Electronic Supplementary Information for:

Hybrid Suture Coating for Dual-Staged Control over Antibacterial Actions to Match Well Wound Healing Progression

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Materials:

Tannic acid (TA) was purchased from Aladdin Industrial Corporation. Potassium hydroxide (KOH), disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$), sodium dihydrogen phosphate dihydrate ($NaH_2PO_4 \cdot 2H_2O$) were purchased from Sinopharm Chemical Reagent Co., Ltd. Dichloromethane (CH_2Cl_2) and dimethylsulfoxide (DMSO) were purchased from Shanghai Reagent Chemical Co. (China). Poly (lactic-co-glycolic acid) (PLGA-COOH) (M_W=50k) was purchased from Xi'an ruixi Biological Technology Co., Ltd. PGLA suture was obtained from Shanghai Pudong Jinhuan Medical Products Co., Ltd. and Catgut sutures were purchased from Shandong Boda Mecical Products Co., Ltd. FITC and was bought from Aladdin. DiR. Agar and Luria-Bertani (LB) broth were purchased from HuanKai Microbial Co. Ltd. (China). Ampicillin (98%) was purchased from Macklin Biochemical Co., Ltd. Propidium iodide (PI) was obtained from Sigma-Aldrich. MycoLight Green JJ98 was purchased from AAT Bioquest, Inc. Phosphate-buffered saline (PBS), Dulbecco's Modified Eagle Medium (DMEM) and penicillin–streptomycin were purchased from Gibco Invitrogen Corp. Fetal bovine serum (FBS) was purchased from Biological Industries. 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium-bromide (MTT) were supplied by Beyotime Biotechnology Co., Ltd. (China). General tissue fixative (4% paraformaldehyde) was obtained from Wuhan Servicebio technology Co., Ltd. Enzyme linked immunosorbent assay (ELISA) kit for Recombinant murine IL-6 and TNF-α was purchased from 4A Biotech Co., Ltd (Beijing, China), recombinant murine IL-10 kit was purchased from Shanghai Zeye Biotechnology Co., Ltd. BCA protein assay kit was obtained from Thermo Fisher Scientific.

Bacterial strains

Escherichia coli (E. coli) ER2738 strain and *E. coli* (ATCC 25922) strain were collected from the American Type Culture Collection (ATCC). *E. coli* mBL21 was achieved by introducing two plasmids into *E. coli* ER2738, which confers *E. coli* mBL21 with ampicillin resistance and mCherry protein expression.

Cell line and cultured conditions

3T3 murine embryonic fibroblast cells were obtained from the China Centre for Type Culture Collection (CCTCC) and cultured in DMEM, containing 10% FBS, 100 U mL^{-1} of penicillin-streptomycin, and incubated at 37 ^{o}C with 5 % CO_2.

Characterization methods

Scanning electron microscope (SEM) images were obtained by Zeiss SIGMA scanning electron microscope (Carl Zeiss, UK). ^{1}H-NMR spectra were obtained on a Mercury VX-300 spectrometer (Varian) with CD_3OD as the solvent. FT-IR spectrum was recorded on a Spectrum Two FT-IR Spectrophotometer (Perkin-Elmer). The TA absorbance spectra were measured by UV−vis spectrophotometer (Lambda Bio40, PerkinElmer, USA). The bacterial and cell absorbance at different wave length was measured by microplate reader (VersaMax and SpectraMax 340PC). The fluorescence and bright imaging were performed with a living image IVIS® spectrum (Perkin-Elmer), inverted fluorescence microscope (IX53, Olympus), and upright fluorescence microscope (BX53, Olympus).

ELISA assay

To quantify cytokines in wound, the fresh wound tissues were collected and homogenized in PBS buffer solution (10 mg tissue/ 1 mL PBS). After, the supernatants were collected and then

quantified protein concentration for each specimen by BCA protein assay kit. Concentration of different cytokines (IL-6, TNF-α, IL-10) was assessed by ELISA kits and the absorbance was measured at 450 nm by microplate reader.

Histological evaluation

Healthy 6-8 weeks female BALB/C mice were treated sutures (S_{PGLA}, $S_{PGLA+TA}$, $S_{PGLA@TA}$), the untreated wound group was taken as the blank control. After 4 days, the wound surrounding tissues were excised and fixed with 10% paraformaldehyde and embedded in paraffin wax. After that, the samples were sectioned for hematoxylin and eosin (H&E) staining, Masson's trichrome staining analyses, IL-6 and TNF-α immunohistochemical staining. The CD31 immunohistochemical staining was employed on day 7.

Determination of MPTA content on S_{PGLA} and S_{Catgut}

In the experiment, we firstly measured the weight and length of the raw PGLA and Catgut suture. Then, PGLA/Catgut sutures were immersed in 9 mL of TA solution (pH = 8) to allow standing for 6 h to form MPTA coating on suture surface. The sutures were taken out and washed with deionized water twice, and then dried in vacuum and stored to constant weight to produce MPTA-coated sutures ($S_{PGLA@MPTA}$ and $S_{Catgut@MPTA}$). $S_{PGLA@MPTA}$ and $S_{Catgut@MPTA}$ were weighed. The contents of MPTA on sutures were calculated as following:

MPTA content (μg / cm) = M (sutures after coating) -M (sutures before coating) / l (sutures)

M (X) represents the weight of X, and l (Y) represents the length of Y.

Animal feeding

All animal experiments were conducted in accordance with the "Guiding Principles for the Care and Use of Laboratory Animals". Before experiment, the means of transport, padding materials

or packing materials for animals and animal products were cleaned and disinfected according to the relevant state provisions in advance, animals were fed for several days before experiments in order to adapt to the environment. BALB/C mice were fed in the sterilized independent ventilation cage (IVC) with high-efficiency filter and the animal bedding were replaced every two days, which could keep the air in the feeding cage clean. For the feeding room, artificial 12/12 or 10/14 day and night alternation of light and dark is carried out for animal lighting. Besides, routine feeding and daily activity inspections were carried out before/after the operation and all animals were free to have water and food during the whole period of time.

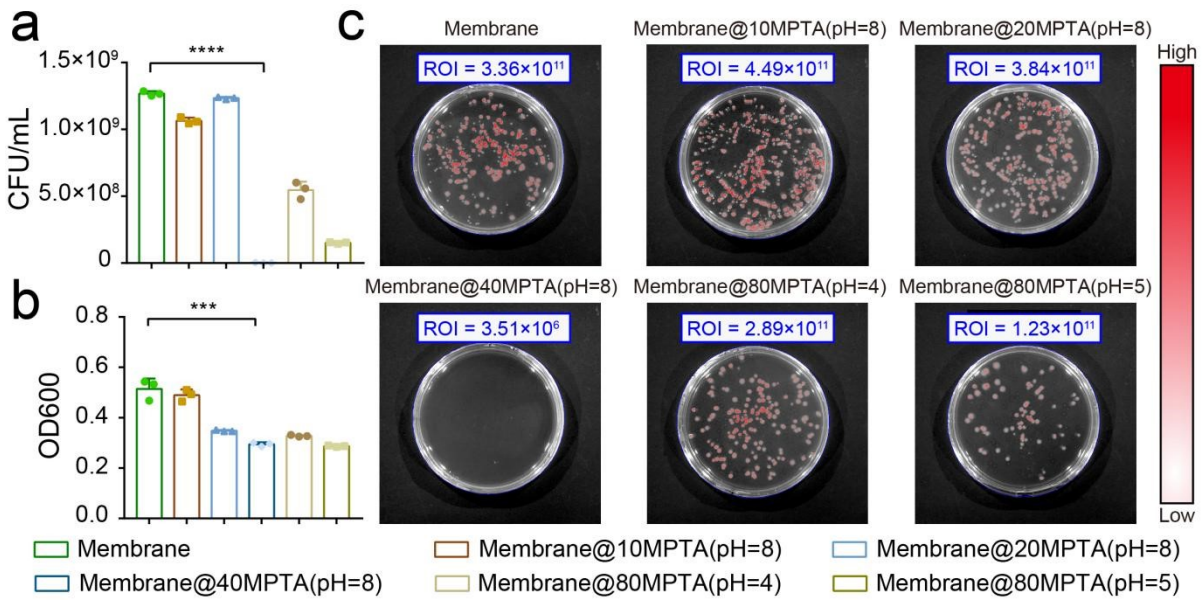

Fig S1. Optimization of TA concentration and pH. (a) The number of co-cultured *E. coli* mBL21 colony-forming units on LB agar plate. Six kinds of membranes were incubated with bacteria for 12 h. The incubated medium was diluted and spread on agar plate for bacteria counting. Membrane@10MPTA(pH=8) referred to that PLGA membrane was incubated with 10 mg/mL TA solution under pH 8. The others followed the same naming rule. (b) Mean optical density (OD) of co-cultured bacteria mediums measured at 600 nm. (c) Representative fluorescence images of membranes co-cultured with bacteria. The fluorescence intensity represented the mCherry protein expressed by co-cultured *E. coli* mBL21. All data are presented as mean with SD. Statistical significance was calculated by one-way ANOVA. ***$P < 0.001$, ****$P < 0.0001$.

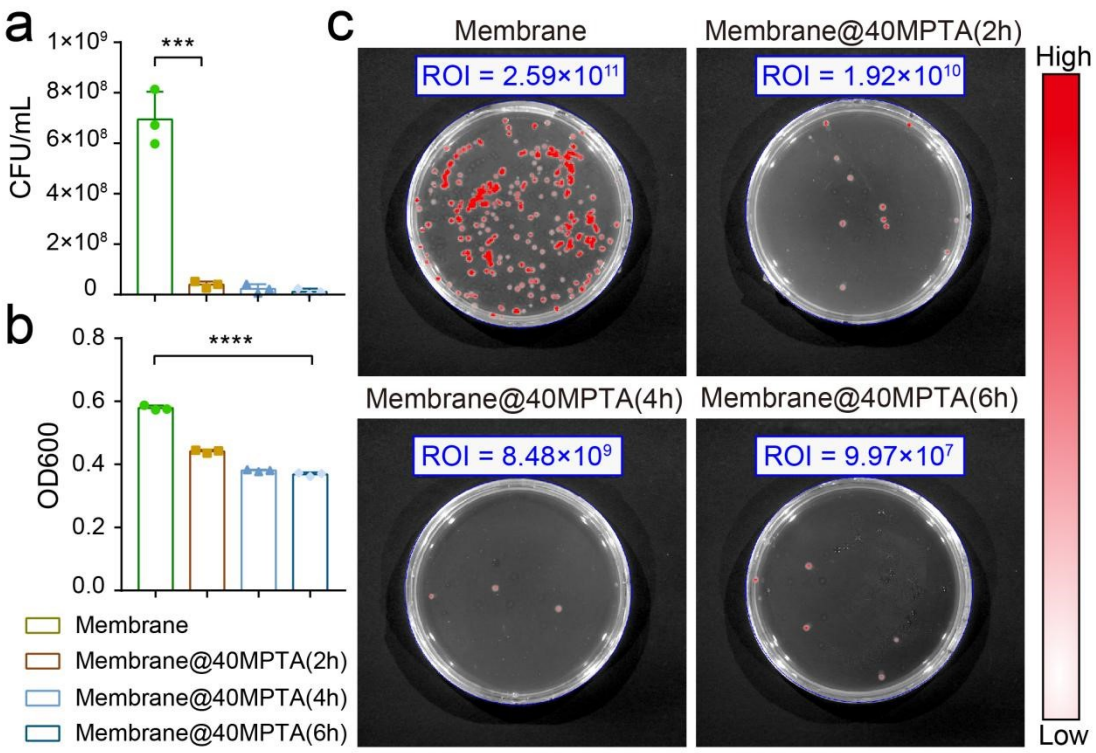

Fig S2. Optimization of incubation time. (a) The number of co-cultured *E. coli* mBL21 colony-forming units on LB agar plate. Four kinds of membranes were incubated with bacteria for 12 h. The incubated medium was diluted and spread on agar plate for bacteria counting. Membrane@40MPTA(2h) means PGLA membrane was incubated in 40 mg/mL TA solution for 2 h. The others followed the same naming rule. (b) Mean optical density (OD) of co-cultured bacteria medium measured at 600 nm. (c) Representative fluorescence images of membranes co-cultured with bacteria. The fluorescence represented the mCherry protein expressed by the co-cultured *E. coli* mBL21. All data are presented as mean with SD. Statistical significance was calculated by one-way ANOVA. ***$P < 0.001$, ****$P < 0.0001$.

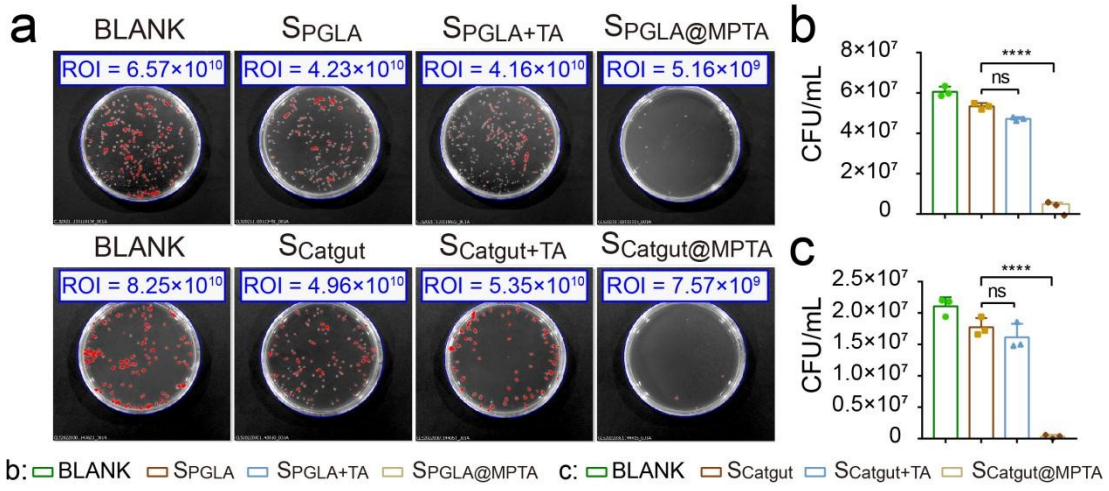

Fig S3. (a) Representative fluorescence images of adherent bacteria on the surfaces of six sutures. Sutures were co-incubated with *E. coli* mBL21 for 2 h; the incubated mediums were diluted and spread on agar plate for imaging. The fluorescence represented the mCherry protein expressed by the co-cultured *E. coli* mBL21. (b c) Numbers of *E. coli* mBL21 colony-forming units on LB agar plate, bacteria were cultured with six sutures for 2 h. Blank group represent the bacteria cultured in the absence of sutures. All data are presented as mean with SD. Statistical significance was calculated by one-way ANOVA. ****$P < 0.0001$, ns $>$ 0.1.

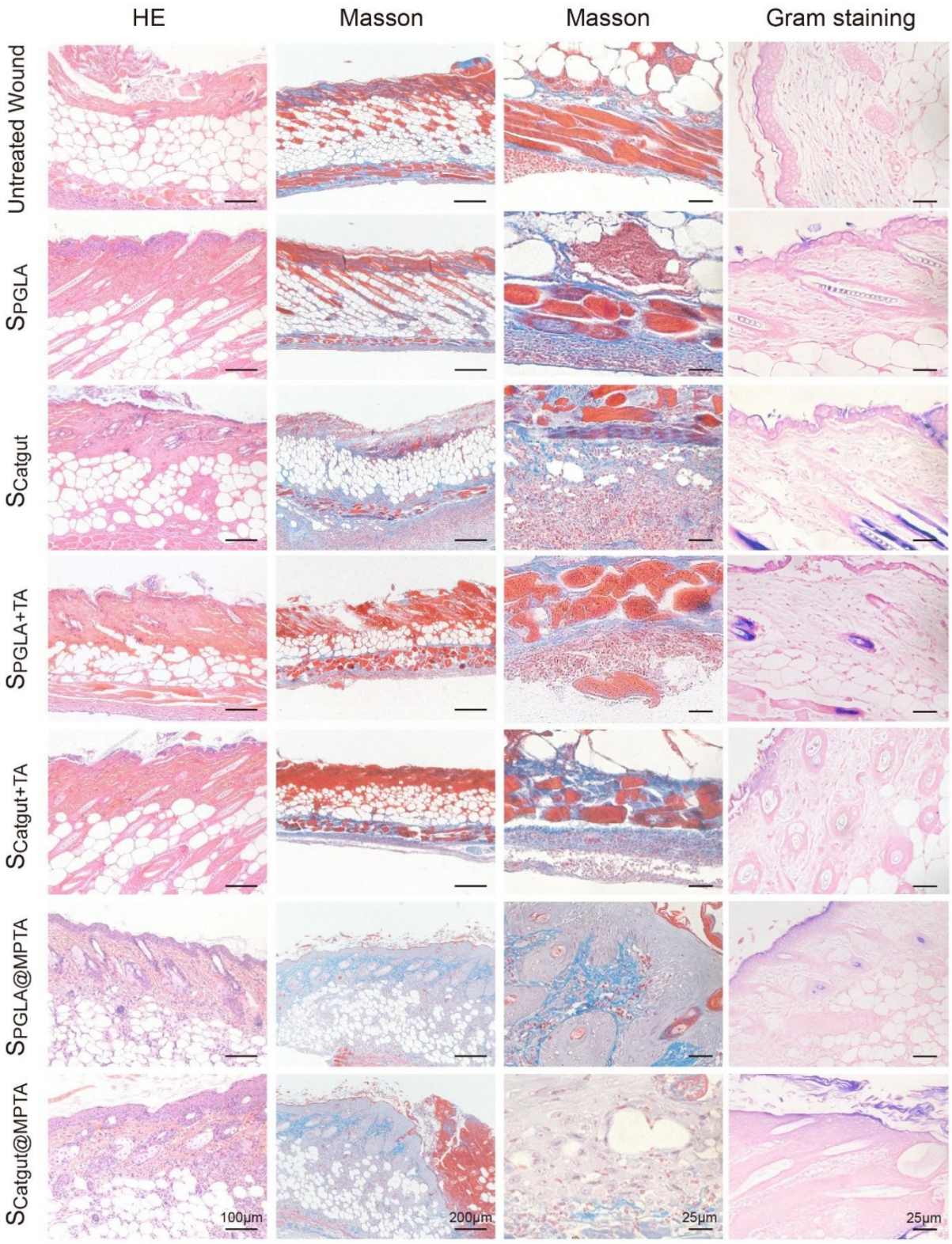

Fig S4. Representative images of H&E staining, Masson's trichrome staining and Gram staining of skin tissues at *E. coli* 25922 infected sites after treatments with various sutures: Untreated Wound, S_{PGLA}, S_{Catgut}, $S_{PGLA+TA}$, $S_{Catgut+TA}$, $S_{PGLA@MPTA}$ and $S_{Catgut@MPTA}$.