Electronic Supplementary Material (ESI) for Nanoscale.

An antioxidant and antibacterial polydopamine-modified thermo-sensitive hydrogel dressing for *Staphylococcus aureus*-infected wound healing

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Methods

^{1}H NMR and Gel Permeation Chromatography

^{1}H NMR spectroscopy was performed on a 400 MHz ^{1}H NMR spectrometer (AVANCE III HD, Bruker). All spectra were recorded at room temperature using $CDCl_3$ (Sinopharm Chemical Reagent, China) as solvent. A gel permeation chromatography (GPC) system (Agilent 1260) was used to analyze the molecular weights and molecular weight distributions of copolymers, where tetrahydrofuran was used as eluent at a flow rate of 0.6 mL/min at 40 °C and polystyrene was used as standards.

Differential scanning calorimetry

The thermal properties of copolymers were studied by a differential scanning calorimeter (DSC 214 Polyma, Netzsch). About 5 mg sample loaded into an aluminum was first heated to 80 °C and then held at this temperature for 5 min to eliminate the thermal history. Then, the cooling curves were recorded when the sample was cooled from 80 to −70 °C. After the sample was kept at −70 °C for 5 min, it was heated to 80 °C to obtain heating curves. Both cooling and heating rates were 20 ℃/min.

UV-vis spectroscopy and dynamic light scattering

The UV-vis absorption spectra were determined by UV-visible spectrophotometer (L650, Perkin Elmer). The sizes and distributions of solutions were measured by dynamic light scattering (DLS, Malvern Zetasizer Nano ZS).

XRD and FTIR

X-Ray diffraction (XRD) measurements were performed in a diffractometer (Shimadzu, XRD-6000) equipped with a CuKα source at room temperature. The diffraction patterns of a scan

range between 5° to 50° were recorded with a scanning rate of 5°/min. The voltage was set at 40kV, and the current was fixed at 40 mA.

The structure of the polymers was characterized by Fourier Transformation Infrared Spectrum (iS50, Thermo Fisher).

Determination of PDA content in PDA/P2 polymers

Dopamine hydrochloride (40 mg) was dissolved in pH 8.5 Tris-HCl solution (2 mL), stirred overnight at room temperature, then dialyzed for 2 d and freeze-dried to obtain polydopamine (PDA). The UV absorbances at 828 nm of PDA-DMSO solutions with a concentration range of 30~200 μg/mL were measured. The standard curve of PDA was obtained by linear fitting between the absorption values and the concentrations of PDA. PDA/P2 was weighted and dissolved in DMSO. The UV absorbance of polymer solution at 828 nm weas measured. The PDA concentrations of PDA/P2 polymer solution were calculated according to the PDA standard curve.

***In vitro* Ag release behavior**

500 μL of PDA/P2-4@Ag solution (polymer: 30wt%, AgNPs: 500 μg/mL) was incubated at 37 ℃ for 10 min to form a gel. Then 5 mL of PBS as the release medium was added to hydrogel. And the PDA/P2-4@Ag gel was placed in a shaker (37 ℃, 50 rpm). 2 mL of release medium was removed for ICP detection at the predetermined time intervals, and 2 mL of fresh medium was added for replacement. Three parallel samples were set for each sample.

Cytotoxicity test

L929 cells were planted in 96-well plates at a proper density and cultivated with 200 μL of DMEM contained P2 and PDA/P2-4. The concentrations of polymers were 100, 200, 300, 400, and 500 μg/mL. After 48 h and 72 h of incubation, the cell culture medium was changed as 100

μL of fresh DMEM culture medium with MTT (10%, v/v). After incubating at 37 ℃ for 4 h, the cell culture medium was placed to 150 μL of DMSO. The cell viability was quantified by a 490 nm microplate reader after the solution in the 96-well plate was mixed well. Cell survival percentage was calculated according to the following formula:

$$\text{Cell viability (\%)} = [(A_x - A_b) / (A_c - A_b)] \times 100\%$$

, where A_x is the absorbance of DMSO solutions with polymers, A_c is the absorbance of DMSO solutions without polymers, and A_b is the absorbance of DMSO solutions without cells.

According to the manufacture's instructions, cell survival is measured using an AO/EB cell staining kit (Bioengineering (Shanghai) Co., Ltd). After the incubation of 48 hours and 72 hours, the stained cells (green in living cells and red in dead cells) were imaged with inverted fluorescence microscope.

Blood compatibility assessments

The fresh rat blood was centrifuged at 4 ℃ at 1000 rpm for 3 min. After the supernatant was discarded, the blood cells were washed with saline for 3 times. Appropriate amounts of polymer P2, PDA/P2-4 and AgNPs were dissolved in saline. The polymer solution with a mass concentration of 100 mg/mL was prepared, in which the concentration of AgNPs was 167 μg/mL. 0.1 mL of red bloods were added into 0.8 mL of saline, then 50 μL of polymer solution was added. The mixture was incubated at 37 ℃ for 1 h. H_2O was used as the positive control while saline was used as the negatively control. After that, the microplate well contents were centrifuged for 3 min and the supernatant (100 μL) was then introduced into a 96-well microplate. The absorbance of the solution was recorded at 540nm by a microplate reader. The hemolysis percentage was calculated from the formula:

$$\text{Hemolysis (\%)} = [\ (A_s - A_c)/(A_p - A_c)] \times 100\%$$

, where A_s was the absorbance value for a copolymer solution with confirmed concentration. A_p was the absorbance value for the positive control and A_c was the absorbance value for saline control.

Table S1. Molecular properties of PCLGA-PEG-PCLGA triblock copolymers.

Samples	Feeding molar ratios of CL/GA/PEG	CL/GA in polymers[a]	M_n[a]	M_n[b]	M_w[b]	(M_w/M_n)[b]
P1	27:2.7:1	10.1:1	1664-1500-1664	6579	9236	1.41
P2	30:3:1	10.58:1	1846-1500-1846	6904	9728	1.41
P3	32:1.6:1	20.32:1	1773-1500-1773	6762	8820	1.30

[a] The M_n of the central block PEG was provided by Sigma-Aldrich. The M_n of polyester block and molar ratios of CL and GA units were calculated by ^{1}H NMR.

[b] Measured by GPC, relative to polystyrene standards.

Table S2. Ingredients for the synthesis of the PDA/P2 polymers.

Polymers	P2 (mg)	DA (mg)	Tris-HCl (mL)
PDA/P2-1	500	250	12.50
PDA/P2-2	500	250	6.25
PDA/P2-3	500	250	5.00
PDA/P2-4	500	250	2.50

Table S3. The gelation properties of different PDA/P2 polymers (30 wt%).

Polymers	Gelation Temperature/Time
PDA/P2-1	37.8 ℃/5.5 min
PDA/P2-2	37.2 ℃/5.0 min
PDA/P2-3	37.0 ℃/5.0 min
PDA/P2-4	34.0℃/9.5 min[a]

a. Although the critical gelation temperature of PDA/P2-4 was 34 ℃, the gelation time at 37 ℃ was significantly shortened to 2.0 min.

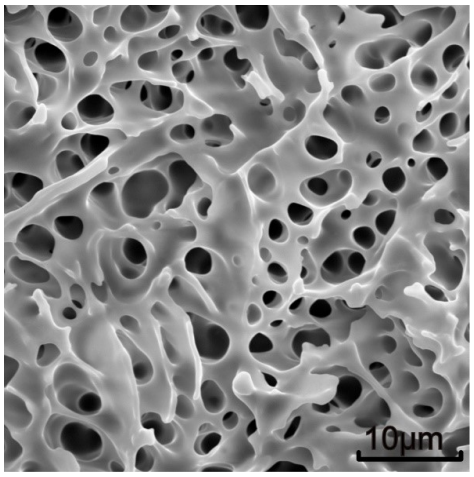

Figure S1. SEM image of P2 gel.

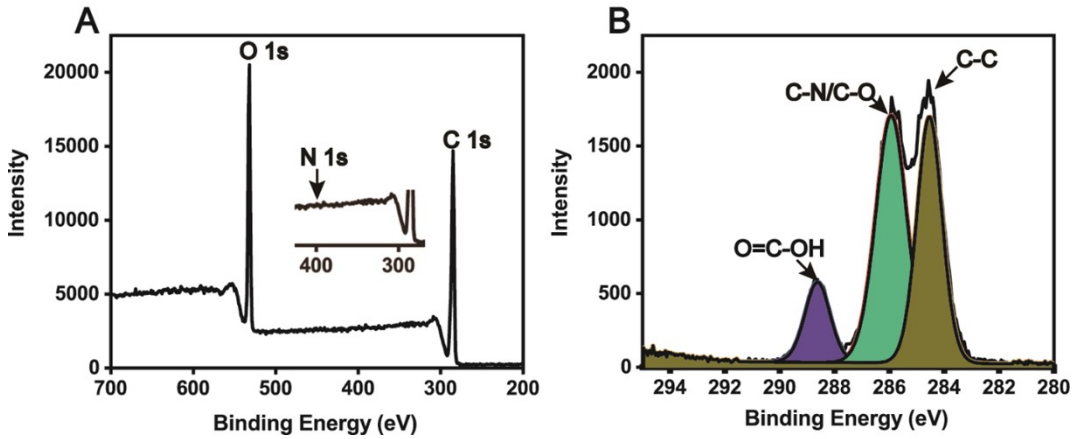

Figure S2. XPS analysis of PDA/P2-4.

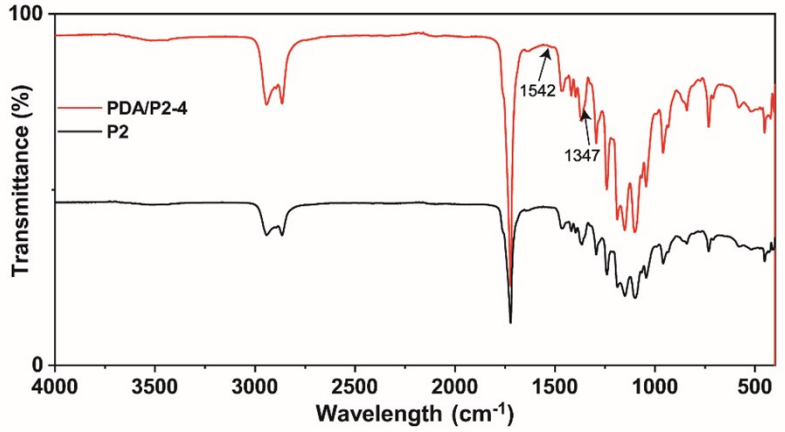

Figure S3. FT-IR spectra of P2 and PDA/P2-4.

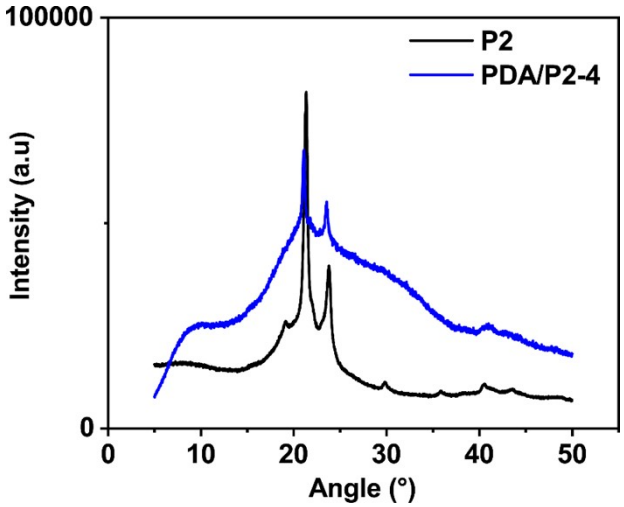

Figure S4. The XRD analysis of P2 and PDA/P2-4.

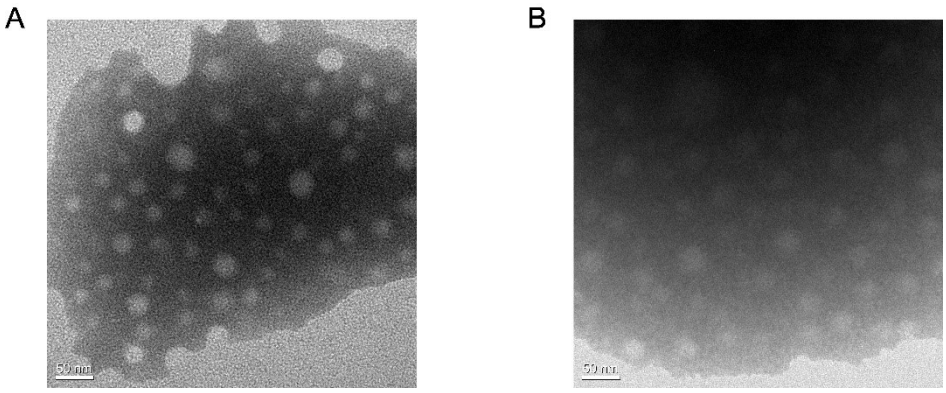

Figure S5. The TEM images of P2 (A) and PDA/P2-4 (B), the particle size of P2:24.30±2.78 nm, the particle size of PDA/P2-4: 29.53 ± 2.37 nm, n = 30. (Stained with 3% phosphotungstic acid solution for 30 s)

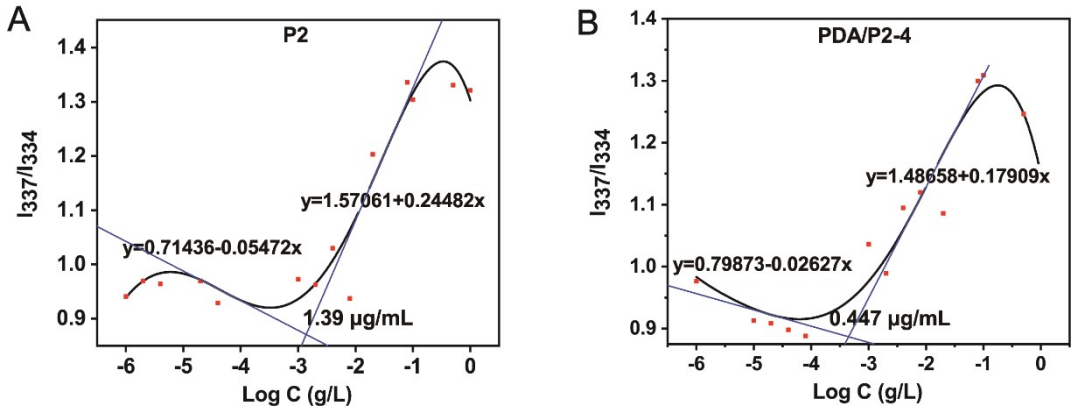

Figure S6. CMC values of P2 and PDA/P2-4 polymers.

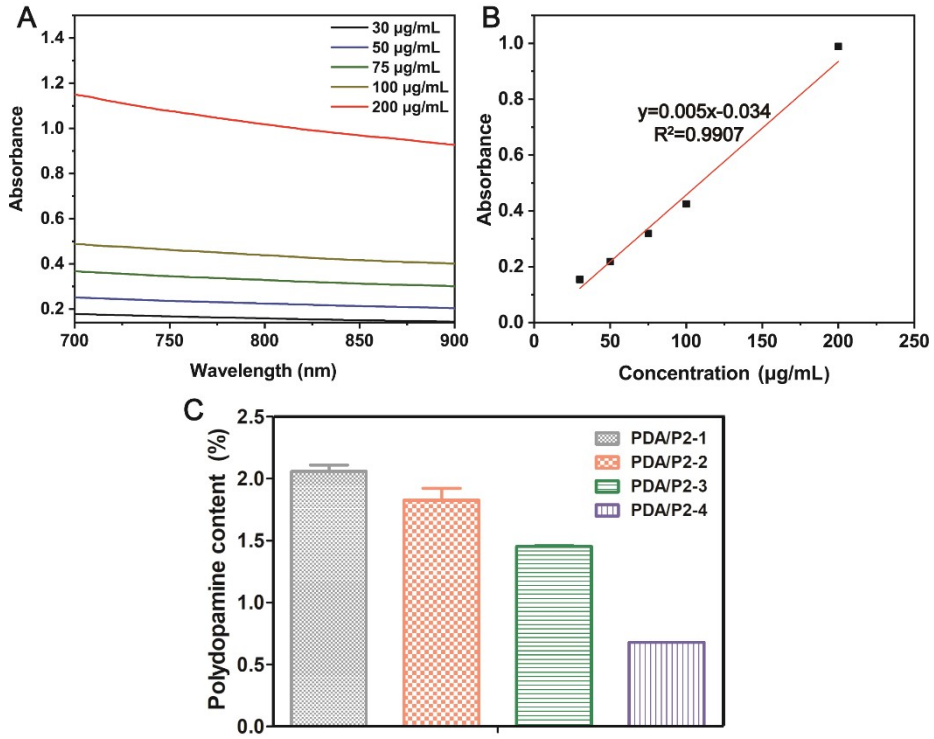

Figure S7. (A) The UV-Vis absorption spectra of PDA solutions at different concentrations. (B) Standard curve of PDA measured at a series of concentrations. (C) The PDA contents of PDA/P2 polymers.

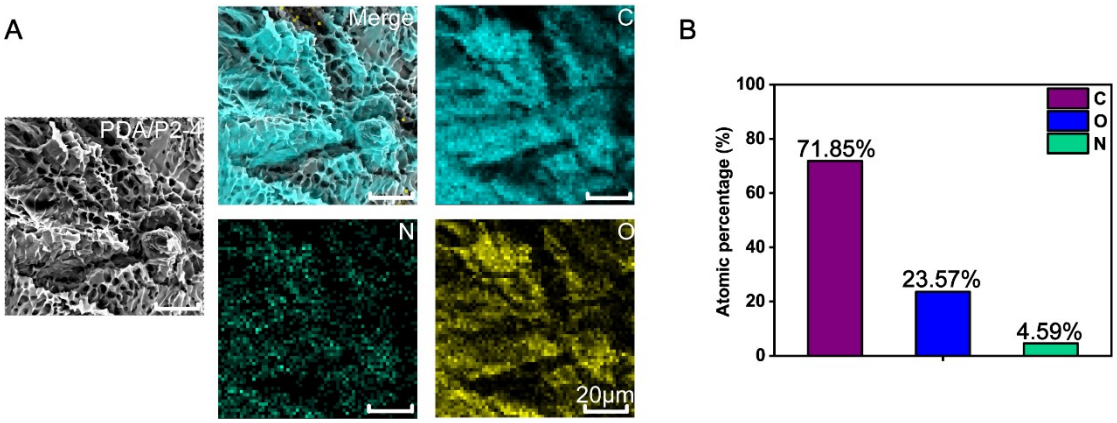

Figure S8. (A) The SEM-EDS analysis of PDA/P2-4 gel. (B) Quantitative elemental analysis of PDA/P2-4 by (A).

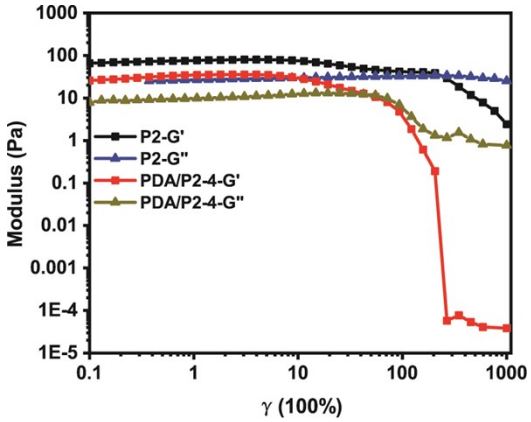

Figure S9. Strain-sweep measurements of P2 and PDA/P2-4 solutions (30 wt%) at 37 ℃.

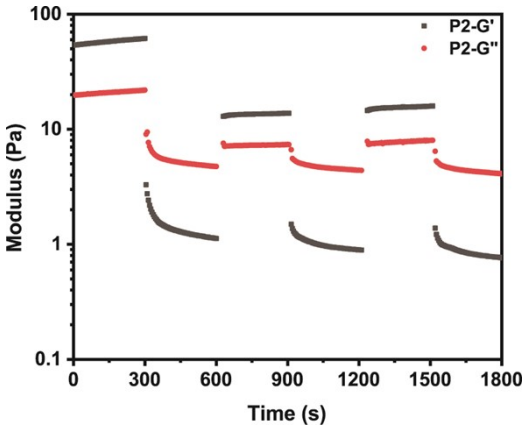

Figure S10. Repeated dynamic strain step testing (γ = 1% or 1000%, 10 rad s^{-1}) of the P2 solution (30 wt%) at 37 ℃.

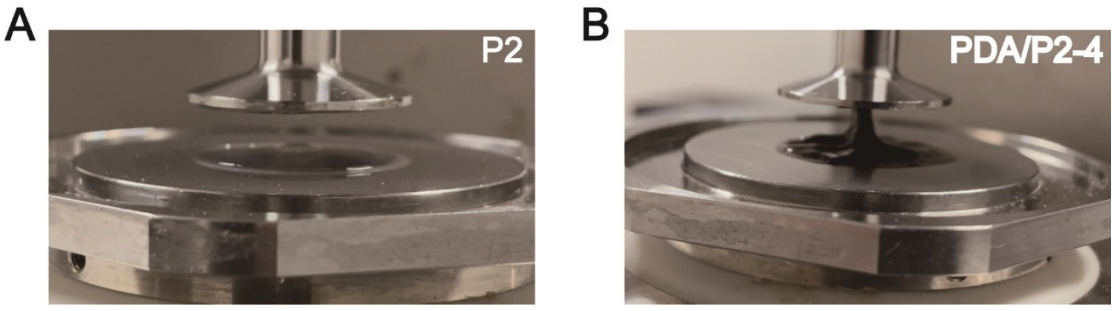

Figure S11. (A), (B) Images of plate rising after the rheological test of P2 and PDA/P2-4 gels, the temperature of the plate was 37 ℃, the rising height of the plate was 20 mm.

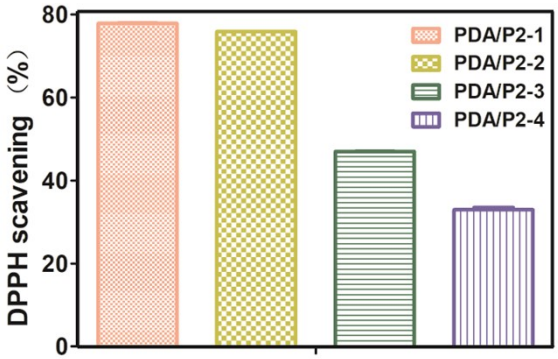

Figure S12. ROS-scavenging efficiency of PDA/P2-1, 2, 3, and 4 at a concentration of 0.32 mg/mL by a DPPH method.

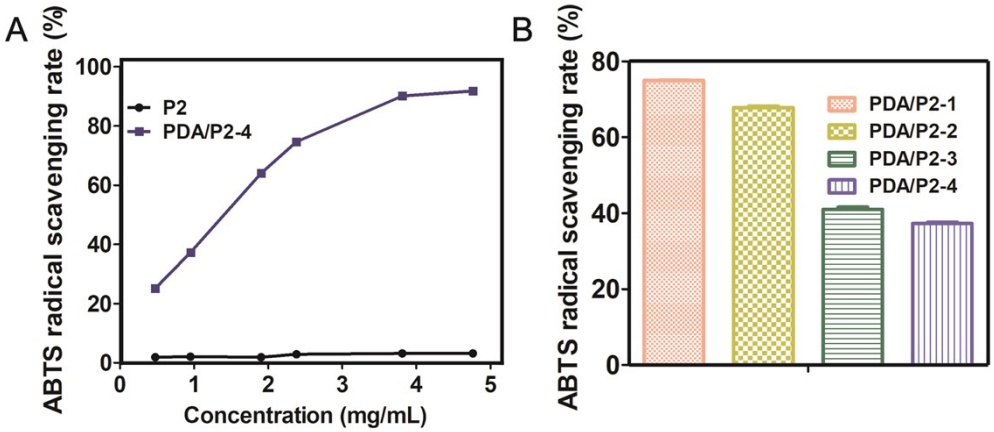

Figure S13. (A) The ABTS radical scavenging rates of polymer P2 and PDA/P2-4. (B) The ABTS radical scavenging rate of different PDA/P2 polymers at the same reaction concentration at 0.95 mg/mL.

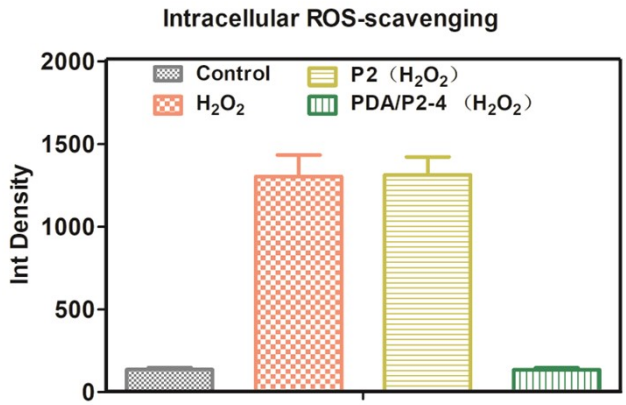

Figure S14. Quantitative statistics of fluorescence intensity of intracellular ROS-scavenging, n=3, *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

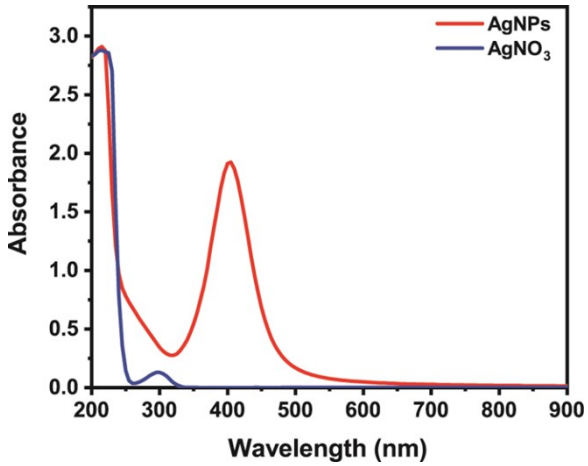

Figure S15. UV-spectra of $AgNO_3$ and AgNPs.

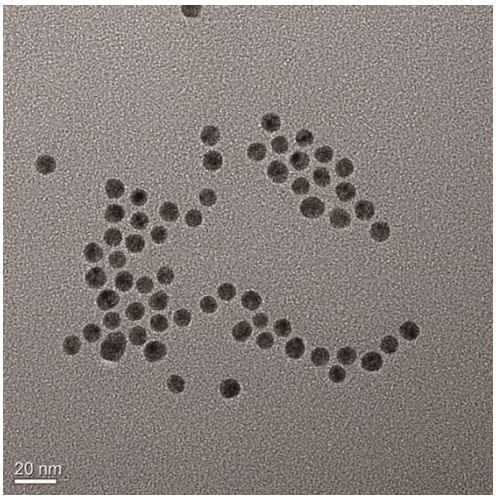

Figure S16. TEM image of AgNPs.

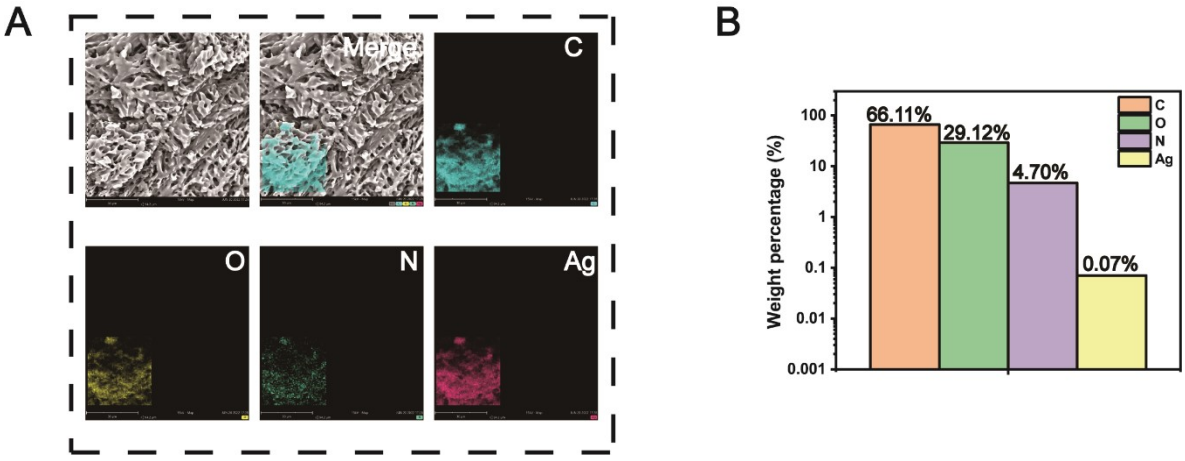

Figure S17. (A) The SEM and EDS analysis of PDA/P2-4@Ag gel. (B) Quantitative statistics of elemental analysis of PDA/P2-4@Ag gel**.**

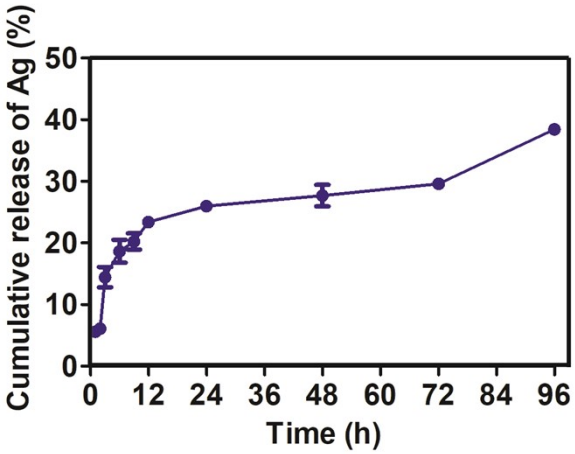

Figure S18. Cumulative release curve of Ag from PDA/P2-4@Ag hydrogel for 96 h.

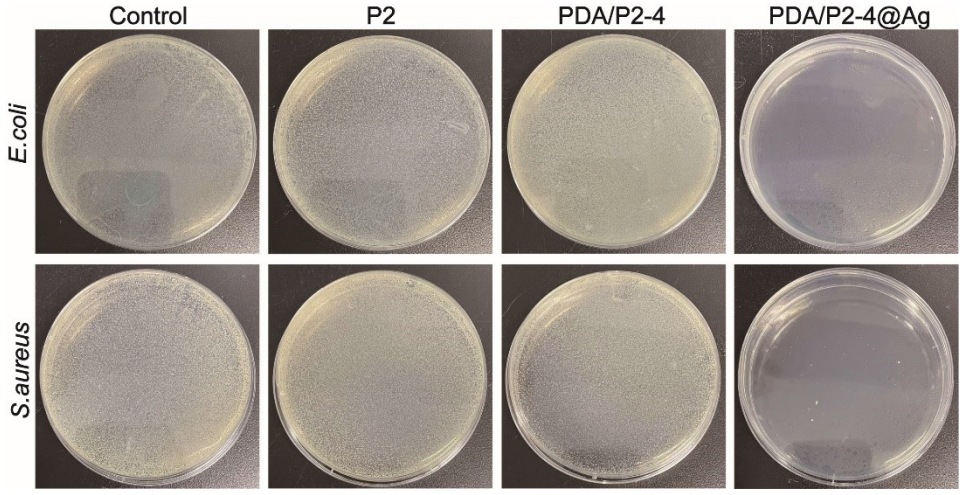

Figure S19. The images of bacteriostatic results obtained by a spread plate method.

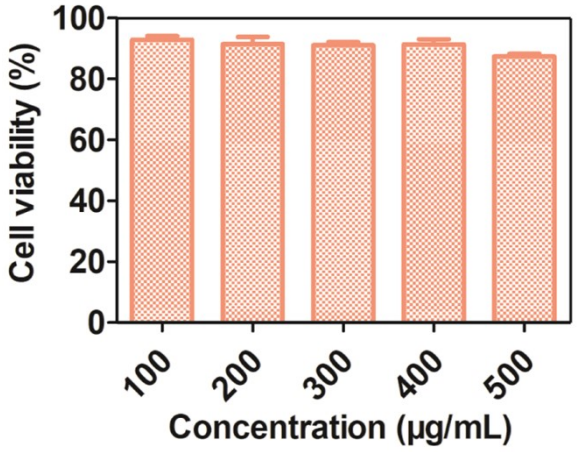

Figure S20 Viability of L929 cells after co-incubation with PDA/P2-4@Ag for 48 h.

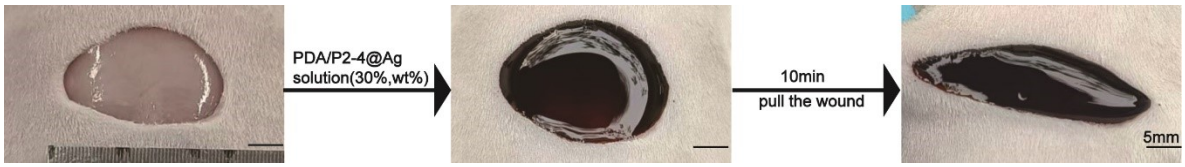

Figure S21. Photographs displaying PDA/P2-4@Ag injection at the wound and formation of a gel within 10 min.