Supporting Information

Chitosan-based self-healing hydrogel for accelerating infected wound healing

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Experimental section

Characterization

Fourier transformed infrared spectroscopy (FTIR) was performed to confirm the successful synthesis of a targeted polymer. And each spectrum was recorded in the wavenumber region of 4000-500 cm^{-1} at room temperature, of which the image of A was shown only in the frequency of 2000-500 cm^{-1}. The nuclear magnetic resonance (^{1}H NMR) of corresponding materials was recorded at 25° using D_2O as solvent and dialdehyde terminated PEG (PEGDA) was recorded using $CDCl_3$ as solvent.

Scanning Electron Microscopy (SEM) analysis

Dynamic hydrogels developed by the combination of two different precursor solutions, denoted QP-1, QP-2, and QP-3, were prepared as the methods described and lyophilized (Table 1). These dehydrated samples were attached to an SEM stage, followed by gold-spraying for observation at an acceleration voltage of 10 kV. The morphology of the dehydrated hydrogels and their pores were visualized and recorded.

Rheological studies

To assess the mechanical performance of QP hydrogels, the oscillatory rheological analysis was carried out by a TA rheometer. The moderate volume of QP samples was placed between the parallel plates of 40 mm diameter to form a gap of approximately 1000 microns thickness. The amplitude sweep test and frequency sweep test were performed to determine appropriate shear frequency and applied strain. And in the cyclic strain tests, the oscillatory strains were alternatively switched as a predefined routine. All the experiments were measured at 25°C and the viscoelastic property of the hydrogels was displayed by measuring the storage modulus (G') and the loss modulus (G").

Swelling behavior

A swelling test was used to determine the equilibrium swelling ratio (ESR) of the hydrogels. In short, freeze-dry hydrogels (W_0) were put into PBS until swelling equilibrium. After carefully removing the residual fluid with filter paper, the weight of hydrogels (W_d) was measured. The ESR was calculated as:

$$ESR\ (\%) = (W_d - W_0) / W_0 \times 100\%$$

Antimicrobial capacity evaluation

Staphylococcus aureus (*S. aureus*) and Escherichia coli (*E. coli*) were used to evaluate the antibacterial properties of the materials. The minimum inhibitory concentration (MIC) was determined in terms of the MTT assay.[1] In brief, precursor QCS was diluted with different concentrations and inoculated into a 96-well plate containing bacterial MHB suspension (10^7 CFU/mL) and CS as a positive group and bacterial MHB suspension as a negative group. The cultures were incubated at 37°C for 12 h and 5 μL of Thiazole Blue (MTT) solution (5 mg/mL) was added. The optical density (OD) value at 570 nm was determined and marked as OD_{0h}. And after 4 h of incubation, the OD value was measured again and marked as OD_{4h}. The minimum inhibitory concentration was calculated as the following equation:

$$MIC\ (\%) = (OD_{sample\ 4h} - OD_{sample\ 0h}) / (OD_{control\ 4h} - OD_{control\ 0h}) \times 100\%$$

And MIC was defined as the minimum concentration at which bacterial survival rate was below 10%.

The plate counting method was also employed to test the surface antimicrobial activity of the hydrogels. First, the prepared hydrogels were added to a 48-well plate, and bacteria were diluted with normal saline (10^8 CFU/mL) to form a bacterial suspension. Then 10 μL bacterial suspension was added onto the hydrogel disks and transferred into an incubator at 37 °C with shaking at 120 rpm for 2 h. After 2 h of incubation, 1 mL of sterilized PBS was added to each well to resuspend bacterial survivors and the control group was performed by adding 10 μL bacterial suspension into 1mL sterilized PBS. Subsequently, 100 μL of the bacterial solution was inhaled and added to the blood plate culture medium so that distinct bacterial colonies can form on the disks.

Live/Dead Bacteria Staining Assay was conducted to study the antibacterial property of the hydrogels.[2] In short, after different treatments, the bacteria were centrifuged and washed thrice with PBS, followed by staining by SYTO9 and PI for 15 min in the dark. All bacteria were labeled and appeared green

for live and red fluorescence for dead. Finally, fluorescence images were observed and captured using fluorescence microscopy.

SEM analysis was also performed to observe the bacteria's morphological characteristics. After different treatments, *S. aureus* and *E. coli* cells were centrifuged and washed with PBS buffer, and then fixed with 4% polyoxymethylene for 15min. After fixation, these specimens were dehydrated in ascending series of ethanol and the dehydrated bacteria were transferred to cleaned silicon wafers and dried. Afterward, these bacteria were gold sputter-coated and observed by SEM.

Biosafety assays

The biosafety of QCS with different degrees of grafting was evaluated in L929 cell lines by CCK-8 as previously described.[3-5] In brief, L929 cells were cultured in Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin at 37°C under a humidified atmosphere containing 5% CO_2. While reaching about 90% confluence, the cells were harvested and seeded in 96-well plates at a density of 1×10^4 per well and incubated for 24 h. Then, the medium was replaced by a 100 μL fresh medium containing different concentrations of QCS. Subsequently, the cells were cultured for another 24 h and then CCK8-solutions were added to measure the cytotoxicity. The absorbance of 450 nm was recorded by a microplate reader. Cell viability was defined as:

$$\text{Cell viability (\%)} = A_s / A_c \times 100\%$$

where A_s and A_c represent the absorbance values of the hydrogel treatments group and the control group at 450 nm, respectively.

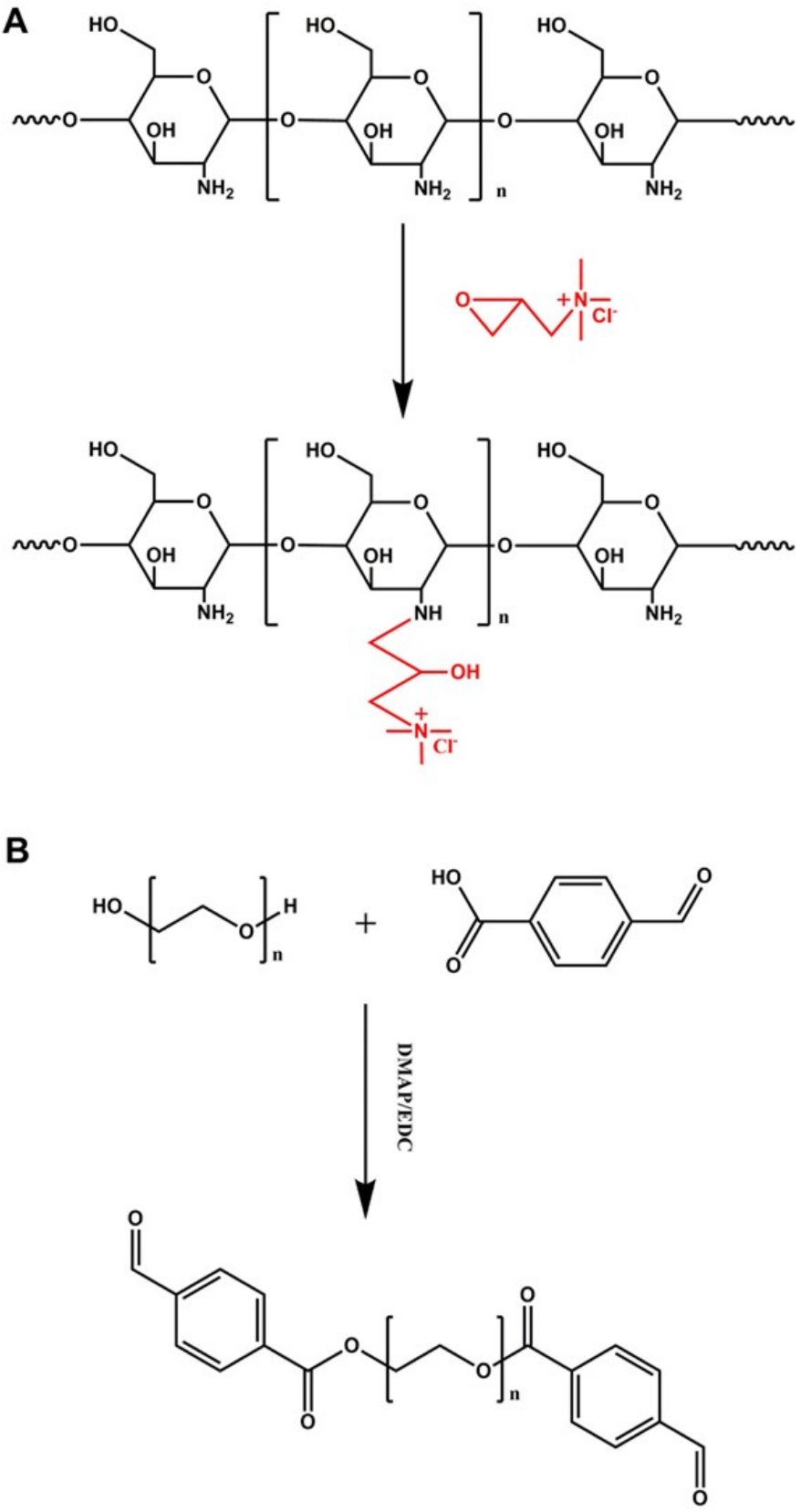
***In vivo* infected full-thickness skin defect model**

Ethics approval was obtained from Henan Eye Hospital (Permit number: HNEECA-2022-17). Female Balb/ c rats' weights of about 20 g and 7-8 weeks were used for the study. All mice were fed and acclimatized for one week before surgery. For the surgery part, all mice were divided randomly into 4 groups including control, Erythromycin ointment ERY, quaternized chitosan

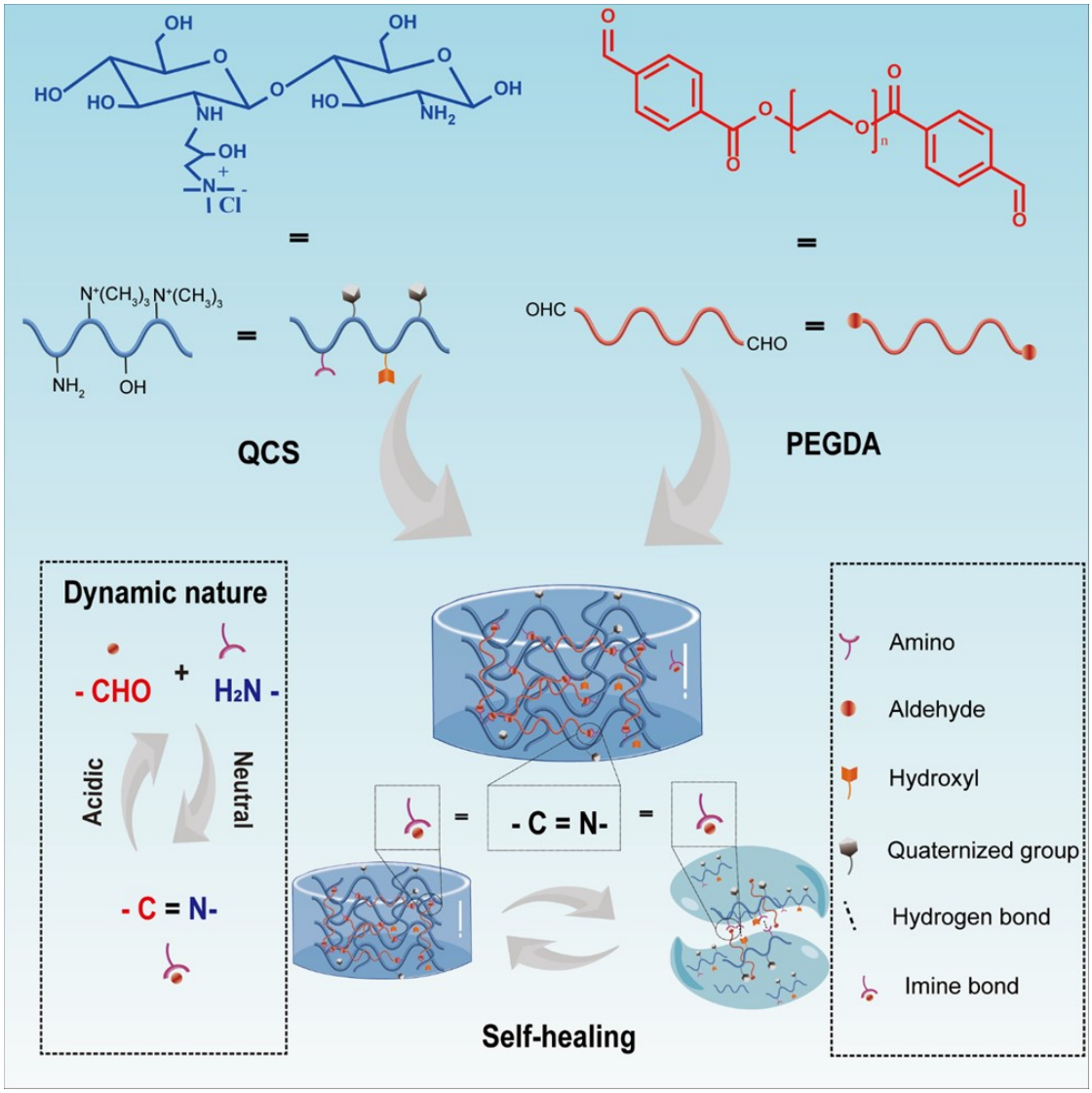
(QCS) solution, and QP-3 hydrogel. The animals were anesthetized with an intraperitoneal injection of chloral hydrate (0.16 g/kg). Afterwards, the hair on the back of the animals was shaved to prepare for surgery. A diameter of 10 mm full-thickness circular wound was created by a trephine and 50 μL bacterial suspension (10^9 CFU/mL) was dripped onto the region of wounds to realize wound infection. A day after the surgery, different wound groups were covered with nothing, ERY, QCS and QP-3 hydrogel. All tissues were monitored on day 3, 7, and 9 after the operation. On day 9, the rats were sacrificed by excessive anesthesia, and the wound area was collected.

For histological analysis, the obtained tissues were fixed in 4% polyoxymethylene and then embedded in paraffin and cross-sectioned to 4 μm thickness slices for hematoxylin and eosin (H&E) staining and Masson staining. As for the in vivo host response estimation, immunohistochemical (TNF-α and IL-6) analyses were performed to assess tissue regeneration. Besides, on day 9, tissue leaching solution was collected, diluted, and spread on the blood plate culture medium.

All animal experiments followed the guidelines of the China Animal Authority and the Vision and Ophthalmology Research Society's Statement on the Use of Animals in Ophthalmic and Vision Research.



Scheme S1. Schematic diagram of the synthesis of QCS (A), and PEGDA (B).



Scheme S2. Illustration of the nature of self-healing hydrogels.

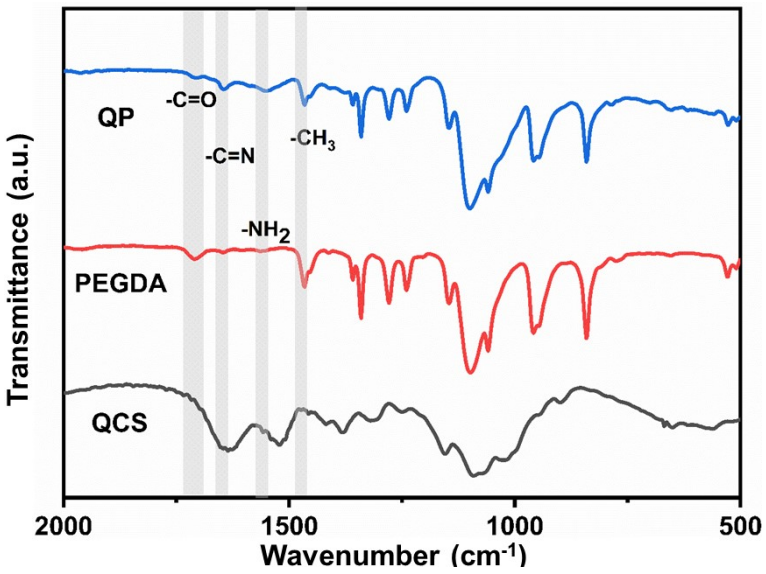


Figure S1. Fourier transform infrared (FTIR) spectra results of QCS, PEGDA and QP hydrogel.

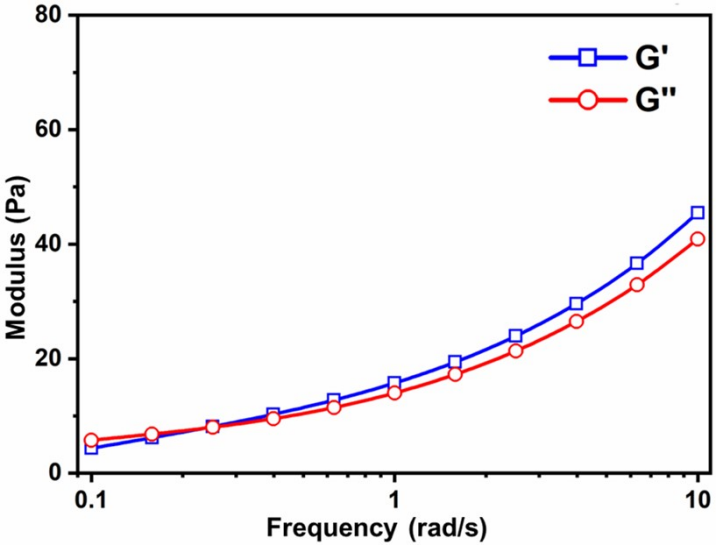


Figure S2. G′ and G″ of QP-1 on frequency sweeps at 25 °C.

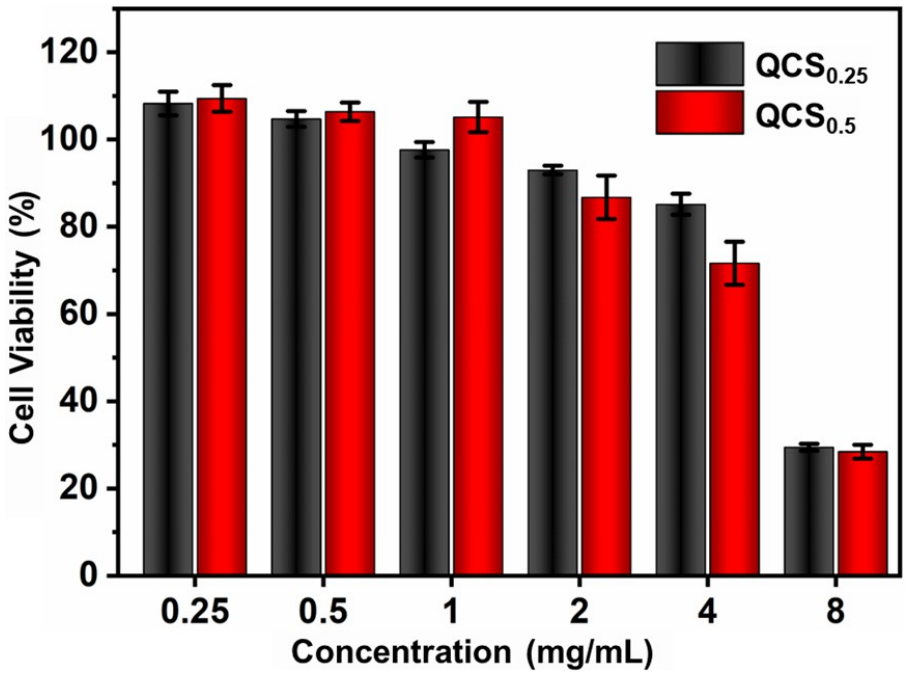


Figure S3. Cytotoxicity of L929 cells incubated with $QCS_{0.25}$ and $QCS_{0.5}$ (means ± SD, n =3; incubation time 24 h).

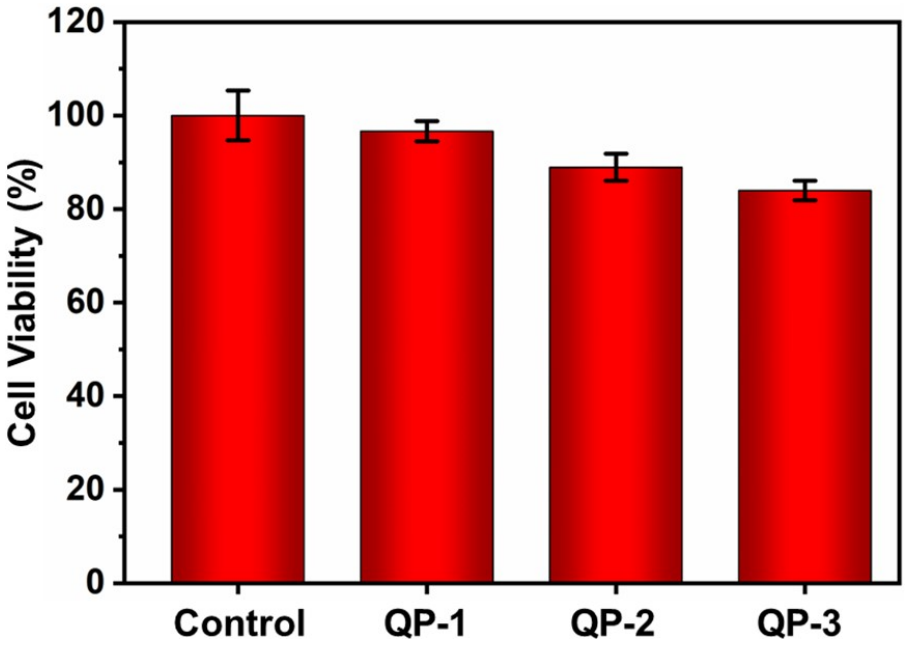


Figure S4. Cytotoxicity of L929 cells incubated with the hydrogels (means ± SD, n =3; incubation time 24 h).

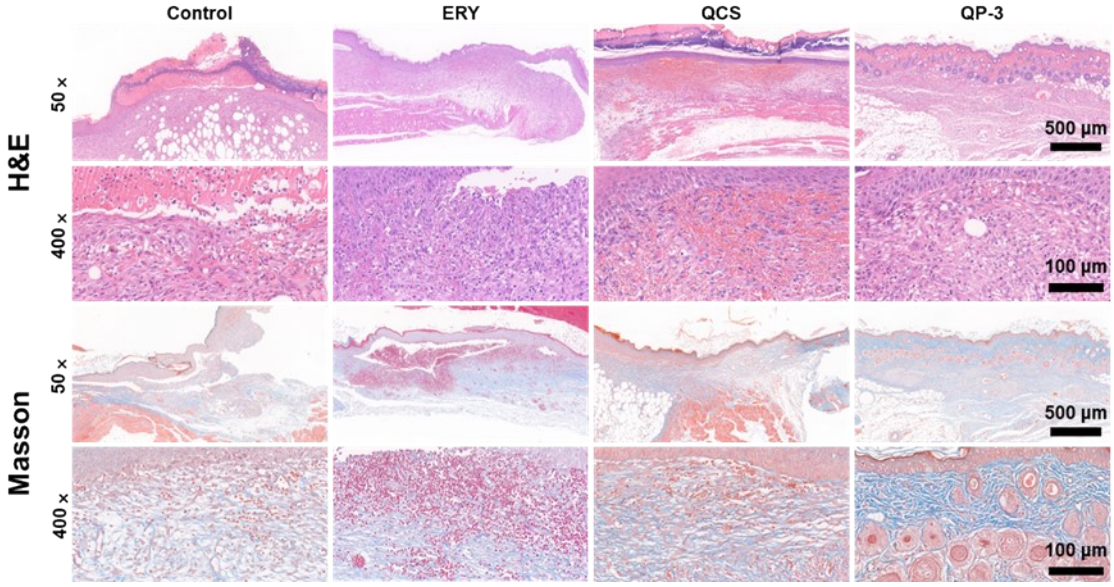


Figure S5. Representative images of the incisional skin wounds with different treatment.

Table S1. Functional differences between this work with previous researches.

Properties	Corresponding studies	This work
biocompatible	Ref. 7-11, 14, 16-21	√
Self-healing	Ref. 7-10, 13, 14, 19-21.	√
Antibacterial	Ref. 6-8, 11-15, 17, 18, 21.	√
biosafe	Ref. 2, 9-13, 15-20.	√

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