Supplementary Information (SI) for Journal of Materials Chemistry B.
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Support Information

Accelerated Scarless Wound Healing by Dynamical Regulation of Angiogenesis and Inflammation with Immobilized Asiaticoside and Magnesium Ions in Silk Nanofiber Hydrogels

Gongwen Yang[a,#], Lutong Liu[b,#], Liying Xiao[a, c], Shiyu Ke[c], Huaxiang Yang[c], Qiang Lu[a,*]

[a]State Key Laboratory of Radiation Medicine and Radiation Protection, Institutes for Translational Medicine, Soochow University, Suzhou 215123, People’s Republic of China

[b]Beijing Allgens Medical Science and Technology Co., Ltd., Beijing 100176, People’s Republic of China

[c]National Engineering Laboratory for Modern Silk & Collaborative Innovation Center of Suzhou Nano Science and Technology, Soochow University, Suzhou 215123, People’s Republic of China

1.1 **Preparation of fresh silk solution**

Briefly, 42.4 g Na_2CO_3 (Sinopharm Chemical Reagent Co. Ltd, China) was dissolved in distilled water (20 L) and used to degum raw silk (50 g, Zhejiang, China) at 100 ^{o}C for 30 min. The degummed silk was dissolved in 9.3 mol/L LiBr (Sinopharm Chemical Reagent Co. Ltd, China) at 60 ^{o}C and then dialyzed against distilled water for 3 days to remove the salt. After centrifugation at 9,000 rpm, a fresh silk solution with a concentration of about 6 wt% was obtained.

1.2 **Preparation of SNF-AC hydrogels**

The SNF-AC hydrogels were prepared using silk nanofibers as drug shuttles. Hydrophobic AC (MCE, NewNishizawa, USA) dissolved in methanol was blended with silk nanofiber hydrogels (2 wt%) at a volume ratio of 1:1. After stirring for 24 h at 200 rpm, the mixed solution was centrifuged at 10,000 rpm for 30 min to separate the AC-laden silk nanofibers and methanol, obtaining AC-laden hydrogels. The hydrogels were washed with distilled water three times to remove the residual methanol, and termed SNF-AC. The centrifuged supernatant was collected to calculate the content of unloaded AC. The content of AC in solution was determined with high-performance liquid chromatography (HPLC, ThermoFisher, USA) with a C^{18} column(5 μm, 250 mm ×4.6 mm, Thermo Fisher, USA). The mobile phase consisted of acetonitrile/water (25 :75 up to 2 min; 90 :10 up to 8 min; 25 :75 upto 10 min) and the flow rate was 1.0 ml/min. The detection was 205 nm. The loading efficiency (LE) and loading capacity (LC) of AC were calculated by the following formulas:

$$LE(\%) = \frac{W_T - W_F}{W_T} \times 100\%.$$

$$LC(\%) = \frac{W_T - W_F}{W_S} \times 100\%.$$

The final SNF-AC hydrogel contained 1.5 mg/mL of AC^+ for control and those contained 3 mg/mL of AC for preparation of the SNF-AC-Mg hydrogel.

1.3 Preparation of SNF-Mg hydrogels

The SNF-Mg hydrogels were treated with ultrasonic homogenization (Ultrasonic Homogenizer JY92-IIN, SCIENTZ, China) for 10 min at room temperature (390W). The hydrogels were transformed into solutions due to the disruption of the silk nanofibers. The Mg^{2+} concentration was fixed to 30 and 60 mM. Thus, magnesium chloride solution (300 and 600 mM, 10mL) was added dropwise into the silk nanofiber solutions (90 mL) and stirred at 2,000 rpm for 12 h at room temperature. The hydrogels formed following the incorporation of Mg^{2+} due to the electrostatic coordination of Mg^{2+} and the silk nanofibers. The final SNF-Mg hydrogel obtained contained 30 mM of Mg^{2+} for control and those contained 60 mM of Mg^{2+} for preparation of the SNF-AC-Mg hydrogel.

1.4 *In vitro* cytocompatibility of SNF-AC-Mg hydrogels

At specific intervals (24 h, 48 h, 72 h), DMEM contained 10% CCK-8 solution was added to each well and incubated for 3 h, Then the solutions were transferred to new 96-well plates and measured at 450 nm with multiscan spectra (Biotek Synergy 4,

Winooski, VT, USA). At the corresponding intervals, these cells were washed with PBS three times and fixed with 4% paraformaldehyde solution (Sigma-Aldrich, St Louis, Missouri) at room temperature for 15 min. After 10 min penetration of 0.1% (v/v) Triton X-100 (Sigma, St.Louis, USA), actin, and nuclei were stained with FITC-phalloidin (Thermofisher, Waltham, USA) and DAPI, respectively. Confocal microscopy (Olympus FV10 inverted microscope, Nagano, Japan) was used to observe fluorescence images.

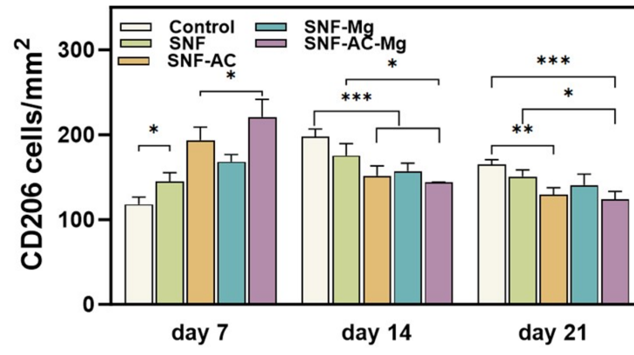

Fig S1. CD206 macrophages per mm^2 at wound sites in immunofluorescence images via ImageJ.