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A poly(γ-glutamic acid)-based hydrogel loaded with superoxide dismutase for wound healing

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ABSTRACT: The purpose of this study was to develop a poly(γ-glutamic acid) (γ-PGA)-based hydrogel loaded with superoxide dismutase (SOD) to accelerate wound healing. First, γ-PGA was modified with taurine (γ-PGAS), and then the SOD-loaded γ-PGAS/γ-PGA hydrogel (SOD-PGAS/PGA-H) was prepared by cross-linking of ethylene glycol diglycidyl ether. The swelling behavior and water vapor transmission rate revealed that PGAS/PGA-H could create a moist environment for wound surface. *In vitro* kinetics of SOD release showed that SOD released from PGAS/PGA-H maintained high activity and SOD-PGAS/PGA-H effectively scavenged the superoxide anion. The results of our fibroblast proliferation experiments showed that PGAS/PGA-H had good cytocompatibility. The effects of SOD-PGAS/PGA-H on wound healing were examined in a Type I diabetic rat model with full-thickness wounds. Twenty-one days after grafted to wounds, SOD-PGAS/PGA-H exhibited a higher rate of wound healing than control group and showed increased collagen deposition and epithelialization. SOD-PGAS/PGA-H seems to promote better wound healing and thus might be a promising candidate for wound healing management. © 2015 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 42033.

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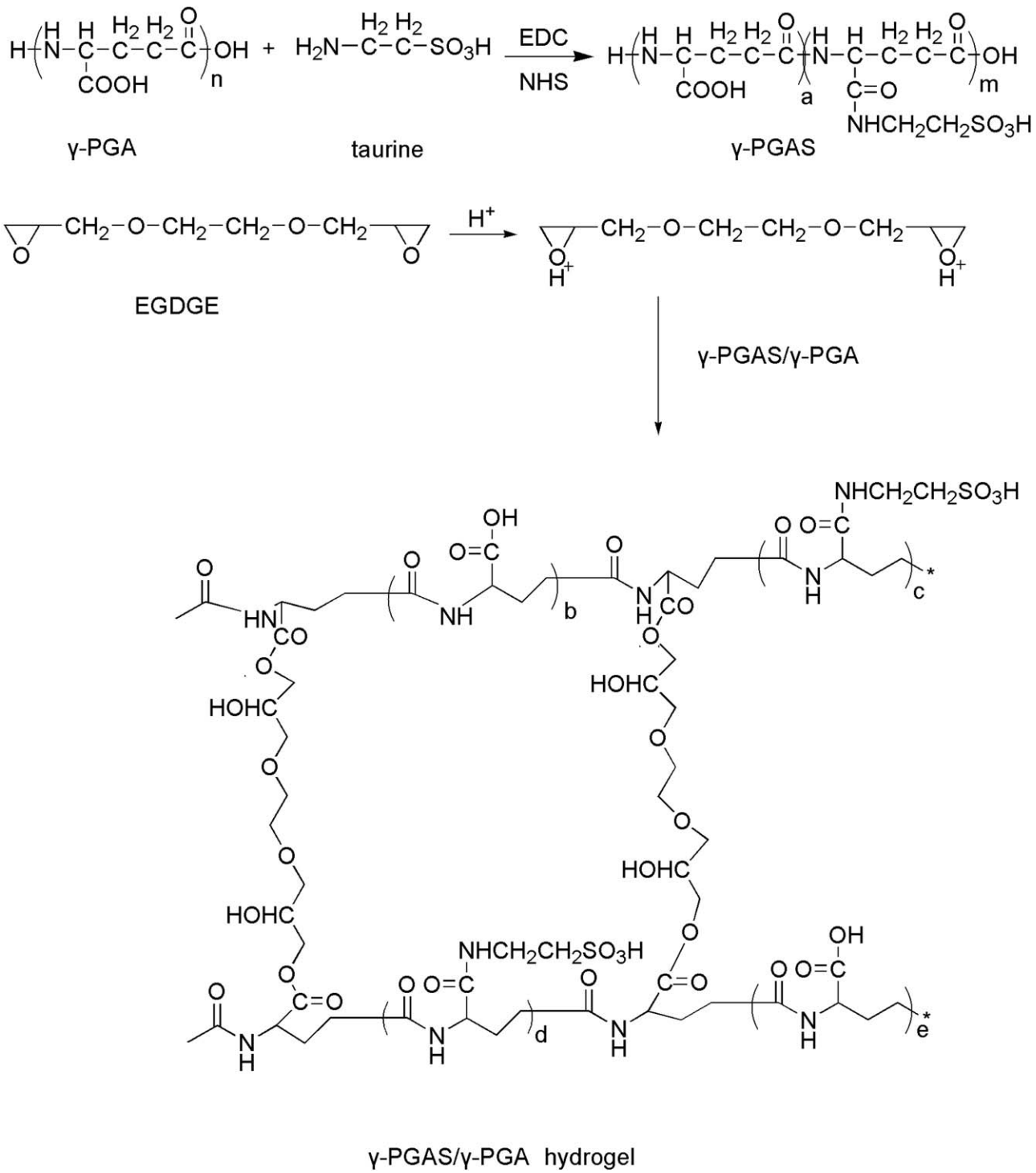
INTRODUCTION

Wound healing is a complex biological process aimed at reconstructing damaged tissue, and it requires precise coordination of connective tissue repair, re-epithelialization, and angiogenesis.[1] In general, the process is very orderly and efficient, characterized by three interrelated dynamic and overlapping phases: the inflammatory phase (minutes to days), proliferative phase (days to weeks), and remodeling phase (weeks to months).[2] Among these phases, a prolonged inflammation is detrimental to the outcome of healing. Chronic inflammation is characterized by abundant neutrophil infiltration with the associated release of inflammatory mediators including reactive oxygen species (ROS), reactive nitrogen species (RNS), and their derivatives.[3–5] The excessive production of these radicals results in oxidative stress, which is one of the major factors causing non-healing ulcers, such as diabetic wound healing.[6] The remedy for these harms would be the use of wound dressings with antioxidant properties.[7]

Superoxide dismutase (SOD), a non-scavenger enzyme capable of catalyzing the dismutation of superoxide into the less toxic hydrogen peroxide and molecular oxygen, has been widely used as an antioxidant for decreasing reactive oxygen species (ROS) content in injured tissues.[8–10] SOD constitutes the first line of defense against ROS and plays an important protective role against the indirect deleterious effects of these radicals.[11,12]

An ideal wound dressing should protect the wound from bacterial infection, control evaporative water loss, remove wound exudates, and promote the establishment of the best milieu for natural healing. At present, high-quality wound dressings are designed to create a moist environment to promote healing.[13] The hydrogel-based wound dressing has recently attracted considerable interest, as they can maintain a moist environment at the wound interface, allow gaseous exchange, absorb wound exudates, and supply a barrier to microorganisms. Poly(γ-glutamic acid) (γ-PGA) is a naturally occurring biopolymer that is water soluble, non-toxic, edible, and biodegradable,[14–16] which has shown to promote cell migration and enhance cell adhesion.[17,18] Besides, γ-PGA has been reported to prevent postsurgical tissue adhesion and the γ-PGA drug-loaded hydrogel to promote wound healing.[19]

In this contribution, we developed a kind of SOD-loaded γ-PGA hydrogel to promote wound healing. First, taurine was grafted to the side chain of γ-PGA to obtain sulfonated γ-PGA (γ-PGAS). The interaction between the sulfonic acid groups in γ-PGAS and the amino groups in SOD can increase the load of



Scheme 1. Synthetic route to γ-PGAS/γ-PGA hydrogel (PGAS/PGA-H).

SOD.[20–22] Then a SOD-loaded γ-PGAS/γ-PGA hydrogel (SOD-PGAS/PGA-H) was prepared for wound healing. The swelling ratio in PBS and the water vapor transmission rate (WVTR) of PGAS/PGA-H were evaluated. The *in vitro* kinetics of SOD release from PGAS/PGA-H and the enzyme activity of the released SOD were examined. In addition, the anti-oxidation ability and cytotoxicity of SOD-PGAS/PGA-H were tested. Finally, the *in vivo* performance of wound healing was evaluated using Type I diabetic rat models. We hypothesized that the work can integrate the advantages of SOD and PGA-based hydrogel by reducing oxidative stress and creating moist micro-environment, which can promote the chronic wound healing. Scheme 1 illustrates the synthetic route to γ-PGAS/γ-PGA hydrogel (PGAS/PGA-H).

EXPERIMENTAL

Materials

γ-PGA (M_w = 1,200,000) was provided by Vedan (Taichung, Taiwan). Ethylene glycol diglycidyl ether (EGDGE) was purchased from Tokyo Chemical Industry (Tokyo, Japan). 2-Aminoethanesulfonic acid (taurine) was obtained from Alfa Aesar. 1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) was obtained from J&K Scientific (Beijing, China). *N*-Hydroxysuccinimide (NHS) was purchased from Aladdin Chemistry (China). Cu–Zn superoxide dismutase (SOD) was purchased from Zhejiang Conkey Biological Technology (Hangzhou, China) and Streptozotocin (STZ) was purchased from Sigma–Aldrich (St. Louis, MO). Tegaderm™, a commercial product for wound healing, was purchased from 3M (3M Health Care) for comparative purposes.

Synthesis of γ-PGAS

Briefly, 100 mg of γ-PGA, 594.4 mg of EDC, and 356.8 mg of NHS were dissolved in deionized water for 15 min at 4°C. Taurine (97 mg) was added to the solution, and then the reaction was maintained for 24 h at room temperature. The resulting solution was dialyzed against water for 3 days. γ-PGAS was obtained by lyophilization.

Preparation of PGAS/PGA-H

γ-PGAS and pure γ-PGA with different molar ratios (0 : 1, 0.5 : 1, 1 : 1, total weight 0.5 g) were dissolved in 1.2 mL deionized water by ultrasonication. EGDGE (equal molar weight as γ-PGA) as the cross-linker was then added under stirring to the reaction at room temperature. The pH of the solution was adjusted to 4.5–5.0 by 2*M* HCl solution. Then the reactive solution was maintained at 60°C for 6 h. The remaining compounds in the hydrogel were removed by dialyzing them in deionized water for 7 days. The PGAS/PGA-H was obtained by lyophilization and the dried hydrogel was weighed.

Compressive Testing of PGAS/PGA-H

A universal testing machine was used to determine the compressive modulus of the PGAS/PGA-H in both dry and swollen states, by compressing the sample discs (10 mm diameter and 4 mm thickness) at a constant rate of 5 mm/min. The swollen hydrogel was prepared by immersion in PBS (pH = 7.4) for 6 h. The slopes of the compressive stress–strain curves from 5% to 35% deformation were used to calculate the compressive modulus.[23]

Calculation of Swelling Ratio

To test the swelling ratio of the hydrogel, the dry hydrogel was weighed and dipped in PBS (pH = 7.4) at 25°C. The swollen hydrogel was then weighted and the water absorption (g/g) was calculated as:

$$\text{Water absorbtion} = \frac{W - W_0}{W_0},$$

where W_0 is the weight of the dry hydrogel and W is the weight of the swelled hydrogel.

Determination of WVTR

The WVTR of PGAS/PGA-H (0 : 1) and PGAS/PGA-H (1 : 1) was determined as previously described.[13] A circular piece of specimen was placed over the top of a glass vial of 3 cm in diameter containing 15 g of $CaCl_2$. The vial was then placed in an incubator at a relative humidity of 90 ± 5% and a temperature of 40 ± 2°C. The WVTR was calculated as:

$$\text{WVTR} = \frac{W_2 - W_1}{S} \times 24 \left(\text{g day}^{-1}\text{cm}^{-2}\right),$$

where W_1 and W_2 are the weights of the whole vial after 1 and 2 h, respectively, and S is the transmitting area of the specimen.

Preparation of SOD-PGAS/PGA-H

Dry PGAS/PGA-H (15 mg) samples with different ratios were dipped in 0.2% SOD/PBS solution at 4°C for 24 h. The hydrogels were then rinsed three times with PBS to remove excess SOD solution.

Determination of *In Vitro* SOD Release Kinetics

SOD-PGAS/PGA-H prepared with various ratios were immersed into 10 mL of PBS (pH = 7.4) at room temperature. At predefined time points, 10 mL of samples were collected and equal volumes of PBS added. The concentration of the released SOD was spectrophotometrically determined at 595 nm according to the Bradford method.[24]

Measurement of the Scavenging Effect on Superoxidant Radicals and *In Vitro* SOD Activity

0.5 mL of released SOD solution prepared in above section (0.5 mL of PBS as the control) was mixed with Tris–HCl (0.24 m*M*) and pyrogallol (9 μ*M*) for 5 min in the dark, and then 0.05 mL of HCl (0.4 *M*) was added to the mixture to stop the reaction.[25,26] The absorbance was measured by spectrophotometer at 299 nm, and the ability to scavenge superoxide radicals was calculated as:

$$\text{Scavenging effect (\%) } \alpha = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}},$$

where A_{control} is the absorbance of the control group, A_{sample} is the absorbance of the sample group.

$$\text{SOD activity (U/mL)} = \alpha \times \frac{100\%}{50\%} \times \frac{V}{v},$$

where V is the volume of the whole solution, v is the volume of the sample solution.

Evaluation of Cytocompatibility

The cytocompatibility of the hydrogels was measured using 3T3 fibroblasts. According to the national standard GB/T 16886.5–2003, the extraction media were prepared by immersing 100 mg of hydrogel (under UV for 24 h) in 10 mL of DMEM media at 37°C for 24 h. 3T3 fibroblasts were cultured with DMEM media (comprised of 10% fetal bovine serum, 1% antibiotic) in 48-well plates at a density of 1×10^4 cells/well for 12 h. Then the media were replaced by 200 μL of hydrogel extract solution, and cells were incubated in 5% CO_2 humidified atmosphere at 37°C for 1, 3, and 5 days. Media were changed every 2 days. Cell viability was measured by methylthiazol tetrazolium (MTT) assay. 50 μL of 5 mg/mL MTT was added to each well and incubated at 37°C for 4 h. The media with MTT solution were removed and 200 μL of dimethylsulfoxide (DMSO) was added.

The plate was agitated at 37°C for 20 min and the absorbance of the solution at 490 nm was measured.

Creation of Skin Wounds on Diabetic Rats

The Type I diabetic rat model was established as previously described.[27] Male Sprague Dawley rats weighing 250–300 g were given single intraperitoneal injections of 60 mg/kg STZ (30 mg/mL in sodium citrate buffer, pH = 4.0–4.5). All animal procedures were approved by the Animal Care Committee and followed the regulations of the Administration of Affairs Concerning Experimental Animals in Nankai University (Tianjin, China). After 3 days, glucose levels were monitored using a blood glucose monitor (Roche Diagnostics [Shanghai], China). STZ-treated rats with whole-blood glucose levels above 16.7 mmol/L were considered diabetic. After anesthetizing the rats with 10% chloral hydrate (300 mg/kg), their dorsal areas were completely shaved by 8% Na_2S, and two full-thickness circular wounds (diameters of 1 cm) were created on the back of each rat. The diabetic rats were divided into three groups: control (treated with 3M wound dressing and gauze only), hydrogel (treated with PGAS/PGA-H) and SOD-loaded hydrogel (treated with SOD-PGAS/PGA-H). The hydrogels were covered with 3M wound dressing and gauze.

Evaluation of Wound Healing *In Vivo*

Wound closure was measured at days 0, 7, 14, and 21 after wounding. The sizes of the healed and the remaining unhealed wounds were evaluated by photographing the wound areas at a close and fixed distance and by analyzing the images using Adobe Photoshop CS4. The rats were sacrificed at days 7, 14, and 21 for histological observation. The trauma samples were fixed in 4% paraformaldehyde, dehydrated with 30% sucrose solution, and frozen and cut into 6 μm-sections for hematoxylin eosin (H&E) staining (to observe inflammatory reaction and cell proliferation) and Masson's trichrome staining (to observe blood vessel formation and collagen secretion).

Statistical Analysis

Values are expressed as mean ± standard deviation (SD). Statistical significance of the difference between wounds treated with gauze versus hydrogels was determined by the Student's *t*-test. A P value of <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Synthesis of γ-PGAS

The characteristics of γ-PGAS were shown as FTIR and ^{1}H-NMR.[21] Figure 1 showed the FTIR spectra of γ-PGAS. The peaks at 1100–1200 and 1350 cm^{-1} for γ-PGAS showed the asymmetric and symmetric stretching vibration modes of S (=O_2), respectively. These peaks were not observed for γ-PGA. The peaks at 1600 cm^{-1} of amide I and 1650 cm^{-1} of amide II were similarly observed for γ-PGAS and γ-PGA. These results indicated that γ-PGAS was prepared by the reaction of taurine with γ-PGA.

Figure 2 showed the ^{1}H-NMR spectra of γ-PGAS, γ-PGA, and taurine. All the peaks for γ-PGAS corresponding to γ-PGA and the CH_2 of taurine were observed after the reaction. The peak of a CH_2 next to a nitrogen atom was shifted from 3.3 ppm in taurine to 3.4 ppm in γ-PGAS, indicating that taurine was

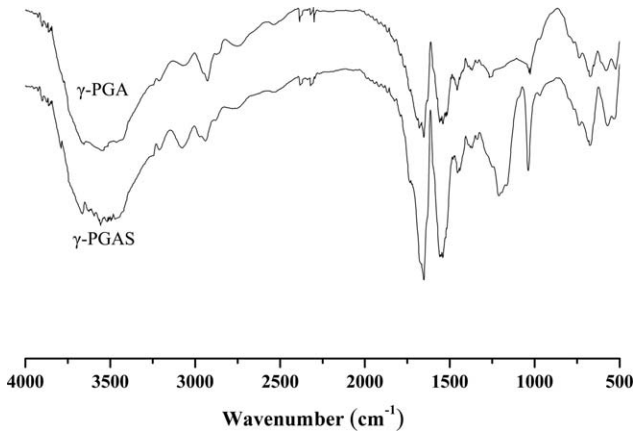


Figure 1. FT-IR spectra of γ-PGAS and γ-PGA.

introduced into γ-PGA by an amide linkage. The peak area of CH ("a" in ^{1}H-NMR spectra) was defined as 1, and the peak area of CH_2 ("d" in ^{1}H-NMR spectra) next to a nitrogen atom was about 1.7. The sulfonate content against total carboxyl groups was estimated by ^{1}H-NMR spectrum was 85%.

Compressive Modulus of PGAS/PGA-H

The mechanical properties of various ratios of PGAS/PGA-H in both dry and swollen states were illustrated as compressive modulus. Figure 3 showed stress–strain curves of hydrogels under the compressive force. The compressive modulus of dry hydrogels was larger than that of swollen hydrogels (Table I). The compressive modulus decreased as amount of γ-PGAS increased, because of the reduced crosslinking density of PGAS/PGA-H.

Characteristics of PGAS/PGA-H and SOD-PGAS/PGA-H

The swelling behavior is one of the most important properties of hydrogels for wound dressing. The different swelling ratios of the hydrogels in PBS (pH = 7.4) are shown in Figure 4(A). The swelling ratios of the fully swollen hydrogels in this study were above 40 g/g. The swelling ratio of PGAS/PGA-H (0 : 1) was generally lower than that of 0.5 : 1 and 1 : 1. This indicated that, in case of a full cross-linking, the swelling ratio increased with the increase of the molar proportion of γ-PGAS. This was probably because the total amount of γ-PGAS and γ-PGA was constant and the molar weight of cross-linker was equal to γ-PGA. The increase of γ-PGAS decreased the cross-linking density, which increased the swelling ratio.

The therapeutic efficiency of a drug-loaded hydrogel primarily depends on the dose and release of the drug at the wound site. We observed a sustained release of the entrapped SOD from hydrogels of different formulations, which could provide very accurate dosing and more availability of the drug at the wound site [Figure 4(B)]. Furthermore, the amount of SOD released from the hydrogel increased with increase of γ-PGAS, which indicated that sulfonate groups of taurine could increase the load of SOD in the blended hydrogel system. It is known that heparin, a sulfated polysaccharide, has numerous important biological activities, associated with its interaction with diverse proteins.[20] In our study, the interaction between γ-PGAS and

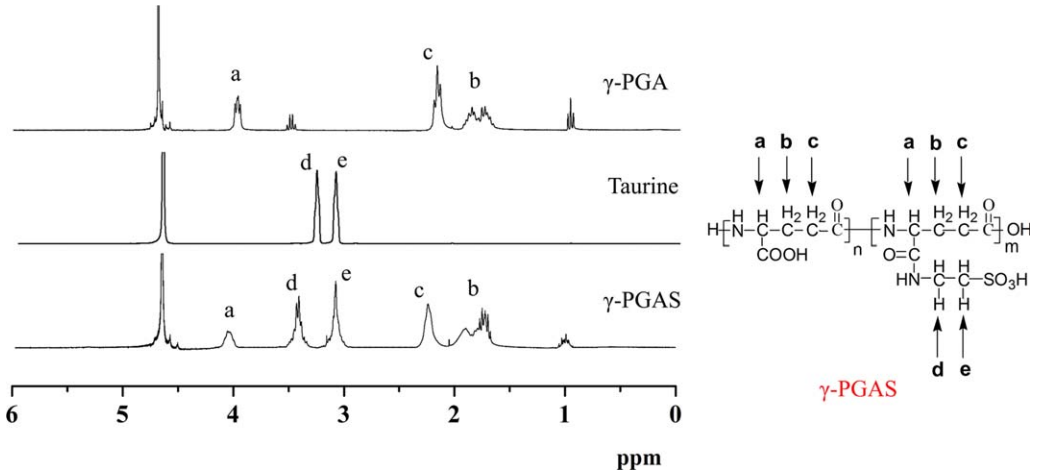


Figure 2. ^{1}H-NMR spectra of γ-PGAS, γ-PGA, and taurine. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

SOD imitate the heparin–protein interaction, which increased the amount of SOD loaded in the hydrogel.

To determine the antioxidative property of SOD released from the hydrogels, we studied the accumulated scavenging effect on superoxide radicals [Figure 4(C)]. Forty-eight hours later, the scavenging effect of SOD-PGAS/PGA-H 0 : 1, 0.5 : 1, and 1 : 1 was 49.37%, 56.74%, and 61.97%, respectively. The scavenging effect of the hydrogels increased with SOD release. This was consistent with the results of Figure 4(B). The amount of SOD released from the hydrogel increased with increase of γ-PGAS thus scavenged more superoxide radicals.

To test the activity of the released SOD, hydrogels of different ratios were immersed in PBS, and the activity was tested using a modified pyrogallol autoxidation-Vc method.[25] Figure 4(D) depicted the activity of SOD released from the hydrogels remained at the same level between each time point. Forty-eight hours later, SOD activity retention rate was 93.1%. There was no statistically significant difference between each group. This indicated that the hydrogel system in our study could protect the structure of SOD, and the method used to load SOD was mild enough to guarantee a high activity of SOD.

WVTR of the Hydrogels

The WVTR of wound dressings is one of the most important parameters.[15] A high WVTR would lead to the dehydration of the wound. The ideal wound dressing can create a humid environment. It has been reported that a WVTR in the range of 0.19–0.58 g day^{-1} cm^{-2} is suitable for wounds with low exudates, and a WVTR of 0.58–0.78 g day^{-1} cm^{-2} and 0.78–0.98 g day^{-1} cm^{-2} would be suitable for moderately and heavily exuding wounds, respectively.[28–30] As shown in Table II, PGAS/PGA-H (0 : 1) with a WVTR of 0.69 g day^{-1} cm^{-2} and PGAS/PGA-H (1 : 1) with a WVTR of 0.71 g day^{-1} cm^{-2} may be useful for treating moderately exuding wounds and keeping a humid environment for wound healing.

Hydrogel Cytotoxicity

Low cytotoxicity is one of the most important properties for biomaterials. Figure 5 showed the cytotoxicity of PGAS/PGA-H evaluated via MTT assay. The cell viability of the specimens was not statistically different from that of the control group (TCPS, tissue-culture polystyrene surfaces) at days 1, 3, and 5 and thus could be considered non-toxic. The excellent cytocompatibility of the hydrogels was partly ascribed to the biocompatibility of

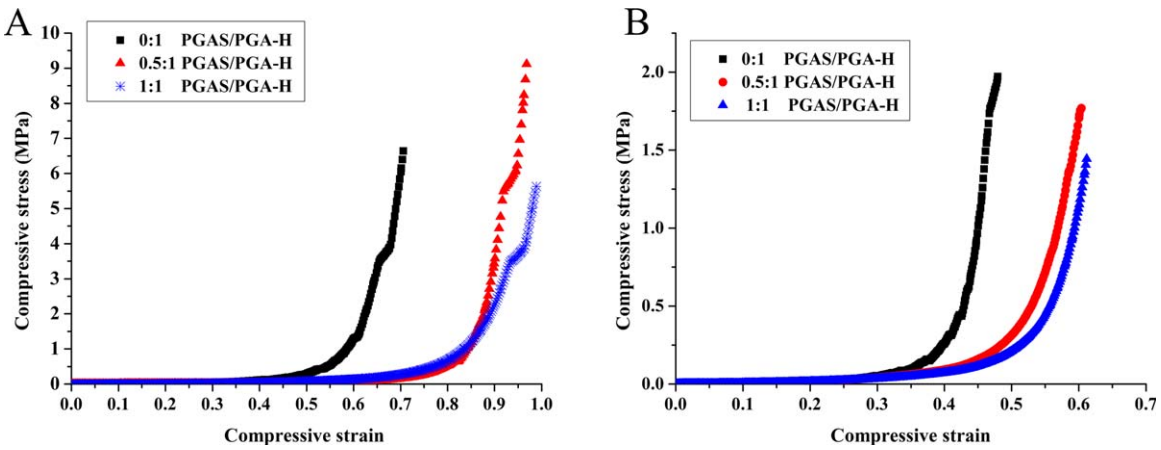


Figure 3. Representative stress–strain curves of dry (A) and swollen (B) hydrogels under the compressive force. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table I. Compressive Modulus of the Hydrogels

	Compressive modulus (MPa)	
PGAS/PGA-H	Dry	Swollen
0 : 1	1.105 ± 0.026	0.080 ± 0.0079
0.5 : 1	0.717 ± 0.037	0.048 ± 0.0067
1 : 1	0.461 ± 0.012	0.038 ± 0.0082

Table II. Water Vapor Transmission Rate of the Hydrogels

Wound application	PGAS/PGA-H (0 : 1)	PGAS/PGA-H (1 : 1)
WVTR (g day $^{-1}$ cm $^{-2}$)	0.69	0.71

γ-PGA and taurine. γ-PGA is a biocompatible material for various types of cells,[1,14,31] and γ-PGAS has proven biocompatibility.[32] Thus, according to our results, taurine linked to the side chain of γ-PGA had little adverse effect on cellular compatibility. In addition, the results in Figure 5 indicated that the synthetic method of preparing our hydrogel was mild, and dialysis after the reaction was necessary and effective to improve its cytocompatibility. Among the three different PGAS/PGA-H compositions, cytocompatibility of the 1 : 1 ratio was slightly higher at day 5 than cytocompatibility of the other two hydrogel compositions. The PGAS/PGA-H 1 : 1 was also tested *in vivo* due to the better results *in vitro* comparing with other molar ratios.

Wound Healing Effects of the Hydrogels

During the process of wound healing, ROS in lower concentrations are beneficial to wound healing in early stage. They promote vascular endothelial growth factor (VEGF) expressions in keratinocytes in homeostatic conditions.[33] However, they will result in oxidative stress if produced excessively and persist for longer periods of time, which is one of the major factors

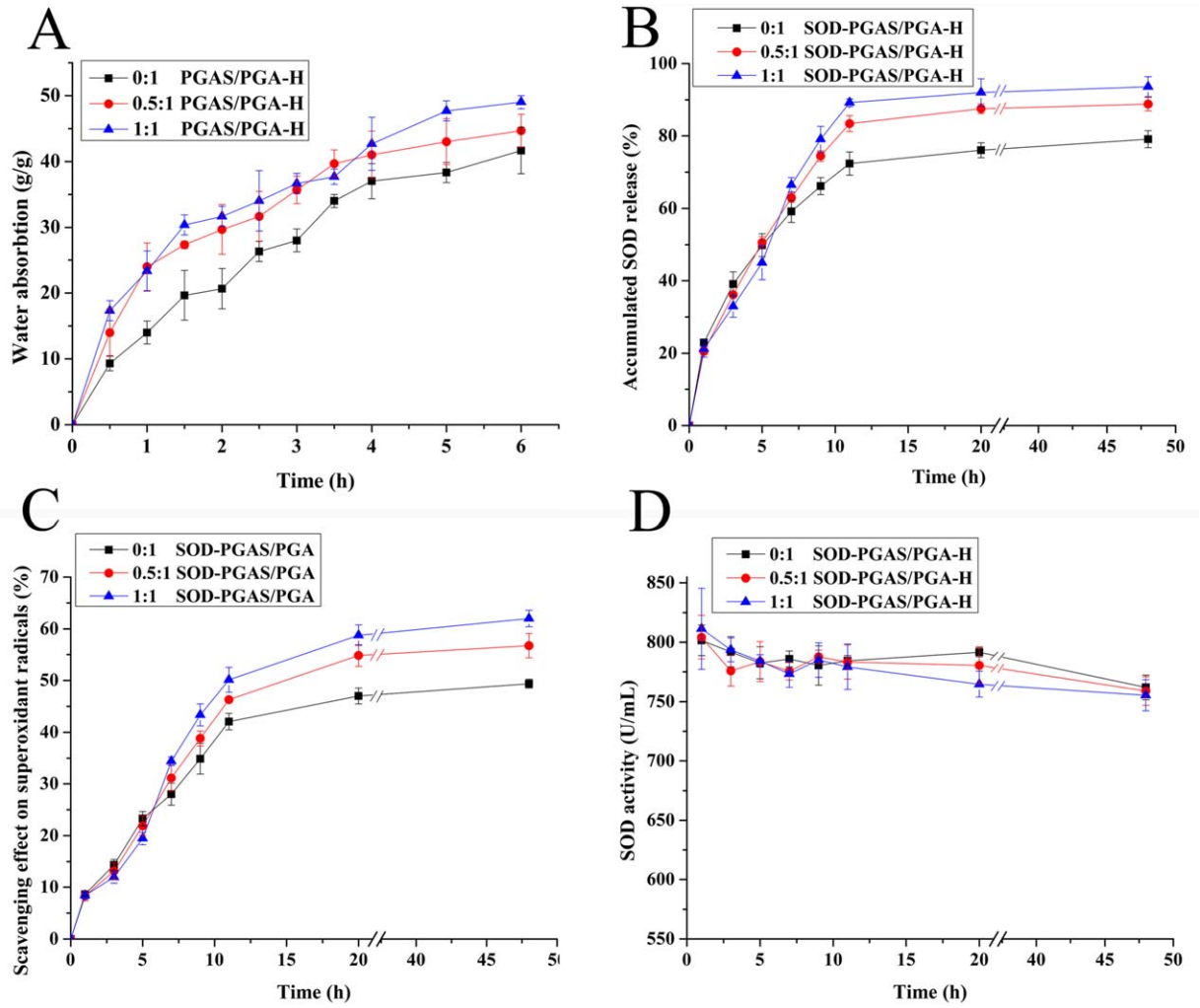


Figure 4. Characteristics of PGAS/PGA-H and SOD-PGAS/PGA-H. (A) Swelling behavior of hydrogels in PBS (pH = 7.4). (B) The kinetics of SOD released from hydrogels in PBS. (C) Accumulated scavenging effect on superoxide radicals of hydrogels for 12 h. (D) Activity of released SOD. Values are expressed as mean ± SD. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

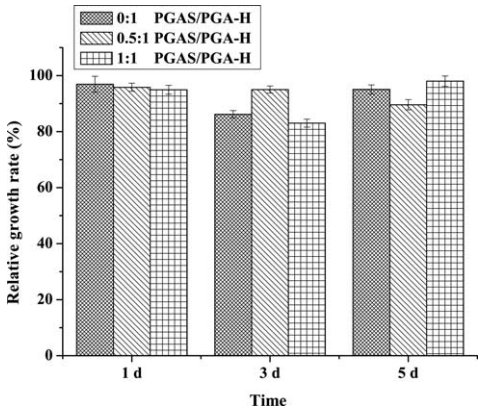


Figure 5. Cytotoxicity of hydrogels. Values are expressed as mean ± SD. The statistical significance of the difference between the control (TCPS, tissue-culture polystyrene surfaces) and hydrogels was determined by the Student's *t*-test. A P value <0.05 was considered statistically significant.

causing chronic wounds, including diabetic ulcer.[6] Therefore, to test the healing effects of hydrogels on chronic wounds, animal model with diabetic rat trauma was used. STZ selectively destroys pancreatic β-cells, inhibits the synthesis and release of insulin, and causes the onset of Type I diabetes at days 2 or 3 after a single injection.[27] In our study, SD rats were induced by STZ to develop Type I diabetes, and the full-thickness circular wounds were formed (Figure 6 for wound healing results and Figure 7 for wound healing rates).

Figure 6 showed representative animals from each group (control, PGAS/PGA-H [1 : 1], SOD-PGAS/PGA-H [1 : 1]) at days 0, 7, 14, and 21. The results from each group showed that the wound areas treated by SOD-PGAS/PGA-H (1 : 1) were smaller than those of other applications at days 7 and 14 after wounding. These results indicated that SOD encapsulated in hydrogel can promote wound healing. Furthermore, the subcutaneous aspect appeared grossly normal for the test groups (PGAS/PGA-H [1 : 1], SOD-PGAS/PGA-H [1 : 1]), and the wound site appeared uninfected. It is known that epithelialization is accelerated if the wound is kept moist.[34] In our experiments, the wound areas treated by hydrogels were smaller than those treated by 3M wound dressing and gauze only. This was probably because keratinocytes migrated more easily over a moist wound surface than underneath a scab.[35]

Figure 7 depicted the wound closure rates of wounds treated with control, PGAS/PGA-H and SOD-PGAS/PGA-H. After treatments for 7, 14, and 21 days, the rates of wound closure were significantly higher in the SOD-PGAS/PGA-H group than in the control group: the wound closure rate in the control group was 60% after 21 days, while in the PGAS/PGA-H group was 70% and in the SOD-PGAS/PGA-H group was 90%.

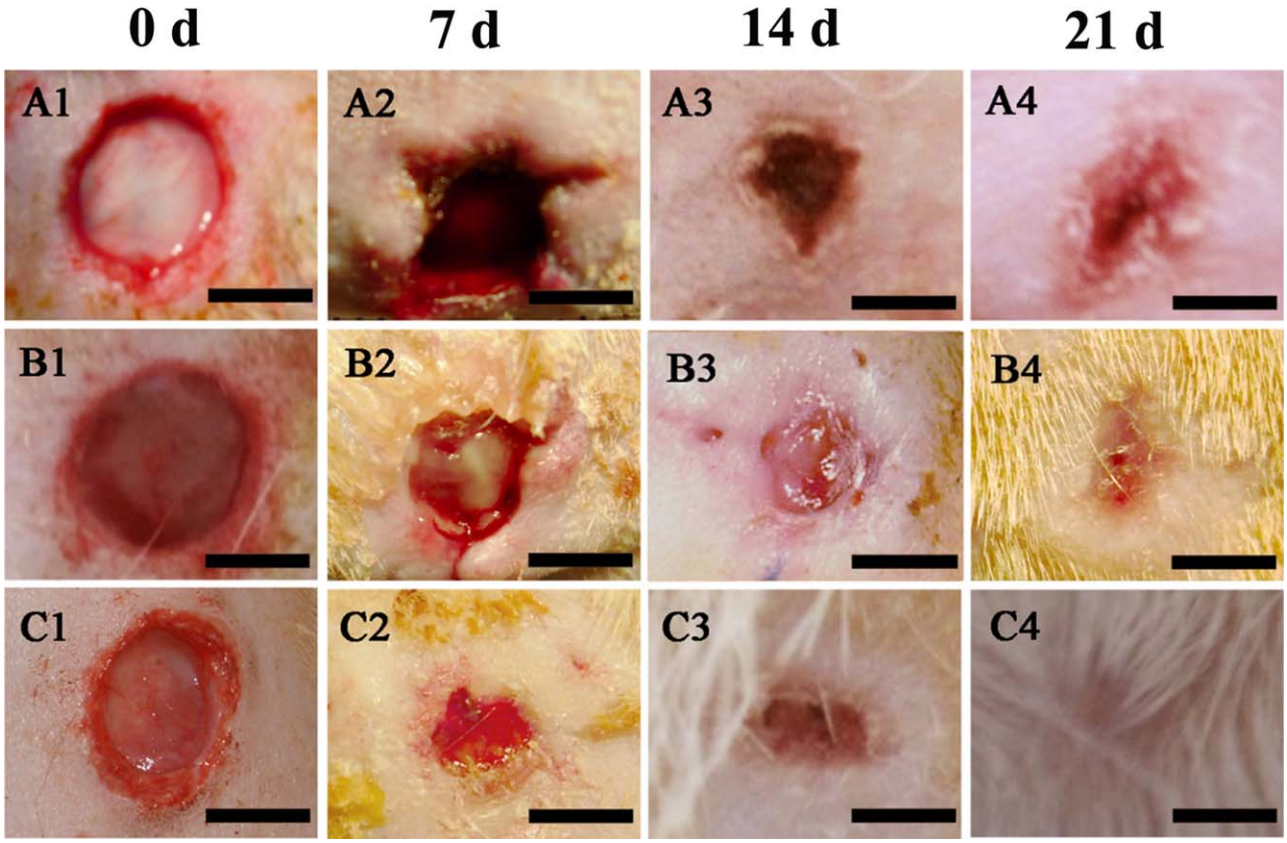


Figure 6. Photographs of wound treated with control group (A1–A4), PGAS/PGA-H 1 : 1 (B1–B4), SOD-PGAS/PGA-H 1 : 1 (C1–C4) at days 0, 7, 14, and 21, respectively; All wounds were covered with 3M wound dressing and gauze. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

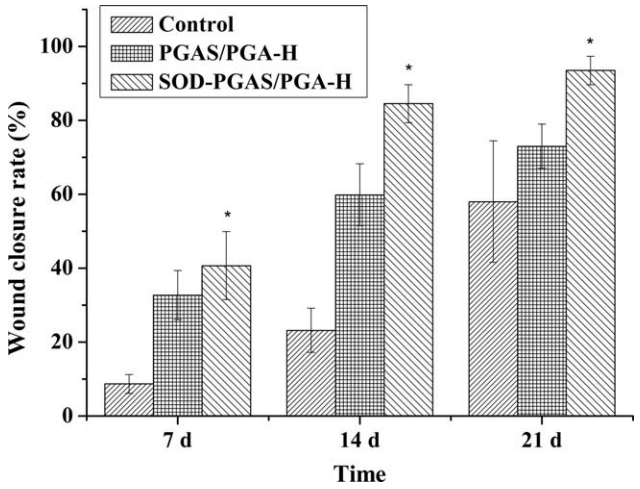


Figure 7. Percentages of wound closure after distinct time intervals. Values are expressed as mean ± SD. The statistical significance of the difference between wounds treated with PGAS/PGA-H and SOD-PGAS/PGA-H was determined by the Student's *t*-test. Results are indicated as $^{*}P < 0.05$.

Compared with other groups, the wounds treated with SOD-PGAS/PGA-H exhibited the fastest wound closure.

Our results indicated that both PGAS/PGA-H and SOD-PGAS/PGA-H accelerated wound healing, but SOD-PGAS/PGA-H promoted wound healing faster. These results are consistent with previous reports.[36] Chiumiento *et al.* showed that their prepared carboxymethylcellulose hydrogel loaded with SOD could promote wound healing.[37] It has also been reported that nitric oxide (NO) promotes processes central to wound healing, but excessive production of superoxide anion (O_2^-) could inactivate NO and delay wound healing.[38] SODs are enzymes that catalyze the dismutation of O_2^- into oxygen and H_2O_2. It is known that treatment with SOD decreases ROS generation and oxidative stress.[39] This may be an explanation for the fact that hydrogel loaded with SOD promotes wound healing faster. Besides, we used γ-PGA-based hydrogel, which is high water absorption and biocompatible, as the carrier to protect SOD from inactivation and guarantee that SOD exerts its best effect on wound healing.

Histological Analysis

The wound healing effects of our hydrogels were estimated from wound inflammatory reactions and the epidermis repair effect by H&E staining of the wound tissue (Figure 8). At day 7, inflammation of the wound treated with 3M wound dressing and gauze only was more severe than the wounds treated with PGAS/PGA-H and SOD-PGAS/PGA-H. Furthermore, more inflammatory cells had penetrated the intramuscular tissue beneath the wound in control group. At day 14, inflammation of the wounds treated with PGAS/PGA-H and SOD-PGAS/PGA-H was less severe than inflammation at day 7, while the degree of inflammation of the control group remained high. At day 21, re-epithelialization in the wounds treated with SOD-PGAS/PGA-H was more complete than re-epithelialization in

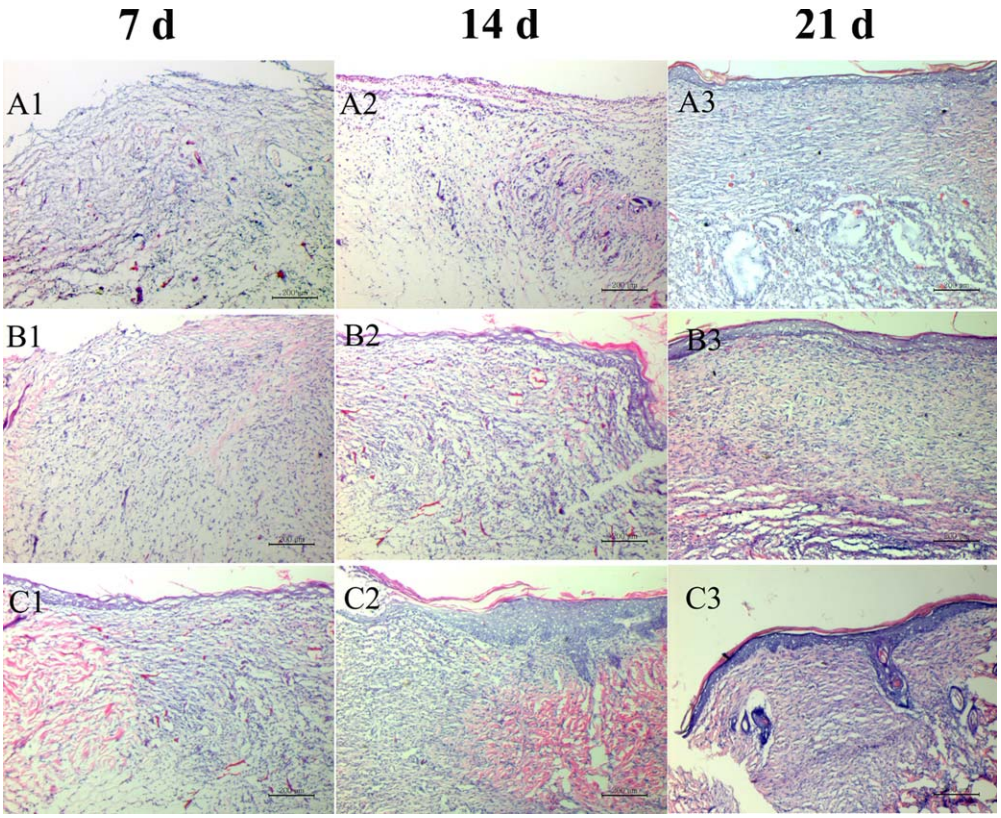


Figure 8. Images of the H&E-stained slices of wound sites treated with control group (A1–A3), PGAS/PGA-H 1 : 1 (B1–B3), SOD-PGAS/PGA-H 1 : 1 (C1–C3) at days 7, 14, and 21, respectively; all wounds were covered with 3M wound dressing and gauze. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

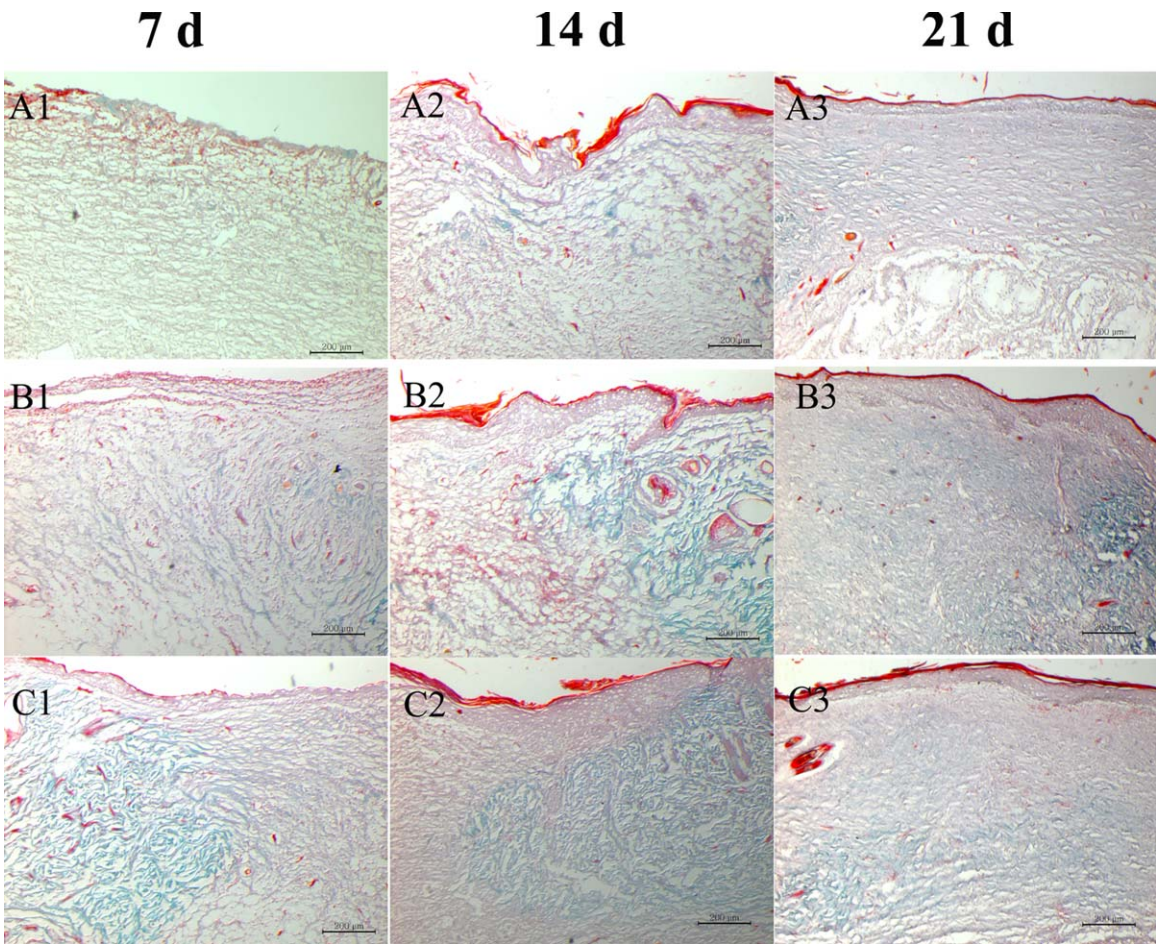


Figure 9. Masson's trichrome staining for collagen. Tissue sections from wound skin of various groups were stained with Masson's trichrome staining for collagen formation (A1–A3: control; B1–B3: PGAS/PGA-H 1 : 1; C1–C3: SOD-PGAS/PGA-H 1 : 1; sampling time: 7 days, 14 days, 21 days, respectively; all wounds were covered with 3M wound dressing and gauze). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

those treated with PGAS/PGA-H, while the surface of the wounds in control group was covered with underlying granulated tissue that had not yet epithelialized. Previously reported results have shown that appropriate inflammation is necessary for wound healing, but prolonged inflammation is detrimental to its outcome.[23,40] This observation is consistent with our results, where wound healing in the test groups (PGAS/PGA-H and SOD-PGAS/PGA-H), especially in the SOD-PGAS/PGA-H group, was faster and more complete than in the control group, probably due to the fact that wounds re-epithelialize more rapidly under moist conditions.[41] Furthermore, also the antioxidant properties of SOD seem to have contributed to the faster acceleration of wound healing in the SOD-PGAS/PGA-H group compared with the PGAS/PGA-H group.

Type I collagen, the predominant collagen form in human skin, is produced mainly by fibroblasts.[42–44] Collagen is a major determinant of the increase in tensile strength of healing wounds.[45] Collagen deposition and granulation tissue formation play an important role in wound healing and reconstruction of tissue.[46,47] The histological analysis of granulated rat tissue from our treated groups at different days was shown in Figure 9. The results indicated that wounds treated with SOD-PGAS/PGA-H exhibited more abundant mature and compact collagen than the wounds of other groups. Polymers with three-dimensional structure have been reported to promote cell growth, cell adhesion, and cell function in an extracellular matrix.[48] Therefore, another reason for the more efficient wound healing in our SOD-PGAS/PGA-H group may be the three-dimensional structure of the hydrogel, promoting fibroblast growth, migration to the wound site, and secretion of fibronectin and collagen.

CONCLUSIONS

We prepared SOD-PGAS/PGA-H for wound healing. SOD was loaded into hydrogels to scavenge the superoxide anion and γ-PGA was modified with taurine to load more SOD. PGAS/PGA-H had high water absorption properties delivering the important moist environment. SOD released from the hydrogel maintained high enzyme activity and SOD-PGAS/PGA-H could scavenge the superoxide anion effectively. *In vivo* results showed that SOD-PGAS/PGA-H could promote collagen deposition, epithelialization, and accelerate the healing of moderately exuding wounds. Therefore, SOD-PGAS/PGA-H would be a good candidate for wound healing applications. Our findings suggest that the combined antioxidant and hydrogel by reducing

oxidative stress and creating moist microenvironment should promote the chronic wound healing. The approach should help with better design of next generation of wound dressing.

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