

Supporting Information

Transparent silk fibroin films facilitate infected wound healing through antibacterial, improved fibroblast adhesion and immune modulation

Experimental section

Materials and animals: The cocoons were provided by the State Key Laboratory of Silkworm Genome, Southwest University (Chongqing, China), and dialysis bags with molecular mass of 8000-14000 Da were purchased from Solarbio Science & Technology Co., Ltd. (Beijing, China). Unless otherwise stated, all biological dyes and antibody reagents were from Beyotime (Shanghai, China) and all other chemical reagents were bought from Aladin Reagent (Shanghai, China).

Female Sprague-Dawley rats (aged 6 weeks, 200 ± 10 g) were provided by Byrness Weil biotech Ltd (Chongqing, China). All animal protocols in this study were approved by the Institutional Animal Care and Use Committee of Southwest University (No.IACUC-20230512-03).

Characterization of the modified RSF films: The tensile properties of the samples were measured by the universal mechanical testing machine, GTM-2100 (Xieqiang Instrument Manufacturing Co., Ltd., Shanghai, China), with a tensile rate of 2 mm/min. The Fourier transform infrared spectrum was recorded by a Nicolet iS10 spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) with the wavenumber ranging from 1750 to 1400 cm^{-1} . The frontal and cross sections of the samples were sprayed with gold for observation by scanning electron microscope (JEOL Ltd., Tokyo, Japan). The samples were measured by an ultraviolet-visible spectrophotometer (Lengguang Technology Co., Shanghai, China) with the full wavelength scanning mode from 300 to 900 nm. The contact angles of the samples were tested by contact angle measurement instrument OCA15EC (Dataphysics Instrument GmbH,

Stuttgart, Germany). The X-ray diffraction spectrum of the samples was recorded using a device TD-3500 (Dandong Tongda Science & Technology Co, Liaoning, China) with a diffraction angle range from 5 to 80 ° and a scanning rate of 1.2 °/min.

In vitro degradation of RSF_{PF127}: RSF_{PF127} with or without drugs was accurately weighed and immersed into the PBS solution or 0.1 μ/mL papain solution at 37 °C, respectively. At predetermined time points, these films were rinsed with deionized water, dried and accurately weighed, and the percentage of residues can be calculated from the residual weight and initial weight.

In vitro cytotoxicity of RSF_{PF127}: RSF solutions (100 μL) with gradient drugs (0.6, 1.3, 2.5 and 5 μg/mL) were added into 96-well plates to prepare the medicated RSF_{PF127}. L929 fibroblasts with the basal medium were introduced into the 96-well plate (10⁵ cells per well) and co-incubated with the RSF_{PF127} for 4 h at 37 °C. Subsequently, the complete medium was added, and the cells were finally incubated for 24 and 48 h. Finally, the cell vitalities were evaluated by the MTT method. Besides, the cells were dyed by Calcein AM/PI and subsequently observed by SRLSCM (Olympus Corporation, Tokyo, Japan) to evaluate the survival percentage.

Cell adhesion in Vitro: L929 fibroblasts were transferred to 6-well plates (5 × 10⁵ cells/well) and respectively co-incubated with 3M medical dressing, RSF_{PF127}, RSF_{PF127}@CUR, RSF_{PF127}@AgN and RSF_{PF127}@KR-12 for 12 h at 37 °C. Subsequently, the cells were fixed with 4% paraformaldehyde and washed with PBS with 0.1% Triton-X. The fixed cells were incubated with rhodamine at room temperature for 1 h, and the cell nuclei were stained by DAPI. Finally, the adhesion of cells was evaluated *via* SRLSCM (Olympus Corporation, Tokyo, Japan)

Modulation of the polarization of macrophage in vitro: RAW 264.7 macrophages were induced by lipopolysaccharide for 12 h to polarize into M1-type. The induced macrophages

were separately co-cultured with 3M medical dressing, RSF_{PF127}@CUR, RSF_{PF127}@AgN and RSF_{PF127}@KR-12 in the basal medium at 37 °C for 4 h, and next incubated in the complete medium at 37 °C for 20 h. After being fixed by paraformaldehyde and washed with PBS (containing 0.1% Triton-X), the macrophages were incubated with the primary rabbit polyclonal antibodies for CD206 (Beyotime, Shanghai, China) at 4 °C overnight. Finally, the macrophages were incubated with the goat anti-rabbit antibodies (Cy3, Beyotime, Shanghai, China) at room temperature for 1 h. Meanwhile, the cell nuclei were stained by DAPI. The stained macrophages were imaged by SRLSCM (Olympus Corporation, Tokyo, Japan).

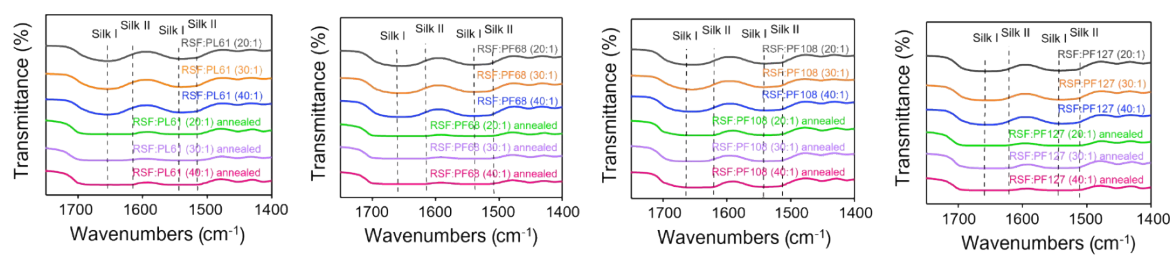


Figure S1. FTIR spectrum of RSF_{PL61} film, RSF_{PF68} film, RSF_{PL108} film and RSF_{PF127} film with different ratios of RSF and PF.

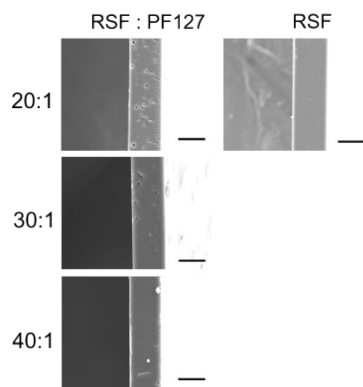


Figure S2. SEM of the side of $\text{RSF}_{\text{PF127}}$ film with different ratios of RSF and PF. Scale bar = 6 μm .

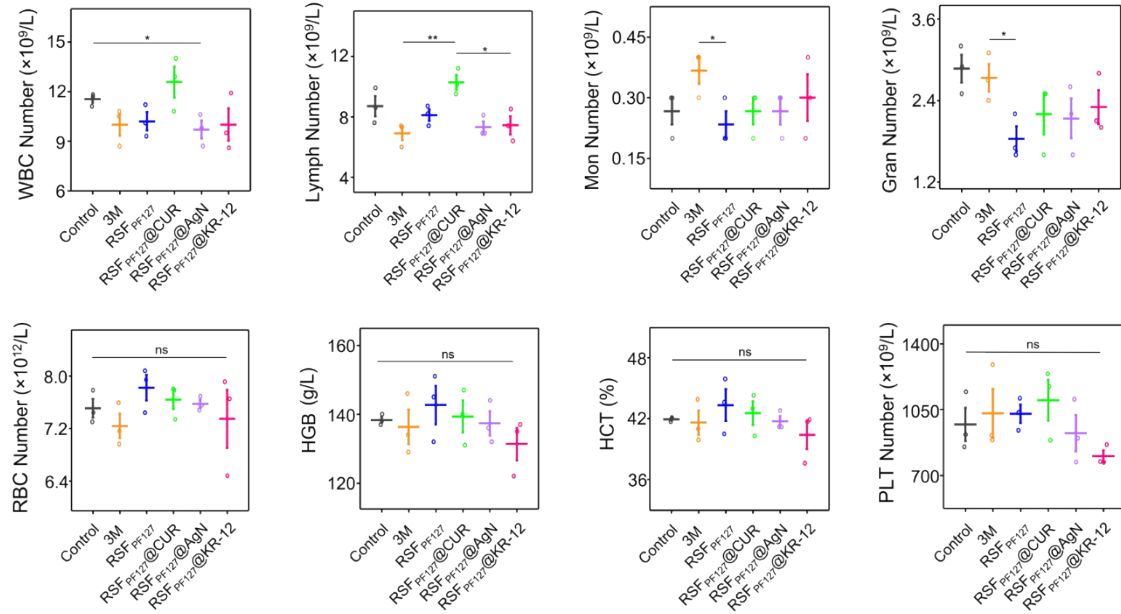


Figure S3. Hematological examinations about WBC (red blood cell count), Lymph (lymphocyte), Mon (monocyte), Gran (neutrophil), RBC (white blood cell count), HGB (hemoglobin), HCT (red blood cell-specific volume) and PLT (platelet count) of wound tissues for SD rats on day 14.

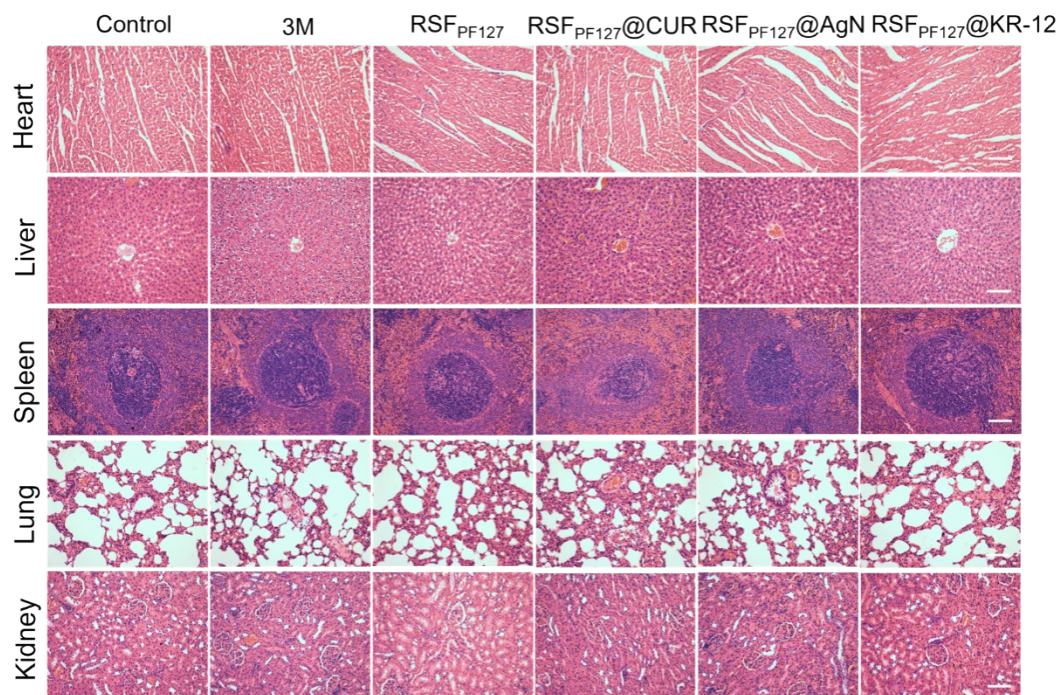


Figure S4. H&E staining of major organs (heart, liver, spleen, lungs and kidneys) of wound tissues for SD rats on day 14. Scale bar = 100 μ m.