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Supporting Information for:

A hybrid hydrogel with in situ formed Ag-nanoparticles within 3D networks that exhibits broad antibacterial activities

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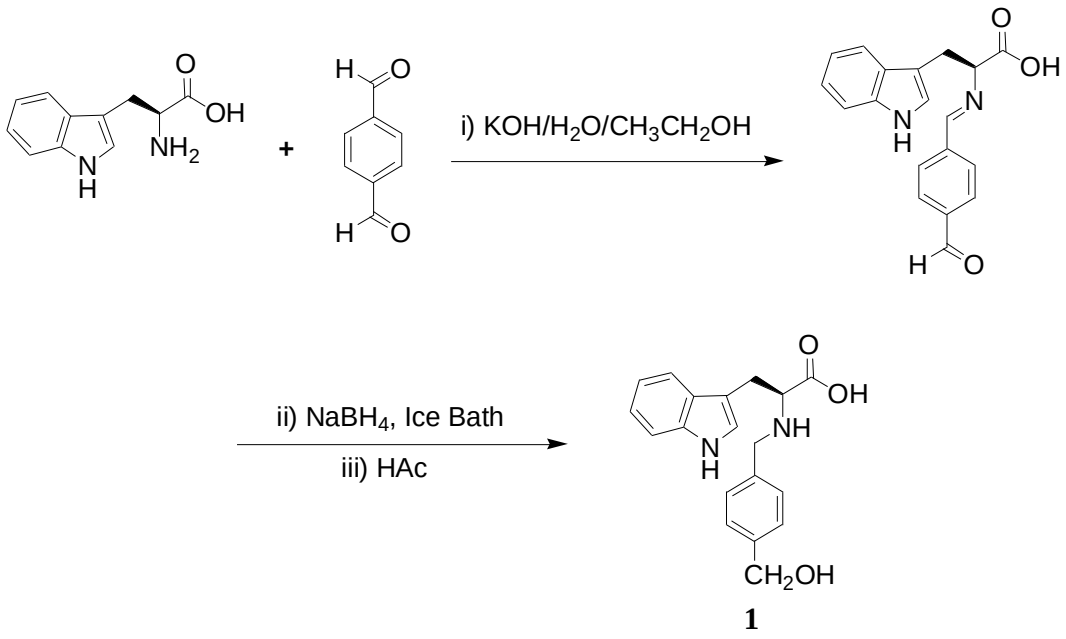
1. Experimental Section

1.1 Reagents

Tryptophan, *p*-Phthalaldehyde and sodium borohydride ($NaBH_4$) were purchased from Aladin Reagent (Shanghai, China). These reagents were used without further purification. All other reagents were of analytical grade, which include HNO_3, KOH and $AgNO_3$, *etc*. Deionized water (MillQ, 18.2 MΩ) was used.

1.2 Synthesis of gelators

The synthesis of compound 1:



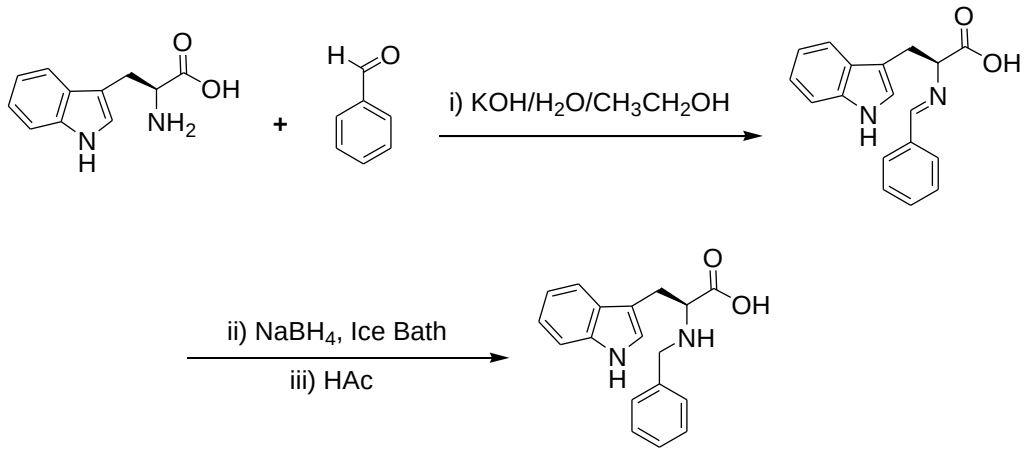
Scheme S1: Synthetic route of 1.

Preparation of compound 1 (HAIP): HAIP was prepared following a modified procedure in literature.[1] To an aqueous solution (10 mL) of L/D-Tryptophan (1 g, 5 mM) containing KOH (0.28 g, 5 mM), 1,4-Benzenedicarboxaldehyde Terephthalaldehyde (0.67 g, 5 mM) in ethanol (5 mL) was added slowly. The solution was stirred for 3 h at room temperature, and during this period the color of the solution was darker. Then the solution was cooled in an ice bath. $NaBH_4$ (0.23 g, 6 mM) was added to the solution slowly. The mixture was stirred for 3 h, and 50% acetic acid was used to neutralize the basic (pH~10) reaction mixture and adjusted the pH to 4.0-5.0. The mixture system was stirred further for 3 h. The resulting solid was filtered off, and washed with ethanol and water, dried, and recrystallized from water/ethanol (3:1). Yield: 1.15 g, 68.9%.

1H NMR (500 MHz, $(CD_3)_2SO$, ppm): $-CH_2$ (1.88, s, 2H), $-CH_2OH$ (4.45, s, 2H), In-H and Phe-H (6.94-7.50, m, 10H), -NH(10.79, s, 1H), -COOH(10.89, s, 1H)

MS (ESI): calc. for $C_{19}H_{20}N_2O_3$ 324.15; observed 323.20 $[M - H]^-$.

The synthesis of compound 2:



2

Scheme S1: Synthetic route of 2.

Preparation of compound 2: The synthetic procedure of compound 2 is similar to that of HAIP, except replacing 1,4-Benzenedicarboxaldehyde Terephthalaldehyde with benzaldehyde. Yield (compound 2): 1.2 g, 78.4%.

1H NMR (500 MHz, D2O, ppm): -CH2 (2.82, d, 2H). -CH (3.17, t, J = 6.7 Hz, 1H), -CH2 (3.55, d, 2H), In-H and Phe-H (6.94-7.44, m, 11H)

MS (ESI): calc. for $C_{18}H_{18}N_2O_2$ 294.15; observed 293.35 $[M - H]^+$.

1.3 Preparation of HAIP and Ag NPs-HAIP gels

HAIP gel preparation

Firstly, HAIP gelator was dissolved in a basic solution. And then, it was adjusted to moderate pH by addition of HNO_3. White HAIP hydrogel was generated at pH 1-7 with the help of sonication after 40 minutes.

Ag NPs-HAIP hydrogel preparation

Ag NPs-HAIP hybrid hydrogel was prepared by mixing HAIP solution (pH 8-9) and silver nitrate solution, and the final concentrations of HAIP and Ag^+ were 10 mM and 1 mM, respectively. The

mixture was gradually changed into a yellow metallohydrogel under diffused sunlight at room temperature after several minutes.

1.4 UV-Vis studies

UV-Vis spectral changes of the mixture of $AgNO_3$ and HAIP were monitored on a Hewlett-Packard 8453 diode array spectrometer within 20 min.

1.5 SEM, TEM, ESI-MS and NMR studies

Scanning electron microscope (SEM) images were obtained on a FEI HELIOS NanoLab 600i SEM (America). Transmission electron microscope (TEM) images were obtained from a FEI Titan microscope (America). The mass spectrum measurements of HAIP and Ag NPs-HAIP were obtained by using Xevo G2-XS QTof mass spectrometer (Waters, America). NMR experiments were performed by using AMX-500 (Bruker, Switzerland).

1.6 Fluorescence spectral studies

Fluorescence spectral measurements of HAIP and Ag NPs-HAIP were performed on a Fluorescence spectrometer (F-7000, Hitachi). (C_{HAIP} = 2 mM, C_{Ag} = 0.2 mM, λ_{ex} = 305 nm).

1.7 Antibacterial activity studies:

The Escherichia coli (*E. coli*), *S. albus* and *S. aureus* strains were applied to evaluate the antibacterial activity of Ag NPs-containing hybrid hydrogels. Addition of 1.0 g peptone, 0.5 g yeast extract and 1.0 g sodium chloride to a beaker, the mixture was dissolved in ultrapure water and was transferred to conical flask with sodium hydroxide to adjust pH to 7.4, and diluted with ultrapure water to 100 mL. Thus, a liquid medium was finished. In the same way, 2.4 g agar was added to prepare a solid medium. These mediums were tightly sealed and then autoclaved at 110 ℃ for 30 minutes. The shaking flask method was used to investigate the antibacterial ability of the as-prepared materials.

In sterile conditions, 100 μL of cryopreservation bacterial suspension (4 ℃) and 8.9 mL liquid medium were dispersed into 3 centrifuge tubes. 1.0 mL of HAIP gels, Ag NPs-HAIP gels, and ultrapure water were added into the above centrifuge tubes, respectively. The untreated bacteria

were used as the control group. These mixtures were shaken up and were incubated in the constant temperature air shock incubator at 37 ℃ for 3 h. The following step was to melt the solid medium; 10 mL of the solid medium was taken into 3 culture dishes before solidification, respectively. 10 μL of mixed bacterial suspension was taken out from centrifuge tubes and was inoculated evenly to the solid medium. After solidification of the culture medium, the solid medium was turned over and cultured at 37 ℃ to form bacterial colonies for 24 h. Finally, the growth of the strains was photographed and examined under a microscope.

1.8 Cell cytotoxicity studies:

The cytotoxicity in cell was obtained using a standard methyl thiazolyl tetrazolium 17 (MTT) assay in MCF7 living cell lines. The MCF7 cell were seeded into a 96-well cell culture plate. HAIP, Ag NPs-HAIP, with different concentrations (0, 50, 75, 100, 200, 300 μM, respectively), were injected to the wells of the treatment group, and the cells were incubated for 24 hours at 37 ℃. The MTT solution was further added to each well of the 96-well assay plate, and continued to incubate for 4 h. In addition, we assessed the cell viability at different concentrations (0.2 mg/mL, 0.6 mg/mL, 1 mg/mL, 2 mg/mL, 5 mg/mL, 10 mg/mL, 20 mg/mL). An enzyme-linked immunosorbent assay (ELISA) reader (infinite M200, Tecan, Austria) was utilized to obtain the OD 490 (absorbance value) of each well.

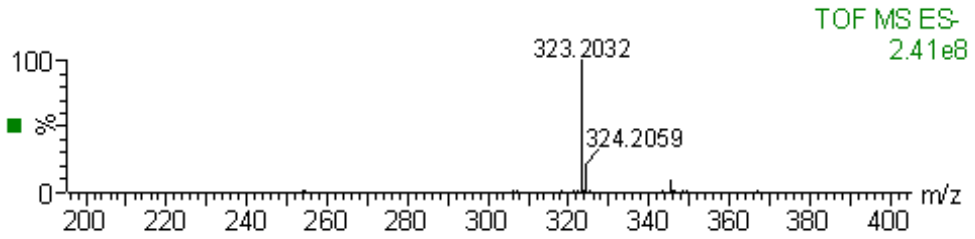


Fig. S1 Electrospray ionization mass spectra of HAIP.

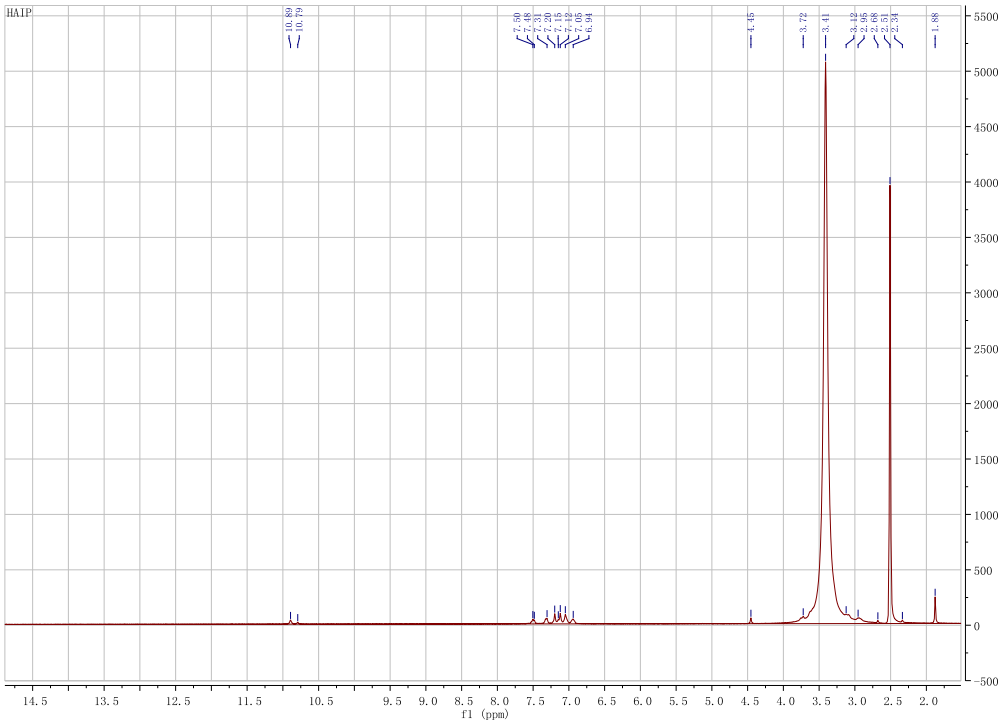


Fig. S2 ^{1}H NMR (500 MHz) Spectra of HAIP in $(CD_3)_2SO$.

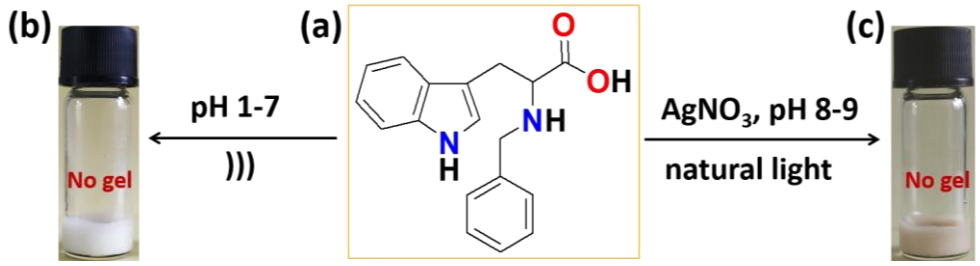


Fig. S3 (a) The structure of compound 2, the gelling behaviors of (b) compound 2, (c) compound 2-$AgNO_3$.

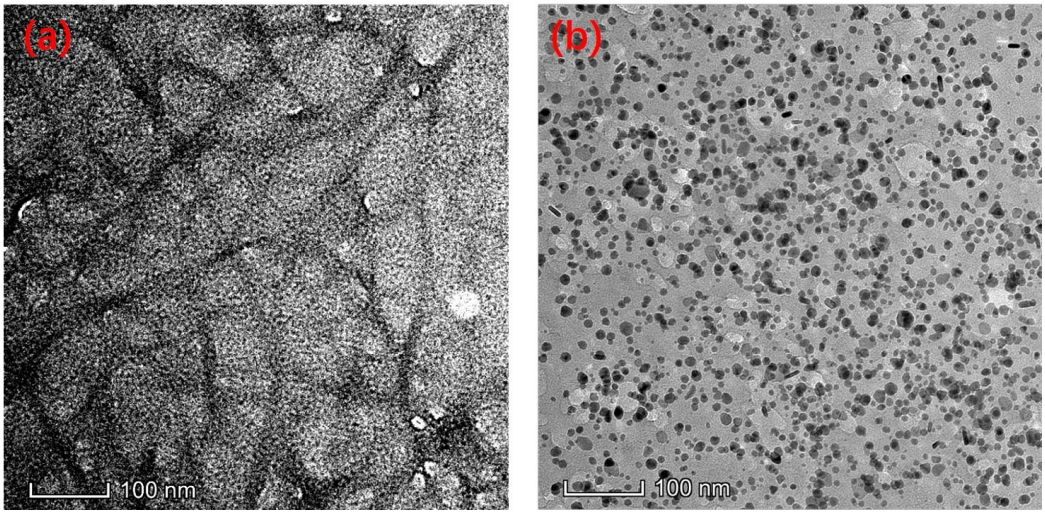


Fig. S4 TEM images of HAIP and Ag NPs-HAIP xerogels.

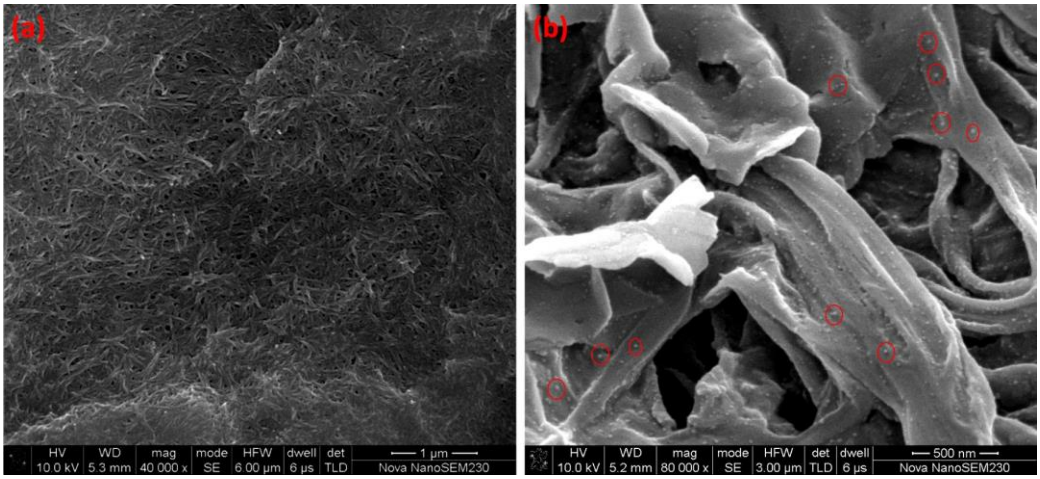


Fig. S5 SEM images of HAIP and Ag NPs-HAIP xerogels.

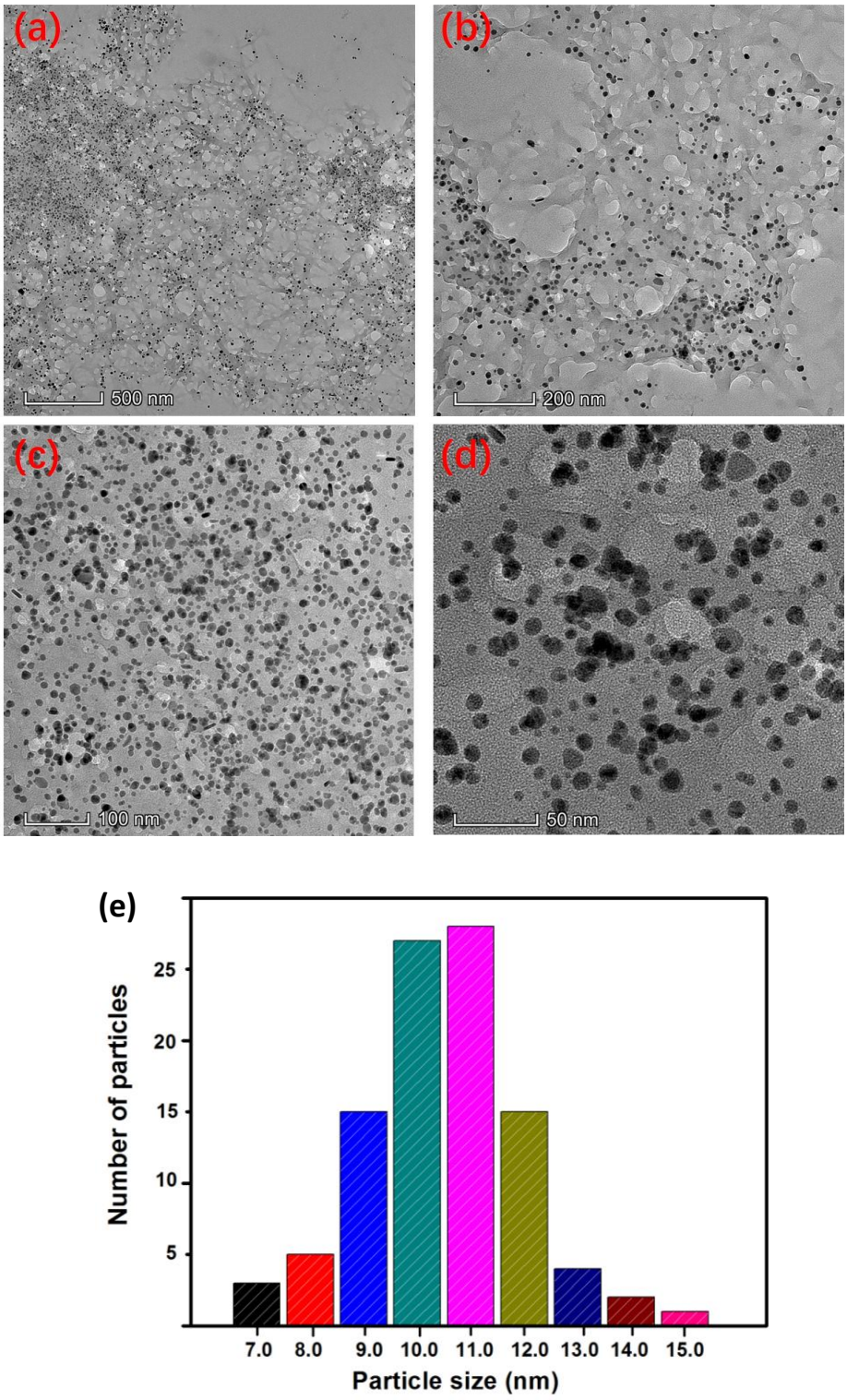


Fig. S6 TEM images of (a-d) Ag NPs-HAIP xerogels (scale bars are (a) 500 nm, (b) 200 nm, (c) 100 nm, (d) 50 nm, respectively), and (e) diameter histogram of Ag NPs.

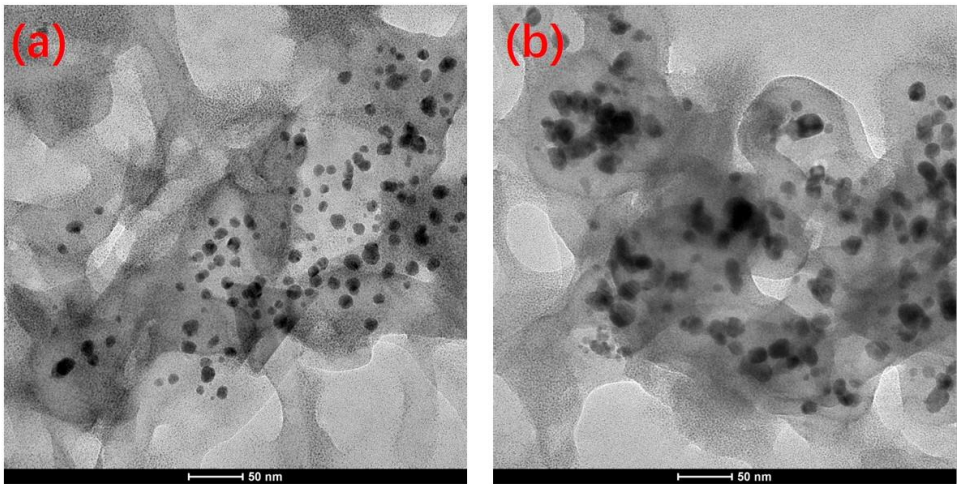


Fig. S7 TEM images of Ag NPs-HAIP xerogels containing the different silver concentrations ((a)1 mM, (b) 5 mM, respectively).

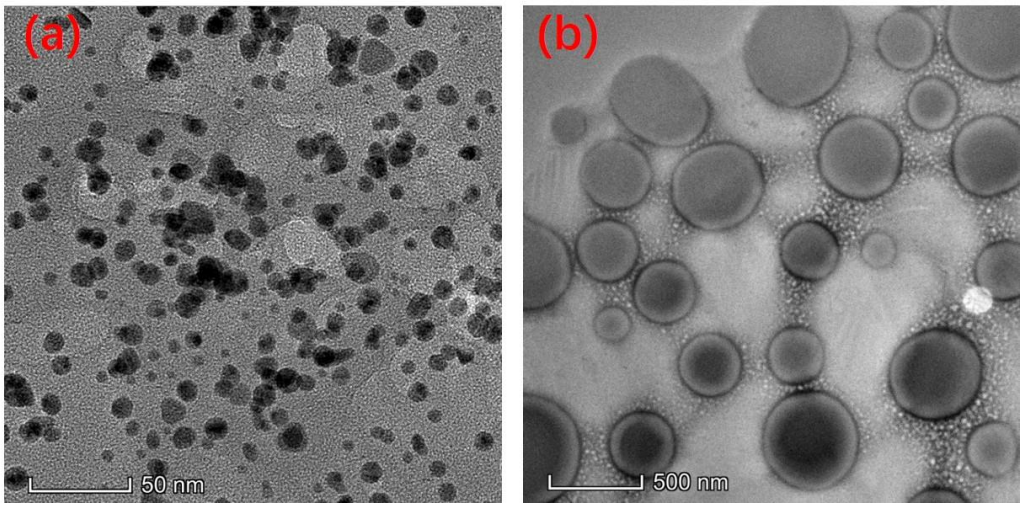


Fig. S8 TEM images of Ag NPs-HAIP xerogels containing the same silver concentration (1 mM) formed at (a) 15 min, (b) one week.

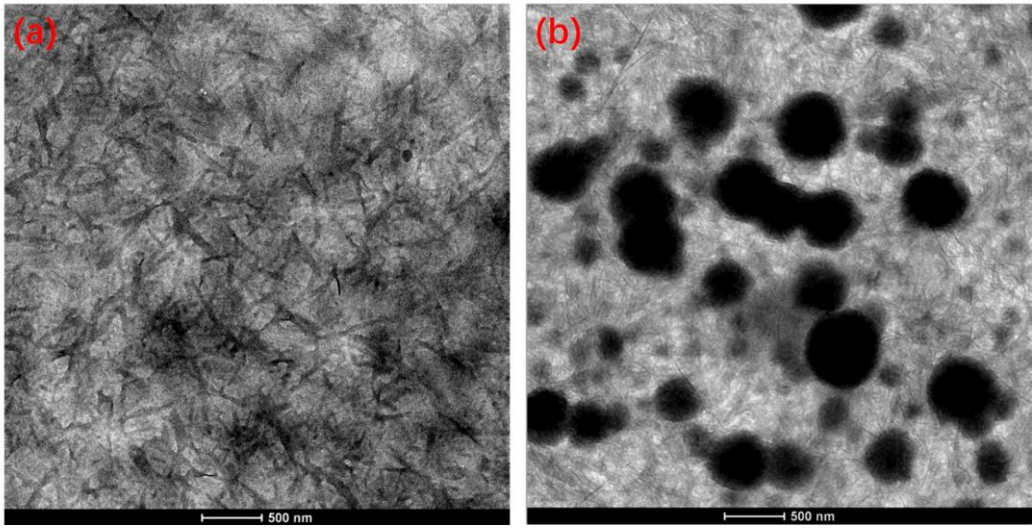


Fig. S9 TEM images of (a) (L+D)-HAIP, (b) Ag NPs-(L+D)-HAIP xerogels.

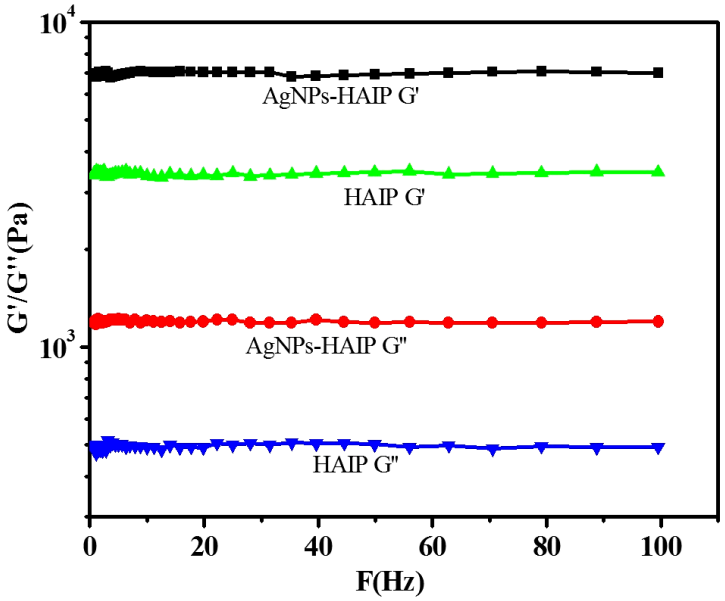


Fig. S10 Dynamic frequency sweep of fresh HAIP gel and Ag NPs-HAIP gel at their respective MGC, measured at 0.1% strain.

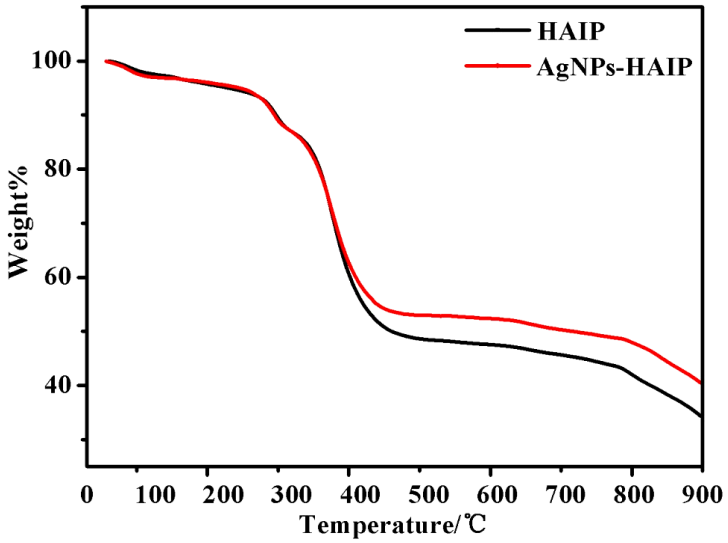


Fig. S 11 TG curves of HAIP, and Ag NPs-HAIP xerogels. Thermal analysis system in a dynamic nitrogen atmosphere (heating rate: 10 ℃/min, mass 1-3 mg, temperature range from room temperature up to 800 ℃).

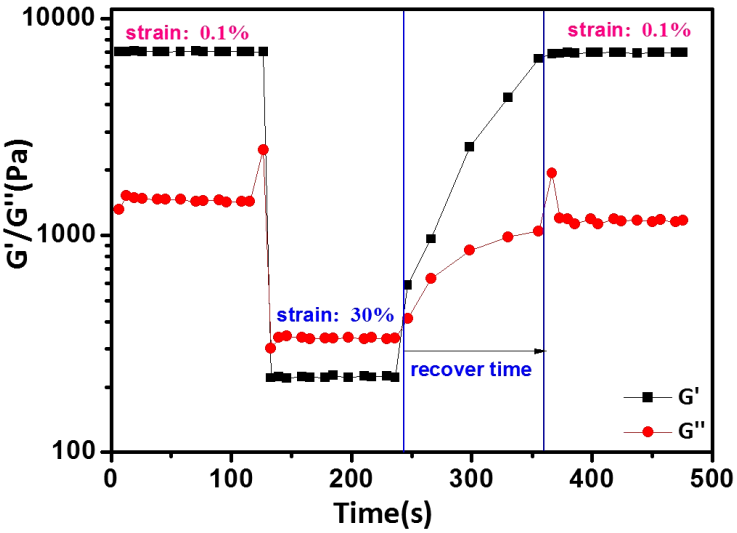


Fig. S12 The step strain experimental data obtained from Ag NPs-HAIP gels at a constant frequency of 1 Hz.

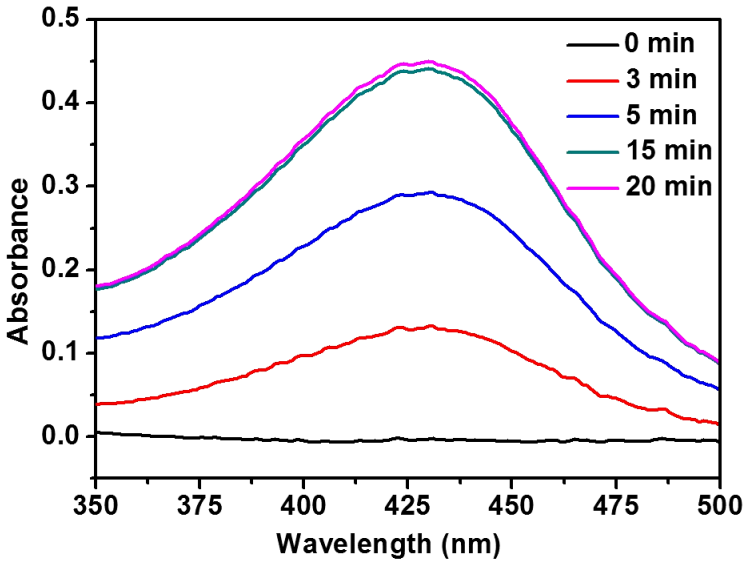


Fig. S13 UV-vis absorption of deluted Ag NPs-HAIP xerogels formed at different time (dispersed in DMSO).

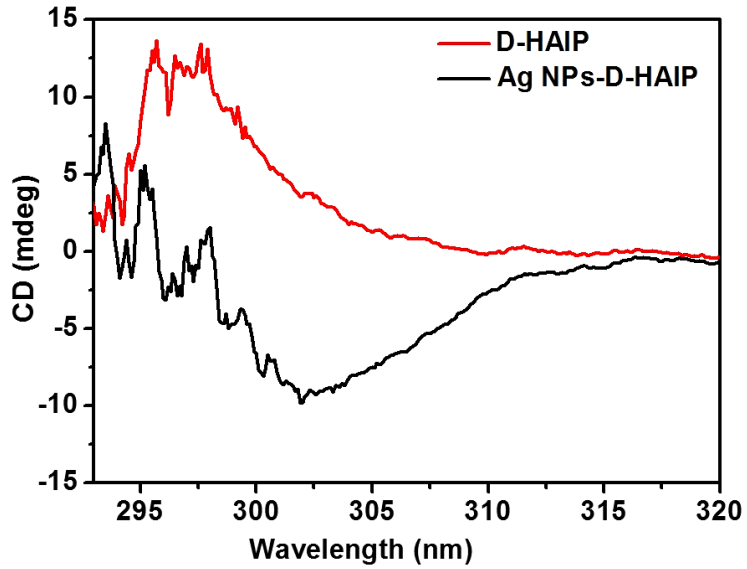


Fig. S14 CD spectra of D-HAIP and Ag NPs-D-HAIP (C_{HAIP} = 2 mM).

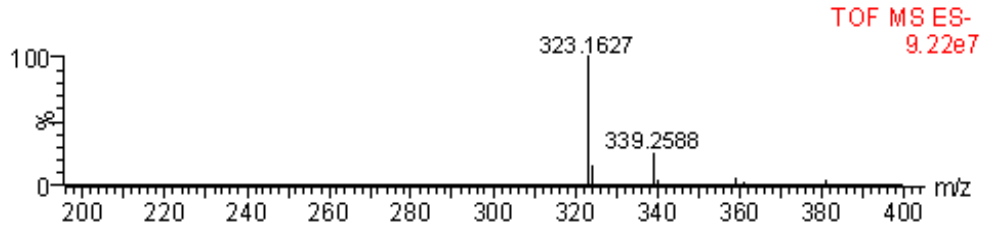


Fig. S15 Electrospray ionization mass spectra of Ag NPs-HAIP.

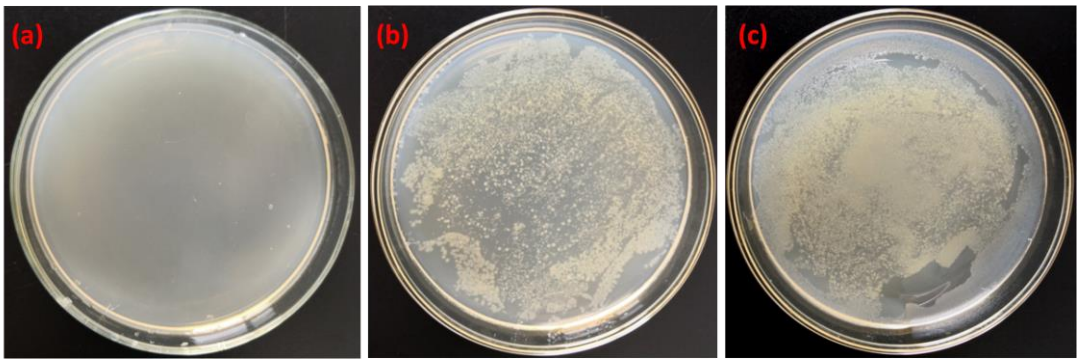


Fig. S16 The digital photos of colonies from E. coli treated with (a) Ag NPs-HAIP (C_{Ag} = 0.1 mM), (b) HAIP gel, (c) untreated, respectively.

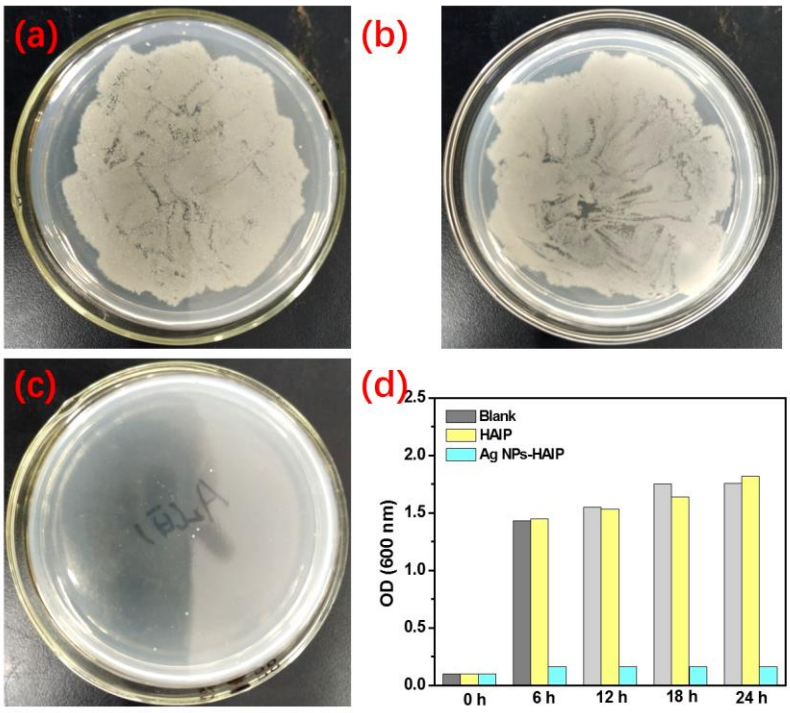


Fig. S17 The digital photos of colonies from S.albus (a) untreated, treated with (b) HAIP gel, (c) Ag NPs-HAIP (C_{Ag} = 75 μM), respectively, (d) Graphical representation of the OD measurements in S.albus at different time (0 h, 6 h, 12 h, 12 h, and 24 h, respectively).

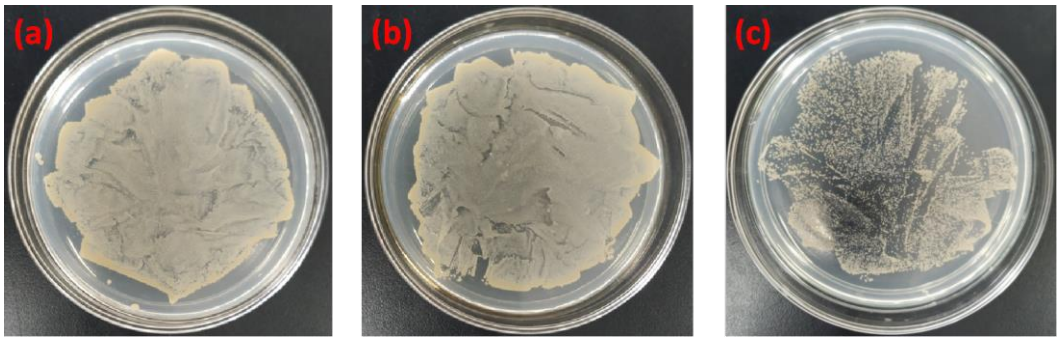


Fig. S18 The digital photos of colonies from S.aureus (a) untreated, treated with (b) HAIP gel, (c) Ag NPs-HAIP (C_{Ag} = 0.1 mM), respectively.

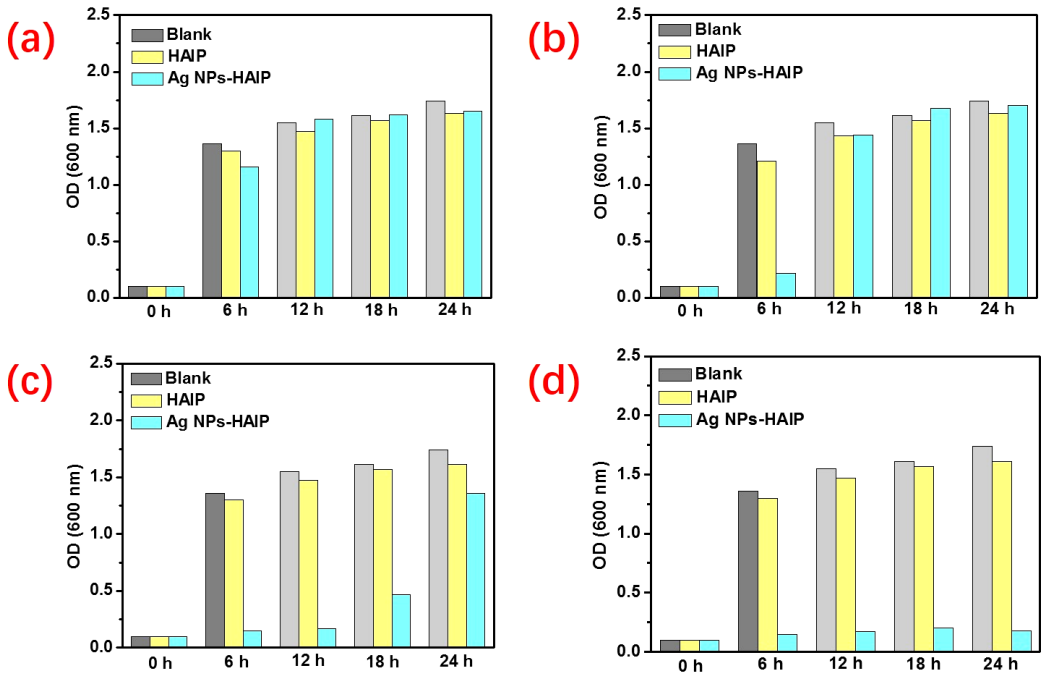


Fig. S19 Graphical representation of the OD measurements in *S.aureus* at different concentrations of Ag (a) 50 μM, (b) 100 μM, (c) 200 μM, and (d) 300 μM.

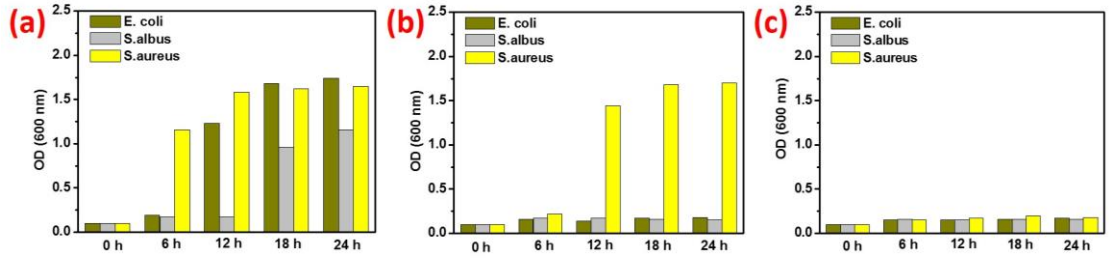


Fig. S20 Graphical representation of the OD measurements in E.coli, S.albus, S.aureus at different concentrations (a) 50 μM, (b) 100 μM, and (c) 300 μM (0 h, 6 h, 12 h, 18 h, and 24 h, respectively).

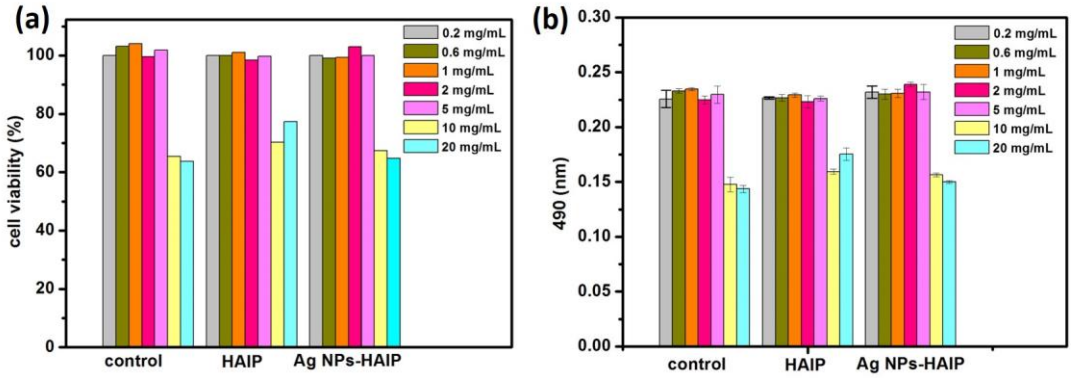


Fig. S21 Graphical representation of the biocompatibility assays: (a) Cell viability, (b) OD measurements at 490 nm (MTT concentrations: 0.2 mg/mL, 0.6 mg/mL, 1 mg/mL, 2 mg/mL, 5 mg/mL, 10 mg/mL, 20 mg/mL, respectively).