

Accelerated Cutaneous Wound Healing Using an Injectable Teicoplanin-loaded PLGA-PEG-PLGA Thermogel Dressing

Xu Wei-Ke, Tang Jing-Yu, Yuan Zhang, Cai Cai-Yun, Chen Xiao-Bin, Cui Shu-Quan, Liu Peng, Yu Lin, Cai Kai-Yong, Ding Jian-Dong

Cite this article as:

Xu Wei-Ke, Tang Jing-Yu, Yuan Zhang, Cai Cai-Yun, Chen Xiao-Bin, Cui Shu-Quan, Liu Peng, Yu Lin, Cai Kai-Yong, Ding Jian-Dong. Accelerated Cutaneous Wound Healing Using an Injectable Teicoplanin-loaded PLGA-PEG-PLGA Thermogel Dressing[J]. *Chinese J. Polym. Sci.*, 2019, 37(6): 548-559. doi: 10.1007/s10118-019-2212-5

View online: <https://doi.org/10.1007/s10118-019-2212-5>

Articles you may be interested in

[Doxorubicin-loaded PLGA Microparticles with Internal Pores for Long-acting Release in Pulmonary Tumor Inhalation Treatment](#)

Chinese J. Polym. Sci. 2015, 33(7): 947 <https://doi.org/10.1007/s10118-015-1642-y>

[Controlled Release of Doxorubicin from Electrospun MWCNTs/PLGA Hybrid Nanofibers](#)

Chinese J. Polym. Sci. 2016, 34(9): 1047 <https://doi.org/10.1007/s10118-016-1827-z>

[Fabrication of Macroporous Protein-containing Films through the Reverse Emulsions Approach Featuring \$\beta\$ -Cyclodextrin-conjugated PEG-PLGA Copolymers](#)

Chinese J. Polym. Sci. 2016, 34(6): 730 <https://doi.org/10.1007/s10118-016-1789-1>

[超临界溶液浸渍法制备负载磺胺嘧啶银不对称壳聚糖膜伤口敷料研究](#)

Preparation of Drug-loaded Asymmetric Chitosan Films towards Wound Dressing Using Supercritical Solution Impregnation
高分子学报. 2014(6): 774 <https://doi.org/10.3724/SP.J.1105.2014.13363>

[PLGA-磷酸钙复合载药纳米粒的制备](#)

Fabrication of Drug Loaded Nanoparticles of PLGA/Calcium Phosphate Composite
高分子学报. 2016(10): 1383 <https://doi.org/10.11777/j.issn1000-3304.2016.16032>

[Lys调节PLGA纳米纤维的体外降解产物酸度的研究](#)

Effect of Lysine on Regulating Acidity of PLGA Nanofiber Degradation in vitro
高分子学报. 2014(5): 604 <https://doi.org/10.3724/SP.J.1105.2014.13328>

Accelerated Cutaneous Wound Healing Using an Injectable Teicoplanin-loaded PLGA-PEG-PLGA Thermogel Dressing

Wei-Ke Xu^a, Jing-Yu Tang^a, Zhang Yuan^b, Cai-Yun Cai^a, Xiao-Bin Chen^a, Shu-Quan Cui^a, Peng Liu^b, Lin Yu^{a*}, Kai-Yong Cai^b, and Jian-Dong Ding^a

^a State Key Laboratory of Molecular Engineering of Polymers, Department of Macromolecular Science, Fudan University, Shanghai 200438, China

^b Key Laboratory of Biorheological Science and Technology Ministry of Education, College of Bioengineering, Chongqing University, Chongqing 400044, China

Abstract Bacterial infection is a very troublesome issue in wound treatment, which stimulates exudate formation and severely delays the healing process. Herein, a thermogelling dressing system composed of two triblock copolymers of poly(D,L-lactic acid-co-glycolic acid)-*b*-poly(ethylene glycol)-*b*-poly(D,L-lactic acid-co-glycolic acid) (PLGA-PEG-PLGA) with different block lengths was developed to deliver teicoplanin (TPN), a glycopeptide antibiotic, for cutaneous wound repair. The TPN-loaded thermogel was a free-flowing sol at room temperature and formed a semi-solid gel at physiological temperature. *In vitro* studies demonstrated that the TPN-loaded thermogel system exhibited desired tissue adhesiveness and realized the sustained release of TPN in a fast-followed-slow manner for over three weeks. Furthermore, a full-thickness excision wound model in Sprague-Dawley (SD) rats was constructed to assess the efficacy of TPN-loaded thermogel formulation. Gross and histopathologic observations implied that treatment with the thermogel formulation reduced inflammation response, promoted disposition of collagen, enhanced angiogenesis, and accelerated wound closure and maturity of SD rats. The combination of the bioactivity of TPN and the acidic nature of the thermogel matrix was responsible for such an enhanced wound healing process. Consequently, the TPN-loaded PLGA-PEG-PLGA thermogel is a good candidate of wound dressing for full-thickness excision wound healing.

Keywords Wound dressing; PLGA-PEG-PLGA thermogel; Anti-infection; Teicoplanin; Sustained release

Citation: Xu, W. K.; Tang, J. Y.; Yuan, Z.; Cai, C. Y.; Chen, X. B.; Cui, S. Q.; Liu, P.; Yu, L.; Cai, K. Y.; Ding, J. D. Accelerated cutaneous wound healing using an injectable teicoplanin-loaded PLGA-PEG-PLGA thermogel dressing. *Chinese J. Polym. Sci.* 2019, 37, 548–559.

INTRODUCTION

Skin plays an important role in protecting the body from damage and microbial invasion. However, it often suffers different levels of defects owing to injuries, burns, or physical trauma.^[1,2] In some serious cases such as large full-thickness skin defect, it takes a long time to achieve a complete wounding repair. Generally speaking, the longer the time for a healing process, the worse the outcome obtained. Therefore, a variety of wound dressings that include but are not limited to rubber, foam, membrane, and electrospun nanofiber have been developed to accelerate wound closure, promote wound healing, and reduce scar formation.^[3–8]

Among them, hydrogel dressings have drawn much attention in view of their superior performance such as maintaining a moist wound bed, providing gas exchange, and absorbing wound fluids.^[9–15] In particular, injectable *in situ* forming hydrogel is a more desirable candidate of wound dressing and other biomedical applications.^[9,16–23] These materials, usually in low-viscous sol states before application, read-

ily entrap various drugs through simple physical mixing, and then fill irregular defects after administration, transform into hydrogels *in situ* and adhere to wounds.

As a representative type of injectable *in situ* forming hydrogels, thermogelling and biodegradable poly(D,L-lactic acid-co-glycolic acid)-*b*-poly(ethylene glycol)-*b*-poly(D,L-lactic acid-co-glycolic acid) (PLGA-PEG-PLGA) triblock copolymers have been extensively employed for drug delivery, tissue engineering, submucosal injection, and so on; they are soluble in water at low or room temperature and their solutions undergo sol-gel transitions upon heating.^[24–35] Besides, the degradation products of PLGA-PEG-PLGA hydrogel contain lactate and lactic acid, which could stimulate angiogenesis as well as aid proliferation and migration of keratinocytes and fibroblasts.^[36,37]

Nevertheless, the narrow composition window of PLGA-PEG-PLGA triblock copolymers to form a thermogel^[38,39] does not well meet the various demands of biomedical applications. Once out of the composition window scope, the polymers are just soluble or precipitated in water over the whole temperature range of application. To overcome this significant shortcoming and broaden the applicable window of relevant polymers, our group has developed a practical blending approach to achieve a thermogelling system with

* Corresponding author: E-mail yu_lin@fudan.edu.cn

Received November 17, 2018; Accepted December 11, 2018; Published online January 11, 2019

easy-to-modulate gel properties based on blending two PLGA-PEG-PLGA triblock copolymers with different PEG/PLGA ratios, in which one was water-soluble while the other precipitated in water.^[40–43] Meanwhile, both *in vitro* and *in vivo* tests confirmed that the PLGA-PEG-PLGA mixture thermogels had adjustable biodegradability and good biocompatibility.^[41] To date, the application of either single PLGA-PEG-PLGA thermogel or mixing system for wound repair has not been reported to the best of our knowledge.

In addition, an ideal wound dressing should have antimicrobial performance against the infection. In fact, bacterial infection is a serious problem in wound treatment, which not only stimulates exudate formation, but also severely delays the healing process, even may lead to sepsis, thus increasing the risk of amputation and mortality.^[37,44] By far, Gram-positive bacteria, particularly *Staphylococcus aureus* (*S. aureus*), are the predominant cause of skin infections.^[45,46] To resolve this issue, a common method is to incorporate antibiotics or antiseptics into wound dressings.^[47,48] Topical and controlled delivery of antibiotics may significantly enhance antibacterial efficacy while minimizing the risk of bacterial resistance as well as decreasing cytotoxic and systemic side effects.

As a glycopeptide antibiotic with a spectrum of activity similar to vancomycin, teicoplanin (TPN) has a better anti-infection effect against Gram-positive bacteria including methicillin-resistant *S. aureus* and *Enterococcus faecalis* but less toxic side effects.^[49–51] Unlike the discouraged use of vancomycin in prophylaxis, TPN has been widely utilized in the prophylaxis and treatment of serious infections of skin and soft tissue, osteomyelitis, sepsis, endocarditis, and so on.^[50–52] The actually used TPN is a mixture of several compounds with the same glycopeptide core but different hydrophobic side chains, as presented in Fig. 1(a).

In the present study, an injectable thermogel system was fabricated based on the blending approach, and the resultant hydrogel acted as both dressing and depot of the antibiotic for wound healing. Two PLGA-PEG-PLGA triblock copolymers with different behaviors in water were synthesized, and their mixtures with appropriate mixing ratios exhibited sol-gel transitions upon heating, as presented in Fig. 1(b). Then, the thermogel formulations containing TPN were prepared by dissolving TPN powder into the mixture aqueous solutions; their dynamic rheological properties, skin adhesiveness, *in vitro* release profiles, and antibacterial effects were detected. Finally, the *in vivo* wound healing efficacy of the TPN-loaded thermogel formulation was evaluated *via* gross observation and histopathologic examination in a full-thickness excision wound model in Sprague-Dawley (SD) rats, as illustrated in Fig. 1(c).

EXPERIMENTAL

Materials and Experimental Animals

Materials

Poly(ethylene glycol) (PEG) with molecular weight (MW) of 1000 and 1500, and stannous octoate ($\text{Sn}(\text{Oct})_2$, 95%) were purchased from Sigma-Aldrich. D,L-lactide (LA) and glycolide (GA) were products of Medzone Biotech Co., Ltd

(China). TPN was kindly supplied by Zhejiang Medicine Co., Ltd of Xinchang Pharmaceutical Factory (China). Chloral hydrate was bought from Aladdin. 4% Buffered paraformaldehyde was purchased from Boster, INC. (China).

Experimental animals

Male SD rats weighing (200 ± 20) g were purchased from Shanghai Institute of Pharmaceutical Industry. The animals were separately housed in different cages to avoid mutual interference in a light-controlled animal house (12 h light/dark cycle) with appropriate humidity and temperature, and were free to uptake food and water. All animal experimental procedures were in accordance with the protocols approved by the Ethics Evaluation Board of Fudan University.

Synthesis and Characterization of Triblock Copolymers

Two PLGA-PEG-PLGA triblock copolymers (copolymer A and copolymer B) with different PEG blocks (MW 1000 and 1500) and various PEG/PLGA ratios but a fixed LA/GA ratio (8/1, mol/mol) were synthesized by ring-opening copolymerization of LA and GA in the presence of PEG as the macro-initiator and stannous octoate as the catalyst. The detailed synthesis process refers to our previous works.^[40,43]

¹H-NMR spectra of the resultant triblock copolymers in CDCl_3 measured by a 400 MHz spectrometer (Bruker, AVANCE III) were used to determine their compositions and MWs. The values of molar mass dispersity (D_M) of the copolymers and their mixtures were further detected by gel permeation chromatography (GPC, Agilent1260) equipped with a differential refractometer as detector. Tetrahydrofuran (THF) and monodispersed polystyrenes (PS) were used as the eluent at a flow rate of 1.0 mL/min and the MW standards, respectively. The measurements were carried out at 35 °C.

Phase Diagram and Dynamic Rheological Measurement

The sol-gel transition temperature was determined *via* a vial inverting approach.^[39] First, polymer aqueous solutions with different concentrations were prepared and stored at 4 °C. The 2-mL vials containing 0.5 mL polymer solutions were immersed in a water bath and equilibrated at each temperature for 10 min. A gel state was determined if no visual flow was detected within 30 s upon inverting the vial. The measurements were performed from 20 °C to 55 °C with a temperature increment of 1 °C per step.

Rheological properties of the polymer aqueous solutions with or without drugs were measured by a dynamic rheometer (Kinexus, Malvern) equipped with a Peltier cone-plate (1°, 60 mm diameter, 0.03 mm gap). Samples were loaded and sealed with a thin layer of silicone oil to prevent water evaporation. The measurements were carried out at an oscillatory frequency of 10 rad/s and a heating rate of 0.5 °C/min from 15 °C to 45 °C.

Preparation of TPN-loaded Thermogel Systems

The copolymer mixture aqueous solution (25 wt%) was obtained by dissolving copolymer A and copolymer B with the pre-designed mixing ratios of weight in ultrapure water. The TPN-loaded thermogel systems at different drug loading amounts were prepared by adding TPN powder into the mixture aqueous solutions and then continuous stirring at 4 °C.

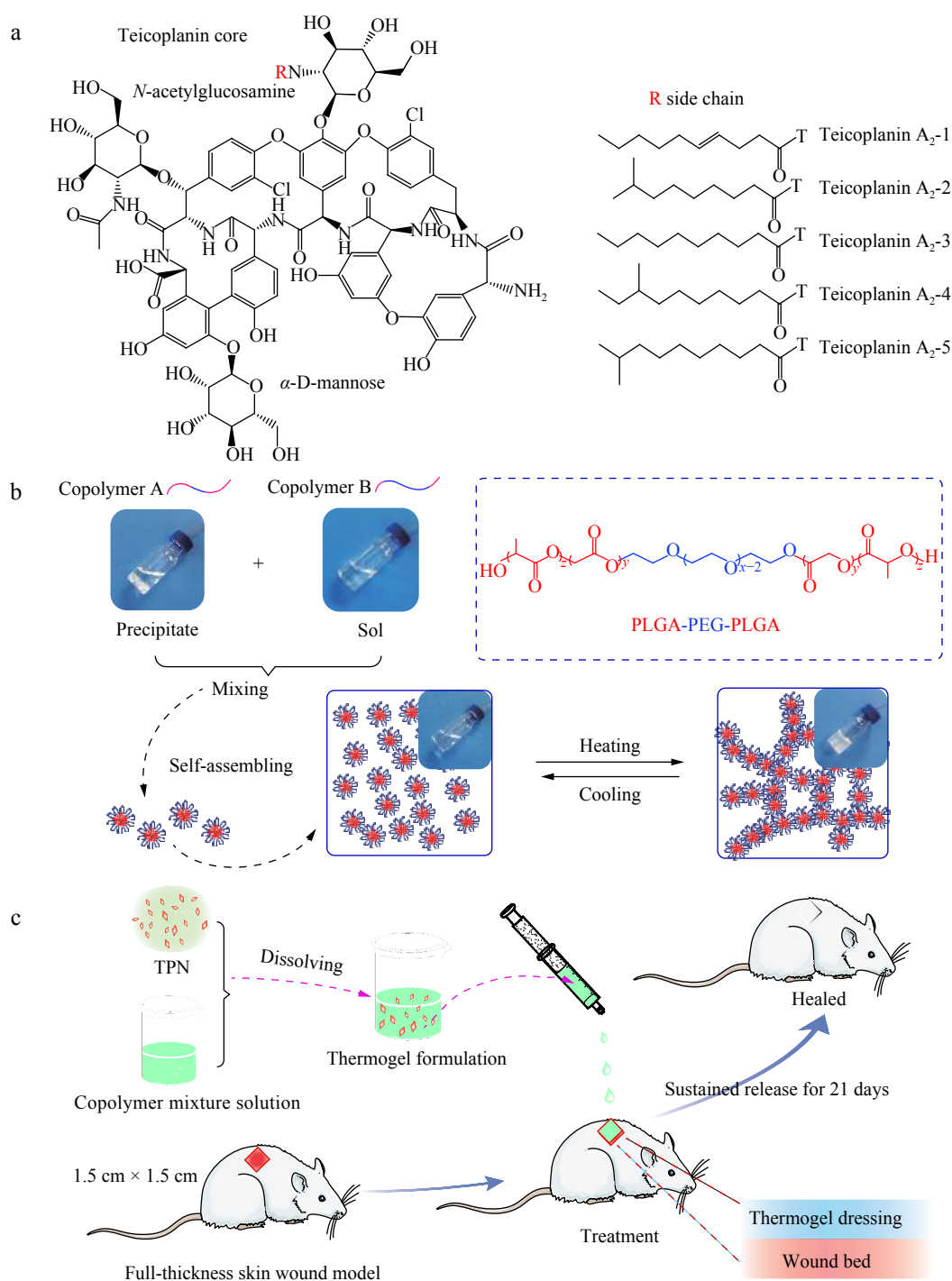


Fig. 1 (a) The molecular structure of TPN. “T” means teicoplanin core; (b) Schematic diagram presenting the fabrication of a thermogel system using the practical mixing approach. After mixing the insoluble copolymer A with the water-soluble copolymer B, the polymer mixture self-assembled into micelles in water. Upon heating, the mixture aqueous solution with a suspension of micelles turned into an *in situ* thermogel with a percolated micelle network; (c) Schematic diagram presenting the application of TPN-loaded thermogel formulation for wound healing. The TPN-loaded polymer solution spontaneously transformed into an *in situ* hydrogel at the wound site after administration.

***In Vitro* Release of TPN**

The mixture aqueous solutions (0.5 mL, 25 wt%) containing various amounts of TPN were transferred into 10-mL vials (internal diameter 17 mm), and the samples were then incubated in a water bath at 37 °C with a fixed shaking rate of 50 r/min for 10 min to form *in situ* hydrogels. Next, 5.0 mL

of phosphate buffered saline (PBS) solution containing 0.025% NaN₃ (pH 7.4, 37 °C) was added into the vial as the release medium. At predetermined time intervals, 1.0 mL of release medium was withdrawn and replaced immediately with the same volume of pre-warmed fresh PBS solution. The collected release media were stored at –20 °C until

analysis. The experiments were performed in triplicate. The amount of TPN in the released medium was determined by high performance liquid chromatography (HPLC, Waters e2695) with a UV-Vis detector (Waters 2489) and a reverse C-18 column (150 mm × 4.6 mm, particle size 5.0 μm, Sunfire Analysis column, YMC Inc.). The column temperature was kept at 25 °C. The mobile phase was water/acetonitrile (70/30, *V/V*) at a flow rate of 0.8 mL/min.

Evaluation of Antimicrobial Activity

The surface antimicrobial activities of the TPN-loaded thermogel systems against Gram-positive strain *S. aureus* (*Staphylococcus aureus*, ATCC 25923) and Gram-negative strain *E. coli* (*Escherichia coli*, ATCC 25922) were evaluated via Bauer-Kirby Disc Diffusion Test. Both *S. aureus* and *E. coli* bacterial strains were cultured in a Mueller-Hinton broth (MHB) medium. The bacteria suspension (200 μL, 10⁶ CFU/mL) was evenly bedded on different nutrient agar plates using a sterile glass-based bacterial coater. Then 20 μL of thermogel systems with or without TPN were added on different regions of the *S. aureus* or *E. coli* growth agar plate and incubated at 37 °C for 24 h. Blank thermogel was regarded as the control group. The inhibition zone was finally recorded by a CCD camera.

Besides, the antimicrobial activities of the TPN-loaded thermogel systems were also tested by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Typically, 200 μL of thermogel systems with or without TPN were added in 48-well plates. After the complete gelation, 500 μL of bacterial suspension in MHB medium (10⁶ CFU/mL) was transferred onto each hydrogel surface in 48-well plates. After incubation for different time periods (6 and 24 h), the viability of planktonic bacteria in medium was evaluated, respectively. Briefly, 180 μL of bacteria suspension and 20 μL of MTT solution (5 mg/mL) were co-incubated at 37 °C for 6 h. After forming formazan crystals, 200 μL of DMSO was employed to dissolve the crystals and the optical density (OD) was measured with a spectrophotometric microplate reader (BioRad 680, USA) at a wavelength of 490 nm.

Excision Wound SD Rat Model

A full-thickness excision wound model^[53] was constructed to estimate the efficacy of wound closure with different treatments. First, a 1.5 cm × 1.5 cm of full-thickness skin was excised on the back of rats. Then, the animals were randomly divided into four groups (5 rats per group): (1) control group (only treatment of normal saline (NS)); (2) TPN solution group (once every two days, 0.5 mL of 1.0 mg/mL TPN solution per time, 4 times in all); (3) blank gel group (25 wt% mixture-1 (copolymer A:copolymer B = 1:2, *W:W*) aqueous solution, 1.0 mL); (4) TPN-loaded thermogel formulation (25 wt% mixture-1 aqueous solution containing 2 mg/mL TPN, 1.0 mL).

The mixture-1 aqueous solution with or without TPN was filtered using a 0.22 μm syringe filter (Millex-LG, Millipore Co., USA) before use. To prevent the potential adhesion between the wound tissue and the cover material, the wound sites were open and no other cover materials were used. After the completion of different treatments, all the SD

rats were kept separately in independent cages to avoid mutual interference, and free to receive standard diet and drank water.

To evaluate the wound closure rate as a function of time, the wound area was copied by a filter paper on days 3, 7, 10, 14, 17, and 21 and then analyzed by Image-J software. The detailed process was as follows: first, a transparent filter paper was chosen to cover the wound carefully and the wound outline was delineated by a pen; next, the filter paper containing the wound outline was photographed under a ruler; finally the photograph with the ruler was uploaded on the Image-J software and the wound area was calculated by the Image-J software. Wound closure percentage was calculated according to the following equation:

$$\text{Wound closure percentage (\%)} = \frac{(A_0 - A_n)}{A_0} \times 100\%$$

here A_0 is the wound area on day 0 and A_n is the wound area on day n . Taking into consideration that an inflammatory response mainly occurred at the initial stage post-operation according to the previous contribution,^[36] one rat of each group was scarified on 10 days and the wounds of rats were harvested for histopathological examination. The remaining animals were scarified on 21 days and the harvested samples of wounds were used to evaluate the granulation tissue growth, the deposition of collagen fibers, and the vascular regeneration of skin in wound healing.

Histological and Immunohistochemical Analysis

The removed skins including epidermis, dermis, and hypodermis were fixed in 4% buffered paraformaldehyde and then embedded in paraffin for tissue section. The slice sections (4 μm in thickness) were stained with hematoxyline-eosin (H&E) and Masson's trichrome according to the standard protocols. The stained sections were photographed using an optical microscope (XSP-C204, CIC).

Based on the images of Masson's trichrome staining, the thickness of the epidermal and dermal layer was measured referring to the previous publication with modifications.^[54] In brief, the thicknesses of 10 sites which were designated every 250 μm were measured using the software CaseViewer. Epidermal or dermal thickness for each sample was obtained via calculating the average of the thickness.

The slice sections were immunostained by CD31 primary antibody to evaluate angiogenesis.^[55] The detailed operation process was according to the previous publication.^[56] Then, the CD31 stained sections were photographed using an optical microscope (XSP-C204, CIC).

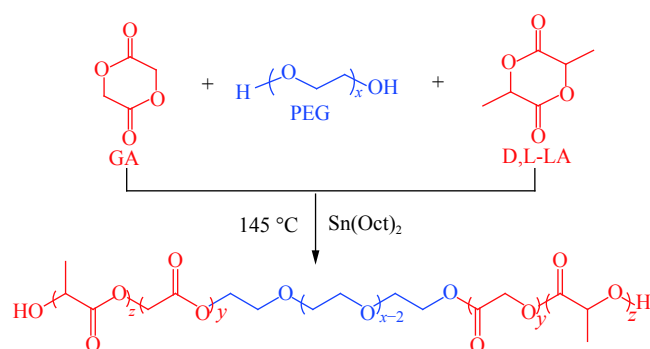
Statistical Analysis

All data were presented as mean ± standard deviation (SD). For group comparison, the statistical significance was analyzed by two-sample Student's *t*-test. A *p* value less than 0.05 was considered as statistical significance.

RESULTS AND DISCUSSION

Synthesis and Characterization of PLGA-PEG-PLGA Triblock Copolymers

As shown in Scheme 1, two PLGA-PEG-PLGA triblock copolymers were synthesized via a ring-opening copolymer-



Scheme 1 Synthesis route to PLGA-PEG-PLGA triblock copolymer

ization method. The compositions of the two PLGA-PEG-PLGA triblock copolymers were determined by $^1\text{H-NMR}$ measurements. Fig. 2 displays the $^1\text{H-NMR}$ spectrum of a PLGA-PEG-PLGA triblock copolymer. All the characteristic peaks were well assigned; the peak areas at 5.20 ($-\text{CH}(\text{CH}_3)\text{COO}-$ of LA, peak a), 4.80 ($-\text{CH}_2\text{COO}-$ of GA, peak c), and 3.60 ($-\text{CH}_2\text{CH}_2\text{O}-$ of EG, peak d) ppm were used to calculate the number-average MW of PLGA-PEG-PLGA triblock copolymers.^[31] The results showed that the two polymers had different block ratios of PEG/PLGA, but similar total MWs. Their MWs and $\bar{D}_\text{M}$ s were further confirmed by GPC analysis (Fig. 3). The two specimens exhibited a unimodal distribution in GPC traces, indicating that the desired polymers were well synthesized. Interestingly, their mixtures with varied mixing ratios of weight (2:1, 1:1, and 1:2) also presented a unimodal manner and their $\bar{D}_\text{M}$ s just located between the two polymers, as presented in Fig. 3. The detailed data of molecular parameters of the two polymers and their indicated mixtures are listed in the Table 1.

Sol-Gel Transition of Copolymer Mixture Aqueous Solutions

The two specimens synthesized exhibited entirely different behaviors in water: copolymer A was too hydrophobic to be dissolved in water, while copolymer B could be easily dissolved. Neither of them could form an *in situ* physical hydrogel at any concentration or temperature. However, their mixtures with appropriate mixing ratios were soluble in water at ambient temperature and transformed into semi-solid thermogels with an increase of temperature. The phase diagrams of the indicated mixture/water systems were determined *via* the vial inverting method. As shown in Fig. 4, the mixture/water systems underwent a sol-gel transition as

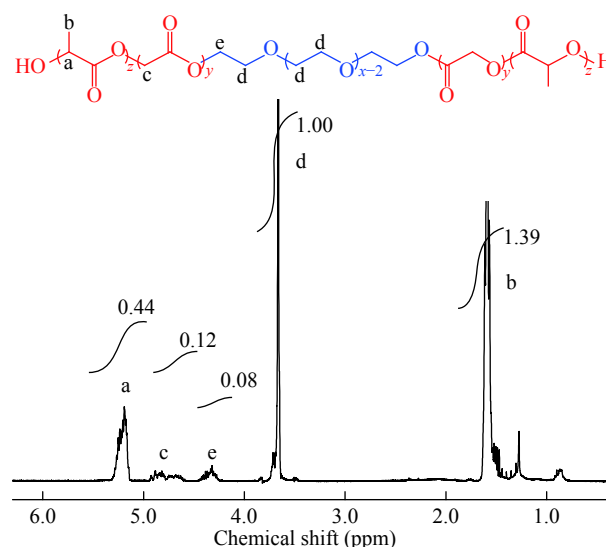


Fig. 2 $^1\text{H-NMR}$ spectrum of PLGA-PEG-PLGA triblock copolymer (Copolymer A)

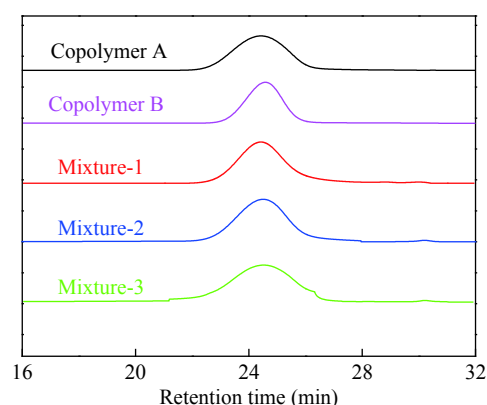


Fig. 3 GPC traces of PLGA-PEG-PLGA triblock polymers and their mixtures with varied mixing ratios of weight

the temperature increased. With the further increasing temperature, a gel-sol (suspension) transition was observed and this transition temperature was significantly dependent on the polymer concentration compared with that of sol-gel transition. Meanwhile, the mixing ratios of the initial two polymers significantly affected their phase transition behaviors. The critical gel concentration (CGC) as well as the sol-gel transition temperature decreased with the increasing mixing ratio. An increase in the total hydrophobicity of mixture/water system due to a higher content of copolymer A was responsible for the decreased CGC and lowered sol-gel transition temperature.

Table 1 Molecular parameters of PLGA-PEG-PLGA copolymers and their mixtures in this study

Sample	Mixing ratio ^c	PEG M_n ^d	M_n ^d	LA/GA ^d (mol/mol)	M_n ^c	$\bar{D}_\text{M}$ ^c
Copolymer A ^a	—	1000	1530-1000-1530	8.0	4270	1.27
Copolymer B ^b	—	1500	1380-1500-1380	8.0	4340	1.14
Mixture-1	1:2	—	—	8.0	4340	1.23
Mixture-2	1:1	—	—	8.0	4210	1.24
Mixture-3	2:1	—	—	8.0	4290	1.25

^a Insoluble in water; ^b Dissolved in water as a sol in a broad temperature region; ^c Mixing ratio by weight of copolymer A and copolymer B; the mixtures exhibited a temperature-induced sol-gel transition in water as the temperature increased; ^d Number-averaged MW, M_n of the central block PEG was provided by Aldrich. Molar ratio of lactide/glycolide (LA/GA) and M_n of each PLGA block were calculated by $^1\text{H-NMR}$ spectra; ^e Confirmed by GPC

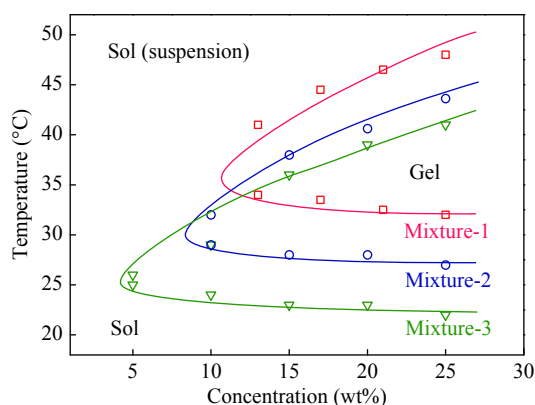


Fig. 4 Phase diagrams of the indicated mixture/water systems. The transition temperature was obtained from the vial inverting method.

Further, the changes in modulus (G') and viscosity (η) of aqueous solutions of the copolymer mixtures with fixed mixing ratios as a function of temperature were monitored *via* dynamic rheological measurements. As shown in Fig. 5(a), G' of the mixture/water systems was very low at low or room temperature, indicating that they were in free-flowing sol states. As the temperature increased, G' increased 4 orders of magnitude rapidly, reflecting the occurrence of *in situ* gelation. As the mixing ratio of the two polymers varied from 1:2, 1:1, to 2:1, the sol-gel transition temperature decreased while the corresponding maximum of G' increased. This finding was consistent with the results obtained from the vi-

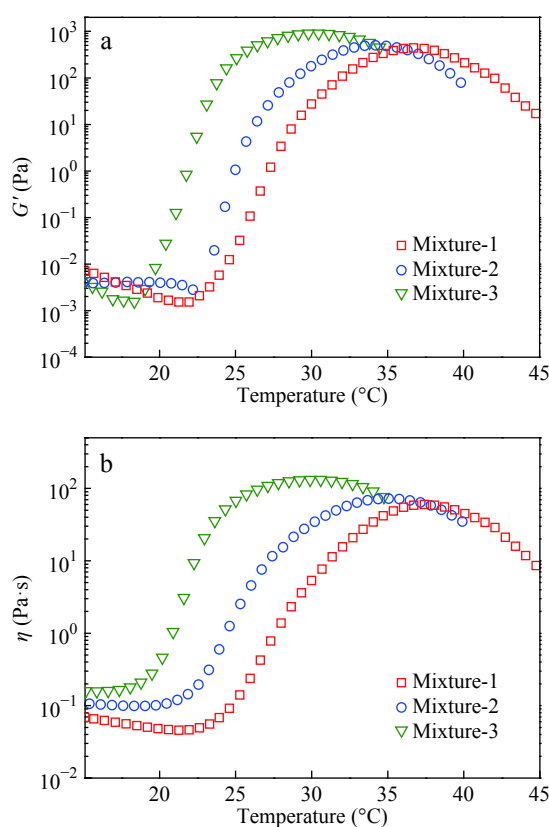


Fig. 5 (a) Storage modulus (G') and (b) viscosity (η) of the mixture aqueous solutions with fixed mixing ratios as a function of temperature

al inverting method. η of the mixture/water systems exhibited a similar variation tendency as a function of temperature (Fig. 5b).

Temperature-induced Gelling Behavior of Mixture-1 Aqueous Solutions with or without Drugs

The TPN-loaded thermogel systems were conveniently prepared by dissolving TPN in the mixture aqueous solution under continuous stirring at 4 °C and the drug encapsulation efficiency was almost 100% in view of little mass loss during such a loading process. Moreover, the rheological tests demonstrated that no significant change in the sol-gel transition was observed before and after the introduction of various amounts of TPN into the mixture-1 aqueous solution (Fig. 6). This feature indicated that the loading of TPN hardly impacted the injectability and temperature-induced gelation of the mixture-1/water system.

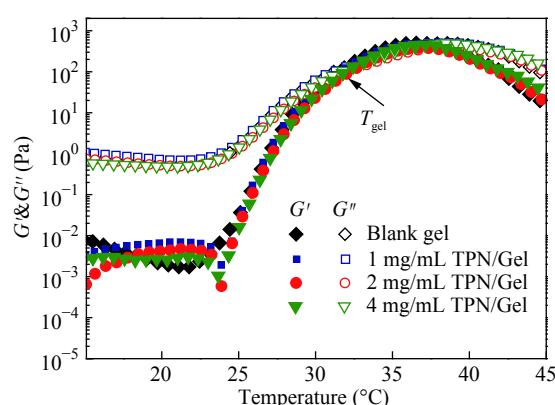


Fig. 6 G' and G'' of mixture-1/water systems with or without TPN as a function of temperature

In Vitro Drug Release

The *in vitro* release profiles of TPN-loaded thermogel systems with various polymer mixing ratios were first evaluated. As shown in Fig. 7(a), an initial burst release on the first day was observed, and subsequently the TPN-loaded thermogel systems exhibited a sustained drug release manner for over three weeks. The mixing ratio had a significant effect on the drug release rate. With changing mixing ratio from 1/2 to 2/1, the release rate of TPN decreased in turn. This feature was attributed to the enhanced mechanical strength of thermogel matrix with an increasing content of copolymer A. It is worth pointing out that a large part of TPN could not be released even up to three weeks. In fact, TPN, as an amphiphilic molecule, contains both hydrophilic groups and hydrophobic groups, such as a hydrophobic fatty acid side chain, as shown Fig. 1(a). The hydrophobic fatty acid and other hydrophobic segments could interact with amphiphilic PLGA-PEG-PLGA polymers. The hydrophobic interaction between TPN and carrier polymer inhibited the drug diffusion from the hydrogel matrix, resulting in an incomplete release of TPN within the three-week examined period. However, we believe that the remaining TPN could be completely released after the disintegration of PLGA-PEG-PLGA hydrogel matrix due to the sustained hydrolysis of PLGA segments.

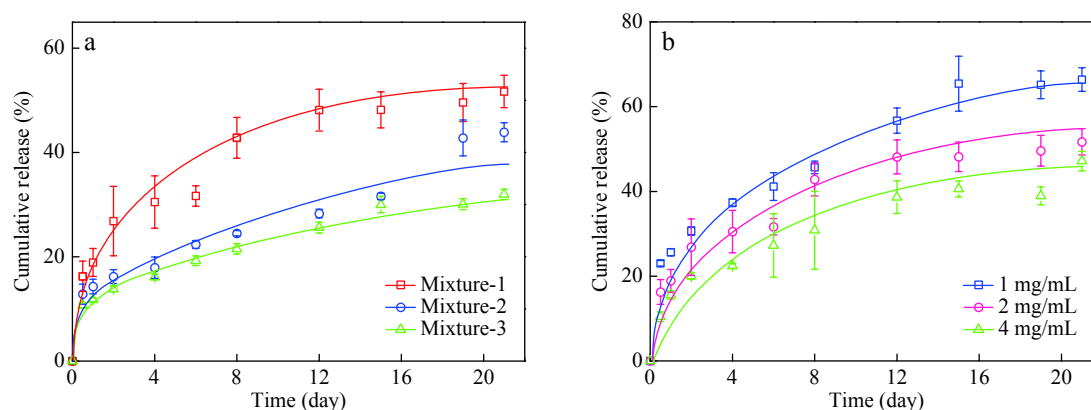


Fig. 7 (a) *In vitro* release profiles of TPN-loaded mixture thermogels with the indicated mixing ratios in PBS at 37 °C. Polymer concentration was 25 wt% and TPN concentration was 2 mg/mL. (b) *In vitro* release profiles of TPN-loaded mixture-1 thermogels at various drug loading amounts in PBS at 37 °C. Polymer concentration was 25 wt%.

We further evaluated the effect of drug loading amount on the release profiles of mixture-1 thermogels containing TPN. As presented in Fig. 7(b), both the initial burst amount and the cumulative release amount relied on the TPN concentration. With increasing drug concentration from 1 mg/mL to 4 mg/mL, the initial burst on the first day decreased from 25.7% to 15.6% and the cumulative release amount lowered from 66.4% to 41.2% within three weeks. Actually, drug release mainly happened in the first week. On 8 days, TPN cumulative release of the TPN-loaded mixture-1 thermogel systems with varying drug concentration from 1 mg/mL to 4 mg/mL reached 45.6%, 42.8%, and 30.8% of TPN, respectively.

As is well known, infection prolongs the inflammatory process and thus delays wound healing. In general, due to the disruption of the skin barrier, microbial invasion to wounds readily occurs in the initial one week post-injury.^[36,57] Therefore, the first week after skin injuries constitutes the most sensitive period of wound infection. It is obvious that the drug release profile of the mixture-1 thermogel system containing TPN well matched with such a process. Moreover, the sustained release of TPN with a relatively low drug dose at the late stage also benefited the prevention of wound infection at the other healing stages.

Meanwhile, the release data of TPN from the thermogel systems at various polymer mixing ratios and drug concentrations were fitted via Higuchi equation $Q = kt^{1/2}$ ($Q < 0.6$), where Q is the cumulative release amount, t is release time, and k is a constant. As is well known, Higuchi equation indicates a diffusion-controlled mechanism.^[26] As illustrated in Table 2, the fitted results basically matched with Higuchi equation, suggesting that the diffusion-controlled mechanism governed the release of TPN.

Table 2 Kinetic assessment of the *in vitro* release data of TPN from the thermogel systems at various mixing ratios or drug concentrations via Higuchi equation

Carrier polymer	Drug concentration	k	R^2
Mixture-1	2 mg/mL	0.11	0.94
Mixture-2	2 mg/mL	0.12	0.93
Mixture-3	2 mg/mL	0.17	0.98
Mixture-1	1 mg/mL	0.13	0.96
Mixture-1	4 mg/mL	0.09	0.97

Antimicrobial Activity

The antimicrobial activities of the TPN-loaded mixture-1 thermogel systems against both *S. aureus* (Gram-positive bacterium) and *E. coli* (Gram-negative bacterium) were investigated using the disc diffusion approach. As shown in Fig. 8(a), the clear bacterial inhibition rings against *S. aureus* were observed on the TPN-loaded thermogel systems at various drug concentrations, while the bacterial inhibition rings against *E. coli* were obviously smaller than the former.

Quantitative MTT analysis further revealed that the TPN-loaded thermogel systems could maintain the long-term antimicrobial efficacy against *S. aureus* due to the sustained release of TPN (Fig. 8b). Interestingly, the administration of a blank thermogel showed a certain antimicrobial activity against both *S. aureus* and *E. coli*. This finding was assigned to the acidic nature of the thermogel matrix ($\text{pH} \approx 2.8$) since the acidic environment is beneficial to inhibiting bacterial growth.^[58,59] As is well known, TPN is only active against Gram-positive aerobic and anaerobic bacteria.^[50,52] As a result, similar antimicrobial effects against *E. coli* were detected in both the blank thermogel and the TPN-loaded thermogel groups (Fig. 8b).

Wound Healing Effect of TPN-loaded Thermogel in an Excise Wound Model

Before the *in vivo* application, the tissue adhesiveness of mixture-1 thermogel itself was first investigated. As presented in Fig. 9, when applied on the skin, the TPN-loaded mixture-1 aqueous solution rapidly turned into an *in situ* hydrogel due to contacting body heat and well adhered to the skin, indicating that the thermogel matrix had a good tissue adhesiveness, which is beneficial to wound closure. The good tissue adhesiveness of mixture-1 hydrogel system was attributed to its high viscosity at body temperature, as presented in Fig. 5(b).

We next evaluated the efficacy of TPN-loaded mixture-1 thermogel system (2 mg/mL) in the healing process of full-thickness wounds in SD rats. A 1.5 cm $\times$ 1.5 cm of full-thickness skin wound was established on the dorsal middle of the animals, followed by applying different treatments. The progress of wound closure for all groups as a function of time was monitored and the planetary photo-

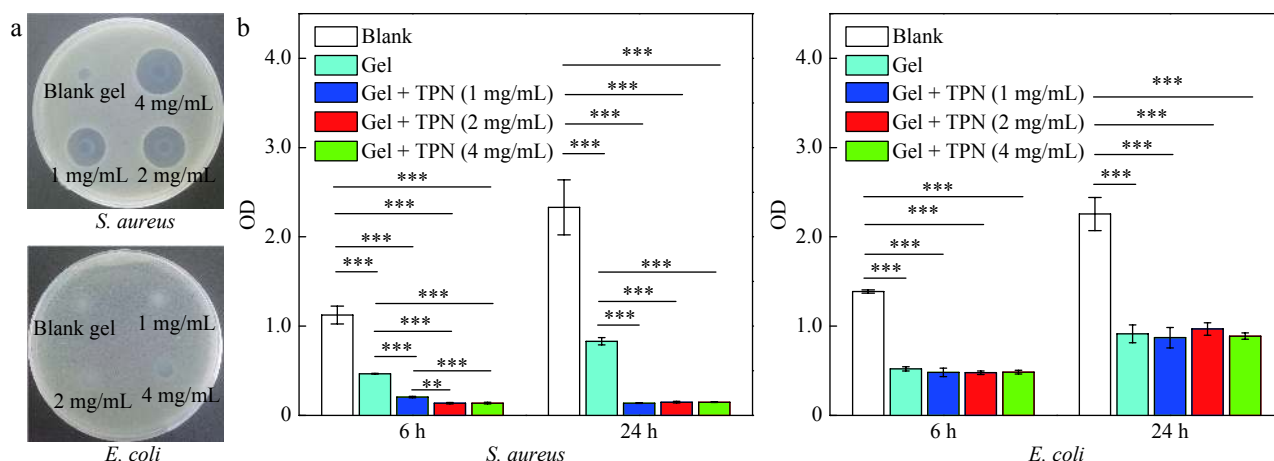


Fig. 8 (a) The bacterial inhibition rings against both *S. aureus* and *E. coli* on agar plates after contacting with the TPN-loaded mixture-1 thermogel systems at the indicated drug concentrations for 24 h; (b) The antimicrobial activity of the TPN-loaded mixture-1 thermogel systems with the indicated drug concentrations against both *S. aureus* and *E. coli* at two different treatment time periods. Significant differences among all the groups are specifically marked: ** $p < 0.01$; *** $p < 0.001$.



Fig. 9 Dermal adhesiveness of prepared mixture-1 thermogel system containing 2 mg/mL TPN

graphs were taken on 3, 7, 10, 14, 17, and 21 days (Fig. 10a). Overall, the animals receiving the treatment of TPN-loaded thermogel formulation exhibited the fastest wound closure rate, while the NS group showed the slowest wound closure rate. Meanwhile, the differences in the wound closure rate, which was reflected by the reduction percentage of wound area as a function of time, were further analyzed quantitatively.

As shown in Fig. 10(b), the wound closure rate showed little difference among the four groups in the initial three days. However, significant differences between the group of thermogel formulation ($(41.3 \pm 13.5)\%$ on 7 days, $p < 0.05$; $(77.3 \pm 2.4)\%$ on 10 days, $p < 0.05$; $(90.4 \pm 1.5)\%$ on 14 days, $p < 0.01$; $(96.2 \pm 1.5)\%$ on 17 days, $p < 0.01$) and the NS group ($(24.4 \pm 18.6)\%$ on 7 days; $(69.6 \pm 5.8)\%$ on 10 days; $(83.6 \pm 2.9)\%$ on 14 days; $(89.5 \pm 3.1)\%$ on 17 days) were observed from day 7 to day 17. The feature suggested that the treatment of TPN-loaded thermogel formulation accelerated the wound healing. Also, the blank thermogel-treated animals showed faster wound closure ($(45.1 \pm 14.4)\%$ on 7 days, $p < 0.05$; $(94.3 \pm 2.3)\%$ on 17 days, $p < 0.05$) than the NS group did ($(24.4 \pm 18.6)\%$ on 7 days; $(89.5 \pm 3.1)\%$ on 17 days) on 7 days and on 17 days. On 21 days, there were no statistical differences among the four groups due to the basic recovery of wounds in all groups.

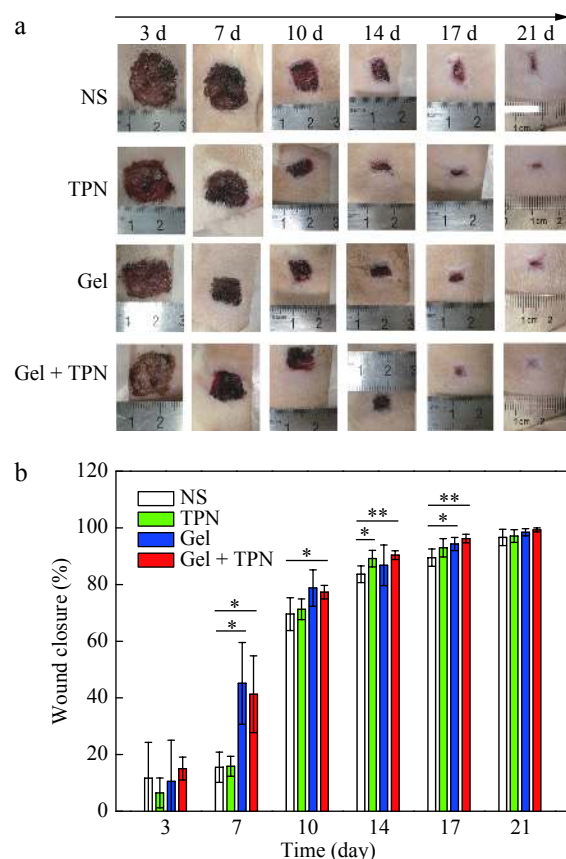


Fig. 10 (a) Optical photographs of skin wounds treated with different formulations as a function of treatment time in an excision wound SD rat model; (b) Closure percentages of skin wounds treated with different formulations as a function of treatment time in an excision wound SD rat model. Significant differences between the group of NS and the other three groups are specifically marked: * $p < 0.05$; ** $p < 0.01$. The NS group only received the treatment of NS; the TPN group received the treatment of TPN solution once every two days and the dose was 0.5 mL of 1.0 mg/mL TPN solution per time, 4 times in all; the Gel group received the single treatment of 1.0 mL of 25 wt% mixture-1 aqueous solution; the Gel + TPN group received the single treatment of 1.0 mL of 25 wt% mixture-1 aqueous solution containing 2 mg/mL TPN.

HE Staining and Masson's Trichrome Staining

Histopathological evaluation on wound regeneration was performed first by HE staining. As shown in Fig. 11(a), on day 10 post-injury, a significantly enhanced infiltration of inflammatory cells was observed in the NS group, while there were only a small number of inflammatory cells both in the TPN and thermogel formulation groups, suggesting that both the multiple administrations of TPN solution and the sustained release of TPN from the thermogel matrix remarkably suppressed inflammatory cells infiltration. Mean-

while, much more fibroblasts migrated into the impaired region of animals receiving the treatment of the thermogel formulation, indicating an accelerated re-epithelization and formation of granulation tissue. Interestingly, the blank thermogel-treated group also showed reduced inflammatory response compared with the NS group.

On day 21 post-wounding, obvious inflammatory response was still detected in both the NS and thermogel groups, as presented in Fig. 11(b). Meanwhile, the healed wounds were just at the stage of developing granulation tissue in the NS,

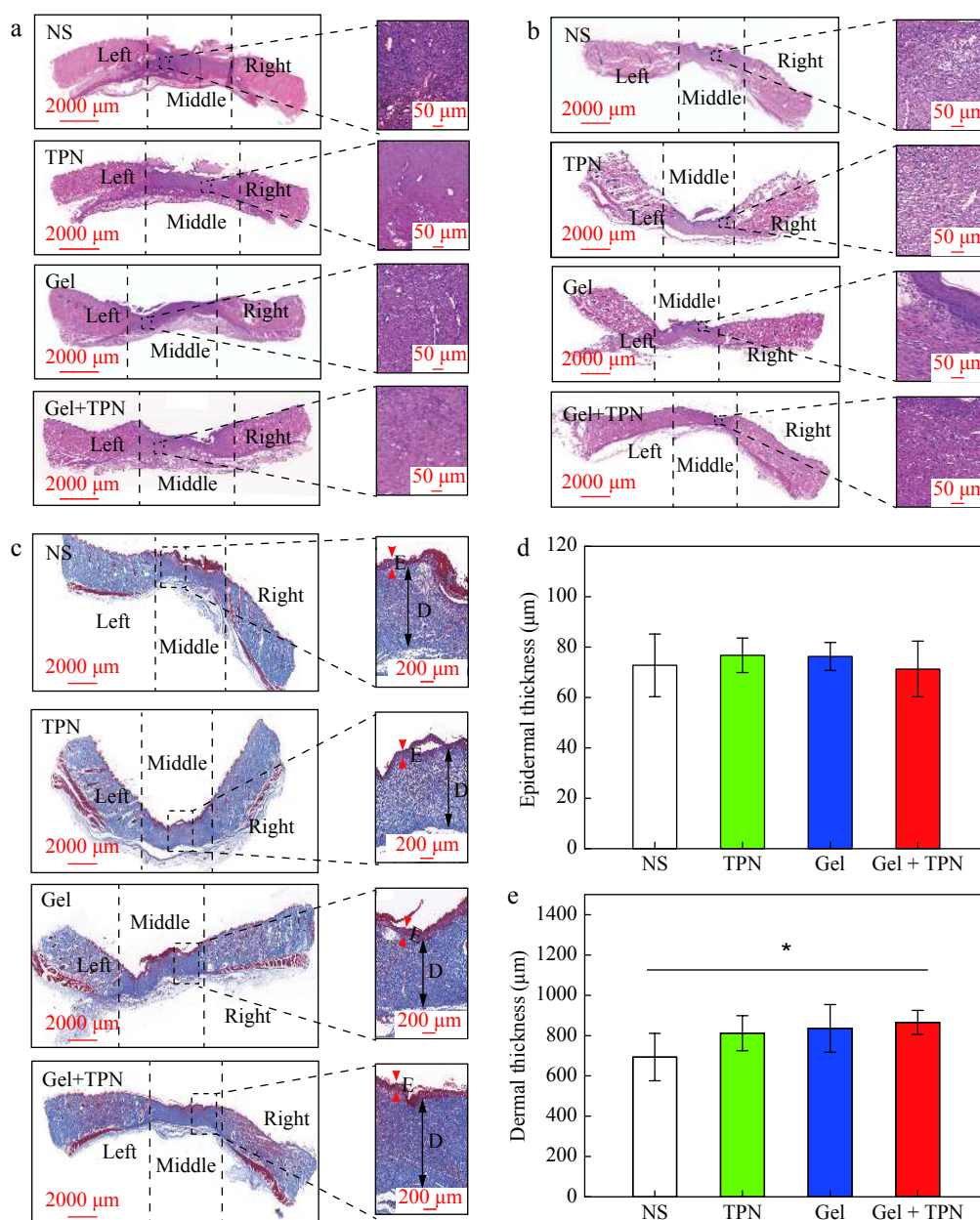


Fig. 11 (a) Representative images of HE staining of skin wounds showing the inflammatory response in different groups on day 10 post-injury. Left and Right represent the normal tissues, Middle represents the wounds. (b) Representative images of HE staining of regenerated skin in different Groups on day 21 post-injury. Left and Right represent the normal tissues, Middle represents the healed tissue. (c) Representative images of Masson's trichrome staining of regenerated skin for evaluating the deposition of collagen fibers in different groups on day 21 post-injury. Left and Right represent the normal tissues, Middle represents the healed tissue. Red and black arrows show the epidermis and dermis layers, respectively. Quantification of (d) epidermal and (e) dermal layer thickness on day 21 post-injury in 10 different locations per slide.

TPN, and thermogel groups. Different from the three groups, the TPN-loaded thermogel-treated wounds developed a relatively mature connective tissue and/or scar with significant fibroblastic deposition. This feature indicated that the administration of the thermogel formulation and subsequent sustained release of TPN promoted wound healing process.

For further evaluation of the quality and maturity of the healed skin, Masson's trichrome staining analysis for collagen evaluation was also carried out. After three weeks of treatment, the healed skin seemed to be intact, yet the regenerated tissue underneath was still vulnerable to damage due to the low tensile strength. It is well known that the increased deposition of collagen fibers improves the wound strength.^[36] As presented in Fig. 11(c), the healed tissue receiving the treatment of TPN-loaded thermogel formulation exhibited densely bunched collagen fibers with parallel arrangement. By contrast, deposited collagen fibers in the healed tissues were loose and irregularly arranged in the NS and TPN groups. Besides, relatively densely packed collagen fibers were observed in the blank thermogel group compared with the NS and TPN groups. This finding might be attributed to the acidic environment of thermogel matrix accompanying the production of lactate or lactic acid through the hydrolysis of ester bonds in PLGA segments.^[41]

The previous study has demonstrated that lactate can adjust the concentration of various enzymes involved in cell energy metabolism and transcription, and collagen synthesis can be enhanced as lactate levels increase within the wound.^[36] Hence, despite the insignificant differences between apparent wound closure among the four groups on day 21 post-wounding, the wounds treated with TPN-loaded thermogel formulation exhibited higher tissue maturity due

to the acidic nature of thermogel matrix and the sustained release of TPN.

Finally, the epidermal and dermal layer thickness was quantified after the three-week treatment. As shown in Fig. 11(d), there were no statistical differences among the various treatments regarding epidermis thickness, while the treatment of the TPN-loaded thermogel formulation enhanced dermal regeneration with a thicker dermis compared with NS-treated wounds.

CD31 Immunohistochemical Analysis

CD31 immunohistochemical staining is extensively utilized for marking angiogenesis at the molecular level.^[56] Fig. 12 shows that more CD31-positive capillaries were observed in the wounds treated with thermogel or TPN-loaded thermogel formulation compared with NS or TPN-treated wounds, indicating the increased wound vasculature. A previous study has revealed that lactic acidosis is beneficial for angiogenesis.^[36] Therefore, we speculated that the increased angiogenesis in the blank thermogel and thermogel formulation groups was attributed to the acidic nature of the thermogel matrix and the generated lactates and lactic acids following its degradation.

CONCLUSIONS

In this study, we demonstrated that the TPN-loaded thermogel system as a wound dressing promoted the wound healing process in a full-thickness skin defect model. Two PLGA-PEG-PLGA triblock copolymers with varying PEG/PLGA block ratios were synthesized and used to construct the blend thermogels with adjustable gelation performance. The TPN-loaded thermogel formulations that were conveniently prepared by dissolving TPN powder into the co-

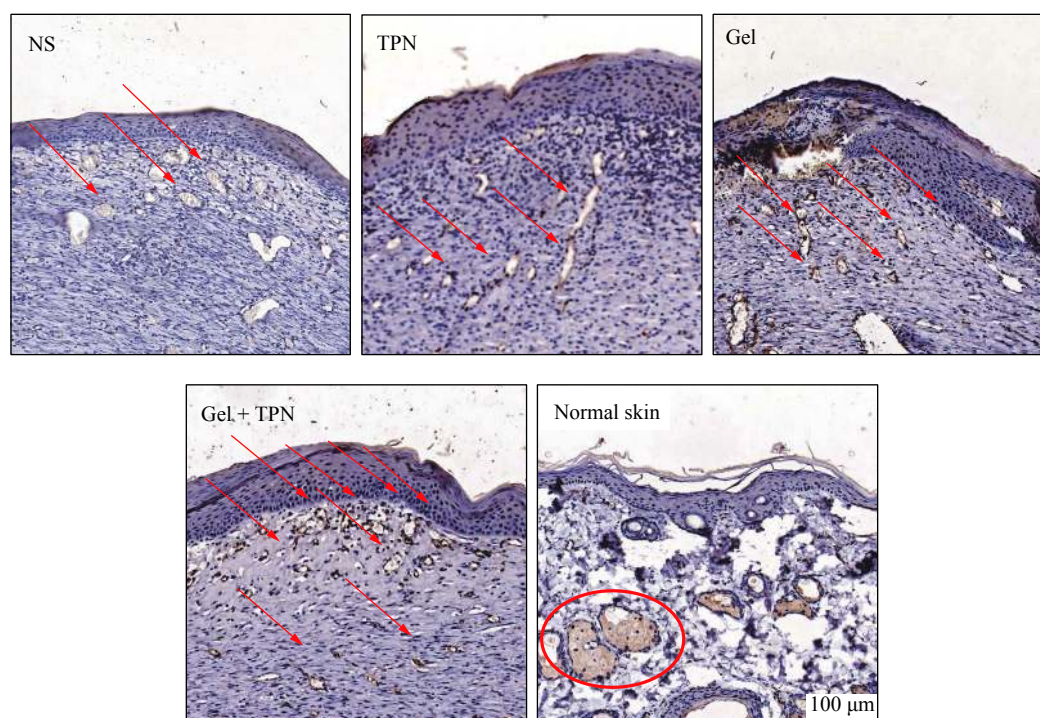


Fig. 12 Representative immunohistochemistry images of CD31 of regenerated skin for angiogenesis evaluation in different groups on day 21 post-injury. The arrows represent new capillaries and the circle means the vessels of normal skin tissue.

polymer mixture aqueous solutions at room or low temperature could form *in situ* thermogel depots at body temperature, showed good adhesiveness to skin tissue, and exhibited a sustained release manner *in vitro* for over three weeks. Meanwhile, the drug release profile was readily modulated *via* changing the mixing ratio of carrier polymer as well as the drug loading amount. In the *in vivo* tests, the administration of thermogel formulation inhibited inflammatory response, accelerated re-epithelization, promoted synthesis and disposition of collagen, and stimulated angiogenesis, thereby enhancing the wound healing process. This feature was attributed to the combination of the bioactivity of TPN and the acidic nature of thermogel matrix. Overall, our results indicated that the TPN-loaded PLGA-PEG-PLGA thermogel is a promising wound dressing for cutaneous wound healing.

ACKNOWLEDGMENTS

The work was financially supported by the National Natural Science Foundation of China (Nos. 51773043, 81772363, and 21474019), National Key R&D Program of China (No. 2016YFC1100300), and China Postdoctoral Science Foundation (No. 2018M632020).

REFERENCES

- Diegelmann, R. F.; Evans, M. C. Wound healing: An overview of acute, fibrotic and delayed healing. *Front. Biosci.* **2004**, *9*, 283–289.
- Boateng, J. S.; Matthews, K. H.; Stevens, H. N. E.; Eccleston, G. M. Wound healing dressings and drug delivery systems: A review. *J. Pharm. Sci.* **2008**, *97*, 2892–2923.
- Xu, R.; Luo, G. X.; Xia, H. S.; He, W. F.; Zhao, J.; Liu, B.; Tan, J. L.; Zhou, J. Y.; Liu, D. S.; Wang, Y. Z.; Yao, Z. H.; Zhan, R. X.; Yang, S. S.; Wu, J. Novel bilayer wound dressing composed of silicone rubber with particular micropores enhanced wound re-epithelialization and contraction. *Biomaterials* **2015**, *40*, 1–11.
- Malmsjö, M.; Ingemansson, R.; Martin, R.; Huddleston, E. Negative-pressure wound therapy using gauze or open-cell polyurethane foam: Similar early effects on pressure transduction and tissue contraction in an experimental porcine wound model. *Wound Rep. Reg.* **2009**, *17*, 200–205.
- Chen, L.; Cheng, H. H.; Xiong, J.; Zhu, Y. T.; Zhang, H. P.; Xiong, X.; Liu, Y. M.; Yu, J.; Guo, Z. X. Improved mechanical properties of poly(butylene succinate) membrane by co-electrospinning with gelatin. *Chinese J. Polym. Sci.* **2018**, *36*, 1063–1069.
- Vargas, E. A.; do Vale Baracho, N. C.; de Brito, J.; de Queiroz, A. A. Hyperbranched polyglycerol electrospun nanofibers for wound dressing applications. *Acta Biomater.* **2010**, *6*, 1069–1078.
- Bu, Y. Z.; Sun, G. Z.; Zhang, L. C.; Liu, J. H.; Yang, F.; Tang, P. F.; Wu, D. C. POSS-modified PEG adhesives for wound closure. *Chinese J. Polym. Sci.* **2017**, *35*, 1231–1242.
- Ishihara, J.; Ishihara, A.; Fukunaga, K.; Sasaki, K.; White, M. J. V.; Briquez, P. S.; Hubbell, J. A. Laminin heparin-binding peptides bind to several growth factors and enhance diabetic wound healing. *Nat. Commun.* **2018**, *9*, 2163.
- Qu, J.; Zhao, X.; Liang, Y.; Zhang, T.; Ma, P. X.; Guo, B. L. Antibacterial adhesive injectable hydrogels with rapid self-healing, extensibility and compressibility as wound dressing for joints skin wound healing. *Biomaterials* **2018**, *183*, 185–199.
- Vukovic, J. S.; Babic, M. M.; Antic, K. M.; Miljkovic, M. G.; Peric-Grujic, A. A.; Filipovic, J. M.; Tomic, S. L. A high efficacy antimicrobial acrylate based hydrogels with incorporated copper for wound healing application. *Mater. Chem. Phys.* **2015**, *164*, 51–62.
- Zhao, X.; Guo, B. L.; Wu, H.; Liang, Y. P.; Ma, P. X. Injectable antibacterial conductive nanocomposite cryogels with rapid shape recovery for noncompressible hemorrhage and wound healing. *Nat. Commun.* **2018**, *9*, 2784.
- Li, S.; Dong, S.; Xu, W.; Tu, S.; Yan, L.; Zhao, C.; Ding, J. X.; Chen, X. S. Antibacterial Hydrogels. *Adv. Sci.* **2018**, *5*, 1700527.
- Zou, Y. J.; He, S. S.; Du, J. Z. ϵ -Poly(L-lysine)-based hydrogels with fast-acting and prolonged antibacterial activities. *Chinese J. Polym. Sci.* **2018**, *36*, 1239–1250.
- Zhao, X.; Lang, Q.; Yildirim, L.; Lin, Z. Y.; Cui, W.; Annabi, N.; Ng, K. W.; Dokmeci, M. R.; Ghaemmaghami, A. M.; Khademhosseini, A. Photocrosslinkable gelatin hydrogel for epidermal tissue engineering. *Adv. Healthc. Mater.* **2016**, *5*, 108–118.
- Zhao, X.; Sun, X.; Yildirim, L.; Lang, Q.; Lin, Z. Y.; Zheng, R.; Zhang, Y.; Cui, W.; Annabi, N.; Khademhosseini, A. Cell infiltrative hydrogel fibrous scaffolds for accelerated wound healing. *Acta Biomater.* **2017**, *49*, 66–77.
- Gong, C. Y.; Wu, Q. J.; Wang, Y. J.; Zhang, D. D.; Luo, F.; Zhao, X.; Wei, Y. Q.; Qian, Z. Y. A biodegradable hydrogel system containing curcumin encapsulated in micelles for cutaneous wound healing. *Biomaterials* **2013**, *34*, 6377–6387.
- Hong, J. H.; Lee, H. J.; Jeong, B. Injectable polypeptide thermogel as a tissue engineering system for hepatogenic differentiation of tonsil-derived mesenchymal stem cells. *ACS Appl. Mater. Interfaces* **2017**, *9*, 11568–11576.
- Yun, E. J.; Yon, B.; Joo, M. K.; Jeong, B. Cell therapy for skin wound using fibroblast encapsulated poly(ethylene glycol)-poly(L-alanine) thermogel. *Biomacromolecules* **2012**, *13*, 1106–1111.
- Li, X. L.; Fan, R. R.; Tong, A. P.; Yang, M. J.; Deng, J. J.; Zhou, L. X.; Zhang, X. N.; Guo, G. *In situ* gel-forming AP-57 peptide delivery system for cutaneous wound healing. *Int. J. Pharm.* **2015**, *495*, 560–571.
- Cui, S. Q.; Yu, L.; Ding, J. D. Injectable thermogels based on block copolymers of appropriate amphiphilicity. *Acta Polymerica Sinica* (in Chinese) **2018**, *8*, 863–881.
- Fu, S. Z.; Ni, P. Y.; Wang, B. Y.; Chu, B. Y.; Zheng, L.; Luo, F.; Luo, J. C.; Qian, Z. Y. Injectable and thermo-sensitive PEG-PCL-PEG copolymer/collagen/n-HA hydrogel composite for guided bone regeneration. *Biomaterials* **2012**, *33*, 4801–4809.
- Zhao, X.; Wu, H.; Guo, B. L.; Dong, R.; Qiu, Y.; Ma, P. X. Antibacterial anti-oxidant electroactive injectable hydrogel as self-healing wound dressing with hemostasis and adhesiveness for cutaneous wound healing. *Biomaterials* **2017**, *122*, 34–47.
- Zheng, Y.; Cheng, Y.; Chen, J.; Ding, J. X.; Li, M.; Li, C.; Wang, J. C.; Chen, X. S. Injectable hydrogel-microsphere construct with sequential degradation for locally synergistic chemotherapy. *ACS Appl. Mater. Interfaces* **2017**, *9*, 3487–3496.
- Moon, H. J.; Ko, D. Y.; Park, M. H.; Joo, M. K.; Jeong, B. Temperature-responsive compounds as *in situ* gelling biomedical materials. *Chem. Soc. Rev.* **2012**, *41*, 4860–4883.
- Zhang, Y. B.; Zhang, J.; Chang, F.; Xu, W. G.; Ding, J. X. Repair of full-thickness articular cartilage defect using stem cell-encapsulated thermogel. *Mater. Sci. Eng.: C Mater. Biol. Appl.* **2018**, *88*, 79–87.
- Chen, Y. P.; Li, Y. Z.; Shen, W. J.; Li, K.; Yu, L.; Chen, Q.; Ding, J. D. Controlled release of liraglutide using thermogelling polymers in treatment of diabetes. *Sci. Rep.* **2016**, *6*,

- 31593.
- 27 McKenzie, M.; Betts, D.; Suh, A.; Bui, K.; Tang, R.; Liang, K. X.; Achilefu, S.; Kwon, G. S.; Cho, H. Proof-of-concept of polymeric sol-gels in multi-drug delivery and intraoperative image-guided surgery for peritoneal ovarian cancer. *Pharm. Res.* **2016**, *33*, 2298–2306.
- 28 Li, K.; Yu, L.; Liu, X.; Chen, C.; Chen, Q.; Ding, J. D. A long-acting formulation of a polypeptide drug exenatide in treatment of diabetes using an injectable block copolymer hydrogel. *Bio-materials* **2013**, *34*, 2834–2842.
- 29 Chen, Y. P.; Luan, J. B.; Shen, W. J.; Lei, K. W.; Yu, L.; Ding, J. D. Injectable and thermosensitive hydrogel containing liraglutide as a long-acting antidiabetic system. *ACS Appl. Mater. Interfaces* **2016**, *8*, 30703–30713.
- 30 Shen, W. J.; Chen, X. B.; Luan, J. B.; Wang, D. N.; Yu, L.; Ding, J. D. Sustained codelivery of cisplatin and paclitaxel via an injectable prodrug hydrogel for ovarian cancer treatment. *ACS Appl. Mater. Interfaces* **2017**, *9*, 40031–40046.
- 31 Cao, L. P.; Li, Q. L.; Zhang, C.; Wu, H. C.; Yao, L. Q.; Xu, M. D.; Yu, L.; Ding, J. D. Safe and efficient colonic endoscopic submucosal dissection using an injectable hydrogel. *ACS Biomater. Sci. Eng.* **2016**, *2*, 393–402.
- 32 Li, X. Z.; Ding, J. X.; Zhang, Z. Z.; Yang, M.; Yu, J. K.; Wang, J.; Chang, F.; Chen, X. S. Kartogenin-incorporated thermogel supports stem cells for significant cartilage regeneration. *ACS Appl. Mater. Interfaces* **2016**, *8*, 5148–5159.
- 33 Zhang, Y. B.; Ding, J. X.; Sun, D. K.; Sun, H.; Zhuang, X. L.; Chang, F.; Wang, J. C.; Chen, X. S. Thermogel-mediated sustained drug delivery for *in situ* malignancy chemotherapy. *Mater. Sci. Eng.: C Mater. Biol. Appl.* **2015**, *49*, 262–268.
- 34 Zhang, W.; Ning, C.; Xu, W.; Hu, H.; Li, M.; Zhao, G.; Ding, J. X.; Chen, X. S. Precision-guided long-acting analgesia by Gel-immobilized bupivacaine-loaded microsphere. *Theranostics* **2018**, *8*, 3331–3347.
- 35 Zhang, W.; Xu, W.; Ning, C.; Li, M.; Zhao, G.; Jiang, W.; Ding, J. X.; Chen, X. S. Long-acting hydrogel/microsphere composite sequentially releases dexmedetomidine and bupivacaine for prolonged synergistic analgesia. *Biomaterials* **2018**, *181*, 378–391.
- 36 Strodtbeck, F. Physiology of wound healing. *Newborn Infant Nurs. Rev.* **2001**, *1*, 43–52.
- 37 Kruse, C. R.; Nuutila, K.; Lee, C. C. Y.; Kiwanuka, E.; Singh, M.; Caterson, E. J.; Eriksson, E.; Sorensen, J. A. The external microenvironment of healing skin wounds. *Wound Rep. Reg.* **2015**, *23*, 456–464.
- 38 Yu, L.; Chang, G. T.; Zhang, H.; Ding, J. D. Temperature-induced spontaneous sol-gel transitions of poly(D,L-lactic acid-co-glycolic acid)-*b*-poly(ethylene glycol)-*b*-poly(D,L-lactic acid-co-glycolic acid) triblock copolymers and their end-capped derivatives in water. *J. Polym. Sci., Part A: Polym. Chem.* **2007**, *45*, 1122–1133.
- 39 Shim, M. S.; Lee, H. T.; Shim, W. S. Poly(D,L-lactic acid-co-glycolic acid)-*b*-poly(ethylene glycol)-*b*-poly(D,L-lactic acid-co-glycolic acid) triblock copolymer and thermoreversible phase transition in water. *J. Biomed. Mater. Res. B* **2002**, *61*, 188–196.
- 40 Yu, L.; Zhang, Z.; Zhang, H.; Ding, J. D. Mixing a sol and a precipitate of block copolymers with different block ratios leads to an injectable hydrogel. *Biomacromolecules* **2009**, *10*, 1547–1553.
- 41 Yu, L.; Zhang, Z.; Zhang, H. A.; Ding, J. D. Biodegradability and biocompatibility of thermoreversible hydrogels formed from mixing a sol and a precipitate of block copolymers in water. *Biomacromolecules* **2010**, *11*, 2169–2178.
- 42 Yu, L.; Li, K.; Liu, X.; Chen, C.; Bao, Y. C.; Ci, T. Y.; Chen, Q. H.; Ding, J. D. *In vitro* and *in vivo* evaluation of a once-weekly formulation of an antidiabetic peptide drug exenatide in an injectable thermogel. *J. Pharm. Sci.* **2013**, *102*, 4140–4149.
- 43 Zhang, L.; Shen, W. J.; Luan, J. B.; Yang, D. X.; Wei, G.; Yu, L.; Lu, W. Y.; Ding, J. D. Sustained intravitreal delivery of dexamethasone using an injectable and biodegradable thermogel. *Acta Biomater.* **2015**, *23*, 271–281.
- 44 Bowler, P. G. Wound pathophysiology, infection and therapeutic options. *Ann. Med.* **2002**, *34*, 419–427.
- 45 Zanger, P.; Holzer, J.; Schleucher, R.; Scherbaum, H.; Schitteck, B.; Gabrys, S. Severity of *Staphylococcus aureus* infection of the skin is associated with inducibility of human beta-defensin 3 but not human β -defensin 2. *Infect. Immun.* **2010**, *78*, 3112–3117.
- 46 Bernard, P. Management of common bacterial infections of the skin. *Curr. Opin. Infect. Dis.* **2008**, *21*, 122–128.
- 47 Ye, S.; Jiang, L.; Wu, J. M.; Su, C.; Huang, C. B.; Liu, X. F.; Shao, W. Flexible amoxicillin-grafted bacterial cellulose sponges for wound dressing: *In vitro* and *in vivo* evaluation. *ACS Appl. Mater. Interfaces* **2018**, *10*, 5862–5870.
- 48 Lee, Y. M.; Kim, S. S.; Park, M. H.; Kim, K. W.; Sung, Y. K.; Kang, I. Y. β -Chitin-based wound dressing containing silver sulfadiazine. *J. Mater. Sci.: Mater. Med.* **2000**, *11*, 817–823.
- 49 Wang, Y.; Cui, R.; Li, G.; Gao, Q.; Yuan, S.; Altmeyer, R.; Zou, G. Teicoplanin inhibits Ebola pseudovirus infection in cell culture. *Antiviral Res.* **2016**, *125*, 1–7.
- 50 Gocer, H.; Onger, M. E.; Kuyubasi, N.; Cirakli, A.; Kir, M. C. The effect of teicoplanin on fracture healing: An experimental study. *Ekleml Hastalik Cerrahisi.* **2016**, *27*, 16–21.
- 51 Kester, R. C.; Antrum, R.; Thornton, C. A.; Ramsden, C. H.; Harding, I. A comparison of teicoplanin versus cephradine plus metronidazole in the prophylaxis of post-operative infection in vascular surgery. *J. Hosp. Infect.* **1999**, *41*, 233–243.
- 52 Rybak, M. J.; Lerner, S. A.; Levine, D. P.; Albrecht, L. M.; Mcneil, P. L.; Thompson, G. A.; Kenny, M. T.; Yuh, L. Teicoplanin pharmacokinetics in intravenous drug-abusers being treated for bacterial-endocarditis. *Antimicrob. Agents Chemother.* **1991**, *35*, 696–700.
- 53 Peng, L. H.; Wei, W.; Qi, X. T.; Shan, Y. H.; Zhang, F. J.; Chen, X.; Zhu, Q. Y.; Yu, L.; Liang, W. Q.; Gao, J. Q. Epidermal stem cells manipulated by pDNA-VEGF165/CYD-PEI nanoparticles loaded gelatin/ β -TCP matrix as a therapeutic agent and gene delivery vehicle for wound healing. *Mol. Pharmacol.* **2013**, *10*, 3090–3102.
- 54 Luan, J. B.; Zhang, Z.; Shen, W. J.; Chen, Y. P.; Yang, X.; Chen, X.; Yu, L.; Sun, J.; Ding, J. D. Thermogel loaded with low-dose paclitaxel as a facile coating to alleviate periprosthetic fibrous capsule formation. *ACS Appl. Mater. Interfaces* **2018**, *10*, 30235–30246.
- 55 Faust, S. N.; Levin, M.; Harrison, O. B.; Goldin, R. D.; Lockhart, M. S.; Kondaveeti, S.; Laszik, Z.; Esmon, C. T.; Heyderman, R. S. Dysfunction of endothelial protein C activation in severe meningococcal sepsis. *New Engl. J. Med.* **2001**, *345*, 408–416.
- 56 Zhang, C. Z.; Niu, J.; Chong, Y. S.; Huang, Y. F.; Chu, Y.; Xie, S. Y.; Jiang, Z. H.; Peng, L. H. Porous microspheres as promising vehicles for the topical delivery of poorly soluble asiaticoside accelerate wound healing and inhibit scar formation *in vitro* & *in vivo*. *Eur. J. Pharm. Biopharm.* **2016**, *109*, 1–13.
- 57 Van Staden Adu, P.; Heunis, T.; Smith, C.; Deane, S.; Dicks, L. M. Efficacy of lantibiotic treatment of staphylococcus aureus-induced skin infections, monitored by *in vivo* bioluminescent imaging. *Antimicrob. Agents Chemother.* **2016**, *60*, 3948–55.
- 58 Hrabalíková, M.; Merchan, M.; Ganbold, S.; Sedlárik, V.; Valasek, P.; Saha, P. Flexible polyvinyl alcohol/2-hydroxypropanoic acid films: Effect of residual acetyl moieties on mechanical, thermal and antibacterial properties. *J. Polym. Eng.* **2015**, *35*, 319–327.
- 59 Ricke, S. C. Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. *Poultry Sci.* **2003**, *82*, 632–639.