro experimental evaluation shows that the AG-PEG gel combines superior mechanical properties with rapid biodegradability, practical applicability, and good biosafety, enabling efficient promotion of tissue repair.

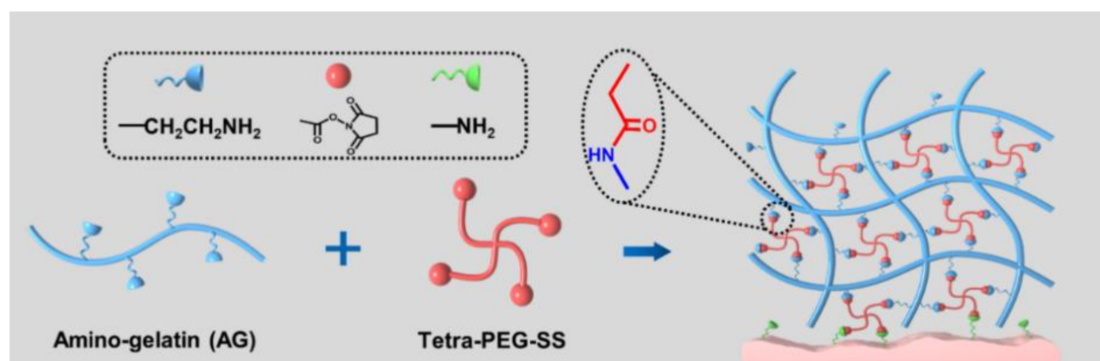

Figure 1. Schematic depiction illustrating the preparation strategy and wound sealing mechanism of the medical hydrogel adhesive.

2. Experimental Section

2.1. Main Materials and Reagents

Gelatin (*Mw* = 60 kDa) was purchased from Sigma-Aldrich. Four-arm polyethylene glycol activated ester (Tetra-PEG-NHS, *Mw* = 10 kDa, *Mw/Mn* = 1.02) was obtained from Xiamen Sino-Biological Technology Co., Ltd.

2.2. Preparation of AG-PEG Hydrogel

Two differently functionalized polyethylene glycols (aminated gelatin and four-arm polyethylene glycol activated ester) were mixed at an equimolar ratio to construct a series of four-arm polyethylene glycol hydrogels with strong water absorption capacity, good tissue compatibility, and strong tissue adhesive properties.

2.3. Scanning Electron Microscopy

Scanning electron microscopy (SEM) observations were performed on a JSM-6700F microscope (JEOL, Japan) at an accelerating voltage of 5 kV. The Tetra-PEG hydrogels prepared were freeze-dried at −80 °C for 3 days. Prior to observation, the freeze-dried hydrogels were sputter-coated with a Pt layer using an E-1010 ion sputter coater, with a coating time of 120 s.

2.4. Compressive Property Test

Compression tests were carried out using a universal testing machine (Instron 3365, USA). AG-PEG gels were prepared in cylindrical molds with a diameter of 10 mm and a height of 4 mm. The compression rate was set to 3 mm/min until sample failure (n = 3). The compressive stress was calculated by dividing the applied force by the cross-sectional area.

2.5. Adhesive Performance Test

AG-PEG gel was prepared in situ on porcine skin strips and stained with rhodamine B. After standing for 2 min, the porcine skin strips were subjected to twisting, squeezing, and other manipulations to evaluate the adhesive performance of the gel on skin surfaces.

For quantitative evaluation of the adhesive strength of the AG-PEG gel to skin, two porcine skin strips with a width of 2 cm were selected. AG-PEG gel was prepared *in situ* on a 2 × 2 cm area at the front end of each strip, and the two strips were overlapped with the overlap area identical to the gel-coated area. After gentle compression for 10 min, the samples were tested using a universal testing machine (Instron 3365, USA). All tests were conducted at a constant tensile rate of 50 mm/min. The adhesive strength was determined by dividing the maximum force by the adhesion area. A commercially available fibrin glue was used as a control for comparison.

2.6. Cytotoxicity Assay

Mouse fibroblasts (NIH-3T3) were employed to evaluate the cytotoxicity of the degradation products of the AG-PEG gel by a CCK-8 assay. NIH-3T3 cells were cultured at 37 °C in DMEM supplemented with 10% fetal bovine serum in an incubator with a CO_2 concentration of 5%. Cells were seeded into 96-well plates at a density of 1×10^4 cells per well and cultured for 24 h. The culture medium in each well was then replaced with 200 μL of fresh medium, followed by the addition of 20 μL of degradation solution to achieve final concentrations of 0.01, 0.1, and 1 mg/mL. After further incubation for 24 h, the supernatant was removed, and 100 μL of fresh medium and 10 μL of CCK-8 solution were added to each well, followed by incubation for an additional 3 h. The absorbance of each well was measured at 450 nm. Cell viability was calculated by comparing the absorbance of the treated groups with that of the control group containing untreated cells.

Cell viability of NIH-3T3 cells was also visually evaluated using a live/dead staining assay. Cells were stained with a Calcein-AM/propidium iodide (PI) staining kit after 2 days of culture. Cell morphology was observed using an inverted fluorescence microscope.

3. Results and Discussion

3.1. Preparation of the Hydrogel

AG solution and Tetra-PEG-NHS solution were mixed at equal volumes in small sample vials to observe the gelation time of the hydrogel. As illustrated in Figure 2, a transparent hydrogel was immediately formed after mixing the solutions. The hydrogel was able to recover its original shape even after vigorous compression, indicating that the hydrogel possesses good mechanical properties.

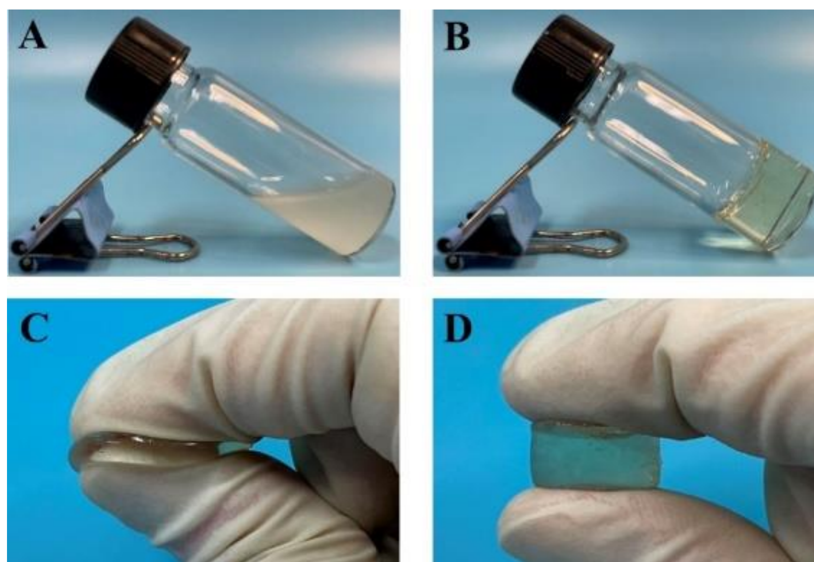

Figure 2. Preparation of the AG-PEG gel and photographs showing its mechanical compression behavior.

3.2. Structural and Mechanical Characterization of the Hydrogel

The microstructure of the hydrogel was observed by scanning electron microscopy. As shown in Figure 3A, the AG-PEG gel exhibits a relatively uniform porous microstructure. The AG-PEG gel shows good compressibility and can fully recover its original shape even under 95% compressive deformation (Figure 3B). In addition, as illustrated in Figure 4, analysis of the stress–strain behavior reveals that the compressive strength of the AG-PEG gel reaches 2.6 MPa, substantially surpassing that of commonly used clinical adhesives, including fibrin glue.

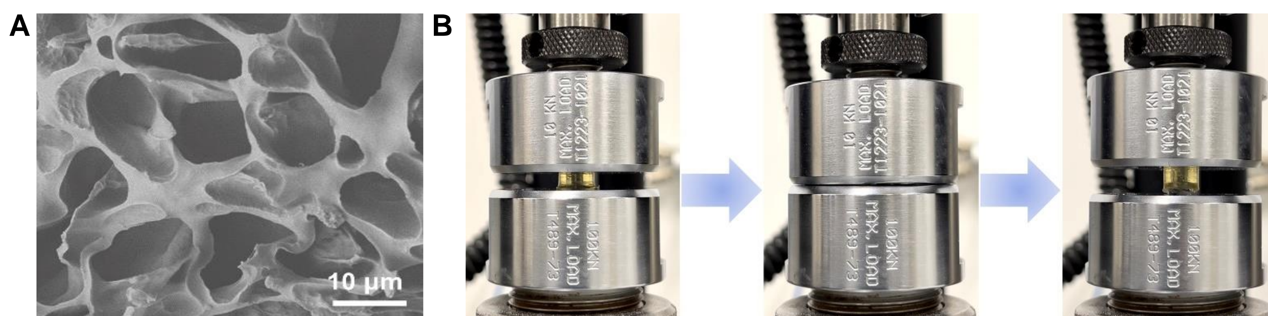

Figure 3. Internal network structure and compression test images of the AG-PEG gel.

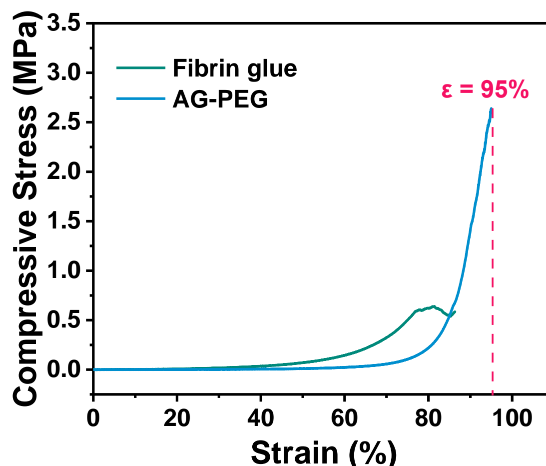

Figure 4. Stress–strain curves of the AG-PEG gel and fibrin glue.

3.3. Adhesive Performance of the Hydrogel

The gel precursor contains a large number of succinimidyl succinate groups, and the unreacted succinimidyl succinate groups can rapidly interact with amino groups in tissues, thereby forming tight adhesion to the tissue surface. To verify this adhesive capability, the AG-PEG gel was injected in situ onto the surfaces of a human finger and porcine skin. As shown in Figure 5, the gel remained attached to the finger surface without detachment after repeated bending and extension of the finger. After twisting and bending were applied to the porcine skin, the AG-PEG gel still adhered tightly to the skin surface.

Most application scenarios for hemostatic gels involve moist or even fully submerged environments, which requires the gel to be able to gel and adhere to tissue surfaces under wet conditions or even underwater. Therefore, we simulated extreme application conditions in water. After injecting the gel onto the surface of porcine skin underwater, the hydrogel was still capable to gel and adhere to the surface of skin, and it maintained strong adhesion even after being subjected to vigorous water flow. These results demonstrate that the AG-PEG gel possesses strong wet-tissue adhesion and mechanical performance.

Figure 5. Adhesive ability of the AG-PEG gel to skin tissue.

3.4. Cytocompatibility of the Hydrogel

The cytotoxicity of the hydrogel was evaluated using 3T3 mouse fibroblasts. The 3T3 mouse fibroblasts were co-cultured with the medium extract and degradation solution of the AG-PEG gel for 1, 3, and 5 days. As shown in Figure 6A, compared with the blank control group, almost no dead

cells exhibiting red fluorescence were observed in the gel-treated groups, and the cells maintained good morphology and high density, indicating that the AG-PEG gel has good cytocompatibility. The CCK-8 assay results showed that the cell viability remained above 85% after co-culture (Figure 6B), further demonstrating the good biocompatibility of this hydrogel. In summary, the hemostatic hydrogel exhibits good cellular safety.

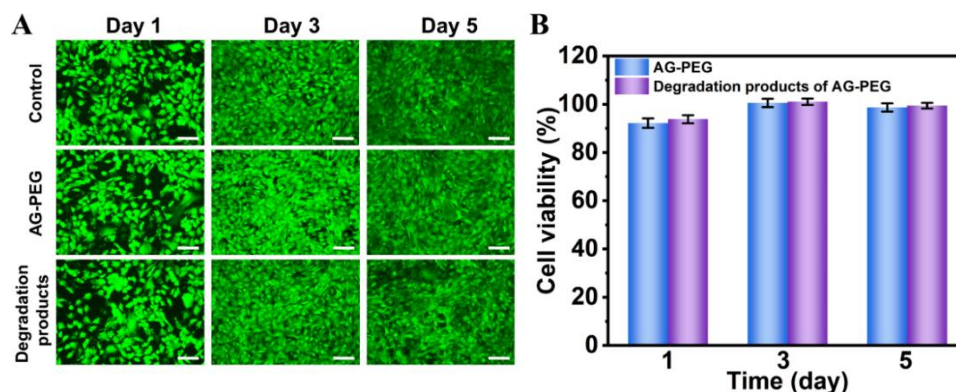

Figure 6. (A) Live/dead staining of NIH-3T3 cells (scale bar: 100 μm); (B) cell viability.

4. Conclusion

In the present study, an injectable, *in situ* crosslinked tough hydrogel, which based on gelatin, derived from cold-water fish skin and PEG derivatives was invented as a material for skin wound repair. The hydrogel exhibits enhanced mechanical performance, adhesive properties, and operability, effectively overcoming the limitations of traditional sealants with poor mechanical strength and unsatisfactory sealing performance. In addition, the AG-PEG gel demonstrates excellent performance in wound management and shows promising potential for clinical applications.

References

[1] Winter GD. Formation of the scab and the rate of epithelization of superficial wounds in the skin of the young domestic pig. Nature, 1962, 193(4812): 293-294.

[2] Hu H, Xu FJ. Rational design and latest advances of polysaccharide-based hydrogels for wound healing. Biomater Sci, 2020, 8(8): 2084-2101.

[3] Liang Y, He J, Guo B. Functional hydrogels as wound dressing to enhance wound healing. ACS Nano, 2021, 15(8): 12687-12722.

[4] Chen Y, Wu L, Li P, et al. Polysaccharide based hemostatic strategy for ultrarapid hemostasis. Macromol Bio Sci, 2020, 20(4): e1900370.

[5] Khan MA, Mujahid M. A review on recent advances in chitosan-based composite for hemostatic dressings. Int J Biol Macromol, 2019, 124: 138-147.

[6] Yang X, Liu W, Li N, et al. Design and development of polysaccharide hemostatic materials and their hemostatic mechanism. Biomater Sci, 2017, 5(12): 2357-2368.

[7] Zhong Y, Hu H, Min N, Wei Y, Li X, Li X. Application and outlook of topical hemostatic materials: a narrative review. Ann Transl Med, 2021, 9(7): 577.

[8] Zhang Z, Zhang L, Li C, et al. Research progress of chitosan-based biomimetic materials. Mar Drugs, 2021, 19(7): 372.

[9] Jayakumar R, Prabaharan M, Sudheesh Kumar PT, Nair SV, Tamura H. Biomaterials based on chitin and chitosan in wound dressing applications. Biotechnol Adv, 2011, 29(3): 322-337.