Natural polyphenolic antibacterial bio-adhesives for infected wound healing

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2. Experimental section

2.1 Characterization.

FTIR spectrometer (Nicolet iS50, Thermo Fisher Scientific) was available to demonstrate the successful synthesis of the bio-adhesive. The FEI Quanta 250 scanning electron microscope allows the surface morphology of the bio-adhesive to be obtained. Electrospray ionization mass spectrometry (ESI-MS) spectra were validated by API 2000 from Applied Biosystems. The rheological measurement at different temperature of bio-adhesive could be monitored by a rotational rheometer (AR1500ex, TA Instruments, USA). The self-healing properties and injectability of bio-adhesive were tested using needles and cylindrical silicone molds. XPS measurements were performed with a VG ESCALAB MKII spectrometer. The XPSPEAK software (version 4.1) allows deconvolution of narrow-scan XPS spectra of C 1s, and O 1s, and baseline calibration before use.

2.2 Hemostatic testing.

All rats' procedures were approved by the Animal Care and Experiment Committee of West China Hospital of Stomatology affiliated with the School of Medicine, Sichuan University (WCHSIRB-D-2017-263). The hemorrhage models were established by partly cutting the rat's tail, heart, and liver with scissors. Firstly, the heart and liver were destroyed by scissors, then the MTA adhesives was quickly injected, then they were pressed and the bleeding was stopped. Then, the tail of the rats was cut by scissors, and the prepared MTA natural bio-adhesives was quickly injected and pressed, then the amount of blood loss was collected on a filter paper and the time of blood was collected with a timer.

2.3 Free Radical Scavenging testing.

The 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2′-azinobis-(3-ethylbenzthiazoline-6-sulphonate) (ABTS) were measured the antioxidant activity of natural bio-adhesives. For the assay of DPPH, the solution of fresh 0.1 mM DPPH/ethanol was used for the measurements. Firstly, 100 μL 1 mg/mL MTA natural bio-adhesives aqueous solution, and corresponding MA and TA concentration with were added into 300 μL DPPH solution and 2600 μL ethanol, then the scavenging activity was evaluated by measuring the absorbance change at 517 nm after mixing the bio-adhesives solution with DPPH for different time. Additionally, the ABTS solution was obtained by mixing ABTS (7 mM) and $K_2S_2O_8$ (3mM) in water for 24 h. Then, 100 μL 1 mg/mL MTA natural bio-adhesives aqueous solution, and corresponding MA and TA concentration with were added into 100 μL diluted ABTS solution and 2900 μL water. Scavenging activity was evaluated by measuring the absorbance change at 734 nm after mixing the natural bio-adhesives aqueous solution with ABTS for different time. All tests were conducted three times. Finally, the radical scavenging activity was calculated according to previous articles.[1]

2.4 Cytocompatibility testing.

The cell viability of natural bio-adhesives was evaluated by Alma Blue assay and live/dead staining. Briefly, the prepared MTA bio-adhesives were soaked in the culture medium for 24 h. Then, NIH 3T3 cells were incubated in a 96-well plate (4000 cells per well) and 6-well plate (250000 cells per well) for 24 h. Then, after treating with leaching solution in different concentrations (62.5, 125, 250, 500 and 1000 μg/mL) for another 24 h, then both Alma Blue assay and live/dead staining were used to evaluate the cell viability and morphology.

2.5 Biodegradability testing.

The biodegradability of natural bio-adhesives was evaluated by injecting the MTA bio-adhesives into subcutaneous of rats. In short, ~100 μL bio-adhesives was injected into subcutaneous tissue of rats, then feed rats normally for 8 days, and biodegradability of glues was evaluated by testing its area and size in different time. Then, after 8 days, the injection site was removed from the rats for histopathological examination, including hematoxylin-eosin (H&E) and interleukin-6 (IL-6) staining.

2.6 Bacterial infection wound healing.

To evaluate the bacterial infection wound healing application of MTA natural adhesives, a wound healing model was established, and conducted on a rat skin with a length of 10 mm and many bacteria. In short, the back skins of rats (20 ± 5 g) were shaved and disinfected, and the wound was prepared with a scalpel, then bacterial (200 μL, 10^8 CFU·mL^{-1}) was injected for 24 h. Firstly, the rats were randomly divided into 4 groups (surgical sutures, MTA adhesives, MA and TA powder, and control). Then, ~50 μL MTA adhesives were dropped onto the wound, and press to product adhesion. The suture treatment was also used, and the untreated rats were used as the control group. Then the rats were fed normally for 8 days, and the wound area was photographed and recorded. Noting that the tissues were collected after the different time and cultured for bacteria growth. Moreover, all animals were sacrificed after 8 days, and the skin tissues were obtained to perform the histopathological examination, including H&E staining, Masson's trichrome, platelet endothelial cell adhesion molecule1 (CD-31), tumor necrosis factor-α (TNF-α), IL-6 staining and Gram's staining.

2.7 Statistical analysis

Data analysis results were described as the mean ± standard deviation (SD) and quantified for each experimental group. One-way analysis of variance was used for comparisons between the two groups, and $p<0.05$ was statistical significance.

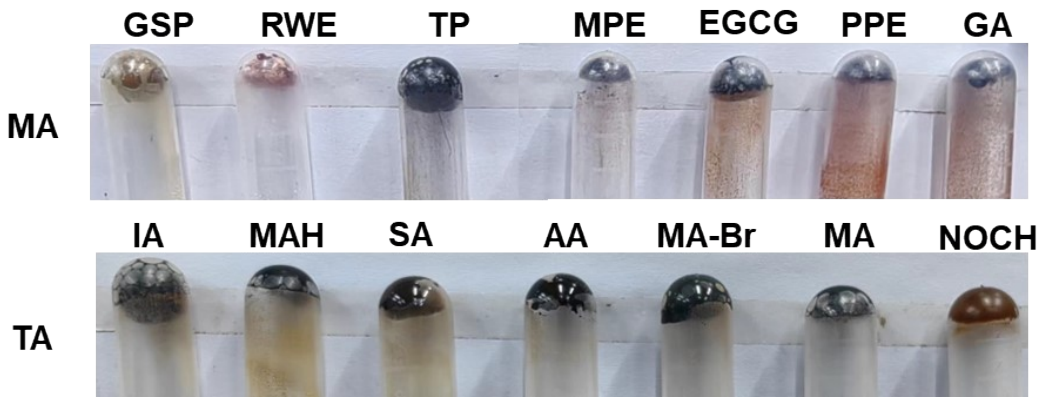

Figure S1. The optical images and reaction process of TA with different anhydrides, MA with different natural polyphenols (extracts).

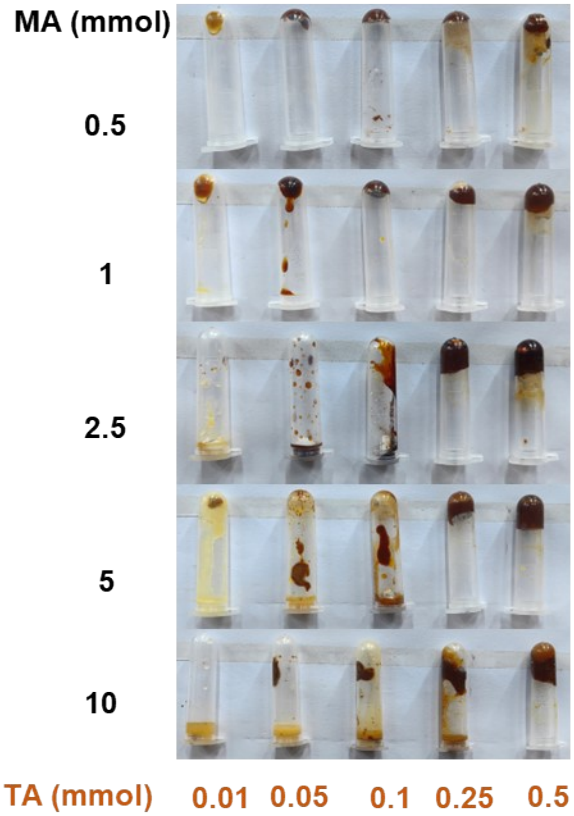

Figure S2. The optical images and reaction process of TA with MA at different ratio.

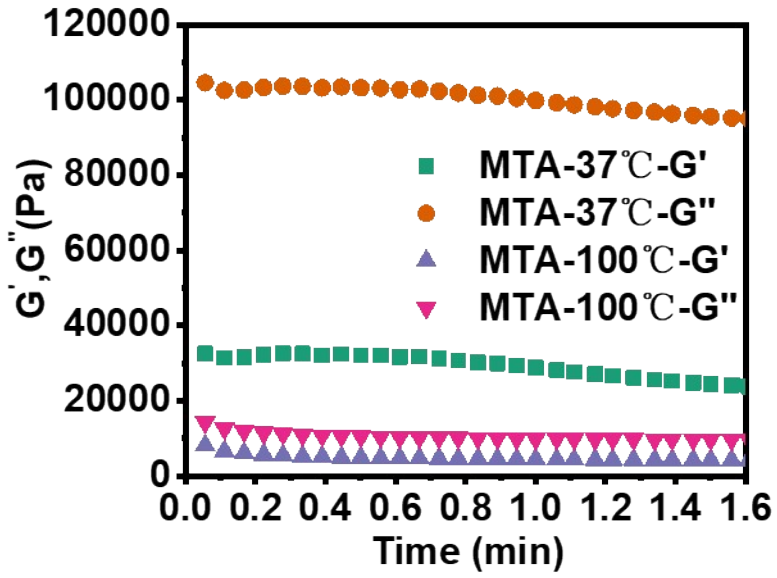

Figure S3. The energy storage modulus G' and loss modulus G'' of MTA adhesives at 37℃ and 100℃, respectively.

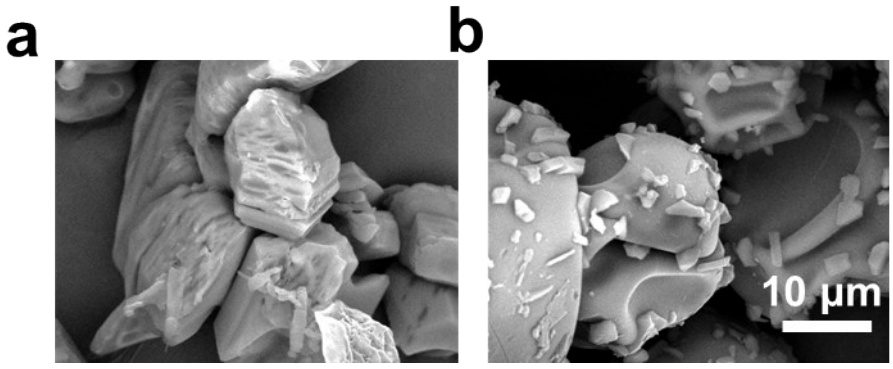

Figure S4. SEM images of (a) MA and (b) TA.

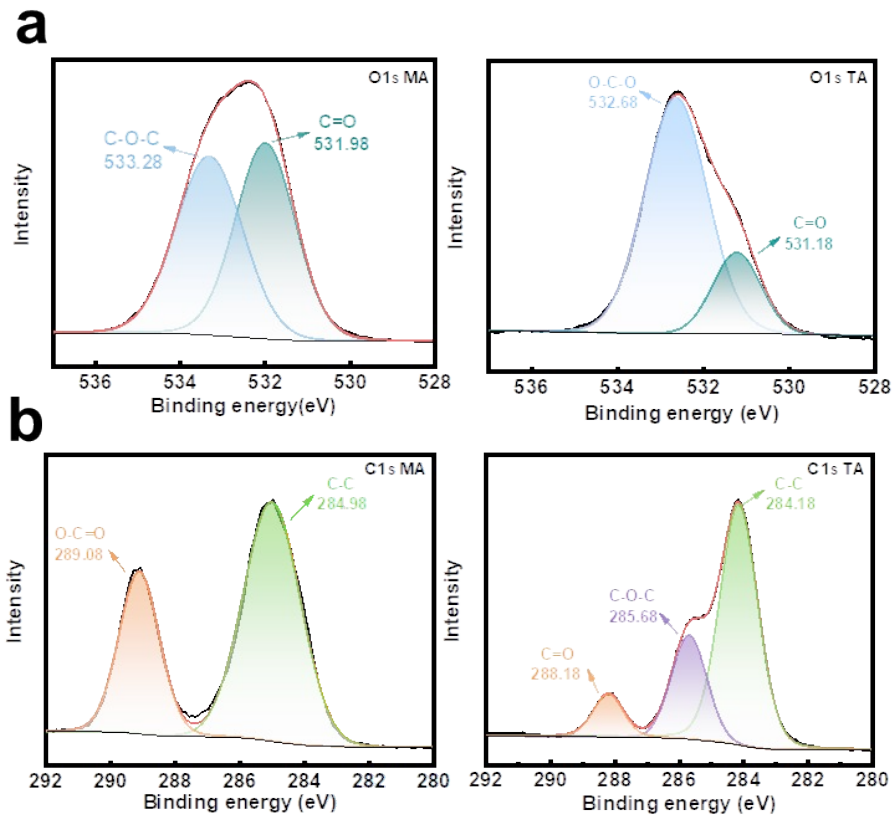

Figure S5. (a) O 1s peaks in XPS spectra of MA and TA. (b) C 1s peaks in XPS spectra of MA and TA.

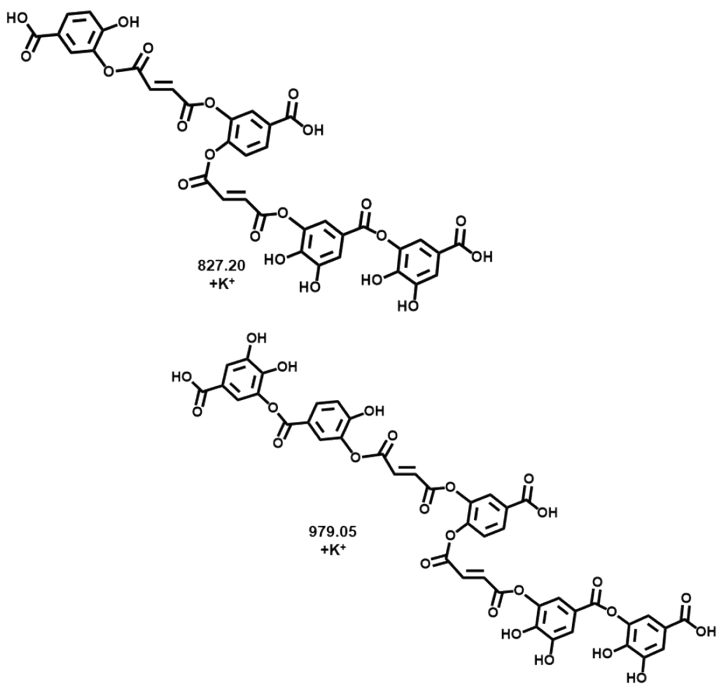

Figure S6. The chemical structure of MTA green adhesives was determined by ESI-

MS.

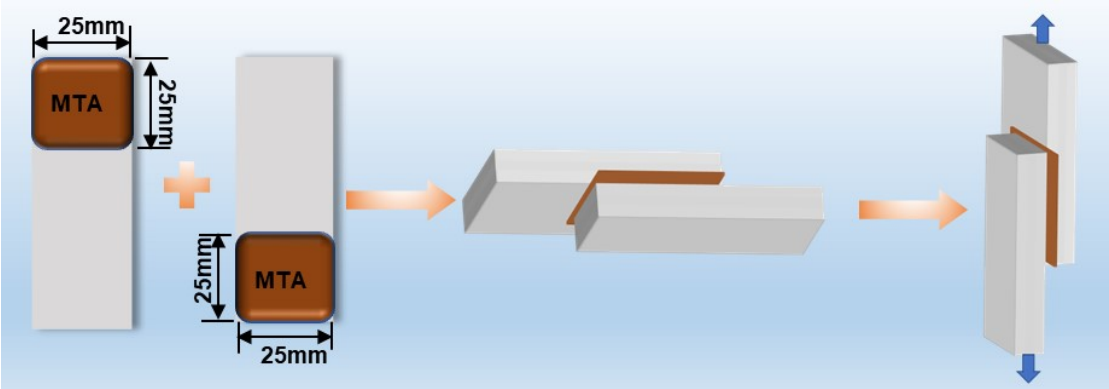

Figure S7. Diagram of adhesion strength of MTA green adhesives.

Figure S8. Diagram of bleeding area after control, gauze, TA/MA powder, MTA green bio-adhesives treatment.

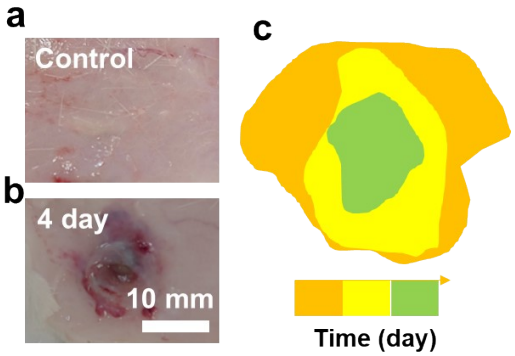

Figure S9. (a) Optical images of the control dorsal subcutaneous space. (b) Optical images of the biodegradability in the dorsal subcutaneous space of mice at 4 day. (c) Diagram of the biodegradability in the dorsal subcutaneous space of mice at different

time.

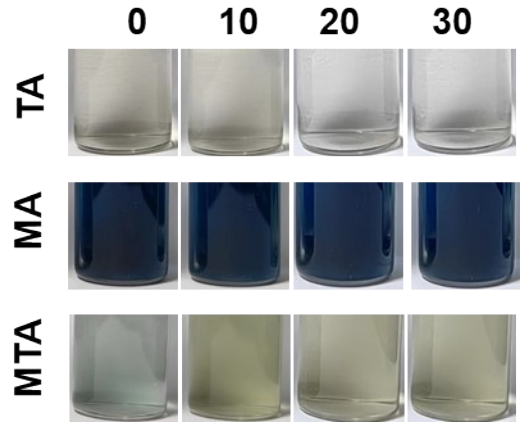

Figure S10. Optical image of color change of ABTS free radical solution scavenged by TA, MA, and MTA adhesives group.

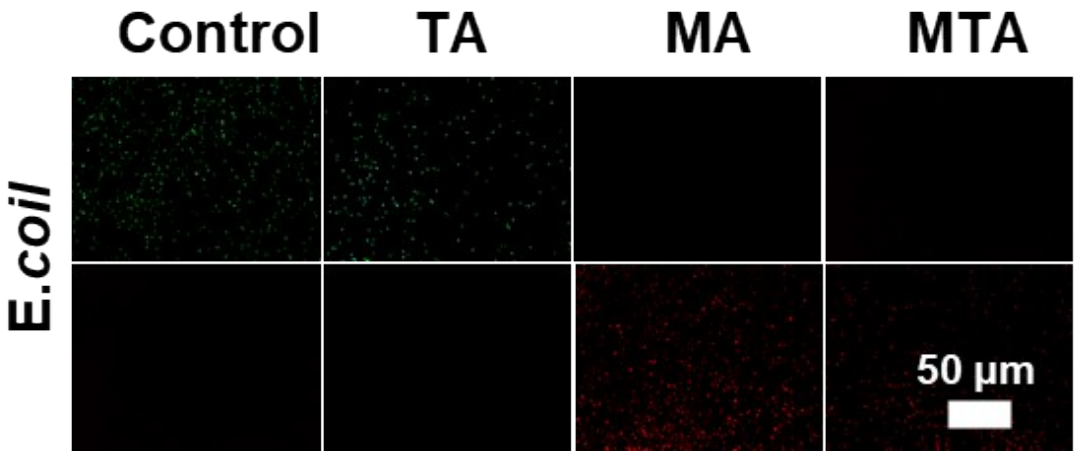

Figure S11. Live/Dead staining of *E. coli* treated with TA, MA, and MTA adhesives group.

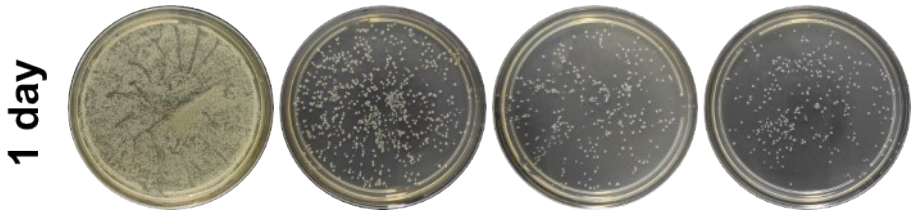

Figure S12. Bacterial plate counting in bacterial infection wound of different groups at 1 day.

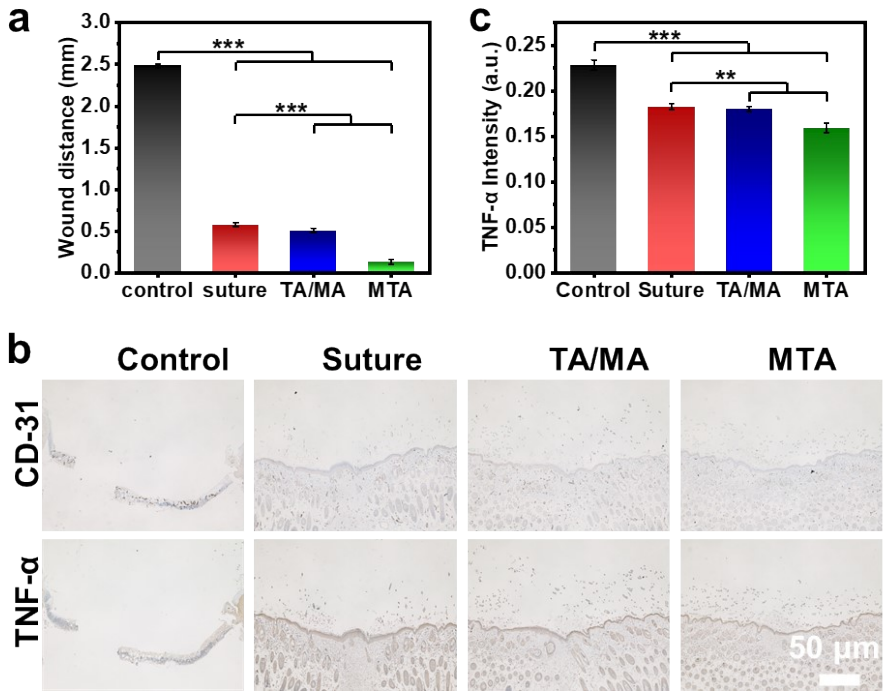

Figure S13. (a) Relative wound distance from HE staining with different treatment after 8 days. (b) Representative histological CD-31, and TNF-α staining after 8 days. (c) Relative level of inflammatory responses of TNF-αstaining after 8 days. (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.)

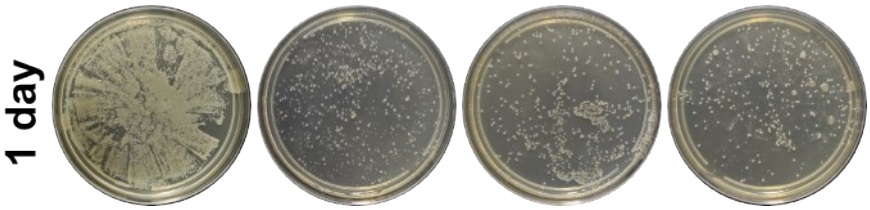

Figure S14. Bacterial plate counting in hot burn infection wound of different groups at 1 day.

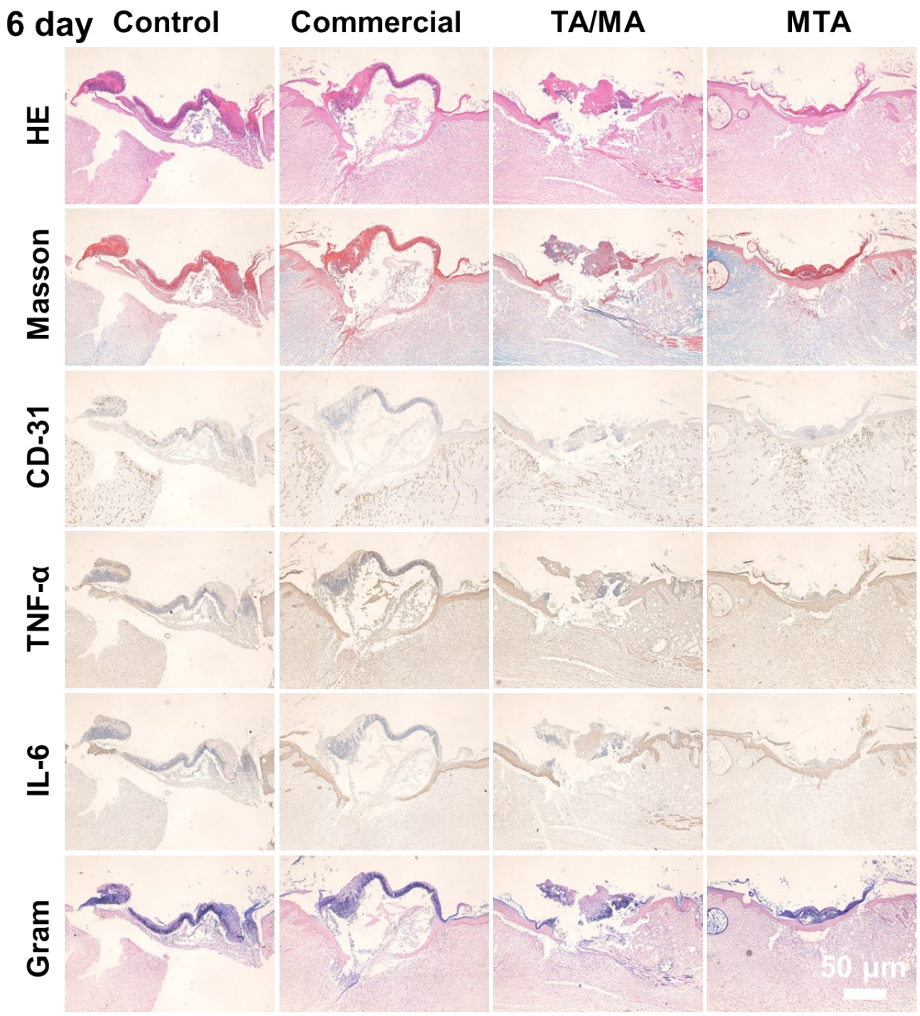

Figure S15. Representative histological H&E staining, Masson's trichrome, CD-31, TNF-α staining, IL-6, and gram staining after 6 days.

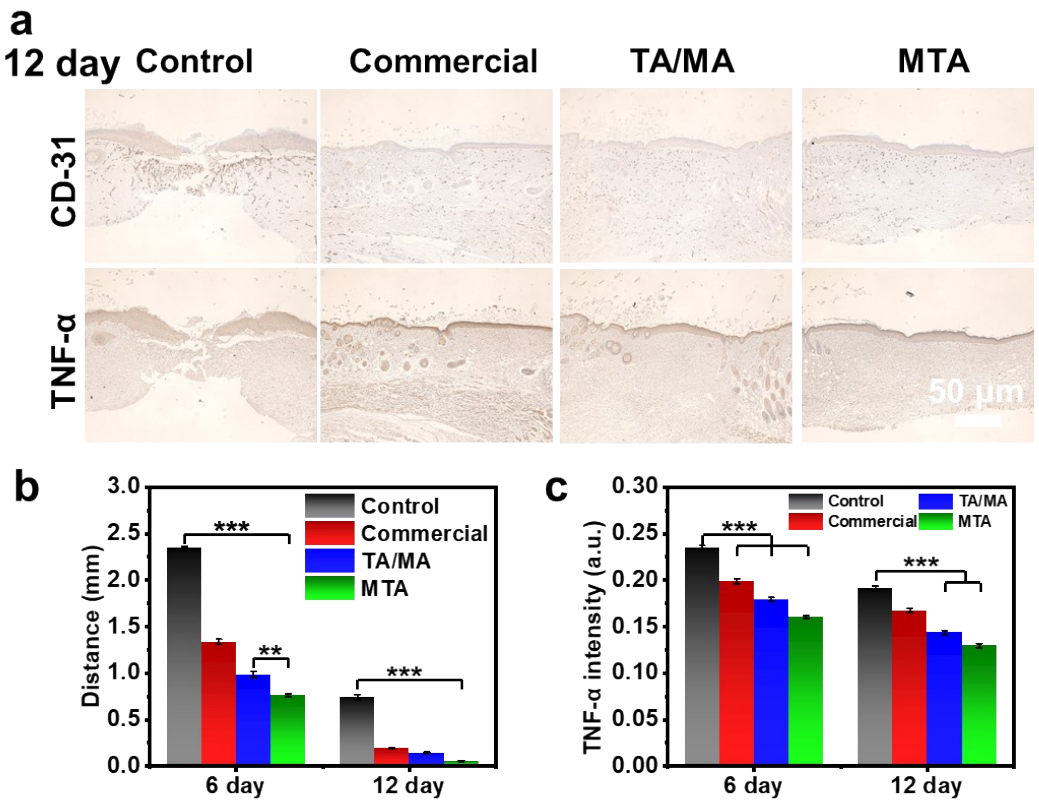

Figure S16. (a) Representative histological CD-31, and TNF-α staining after 12 days. (b) Relative wound distance from HE staining with different treatment after 6 and 12 days. (c) Representative histological CD-31, and TNF-α staining after 6 and 12 days. (*p < 0.05, **p < 0.01, ***p < 0.001.)

1. H. Zhang, C. Huang, J. Zhang, C. Wang, T. Wang, S. Shi, Z. Gu and Y. Li, *Giant*, 2022, **12**, 100120.