

Injectable antibacterial polyurethane hydrogel for infected wound therapy

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Received November 5, 2025; accepted January 26, 2026; published online March 18, 2026

The fabrication of injectable hydrogels with multiple functions and effective promotion of wound repair has a great prospect in treatment of bacterial infected wounds. Herein, we fabricated polyurethane hydrogel *via* a catalyst-free click reaction, in which aminoporphyrin (TPP-NH₂) being covalently incorporated into a polyurethane network crosslinked with L-cystine (*L*-Cys) to form an injectable, self-healing levofloxacin-loaded porphyrin polyurethane hydrogel (APU-TPP-Lev gel). In weakly acidic environments, the gel released antibiotics and generated reactive oxygen species (ROS) under red light irradiation, synergistically eliminating *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) to demonstrate highly effective contact antimicrobial activity. Furthermore, the hydrogel exhibits excellent biocompatibility, significantly promoting the healing of infected wounds after injection and light exposure, indicating broad application prospects in the treatment of bacterial infected wounds.

polyurethane; hydrogel; antibacterial; photosensitizer

Citation: CHEN X., XIA Y., PAN S., CHEN L., Injectable antibacterial polyurethane hydrogel for infected wound therapy, J. Mol. Sci. 42, 26010508 (2026), <https://doi.org/10.3724/jmolsci.2025110002>

1. Introduction

With the incidence of chronic wounds such as diabetic foot and pressure ulcers rising year by year, their healing process is often severely impeded by bacterial infection and biofilm formation. Against the backdrop of the global antibiotic resistance crisis, traditional therapies are increasingly inadequate, creating an urgent need for smart dressings that combine excellent mechanical properties, biocompatibility, and novel antimicrobial mechanisms. Among these, injectable, self-healing, biodegradable hydrogel dressings capable of conforming to irregular wound surfaces show tremendous potential. However, the core challenge lies in simultaneously meeting the three critical requirements of mechanical toughness, biocompatibility, and effective antimicrobial activity^[1-3].

The preparation of antibacterial hydrogel dressings hinges on selecting suitable raw materials tailored to the specific wound environment, designing rational antibacterial mechanisms, and constructing structurally stable crosslinked networks. Various strategies have been proposed for con-

structing functionalized hydrogels with enhanced antibacterial performance. For example, in 2021, Wang et al. developed a cellulose/polyacrylamide composite hydrogel loaded with silver nanoparticles. This material exhibited antibacterial properties while maintaining good flexibility, and its application was extended to the field of sensors^[4]. In 2021, Liu et al. designed an acrylic-based hydrogel coating embedded with antimicrobial peptides, which demonstrated significant inhibitory effects against both Gram-positive and Gram-negative bacteria^[5]. In 2021, Wei et al. constructed a multifunctional hydrogel based on oxidized dextran, hyaluronic acid-antimicrobial peptide conjugates, and platelet-rich plasma. This hydrogel effectively promoted the healing and skin regeneration of diabetic infected wounds^[6]. Additionally, in 2023, Ran et al. reported an intelligent responsive hydrogel that combined polylysine derivatives with porphyrin photosensitizers. This system exhibited a synergistic photodynamic antibacterial effect and significantly accelerated the repair process of infected wounds^[7].

Polyurethane (PU), with its main-chain structure resembling peptide bonds, offers both excellent blood compatibility and molecular tailorability, making it an ideal matrix material for such dressings^[7]. However, this material inherently lacks antimicrobial sites. Externally loading anti-

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Executive editor: YUAN Haiyan

biotics or silver ions risks antibiotic resistance and metal accumulation toxicity^[8–11]. Antimicrobial photodynamic therapy (APDT) utilizes “light-oxygen-sensitizer” interactions to generate reactive oxygen species (ROS), offering broad-spectrum efficacy, low resistance potential, and spatiotemporal controllability, making it a promising alternative to antibiotics^[12–15]. Although porphyrin photosensitizers exhibit excellent performance, their hydrophobic macrocycles readily undergo π - π stacking and self-quenching in physiological media, while free molecules are easily cleared by exudate, leading to a sharp decline in ROS yield^[16–19]. While covalent immobilization can suppress aggregation, it often faces new bottlenecks such as low loading capacity and decoupling from the infectious microenvironment response^[20–22].

To address these issues, this study retains the superior properties of the original PU prepolymer while employing a catalytic-free click chemistry approach to covalently embed aminoporphyrin (TPP-NH₂) into the network, thereby completely suppressing aggregation. Simultaneously, *L*-cysteine (*L*-Cys) was employed as the crosslinker to enable rapid dissociation and levofloxacin (Lev) release within the microacidic infection environment (pH $\approx$ 5). This achieves a cascading antibacterial effect: acid-triggered drug release $\rightarrow$ photodynamic bactericidal action $\rightarrow$ antibiotic synergy. The resulting levofloxacin-loaded porphyrin polyurethane hydrogel integrates injectable self-healing, tissue adhesion, controlled degradation, and fluorescent tracing capabilities, offering a novel approach for developing advanced wound dressings with antibiotic-free and low-resistance properties.

2. Result and discussion

2.1 Synthesis and characterization of TPP-NH₂ and antibacterial polyurethane (APU)

The chemical structures of TPP-NH₂ and antibacterial polyurethane (APU) were confirmed by ¹H NMR and Fourier transform infrared (FT-IR) spectroscopy. For TPP-NH₂, a distinct —NH₂ characteristic peak appeared in its ¹H NMR spectrum (See Figure S1a); while the FT-IR

spectrum (See Figures S1b and S1d) exhibited characteristic —NH₂ absorption doublets at 3436 and 3353 cm^{−1}. Absorption peaks in the range of 3215 to 3027 cm^{−1} were attributed to C—H stretching vibrations of the porphyrin macrocycle and benzene rings, collectively confirming the successful synthesis of TPP-NH₂. For the polyurethane precursor APU, its ¹H NMR spectrum (See Figure S1c) exhibited characteristic peaks of methyl and methylene groups from isophorone diisocyanate (IPDI) at 0.97 to 1.09. FT-IR analysis (See Figure 1a) revealed that absorption peaks at 1697 cm^{−1} correspond to the carboxyl group of the acetylenedicarboxylic acid (AA) residues, while peaks at 1656 and 1433 cm^{−1} correspond to C—N bonds in the urethane linkages, confirming the successful construction of the polyurethane backbone. These results provide the key precursor confirmed for subsequent construction of functionalized gels in this study.

2.2 Preparation and characterization of porphyrin polyurethane gels

An ethanol solution of TPP-NH₂ was mixed with a PBS solution of APU, followed by the addition of *L*-Cys as a crosslinking agent. Through a click reaction between the amino/thiol groups and the carbon-carbon triple bond in the backbone, a stable gel formed at room temperature within approximately 2 hours. The successful crosslinking of this gel is attributed to the multiple reactive sites provided by the APU backbone interacting with *L*-Cys. Scanning electron microscopy (SEM) revealed that the porphyrin polyurethane hydrogel (APU-TPP gel) exhibits a non-uniformly distributed porous network structure (See Figure 1b), with pore diameters of approximately 8 μ m. This structure lays the foundation for its subsequent water absorption, drug loading, and mass transport functions. In the FT-IR spectrum, a broad absorption band at 3300 to 3700 cm^{−1} indicates abundant hydrogen bonds within the gel (See Figure 1a), closely related to the formation of the crosslinked network. The aforementioned experimental results collectively confirm the successful preparation of the porphyrin poly-

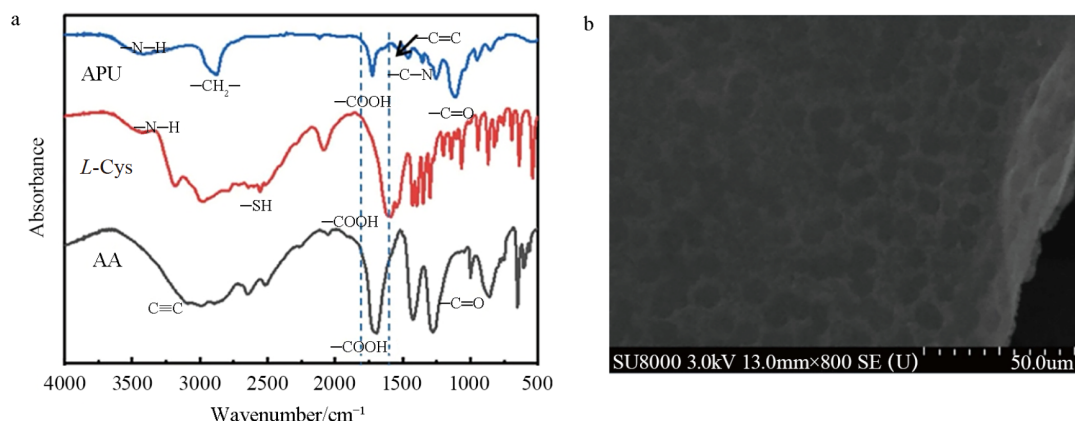


Figure 1 FT-IR spectra of AA, *L*-Cys and APU (a) and SEM image of APU-TPP gel (b)

urethane gel and the construction of its three-dimensional crosslinked network, both at the macroscopic formation and microscopic structural levels.

2.3 H/ROS dual responsiveness and drug release kinetics of porphyrin polyurethane gels

The design of APU-TPP gel combines the pH-sensitive crosslinking of *L*-Cys with the photosensitive properties of amino porphyrin, enabling dual responsiveness to both the acidic microenvironment at wound sites and light stimulation. We systematically evaluated its drug release behavior and ROS generation capacity. Controlled drug release tests (See Figures 2a and 2b) revealed that the gel exhibited the highest levofloxacin loading capacity ($12.67 \mu\text{g}\cdot\text{mL}^{-1}$) under neutral conditions (pH = 7.4), while achieving a release rate of 85.3% under acidic conditions (pH = 5), attributed to the dissociation of the crosslinked network near the isoelectric point of *L*-Cys, enabling environmentally triggered controlled drug release. Concurrently, we monitored singlet oxygen generation under illumination using 9,10-anthracenediyl-bis(methylene)dimalonic acid (ABDA) as a ROS indicator *via* UV-Visible spectroscopy (See Figure 2c). As shown in Figure 2d, the absorbance of the light-irradiated porphyrin polyurethane hydrogel (L-APU-TPP gel) group decreased significantly over time in acidic PBS (pH = 5). The light-irradiated porphyrin polyurethane hydrogel loaded with levofloxacin (L-APU-TPP-Lev gel) exhibited a similar ROS generation trend (See Figure 2e), confirming that acidic conditions facilitate porphyrin release from the gel network and activate photodynamic activity. Under neutral conditions at pH = 7.4, absorbance showed no significant change (See Figure 2f), indicating marked pH dependence of ROS generation. Collectively, these results demonstrate

that porphyrin polyurethane gels can synergistically achieve rapid antibiotic release and activation of photodynamic antimicrobial agents under acidic and light-irradiated conditions at infected wounds, exhibiting intelligent dual-responsive therapeutic functionality.

2.4 Degradation test of porphyrin polyurethane gels

An ideal wound dressing should possess controllable biodegradability to ensure it can be metabolized or cleared by the body after fulfilling its function, thereby avoiding damage caused by secondary surgery. To evaluate the degradation performance of APU-TPP gel, we conducted degradation tests of both *In vitro* enzymatic solutions and oxidative hydrolysis environments. As shown in Figure 3a, in lipase solution, the degradation rates of the gels APU-TPP-1, APU-TPP-2, and APU-TPP-3, which contain different IPDI contents, were recorded as 29.4%, 33.8%, and 32.9%, respectively, within 16 days, indicating the material is susceptible to enzymatic degradation. In the simulated bodily fluid oxidation environment (See Figure 3b), the degradation rates further increased to 46.4%, 38.3%, and 38.4%, respectively, suggesting the material possesses accelerated responsive degradation potential within complex *In vivo* environments.

In vitro degradation results indicate that the degradation behavior of APU-TPP gel is closely related to its crosslinking density and hydrophilicity, which can be regulated by adjusting the IPDI ratio. Specifically, IPDI, as a diisocyanate component, directly influences the proportion of hard segments and the overall hydrophobicity of the polymer network. An increase in IPDI content generally enhances the hydrophobicity of the gel, which may slow down the infiltration and diffusion of water molecules and en-

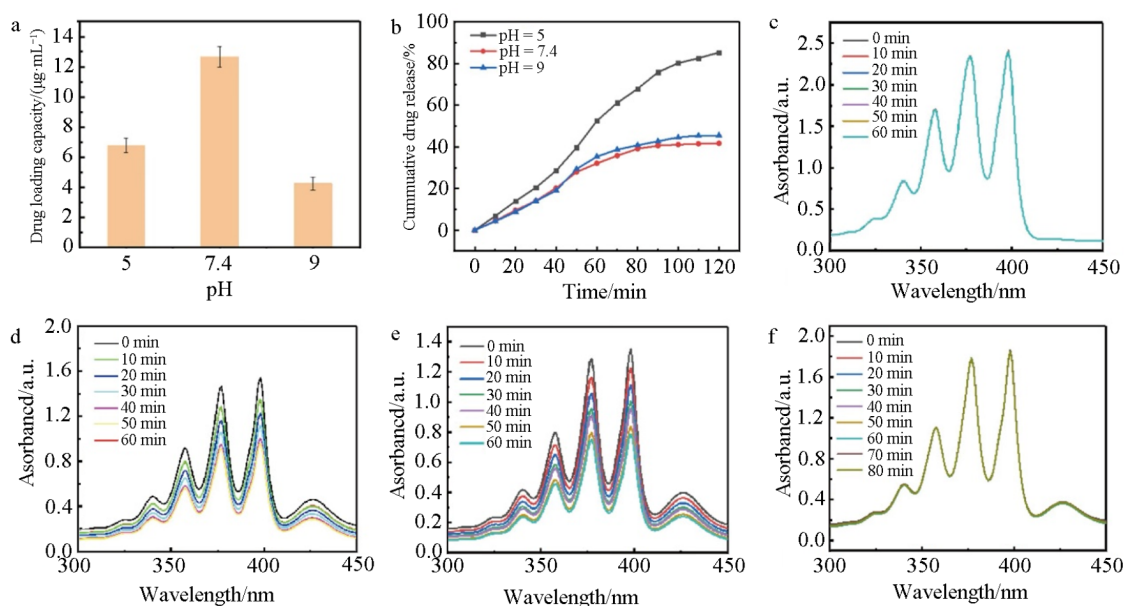


Figure 2 Drug loading and release performance of APU-TPP gel under different pH conditions (a and b), time-dependent absorbance curve of ABDA (c), L-APU-TPP gel (d), L-APU-TPP-Lev gel at pH = 5 (e) and 7 (f)

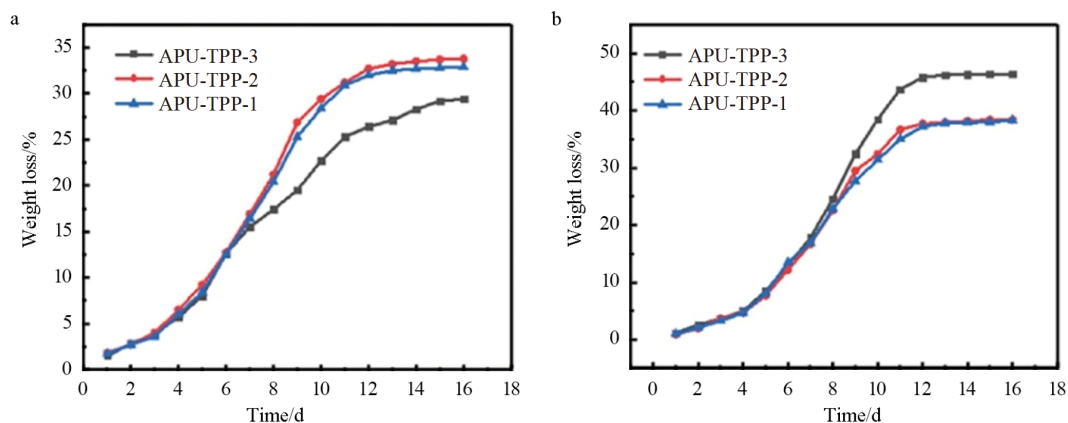


Figure 3 *In vitro* lipase degradation of polyurethane gels at different IPDI concentrations (a), and *In vitro* bio-hydrolysis conditions (b)

zymes, thereby affecting the rates of hydrolysis and enzymatic cleavage. Concurrently, a higher IPDI content typically leads to a higher crosslinking density, forming a more compact three-dimensional network. This denser structure can restrict the access of degradation media to some extent, resulting in the observed non-monotonic variation in degradation rates (e.g., the similar yet slightly lower degradation rates of APU-TPP-2 and APU-TPP-3 compared to APU-TPP-1). Therefore, by modulating the IPDI content, the degradation kinetics of the gel can be finely tuned to meet the requirements of different wound healing stages. Furthermore, observation of the material changes over time in physiological conditions (See Figure S2) reveals gradual structural disintegration within 50 days, further confirming an excellent biodegradability of the gel.

2.5 Antibacterial activity of porphyrin polyurethane gels

Bacterial infection poses a major challenge during wound healing, as the moist environment created by wound exudate readily supports microbial growth and secondary infections. Therefore, developing wound dressings with highly effective antimicrobial properties is crucial. The APU-TPP gel prepared in this study achieves synergistic antibacterial effects by combining antibiotic therapy with photodynamic therapy. In an acidic microenvironment, the gel controls the release of the antibiotic levofloxacin, which kills Gram-negative bacteria such as *E. coli* by inhibiting bacterial DNA gyrase. Simultaneously, the photosensitizer TPP-NH₂ released from the gel is activated upon illumination, generating ROS. These ROS oxidatively damage bacterial cell membranes, effectively targeting both Gram-positive and Gram-negative bacteria. To comprehensively evaluate its antimicrobial performance, we employed the zone of inhibition method to compare the inhibitory effects on *E. coli* and *S. aureus* of this gel under different treatment conditions. As shown in Figure S3, the APU-TPP gel without drug loading and without light exposure did not form a distinct inhibition zone, indicating it lacks intrinsic antibacterial activity. However, the L-APU-TPP gel produced inhibition zones against both bacteria, with a particularly

pronounced effect against *S. aureus* (15 mm radius), attributed to ROS generated by photodynamic action. The levofloxacin-loaded porphyrin polyurethane hydrogel (APU-TPP-Lev gel) produced a 12.2 mm inhibition zone specifically against *E. coli* through antibiotic release. Most importantly, the L-APU-TPP-Lev gel exhibited the strongest synergistic antibacterial effect, with inhibition zones of 12.4 and 15.8 mm against *E. coli* and *S. aureus*, respectively (See Figure 4a).

Under light irradiation, the significantly enhanced antibacterial effect observed with the L-APU-TPP-Lev gel indicates a potent synergistic interaction between photodynamically generated ROS and the released levofloxacin. This synergistic mechanism can be understood through complementary actions in both temporal and spatial dimensions. Temporally, upon light exposure, the rapid production of ROS immediately inflicts oxidative damage to bacterial membranes, increasing membrane permeability. This compromise of the primary cellular barrier can facilitate the subsequent uptake and intracellular accumulation of levofloxacin, thereby enhancing its inhibitory effect on DNA gyrase. Spatially, the two agents attack distinct targets: ROS causes broad, non-specific damage to membrane lipids, proteins, and other cellular components, whereas levofloxacin acts specifically on the intracellular enzyme DNA gyrase to inhibit DNA replication. This combined multi-target strategy—simultaneously disrupting structural integrity and genetic function—not only enhances immediate bactericidal efficacy but also significantly raises the barrier to resistance development, as bacteria would need to concurrently evolve defenses against two fundamentally distinct mechanisms. The superior inhibition zones produced by L-APU-TPP-Lev against both *E. coli* and *S. aureus* (See Figure 4a) provide compelling experimental support for this spatiotemporally coordinated synergy. These results demonstrate that porphyrin polyurethane gels successfully combines drug-based antibacterial action with photodynamic antibacterial effects. It can activate corresponding bactericidal mechanisms under different conditions, thereby exhibiting broad-spectrum and highly effective antibacterial activity. This provides dual assurance for addressing complex wound infections.

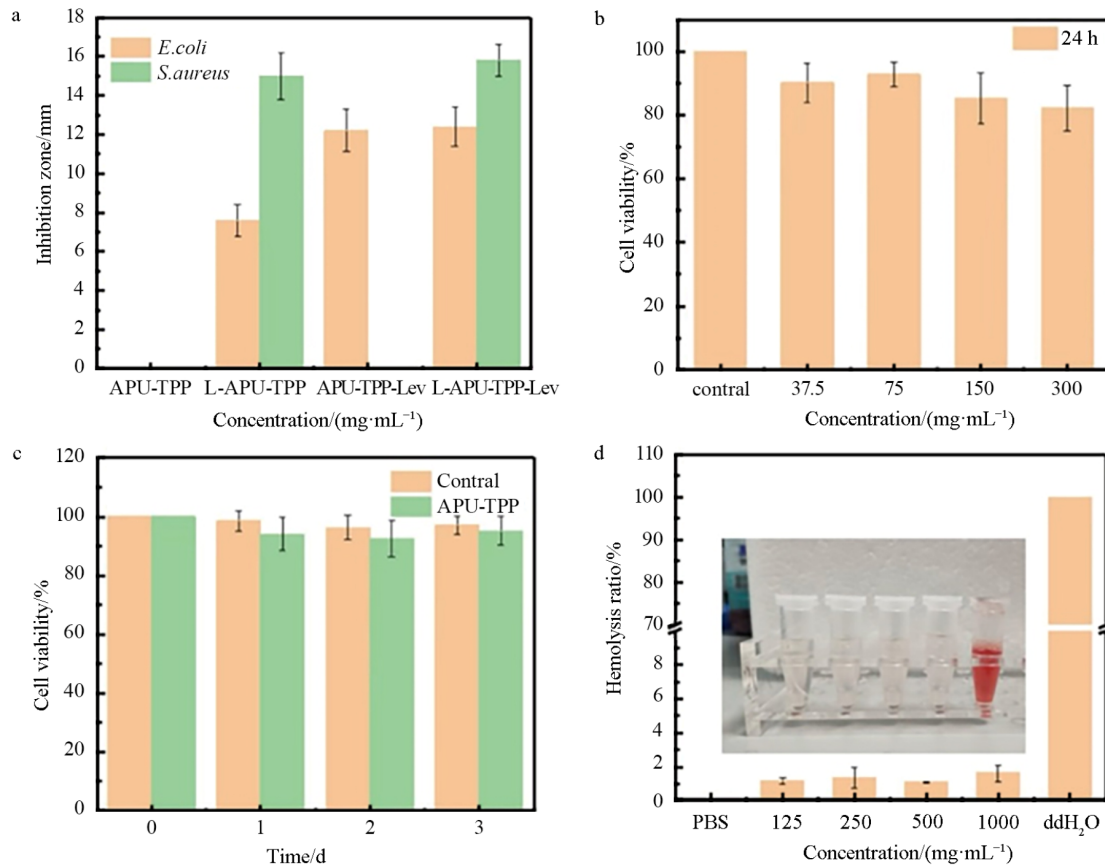


Figure 4 Antibacterial zone radii of porphyrin polyurethane gels with different treatments against *E. coli* and *S. aureus* (a), NIH-3T3 cell viability assay of porphyrin polyurethane gels at different concentrations (b), cell viability over time (c), and acute hemolysis test of porphyrin polyurethane gels at different concentrations (d)

2.6 Cytocompatibility and hemocompatibility of porphyrin polyurethane gels

For wound dressings in direct contact with the wound bed, excellent biocompatibility is an indispensable and critical property. To systematically evaluate the biocompatibility of APU-TPP gel, we examined its cellular compatibility and hemocompatibility separately. The cytotoxicity of APU-TPP gel extracts was studied using the CCK-8 assay. As shown in Figure 4b, NIH-3T3 cell viability remained above 80% after 24 hours exposure to extracts at various concentrations, indicating no significant concentration-dependent cytotoxicity within the tested range. To further evaluate long-term contact safety, we monitored cell survival rate changes during co-culture with the material. As shown in Figure 4c, cell viability remained consistently high without significant decline throughout the multi-day culture period, demonstrating the excellent long-term biocompatibility of APU-TPP gel. Live/dead cell staining provided more intuitive evidence. As shown in Figure S4, cells in the APU-TPP gel-treated group exhibited healthy spindle shapes and emitted bright green fluorescence, with minimal dead cells (red fluorescence). During the culture period, cell density increased significantly, which fully confirms that this material supports normal cell adhesion and proliferation.

Subsequently, we evaluated the hemocompatibility of the

APU-TPP gel through a hemolysis assay. As shown in Figure 4d, even at high concentrations (1000 $\mu\text{g}\cdot\text{mL}^{-1}$), the hemolysis rate remained below 10% across all test groups, meeting safety standards for biomedical materials. These results demonstrate the excellent biocompatibility of the APU-TPP gel, providing crucial evidence for its application as a safe and reliable wound dressing.

2.7 Infected wound healing effects of porphyrin polyurethane gels

By establishing a mouse model of full-thickness skin excision wound healing, different gel materials were applied to mouse wounds to evaluate wound healing efficacy. The auxiliary healing function of this gel was validated through *In vivo* healing experiments. Mice were divided into four groups, each containing four animals. The experiments employed porphyrin polyurethane gel and porphyrin polyurethane gel loaded with levofloxacin to treat mouse wounds. Figure 5a shows electron micrographs of wound recovery at 0, 3, 7, 10, and 14 days post-treatment. Figure 6a depicts wound recovery in mice treated with polyurethane gel. After treating mouse wounds with four conditions and three different gel materials, it can be observed that the wound in the untreated control group healed very slowly after 14 days. At 14 days, the wound surface still had ap-

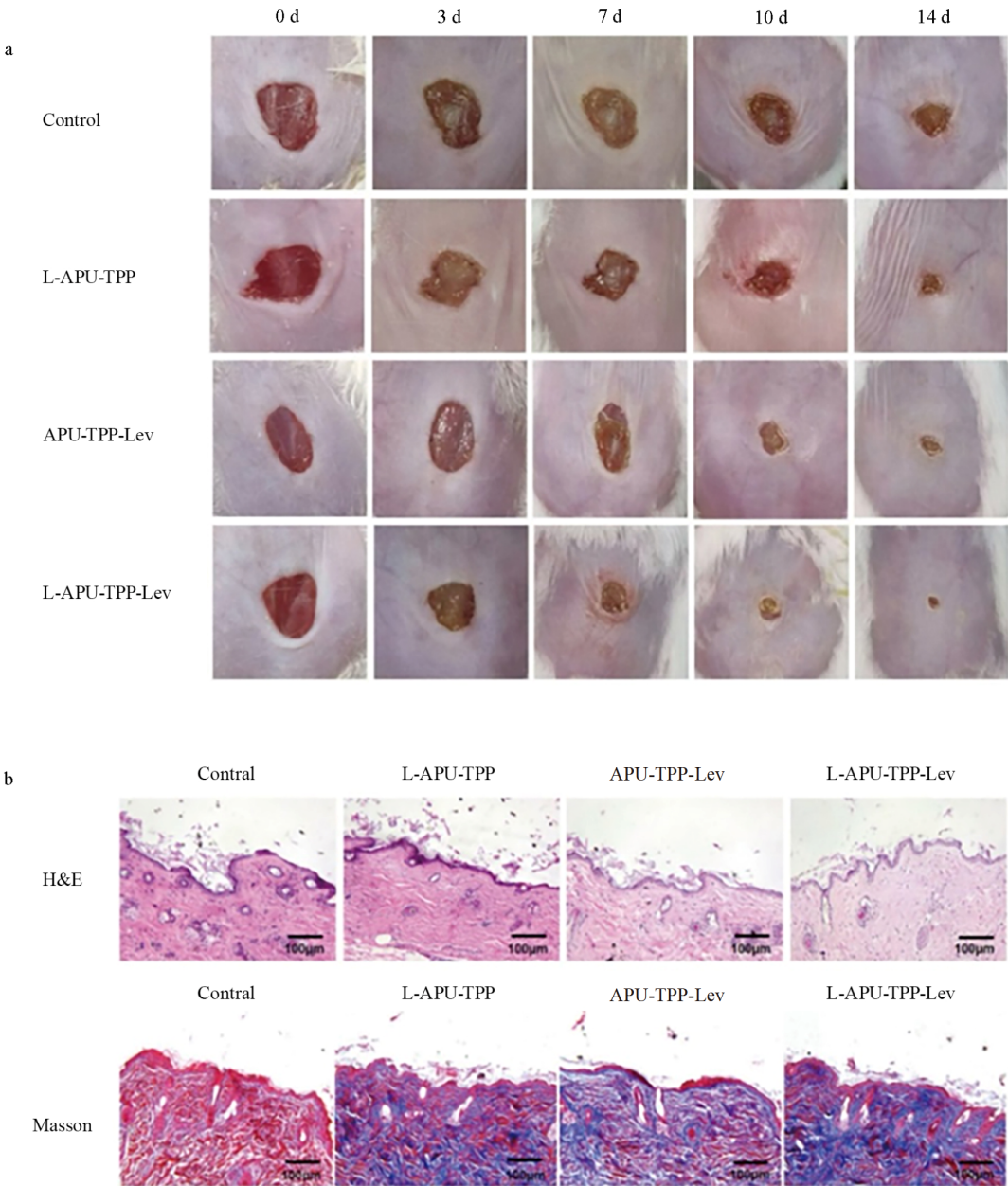


Figure 5 Wound healing progression in mouse wound surfaces treated with blank hydrogel, L-APU-TPP gel, APU-TPP-Lev gel, and L-APU-TPP-Lev gel from days 0 to 14 (a), histological analysis of wound tissue after H&E staining and Masson's trichrome staining following treatment with different porphyrin polyurethane gels at 14 days post-treatment (b)

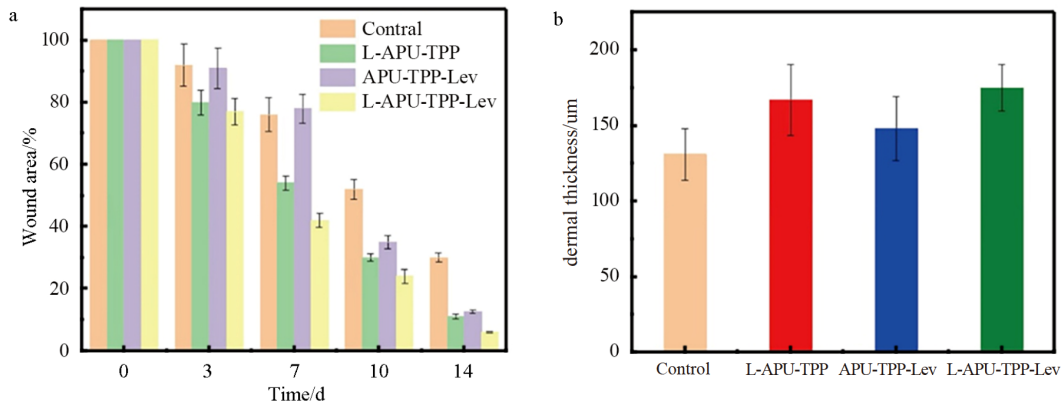


Figure 6 Recovery of wound area in mouse wounds treated with various materials (a) and dermal thickness of skin following wound healing with different gel treatments (b)

proximately 0.5 mm of unhealed tissue, with an unhealed rate of 30%. In contrast, Group 2 (treated with L-APU-TPP) showed minimal healing during the first week but demonstrated significant improvement after day 10, ultimately achieving an 87% healing rate. This initial lag phase may be attributed to the time required for the clearance of bacterial debris following photostimulation. Group 3 (treated with APU-TPP-Lev) displayed improved recovery compared to the control. The material effectively released antibiotics to prevent infection, with inflammation subsiding almost completely by day 10. By day 14, a 2 mm diameter area remained unhealed, yielding an 89% recovery rate. Notably, wounds in Group 4 (treated with L-APU-TPP-Lev) exhibited partial scab formation by day 7, indicative of the material's potent bactericidal, anti-infective, and hemostatic properties. By day 14, these wounds were nearly completely healed, achieving a 95% healing rate. These findings demonstrate that L-APU-TPP-Lev effectively promotes wound healing through the synergistic action of levofloxacin and the photosensitive antimicrobial amino porphyrin, suggesting it is a promising candidate for antimicrobial wound dressings.

Visual inspection alone cannot accurately assess wound healing. To better understand the wound healing process in mice, histological evaluation was performed. Tissue samples were collected from mouse wounds on day 14 and stained using hematoxylin and eosin (H&E) and Masson's trichrome stains. As shown in Figure 5b (H&E staining), H&E sections revealed newly formed skin tissue in all experimental groups, indicating substantial epithelial cell coverage of the wound surface. Fibroblast formation was observable in each group, suggesting that bacterial infection and inflammatory response at the wound site had largely subsided. As shown in Figure 6b, the phototherapy group treated with L-APU-TPP-Lev exhibited the greatest dermal thickness. Masson's staining revealed collagen deposition in the wound surfaces of mice treated with L-APU-TPP, APU-TPP-Lev, and L-APU-TPP-Lev gels, and significantly higher collagen deposition in the L-APU-TPP-Lev-treated group compared to others. This indicates that L-APU-TPP-Lev can eliminate wound bacteria, maintain good skin condition at the wound site, and promote sufficient collagen deposition. Additionally, partial hair follicle structures were formed, demonstrating no harm to biological tissues and cells. The photodynamic effect exhibits excellent antibacterial properties, accelerating wound healing rates in mice, making it a functional wound dressing.

3. Conclusions

In summary, this study successfully developed an injectable porphyrin polyurethane hydrogel through click chemistry and dynamic covalent crosslinking strategies. This material exhibits synergistic photodynamic-chemical antibacterial activity, controlled drug release, and excellent biocompatibility. This gel enables *in-situ* injection into irregular wound

surfaces via shear-thinning properties and triggers sustained levofloxacin release in acidic and ROS microenvironments while simultaneously releasing porphyrin to generate singlet oxygen. It achieves a 99.9% kill rate against *S. aureus* and *E. coli* within 3 hours of contact. *In vitro* cell experiments and acute hemolysis tests confirmed its low cytotoxicity and excellent hemocompatibility. In a full-thickness infected wound model, the L-APU-TPP-Lev gel achieved a 14-day healing rate of 95%, significantly outperforming conventional dressings. Consequently, APU-TPP gel demonstrates broad application prospects for rapid sealing, efficient antibacterial activity, and accelerated regeneration in bacterial infected wounds. All animal experiments in this work have passed the animal welfare ethics review of the experimental unit (Department of Chemistry, Northeast Normal University, Changchun 130024, P. R. China), and the acceptance number is [2024]Y.Numb.[0066].

Acknowledgements This research was financially supported by the National Natural Science Foundation of China (52222307), and Jilin Province Development and Reform Commission (2023C028-4).

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具有可控药物释放和光动力活性的注射型 抗菌聚氨酯水凝胶用于感染性创面治疗

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摘要 制备具有多重功能且能有效促进伤口修复的可注射水凝胶, 在治疗细菌感染性创面方面具有广阔前景。本研究通过无催化剂的点击反应制备聚氨酯水凝胶, 将氨基卟啉(TPP-NH₂)共价嵌入L-半胱氨酸交联的聚氨酯网络, 形成可注射自愈合型APU-TPP-Lev水凝胶。在弱酸性环境下, 该凝胶可释放抗生素, 并在红光照射下产生活性氧, 协同消灭大肠杆菌和金黄色葡萄球菌, 展现出高效的接触抗菌活性。此外, 该水凝胶具有优异的生物相容性, 经注射与光照后显著促进感染性创面愈合, 表明其在细菌感染性创面治疗中具有巨大的应用潜力。

关键词 聚氨酯; 水凝胶; 抗菌; 光敏剂