Supporting Information

Bioactive Antiinflammatory Antibacterial Antioxidative Silicon-Based Nanofibrous Dressing Enables Cutaneous Tumor Photothermo-Chemo Therapy and Infection-Induced Wound Healing

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MATERIALS AND METHODS

Materials. Citric Acid (CA, 99%), dichloromethane (DCM), Triton X-100, 1,8-Octanediol (OD, 98%), Dimethyl Sulphoxide (DMSO), curcumin, Dopamine hydrochloride (DA) and 3-Aminopropyltriethoxysilane (AS) were purchased from Sigma-Aldrich. poly(L-lactide) (PLLA) was obtained from Jinan Daigang Biomaterial Co. Ltd (China). Phosphate buffered saline (PBS), Dulbecco's Modified Eagle Medium (DMEM), and Alamar Blue kit were bought from Invitrogen. The cells used in this study were obtained from cell bank in Chinese Academy of Sciences.

Mechanical Properties Assessments. Before the tensile test, PP nanofibrous matrices were cut into a rectangular shape of 5 cm in length 6 mm in width and 0.10-0.30 mm in thickness. Briefly, the PP nanofibrous matrices were pulled at a rate of 20 mm/min until the sample broke at 25 ℃ with a relative humidity of 20%. The tensile stress-strain curves were obtained from system software. In order to obtain the mean value and standard deviation, each sample was tested on at least three species.

Hydrophilicity Measurement. The hydrophilicity of PP nanofibrous matrices were assessed by water contact angle. Briefly, a drop of water was put on the PP nanofibrous matrices (2 cm×2 cm) . After 0 second, 30 seconds and 60 seconds, the water drop was captured and the contact angle was measured, each sample was tested on at least three species.

***In vitro* drug release study.** 2 g of PPC and PPCP nanofibrous matrices were placed into 30 mL PBS (pH value =7.4) at 32 ℃ with shaking at 100 rpm, respectively. At predetermined time intervals, 1 mL of release solution was withdrawn, then 1 mL of fresh PBS buffer was added to maintain constant volume. The concentrations of the curcumin released from nanofibrous matrices were analyzed by the UV-vis

spectrophotometer (Lambda950, Perkin Elmer). PBS buffer was used as a control to measure the absorbance at 426 nm.

Antibacterial activity. For the antibacterial activity assay, Gram-negative *E. coli* and Gram-positive *S. aureus* were selected as bacterial models. Briefly, 20 mg nanofibrous matrices were immersed in 5 mL PBS buffer containing 10^4 bacteria mL^{-1} and cultured at 37 °C with 200 rpm min^{-1}. The PBS buffer containing 10^4 bacteria mL^{-1} was used as control group. After 14 h incubation, 10 μL from each group were spread onto LB agar plates, and the plates were incubated for 12 h at 37 °C. Finally, the number of colonies on the LB agar plates was recorded and the antibacterial rate was calculated.

Hemocompatibility Evaluation. Platelets adhesion test: The whole blood (1.8 mL) and anticoagulant sodium citrate solution (38 mg/mL, 0.2 mL) were mixed under stirring, and the platelet solution was separated from the red blood cell portion by centrifuging at 1500 rpm for 15 minutes. Gauze, PP, PPC and PPCP were covered with platelet solution and incubated at 37 °C under 5% CO_2 condition for 1 h. The samples were taken out and slightly rinsed 3 times with PBS to remove non-adherent platelets. Subsequently, the samples were fixed with 2.5% glutaraldehyde aqueous solution for 4 h at 4 °C. Then they were dehydrated with a series of gradient concentration of ethanol solution (50%, 70%, 80%, 90%, 95%, and 100%) and vacuum dried for SEM observation.

Whole blood clotting measurement: The samples were placed into 24-well plate and prewarmed to 37 °C. The whole blood (0.2 mL) mixed with anticoagulant sodium citrate solution (38 mg/mL, 0.02 mL) was slowly dispensed onto the samples until completely covered, and then $CaCl_2$ solution (0.2 M, 10 μL) was added to start coagulation. The 24-well plate was further incubated at 37 °C for 10 min. Subsequently 1 mL deionized water was slightly added into the tube along inside wall. Then the solution was gathered from 24-well plate and centrifuged at 800 rpm for 30 s. The supernatant was transferred to a tube with additional

10 mL deionized water and kept at 37 ℃ for 60 min. The relative absorbance of diluted blood samples at 540 nm was measured by microplate reader (SpectraMax Paragigm, Molecular Devices). The absorbance of 0.2 mL citrated whole blood mixed with 11 mL deionized water at 540 nm was set as 100% as a reference value. The blood clotting index (BCI) of samples can be calculated by the following equation:

$$BCI = \frac{A}{B} \times 100\%$$

where A is the absorbance value of blood which had contacted with samples at 540 nm, B is absorbance value of citrated whole blood in deionized water at 540 nm.

Hemolytic test: Briefly, 1 mL of fresh mice blood was suspended in 10 mL PBS with 10 mg heparin sodium (as an anticoagulant) and then centrifuged at 1000 rpm for 15 min to separate the red blood cells. The cells were washed three times with PBS until the supernatant was clear. The PP, PPC and PPCP nanofibrous matrices were cut into 14 mm diameter circular films, and then put into a tube containing 0.2 mL physiological saline. Subsequently, 0.2 mL of diluted blood was added into each test tube and incubated for 60 min at 37 °C. Similarly, same volume of diluted blood was added into 0.2 mL 0.1% Triton x-100 and PBS as the positive and negative controls, respectively. After incubation, all samples were centrifuged at 1000 rpm for 15 min, the supernatant was taken for absorbance at 540 nm using a microplate reader (SpectraMax Paragigm, Molecular Devices). The hemolysis percentage was calculated as follows:

$$\text{Hemolysis (\%)} = [(OD_t\text{-}OD_n)/(OD_p\text{-}OD_n)] \times 100\%$$

where OD_t, OD_n and OD_p were the absorbance values of samples, negative control (PBS) and positive control (Triton x-100), respectively.

Cytotoxicity Assay. A mouse-derived fibroblast (L929) was employed to investigate the cytotoxicity of PP, PPC and PPCP nanofibrous matrices. Before the test, the PP, PPC and PPCP nanofibrous matrices were cut into 14 mm diameter circular films and fixed on circular slide with Parafilm. The slides with nanofibrous

matrices were sterilized by UV radiation and put into 24-well plates. L929 cells (10000 cells/well) were seeded and cultured under standard conditions (humidity environment, 5% CO_2, 37 °C), including the growth medium (DMEM with 10% fetal bovine serum, GIBCO). On day 1 to day 5, the cell activity was evaluated *via* an Alamar Blue kit (Invitrogen) and the microplate reader (530 nm/600 nm, SpectraMax Paragigm, Molecular Devices). On day 5, cells were washed with PBS and then fixed with 2.5% glutaraldehyde for 2 h at 4 °C. After that, the samples were continuously dehydrated using 25, 50, 75 and 100% ethanol solutions and then sputter coated with Palladium and observed at an accelerating voltage of 10 kV.

Cutaneous Wound Healing Examination. The wound healing investigation *in vivo* for samples was conducted on female Kunming mice (30-35 g). After anesthetization, the round section of skin injury (7 mm) was made with a skin biopsy apparatus from the back of the mouse skin under anesthetization. Kunming mice were randomly divided into four groups (A-D). Group A mice received 3M Tegaderm™ film (3M Health Care, USA) as a control group, group B mice were dressed with the PP nanofibrous matrices, mice in group C were treated with PPC nanofibrous matrices and group D mice received PPCP nanofibrous matrices. After 3, 7, 10 and 14 days, the wounds imaged, the wound area were measured by Image J software according to the equation: wound size (%) = $[W_{(3,7,10,14)}/W_0)] \times 100\%$ where W_0 and $W_{(3,7,10,14)}$ represent the wounds areas on 0 day and 3, 7, 10, 14 days, respectively. The healed skin tissues were further evaluated using the histological analysis. Briefly, the wound site with the surrounding skin was isolated and fixed in 4% neutral formalin solution. The samples were embedded by paraffin, sectioned, stained *via* hematoxylin & eosin (H&E) and Masson's trichrome. All samples were analyzed and photo-captured by microscope (IX53, Olympus, Japan). At 3, 7, 10 and 14 days, the thickness and cell density of the epidermis was measured by H&E stained micrographs and Image J software.

Cutaneous Wound Healing Combined with Anti-Infection Examination. The infection model was slightly modified based on previous study. Female Kunming mice (8 weeks, 30-32 g) were anesthetized and a round wound (7 mm in diameter) was made. *S. aureus* suspension (10 μL) with 10^4 CFU mL^{-1} in PBS was put on the wound, and various materials were used to cover the wound. After 3, 7, 10 and 14 days, the tissue near the wound were taken and then cultured on LB agar plates to check the anti-inflection activity. In addition, the wound area was measured by Image J software, and the regenerated skin tissues were further evaluated using the histological analysis. All animal experiment studies were carried out by the Guidelines for the care and use of laboratory animals in Xi'an Jiaotong University and approved by the animal ethics committee of Xi'an Jiaotong University.

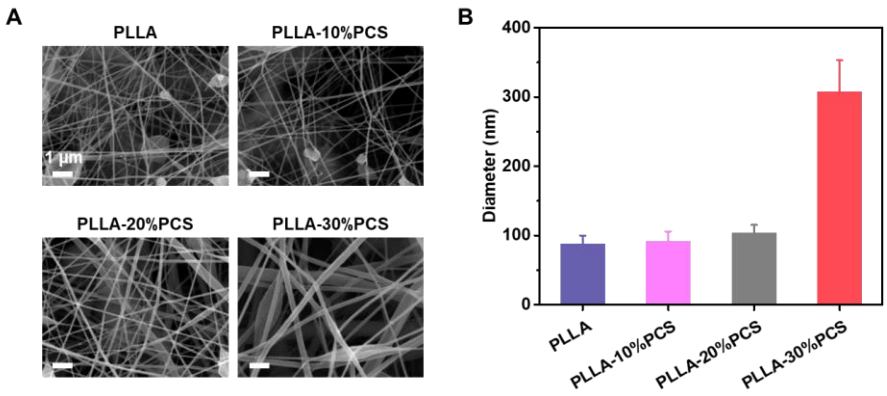

Figure S1. Characterizations of various PP nanofibrous scaffiolds. (A) SEM images of the PLLA, PLLA-10%PCS, PLLA-20%PCS and PLLA-30%PCS nanofibrous matrices (scale bar =1 μm); (B) Diameter distribution of PLLA-10%PCS, PLLA-20%PCS and PLLA-30%PCS nanofibrous scaffold (n=50);

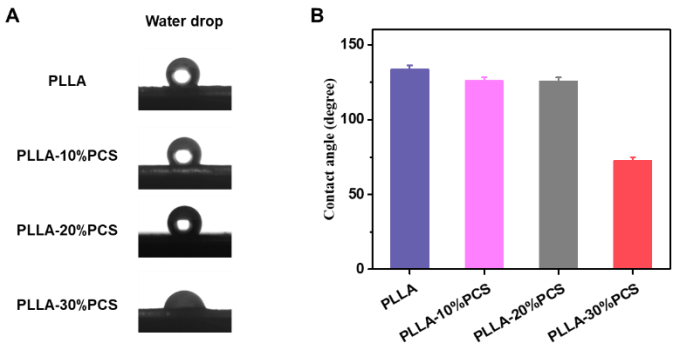

Figure S2. Hydrophilicity of PP nanofibrous matrices. (A) Water drop change on various matrices; (B) Water contact angle test (n=3).

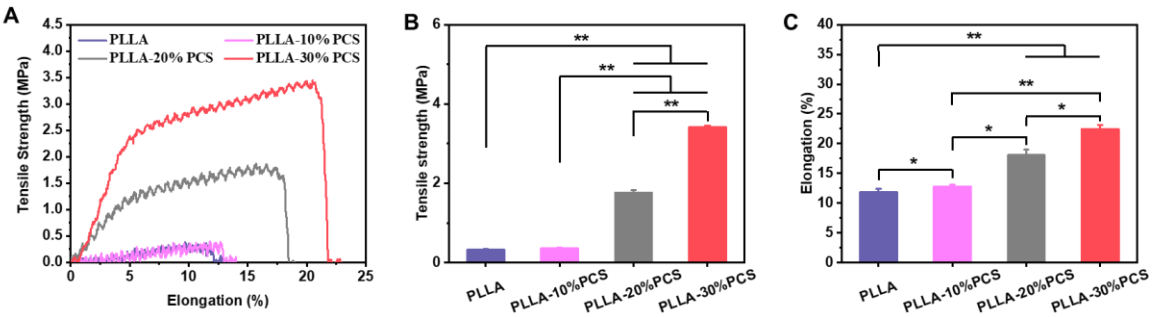

Figure S3. Mechanical properties of PLLA, PLLA-10%PCS, PLLA-20%PCS and PLLA-30%PCS nanofibrous matrices: (A) Stress-strain curves; (B) Tensile strength; (C) Elongation. (n=3; *$p < 0.05$ and **$p < 0.01$).

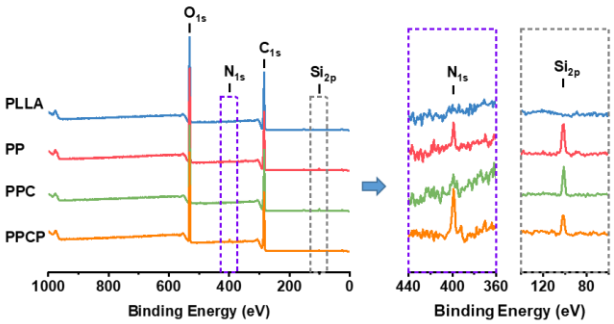

Figure S4. XPS spectrum of PLLA, PP, PPC and PPCP.

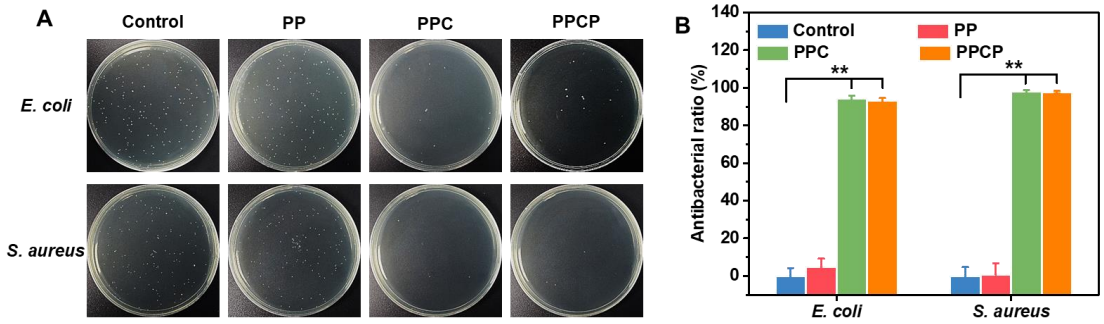

Figure S5. ***In vitro*** **antibacterial activity. (A) Pictures of LB agar plates and (B) corresponding statistical data of colonies of** ***E. coli*** **and** ***S. aureus*** **treated with various nanofibrous matrices. (n=3; Control: PLLA; **$p < 0.01$).**

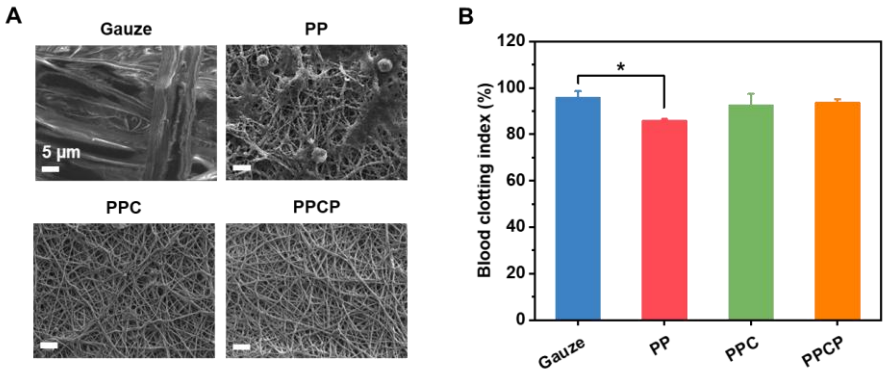

Figure S6. ***In vitro*** **hemocompatibility evaluation. (A) SEM images of platelets adhesion test, (B) whole blood clotting measurement (n=3; ****p*** **< 0.05).**

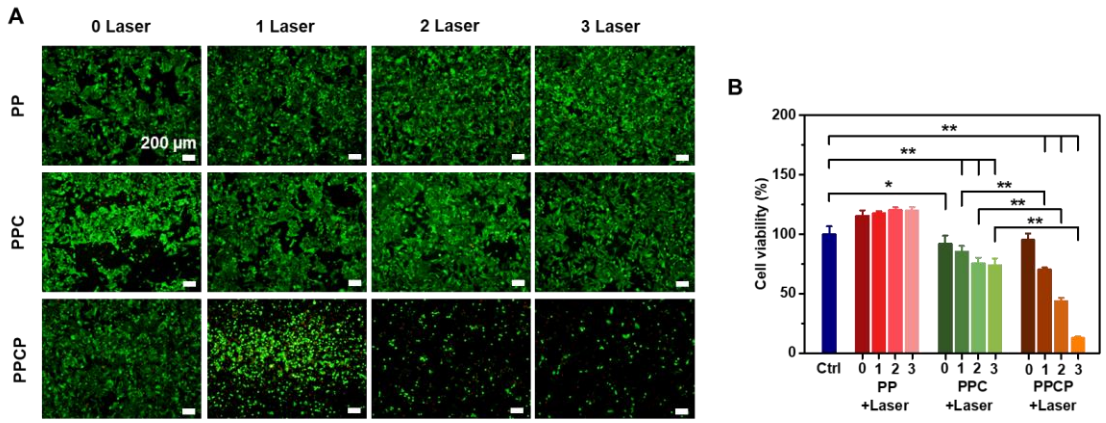

Figure S7. ***In vitro*** **anticancer efficiency. (A) Calcein-AM staining fluorescent images of A375 after treated with various nanofibrous scaffold after irradiated for 0, 1, 2 and 3 times; (B) Cell viability of A375 cells after irradiated for 0, 1, 2 and 3 times. (n=3; ****p*** **< 0.05 and *****p*** **< 0.01).**

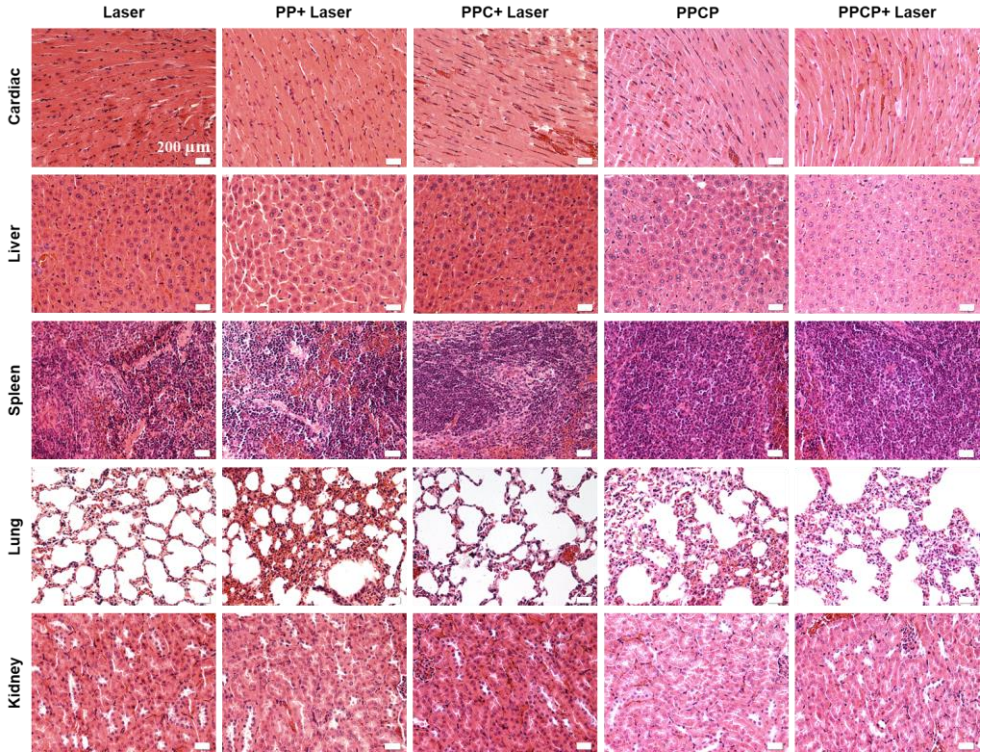

Figure S8. H&E stainings of cardiac, liver, spleen, lung, kidney after various treatments.

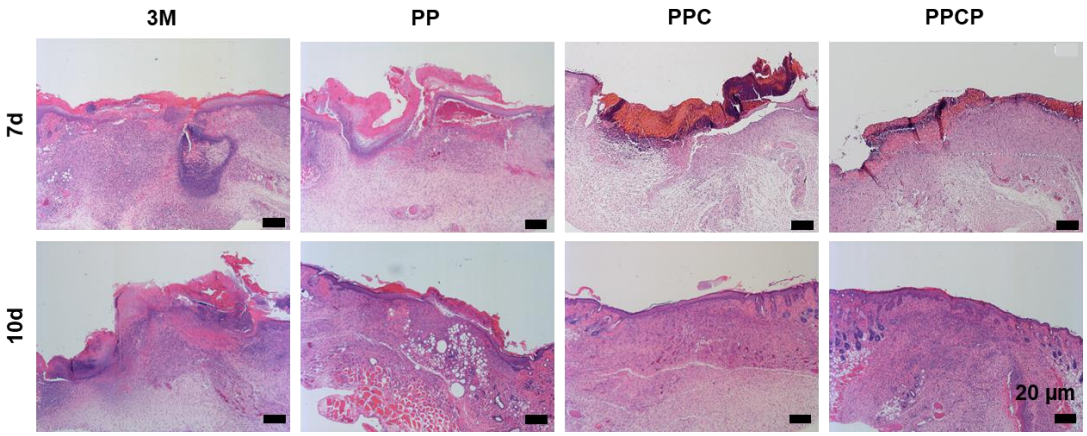

Figure S9. H&E stained images of wounds after infection at 7 d and 10 d.