Feasibility Study of Tissue Transglutaminase for Self-Catalytic Cross-Linking of Self-Assembled Collagen Fibril Hydrogel and Its Promising Application in Wound Healing Promotion

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ABSTRACT: Collagen-based bio-hydrogels are undoubtedly a hot spot in the development of biological dressings for wound healing promotion. Herein, glutamine transaminase (TGase), a biological nontoxic cross-linker with high specific activity and reaction rate under mild conditions, was utilized for the self-catalytic cross-linking of the regenerated collagen (COL) fibril hydrogel fabricated through a molecular self-assembly method. The results showed that the natural triple helical conformation of COL remained completely integrated after self-catalytic cross-linking TGase, which was definitively the fundamental for maintaining its superior bioactivity. It was worth noting that TGase could promote the self-assembly process of COL building blocks into a higher order D-period cross-striated structure. Also, the reconstructed TGase cross-linked COL fibrils exhibited a higher degree of interfiber entanglements with more straight and longer fibrils. Meanwhile, the thermal stability of COL was significantly improved after introducing TGase. Besides, the cytocompatibility analysis suggested that the regenerated COL fibril hydrogel showed excellent cell growth activity and proliferation ability when the dosage of TGase is less than 40 U/g. Further, animal experiments indicated that the targeted COL fibril hydrogel could significantly promote skin wound healing, exhibiting better capacity of skin tissue for regeneration than the COL hydrogel untreated as expected. Therefore, the reconstructed TGase cross-linked COL fibril hydrogel could serve as a novel soft material for wound healing promotion.

INTRODUCTION

Collagen (COL) is a major component of the extracellular matrix and widely presented in connective tissues. Due to its natural and unique triple helical structure, COL has distinct functional properties, such as excellent biocompatibility, hemostatic properties, biodegradability, and induction of cell growth and differentiation, which has been widely used in cosmetics, biomedical materials, tissue engineering, wound healing, and other fields. Studies have shown that, as a biomedical material, COL can activate the expression of characteristic groups of cells and promote the adhesion and growth of cells. Therefore, COL-based medical materials are considered to be the most promising substrates for biomedical applications. As one application, COL hydrogel is a promising scaffold material owing to its excellent properties of water retention and the ability to maintain a spherical morphology of encapsulated cells and promote tissue regeneration, exhibiting a promising soft biomaterial for medical dressing, drug delivery, and tissue engineering. COL could spontaneously self-assemble into hydrogels with aligned fibril structures at physiological conditions, mimicking the structure and biofunctionality of biological tissues from the nanoscopic to macroscopic length scales. However, these spontaneous hydrogels suffer from the relatively weak mechanical properties, inferior stability (thermostability and structural stability) in vivo, inadaptable enzyme degradation, etc. Therefore, an additional cross-linking step is extremely required to enhance the natural physicochemical performances of COL. Many different methods exist for the improvement of the natural physicochemical performances of COL in vivo, including physical modification, chemical modification, and biological modification. Thereinto, chemical cross-linking methods are more diverse and mostly used. Up to date, many synthetic or natural derived cross-linkers, including...
formaldehyde, glutaraldehyde, polyepoxy compounds, genipin, cyanamide, and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride, have been broadly utilized for the chemical modification of collagen-based biomaterials. Unfortunately, the cytotoxicity and ability to significantly improve the physicochemical properties of these cross-linkers could not be met simultaneously, which has restricted their potential applications seriously. Physical methods include dehydrothermal treatment (DHT), and ultraviolet radiation (UV irradiation), among others. DHT treatment universally requires a longer reaction time when compared to UV irradiation, which consequently increases the possibility of COL degradation; however, UV irradiation is only effective for thin or transparent materials. Although all of these methods can increase the tensile strength of the material, they cause the natural structure of the COL to be broken to varying degrees. Furthermore, the physical methods result in weaker bonds compared with the chemical methods and require a long reaction time.\(^1\) Biological modification is a highly efficient and nontoxic method to promote COL stabilization through the addition of enzymes. The biomass enzymes have several advantages, including specificity, mild reaction conditions, and biodegradability. Avoiding the introduction of a toxic cross-linker is important; thus, biological modification is an ideal mode of action for COL-based medical materials.

Transglutaminase (TGase), a nontoxic cross-linker with high specific activity and reaction rate under mild conditions, has been widely used for the cross-linking of protein. To date, it has been applied in the fields of medicine,\(^1\) food,\(^2\) and tissue engineering.\(^3\) TGase, widely found in animals, plants, and microorganisms, is composed of 331 amino acids and has a cysteine residue site as the active center of the monomeric protein.\(^4\) It has been reported that TGase could mainly catalyze the acyl transfer reaction between the γ-carboxamide group (acyl donor) of the glutamine residue and the ε-amino group (acyl acceptor) of the lysine residue in the collagen peptide chains, thus resulting in the intermolecular and intramolecular formation of ε-(γ-glutamyl)-lysine covalent bonds.\(^5\) Furthermore, the cross-linking reaction of microorganism-derived TGase and collagen can improve the stability and mechanical strength.\(^6\) Therefore, TGase could serve as an ideal biological cross-linker to improve the physicochemical properties of collagen. However, the studies rarely evaluate the effects of TGase on the fibrillogenesis of collagen and the microtopography of the resulting regenerated collagen fibrils.\(^7–9\) Meanwhile, the feasibility of collagen after cross-linking TGase for promising applications in wound healing promotion still remains unknown.

Herein, a series of self-assembled COL fibril hydrogels cross-linked by different concentrations of TGase as the nontoxic biocatalyst and cross-linker were obtained. The effects of TGase on the fibrillogenesis process, thermostability, and microstructure of COL were synthetically investigated. Furthermore, the cytocompatibility in vitro and histocompatibility in vivo of the resulting cross-linked collagen fibril hydrogel has been evaluated. Our aim is to fabricate the self-cross-linking COL fibril hydrogel for potential applications as soft materials for wound healing promotion.

## MATERIALS AND METHODS

### Materials

The porcine type I COL was self-prepared according to our previous report,\(^2\) and microbial transglutaminase was purchased from Shanghai Yuanye Biotechnology Co., Ltd., China. Unless noted otherwise, all the reagents used in the experiment are all analytical grade.

### Preparation of the Self-Catalytic Cross-Linked COL Fibril Hydrogel

According to the properties of TGase under aqueous phase,\(^1\) the TGase self-catalytic cross-linked COL conditions are as follows: the COL was prepared into a 2 mg/mL COL solution (pH 7.0), the temperature and reaction time was 25 °C and 6 h, respectively, and the enzyme dosages were 0, 10, 20, 40, 60, and 80 U/g (U/g collagen). After the reaction was complete, the cross-linked COL solution was cultivated at 37 °C for 1 h to prepare the regenerated COL fibril self-assembled hydrogel. In addition, the cross-linked COL sponge was obtained by vacuum freeze-drying under the temperature of −20 °C for further testing.

Scheme 1 shows the molecular interactions between TGase and COL and the effects of TGase on the fibrillogenesis of COL, which indicates that TGase mainly catalyzes the acyl transfer reaction between the γ-carboxamide group (acyl donor) of the glutamine residue and the ε-amino group (acyl acceptor) of the lysine residue in the collagen peptide chains, thus resulting in the intermolecular and intramolecular formation of ε-(γ-glutamyl)-lysine covalent bonds. Scheme 1 also presents that TGase could further promote the self-assembly of COL building blocks into a higher-order D-period cross-striped structure, which is confirmed in the fibrillogenesis process analysis.
Fourier Transform Infrared (FT-IR) Analysis. The secondary structure of the cross-linked COL sponge was analyzed by an FT-IR spectrophotometer in the 4000–500 cm\(^{-1}\) range (PerkinElmer Company, England). One hundred milligrams of KBr crystal and 2 mg of the sample were mixed and then compressed into flakes. The measurements were carried out in a dry atmosphere (relative humidity: \(<65\%\) ) at room temperature (21 ± 1 °C). All spectra were collected in the wavenumber range of 4000–500 cm\(^{-1}\) and recorded by transmission mode with a 4 cm\(^{-1}\) resolution time and 32 scans.

Circular Dichroism (CD) Analysis. The self-catalytic cross-linked COL fibril hydrogel was re-dissolved in a solution of 0.05 M acetic acid and then prepared into a sample liquid with a concentration of 0.1 mg/mL. Before the test, the sample liquid was placed in a biological incubator at 25 °C for 1 h and measured by a circular dichromatic spectrometer (Jasco, J-810, Japan) in the wavelength range of 190–260 nm at 25 °C. The corresponding molar ellipticity was collected at the rate of 20 nm/min.

Differential Scanning Calorimetry (DSC) Analysis. The thermal degradation process and thermal phase transition temperature of the cross-linked COL sponges were analyzed by differential scanning calorimetry (Netzsch DSC 2000PC, Germany). Three to five milligrams of freeze-dried samples was sealed in the DSC aluminum crucible and heated at the speed of 10 °C/min over the range of 20–140 °C under a nitrogen flow of 60 mL/min. Besides, the empty crucible was used as the reference.

Fibrillogenesis Process of TGase-COL Solutions. The COL was dissolved in 10 mM PBS solution (containing 110 mM NaCl) at pH 7.0 and prepared into a COL solution with a concentration of 1 mg/mL. Then, the different concentrations of TGase was added to the COL solution and stirred magnetically for 4 h. After the solution was thoroughly mixed, it was poured into a cuvette. In order to investigate the effect of TGase on the fibrillogenesis process of COL, the turbidity of the mixed solution was monitored at 313 nm by an ultraviolet spectrophotometer (Perkin Elmer Lambda 25 UV/VIS, US) at 37.0 ± 0.1 °C.

Atomic Force Microscopy (AFM) Morphology. The self-catalytic cross-linked COL fibril hydrogel was diluted with 0.1 M acetic acid into a sample solution with a concentration of 20 μg/mL, and 5 μL of sample solution was dropped to the mica tablet. The mica tablets carrying the samples were directly placed on the AFM (SPM-9600, SHIMADZU Co., Ltd.) sample table after drying naturally at room temperature, and the observation was set in a noncontact tapping mode. The force constant is 2.8 N/m, the scanning frequency is 1 Hz, the probe microcantilever length is 180 μm, and the tip radius of curvature is 10 nm.

Scanning Electron Microscopy (SEM) Analysis. The self-catalytic cross-linked COL fibril hydrogels were diluted to a concentration of 5 mg/mL with 0.1 M acetic acid, and 200 μL was paved on a clean coverslip. The SEM test sample was obtained by drying naturally, washing with deionized water repeatedly, fixing with glutaraldehyde, dehydration with gradient dehydration, critical-point drying, and other steps. After the liquid nitrogen was fractured and the surface was sprayed with gold, the test samples were observed in the scanning electron microscope at the acceleration voltage of 5 kV (Hitachi S3000N, Hitachi, Ltd., Japan).

In Vitro Biocompatibility Study. According to the international standard and the method of Streifel, the cytotoxicity of the self-catalytic cross-linked COL fibril hydrogel was evaluated by the MTT method with L929 as the model cell, and further the growth and proliferation of cells on the self-catalytic cross-linked COL fibril hydrogel were observed by confocal laser scanning microscopy (CLSM) and SEM.

In Vivo Study. The rats with a body weight of about 300 g were selected. All experimental animals were handled in accordance with the guidelines formulated by the National Institutes of Health (China) on human use and care of laboratory animals. The Animal Care and Use Committee of Sichuan University has approved all procedures performed for animals. Based on the previous research, a rat model of wound healing was established. Briefly, Sprague–Dawley rats (average weight: 300 g) were all anesthetized by intraperitoneal injection of 3% pentobarbital sodium (1 mL/kg). Then, an area of approximately 1 × 2 cm² full-thickness skin excision wounds was artificially created on both sides of the dorsal surface of the rats (two injuries/rat) under aseptic conditions. Subsequently, COL-GA group, COL-TGase-0U/g, and COL-TGase-40U/g specimens were implanted in the right injured side of the rats, and the left side was regarded as a contralateral control. All the wounds were wrapped with medical gauze, and an interrupted suture was further performed. Finally, the animals were euthanized at 1, 2, and 3 weeks after operation, and the injured and surrounding tissues were taken and fixed by 10% buffered formalin for histopathological analysis. The tissue samples were then obtained after dehydration, defatting and paraffin-embedding, and Masson staining according to our previous report. The appropriate magnification was selected by an optical microscope to observe wound healing and new tissue formation.

RESULTS AND DISCUSSION

FT-IR Analysis. FT-IR spectroscopy can accurately reflect the secondary structure of COL and has been reported to be used to study the characteristic groups and basic skeletons of COL. The triple helical conformation of COL is particularly
imported for maintaining its natural functional characteristics. Figure 1 showed the FT-IR spectra of COL treated with different concentrations of TGase, and the absorption peaks of each characteristic amide band of COL could be still clearly identified. The amide A and B bands of COL appeared at approximately 3326 and 3085 cm\(^{-1}\), respectively, and were the characteristic absorption peaks of the \(-\text{OH}\) and \(-\text{NH}_2\) groups of the molecular side chain, which were caused by the N\(\equiv\)H bond stretching vibration. The amide I band that appeared at about 1654 cm\(^{-1}\) was mainly associated with the stretching vibration of the amide group C\(\equiv\)O of the COL molecular skeleton, while the amide II band appearing at 1560 cm\(^{-1}\) was the absorption peak formed by the bending vibration of the N\(=\)H bond and the telescopic vibration of the C\(\equiv\)N bond. In addition, amide III band at 1240 cm\(^{-1}\) was predominantly attributed to C\(\equiv\)N stretching vibration, N\(=\)H bending vibration, and the wagging vibrations of the CH\(_2\) group in the glycine backbone and proline side chains, which was consistent with observations previously reported.\(^{35-38}\) As shown in Figure 1, the characteristic absorption peaks of COL after treatment with different dosages of TGase were obvious, and it was confirmed that TGase did not destroy the triple helical structure of COL. After the introduction of TGase, the intensity of the absorption peaks of each amide band in the IR spectrum was reduced to varying degrees. With the increasing concentration of TGase, the absorption peaks of amides A and B exhibited widening and underwent a weak blue shift. This may have resulted from the generation of intermolecular or intramolecular covalent cross-linking among COL molecules producing a certain disruption for the hydrogen bonds between the COL molecules chain and increasing the order of the molecules, which also suggested that COL had a certain degree of self-assembly.

**DSC Analysis.** DSC is commonly used to study the thermal stability of COL. The thermal denaturation temperature \(T_d\) is defined as the peak value of the corresponding endothermic process when the triple helical structure of COL is irreversibly damaged. To some extent, it can also be used to measure the degree of cross-linking between molecules\(^{34-36}\) and can reflect the integrity of the triple helical structure of COL. The typical DSC spectrum of COL has a small peak at about 35 °C, which indicates that the three \(\alpha\)-peptide chains of COL undergo relaxation to a certain extent. With the heating temperature reaching \(T_d\), the triple helical structure of COL is completely dissociated into three \(\alpha\)-peptide chains, causing many of the excellent properties of COL to disappear or diminish.\(^{37}\) Figure 2 shows the DSC spectrum of COL treated with different dosages of TGase. As for untreated COL, the \(T_d\) was found to be 60.6 °C. The causes of this heat conversion included the interactions of protein and water, the breaking of hydrogen bonds, and the evaporation and vaporization of combined water.\(^{38}\) When COL was treated with TGase, its \(T_d\) increased to varying degrees from 60.6 to 82.8 °C, indicating that the addition of TGase was conducive to the improvement of the COL thermal stability.\(^{39}\) This is mainly due to the increased dosage of TGase, the improved osmosis binding of enzymes to COL, and the formation of more \((\gamma\text{-glutamyl})\)-lysine covalent cross-linking bonds of intermolecular or intramolecular COL molecules. Consequently, the thermostability of COL was increased to a certain extent, which is significantly beneficial.

![Figure 2. DSC curves of COL cross-linked with different dosages of TGase.](image)

![Figure 3. CD spectra of COL cross-linked with different dosages of TGase.](image)

![Figure 4. The turbidity curves of COL cross-linked with different dosages of TGase on the fibrillogenesis process of collagen at 313 nm ((A) 0 U/g, (B) 10 U/g, (C) 40 U/g, (D) 80 U/g).](image)
for maintaining its biological activity and promoting the use of COL biomedical materials.

**CD Analysis.** CD can be used to detect specific stereostructures of α-helix, β-sheet, β-turns, and random coils in the secondary structure of proteins. It can also be used to characterize the integrity of COL triple helical structures to some extent. The typical CD spectra of COL exhibit positive and negative absorption peaks at 221 and 198 nm, respectively. The ratio of the positive peak intensity to the negative peak intensity (Rpn) is typically used to determine the integrity of the COL triple helical structure in dilute solutions. When its helical structure is destroyed, the positive peak at 221 nm will disappear in the CD spectrum, and the trough at ~198 nm will also shift to a lower wavelength. The CD spectra of COL treated with different dosages of TGase are shown in Figure 3. This spectrum still displayed the typical pattern of natural COL, indicating that the triple helical structure of COL was still kept complete after TGase treatment, which is consistent with the findings of the FT-IR analysis. It is worth noting that the Rpn value of COL after treatment with TGase was significantly greater than that of pure COL. This may suggest

Figure 5. AFM images of collagen cross-linked with different contents of TGase ((A) 0 U/g, (B) 10 U/g, (C) 20 U/g, (D) 40 U/g, (E) 60 U/g, (F) 80 U/g).

Figure 6. SEM images at different magnifications after COL cross-linking different concentrations of TGase ((A, a) 0 U/g, (B, b) 40 U/g, (C, c) 80 U/g).

Figure 7. The effect of TGase dosage on the proliferation of fibroblasts on the COL fibril hydrogel at different time intervals (1, 3, and 5 days).
that the tidiness and orderliness of COL molecules after treatment with TGase are significantly higher when compared with pure COL and may also be related to the slight differences in their secondary structures. Compared with the 80 U/g concentration of TGase, the Rpn exhibited a greater improvement when the concentration of TGase was 40 U/g, which may be because the excessive catalytic cross-linking may have a negative effect on the maintenance of triple helical structure of collagen. Meanwhile, a higher Rpn value may also suggest that the formation of the covalent bond induces the self-aggregation of COL molecules and accelerates the formation of COL fibrils.

**Fibrillogenesis.** Figure 4 displays the turbidity versus time curves of COL treatment with different dosages of TGase. The increase in TGase concentration is expected to have an impact on the turbidity of COL at 313 nm. Turbidity experiments are a common method for studying COL self-assembly in vitro, which refers to the process of COL fiber formation. The self-assembly of COL molecules follows the cooperative nucleation-growth mechanism and is mainly divided into three stages: initial lag period (the growth phase of nucleation), fiber growth period, and growth balance period. During the growth phase of nucleation, the absorbance increases at a slow rate, while the absorbance rapidly increases and COL accumulates into COL fibrils during the fibril growth period. Finally, the absorbance reaches the maximum value in the growth balance period, and the three-dimensional network structure of the COL is formed at this stage.  

As seen from Figure 4, the nucleation period of COL after treatment with different concentrations of TGase was very short, indicating that their nucleation period of fibrillogenesis process was relatively rapid. The total turbidity change ($\Delta h$) and the time reached $\Delta h/2(t_{1/2})$ of pure COL were also calculated in the current study and found to be 0.1607 and 3.49 min, respectively. After the introduction of TGase, the total turbidity increased significantly, and the degree of absorbance was higher with the growth balance period reached. This proved that the formation rate of COL fibrils increased and demonstrated that TGase had a significant promoting effect on the self-aggregation process of COL. This may be directly related to the fact that TGase catalyzes the formation of covalent cross-linking of intermolecular or intramolecular COL molecules.

**AFM Morphology Analysis.** AFM was used to investigate the effect of TGase on the morphology of COL fibrils. As shown in Figure 5, a large number of tortuous COL fibrils were tightly arranged and exhibited dense interfiber tangles. Following the introduction of TGase, the COL fibrils exhibited a higher degree of entanglement, and the interstices between fibrils were gradually decreased, which is advantageous for improving the stability of COL fibrils. In addition, thickening of the fiber diameter of COL fibrils was observed to a certain degree. Furthermore, it was shown that TGase catalyzed the self-cross-linking reaction among COL molecules’ ontology and induced self-aggregation between COL molecules. At the same time, the D-period cross-striated structure of COL fibrils could be clearly observed subsequent to the catalysis of cross-linked COL by different TGase concentrations. These results proved that the natural structure of COL fibrils can still be greatly retained after catalytic cross-linking, which plays a major role in the mechanical and biological functions of COL matrix materials.

**SEM Analysis.** In the abovementioned FT-IR, CD, and AFM analyses, TGase treatment was found to promote the self-aggregation of COL, while the hydrogen bonds between COL molecule chains also provided a certain driving force for the self-aggregation of COL. As shown in Figure 6, the SEM images at different magnifications showed the TGase self-catalytic cross-linked COL fibrils used at different concentrations (0, 40, and 80 U/g). COL fibrils treated with different concentrations of TGase exhibited a typical three-dimensional network structure. COL fibrils were tightly intertwined, and the arrangement and orientation of fibrils were disordered and irregular. However, it can be clearly observed that, with the increase in TGase concentration, the fibers formed closer tangles and were interspersed with each other, resulting in a reduction in voids and a more substantial fiber diameter. It is worth noting that fission occurred in the segmental horizontal stripes of COL fibrils after cross-linking TGase. This may be due to the fact that the self-cross-linking could influence the natural lateral and transverse aggregation of COL simultaneouly during the fibrillogenesis process; therefore, the periodic interval between D-bandning of the fibrils presented expanded and even separated finally.

**In Vitro Cytotoxicity Assay.** Good cell compatibility of COL-based materials is a necessary prerequisite for their use as a medical material.  

Figure 7 illustrates the optical density values acquired in the MTT assay for the L929 fibroblast cultured in the leaching liquor after 1, 3, and 5 days. Obviously, the optical density for the L929 fibroblasts cultured in all the extraction liquids from TGase cross-linked collagen specimens was significantly higher than that of the negative control, indicating their nontoxic nature. It is still worth noting that the optical density of COL-TGase-80U/g is slightly lower than that of COL-TGase-40U/g in the third and fifth day. Combined with the CD and fibrillogenesis analysis, 40 U/g may be the optimal concentration of TGase for the cross-linking of collagen.

Figure 8 displays CLSM diagrams of fibroblasts co-cultured with the COL fibril hydrogel for 3 days. After 3 days of culture, the morphology of fibroblasts on COL-based materials was still...
normal. Nevertheless, fibroblasts had a long spindle rather than a circular shape (apoptotic state), demonstrating that the TGase self-catalytic cross-linked COL was well compatible. With the increase in TGase concentration, an increasing trend in the cell density was also observed. According to the CD and turbidity analyses, the addition of a higher concentration of TGase led to a higher tendency for the formation of COL regenerated fibers. Furthermore, it was illustrated that COL regenerated fibers can better increase the adhesion and proliferation of cells and improve the cell compatibility of COL-based materials to a certain extent.

To further illustrate the compatibility of the COL fibril gel cross-linked by different TGase dosages on the wound healing rates of the rats. SEM images were used to observe the cell growth. As shown in Figure 9, L929 fibroblasts grew well on the specimens, indicating that different concentrations of the TGase cross-linked COL fibril gel exhibited excellent compatibility. The number of cells on the COL fibril gels treated with TGase increased, which may be due to the modified COL fibril gels providing adhesion sites for L929 fibroblasts, thereby increasing the cell proliferation rate. As a biomass enzyme, the nontoxic behavior of TGase enables cells that adhere to the COL matrix to survive. Therefore, the catalytic cross-linked COL material can be applied in biomedicine.

**Figure 9.** SEM images of L929 fibroblasts cultured for 3 days on the COL fibril gel with different contents of TGase treatment ((A) 0 U/g, (B) 40 U/g, (C) 80 U/g).

**Figure 10.** The effect of the COL fibril gel cross-linked by different TGase dosages on the wound healing rates of the rats.

**In Vivo Evaluation.** Figure 10 showed the comparison of the effects of COL-GA, COL-TGase-0U/g, and COL-TGase-40U/g groups on the healing rate of full-thickness cutaneous wounds.
wounds. Both COL-TGase-0U/g and COL-TGase-40U/g could obviously promote wound healing compared with COL-GA. The wound healing rate in both COL-TGase-0U/g and COL-TGase-40U/g was more than 50% at 2 weeks postoperatively, even the wound healing rate in the COL-TGase-40U/g group reached ∼75%. At 3 weeks postoperatively, all the groups were almost completely healed. Figure 11 shows the histological observation of the wound sections stained with Masson for 1, 2, and 3 weeks. One week after healing, the wound was enriched with a large number of fibroblasts, which secrete COL to form COL fibers in order to fill the wound. As shown in Figure 11a1, b1, a greater number of inflammatory cells were detected in the COL-GA group as compared with that in the COL-TGase-0U/g group, while fewer inflammatory cells were observed in the COL-TGase-40U/g group. After 2 weeks, the epidermal cells at the edge of the wound were activated, proliferated, and differentiated, and the epithelial cells of the hair follicle mass in the dermis around the wound migrated to the wound surface, which accelerates the epithelialization of the wound. The wounds of COL-TGase-40U/g and COL-TGase-0U/g groups exhibited complete epithelialization, while small vessels and granulation tissues also existed. The degree of formation of the COL-TGase-40U/g group was relatively larger and denser. After 3 weeks, the epithelialization of the wounds in each group was completed, and the stratum corneum was formed on the outermost surface. Furthermore, the formation of the granulation tissue and small blood vessels was observed in each group, with the highest formation degree observed in the COL-TGase-40U/g group. It is worth noting that the hair root was formed in the COL-TGase-40U/g group, indicating that the wound surface had healed completely. Finally, the results also revealed that the COL-TGase-40U/g group had a significant effect on wound healing.

**CONCLUSIONS**

Herein, the nontoxic glutamine transaminase (TGase) with high specific activity was used for the self-catalytic cross-linking of the regenerated collagen (COL) fibril hydrogel fabricated through a molecular self-assembly method. The results indicated that TGase can catalyze the covalent cross-linking among the intermolecular or intramolecular COL and promote the self-aggregation of COL to assemble into aligned collagen fibrils, mimicking the structure and biofunctionality of biological tissues from the nanoscopic to macroscopic length scales. After introducing TGase, the triple helical structure of natural COL is still remained, and its thermal stability is greatly improved, which plays an important role in maintaining the biological function of the collagen matrix material. Furthermore, the resulting COL fibril hydrogel can significantly increase cell adhesion and proliferation. In addition, the COL fibril hydrogel could meaningfully promote skin wound healing, and its capacity of skin tissue for regeneration was better than the COL hydrogel as expected. Therefore, the COL fibril hydrogel self-catalytic cross-linked by TGase is expected to be a novel soft material for wound healing.

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**Notes**

The authors declare no competing financial interest.

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