Electronic Supplemental Information (ESI)

In situ synthesis of Ag/amino acid biopolymer hydrogels as moldable wound dressing

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Experiment section

Materials:

Silver nitrate and chitosan (MW=140000-220000) were obtained by Sigma Aldrich Chemical Co. Fmoc-Glu-OMe was purchased from GL Biochem (Shanghai, China) Ltd. Dimethyl sulfoxide (DMSO), acetic acid and sodium chloride were provided from Sinopharm Chemical Reagent Co., Ltd. Peptone was supplied from Beijing Aoboxing Bio-tech Co. Yeast extract was purchased from Oxoid (England). Agar was purchased from Beijing Dingduo Changsheng Bio-tech Co., Ltd. Ultrapure water (>18MΩ) was used and all the reagents were analytically pure reagents throughout this study.

Preparation of supramolecular hybrid hydrogels:

Fmoc-Glu-OMe was dissolved in DMSO at a concentration of 100 mg mL^{-1} as Fmoc-Glu-OMe stock solution, followed by sonicating for several minutes. Then the stock solution was diluted to a final concentration of 2 mg mL^{-1}, then, 480 μL of silver nitrate stock solution (4 mmol L^{-1}) was introduced under darkness, and immediately shaken well, after that 480 μL of chitosan stock solution (20 mg mL^{-1}, acetic acid 1%, v/v) was mixed together with quick shake. The mixed solution turned to be stable and transparent hydrogels after an hour of ultrasonication. The water temperature should be paid attention to assure not being too high.

Characterization:

The microstructure of the freshly prepared soft nanocomposite was characterized by microscopes. Scanning electron microscope (SEM) images were carried out on a FEI HELIOS NanoLab 600i SEM (America). 10 μL of samples was placed on a clean silicon slice, and dried overnight. Before

testing SEM images, the sample should be coated with gold. Transmission electron microscope (TEM) images were collected by a FEI Titan G2 60-300 TEM (America). A 300 mesh carbon copper grid was carefully put into the hydrogels and shaken for a few times. Redundant hydrogels were absorbed and the as-prepared sample was dried overnight. High-angle annular dark field scanning transmission electron microscope (HAADF-STEM) was used to acquire further evidences of silver nanoparticles. Atomic force microscopic (AFM) was applied to detect the morphology of the hydrogel and the supramolecular hybrid hydrogels. The high-angle annular dark field scanning TEM/energy-dispersive X-ray spectroscopy (HAADF-STEM-EDS) maps were obtained by FEI Titan G2 60-300 (America). The AFM analysis was performed on a Veeco dimension 3100 AFM (Japan). X-ray photoelectron spectroscopy (XPS) analysis was acquired on an ESCALAB 250Xi XPS from Thermo Fisher-VG Scientific (America). Fourier transforming infrared spectrum (FT-IR) were conducted using a Perkin Elmer Spectrum One instrument (America). The oscillatory rheological data was collected by using rotated rheometer (AR 2000ex, TA Instruments, America). The supramolecular hybrid hydrogels were prepared as mentioned above. The samples were placed on the rheometer stage and the Dynamic frequency sweep was determined from 0.1 to 100 Hz with 5% strain at 25 °C.

Antibacterial activity studies:

The *Escherichia coli* (*E. coli*) strains, as a representative of Gram-negative bacteria, were applied to evaluate the antibacterial activity of the soft nanocomposite. 10.0 g peptone, 5.0 g yeast extract and 10.0 g sodium chloride, which were dissolve in ultrapure water stirring by a glass rods, were added to a beaker. The mixture was transferred to 1 L conical flask with sodium hydroxide to adjust pH to 7.4, and diluted with ultrapure water to 1000 mL. Thus, a liquid medium was finished.

In the same way, 15.0 g agar was added to prepare a solid medium.

The configured medium was tightly sealed and then autoclaved at 110 °C for 30 minutes. The shaking flask method was used to investigate the antibacterial ability of the as-prepared hydrogels. The *E. coli* strain was chosen as the representative of bacteria. In sterile conditions, 100 μL of cryopreservation bacterial suspension (4 °C) and 8.9 mL liquid medium were dispersed into 5 centrifuge tubes. 1.0 mL of supramolecular hybrid hydrogels, Fmoc-Glu-OMe/AgNPs hydrogels, Fmoc-Glu-OMe/Chitosan hydrogels, Fmoc-Glu-OMe hydrogels, 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 °C for 2 h. The following step was to melt the solid medium; 20 mL of the solid medium was taken into 5 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 °C for 12 h to form bacterial colonies. Finally, the growth of the *E. coli* strains was photographed, and examined under a microscope.

In vivo **wound healing measurement:**

The effect of the supramolecular hybrid hydrogels for wound healing was estimated by rats models. The whole experiment was in the Xiangya School of Medicine, Central South University. All the test rats were with approval of the Xiangya School of Medicine. Twelve Sprague Dawley

(SD), aged 7 weeks, male rats, whose weight was approximately 240 g, were exploited in this experiment. The weight of the rats was weighed and recorded, after that the rats were anaesthetized through intraperitoneal injection with 10% chloral hydrate at a dose of per hectogram weight 0.3 mL. The dorsal hair of rats was shaved by a razor blade, and then two centimeters long cross wound was made on the back of the test rats. These rats were equally divided into four groups: hybrid hydrogels treatment, Fmoc-Glu-OMe hydrogels control, commercial antimicrobial control and blank group. For the hybrid hydrogels treatment group, the as-prepared soft nanocomposite was applied on the wound. As for the Fmoc-Glu-OMe hydrogels and commercial antimicrobial control group, Fmoc-Glu-OMe hydrogels and commercial silver nanoparticles antibacterial agent were employed with the wound, respectively. The untreated wound was used as the blank group. Subsequently, they were individually housed in cages and equivalent food and water were supplied to them under constant temperature. Wound healing were taken digital photos and recorded. The rats were successively observed and fed for seven days. The bedding, water and food for rats were replaced and the wound healing process was recorded by taking digital photos every day.

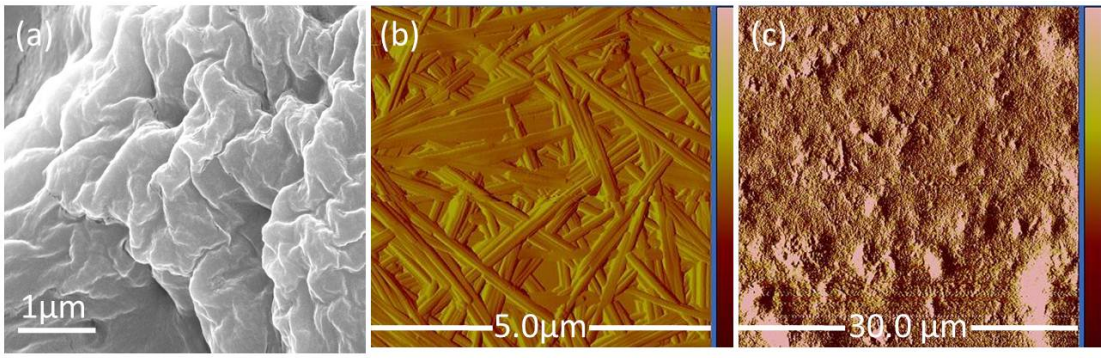

Fig. S1 (a) The SEM images of the Fmoc-Glu-OMe/AgNPs/chitosan hydrogels. (b-c) The AFM images of the Fmoc-Glu-OMe hydrogels and Fmoc-Glu-OMe/AgNPs/chitosan hydrogels, respectively.

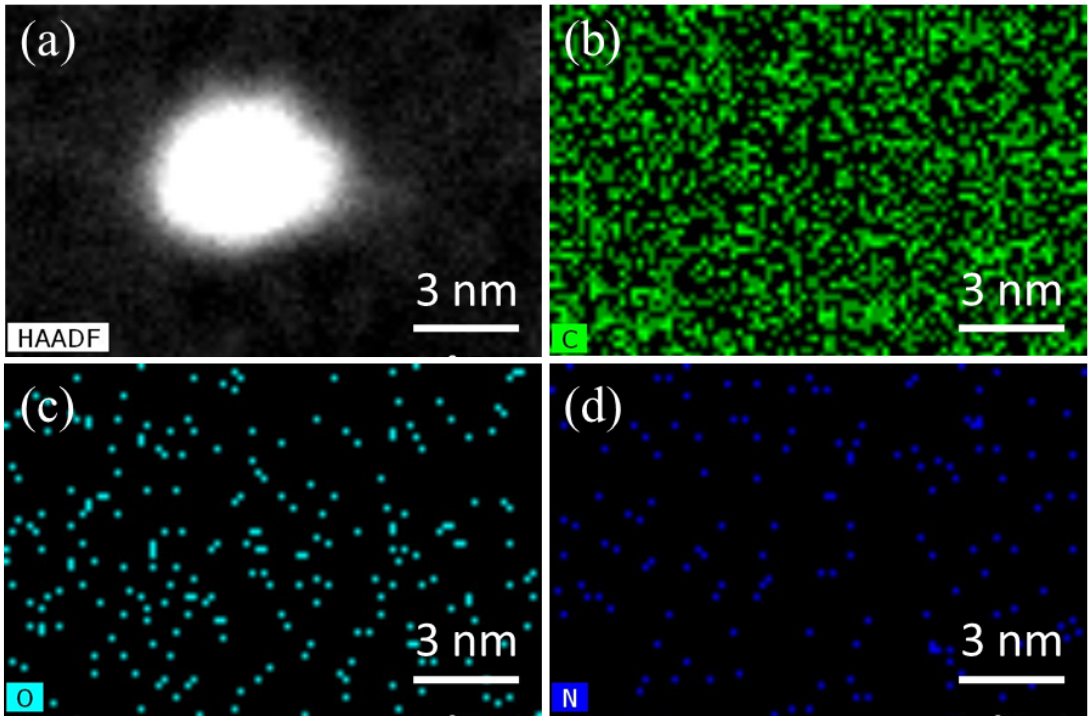

Fig. S2 The HAADF-STEM-EDS maps of Fmoc-Glu-OMe/AgNPs/chitosan hydrogels.

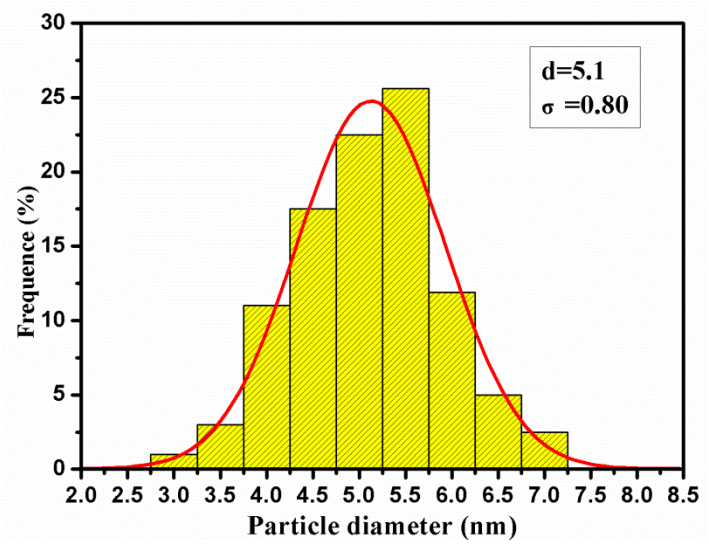

Fig. S3 The size distribution of the AgNPs dispersed in the hybrid hydrogels.

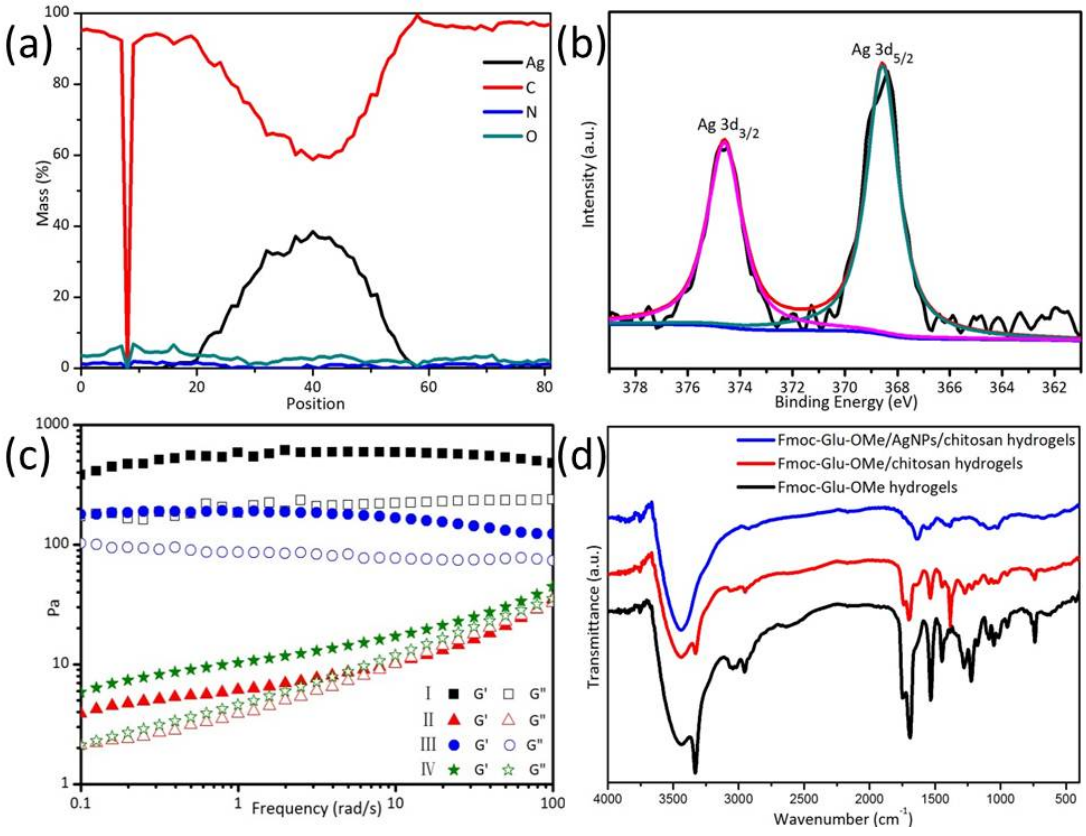

Fig. S4 (a) The cross-sectional compositional line-scanning profile of the silver nanoparticles. (b) The XPS Ag $3d_{5/2–3/2}$ core-level spectra of the supramolecular hybrid hydrogels. (c) The frequency dependence of dynamic storage modulus (G′) and loss modulus (G″) of the hydrogels (I Fmoc-Glu-OMe, II Fmoc-Glu-OMe/AgNPs, III Fmoc-Glu-OMe/chitosan, IV Fmoc-Glu-OMe/ AgNPs/chitosan) with the 5% strain at 25 °C. (d) The FTIR spectra of the hydrogels prepared from Fmoc-Glu-OMe, Fmoc-Glu-OMe/AgNPs, Fmoc-Glu-OMe/ AgNPs/chitosan.

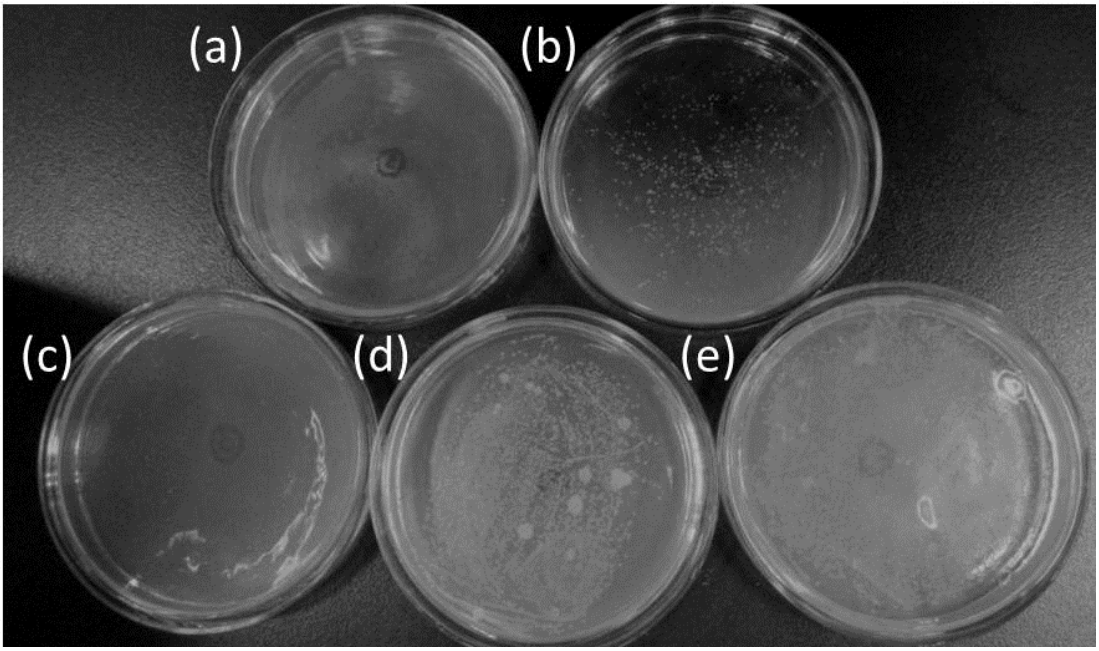

Fig. S5 Photographs of bacterial colonies formed by *E. coli* strains treated with the hydrogels, which were prepared from (a) Fmoc-Glu-OMe/AgNPs/chitosan, (b) Fmoc-Glu-OMe/AgNPs, (c) Fmoc-Glu-OMe/AgNPs/chitosan, (d) Fmoc-Glu-OMe, and (e) the untreated bacteria were used as control, respectively.

Groups	No.	Preoperative weight (g)	Six-days later weight (g)	Weight increase (g)	Average weight increase (g)
Fmoc-Glu-OMe hydrogel	1	235	280	45	48.3
	2	230	280	50	
	3	250	300	50	
Hybrid nanocomposite	4	245	300	55	51.7
	5	235	285	50	
	6	250	300	50	
Commercial antimicrobial	7	250	300	50	46.7
	8	245	285	40	
	9	230	280	50	
Untreated	10	230	260	30	35.0
	11	245	280	35	
	12	250	290	40	

Table. S1 The weight trends statistical data of the four groups of rats before and after the wound healing.

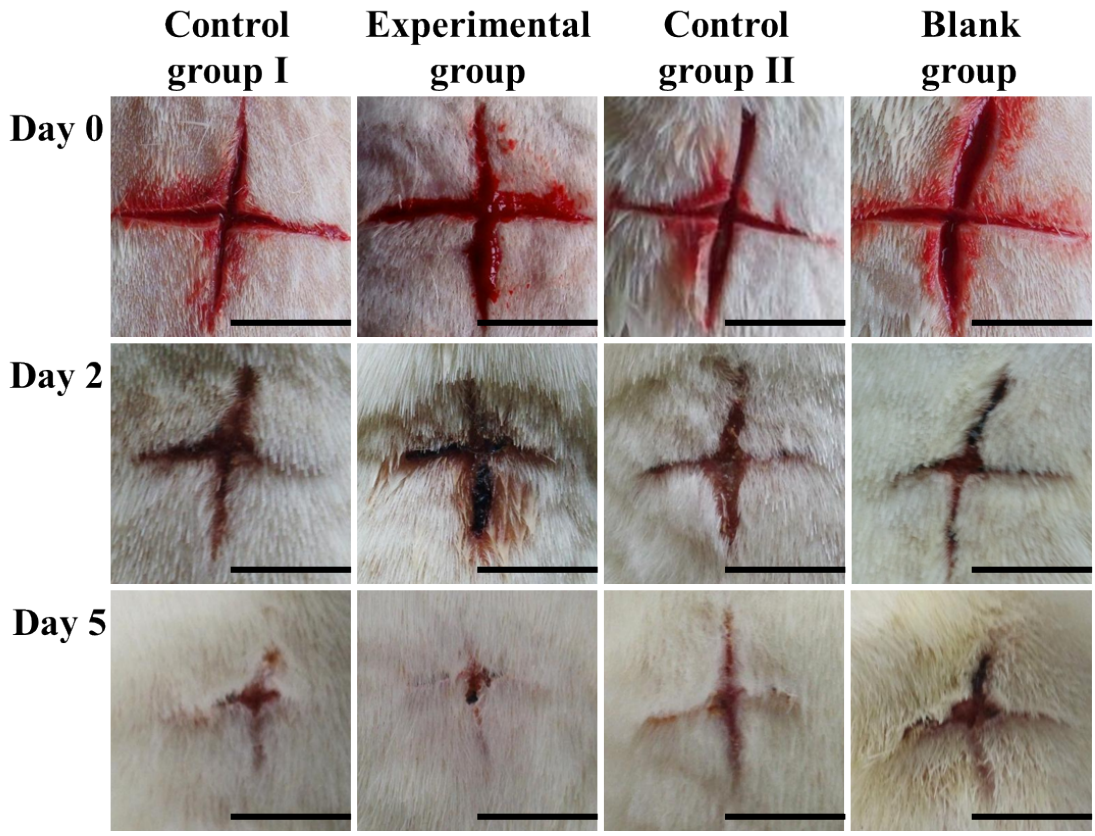

Fig. S6 Visual observations of the rats wound healing (scale bar= 1 cm).

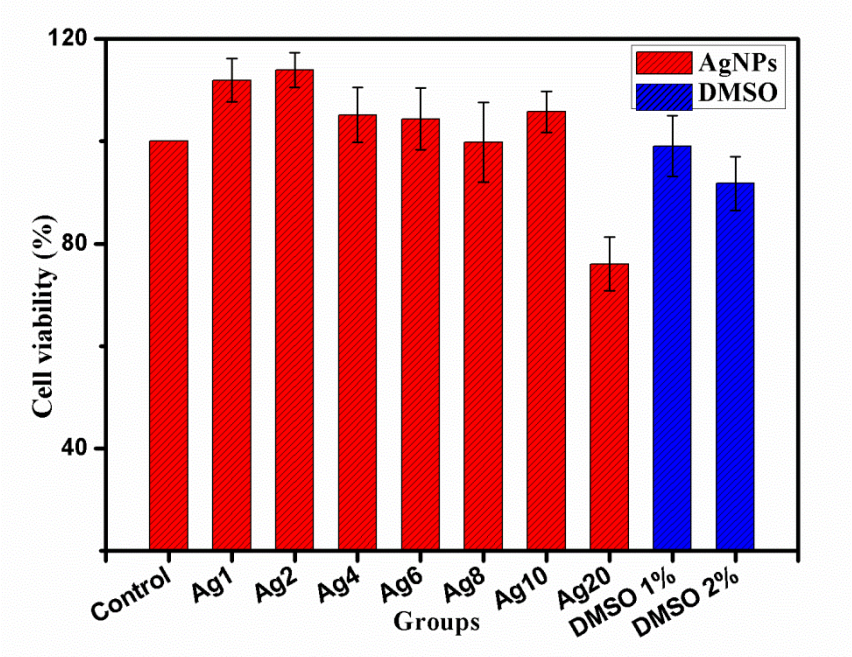

Fig. S7 Cell viability analysis, L929 cells contacted with the AgNPs 1 μM, 2 μM, 4 μM, 6 μM, 8 μM, 10 μM, 20 μM for 24 h, respectively (red bars).The two blue bars represent L929 cells contacted with DMSO 1% and 2% for 24 h, respectively.