

A multifunctional injectable ϵ -poly-L-lysine-loaded sodium-alginate/gelatin hydrogel promotes the healing of infected wounds by regulating macrophage polarization and the skin microbiota

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Abstract

Background: Infected wounds caused by bacteria such as *Escherichia coli* and *Staphylococcus aureus* pose significant challenges during the healing process. Hydrogels have emerged as promising materials for the treatment of such infections, as they have the potential to deliver therapeutic agents while supporting tissue repair. This study aimed to develop ϵ -PLL@SA/Gel (PSG) hydrogels by incorporating varying concentrations of ϵ -poly-L-lysine (ϵ -PLL) into sodium alginate/gelatin (SA/Gel) using calcium chloride as a crosslinking agent, and to evaluate their antibacterial efficacy.

Methods: The mechanical properties, biocompatibility, antibacterial activity of hydrogels were evaluated. Biocompatibility was examined by measuring cell viability and proliferation of human skin fibroblasts in vitro. Antibacterial efficacy against *Escherichia coli* and *Staphylococcus aureus* was quantified using bacterial inhibition assays. The wound healing efficacy of the hydrogels were evaluated in mouse models of infected wounds.

Results: PSG hydrogels exhibited excellent mechanical strength, injectability, and self-adhesive properties. *In vitro*, hydrogel treatment resulted in high cell viability and promoted human skin fibroblast proliferation. PSG15 exhibited the highest antibacterial activity and inhibited *E. coli* and *S. aureus* by 89.53% and 92.21%, respectively. *In vivo*, PSG15 significantly accelerated wound healing, enhanced angiogenesis, and regulated macrophage polarization by increasing CD206 expression and decreasing CD80 expression. Additionally, PSG15 modulated the skin microbiota, reduced pathogenic bacterial abundance and maintained microbiota diversity.

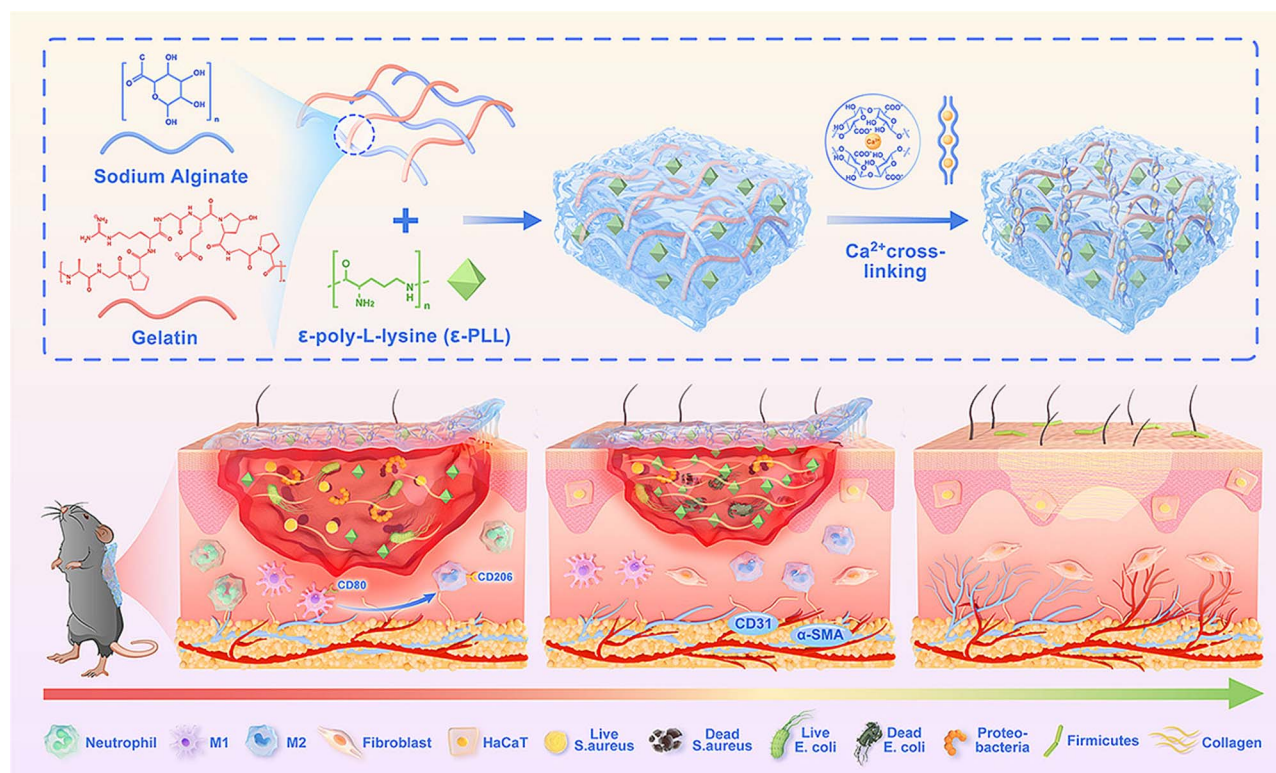
Conclusions: The PSG15 hydrogel is a promising candidate for the treatment of infected wounds because it inhibits bacterial growth, promotes tissue repair, and modulates the wound microbiota.

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Graphical Abstract



Keywords: ϵ -PLL@SA/gel; PSG; Infected wounds; Skin healing; Skin microbiota

Highlights

- This study achieved the synergistic improvement of the antibacterial properties and mechanical strength of ϵ -PLL@SA/Gel (PSG) hydrogel for the first time through gradient embedding of ϵ -PLL.
- This study first clarified the mechanism by which PSG hydrogel promotes the repair of infected wounds by regulating macrophage polarization and microbiome homeostasis.
- This study provides a new idea for the development of multifunctional wound dressings with physical adaptability, antibacterial function and regeneration ability.

Background

An infected wound is a type of refractory wound that commonly occurs after open injuries, burns, or surgical procedures and that is challenging to heal because of bacterial infection and other various factors. Severe infections can impede the healing process, leading to necrosis, sepsis, and potentially life-threatening conditions [1]. Currently, the primary treatment of infected wounds involves local debridement combined with systemic antibiotics [2]. However, the widespread misuse of antibiotics has led to a significant increase in bacterial resistance and difficulties in achieving effective antibacterial concentrations locally without causing systemic toxicity issues such as ototoxicity and nephrotoxicity [3]. Infected wounds have therefore become a substantial burden on social health care systems, which has necessitated an urgent search for novel, effective treatment methods [4].

ϵ -Poly-L-lysine (ϵ -PLL) is an antimicrobial peptide widely used in food, medicine, and other fields [5]. This peptide is characterized by low toxicity, good biocompatibility, a wide antibacterial spectrum, and low bacterial resistance [6]. It is recognized by the U.S. Food and Drug Administration as a food additive [7]. It also has a positive charge and can accumulate on the negatively charged surface of bacterial

cell membranes, which leads to an increase in membrane permeability and loss of integrity, thus mediating its antibacterial effect [8]. The application of ϵ -PLL-loaded hydrogels for infected wounds has been reported. ϵ -PLL incorporated into hydrogels can achieve slow and sustained release of antimicrobial peptides, thereby providing long-term antibacterial protection [9]. In addition, these hydrogels can absorb more water and maintain a moist environment, which is beneficial for wound healing [10].

Sodium alginate (SA) is a naturally degradable polysaccharide biomaterial extracted from algae. It is composed of α -L-guluronic acid (G unit) and β -D-mannuronic acid (M unit) polymerized through 1–4 glycosidic bonds and resembles a biomimetic extracellular matrix component [11]. The wound healing properties of SA dressings lie in their strong exudate absorption ability and their water absorption capacity, which is 5–7 times greater than that of traditional dressings [12]. Upon contact with a wound, SA quickly forms a gel to induce cell migration, proliferation, and regeneration and releases bioactive substances to activate platelets for hemostasis [13]. Gelatin (Gel) is derived from the hydrolysis and denaturation of collagen and has been recognized by the United States Food and Drug Administration (FDA) as a nontoxic and safe

material [14]. One of its most important characteristics is its thermal reversibility: it is soluble in water at temperatures above 35°C, and upon cooling, solidifies into a transparent gel. This is due to the presence of numerous active hydrophilic groups, such as -NH₂, -OH, and -COOH, in the gelatin molecule chains, which allow for gel formation through graft modification [15].

Based on the advantages of the respective compounds mentioned above, we integrated three different concentrations of ϵ -PLL into a stable hydrogel crosslinked with calcium alginate and Gel, which resulted in a multifunctional hydrogel, ϵ -PLL@SA/Gel (PSG). We tested the injectability, self-adhesive properties, compressibility, morphology, swelling ratio, gelation time, mechanical properties, and biocompatibility of PSG. PSG strongly promoted cell proliferation and exhibited antibacterial, anti-inflammatory, and angiogenesis-promoting properties. Furthermore, PSG promoted the healing of infected wounds induced by both *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) by regulating macrophage polarization and modulating the skin microbiota. This study provides a new research approach for the prevention and treatment of infected wounds.

Methods

Materials

SA (#W201502), Gel (#9000-70-8), and calcium chloride (CaCl₂, #C3881) were purchased from Sigma–Aldrich (US). ϵ -PLL was obtained from Meryer (#M60714, Shanghai, China). Human skin fibroblasts (HSFs) and human keratinocytes (HaCaT) were derived from primary cells donated by volunteers at the Chinese PLA General Hospital (Beijing, China). *S. aureus* (#25923) and *E. coli* (#25922) were purchased from ATCC (US). Male C57BL/6 mice, aged 6–8 weeks, that met SPF grade standards, were obtained from GemPharmatech Co. Ltd (Jiangsu, China).

Preparation of ϵ -PLL@SA/gel

Four different concentrations of ϵ -PLL solutions were prepared by dissolving ϵ -PLL powder (0, 5, 10, and 15 g) in 100 ml of deionized water to obtain solutions with concentrations of 0%, 5.0%, 10.0%, and 15.0% (w/v). Subsequently, 4 g of SA and 20 g of Gel were dissolved in 200 ml of PBS at 37°C and 50°C, respectively, to obtain a 2% SA (m/v) solution and a 10% Gel (m/v) solution. The solutions were allowed to stand for 4 hours to remove air bubbles. The SA and Gel solutions were mixed and stirred thoroughly using a magnetic stirrer until a homogeneous SA/Gel solution was formed. The four ϵ -PLL solutions were added to the SA/Gel solution at a 1:50 (v/v) ratio and were stirred at room temperature to form four PSG precursor solutions with different ϵ -PLL concentrations. The PSG solutions were mixed with equal volumes of 2% CaCl₂ solution to induce ionic crosslinking. After incubation for 1 h at room temperature, PSG hydrogels were formed. The hydrogels were rinsed three times with deionized water to remove residual crosslinking agents and were labeled as PSG0, PSG5, PSG10, and PSG15.

Characterization of PSG hydrogels

Injectability and shape adaptability

The injectability and shape adaptability of PSG were tested by loading the gel into a syringe and extruding it through a 10 ml syringe.

Skin compliance and self-adhesive ability

The skin compliance of the hydrogels was observed by placing them on finger joints. After mild external pressure was applied, the joint was flexed to observe the gel's ability to adapt to joint movement.

The self-adhesive behavior of the hydrogels was then investigated. A hydrogel disk was divided into two halves: one half was stained with Rhodamine B, while the other half remained unstained. The two halves were placed in contact for 30 minutes, and the healing process was observed by lifting the gel with forceps.

Scanning electron microscopy and energy-dispersive X-ray spectroscopy

The surface morphology of the hydrogels was observed by scanning electron microscopy (SEM) (ApreoC, Thermo Fisher). Hydrogels (2 ml) were prepared in molds with a 20 mm diameter and freeze-dried. The freeze-dried hydrogel samples were sliced, and the cross-sections were sputtered with a thin layer of gold for SEM observation. The elemental composition and content were analyzed via energy-dispersive X-ray spectroscopy under an accelerating voltage of 20 kV.

Chemical structure analysis

Fourier transform infrared spectroscopy (FTIR, Nicolet 6700, Thermo Nicolet Corporation) was used to investigate the chemical structure of the samples.

Rheological properties

The rheological properties of the hydrogels were evaluated using a rheometer (MCR92, Anton Paar) at 25°C. Disk-shaped hydrogel samples (20 mm diameter, 5 mm thickness) were prepared, and the storage modulus (*G'*) and loss modulus (*G''*) were recorded over a frequency range of 1–100 Hz.

Mechanical properties

The compressive mechanical properties of the hydrogels were measured using a universal testing machine (CMT6103, MTS). The hydrogel samples were prepared as cylindrical shapes (25 mm diameter × 5 mm height) for compression testing. The compression load was applied at 100 N with a compression rate of 0.5 mm/s, and the loading stopped when the compression strain reached 60%. Stress–strain curves were then plotted.

Swelling and degradation measurements

For swelling measurements, freeze-dried PSG hydrogels of the same shape and volume were weighed (*W*₀) and immersed in 10 ml of PBS (pH 7.4) at 37°C. At predetermined intervals, the hydrogels were removed, blotted with filter paper to remove excess surface water, and weighed again (*W*_t) until the weight stabilized, which was an indication of equilibrium swelling. The swelling ratio was calculated as (*W*_t – *W*₀)/*W*₀ × 100% [16]. Each hydrogel sample was tested three times.

For degradation measurements, hydrogels of the same shape and volume were weighed (*V*₀) and immersed in 10 ml of PBS (pH 7.4) at 37°C with shaking (100 rad/s). At predetermined intervals, the hydrogels were removed, washed, dried at 37°C for 48 hours, and weighed again (*V*_t). The remaining mass percentage was calculated as *V*_t/*V*₀ × 100%. Each hydrogel sample was tested three times.

Biocompatibility of the PSG hydrogels

Cytotoxicity detection by CCK-8 assay

PSG0, PSG5, PSG10, and PSG15 hydrogels (1 ml each) were immersed in 10 ml of DMEM containing 10% fetal bovine serum and incubated at 37°C for 24 hours. The resulting extracts were sterilized using a 0.22 µm filter. HSFs and HaCaT cells were seeded into 96-well plates (5000 cells/well) and incubated overnight to allow cell adhesion. The culture medium was replaced with 100 µl of hydrogel extract or fresh complete medium. After 1, 2, and 3 days of incubation, the medium was discarded, and 100 µl of fresh medium containing 10% CCK-8 reagent was added to each well. After a 3-hour incubation at 37°C, the absorbance at 450 nm was measured via a microplate reader. Cell viability was calculated according to the following formula:

$$\left[\frac{(\text{OD}_{\text{sample}} - \text{OD}_{\text{blank}})}{(\text{OD}_{\text{control}} - \text{OD}_{\text{blank}})} \right] \times 100\%.$$

Live/dead staining

A live/dead assay kit (#C542, DOJINDO, Japan) was used to assess cell viability. HSFs and HaCaT cells were seeded into 12-well plates (70 000 cells/well) and incubated overnight. The culture medium was replaced with 2 ml of hydrogel extract or fresh complete medium. After incubation for 1, 2, and 3 days, the cells were washed with PBS, stained with a working solution of calcein-AM (2 µM) and PI (4.5 µM), and incubated at 37°C for 30 minutes. Live cells emit green fluorescence (calcein-AM), while dead cells emit red fluorescence (PI). Images were captured with an inverted fluorescence microscope (Olympus, Tokyo, Japan).

Cell migration ability

A scratch assay was used to assess the effects of the hydrogel extracts on cell migration. HSFs and HaCaT cells were seeded into 12-well plates (100 000 cells/well) and incubated overnight until they reached 80% confluence. A sterile pipette tip was used to generate a scratch wound, after which the cells were washed twice with PBS. The wells were then filled with hydrogel extract or fresh complete medium and incubated for 24 hours. The migration rate was calculated as follows: Migration rate (%) = [(Initial wound area – final wound area)/initial wound area] × 100%.

Blood compatibility evaluation

Fresh mouse blood was collected and centrifuged at 2000 rpm for 10 minutes after which a red blood cell suspension (2 wt%) was prepared. The hydrogels were then incubated with the red blood cell suspension at 37°C for 4 hours, at which point the samples were centrifuged, and the absorbance of the supernatant was measured at 540 nm. Red blood cell morphology was observed by microscopy. The hemolysis rate was calculated as follows: hemolysis rate (%) = [(OD_{sample} – OD_{negative control})/(OD_{positive control} – OD_{negative control})] × 100%.

Antibacterial activity

Bacterial absorbance detection

The antibacterial activities of the four types of PSG hydrogels were evaluated by measuring bacterial absorbance at 600 nm using a microplate reader to assess their inhibitory effects on *E. coli* and *S. aureus*. *E. coli* and *S. aureus* (5 × 10⁵ CFU/ml) were inoculated into LB media. Subsequently, 1 ml of each PSG hydrogel was dissolved in 5 ml of LB media, after

which 100 µl of bacterial suspension (5 × 10⁵ CFU/ml) was added. The cultures were incubated at 37°C with shaking at 150 r/min in a constant-temperature shaker for 24 hours. The bacterial absorbance at the start and end points was measured at 600 nm using a microplate reader. This experiment was repeated three times.

Inhibition zone assay

The inhibition zone assay was performed on LB agar plates by inoculating LB media with 200 µl of *E. coli* or *S. aureus* (1 × 10⁶ CFU/ml) suspensions. The four types of PSG hydrogels (1 cm in diameter) were placed in the center of the Petri dishes. After 24 hours of incubation, the diameters of the inhibition zones were measured to determine the antibacterial activity. This experiment was repeated three times.

Bacterial colony count

The antibacterial properties of the hydrogels were evaluated according to CFU colony counts. One milliliter of each PSG hydrogel was added to an aseptic centrifuge tube, while 1 ml of PBS served as the control. Then, 5 ml of *E. coli* or *S. aureus* bacterial mixture was added, after which the mixtures were incubated at 37°C for 24 h. The bacterial mixture was diluted, 20 µl was added evenly to solid medium, and the total number of colonies was recorded after culture for 24 h.

Live/dead staining

A live/dead staining assay was performed to further evaluate the antibacterial activity of the PSG15 hydrogel against *E. coli* and *S. aureus*. The PSG15 hydrogel (1 ml) was immersed in 1 ml of an *E. coli* or *S. aureus* suspension (1 × 10⁸ CFU/ml) and incubated overnight. As a control, 1 ml of PBS was added to the suspensions. After incubation, the PSG15 hydrogel was removed, and the remaining bacterial suspensions were centrifuged at 3000 r/min for 5 minutes. The bacterial pellets were washed three times with PBS, after which the pellets were stained with SYTO-9 (5 µM) and PI (1 µg/ml) for 15 minutes at 37°C in the dark. After staining, the supernatant was removed by centrifugation (5000 r/min, 5 minutes), and the bacteria were washed three times with saline. Finally, the bacterial suspension was observed under a confocal laser scanning microscope.

Scanning electron microscopy

The morphology of *E. coli* and *S. aureus* incubated with the PSG15 hydrogel was observed via SEM. A mixture of 1 ml of the PSG15 hydrogel and 1 ml of bacterial suspension (1 × 10⁸ CFU/ml) was incubated at 37°C overnight. The bacterial suspensions were then centrifuged at 3000 r/min for 10 minutes, and the pellets were fixed in 4% glutaraldehyde for 5 hours, followed by further fixation in 2.5% glutaraldehyde at 4°C for 1 hour. The bacteria were dehydrated in a graded ethanol series (20%, 50%, 80%, and 100% w/v) for 10 minutes at each step. After dehydration, the bacteria were sputter-coated with a thin layer of gold and observed under a scanning electron microscope (S-4800, 5 kV, Hitachi, Tokyo, Japan).

Animal experimental study

Male C57BL/6 mice (6–8 weeks, 20–22 g) were purchased from GemPharmatech Co. Ltd (Jiangsu, China). All animal experiments were performed in accordance with the guidelines issued by the National Research Council for the Care and Use

of Laboratory Animals. All animal experiments complied with the ARRIVE guidelines.

After one week of acclimatization, the mice were anesthetized via an intraperitoneal injection of 1% sodium pentobarbital (50 mg/kg). The anesthetized mice were placed in a prone position, and the fur on their backs was shaved using electric clippers, which was followed by the application of a depilatory cream to completely remove the fur. After the area was disinfected with 75% ethanol, a full-thickness skin wound (8 mm in diameter) was then generated on the back of each mouse using a skin biopsy punch.

The mice were divided into seven groups via a random number table, with eight mice per group. Except for those in the control group, the wounds in the other groups were infected with *E. coli* or *S. aureus* suspensions (1×10^7 CFU/ml) for 48 hours. From Day 3 onward, the wounds were treated every 3 days. The mice in the control and model groups were gently cleaned with sterile saline. PSG15 and the commercial dressing Urgotul Ag/Silver (Urgo Medical, France) were applied to the experimental group, after which the wounds were covered with sterile medical gauze, which was secured with surgical tape. The mice were housed individually and provided with adequate food and water. A circular template (1 cm in diameter) was placed around the wounds to ensure consistent imaging. The percentage of wound healing (%) was calculated as follows: Wound closure rate (%) = $[(S_0 - S_n)/S_0] \times 100\%$, where S_0 is the initial wound area on Day 0, and S_n is the wound area on the day of the dressing change.

Hematoxylin–eosin and Masson’s trichrome staining

On Day 15, the mice were euthanized, and the wound tissues were excised, fixed in 4% (w/v) paraformaldehyde, and subsequently embedded in paraffin blocks. The tissues were then sectioned for further analysis.

For hematoxylin and eosin (H&E) staining, the paraffin-embedded sections were deparaffinized by two 10-minute immersions in xylene. Rehydration was achieved through a graded ethanol series (100%, 95%, 80%, 70%), followed by a 5-minute rinse in distilled water. The sections were stained with hematoxylin solution for 5 minutes and then washed under running tap water for 10 minutes. Acid alcohol (1% HCl in 70% ethanol) was applied for 3 seconds to differentiate the nuclei, after which the sections were rinsed again under running tap water for 5 minutes. Eosin staining was performed for 1 minute, followed by dehydration through a graded ethanol series (70%, 80%, 90%, 100%) for 1 minute each. The sections were cleared with two 5-minute immersions in xylene and mounted with a coverslip using xylene-based mounting medium, allowing them to dry.

For Masson’s trichrome staining, deparaffinization and rehydration were performed. Then, the tissues were subsequently stained with Weigert’s iron hematoxylin (5 minutes) and Biebrich scarlet-acid fuchsin solution (10 minutes). After differentiation in phosphotungstic–phosphomolybdic acid solution for 10 minutes, the sections were stained with aniline blue for 5 minutes. After acidic differentiation and dehydration, the sections were cleared in xylene twice for 5 minutes each time. Finally, the tissues were mounted with a coverslip using xylene-based mounting medium.

Immunohistochemical staining

After deparaffinization and rehydration, the tissue sections were subjected to heat-mediated antigen retrieval (EDTA

buffer, pH 9.0) in a microwave for 8 minutes. The slides were then cooled naturally and washed with PBS (pH 7.4) three times for 5 minutes each time. The primary antibody diluted in PBS was applied, and the sections were incubated overnight at 4°C in a humidified chamber. After the slides were washed three times with PBS (pH 7.4) on a shaker (5 minutes each time), the secondary antibody was applied, and the samples were incubated at room temperature in the dark for 50 minutes. The slides were incubated with DAPI (4',6-diamidino-2-phenylindole), which stains cell nuclei, in the dark for 10 minutes, followed by three washes with PBS. The slides were then mounted with anti-fade mounting medium. The sections were observed and imaged under a fluorescence microscope.

16S rRNA gene sequencing

16S rRNA gene sequencing was performed according to previous report. [17]. DNA was extracted from the samples. The V3-V4 region of the 16S ribosomal RNA (rRNA) gene was amplified using the specific primers 341F (5'-CCTAYGGGRBGCASCAG-3') and 806R (5'-GGACTACNNGGGTATCTAAT-3') with barcodes. Phusion® High-Fidelity PCR Master Mix (New England Biolabs, USA) was used for PCR amplification. The PCR program consisted of initial denaturation at 98°C for 1 minute, followed by 30 cycles of 98°C for 10 seconds, 50°C for 30 seconds, and 72°C for 30 seconds, with a final extension at 72°C for 5 minutes. The PCR products were purified using a DNA Gel Extraction Kit (Qiagen, Germany). Sequencing libraries were constructed using the NEBNext® Ultra™ II DNA Library Prep Kit (New England Biolabs, USA) and subsequently sequenced in a paired-end configuration on the Illumina NovaSeq platform (Illumina, USA). Raw sequencing reads were demultiplexed according to barcode sequences and subjected to quality control processing with FASTP for adapter trimming and low-quality read filtering, resulting in high-quality clean reads. Potential chimeric sequences were identified and removed by performing similarity-based chimera checking against the Silva database, thereby generating the final set of validated amplicon sequences.

Hydrogel skin irritation test

The skin irritation test of the PSG15 hydrogel was conducted in accordance with the National Standard of the People’s Republic of China GB/T 16886.10-2017/ISO 10993-10:2010. Healthy C57BL/6 mice with undamaged intact skin were selected. Twenty-four hours prior to the experiment, the dorsal area of the mice was depilated, and the administration sites were indicated with a marker (divided into the normal control skin group, the commercial dressing treatment group, and the PSG15 hydrogel treatment groups). Changes at the administration sites were observed and scored according to the evaluation criteria (Table S1).

Statistical analysis

All the data are expressed as the means $\pm$ standard deviations (SDs). Statistical analysis was performed via GraphPad Prism 9 software, while visualization was performed via RStudio (version 4.1.1). For experiments with a single independent variable, one-way ANOVA followed by Tukey’s post hoc test was used to determine significant differences between groups ($P < .05$ or $P < .01$). For experiments involving two independent variables, two-way ANOVA with Tukey’s post hoc test was employed to assess the interaction and individual effects

of these factors. Skin microbiome analysis was performed via MicrobiomeAnalyst 2.0, which included a diversity analysis (Shannon index) and a differential microbial analysis (Lefse analysis, LDA > 2).

Results

Design, preparation, structure, and morphology of the PSG hydrogel

The SA and Gel solutions exhibited stable fluid states at 37°C and 50°C, respectively. When mixed thoroughly, they form a transparent SA/Gel precursor solution at 37°C. Upon crosslinking with a CaCl₂ solution, the mixture gels and remains stable when inverted, which demonstrates its structural integrity (Figure 1a). Ca²⁺ improves the mechanical properties of the hydrogel, which is consistent with the findings of a previous study [18]. The hydrogel could be extruded continuously through a needle to write the letters "ABCD", confirming the hydrogel's injectability and adaptability to different wound shapes (Figure 1b). Furthermore, the hydrogel adhered to finger joints and withstood bending and stretching forces without detaching or rupturing, which indicates good skin compliance (Figure 1c). When two halves of a pink and a transparent circular hydrogel disc were cut symmetrically and placed together without external force, the PSG hydrogel displayed self-adhesive behavior. After 30 min, the broken hydrogel reattached, restoring its integrity and bearing its own weight (Figure 1d).

The PSG0, PSG5, PSG10, and PSG15 hydrogels were all visually transparent and jelly-like, and the peptide content was uniformly distributed throughout the hydrogel, increasing with increasing ϵ -PLL concentration (Figure 1e). However, some white dots were present on the hydrogel. We speculate that these dots may represent localized aggregates or microphase separation of ϵ -PLL. SEM images revealed that all the hydrogels exhibited a 3D interconnected network structure (Figure 1f). The average pore size of the PSG0 hydrogel was 152.9 μ m, that of PSG5 was 151.6 μ m, that of PSG10 was 141.4 μ m, and that of PSG15 was 133.1 μ m (Figure 1g). The decrease in pore size is likely due to the denser structure formed as more ϵ -PLL is incorporated into the hydrogel.

Characterization of the PSG hydrogel

To confirm the successful incorporation of ϵ -PLL into the PSG hydrogels, FTIR analysis was performed on ϵ -PLL, SA, Gel, and the PSG0, PSG5, PSG10, and PSG15 hydrogels. The main elements in the PSG hydrogels were C, O, and Cl, which indicates that some Na⁺ ions in the sodium alginate were successfully replaced by Ca²⁺, confirming successful ionic crosslinking (Figure 2a). The FTIR spectra of the raw materials and the PSG hydrogels presented three distinct characteristic peaks: the O-H stretching vibration peak at 3425 cm⁻¹, the symmetric stretching vibration peak of the C=O bond at 1636 cm⁻¹, and the out-of-plane stretching vibration peak of the C-H bond at 563 cm⁻¹. As the concentration of ϵ -PLL increased, the intensities of these peaks, particularly the O-H peak, significantly changed. This finding indicates that crosslinking reactions occurred among the carboxyl, amine, and Ca²⁺ ions in the hydrogel as the concentration of ϵ -PLL increased, which resulted in peak intensity changes. Additionally, a C-H stretching vibration peak was detected at 2929 cm⁻¹ in the PSG15 sample, which was attributed to the interaction

between ϵ -PLL and SA. These results confirmed the successful incorporation of ϵ -PLL into the hydrogels (Figure 2b).

Compression tests revealed that as the ϵ -PLL concentration increased, the hydrogels became softer. However, all PSG hydrogels at different concentrations were able to withstand maximum stresses exceeding 10 kPa, which indicates that they can endure external compression forces and protect wounds while maintaining their softness (Figure 2c-d). Rheological tests revealed that at 37°C, all PSG hydrogels presented a storage modulus (G') greater than the loss modulus (G''); this suggests the formation of a hydrogel network with both solid and fluid properties, which is crucial for withstanding the dynamic forces on a wound site during healing [19]. The hydrogels exhibited both fluid and solid properties, with good viscosity that helps mitigate external forces on the wound (Figure 2e-f).

Swelling capacity is closely related to the ability of a hydrogel to continuously absorb exudate [20]. Under pH 7.4 conditions, the hydrogels reached equilibrium swelling after 36 hours, with swelling rates exceeding 400% in all groups. PSG5 and PSG10 exhibited higher swelling rates than did PSG15 (Figure 2g-j). We speculate that the interaction between ϵ -PLL and the SA/Gel matrix promoted the formation of a denser network with smaller pore sizes, which restricted the space available for water uptake, thereby lowering the swelling capacity of the hydrogel. Additionally, at pH 7.4, all the hydrogels significantly degraded after 36 h, with lower degradation rates observed at higher drug concentrations. The higher drug content may have resulted in a denser hydrogel structure, and thus the denser gels were more resistant to degradation (Figure 2k-n).

Biocompatibility of hydrogels

The cytotoxicity and biocompatibility of hydrogel dressings are critical for wound healing, as they are in direct contact with damaged skin [21]. In this study, the cytotoxicity of the PSG hydrogels was evaluated by CCK-8 assay. After 3 days of incubation with different concentrations of PSG hydrogel extracts, the cell viability remained ~100%, which complies with the international standard (>80%, ISO 10993-5) and indicates that the PSG hydrogels are nontoxic to a certain extent (Figure 3a-b). Notably, the viability of HSFs increased significantly by Day 3, which suggests that the hydrogels promoted HSF proliferation. Furthermore, live/dead staining (calcein-AM/PI) was used to assess the viability of HaCaT cells and HSFs after 1, 2, and 3 days of incubation. After 72 hours, abundant live cells (green fluorescence) and almost no dead cells (red fluorescence) were observed, which indicates that the hydrogels elicited no significant cytotoxic effects (Figure 3c-d).

A scratch assay was also used to evaluate the migration ability of HaCaT cells and HSFs. After 3 days of incubation with the PSG hydrogel extracts, the scratch distance significantly decreased, which indicates that both cell types exhibited increased migration toward the wound center over time. These findings suggest that PSG hydrogels promote wound healing by enhancing the migration of HaCaT cells and HSFs (Figure 3e-f).

As hydrogel dressings are in direct contact with open wounds, blood compatibility was assessed via a hemolysis assay in which mouse red blood cells were used [22]. The supernatants of the different concentrations of PSG hydrogels and the negative control (0.9% NaCl) remained clear and

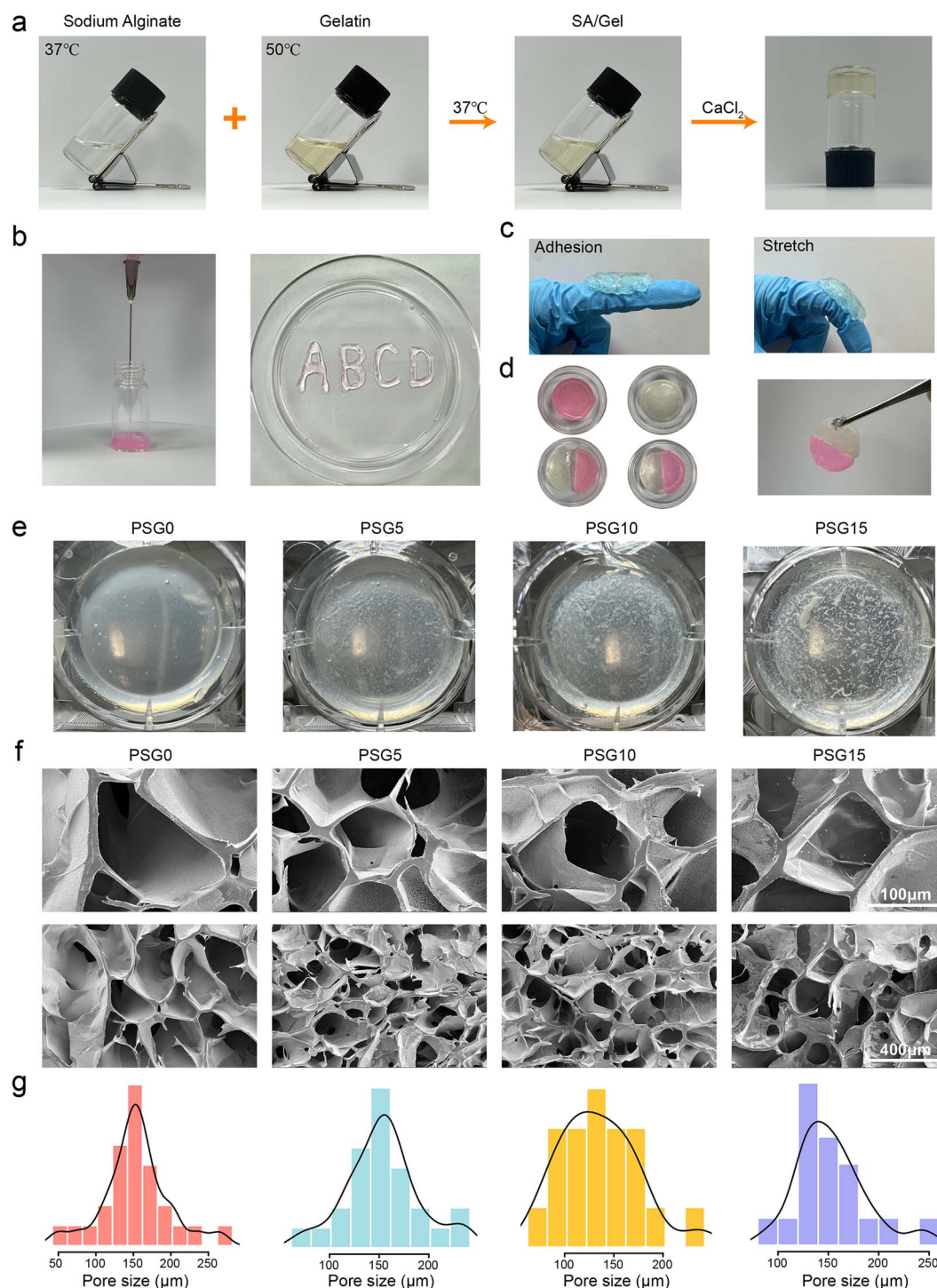


Figure 1. Formation, injectability, skin compliance, self-adhesive, and morphological characterization of PSG hydrogels. (a) The formation of PSG via Ca^{2+} crosslinking of a mixed SA/Gel precursor solution containing various ϵ -PLL concentrations. (b) The hydrogel was extruded through a syringe needle and successfully formed the letters 'ABCD' on a flat surface, demonstrating its potential for application in irregular wound geometries. (c) The hydrogel adhered to a human finger joint and maintained its integrity under bending and stretching forces, which is indicative of its adaptability to dynamic wound sites. (d) Two separately colored halves of the hydrogel were brought into contact without external force, and within 30 minutes, spontaneous reattachment restored continuity, which suggests an intrinsic self-adhesive capability. (e) Macroscopic appearance of PSG hydrogels with 0%, 5%, 10%, and 15% ϵ -PLL. (f) SEM images reveal the 3D interconnected network structure of the hydrogels. (g) Quantitative analysis of average pore sizes across the formulations indicate a reduction in pore size with increasing ϵ -PLL concentration. SA sodium alginate, Gel gelatin

transparent, with no signs of hemolysis (red coloration). In contrast, the positive control (1% w/v Triton X-100) resulted in hemolysis, and the supernatant became red. Further absorbance measurements at 540 nm revealed that

the absorbance values of the PSG hydrogels and the negative control were < 0.1 , whereas the positive control had an absorbance value > 0.6 (Figure 3g). The uniform dispersion of erythrocytes at various absorbance values of the PSG

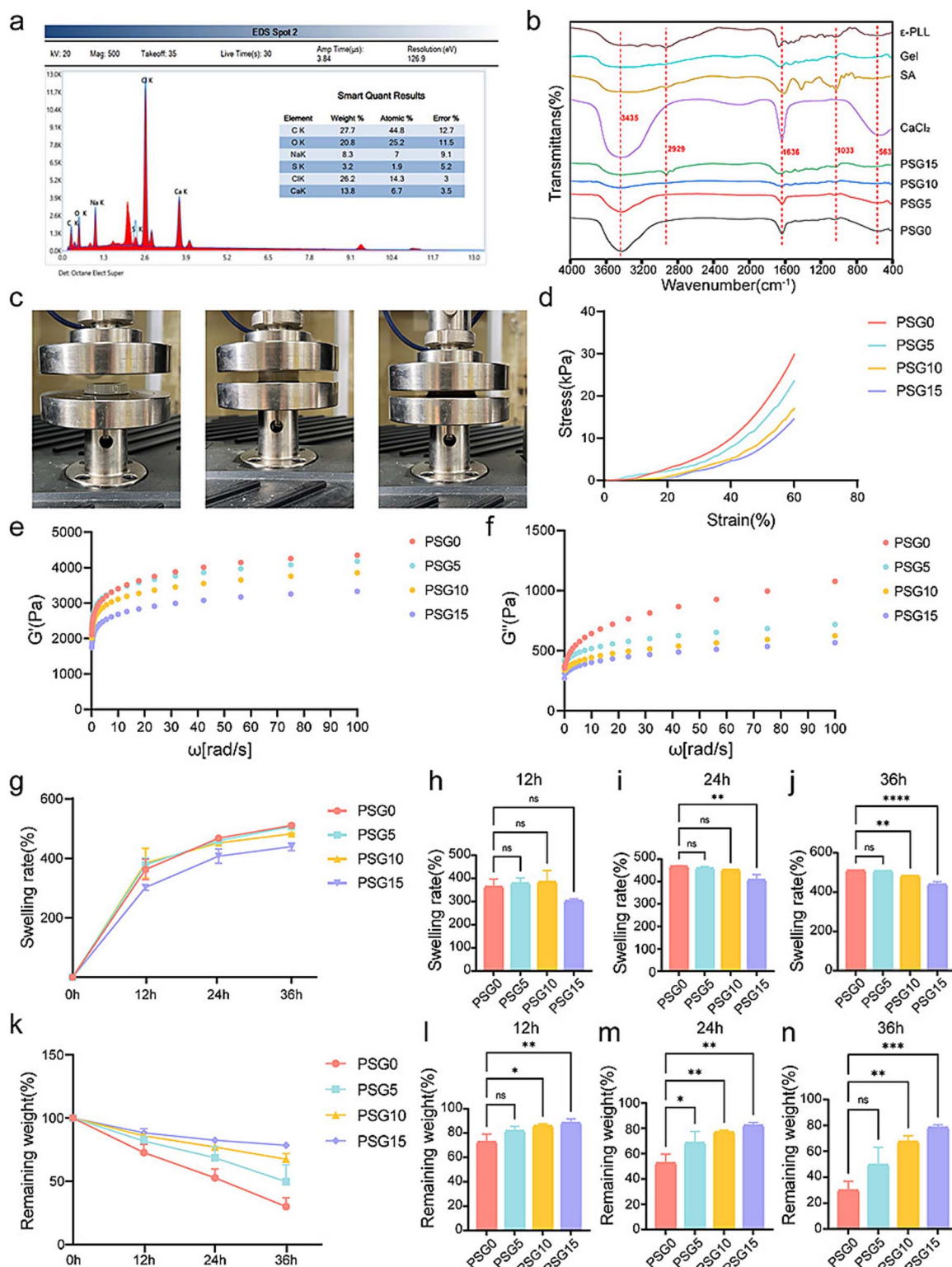


Figure 2. Chemical and mechanical characterization of PSG hydrogels. **(a-b)** FTIR spectra of pure ε-PLL, SA, Gel, and PSG hydrogels confirm the incorporation of ε-PLL by the appearance of characteristic peaks. **(c-d)** Compression test of PSG hydrogels demonstrate their mechanical stability under applied stress. **(e-f)** Rheological properties at 37°C show that the storage modulus (G') was consistently greater than the loss modulus (G''), which indicates a stable hydrogel network. **(g-j)** Swelling behavior of PSG hydrogels in pH 7.4 buffer shows time-dependent water uptake until equilibrium was reached ($n = 3$). **(k-n)** Degradation profiles over time illustrate that hydrogels with higher ε-PLL content exhibited slower degradation ($n = 3$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

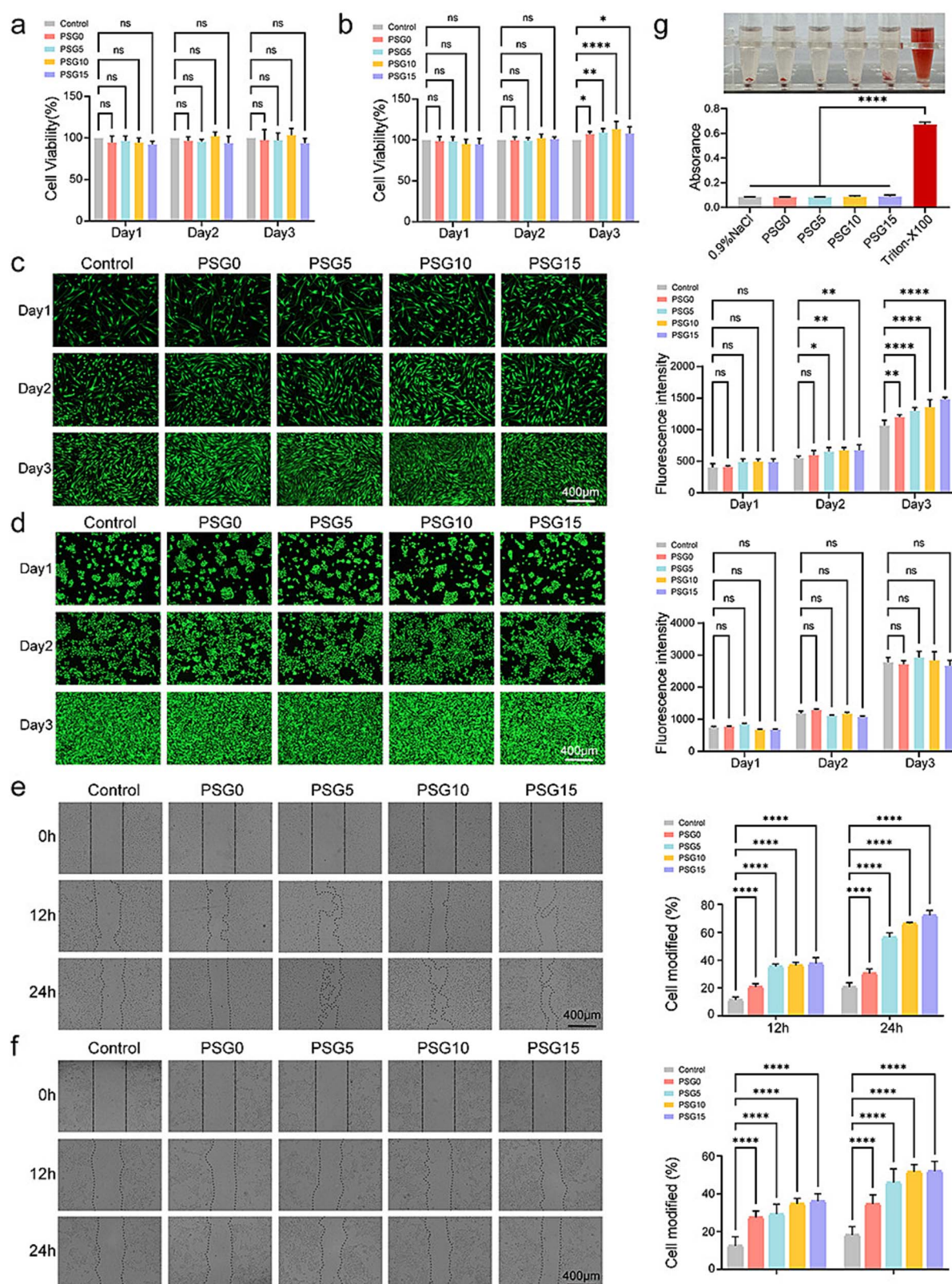


Figure 3. *In vitro* biocompatibility and cytotoxicity evaluation of PSG hydrogels. (a-b) CCK-8 assay results show the viability of HaCaT cells and HSFs after 3 days of incubation with PSG hydrogel extracts ($n = 3$). (c-d) Live/dead staining images of HaCaT cells and HSFs after 72 hours indicate no significant cytotoxicity ($n = 3$). (e-f) Scratch assay results show enhanced migration of HaCaT cells and HSFs toward the wound center after 3 days of incubation with PSG hydrogels ($n = 3$). (g) Hemolysis assay results confirm the blood compatibility of the PSG hydrogels ($n = 3$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$, ***** $P < 0.00001$.

hydrogels and in the negative control was observed under an optical microscope, while significant hemoglobin release was observed in the positive control group treated with Triton X-100 (Figure S1). These findings demonstrate that PSG hydrogels do not induce hemolysis and have good blood compatibility.

Antibacterial properties of the hydrogels

The antibacterial properties of hydrogel dressings are essential for the treatment of infected wounds [23]. *E. coli* (Gram-negative) and *S. aureus* (Gram-positive) are common pathogens in infected wounds [24]. The antibacterial activity of the PSG0, PSG5, PSG10 and PSG15 hydrogels against *E. coli* and *S. aureus* was evaluated via absorbance measurements. PSG15 exhibited 89.53% and 92.21% antibacterial activity against *E. coli* and *S. aureus*, respectively, which indicates excellent antibacterial properties (Figure 4a–b). Inhibition zone experiments were also used to measure the antibacterial efficacy of PSG hydrogels at different concentrations against *E. coli* and *S. aureus*. The results revealed that the PSG hydrogels with ϵ -PLL added exhibited antibacterial effects, with increasing inhibition zone diameters and higher antibacterial efficacy as the ϵ -PLL concentration increased (Figure 4c–e). The antibacterial activity of the four hydrogels against *E. coli* and *S. aureus* was verified via CFU counts. Compared with those in the control group, the numbers of *E. coli* and *S. aureus* colonies in the PSG5, PSG10, and PSG15 groups decreased in a concentration-dependent manner. The antibacterial activity of the hydrogel group with a higher ϵ -PLL content was significantly greater than that of the hydrogel group with a lower ϵ -PLL content, and PSG15 demonstrated the best antibacterial effect (Figure 4f–h). SEM images revealed that untreated *E. coli* and *S. aureus* retained a normal morphology with smooth, intact surfaces. After 12 hours of exposure to the PSG15 hydrogels, the bacteria exhibited severe deformation, wall rupture, and cytoplasmic leakage, which suggests that the antibacterial activity of the PSG15 hydrogels was mediated by disruption of the bacterial wall and loss of membrane integrity (Figure 4i). SYTO-9 and PI were used to stain live and dead bacteria, respectively. Incubation with PSG15 significantly increased the proportion of dead bacteria (Figure 4j). PSG15 hydrogels demonstrated the best antibacterial activity, and thus they are a promising option for the prevention and treatment of wound infections.

PSG15 hydrogels strongly promoted the healing of wounds infected by *E. Coli* and *S. Aureus*

After punch wounds were generated on the backs of the mice, the experimental groups were infected with *S. aureus* and *E. coli* suspensions for 48 hours. By Day 3, the infected wounds exhibited purulent exudation, confirming the successful establishment of a full-thickness infected skin model. Macroscopic observation of wound healing in the infected wounds revealed that, compared with what was seen in the model group, the PSG15 hydrogels significantly promoted the healing of wounds infected with *E. coli* and *S. aureus*. The healing efficiency of PSG15 was similar to that of the commercial product (Figure 5a–c). Histopathological analysis further demonstrated that, compared with the other groups, the PSG15 hydrogels demonstrated better healing efficiency. H&E staining on Day 15 revealed the formation of new blood vessels and follicles in the PSG15 and Urgotul Ag/Silver

groups. Masson's trichrome staining revealed greater collagen deposition in the PSG15 group than in the model group, which indicates enhanced tissue repair (Figure 5d–g). PSG15 hydrogels maintain a moist wound environment, which is beneficial for tissue regeneration [25].

CD31 was used to confirm the presence of vascular endothelial cells, while α -smooth muscle actin (α -SMA) serves as a specific marker of vascular rings, which are associated with tissue fibrosis and wound contraction [26]. CD31 and α -SMA staining was significantly greater in both the hydrogel-treated group and the Urgotul Ag/Silver dressing group than in the other groups (Figure 6a–b). The above conclusions were confirmed by α -SMA and CD31 double labeling (Figure S2A). These findings indicate that the PSG hydrogel promoted subcutaneous angiogenesis in infected wounds, which is crucial for tissue repair and regeneration. We propose that the promotion of neovascularization could be due to a combined effect. Gel can promote angiogenesis and support endothelial cell migration by releasing matrix metalloproteinase-responsive peptides [27]. ϵ -PLL may indirectly support angiogenesis by reducing local infection and inflammation, thereby establishing a favorable environment for new vessel formation and tissue repair.

CD80 and CD206 are used as markers to identify M1 and M2 macrophages, respectively [28]. M1 macrophages are known to trigger immune responses around wounds, which leads to inflammation and tissue damage. In contrast, M2 macrophages help reduce local inflammation, improve the tissue microenvironment, and promote wound repair [29]. Compared with what was observed in the model group, CD206 expression was elevated, while CD80 expression was decreased in both the hydrogel and Urgotul Ag/Silver dressing groups (Figure 6c–f). Further costaining of pathological tissues for the M2 biomarkers CD206 and CD163 revealed that the red–green overlap in fluorescence intensity in the PSG15 hydrogel group was significantly greater than that in the other groups (Figure S2B). Moreover, the mRNA and protein expression levels of M1 (TNF- α , IL-1 β and INOS) and M2 macrophage secretions (IL-10 and ARG-1) were also measured (Figures S3–S4). Significantly lower expression of TNF- α , IL-1 β and INOS and higher levels of IL-10 and ARG-1 was observed in the PSG15-treated group (Figures S3–S4). These findings suggest that the PSG15 hydrogel facilitated the conversion of M1 macrophages to M2 macrophages during the later stages of wound healing, which may contribute to its anti-inflammatory and prorepair effects.

Since the *in vivo* biosafety of hydrogels is crucial for clinical applications, a skin irritation test was performed. At 0, 12, and 24 hours posttreatment, no erythema, eschar, or edema formation was observed in the PSG15 hydrogel-treated skin (Figure S5). These results demonstrate that the PSG15 hydrogel has no irritant effects on normal murine skin.

Dynamics of the skin microbiota during wound healing in the PSG15-treated and control groups

To investigate whether treatment with PSG15 could regulate changes in the skin microbiota during wound healing, skin samples were collected from all groups at three different time points (3, 7, and 14 days after infection). On Day 3 post infection, skin microbiota diversity significantly decreased in both the *E. coli* and *S. aureus* model groups, whereas no significant changes were observed in the other groups compared with the

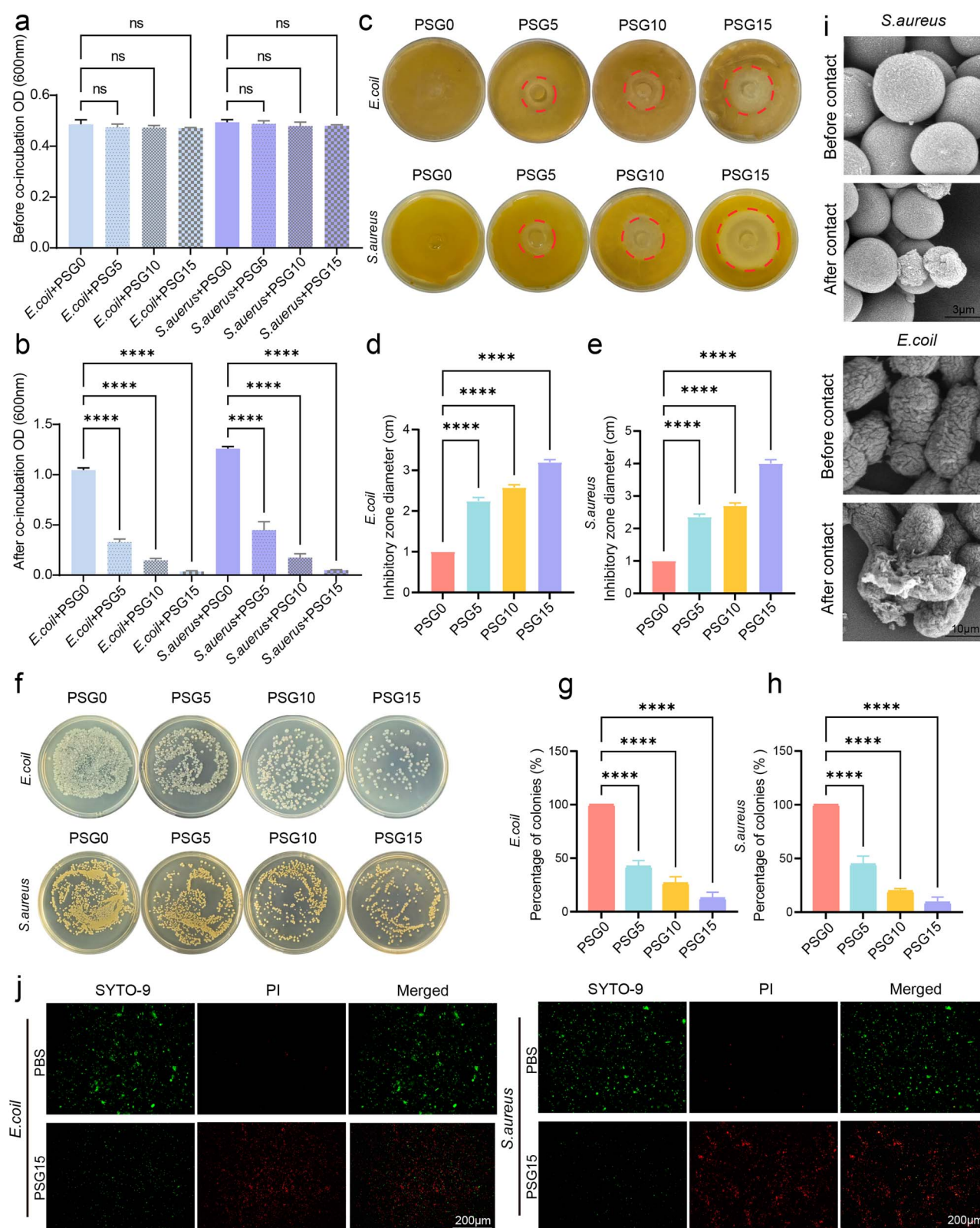


Figure 4. Antibacterial properties of PSG hydrogels. **(a)** Antibacterial activity of the hydrogels against *E. coli* and *S. aureus* on the basis of absorbance measurements at the starting point of incubation ($n = 3$). **(b)** Antibacterial activity of the hydrogel against *E. coli* and *S. aureus* based on absorbance measurements after 24 hours of incubation ($n = 3$). **(c-e)** Inhibition zone experiments demonstrate the antibacterial efficacy of PSG hydrogels at different concentrations ($n = 3$). **(f-h)** Colony forming unit (CFU) counts for *E. coli* and *S. aureus* after coculture with the hydrogels demonstrate a graded reduction in bacterial colonies with increasing ϵ -PLL content ($n = 3$). **(i)** SEM images of *E. coli* and *S. aureus* following 12 hours of exposure to the PSG15 hydrogel, which revealed marked deformation, cell wall rupture, and cytoplasmic leakage indicative of membrane disruption ($n = 3$). **(j)** Fluorescence microscopy images with SYTO-9 (green, live cells) and propidium iodide (red, dead cells) staining of bacteria treated with the PSG15 hydrogel, which confirms a significant increase in bacterial cell death ($n = 3$). *** $P < 0.0001$, **** $P < 0.00001$

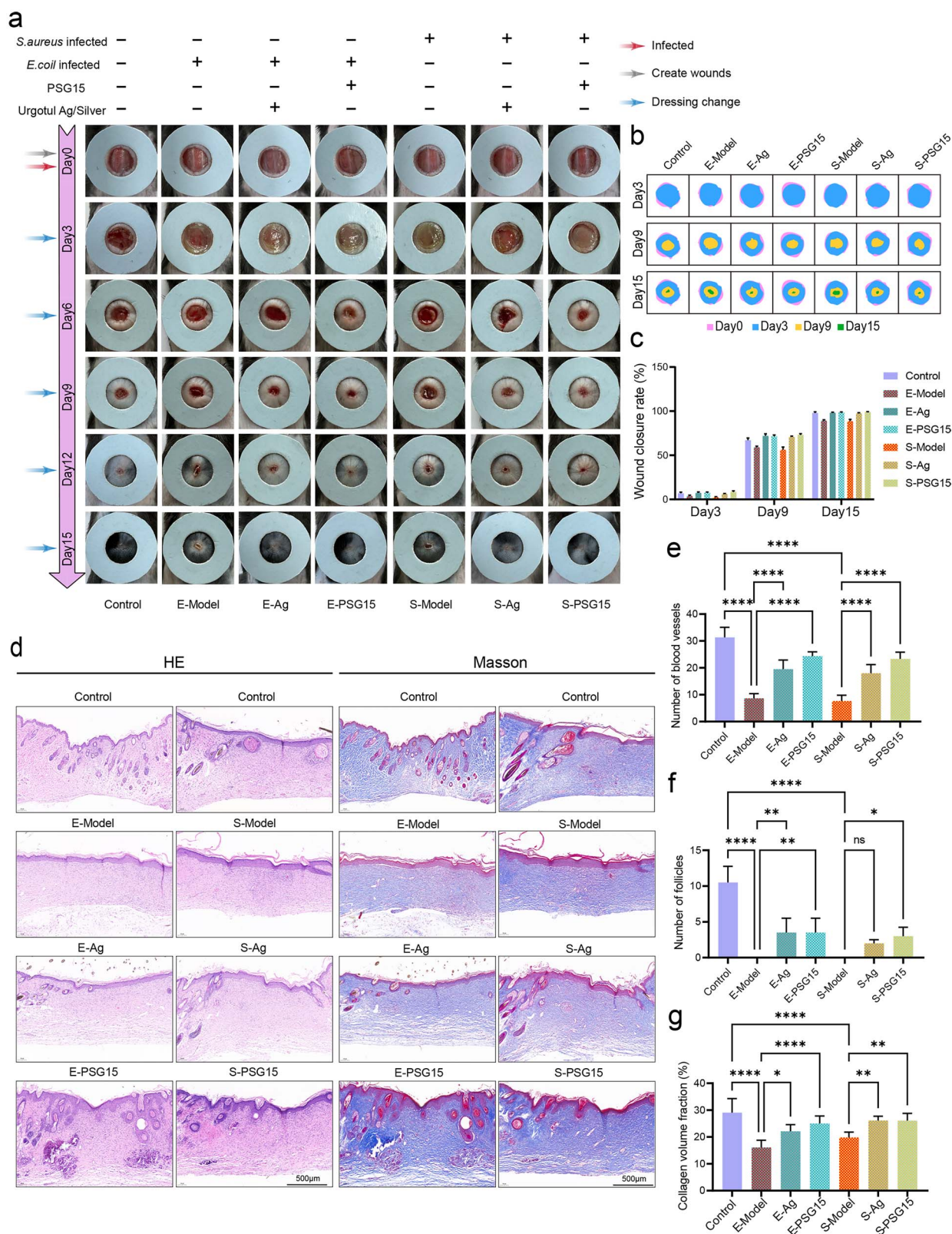


Figure 5. In vivo wound healing and histopathological analysis of infected wounds treated with PSG15 hydrogels. **(a)** Serial photographic images of full-thickness skin wounds infected with *E. coli* and *S. aureus* treated with the PSG15 hydrogel or a commercial product (Urgotul Ag/silver) document the healing process over time (n = 8). **(b-c)** Quantitative analysis of wound closure rates show accelerated healing in the PSG15-treated groups compared with the untreated groups (n = 8). **(d)** H&E and Masson's trichrome staining results on Day 15 show enhanced collagen deposition and new blood vessel formation in the PSG15-treated group (n = 8). **(e)** the formation of new blood vessels in different groups was analyzed (n = 8). **(f)** the number of follicles was quantified (n = 8). **(g)** Collagen density was analyzed (n = 8). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$, ***** $P < 0.00001$. E-model *E. coli* Model, E-Ag *E. coli*-Ag, E-PSG15 *E. coli*-PSG15, S-model *S. aureus* Model, S-Ag *S. aureus*-Ag, S-PSG15 *S. aureus*-PSG15

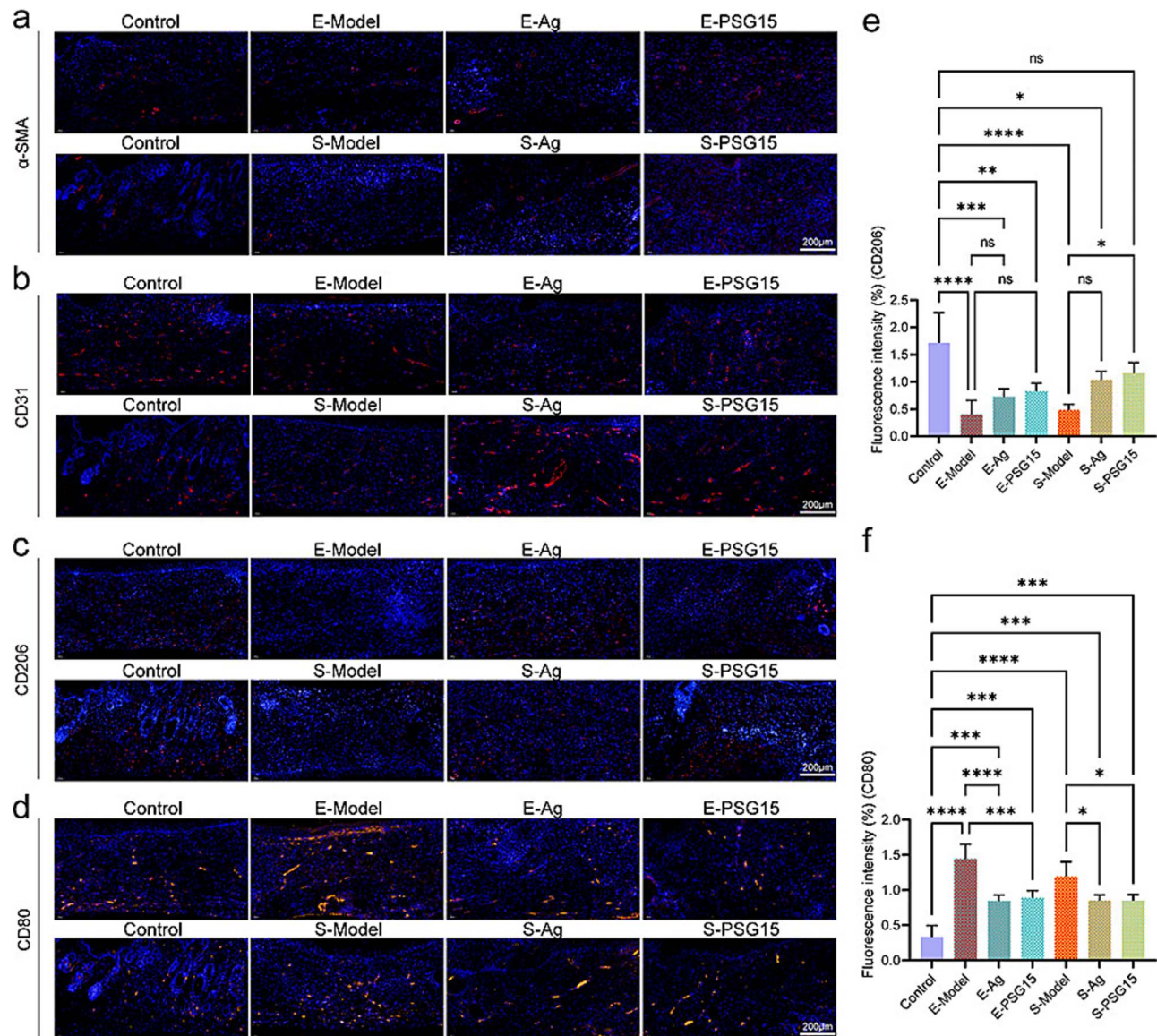


Figure 6. Immunohistochemical analysis of angiogenesis and macrophage polarization in wound tissues. (a-b) CD31 and α -SMA staining confirms enhanced angiogenesis in the PSG15 and Urgotul Ag/silver groups ($n=8$). (c-f) CD80 and CD206 staining indicates a shift from M1 to M2 macrophages in the PSG15-treated wounds, which suggests anti-inflammatory and prorepair effects ($n=8$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$, **** $P < 0.00001$. E-model *E. coli* model, E-Ag *E. coli*-Ag, E-PSG15 *E. coli*-PSG15, S-model *S. aureus* model, S-Ag *S. aureus*-Ag, S-PSG15 *S. aureus*-PSG15

control group. By Day 7, the microbiota diversity in the *E. coli* and *S. aureus* model groups gradually recovered, and by Day 14, no significant difference in diversity was observed between the groups (Figure 7a-b).

At the phylum level, the skin microbiota is primarily composed of Firmicutes and Bacteroidetes. On Days 3 and 7, a notable increase in Proteobacteria was seen in both the *E. coli* and *S. aureus* model groups, and this increase was more pronounced in the *E. coli* group (Figure 7c-d). In contrast, the commercial dressing and PSG15 significantly inhibited this increase. Using linear discriminant analysis (LDA) with an effect size of $|LDA| > 2$ and $P < .05$, we found that on Day 3, the abundance of *Escherichia* (from the family Enterobacteriaceae), which is closely related to *E. coli* infection, was significantly elevated in the *E. coli* model group. Similarly, *Staphylococcus* was significantly elevated in the *S. aureus* model group, which indicates infection by *S. aureus*. These

results confirmed the successful establishment of the infection models (Figure 7e-f).

Skin microbial diversity is important for preventing chronic infection and promoting wound healing [30]. We therefore used a heatmap to analyze the relative abundance of the top 10 bacterial genera that were significantly different on Day 3. The heatmap displays the decrease in bacterial abundance during the healing process of wounds infected with *E. coli* and *S. aureus*. These results revealed that infection with *E. coli* or *S. aureus* significantly reduced the diversity of the skin microbiota in the wound area, which led to an increase in the relative abundance of harmful bacteria (Figure 7g-h). The application of the PSG15 hydrogel markedly alleviated this effect by effectively inhibiting the proliferation of *E. coli* and *S. aureus* and maintaining the stability of the skin microbiota in the wound. These findings demonstrate the beneficial role of the PSG15 hydrogels not only in

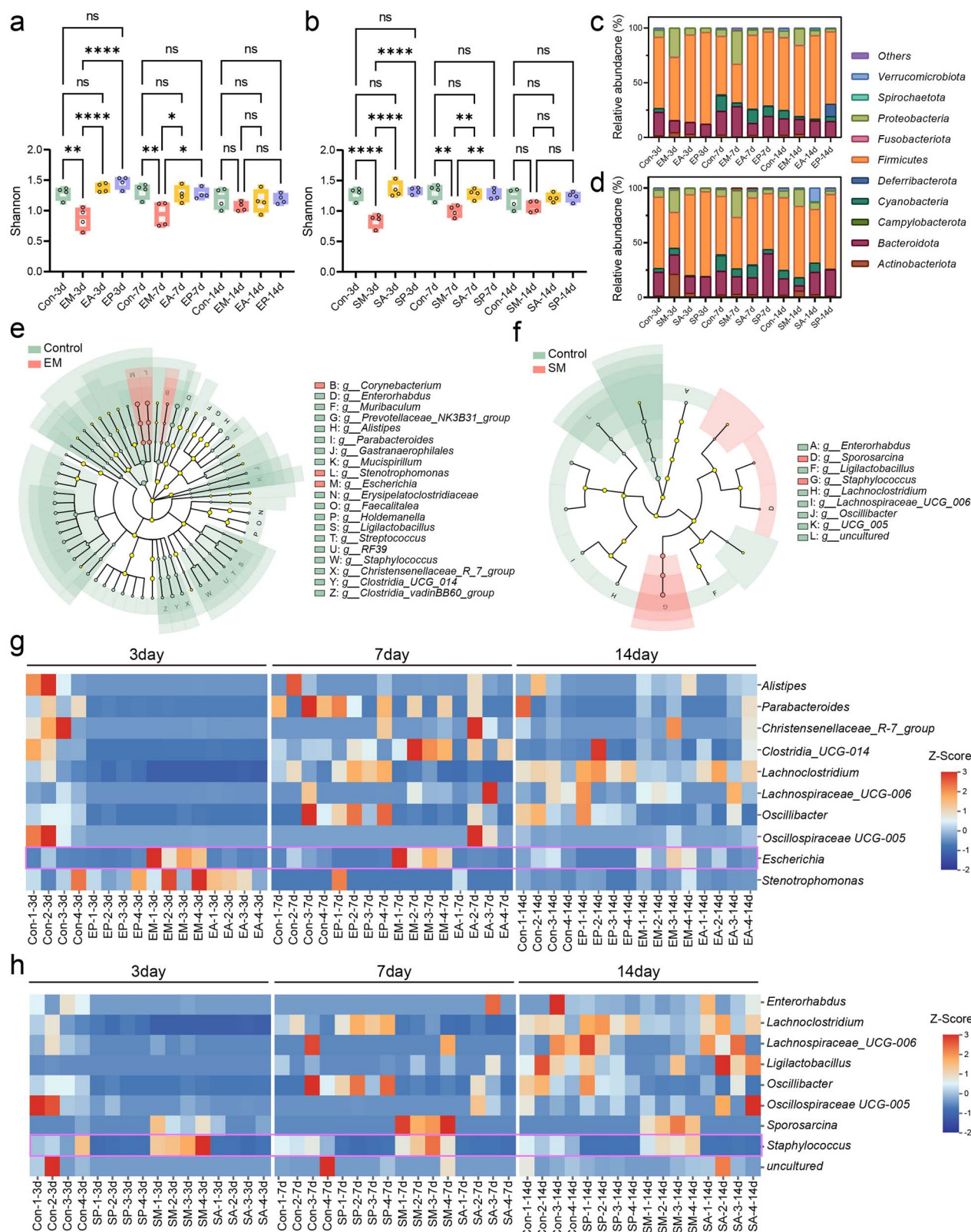


Figure 7. Dynamics of the skin microbiota during wound healing in the PSG15-treated and control groups. **(a-b)** Skin microbiota diversity over time shows significant recovery in the PSG15-treated group by Day 14 ($n = 6$). **(c-d)** Phylum-level composition analysis of the skin microbiota in wound tissues illustrates the dominant bacterial phyla and the impact of PSG15 treatment on the relative abundances of Proteobacteria, Firmicutes, and Bacteroidetes ($n = 6$). **(e-f)** LDA analysis performed on Day 3 identified elevated *Escherichia* and *Staphylococcus* in the *E. coli* and *S. aureus* model groups, respectively ($n = 6$). **(g-h)** Heatmap displays the reduction in pathogenic bacteria during wound healing in the PSG15 group compared with the model group ($n = 6$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$, ***** $P < 0.00001$. Con control, EM *E. coli* model, EA *E. coli*-Ag, EP *E. coli*-PSG15, SM *S. aureus* model, SA *S. aureus*-Ag, SP *S. aureus*-PSG15

suppressing pathogenic bacteria but also in preserving the balance of the wound microbiome, which positively impacts wound healing. The ϵ -PLL in PSG15 provides robust antibacterial activity against common wound pathogens (e.g. *E. coli* and *S. aureus*). By effectively disrupting bacterial membranes and reducing the overall bacterial load, PSG hydrogels minimize the risk of infection and prevent the onset of chronic inflammation. Moreover, by suppressing pathogenic bacteria, the hydrogel establishes a microenvironment that is conducive to the recovery and stability of the native skin microbiota. The moisture-retentive network stabilizes pH and hydration, which supports microbiome equilibrium. A balanced microbial community is essential for healthy wound healing [31] and can support tissue repair processes, such as angiogenesis and collagen deposition. However, further mechanisms should be explored. These findings underscore the dual action of PSG15 hydrogels in both infection control and microbiota stabilization and highlight their potential to enhance wound healing, particularly that of infected wounds.

Discussion

This study demonstrated the successful fabrication of ϵ -PLL-incorporated SA/Gel hydrogels with desirable characteristics for wound healing applications, including excellent antibacterial activity, cytocompatibility, and promotion of tissue regeneration. By integrating ϵ -PLL—a naturally derived antimicrobial polypeptide—into the SA/Gel matrix, the resulting hydrogel exhibits enhanced functional performance compared to conventional hydrogel dressings.

The introduction of ϵ -PLL significantly improved the antibacterial capacity of the SA/Gel hydrogel, as evidenced by *in vitro* inhibition of *E. coli* and *S. aureus* growth and healing promotion of wounds infected by *E. coli* and *S. aureus*. These findings are consistent with previous studies showing ϵ -PLL's potent broad-spectrum antimicrobial activity via membrane disruption mechanisms [32]. However, unlike pure ϵ -PLL coatings or films, which may suffer from burst release and short-lived activity [33], our PSG hydrogel provides a sustained-release matrix that maintains antibacterial effectiveness while minimizing cytotoxicity. Traditional antibacterial strategies often lead to microbial dysbiosis [34]. In this study, microbiota profiling via 16S rRNA sequencing revealed that PSG hydrogel not only reduced pathogenic colonization but also helped restore a more balanced wound microbial community, increasing the relative abundance of beneficial bacteria such as *Lactobacillus*. This aspect is less frequently reported in wound healing studies and represents a unique contribution of our work.

The cytocompatibility of PSG hydrogels was demonstrated through sustained cell viability and proliferation, indicating that the inclusion of ϵ -PLL does not compromise the biocompatibility of the SA/Gel base. This is in line with previous reports that ϵ -PLL, at appropriate concentrations, supports mammalian cell growth while exerting selective antimicrobial activity [35]. In a full-thickness infected wound model, the PSG hydrogels achieved significantly accelerated wound closure, enhanced granulation tissue formation, and more organized collagen deposition compared to controls. These regenerative effects may result from a combination of reduced bacterial burden, balanced inflammatory response, and improved local tissue environment. The synergistic incorporation of ϵ -PLL in our hydrogel provides both antimicrobial protection

and pro-regenerative properties, a combination that addresses a critical need in infected wound management.

Histological and immunohistochemical analyses indicated that PSG hydrogel significantly reduced inflammatory cell infiltration and downregulated pro-inflammatory markers, while promoting collagen maturation and re-epithelialization. These findings are consistent with the hypothesis that controlling infection at early stages reduces prolonged inflammation and facilitates tissue repair. While some ϵ -PLL-based materials have been studied for antibacterial action [36], few have demonstrated such clear immunomodulatory effects within a hydrogel matrix, highlighting the translational potential of our material.

This work highlights a promising strategy for the development of next-generation wound dressings by integrating natural bioactive molecules with biocompatible polymers. The PSG hydrogel addresses key challenges in wound care, including infection control, tissue regeneration, and microbiota balance. Compared to existing dressings such as silver- or iodine-based products, which may cause cytotoxicity and microbial resistance [37], our hydrogel offers a safer, multifunctional alternative that aligns with the principles of sustainable and personalized medicine. Future studies should explore the long-term safety of PSG hydrogels in larger animal models, evaluate their efficacy in chronic wounds (e.g. diabetic ulcers), and investigate the underlying molecular mechanisms of microbiota–host–hydrogel interactions.

Conclusions

In conclusion, the synthesized PSG gels, particularly the PSG15 hydrogel, demonstrated good mechanical properties, injectability, self-adhesive ability, biocompatibility, and excellent antibacterial properties, and thus they are promising candidates for the treatment of infected wounds. *In vitro* tests confirmed that the hydrogels were nontoxic and biocompatible. PSG15 hydrogels significantly accelerated the healing of infected wounds, which resulted in enhanced tissue regeneration, angiogenesis, and collagen deposition. This research provides a novel approach for the prevention and treatment of infected wounds.

Abbreviations

E. coli, *Escherichia coli*; *S. aureus*, *Staphylococcus aureus*; PSG, ϵ -PLL@SA/Gel; ϵ -PLL, ϵ -poly-L-lysine; SA, gelatin (Gel), sodium alginate; FDA, Food and Drug Administration; HSFs, Human skin fibroblasts; HaCaT, Human keratinocytes; SEM, Scanning electron microscopy; EDS, Energy-dispersive X-ray spectroscopy; FTIR, Fourier transform infrared spectroscopy; FBS, Fetal bovine serum; HE, Hematoxylin–eosin; SD, Standard deviation.

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Not applicable.

Authors' contributions

Xiaoran Zu (Conceptualization [equal], Project administration [equal], Software [equal], Supervision [equal], Writing—original draft [equal]), Yudi Han (Data curation [equal], Funding acquisition [equal],

Writing—review & editing [equal]), Yongqiang Zhou (Resources [equal], Software [equal], Supervision [equal]), Long Zhu (Investigation [equal], Methodology [equal], Supervision [equal], Validation [equal]), Yubai Chen (Investigation [equal], Methodology [equal], Supervision [equal], Visualization [equal]), Xi Lu (Investigation [equal], Methodology [equal], Supervision [equal], Validation [equal]), Chenxuan Yang (Formal analysis [equal], Supervision [equal], Validation [equal], Visualization [equal]), Xiaomin Hu (Data curation [equal], Formal analysis [equal]), Tengwen Zhang (Software [equal], Supervision [equal]), Ming Zhang (Data curation [equal], Methodology [equal]), Wei Zhou (Conceptualization [equal], Data curation [equal], Writing—review & editing [equal]), Chaoji Huangfu (Conceptualization [equal], Funding acquisition [equal], Project administration [equal], Writing—original draft [equal], Writing—review & editing [equal]), Yue Gao (Conceptualization [equal], Visualization [equal], Writing—review & editing [equal]), Yan Han (Conceptualization [equal], Writing—review & editing [equal]).

Supplementary data

Supplementary data are available at *Burns & Trauma* online.

Ethical approval and consent to participate

The experimental protocol was approved by the Ethics Committee of Animal Experiments of the Beijing Institute of Radiation Medicine (Approval number: LACUC-DWZX-2024-P606).

Consent for publication

Not applicable.

Conflict of interest: All authors declare that they have no conflicts of interest.

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Data availability

The data and material used to support the findings of this study are included within the manuscript.

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