Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

A moisturizing chitosan-silk fibroin dressing with silver nanoparticles-adsorbed exosomes for repairing infected wounds

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1. Materials and methods

1.1. Test of physical properties

The silver content of the AgNPs-Exo and CTS-SF/SA/Ag-Exo dressings was determined by inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7500ce, USA).

Ink was dripped onto the hydrophilic and hydrophobic surfaces of CTS-SF/SA/Ag-Exo dressing at six different points, and photos of the droplets were recorded. The pro-/hydrophobicity of CTS-SF/SA/Ag-Exo dressing was detected by contact angle goniometer (Han Guang High-tech Company in Tai Wan, application: Magic Droplet). The contact angle was measured at five different points on both the hydrophilic and hydrophobic surfaces.

The dressing was dipped into absolute ethanol until saturation. The weight of the dressing was measured before and after immersion. The porosity (P) was calculated by the following formula.[1]

$$P = \frac{m_2 - m_1}{\rho V} \times 100\%$$

In this equation, m_1 and m_2 were the weights of the dressing before and after immersion in alcohol, respectively. V was the volume of the dressing before immersion, and ρ was the density of alcohol at 25°C, i.e., 0.785g/cm^3. All samples were tested in triplicate.

The swelling degree (DS) of the prepared dressing was measured according to a previously reported method.[2] Briefly, for complete swelling examination, dried samples were immersed in five medias (deionized water, normal saline, phosphate buffer, α-MEM and fetal calf serum) at 37 °C for 1h. The dressings were removed, scrubbed softly, and the samples were

immediately weighed. The degree of swelling (DS) was calculated according to Formula:

$$DS = \frac{m_w - m_0}{m_0} \times 100\%$$

In this equation, m_0 and m_w were the weights of the dressings before and after immersion. All samples were tested in triplicate.

To measure the moisture retention of the dressing, the dressing fully swollen in deionized water was placed in a glassware at room temperature with the modified side up, and DS was measured every 1.5 h. The moisture-retention time was recorded when the value of DS was reduced to 100%。

Sustained release protein of the CTS-SF/SA/Ag-Exo dressing was measured using the BCA-protein quantification assay kit.[3] One milliliter of pH 7.4 PBS buffer was added in 24 aperture plank. The CTS-SF/SA/Ag-Exo dressings (size: 10×10 mm) were floated on PBS buffer surface. The hydrophilic surface of the dressing touched PBS buffer, the hydrophobic surfaces touched air. The dressings were cultured for 2h, 4h, 8h, 12h, 24h, 36h, 48h, 60h and 72h at 37°C.

Next, 25 μL test fluid was added in 96 aperture plank, then 200 μL of the BCA working solution was added to each well quickly at 37°C for 30 minutes. Absorbance was measured at 562 nm with the Microplate Reader. The linear regression equation was obtained by using GraphPad Prism software, and the protein concentration of the liquid to be measured was calculated. Finally, the sustained release of total protein was measured in the dressing.

1.2. Cytotoxicity and subcutaneous sensitization studies

CTS-SF/SA/Ag-Exo, CTS-SF/Ag/SA, CTS-SF/Ag and CTS-SF dressings (Co-60 sterilization processing) were immersed in serum-free α-MEM (100mg of sample in 1ml of medium) and extracted for 24 h at 37°C. L929 cells, HFCs

and HUMSCs were cultured in the medium of the dressings extracts and supplemented with 10% FCS. MTT was used to evaluate cell viability and proliferation. The morphology of HFCs was observed by staining of vimentin (Rabbit anti human, Abcam) with the five dressings extracts at four days. Standard tests for irritation and delayed-type hypersensitivity were performed using CTS-SF/SA/Ag-Exo dressing extracts in New Zealand rabbits (wt: 2.5±0.5 Kg) according to ISO 10993-10:2010: Biological Evaluation of Medical Devices, Part 10.

1.3. Bacterial cultures of exudate and blood sample analysis

On day 3, samples were taken from the above five groups (CTS-SF/SA/Ag-Exo, CTS-SF/Ag/SA, Acosin, gauze-Exo and gauze dressings groups) of animals. The mice were sacrificed, and the body surfaces were disinfected. In a biosafety cabinet, wound excretion was sampled with a sterile swab and added into the LB fluid medium for 8 h at 37°C, and photographed LB pipes. Then, 50 μL of LB fluid medium from each group was spread on an agar plate and cultured for 12 h at 37°C, and photographed the plates.

On days 3, 7, 10 and 12, among five groups of animals, blood samples (using EDTA-K as anticoagulant) were collected in EDTA-K anticoagulation tubes by removing mice eyeballs (Anesthetized by intraperitoneal injection of 1% sodium pentobarbital). The blood samples were analysed by Full automatic blood analyzer (XS-800i, Sysmex Corporation, Japan), monitoring the change of WBC count, NEUT% and LYMPH%.

2. Supplementary data:

Table S1. Silver Content in different Materials

Materials	Silver content
AgNPs-Exo-1	83.84±2.773 (mg/L)
AgNPs-Exo-2	44.41±0.788 (mg/L)
AgNPs-Exo-3	26.53±1.082 (mg/L)
AgNPs-Exo-4	15.41±0.815 (mg/L)
AgNPs-Exo-5	10.47±1.143 (mg/L)
CTS-SF/Ag/SA	101.94±2.991(mg/kg)
CTS-SF/SA/Ag-Exo	111.03±3.077(mg/kg)

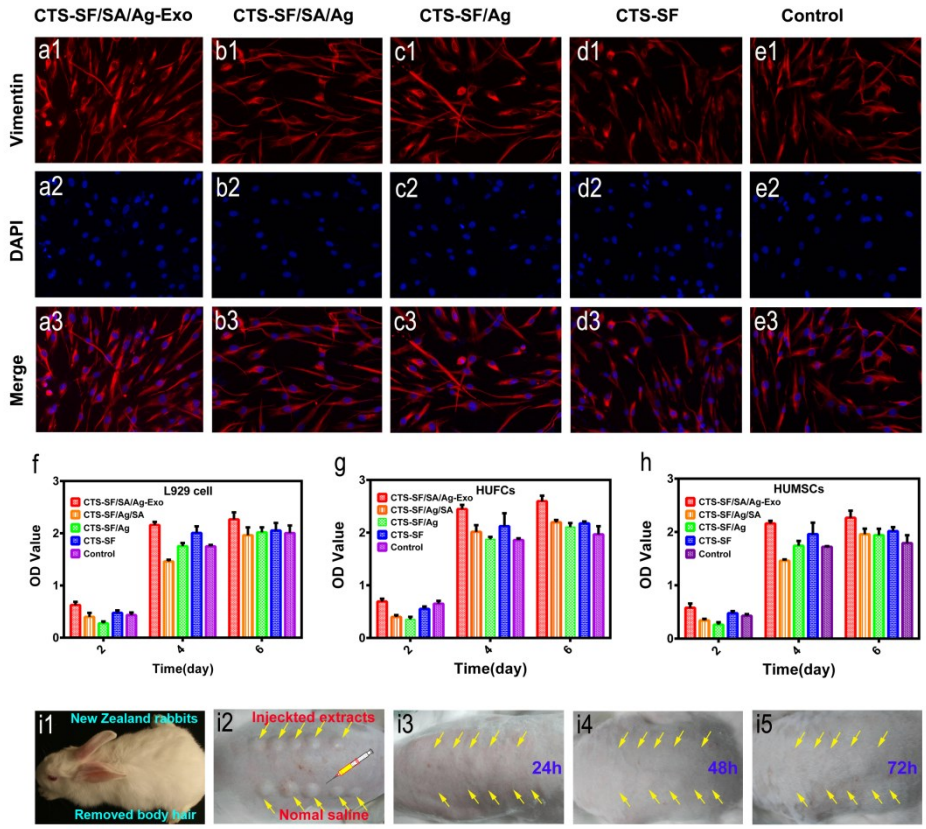

Fig. S1. Evaluation of cytocompatibility and subcutaneous sensitization of rabbits. (a-e) HFCs stained with vimentin (red) and DAPI (blue) cultured in the four dressing extracts on day 4. (f-h) Cytocompatibility test of the dressing extract on L929 cells, HFCs and HUMSCs. (i) Subcutaneous sensitization test of CTS-SF/SA/Ag-Exo dressing extract observation at times of 24, 48, and 72 h.

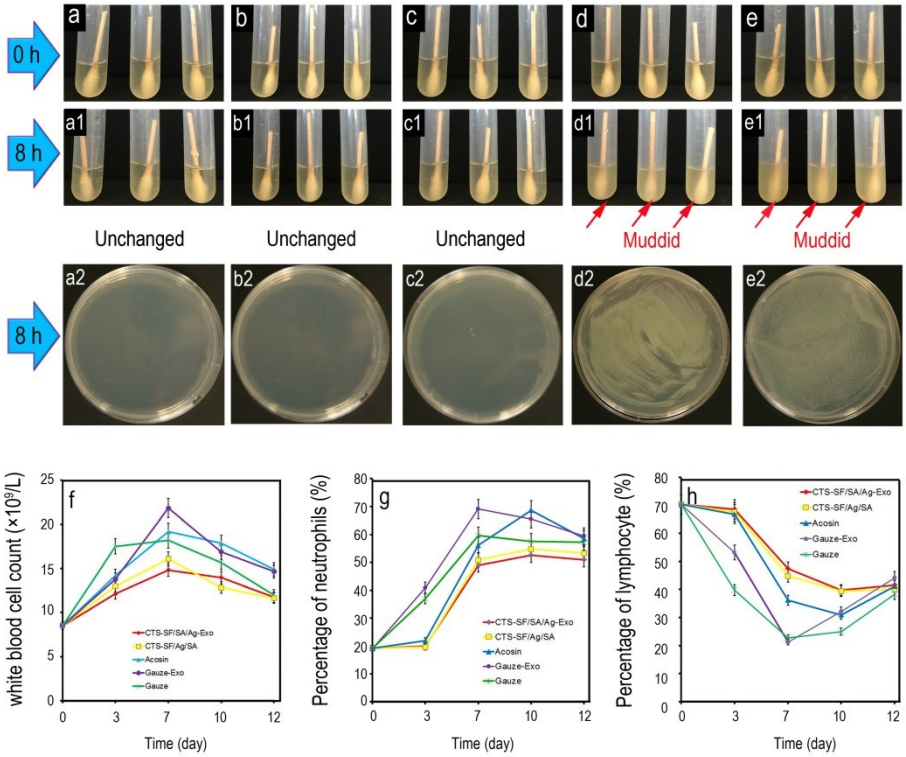

Fig. S2. Bacterial cultures of exudate and blood sample analysis. (a-e) Bacterial culture of wound secretion on day 3, (a) CTS-SF/SA/Ag-Exo group, (b) CTS-SF/Ag/SA group, (c) Acosin group, (d)gauze-Exo group, (e) gauze group. (f) The test of white blood count on days 3, 7, 10 and 12. (g) The test of neutrophil granulocyte percentage on days 3, 7, 10 and 12. (h) The test of lymphocytes percentage on days 3, 7, 10 and 12.

References

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