Supporting Information

One-Pot, Self-Catalyzed Synthesis of Self-Adherent Hydrogels for Photo-Thermal, Antimicrobial Wound Treatment

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1. Experiment

1.1. Materials.

Chitosan powder (CS, Deacetylation degree >90%; Viscosity=50~800 mpa.s) was purchased from Zhejiang Gold·Shell Pharmaceutical Co., Ltd, China. Ferric chloride (FeCl$_3$), Acrylamide (AM), N, N’-Methylene bisacrylamide (MEBA) and Ammonium persulphate (APS), were purchased from KESHI, Chengdu, China. Tannic acid (TA) and N, N, N’, N’-Tetramethylethylenediamine (TMEDA) was purchased from Sigma-Aldrich (USA). Phosphate buffer saline (PBS) was purchased from Hefei new source biological technology co. LTD. Fetal bovine serum (FBS), DMEM medium, 1% penicillin-streptomycin solution and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Hyclone, USA. Epidermal growth factor (EGF) was purchased from Shanghai Primegene Bio-Tech, China. The EGF assay kit was purchased from Wuhan ColorfulGene Biological Technology Co., Ltd, China. Live/dead cell assay kits were purchased from Jiangsu KeyGEN BioTECH Corp., Ltd., China.

1.2. Preparation of various hydrogels.

Six types hydrogels, including PAM, PAM/CS, TA-500, TA-1000, TA-1500 and TA-2000 hydrogels were prepared by radical polymerization. The name and composition of various hydrogels were listed in Table S1.

Table S1. The name and composition of hydrogels.

<table>
<thead>
<tr>
<th>Materials</th>
<th>AM (g)</th>
<th>CS (mg)</th>
<th>TA (mg)</th>
<th>FeCl$_3$·6H$_2$O (mg)</th>
<th>H$_2$O (mL)</th>
<th>APS (mg)</th>
<th>MEBA (mg)</th>
<th>TMEDA (μL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAM</td>
<td>2.6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>25</td>
<td>1.5</td>
<td>20</td>
</tr>
<tr>
<td>PAM/CS</td>
<td>2.6</td>
<td>50</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>25</td>
<td>1.5</td>
<td>20</td>
</tr>
<tr>
<td>TA-500</td>
<td>2.6</td>
<td>50</td>
<td>5</td>
<td>2.5</td>
<td>10</td>
<td>25</td>
<td>1.5</td>
<td>0</td>
</tr>
<tr>
<td>TA-1000</td>
<td>2.6</td>
<td>50</td>
<td>10</td>
<td>5.0</td>
<td>10</td>
<td>25</td>
<td>1.5</td>
<td>0</td>
</tr>
</tbody>
</table>
To measure the self-catalytic property of TA@Fe$^{3+}$ for AM/CS solution with APS and MEBA. First, the AM (2.6 g), CS (50 mg), APS (25 mg) and MEBA (1.5 mg) were uniformly dispersed into acetic acid solution (10 mL, 0.2% v/v.%) to obtain the AM/CS solution with APS and MEBA. Then, TA (15 mg), FeCl$_3$·6H$_2$O (7.5 mg) and TA@Fe$^{3+}$ (TA of 15 mg and FeCl$_3$·6H$_2$O of 7.5 mg) were added to the AM/CS solution with APS and MEBA, respectively. Final, the above mixed solution of 5 mL was transferred separately to sample bottle to record the time and situation of the formation of hydrogel at room temperature.

1.3. Swelling tests.

To measure the swelling ratios, various hydrogels ($\Phi$ 10 × 1 mm) were swollen at 25 °C until the equilibrium state was reached in PBS solutions. The weight of hydrogels was determined at different times (0, 3, 6, 12, 24, 48 and 72 h). The swelling ratio was determined by the following equation:\textsuperscript{1}

\[
Swelling\ ratio = \frac{W_S}{W_0}\]

Equation (1)

$W_0$ was the weight of initial samples and $W_S$ was the weight of the swollen samples.

1.4. Mechanical properties test.

The mechanical properties of various hydrogels, including compressive property and tensile property, were performed on a universal testing machine (Instron 5567, USA) according to previous report.\textsuperscript{2} For compression tests, the hydrogels with a cylindrical shape ($\Phi$ 10 × 12 mm) were compressed at a rate of 5 mm/min. The compression strength was defined as the stress value when the compress strain reached to 80%. For tensile testing, the hydrogels were prepared with 25 mm in width and 3 mm in thickness. The gauge length was 5 mm. A cross-head speed of 20 mm/min was used.

1.5. Adhesion tests.

The adhesion testing was performed to measure the adhesive strength of various hydrogels. The
hydrogels were applied to the surface of the porcine skin with a bonded area of 25 mm × 20 mm. The samples were pulled to failure at a crosshead speed of 5 mm/min until the samples separated, using a universal testing machine. The adhesion strength was calculated by the maximum load divided by the initial bonded area. In addition, the adhesive strength of commercial dressings (Cosmopor®E) was tested using a similar method.

1.6. Cumulative release profile of EGF from hydrogels.

The release profiles of EGF from the hydrogels were characterized by the half-change method in PBS solution (pH 7.4).\(^3\) EGF-loaded PAM/CS hydrogel and TA-1500 hydrogel (30 μg/sample) were immersed in the PBS solution (1 mL) and shaken (100 rpm) at 37 °C. At predetermined intervals (1, 3, 7, 11 and 15 day), fifty percent of the release PBS was collected and replaced by fresh PBS. The amount of released EGF was measured by EGF assay kit, according to the manufacturer’s protocol.

1.7. NIR Assisted antibacterial activity.

The antibacterial activity in vitro of the hydrogels was tested by using Staphylococcus aureus (S. aureus, ATCC 6538) and Escherichia coli (E. coli, ATCC 8739) as the representing gram-positive bacteria and gram-negative bacteria. Six types of hydrogels (Φ 10 × 1 mm), including PAM, PAM/CS, TA-500, TA-1000, TA-1500 and TA-2000 were used for the antibacterial tests. The hydrogels were firstly sterilized in 75% alcohol and purified in sterilized PBS for one day to remove the residue alcohol. A volume of 300 μL of bacterial suspension (10⁵ CFU/mL) was added onto the surfaces of each sample in a 48-well culture plate. Then, the samples were randomly divided into two groups, including the group with NIR irradiation and the group without NIR irradiation. For the irradiation groups, the hydrogels were exposed to the NIR laser (808 nm, 2 W·cm⁻²) for 15 min. Next, 700 μL of Luria-Bertani (LB) broth was added into each well to re-suspend the bacterial, and then the optical density (OD) of the bacterial resuspension was measured at 600 nm after incubation for another 12 h at 37 °C. The antibacterial ratio of various hydrogels was calculated from the following equation:\(^4\)

\[
\text{Antibacterial ratio (%)} = \left(\frac{\text{OD}_{\text{control}} - \text{OD}_{\text{Sample}}}{\text{OD}_{\text{Blank}}}\right) \times 100\% \quad \text{Equation (2)}
\]
The specific operation steps of the LB plates technology were as follows. First, the LB medium (60 °C) was added to petri dish and cooled at room temperature to form LB plates. Second, the bacterial resuspension of blank group was diluted to $1\times10^2$ CFU/mL using PBS solution. Then, the bacterial resuspension of PAM/CS hydrogel and TA-1500 hydrogel groups was diluted using the same dilution multiple as blank group. Final, 100 mL diluted bacteria resuspension was coated to the surface of LB plates and cultured for 24 h at 37 °C.

The antibacterial activity *in vivo* of the hydrogels was evaluated in Sprague-Dawley (SD) rat subcutaneous model. All animal experiments have received the animal ethical and welfare permission from the Animal Ethics Committee of the West China Hospital, Sichuan University, Chengdu, China. All experimental protocols used in this study constitute appropriate and acceptable procedures, and were carried out as requested by the committee. Experiments were performed in eight SD rats (Dashuo, Chengdu, China), weighing 250~300 g. The samples ($\Phi\ 5 \times 1\ mm$), including PAM/CS hydrogel and TA-1500 hydrogel, were implanted into the subcutaneous pocket on the back of each rat. Following implantation of the samples, the subcutaneous pocket was inoculated with an *E. coli* suspension (0.1 mL, $10^8$ CFU/mL). Then, the samples were randomly divided into two groups, including the group with NIR irradiation and the group without NIR irradiation. For the irradiation groups, the hydrogels were exposed to the NIR laser (808 nm, 2 W·cm$^{-2}$) for 15 min. After the operation, the rats were housed individually and given access to food and water ad libitum. At day 7, the rats were euthanized by the cervical dislocation, and the skin was collected and fixed in 10% formalin. Then the skin surface tissues were cut into 4-μm thick vertical slices for cross-sectional observation. Histological observations were performed after hematoxylin-eosin (H&E) staining.

1.8. *In vitro* cell activity evaluation.

*In vitro* cell culture was performed by seeding fibroblasts (*NIH3T3, Stem Cell Bank, Chinese Academy of Sciences, SCSP-515*) on the various hydrogels. Six types of hydrogels ($\Phi\ 10 \times 1\ mm$), including PAM, PAM/CS, TA-500, TA-1000, TA-1500 and TA-2000 were used to evaluate cell behaviors. The blank group without any treatment were used as control groups. Before cell culture,
samples were sterilized in 75% alcohol and purified in sterilized PBS for one day to remove the residue alcohol. 3T3 cells at passage 2-5 were seeded on the hydrogels with density of $5 \times 10^4$ cells/sample. The cell-seeded hydrogels were cultured in DMEM supplemented with 10% of fetal bovine serum and 1% of penicillin-streptomycin solution (HyClone) in a CO$_2$ incubator at 37 ºC. The proliferation of the cells seeded on the hydrogels was evaluated by MTT assay after 3 and 5 days of culture. The morphology and spreading of cells seeded on the hydrogels were examined by CLSM (TCSSP5, Germany) after the cells were stained using the Live/dead cell assay kits on day 3.

1.9. **In vitro gene expression.**

The relative gene expression levels of smooth muscle α-actin and collagen type III (COL III) was quantitatively analyzed by qRT-PCR as pervious papers.$^5$ First, the sterile hydrogels, including PAM/CS hydrogel group and TA-1500 hydrogel group, were placed on 24-well plates, and 3T3 cells at passage 2-5 were seeded on the hydrogels with density of $1 \times 10^5$ cells/sample. The blank group without hydrogel were used as control groups. After 3 days of cultivation, the culture medium was removed from each plate, and the cells were collected with cryopreservation tubes. Final, after RNA extraction, reverse transcription, amplification, the relative gene expression levels of smooth muscle α-actin and COL III was detected by qRT-PCR according to specific procedures.

1.10. **In vivo wound repairing experiments.**

The hydrogels were filled in vivo into repair full-thickness skin defect in a rat model. 4 male SD rats weighing 250-300 g each were used. Briefly, the rats were firstly anesthetized and then hair on the dorsal skin was shaved. Then four circular wounds with diameter of 8 mm were created on the upper back of each mouse by a biopsy punch to construct a full-thickness skin defect model. The PAM/CS hydrogel, TA-1500 hydrogel, and EGF loaded TA-1500 hydrogel (30 μg/sample) were filled into the wound defects (Φ 8 × 1 mm). The blank wounds without any treatment were used as control groups. After implantation, the commercial 3M Tegaderm™ film was applied to the wound site to prevent the samples from falling off. The wound area of the rats was recorded at 0, 7, and 15 days. At day 15, the rats were euthanized by cervical dislocation, and the skin was collected and fixed in 10% formalin. Then
the skin surface tissues were cut into 4-μm thick vertical slices for cross-sectional observation. Histological observations were performed after H&E staining.

**1.11. Statistical analysis.**

The data were analyzed using one-way analysis of variance (ANOVA) followed by the Tukey multiple-comparison post hoc test to determine any statistical significance of observed differences between the test groups. The level of statistical significance was set at \( p < 0.05 \).
2. Results

**Figure S1.** The dispersity of TA@Fe\(^{3+}\) in different solvents.

As shown in **Figure S1**, the TA@Fe\(^{3+}\) were formed in different solvent systems, including single AM solvent systems and the AM and CS composite solvent systems. After standing for 30 min, the TA@Fe\(^{3+}\) in AM solvent systems were deposited at the bottom of the sample bottle. However, the TA@Fe\(^{3+}\) in AM and CS composite solvent systems were still evenly dispersed in the sample bottle. The result indicated that the introduction of CS is beneficial to avoid TA@Fe\(^{3+}\) reunion.

**Figure S2.** (a) UV-vis-NIR absorbance of Fe\(^{3+}\), TA and TA@Fe\(^{3+}\). (b) UV-vis-NIR absorbance of different TA@Fe\(^{3+}\) concentration.

As shown in **Fig S2a**, there are no obvious absorption peaks of Fe\(^{3+}\) and TA in 808 nm. However, a distinct absorption peak was observed in 808 nm when Fe\(^{3+}\) and TA form a coordination compound,
which indicated that the TA@Fe\(^{3+}\) could absorb 808 nm laser and convert it into heat. In addition, the intensity of absorption peaks improved with the increase of TA@Fe\(^{3+}\) concentration (Figure S2b), which indicated that the higher the concentration of TA@Fe\(^{3+}\), the stronger the NIR photothermal ability.

Figure S3. (a) The compressive property of PAM/CS hydrogel. (b) The adhesion properties of commercial dressings Cosmopor\(^{\text{RE}}\) and TA-1500 hydrogel. (c) The swelling ratio of various hydrogels.

As shown in Figure S3a, the PAM/CS hydrogel could be compressed to a high strain of 85%. After removing the compression force, the PAM/CS hydrogel could immediately recover to its original shape. In addition, the adhesion properties of commercial dressings (Cosmopor\(^{\text{RE}}\)) and TA-1500 hydrogels were compared (Figure S3b). The intermediate part of Cosmopor\(^{\text{RE}}\) had almost no adhesion. The adhesive tape around it was measured to give an adhesive strength of about 6.2 ± 2.4 kPa on surface of porcine skin, and which was lower than that of TA-1500 hydrogel (15.5 ± 1.2 kPa). The swelling behavior of hydrogels was investigated by immersing them into the PBS solution (Figure S3c). The
swelling of all hydrogel in PBS increased with time until a swelling equilibrium was reached after 75 h. After the CS was incorporated, the equilibrium-swelling ratio of the hydrogels was lower than that of PAM hydrogel. The reduction of swelling ratio was attributed to the formation of interpenetrating PAM and CS networks, which resulted in more rigid polymer network due to chain entanglement. The low swelling ratio allowed the hydrogels to maintain their shape and properties, which could prevent them from shedding or invalid after implanting into body.

Figure S4. The elemental distribution of TA-1500 hydrogel from EDS mapping.

As shown in the EDS mapping (Figure S4), apart from hydrogen element (undetectable), the most important elements in TA-1500 hydrogel were carbon and oxygen. Meanwhile, the trace iron was evenly distributed in the EDS mapping, which showed that the TA@Fe$^{3+}$ was almost no aggregation in the TA-1500 hydrogel network.
Figure S5. (a) Photothermal image of the PAM/CS hydrogel and TA-1500 hydrogel irradiated under an 808 nm NIR laser. Antibacterial ratio against the *S. aureus* suspension (b) and *E. coli* suspension (c) cultured on the hydrogels for 24 h.
Figure S6. (a) The photothermal effect of TA-1500 hydrogel on 3T3 cells. (b) MTT assay (570 nm) of 3T3 cells with TA-1500 hydrogel under NIR free and NIR (808 nm, 2 W·cm⁻²) irradiation for 10 minutes, and then cultured at 37 °C for 1 day and 2 days separately. (c) Fluorescence images of 3T3 cells incubated with TA-1500 under NIR irradiation for 10 minutes, and then cultured for 2 days.

As shown in Figure S6, we tested the NIR photothermal effect of TA-1500 hydrogel on fibroblasts (3T3 cells) with NIR laser and the experimental model was shown in Figure S6a. After 10 min irradiation, the MTT test indicated that the number of cells in TA-1500 hydrogel was lower than that of the control group without NIR irradiation. After 2 days of incubation, there was almost no statistical difference in the number of cells between the two groups (Figure S6b). In addition,
the inversion fluorescence digital imaging showed that the large number of dead cells were observed in TA-1500 hydrogel with NIR laser irradiation. After 2 days of incubation, the dead cells were replaced by living cells that proliferate around them. In short, these results indicated that the photothermal effect of TA-1500 hydrogel indeed could cause apoptosis of the fibroblasts to some extent. Following treatment, fibroblasts could quickly proliferate and reach almost the same number as that of the control group, which indicated that the limited photothermal damage on the normal cells could be rapidly recovered by cellular self-regulation.

**Figure S7.** (a) The photothermal images of different implant site irradiated under an 808 nm NIR laser. (b) The representative photos of implant site after 14 days.
Figure S8. The cumulative release curve of EGF from PAM/CS hydrogel and TA-1500 hydrogel.

The cumulative release curve of EGF from PAM/CS hydrogel and TA-1500 hydrogel was shown in Figure S8. The EGF from the TA-1500 hydrogel showed sustained release without burst release at the beginning, and the release rate gradually decreased over time. In contrast, EGF in PAM/CS hydrogel exhibited a fast release rate with approximately 41% in the first 3 days, indicating that EGF was simply entrapped in the PAM/CS hydrogel. These results demonstrated that the incorporation of TA@Fe^{3+} into the hydrogel facilitated the sustained release of EGF, which was ascribed to the presence of a large number of reactive functional groups on TA@Fe^{3+} and their high affinity to proteins.
References


