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Electronic Supplementary Information for：

Highly Flexible Hydrogel Dressing with Efficient Antibacterial, Antioxidative, and Wound Healing Performances

Ke Shang,[‡[a]] Longxiang Tao,[‡[b]] Shanyue Jiang,[‡[c]] Jinhao Yan[[a]], Shoukui Hu[[a]], Guangwei Yang[[a]], Chao Ma[[a]], Sheng Cheng,[[d]] Xuefu Wang,*[[c]] and Jun Yin*[[a]]

Instruments

The ^{1}H nuclear magnetic resonance (NMR) spectra were recorded using a Bruker 600 MHz spectrometer operated in the Fourier Transform mode. Chemical shifts are reported in delta (δ) units and expressed in parts per million (ppm) downfield from tetramethylsilane using the residual proton solvent as an internal standard. Molecular weights and molecular weight distributions were determined using a size exclusion chromatogram (SEC) equipped with a Waters 1515 pump and a Waters 2414 differential refractive index detector (set at 40 °C). A series of three linear Styragel columns (HR0.5, HR2, and HR4; 3.6 × 300 mm) was used at a temperature of 40 °C. The eluent used was THF at a flow rate of 0.3 mL/min. FT-IR spectra were recorded on Perkin-Elmer Spectrum BX FT-IR system using KBr pellets at 25 °C. UV-vis spectra were performed on UNIC 4802 UV/vis double beam spectrophotometers, quartz cells with 1.0 mm lengths were used in UV-vis measurements. Fluorescence spectra were recorded using a RF-5301/PC (Shimadzu) spectrofluorometer. Transmission electron microscopy (TEM) observations were conducted on a JEM-2100F electron microscope operating at an acceleration voltage of 100 kV. The samples for TEM observation were prepared by casting the corresponding solutions of polymers onto copper mesh grids and drying in air at room temperature. Dynamic light scattering (DLS) measurements were carried on a Nano-ZS90 Zetasizer of Malvern (UK) instrument, all data were averaged over three measurements. Atomic force microscopy (AFM) experiments were performed on a Digital Instruments Dimension 3100 Scanning Probe Microscope in tapping mode at room temperature. The sample for AFM observation was prepared by depositing a hybrid film on a pre-cleaned mica sheet. The rheology test adopts Discovery HR-2 rheometer (TA instrument, USA), with 40mm flat plate and 2.0mm gap. The oscillation frequency sweep measurement is performed at 10% strain, and the shear frequency at 25°C is from 1 to 100 rad/s to determine the storage modulus (G') and loss modulus (G") of the hydrogel. All animal experiments were approved by the Local Ethics Committee for Animal Care and Use at Anhui Medical University, the number LLSC20210651 and the protocols were carried out in accordance with the approved guideline.

Materials.

Polyethylene glycol (PEG; average M_n: 300 g/moL), branched polyethylenimine (PEI; M_n = 10 K, M_w = 25 K), curcumin, 1-thioglycerol, tritvl chloride, triethylsilane, chloroauric acid ($HAuCl_4.4H_2O$), trifluoroacetate (TFA), 1,4-dithiothreitol (DTT), Triton X-100, phosphonitrilic chloride trimer (HCCP), sodium citrate, and oxalyl chloride were purchased from Aladdin or Sigma-Aldrich, and used as received without further purification. Phosphate buffer solutions and 1, 1-diphenyl-2- picrylhydrazyl (DPPH) were purchased from Aladdin. Fetal bovine serum (FBS) and Dulbecco's modified Eagle medium (DMEM) were purchased from GIBCO and used as received. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Aldrich) was used as received. Sodium chloride (NaCl), triethylamine (TEA), anhydrous sodium sulfate (Na_2SO_4), sodium bicarbonate ($NaHCO_3$), and all the other reagents were purchased from Sinopharm Chemical Reagent Co., Ltd. and used as received unless otherwise stated. Staphylococcus aureus (S. aureus, ATCC 25923) and methicillin-resistant Staphylococcus aureus (USA300) were used for antimicrobial studies. The lysogenic broth (LB) medium and deionized water in all bacterial cultures should be autoclaved (120 °C, 60min) before use. Sheep red blood cells (2%) (purchased from Jiangsu Kejing Biological Technology Co., Ltd.). THF was further dried over sodium benzophenone ketyl and distilled onto $LiAlH_4$ under nitrogen just before use. All solvents were obtained from Sinopharm. Co. Ltd. and were purified by the standard procedures before use. Water was deionized with a Milli-Q SP reagent water system (Millipore) to a specific resistivity of 18.0 MΩ cm.

Sample Preparation.

Synthesis of Compound 1 (Trityl Thioglycerin). This compound was synthesized according to previously reported literature.[1] Typically, thioglycerol (2.0 g, 18.49 mmol), triethylamine (2.06 g, 20.36 mmol), and THF (15 mL) were added into a 100 mL round bottom flask. Under a nitrogen atmosphere, 30 mL THF solution of trityl chloride (5.16 g, 18.51 mmol) was added dropwise into the flask in ice bath condition. The mixture was stirred at room temperature for 10 h. The reactant was suction filtered and concentrated under a vacuum pump to remove the solvent. The crude product was re-dissolved in DCM, washed with deionized water and saturated NaCl solution, and dried with anhydrous Na_2SO_4. The solvent was then removed under reduced pressure, and the resulting crude product was purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 3/1, v/v) to yield a white powder of compound **1** (~5.0 g, yielding: ~70%). The ^{1}H NMR spectrum was shown in Figure S1.

Synthesis of Copolymer 1 (P1). This polymer was prepared according to previously reported literature.[2] In a typical run, a 100 mL round bottom flask was charged with PEG (0.51 g, 1.70 mmol), curcumin (0.63 g, 1.71 mmol), tritylthioglycerin (0.6 g, 1.71 mmol), triethylamine (0.87 g, 8.56 mmol), and 15 mL of purified THF. Under the protection of nitrogen, 5.0 mL purified THF solution of oxalyl chloride (0.76 g, 5.99 mmol) was added dropwise into the mixture within 30 min. The mixture was stirred for 8 hours at room temperature. Then, the polymer was concentrated and precipitated with ice mixed solvent (THF/methanol = 1/10, v/v). After fractionation by successive dissolution-precipitation process, the third fraction (monomodal GPC elution peak) was selected for further study. About 0.6 g of pure copolymer was obtained, yielding ~25%. The ^{1}H NMR and UV-vis spectra were shown in Figure 1b and Figure S3a.

Synthesis of Poly(curcumin-co-oxalate-co-SH) Amphiphilic Copolymers (P2). Typically, P1 (250 mg, 0.23 mmol tritylthioglycerin units), triethylsilane (27.5 mg, 0.24 mmol), DTT (30 mg, 0.19 mmol), purified THF (2 mL) were added into a 25 mL round bottom flask. Under a nitrogen atmosphere, TFA (162.5 mg, 1.43 mmol) was added dropwise into the mixture solution. The mixture was stirred at room temperature for 0.5 hour, and the crudes were evaporated in vacuo and redissolved with 1.0 ml of purified THF. The product was washed with ice mixed solvent (THF/ methanol = 1/10, v/v) to obtain ~200 mg of red solid powder (P2, yielding: ~80%), which can be used in the next step without further purification. Each of the above steps is performed in a nitrogen atmosphere.[3] The ^{1}H NMR and UV-vis spectra were shown in Figure 1b and Figure S3a.

Synthesis of Gold Nanoparticles. AuNPs were prepared by citrate reduction of $HAuCl_4$ in aqueous solution according to previously reported literature.[4] Typically, a sodium citrate (57 mg) DI water solution (5 mL) was rapidly injected into a boiling aqueous $HAuCl_4$ (5.0 mg in 50 mL of DI water) solution under vigorous stirring. After boiling for 30 min, the solution was cooled to room temperature and stored for further use.

Fabrication of Organic/Inorganic Hybrid Films at Liquid-Liquid Interface. Into a clean screw bottle, 4.0 mL chloroform solution of P2 (20 mg) was charged. Then, 4 mL of concentrated gold nanoparticles was added dropwise into the bottle slowly to cover chloroform layer. After being left naturally at room temperature for several hours, a visible thin film will appear at the interface of the two phases. After standing for 48 hours, the resulting thin film is deposited on a carbon-coated copper mesh for further characterization.

Photothermal Effect of Organic/Inorganic Hybrid Films. For photothermal efficiency measurements, 20 mg of the hybrid film was charged into a cuvette, the

temperature after being subjected to light (808 nm, 1.0 Wcm^{-2}) irradiation for 10 min was directly measured by thermometer probe. For comparison, pure water, Au NPs, and copolymer (P2) were also tested.

Preparation of Acid-Responsive Hydrogels. Briefly, PEI (400 mg, 0.04 mmol), PEG (60 mg, 0.2 mmol), and TEA (170 mg,1.6 mmol) were dissolved in 1.0 mL of deionized (DI) H_2O and dimethylacetamide (DMAC) mixing solvent (v/v = 9/1). Then, HCCP (83.4 mg, 0.23 mmol) was dissolved in 0.2 mL of DMAC and added into the reaction mixture. The mixture was kept at 25 ^oC 16 hours for the hydrogel formation. The resultant hydrogel was then dialyzed against TEA and DMAC mixing solvent and DI H_2O in turn for 5 days (refreshed five times per day) to remove any possible impurities and stored in a refrigerator prior to use. Curcumin was physically solubilized into the hydrogel according to the need: For Gel 1, no curcumin was added during the preparation process; For Gel 2, curcumin (0.2 g/mL) was added together with PEI and PEG, then, HCCP was introduced to cross-link the mixture. Following the same protocol, other control samples were also prepared by varying the feeding concentrations.

Hydrogel Swelling Behavior. The swelling property of the hydrogel was investigated by immersing dried hydrogels (20 mg) in phosphate buffered saline (PBS) buffers at pH 7.4 and 37 ℃. After a certain time intervals (0-20min), the partially expanded hydrogel was removed and wiped with filter paper to remove the remaining liquid on the surface. Then, calculated the water absorption capacity by weight measurement. After that, the hydrogel was immersed in the new PBS buffer under the same conditions until the expansion ratio remained consistent. Each experiment was carried out three times and the results were reported as average.

Fabrication of Organic/Inorganic Hybrid Films Coated Composite Hydrogels. The targeted composite hydrogels were fabricated by a facile deposition process by immersing a hydrogel slice with regular morphology in the film dispersion, followed by a slow lifting process. The resultant composite hydrogels were left for a while, washed by DI water, and used for further characterization.

Adhesion Performance Investigation of Hydrogels. The adhesion property of hydrogels was tested on different surfaces. The underwater adhesion test of the hydrogel was carried out in a beaker filled with deionized water. A 1 cm × 4 cm hydrogel was attached to a 20 g stainless steel weight to lift another 100 g stainless steel weight underwater. In addition, the adhesion test was performed on other material surfaces (plastic, glass, metal, skin, leaves, paper) using the same method as mentioned above. For the finger joints, when the finger was fully straightened, a 1 cm × 2 cm hydrogel was attached to the joint. Then, the finger was bent to different angles (60°, 90°, and 120°). After reaching the maximum bending state, slowly restored the finger to the original straight state, the adhesion of the hydrogel during the whole process was observed and recorded by digital photo.

Hydrogel Stripping Experiment on the Skin Surface. Onto the hydrogel that was already attached to the skin, a 20 ml of mixed solvent of glycerol and water (v/v = 1:1) was dropped. The time dependent gel shedding was recorded by digital photo. *In Vitro Cargo Release from Hydrogels*. The curcumin release ratio was measured by the dialysis method. Briefly, the hydrogels (50 mg) were incubated in a 10 mL of aqueous solution at pH 5.5 and 37 ^oC for 28 h. The mixture was placed in a dialysis tube (MWCO 3.5 kDa) and then immersed into 500 mL of water with Tween 20 (1.0% total volume) under gentle stirring. At different time intervals, 20 mL of external solution was removed and replaced with equal volume of acid solution. The separated solution was lyophilized and then dissolved in DMSO, the curcumin concentration was quantified by measuring the absorbance against a standard calibration curve.

Antioxidant Property Evaluation. The curcumin loaded hydrogel was lyophilized and cut into several small pieces, and then these hydrogel samples were dispersed in

5 mL of DPPH ethanol solution (100 μM). The experiment was divided into three groups: Gel1, Gel2, and pure curcumin. The experiment was repeated three times for each group (n=3), and the results were averaged. The free radical scavenging efficiency of pure curcumin with the same concentration was used as a positive control. The hydrogel mixture was stirred and incubated in a dark place at room temperature for half an hour. Then, the UV absorption intensity at 517 nm was recorded (A_f). The DPPH scavenging could be calculated by the following equation:

$$\text{DPPH scavenging \%} = \frac{Ai - Af}{Ai} \times 100\%$$

In Vitro Antibacterial Activity Assays. The antibacterial activity of the hydrogels was performed in solid agar Petri dish with methicillin-resistant Staphylococcus aureus (MRSA) and S. aureus. The samples were divided into nine groups: PBS; Au@P2 film/laser off; Au@P2 film/laser on; Gel 1; Gel 1/Au@P2 film/laser off; Gel 1/Au@P2 film/laser on; Gel 2; Gel 2/Au@P2 film/laser off; Gel 2/Au@P2 film/laser on. First, 150 μL of bacterial suspension at a density of 1×10^5 CFU mL^{-1} was spread uniformly onto the agar plates. Thereafter, the hydrogels with a length of 3 mm and a width of 2 mm were gently placed on the MHB agar plates, and the plates were incubated at 37 °C for 24 h. Finally, the growth inhibition zones around the samples of each group were photographed, and the antibacterial activity was determined by measuring the radius of the inhibition zones produced around the samples on the plates. Each group performed three parallel experiments and averaged the three results.

In Vitro Cytotoxicity Assessment. The cytotoxicity of hydrogels was explored using 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl tetrazolium bromide (MTT) analysis of L929 cell line. The samples were divided into four groups: PBS; Au@P2 film; Gel 1; Gel 2. In brief, 2×10^4 cells were inoculated in each well of a 96-well plate containing 100 μL of Dulbecco's modified Eagle's medium (DMEM; containing 10% fetal bovine serum) and cultured for 24 h under typical culture conditions (5% CO_2 and 37 ^oC). PBS, Au@P2 film and hydrogel samples were then added and cultured for 24 hours. Then, 100 μL of fresh DMEM medium was added to each well, followed by 10 μL of MTT (fixed at 0.5 mg/mL). After another 4 hours, the gel sample was removed and 100 μL of DMSO were evenly mixed with the substrate. Microplate reader was used to measure the optical density of the solution at 490 nm to measure the cell activity. Cells incubated without hydrogels were served as positive control. Five parallel experiments were conducted for each group of samples, and the results were averaged.

Hemolysis Assay. The hemocompatibility of the hydrogel was evaluated by the hemolysis ratio assay. Briefly, before the test, Au@P2 hybrid film, cubic Gels 1 and 2 with a side length of 1 mm were soaked in a 24-well plate containing neutral PBS (pH 7.4) for 24 hours. Then, transfer these samples to a centrifuge tube (0.5 mL) and added 0.4 mL of diluted sheep whole blood (with a blood cell concentration of 2%) to each tube. Diluted blood containing surfactant (Triton X-100) and neutral PBS were set as positive and negative control groups, respectively. After incubating in a water bath at 37 °C for 1 hour, each tube was centrifuged at 2000 rpm for 10 min. The optical density of the supernatant was measured at 545 nm using a microplate reader (Thermo Fisher). Five parallel experiments were carried out for all five groups of samples, and the results were averaged. The hemolysis ratio (HR) was determined by the following formulation:

$$HR(\%) = (OD_s\text{-}OD_n)/(OD_p\text{-}OD_n) \times 100\%$$

Where OD_s, OD_n and OD_P refer to the OD values of the samples and the negative and positive controls, respectively. An average of six samples was taken for each group.

In order to study the morphology of red blood cells (RBCs), they were collected and re-suspended in PBS buffer, and dispersed on transparent glass plates. The images of RBCs were captured by using a microscope equipped with a digital camera.

Living Mice Wound Healing. The whole-layer skin defect model was used for wound healing in vivo. Twenty BALB/C male mice (4-6 weeks of age, 20-25g) were randomly divided into 4 groups with 5 replicates per group (n=5). All mice were anesthetized with isoflurane and their back hair was shaved. Two wounds with diameters of 5 mm were made on the back of each mouse using surgical scissors. The gauze, the prepared hybrid film and hydrogel are directly applied to the wound. After surgery, the wound area in Gels 1 and 2 groups was completely covered with hydrogel dressings and fixed with Tegaderm (3 M). Dressing should be changed every 2 days. On days 2, 4, 8, and 12, images of the wound were taken with a digital camera. Observe and record the wound healing in different periods. The results of the four groups of records were averaged.

Similarly, five mice were anesthetized with isoflurane and their back hair was shaved. Two wounds with diameters of 5 mm were made on the back of each mouse using surgical scissors. Then, wrap the two wounds with bandages to keep out of air. Two days later, the wound on the back of the mice became inflamed and festered. Then, Gel 1 and Gel 2 are directly applied to the wound. After surgery, the wound area in Gel 1 and Gel 2 groups was completely covered with hydrogel dressings and fixed with Tegaderm (3 M). Dressing should be changed every 2 days. On days 2, 6, 10, and 14, images of the wound were taken with a digital camera. Observe and record the wound healing in different periods. Five parallel experiments were conducted for this group of experiments, and the measurement results were averaged.

All experiments and feeding programs were approved by the Animal Ethics Committee of Anhui Medical University.

Statistical analysis. All data were analyzed by a paired or unpaired t test or two-wayanalysis of variance(ANOVA) with GraphPad Prism 8.0.A log-rank(Mantel-Cox) test was used for comparison of survival curves.

References

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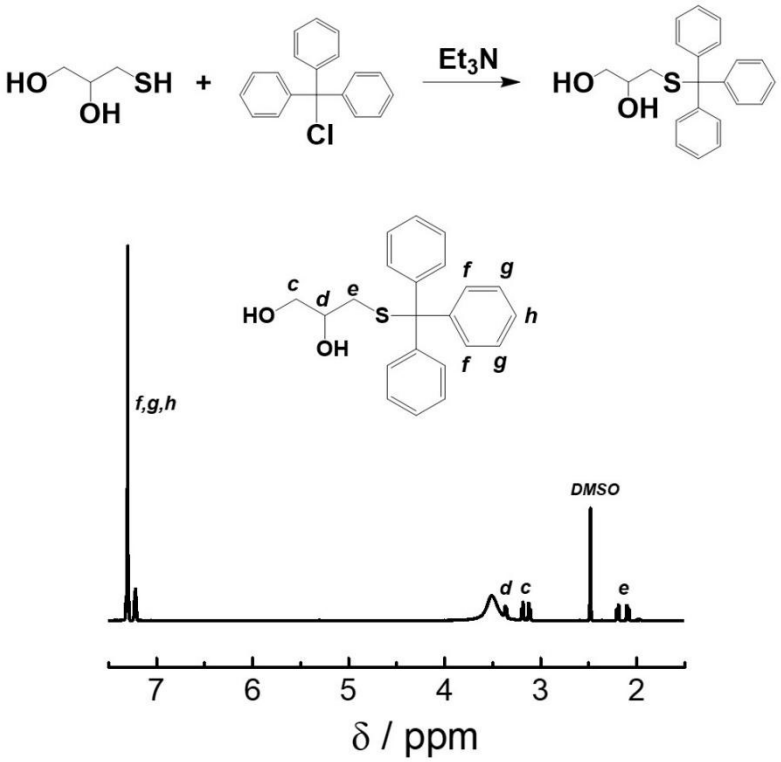

Figure S1. Synthetic route employed for the preparation of trityl thioglycerin (TTG) and corresponding ^{1}H NMR spectrum using DMSO-d_6 as solvent.

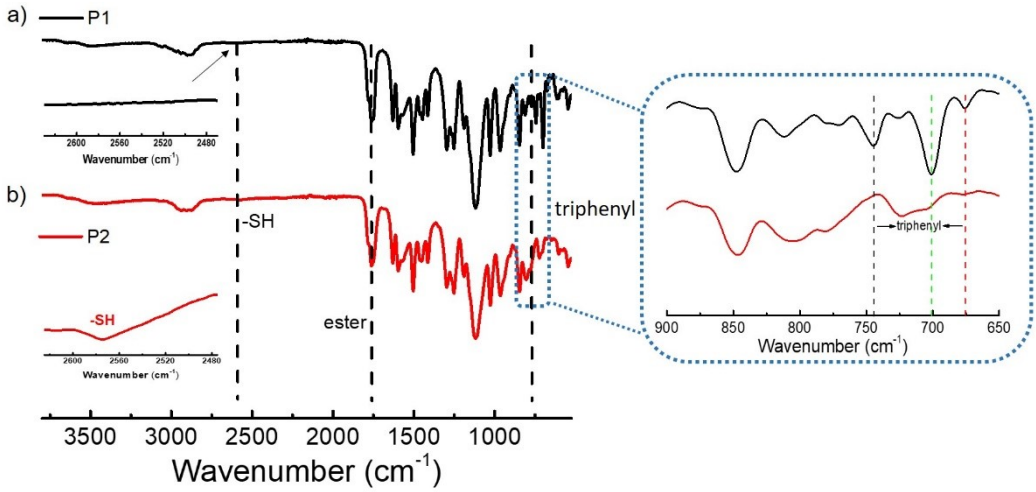

Figure S2. FT-IR spectra obtained for P1 and P2 using KBr pellets.

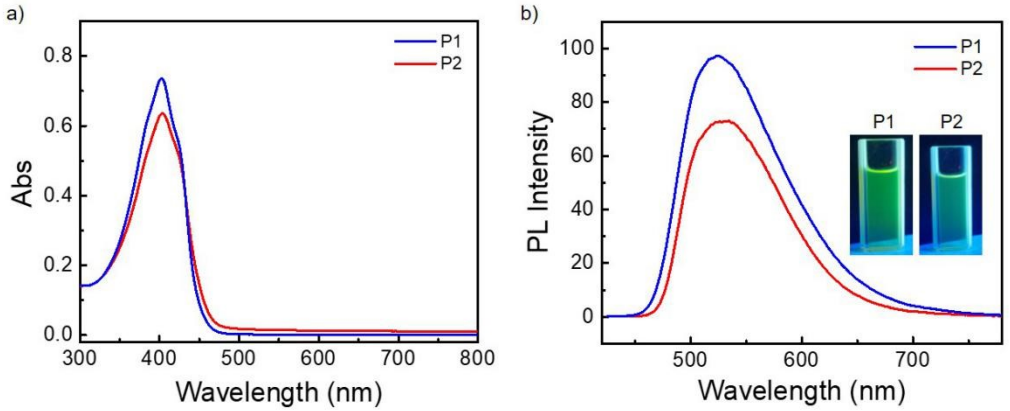

Figure S3. (a) UV-vis and (b) fluorescence spectra recorded for P1 and P2.

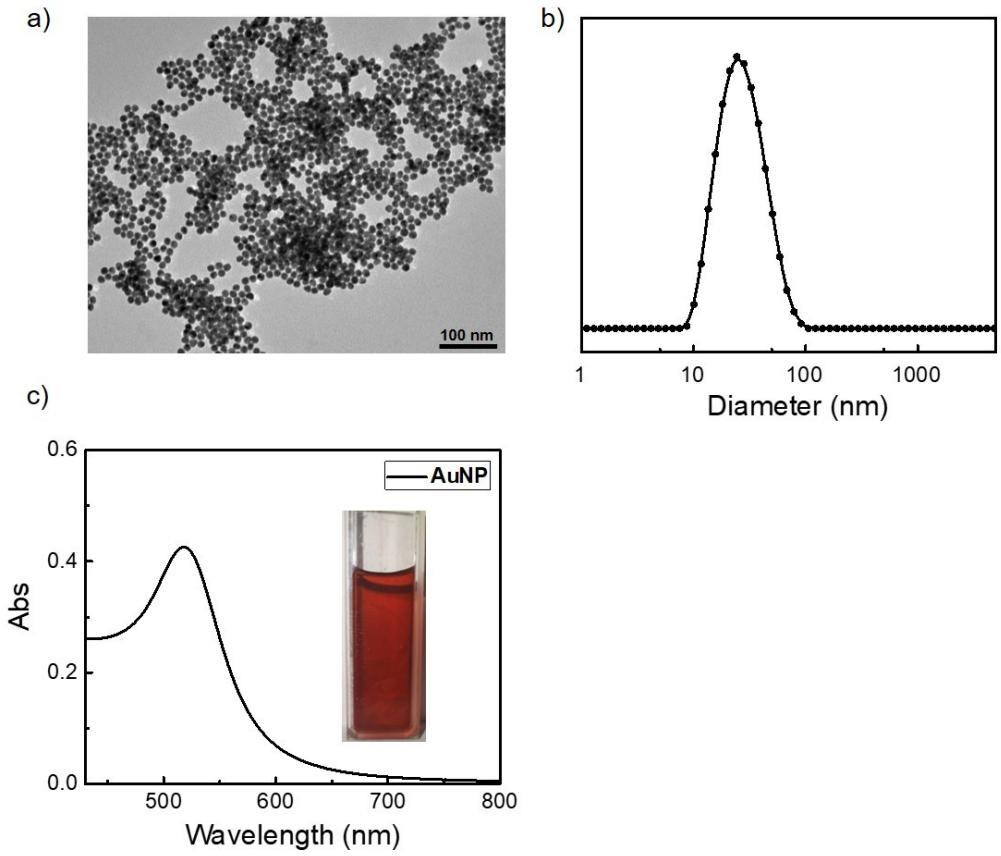

Figure S4. (a) TEM image, (b) DLS trace, and (c) UV-vis spectrum recorded for monodispersed Au nanoparticles.

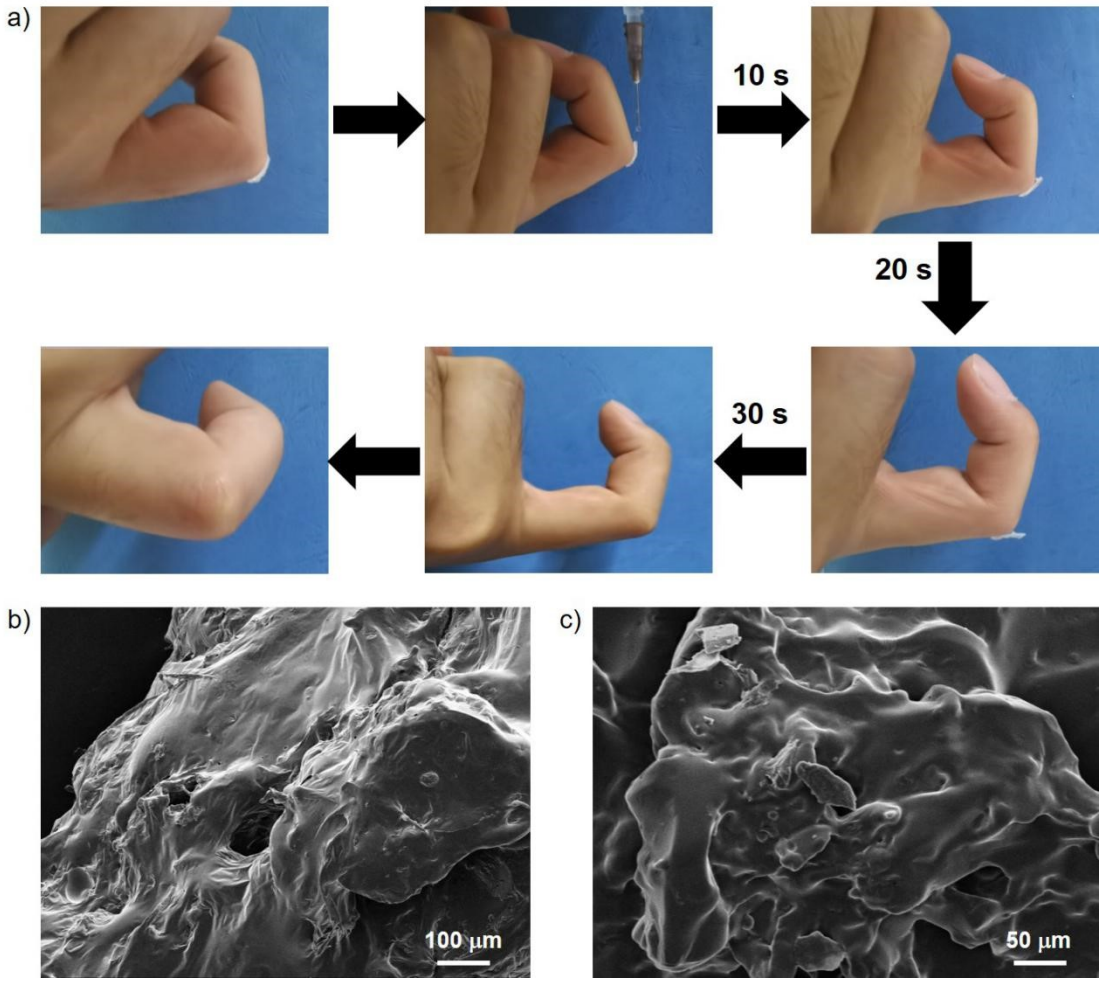

Figure S5. (a) Schematic illustration for the glycerine assisted detachment of Gel 1 from finger joint. (b, c) SEM images obtained for Gel 1 after treatment with glycerine.

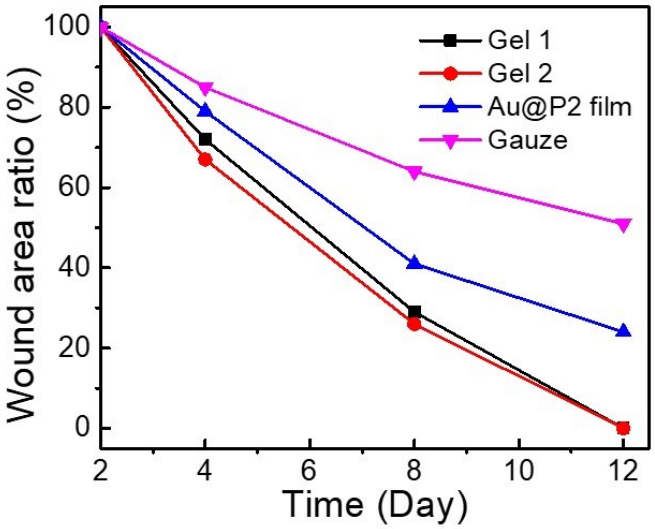

Figure S6. Statistical analysis of wound area at various time points after treated with gauze, Au@P2 film, Gel 1, and Gel 2, respectively.

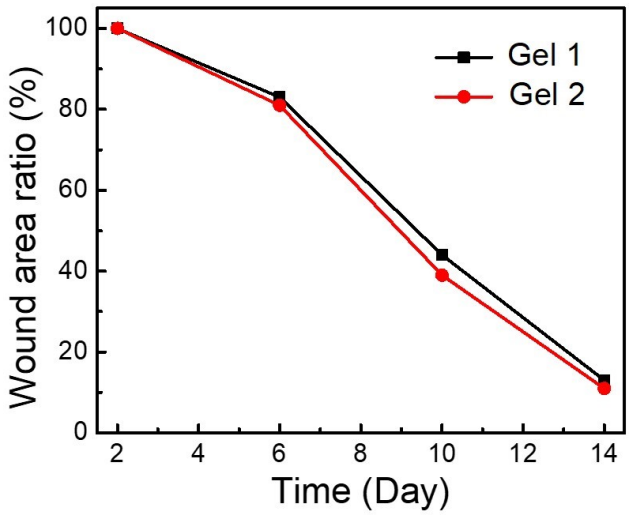

Figure S7. Statistical analysis of wound area (pre-treated to be inflamed) at various time points after treated with Gel 1 and Gel 2.

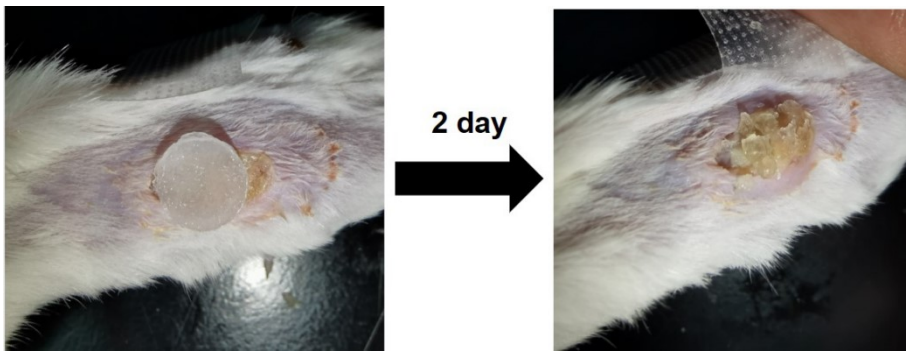

Figure S8. Schematic illustration for the degradation behavior of Gel 1 after covering on the full-thickness skin wound model for 2 days.