Supplementary material

Customizing Spatial Distribution and Release of Silver for Antibacterial by Biomineralized Self-assembling Silver-loaded Hydroxyapatite

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1. Preparation of silver-loaded hydroxyapatite antibacterial nanoparticles

1.1 *Staphylococcus aureus* culture

The traditional microbial experiment technology was applied for Staphylococcus aureus culture. The sterile Luria nutrient broth (Contains 0.5wt% NaCl, 1 wt% Tryptone, 0.3wt% beef extract, pH 7.4） was used as the bacterial culture medium at 37 °C, constant speed 140 rpm/min and continue shaking culture for 24 h. Then 1 L of the above-mentioned Staphylococcus aureus (~10^9 CFU/mL) culture solution was centrifuged at 4500r/min for 10 min, and then resuspend it in 50 mL of phosphate buffered saline (PBS) buffer. Finally, the bacteria pellets were rinsed with sterilized deionized water and centrifuged, and dispersed in 20 mL of deionized water to obtain a high concentration of Staphylococcus aureus solution.

1.2 Biominerlized assembling silver-loaded hydroxyapatite layer-by-layer

Table S1. Reagents added in each step of preparation of Ag-HA-HA, HA-Ag-HA and HA-HA-Ag particles

Step	Ag-HA-HA sample	HA-Ag-HA sample	HA-HA-Ag sample
Step1	**S_1 solution** $AgNO_3$ (10mL, 0.01g/mL) S.aureus(20mL,~10^9 CFU/mL)	**S_1' solution** $Ca(NO_3)_2$(10mL 0.225 M) S.aureus(20mL,~10^9CFU/mL)	**S_1" solution** $Ca(NO_3)_2$(10mL 0.225 M) S.aureus(20mL,~10^9CFU/mL)
Step2	**S_2 solution** Na_2HPO_4 (80 mL, PH=12) +S_1	**S_2' solution** Na_2HPO_4(80 mL, PH=12) +S_1'	**S_2" solution** Na_2HPO_4(80 mL, PH=12) +S_1"
Step3	**S_3 solution** $Ca(NO_3)_2$(10mL 0.225 M)+S_2	**S_3" solution** $AgNO_3$ (10mL, 0.01g/mL)+S_2''	**S_3" solution** $Ca(NO_3)_2$(10mL 0.225 M)+S_2"
Step4	**S_4 solution**	**S_4' solution**	**S_4" solution**

	Na_2HPO_4(10 mL, PH=12) +S_3	Na_2HPO_4(10 mL, PH=12) +S_3'	Na_2HPO_4(10 mL, PH=12) +S_3"
Step5	**S_5 solution** $Ca(NO_3)_2$(10mL 0.225 M) +S_4	**S_5' solution** $Ca(NO_3)_2$(10mL 0.225 M) +S_4'	**S_5" solution** $AgNO_3$ (10mL, 0.01g/mL) S_4"
Step6	Washing, Dispersion, Heating and drying	Washing, Dispersion, Heating and drying	Washing, Dispersion, Heating and drying

2. FT-IR samples

The cultured *Saphylococcus aureus*, S1 and S1' suspension were centrifuged, washed with water for three times, and the precipitate was freeze-dried to obtain powder and was compressed to tablets with 0.16g potassium bromide.

3. XRD step-by-step

The solutions in Step 2 (S_2, S_2', S_2")→Step 3(S_3, S_3', S_3")→Step 4(S_4, S_4', S_4")→Step 5(S_5, S_5', S_5") are centrifuged, washed by ethanol and dried to obtain the corresponding powder samples, which are characterized by XRD.

4. Ag-HA prepared by co-precipitation method

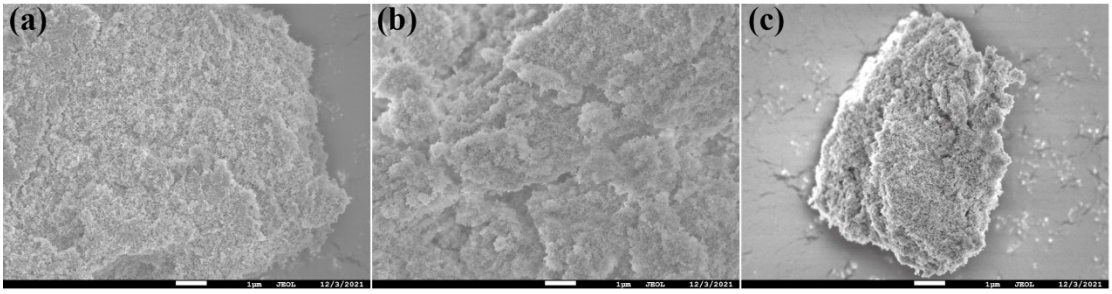

Fig. S1. SEM of Ag-HA prepared by co-precipitation method without *S.aureus*

templates: (a)Ag-HA-HA, (b)HA-Ag-HA, (c)HA-HA-Ag

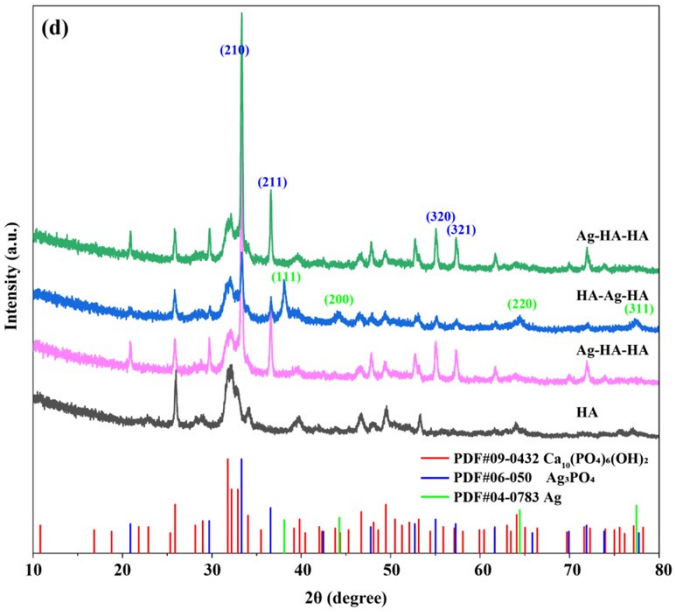

Fig. S2. XRD patterns of Ag-HA prepared by co-precipitation method without *S.aureus* templates: (a)Ag-HA-HA, (b)HA-Ag-HA, (c)HA-HA-Ag

XRD patterns of Ag-HA prepared through the preparation procedure of experimental section without *S. aureus* templates shows that all the three samples contain $AgPO_4$ and HA phase with excellent crystallinity. That's because the ions cannot be anchored without *S.aureus* templates and PO_4^{3-} ions in the solution are sufficient enough to react with both Ca^{2+} and Ag^{+} ions. $AgPO_4$ and HA can nucleate and crystallize independently. Specially, for HA-Ag-HA prepared without *S. aureus* templates, other than $AgPO_4$ and HA phase, there is still elemental Ag because Ag^{+} tend to be reduced without anchoring effect of templates.

5. Silver ions release

(1) Ag^{+} concentration with different soaking times

The three particles (Ag-HA-HA, HA-Ag-HA, and HA-HA-Ag) with the same

mass were immersed in the same amount of deionized water. Every seven days, the solutions were centrifuged to collect the supernatant and the precipitation was immersed in the same amount of deionized water again. The concentration of Ag^+ ions in the supernatant was tested by atomic absorption spectrometry.

(2) **Ag^+ concentration with duration**

The three particles (Ag-HA-HA, HA-Ag-HA, and HA-HA-Ag) with the same mass were placed in the same amount of deionized water. The same amount of supernatant was collected every seven days, in which the concentration of Ag^+ ions in the supernatant was tested by atomic absorption spectrometry.

6. Inhibition zone experiment

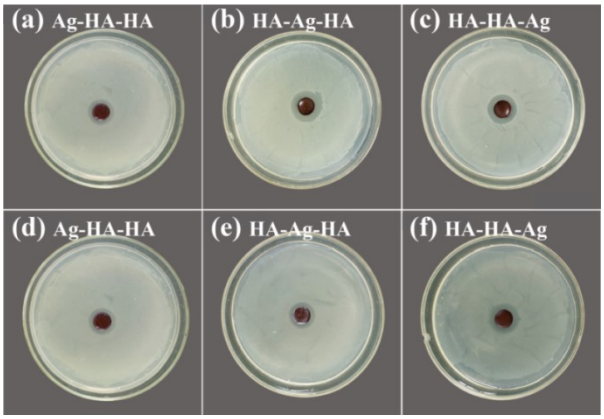

Fig. S3. Bacteriostatic zone test of silver-loaded hydroxyapatite antibacterial agents: S. **aureus ((a),(b),(c)) and Escherichia coli ((d),(e),(f)).**