

Supplementary Information for Biomaterials Science

# Non-sticky and Antimicrobial Zwitterionic Nanocomposite Dressings for Infected Chronic Wounds

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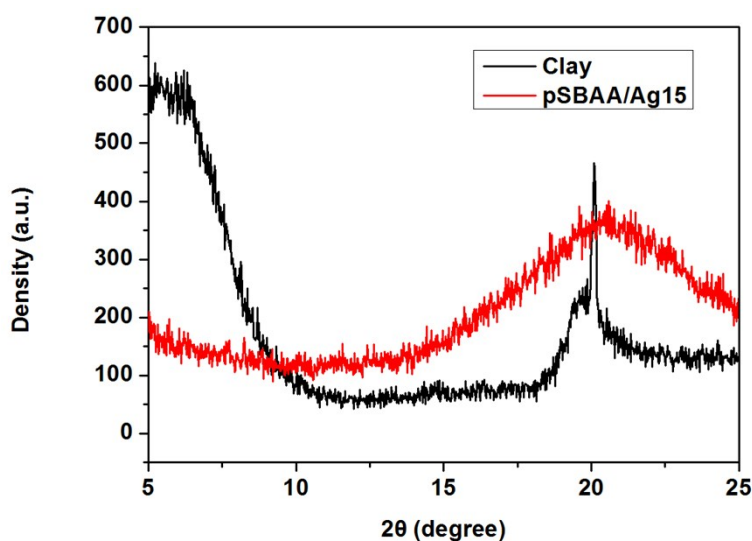
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## **XRD pattern of polymer/clay interaction:**

The formation of polymer–clay networks in the pSBAA/Ag15 hydrogel was

confirmed using X-ray diffraction (XRD). To prepare samples for XRD, the freeze-dried hydrogels were ground into fragments carefully. XRD analyses were carried out with a single-crystal X-ray diffractometer (Bruker KAPPA APEX II, Germany) using Cu K $\alpha$  X-rays,  $2\theta = 5^\circ$ – $25^\circ$ . In the XRD pattern of the clay, a strong X-ray diffraction peak was observed at around  $2\theta = 7^\circ$  in Figure S1. The result corresponded to a layer spacing of 1.5 nm for regularly stacked clay sheets, which was consistent with a literature published by Haraguchi et al.<sup>1</sup> However, the peak was not observed for the dried pSBAA/Ag15 gel, indicating that the clay was sufficiently exfoliated in the pSBAA/Ag15 sample.



**Figure S1.** XRD profiles for pSBAA/Ag15 sample and nanoclay.

#### **The efficiency of silver nitrate reduction:**

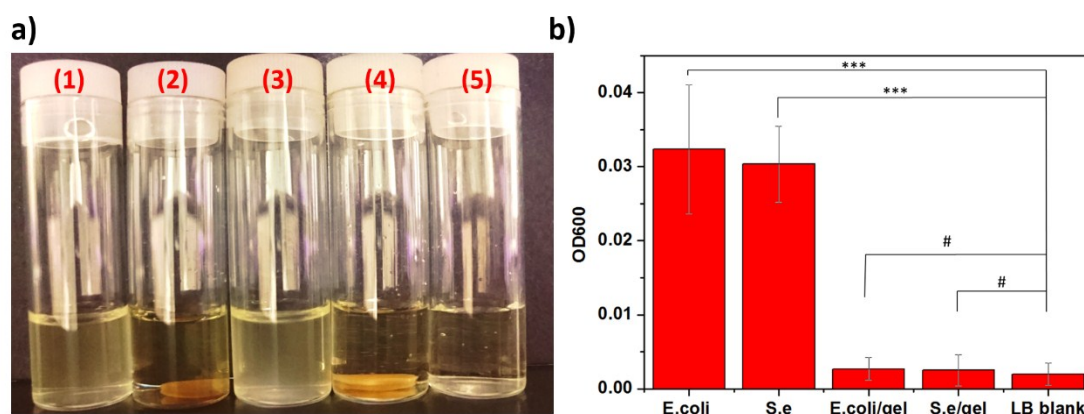
The efficiency of silver nitrate salt reduction in the pSBAA/Ag5 and pSBAA/Ag15 hydrogel was calculated by the percentage of ratio of the total silver ions in the hydrogel after reduction and the initial addition of silver ions. The total silver ions in hydrogels after reduction was measured with inductively coupled plasma-mass spectrometer (ICP-MS, Agilent 7500ce, Japan) by dissolving all reduced silver by nitric acid in the pSBAA/Ag15. The efficiency of silver nitrate reduction in hydrogels is shown in Table S1. The efficiency of silver nitrate reduction in pSBAA/Ag5 was 0.2%, and that of pSBAA/Ag15 increased to 0.49%. The increasing of the efficiency of silver nitrate can be ascribed to the exfoliated nanoclay as stabilizer to reduce silver nanoparticles.

**Table S1.** Efficiency of silver nitrate reduction in hydrogels.

Sample code	Nanoclay (wt%)	AgNO <sub>3</sub> (mg/mL)	Addition of silver ions (ppm)	Total silver ions after reduction (ppm)	Efficiency of silver nitrate reduction (%)
pSBAA/Ag5	3.81	1.7	1046	2.136	0.2
pSBAA/Ag15	11.43	1.7	1046	5.121	0.49

### Bacterial inhibition effect in liquid medium:

The bacteria inhibition effect in liquid medium were tested by measurement of optical density (OD600) in liquid media with Gram negative *Escherichia coli* (E. coli, ATCC® 25922™) and Gram positive *Staphylococcus epidermidis* (S. e, ATCC® 12228™). The bacterium was incubated in sterile Luria-Bertani (LB) liquid culture medium at 37 °C for 16 h at a shaking speed of 200 rpm. After, the bacteria solution was diluted to a 0.01 optical density at 600 nm (OD600) with fresh LB solution. Then, 10 μL of diluted bacterial solution was dropped into 990 μL of fresh LB solution. The sterile pSBAA/Ag15 hydrogels were cut into circles with 8 mm in a diameter and immersed in the final bacterial solution. The fresh LB broth solution was serve as negative control and the bacteria solution without addition of gel was serve as a positive control. All samples were then placed in an incubating oven at 37 °C for 1 day. After 1 day, the liquid samples were photographed in Figure S2a and the OD600 of liquid samples were measured by a spectrophotometer (Synergy 2, BioTek) in Figure S2b. Obviously, the bacteria-containing LB solution with pSBAA/Ag15 hydrogel was yellowish transparent after incubation as the same as the fresh LB broth solution in Figure S2a. In contrast, the bacterial solution without pSBAA/Ag15 hydrogel became turbid. The results of bacterial inhibition effect in liquid medium support the great germicidal effect of pSBAA/Ag15 for infected wound treatment.



**Figure S2. a)** The photo images of E.coli-contained LB solution (1), E.coli-contained LB solution with pSBAA/Ag15 gel (2), S. e-contained LB solution (3), S. e-contained LB solution with pSBAA/Ag15 gel (4), and fresh LB solution (5) at 37°C incubation

after 1 day. **b)** OD600 for each conditions of LB solution, #p > 0.05, \*\*\*p < 0.001.

**Reference:**

1. J. Ning, G. Li and K. Haraguchi, *Macromolecules*, 2013, **46**, 5317-5328.