Supporting Information for

Sensing-Coupled Sprayable Hydrogel Dressing Enable Rapid Wound Inflammation Indication and Antibacterial Therapy

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Methods:

Bacterial culture: *S. aureus* (ATCC 29213) and *E. coli* (ATCC 8739) were amplified in LB liquid medium (Solarbio) under aerobic conditions at 37°C. The concentration of the bacterial suspensions was then adjusted to approximately 10^6 for subsequent antibacterial experiments. The *S. aureus* and *E. coli* suspensions were spread onto LB agar plates. The control, 10T, 20T, 30T, and 40T GCTC hydrogels were designed to compare their antibacterial capabilities.

Antibacterial assay: For Live & Dead bacterial staining, DMAO (1 volume) was combined with EthD-III (2 volumes), followed by the addition of 0.85% NaCl solution (8 volumes) to obtain 100× dye solution. Subsequently, the 100× dye solution (1 μL) was added to bacterial suspension (100 μL) co-cultured from each group. The mixture was incubated at room temperature, shielded from light, for 15 min. Finally, the stained bacterial suspension (5 μL) was then deposited onto a glass slide and imaged by a fluorescence microscopy.

The morphology of bacteria was observed by SEM (G300, Zeiss, Germany). *S. aureus* and *E. coli* were co-cultured with 10T, 20T, 30T, 40T GCTC hydrogels for 12 h. Then, the LB liquid medium with GCTC hydrogels were washed by PBS and the bacteria were then fixed with 2.5% glutaraldehyde for 24 h at 4 °C. After fixation, the samples were dehydrated by using a series of ethanol solutions with concentrations of 30%, 45%, 60%, 70%, 80%, 90%, and 100%, each for 15 min. Finally, the samples were observed using a SEM.

For the inhibition zone test, the 10^6 CFU mL^{-1} *S. aureus* and *E. coli* suspensions were diluted 10 times, and a sterile cotton swab was dipped into the suspensions (500 μL) and evenly spread onto the plate. Then, five pieces of sterile paper (diameter 6 mm, thickness 0.7 mm), added dropwise with 25μL extraction solution from 10TGCT, 20TGCT, 30TGCT, 40TGCT, were placed on LB agar plates coated with bacteria. The bacterial inhibition zone formed around the sterile paper were observed after 8h 37 °C incubation, and the area of the antibacterial zones was measured (n=3 per group, biological replicates).

Rheological measurement: The rheological properties of the hydrogels were measured using a rotational rheometer (Netzsch, Germany). Measurements were performed with a parallel plate rotor of 20 mm in diameter, and the test gap was set to 1 mm. Specifically, 0.5 mL of GC solution and 0.5 mL of TC solution were sequentially added onto the rheometer plate. After complete crosslinking to form a gel, the apparent

viscosity of the hydrogel was recorded as a function of shear rate at a constant temperature of 37°C. The shear rate (angular frequency) sweep was conducted over a range of 0.1 to 100 rad/s.

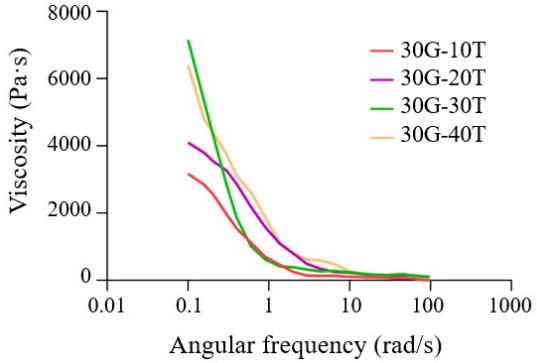


Figure S1: Rheological characterization of GCTC hydrogel.

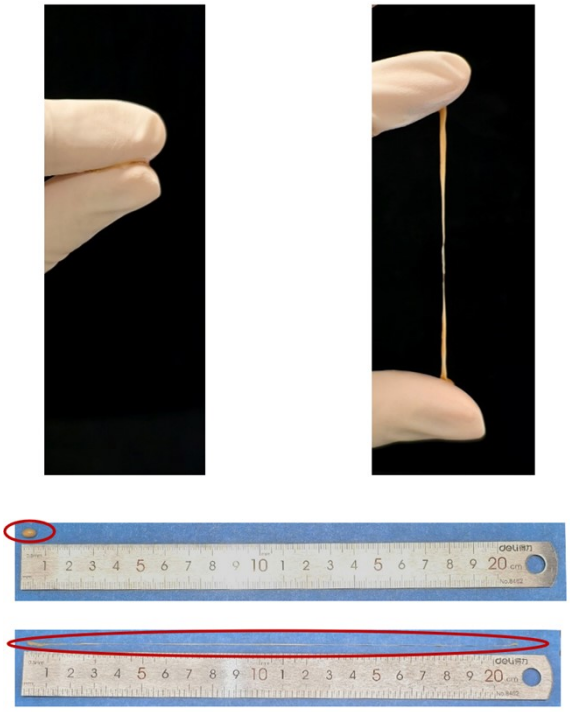


Figure S2: Tensile properties of the GCTC hydrogel.

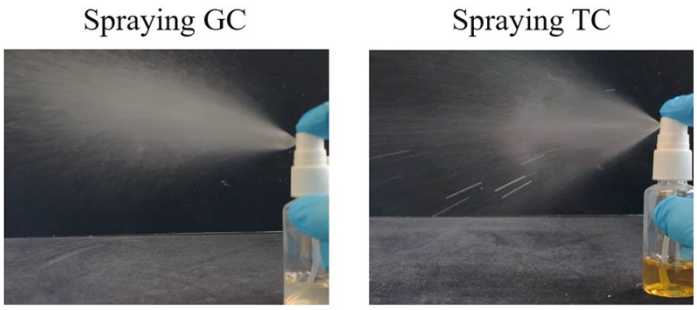


Figure S3: Demonstration of sprayability.

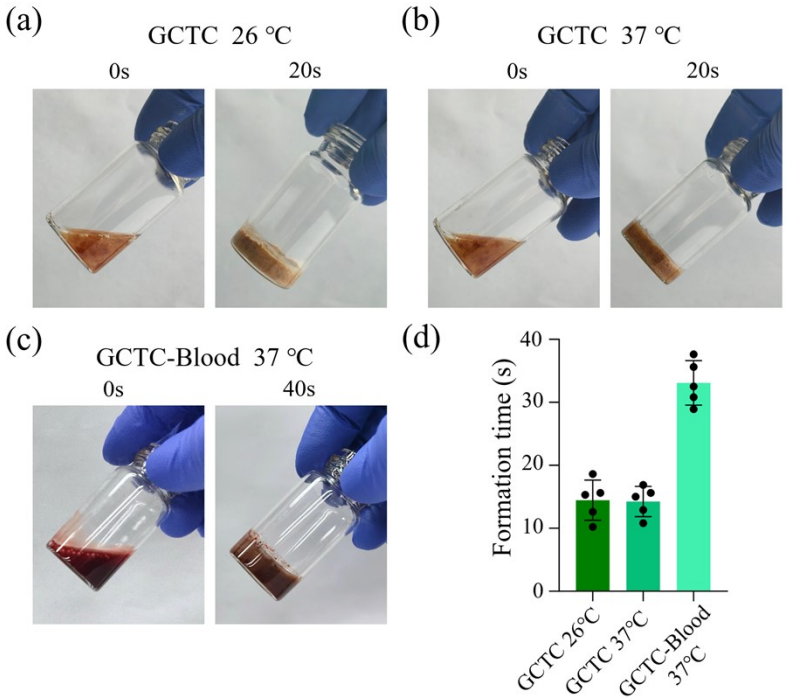


Figure S4. In vitro gelation time characterization of the GCTC hydrogel. (a) Gelation process of the GCTC hydrogel at 26°C. (b) Gelation process of the GCTC hydrogel at 37°C. (c) Gelation process of the GCTC hydrogel mixed with blood at 37°C. (d) Statistical analysis of the gelation time.

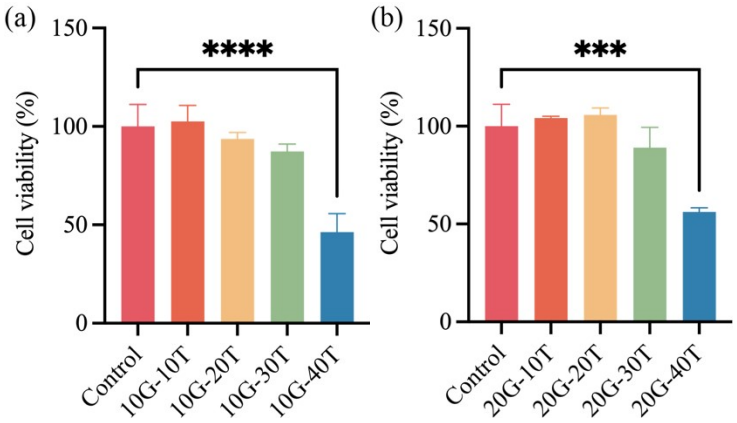


Figure S5: Cell viability of L929 cells after 24 hours of co-culture. (a)10% Gel; (b)20% Gel.

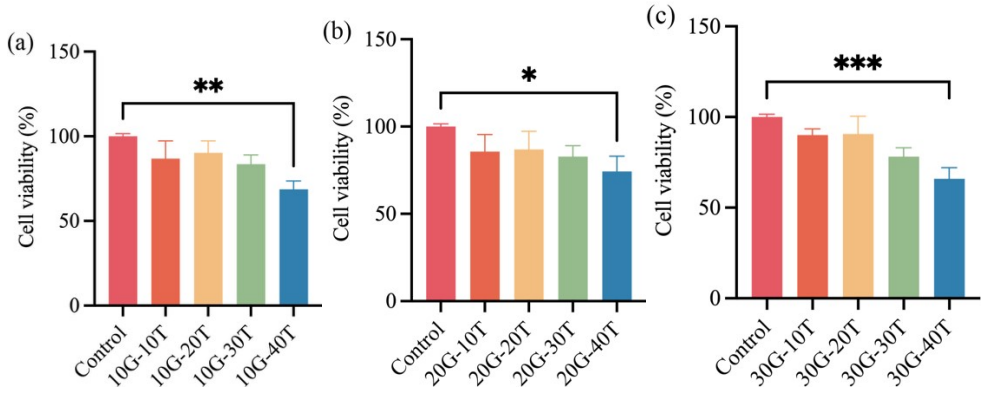


Figure S6: Cell viability of L929 cells after 72 hours of co-culture. (a)10% Gel; (b)20% Gel; (c)30% Gel.

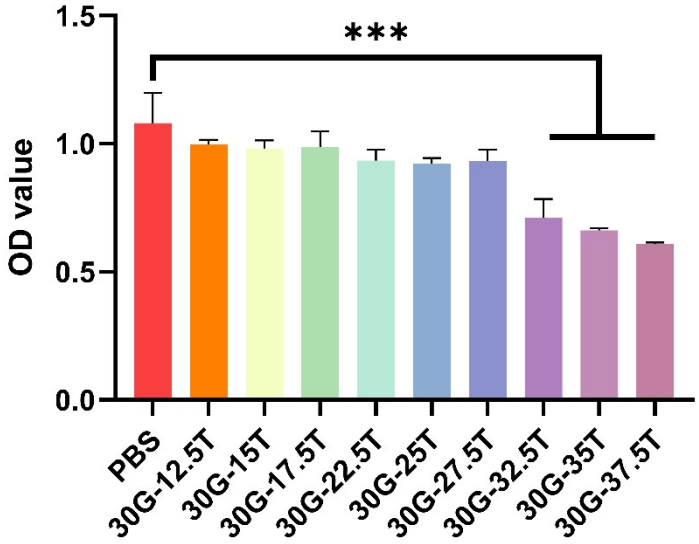


Figure S7: Cell viability of L929 cells after 72 hours incubation in 30% gelatin hydrogels containing varying concentrations of tannic acid.

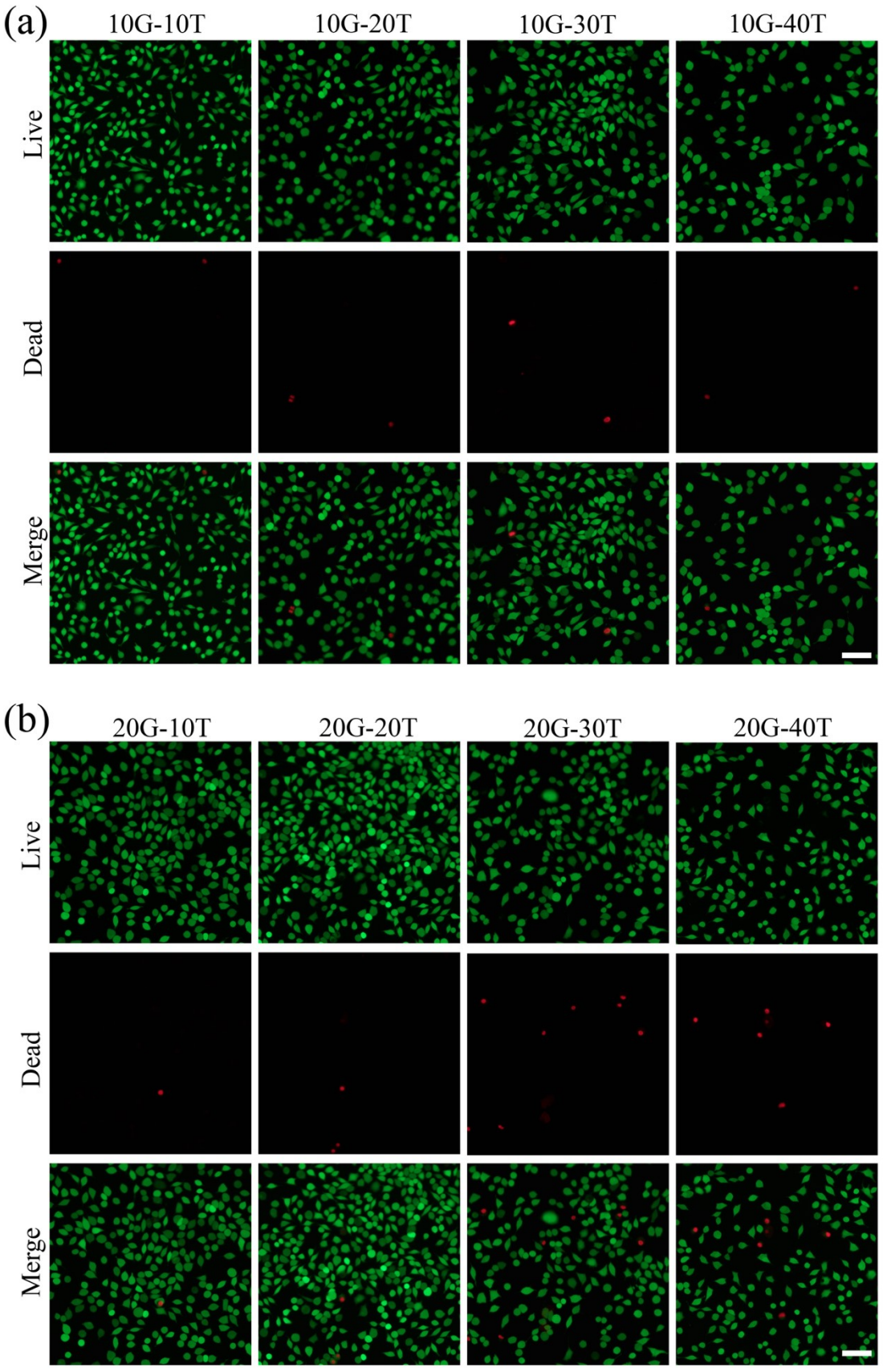
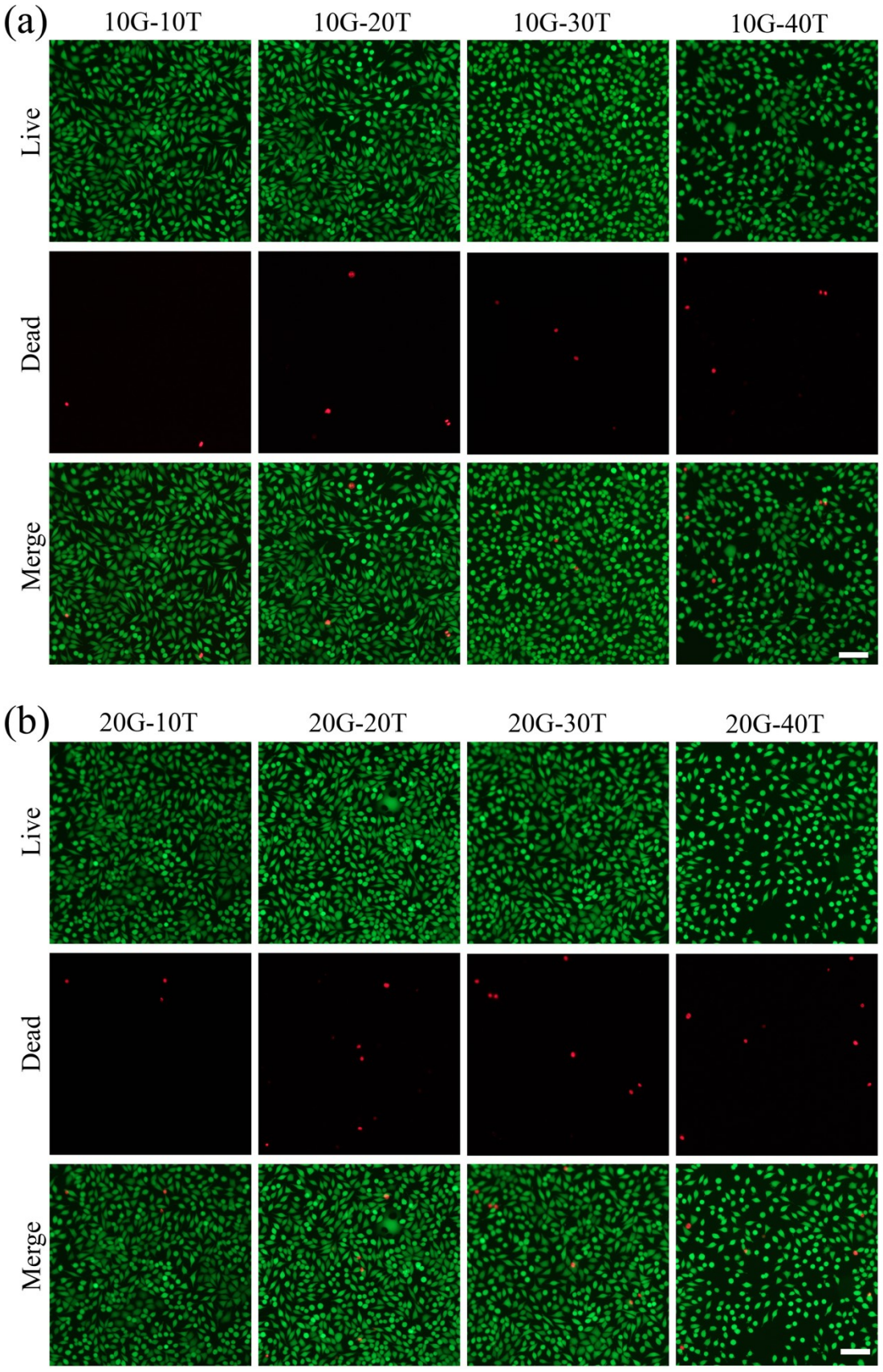


Figure S8: Fluorescent live/dead staining of L929 cells after 24 hours of co-culture.

Scale bar: 200 μm. (a)10% Gel; (b)20% Gel.



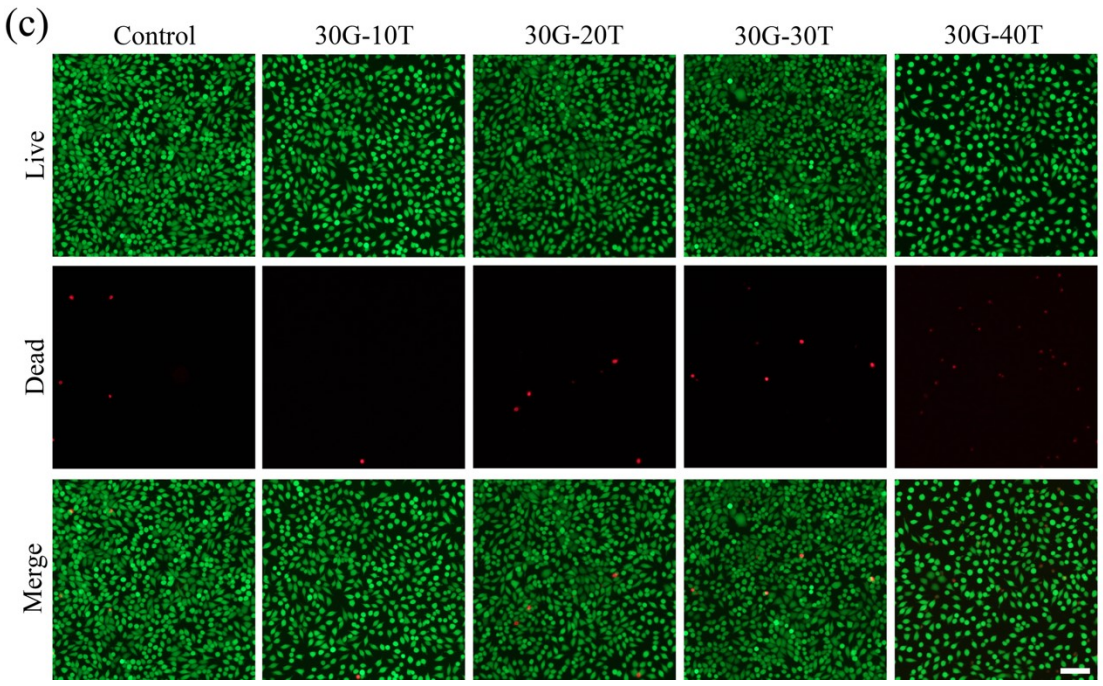


Figure S9: Fluorescent live/dead staining of L929 cells after 72 hours of co-culture. Scale bar: 200 μm. (a)10% Gel; (b)20% Gel; (c)30% Gel.

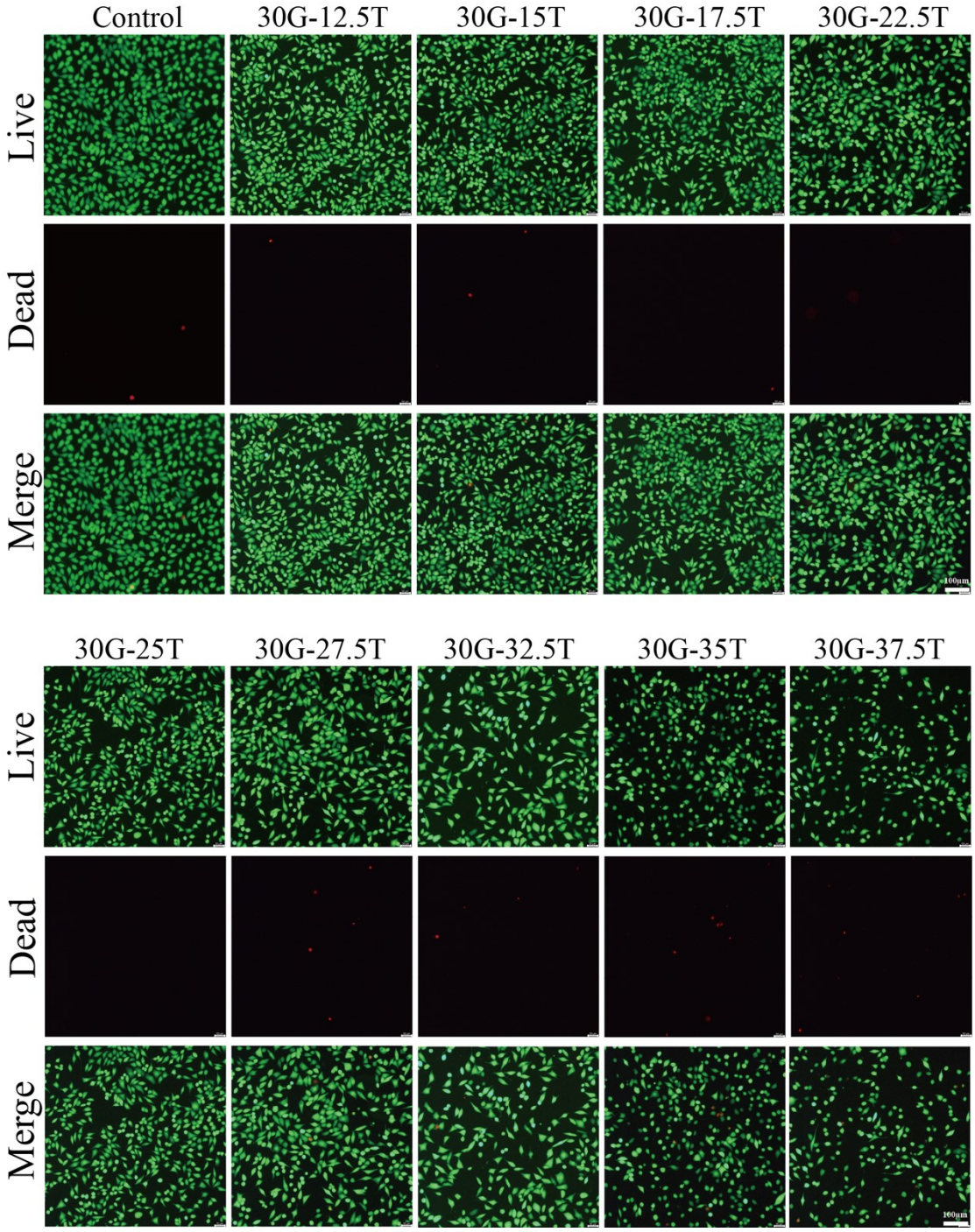


Figure S10: Live/dead staining of L929 cells cultured for 72 hours in 30% gelatin with different tannic acid concentrations.

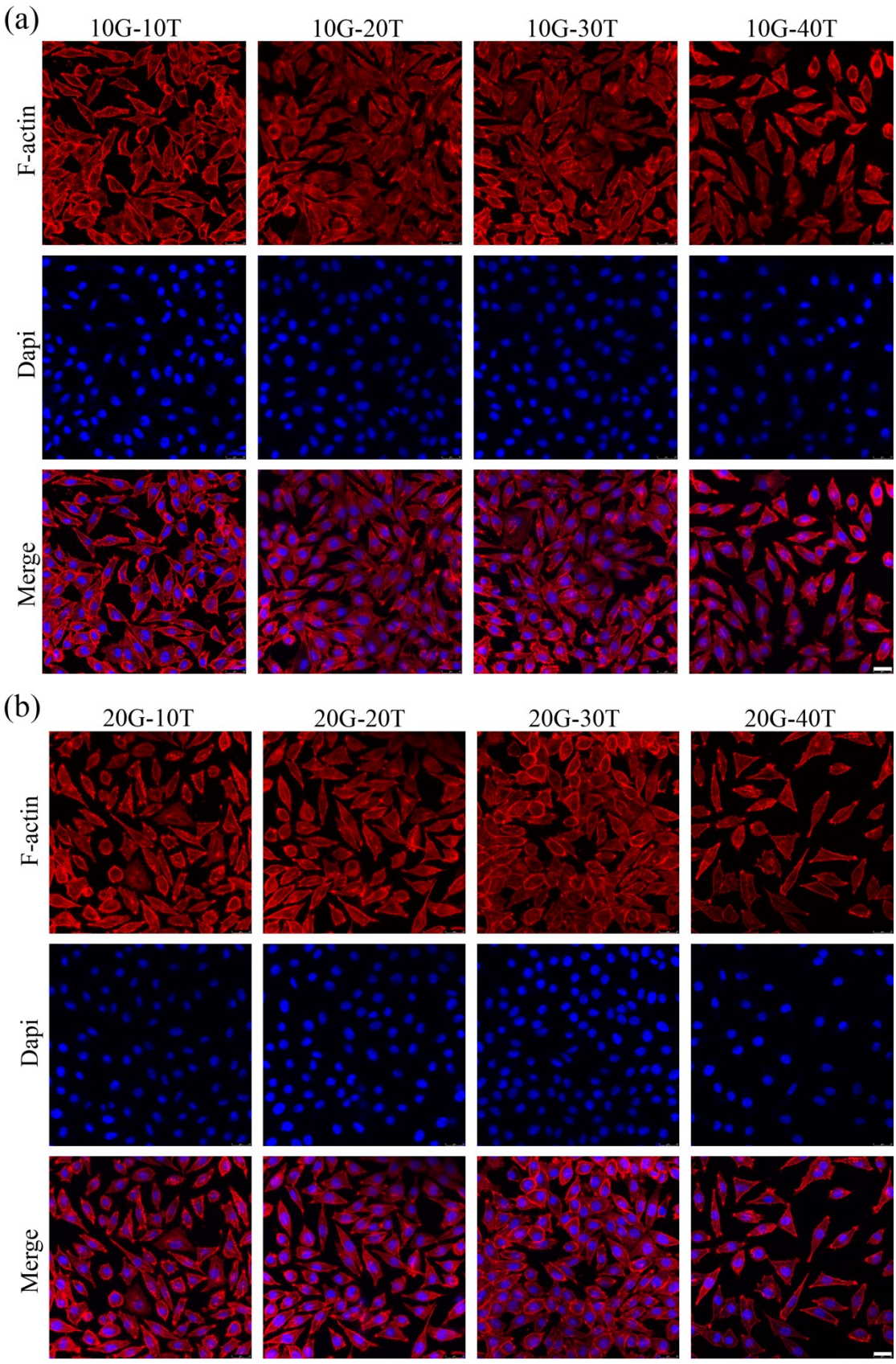
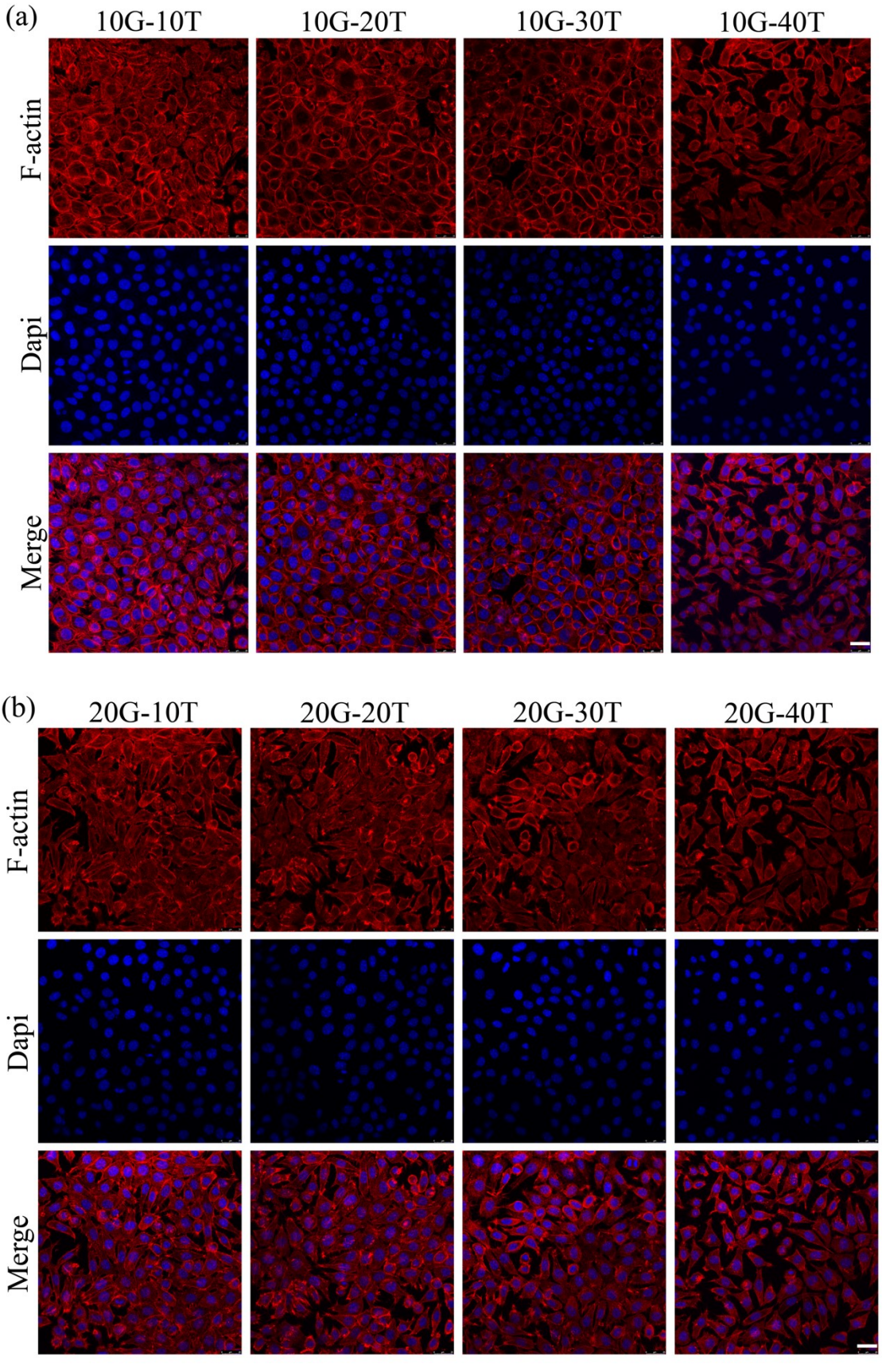


Figure S11: Cytoskeleton staining (Scale bar: 25 μm) of L929 cells after 24 hour co-culture. (a)10% Gel; (b)20% Gel.


(a)
10G-10T
10G-20T
10G-30T
10G-40T
F-actin
Dapi
Merge
(b)
20G-10T
20G-20T
20G-30T
20G-40T
F-actin
Dapi
Merge

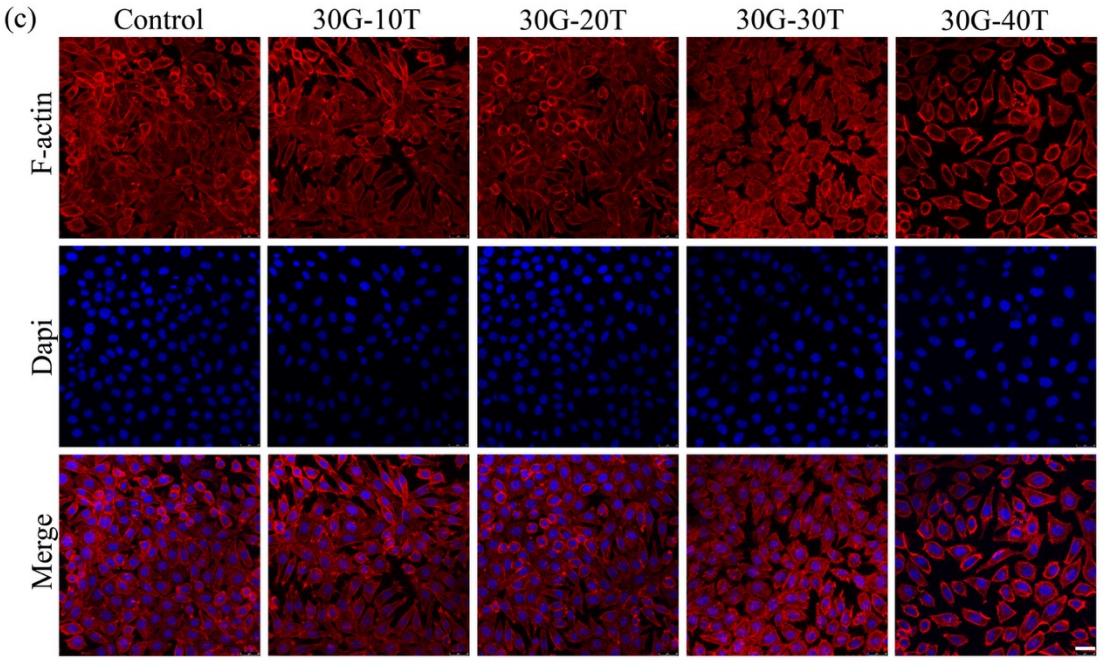


Figure S12: Cytoskeleton staining (Scale bar: 25 μm) of L929 cells after 72 hour co-culture. (a)10% Gel; (b)20% Gel; (c)30% Gel.

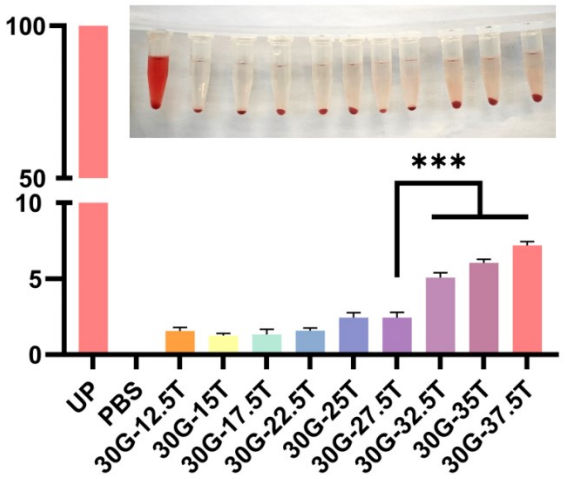


Figure S13: Hemolysis evaluation of 30% gelatin hydrogels with varying concentrations of tannic acid.

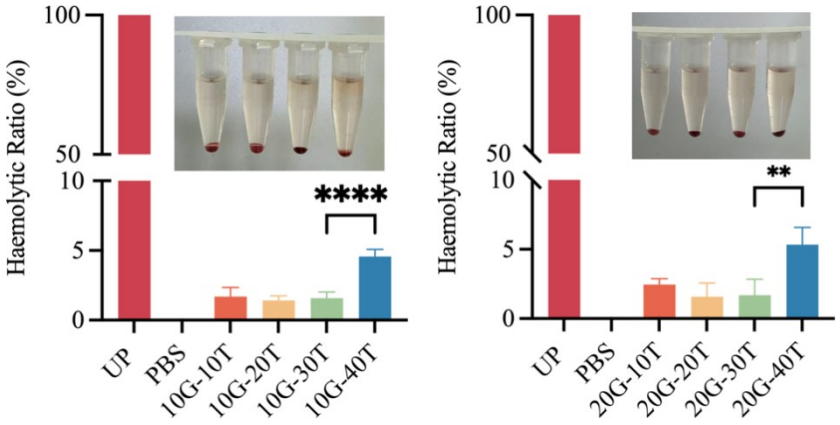


Figure S14: Evaluation of hemocompatibility.

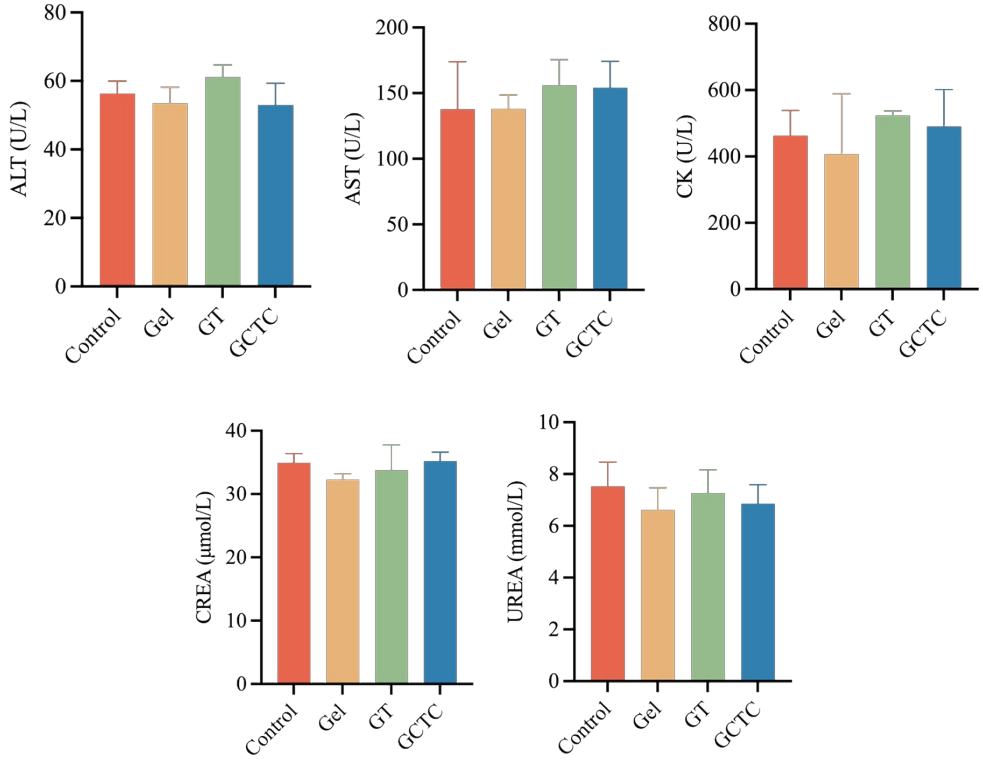


Figure S15: Main serum biochemical indicators of rats 14 days after hydrogel implantation on the back.

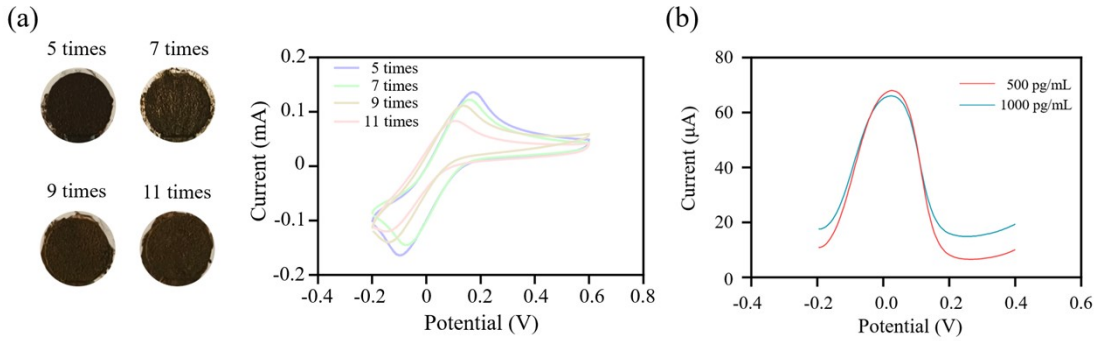


Figure S16: Characterization of gold deposition cycle optimization and DPV curves at 500 pg/mL and 1000 pg/mL. (a) Images under different gold deposition cycles and CV curves under different gold deposition cycles; (b) DPV curves at 500 pg/mL and 1000 pg/mL.

Figure S17: Portable electrochemical workstation demonstration.