

Supporting Information

A multi-responsive self-healing hydrogel for control-release of curcumin

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Experiment Section

1. Materials

Poly(ethylene glycol) methyl ether methacrylate (PEGMA, $M_n \sim 950 \text{ g mol}^{-1}$, Sigma Aldrich), (4-(3-((2-(Methacryloyloxy) ethoxy) carbonyl)-2,7,7-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinolin-4-yl) phenyl) boronic acid (J&K, 99%), 2,2'-azobis(2,4-dimethyl) valeronitrile

(ABVN, Energy Chemical, 98%), curcumin (Heowns, 97%), phosphate buffered saline (PBS, pH ~ 7.2-7.4, 0.01 M, Solarbio), poly(vinyl alcohol) (PVA-1788, Anhui Wanwei Ltd.), fetal bovine serum (FBS, Gibco), 1640 RPMI culture medium (Gibco), penicillin-streptomycin solution (Gibco), immunol staining fix solution (Beyotime), trypsin-EDTA (Gibco, 0.25%), cell counting kit-8 solution (Beyotime), fluorescein diacetate (FDA, Sigma), propidium iodide (PI, Sigma, 94%) and lectin (MACKLIN) were used as purchased. Solvents such as acetonitrile, ethanol and diethyl ether were purchased from Sinopharm Chemical Reagent and used directly without further purification.

2. Instrumental Analysis

The ^1H NMR spectra of all samples were obtained using a JEOL JNM-ECA400 spectrometer. Gel permeation chromatography (GPC) system included a Shimadzu LC-20AD pump 45 system consisting of an auto injector, a MZ-Gel SDplus 10.0 μm guard column ($50 \times 8.0 \text{ mm}$, 10^2 \AA), a MZ-Gel SDplus 5.0 μm bead-size column ($50 - 10^6 \text{ \AA}$, linear) and a Shimadzu RID-10A refractive index detector. Narrow molecular weight distribution polystyrene standards ranging from 200 to 10^6 g mol^{-1} were calibrated for this system, and *N, N*-dimethyl formamide (DMF) was used as the eluent. Rheology analyses were performed on a TA-AR G2 rheometer with parallel plate geometry (20 mm in diameter) at 25°C. Scanning electron microscope (SEM) photos were taken from HITACHI SU-8010. UV analyses were carried out by a PerkinElmer Lambda 750 UV/Vis/NIR spectrophotometry. The orbital shaker (ZD-9566, HLD laboratory equipment Co., China) were used for the study. Confocal images were collected by a Zeiss LSM-710 confocal microscopic system. Ethical approval was obtained from the Animal Care and Use Committee of Tsinghua University. All experimental animal procedures were performed under anesthesia, and all efforts were made to minimize suffering.

3. Methods

3.1 Preparation of P1

The polymers were synthesized by convenient radical polymerization as we reported previously. Firstly, the monomer (4-(3-((2-(Methacryloyloxy) ethoxy) carbonyl)-2,7,7-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinolin-4-yl) phenyl) boronic acid (M1) was purchased directly. Then, M1 (1.84 g, 4 mmol), PEGMA (7.60 g, 8 mmol), ABVN (0.06 g, 0.24 mmol) were dissolved in 10 mL of *N,N*-dimethylformamide. The mixture was purged with nitrogen flow to remove oxygen followed by keeping in a 70 °C oil bath for 8 hours. The polymerization was quenched in an ice-water bath. The final polymer **P1** was obtained by precipitation in diethyl ether three times as a white powder (8.22 g, 87.1% yield).

3.2 Preparation of P1-curcumin

The P1 were dispersed in ethanol/sodium phosphate buffer mixture (P1: 12 wt%, ethanol: buffer = 5/95 (v/v), pH ~7.4, 0.10 M), and the curcumin powder was added to form P1-curcumin suspensions (curcumin 1 mg/mL) were treated by ultrasonic dispersion for 30 minutes to make the binding completely.

3.2 Hydrogel preparation

The solution of PVA in PBS (6 wt%, pH ~ 7.4) was prepared by dissolving PVA powders (2.8 g) in PBS solution (37.2 g, pH ~ 7.4). A series of P1 solutions in PBS solution (8 wt%, 10 wt%, and 12 wt%) were prepared by dissolving P1 (0.24 g, 0.30 g, 0.36 g) and curcumin (1 mg/mL) in PBS/ethanol (ethanol: buffer = 5/95 (v/v), pH ~7.4, 0.10 M) solution (2.76 g, 2.70 g, and 2.64 g, pH ~ 7.4) respectively. As a typical example, Gel12-Cur. was prepared by mixing equal volume

solutions of P1-Cur. (12 wt%) and PVA (6 wt%), and a hydrogel was quickly formed in ~ 13 s. Gel8-Cur. and Gel10-Cur. were prepared similarly.

Gel12, Gel10, Gel8 were prepared as the same way without curcumin. Gelatine hydrogel (6 wt%) was prepared by dissolving gelatine (0.18 g) in a PBS solution (pH~7.4, 2.82 g) with trace bromocresol green dye.

3.3 Rheology analysis

Typically, a piece of hydrogel (~ 0.6 mL, ~ 2.0 cm diameters) was put on the measuring plate of a rheometer. The storage modulus (G') and loss modulus (G'') of the hydrogel were recorded under frequency sweep step (strain = 1%). All hydrogels were tested through the same process.

3.4 Self-adaptability study of the hydrogel

To test the self-adaptability of obtained hydrogels, the shear-thinning property of these hydrogels was studied via mobility experiment and step-changing strain experiment. Typically, a hydrogel Gel12-Cur. (1 mL, orange) was placed on the top of two layers of steel balls (diameter: 1 mm), the movement of this hydrogel driven by gravity was photographically recorded. A gelatine hydrogel (1 mL, blue) with a fixed skeleton was prepared and used as a control.

Rheology analyses were further carried out to test the shear-thinning property of the hydrogels. Typically, Gel12-Cur. was prepared as previously described. The moduli of Gel12-Cur. under different strains were tested (frequency = 1 Hz). Then, alternative changed strains (1%, 400%) under the same frequency (1.0 Hz) were applied to the hydrogel while G' and G'' values of the hydrogel under different strains were recorded.

The shear-thinning properties of Gel10-Cur., Gel8-Cur. and Gel12, Gel10, Gel8 were tested similarly.

3.5 Curcumin release study

A Gel12-Cur. hydrogel (40 mL) was prepared by previous method and added to a dialysis bag (MWCO: ~ 3.5 K). The dialysis bag was put into a beaker (500 mL) containing PBS/ethanol solution (360 mL, ethanol: buffer (pH ~7.4, 0.10 M) = 5/95 (v/v)) on an orbital shaker (90 rpm). Samples (2 mL) were taken from the outside PBS solution at different time points to measure released curcumin (UV: 359 nm), then put back for continue dialysis. A P1-Cur. conjugate solution (12 wt%) was used as a control.

3.6 Multiple stimulus response study

The hydrogel Gel12-Cur. (1.6 mL) was used as a typical example to study the multi-responsibility of hydrogels.

3.6.1 Acid-base response. A HCl solution (100 μ L, 6 M) was added into the vial to mix with Gel12-Cur. The vial was shaken for ~2 min, and the hydrogel changed from a solid to a liquid. Then, a NaOH solution (100 μ L, 6 M) was added into the vial. The hydrogel regenerated after ~ 2 min shake. This process was repeated four times.

3.6.2 Multiple stimulus response. H₂O₂ (1.6 mL, 0.1 M), HCl (1.6 mL, 0.1 M), fructose (1.6 mL, 0.1 M), mannitol (1.6 mL, 0.1 M) and PBS (1.6 mL, 0.1 M) were added into the vials with Gel12-Cur. hydrogel, respectively. These vials were put on an orbital shaker (60 rpm) for 8 h. Photos were taken every 30 min, samples (0.1 mL) were taken from the upper solution at different time points to measure released curcumin (UV: 359 nm) then put back.

3.7 Cell experiments

Cell culture was maintained in a 37 °C incubator with 5% CO₂, culture medium was changed every one or two days for maintaining the exponential growth of the cells. L929 cells (a murine-derived fibroblast cell line) were purchased from ATCC (USA), they were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin and streptomycin.

3.7.1 3D cell culture. L929 cells (1×10^6 cells/mL) were resuspended in a culture medium containing P1-Cur. (12 wt%). The L929/P1-Cur. (500 μ L) was pipetted into a petri-dish, then PVA in culture medium (6 wt%, 500 μ L) was pipetted into the same dish. The dish was gently shaken to form Gel12-Cur.-L929 evenly. This gel was incubated at 37°C, 5% CO₂, and imaged either after 24 h or 48 h. To investigate the cell viability, PBS-FDA-PI solution (FDA: 5 μ g/mL; PI: 5 μ g/mL, 1 mL) was added to the surface of Gel12-Cur.-L929. After stored in the dark for 10 minutes, the cells were monitor by a confocal microscopy. The live and dead cells were observed through 450-490 nm and 515-560 nm band-pass excitation filters, respectively. The z-stacks were taken through the depth of the gels to detect the distribution of cells throughout. Viability is reflected by the percentage of FDA-stained cells to total cells.

3.7.2 In vitro wound-healing tests. To evaluate the wound-healing ability of the Gel12-Cur. hydrogel, an *in vitro* wound-healing assay was carried out as literatures¹⁻⁴. L929 cells (1×10^6 cells/mL) were cultured in a Petri dish (diameter 3.5 mm); then, an artificial wound was created by scratching the bottom of the dish with a 200 μ L pipette tip. The cells were washed with PBS. The artificial wound was covered by Gel12-Cur. hydrogel (1 mL) followed by a 48-h observation. Optical images were taken at different time points to observe the healing process. Gel12 hydrogel (1 mL) and RPMI 1640 culture media were used as a control and a blank, respectively.

3.8 *In vivo experiments.* The bio-safety of the Gel12 and Gel12-Cur. hydrogel was tested using living mice model (six Balb/c mouse/group, 6–8-week-old female, 18–21 g). In this research, all animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Tsinghua University and approved by the Animal Ethics Committee of Tsinghua University. All experimental animal procedures were performed under anesthesia, and all efforts were made to minimize suffering. Briefly, hair on mice's waists was removed by a depilatory paste, P1-Cur. (200 μ L, P1 12 wt%, curcumin 1 mg/mL) and PVA (200 μ L, 6 wt%) solutions were mixed in a 1 mL syringe to form a Gel12-Cur. hydrogel. This hydrogel (400 μ L) was injected under the skin of a Balb/c mouse. After 24 h and 72 h, the inflammation and oedema of the skin was observed and recorded by photograph. After 3 days, mice were euthanized by carbon dioxide asphyxia respectively. These mice were dissected to excavate the Gel12-Cur. hydrogel.

Gel12 was similarly tested.

Supporting Data

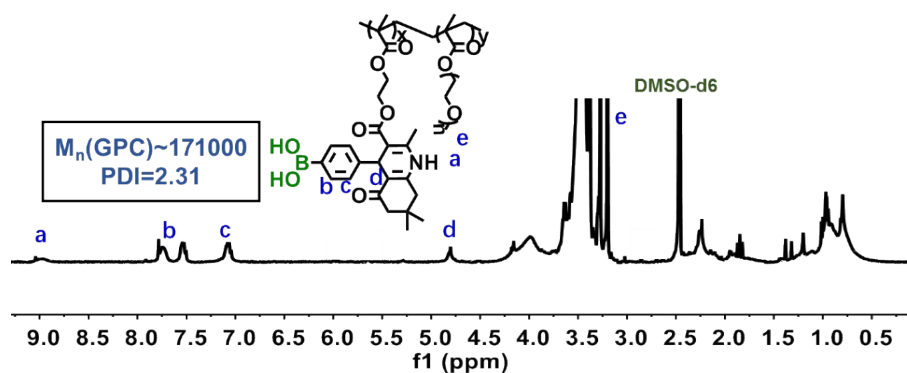


Figure S1. ^1H -NMR spectrum (400 MHz, DMSO- d_6) of polymer (P1).

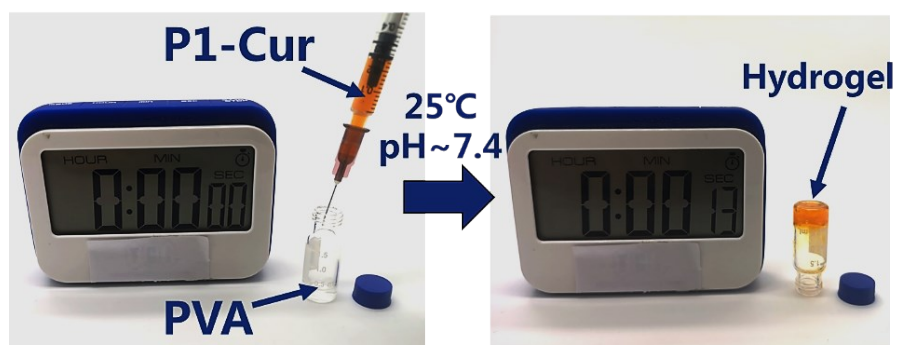


Figure S2. Preparation of hydrogels by mixing equal volumes of P1-Cur. conjugate and PVA solutions within 13 s.

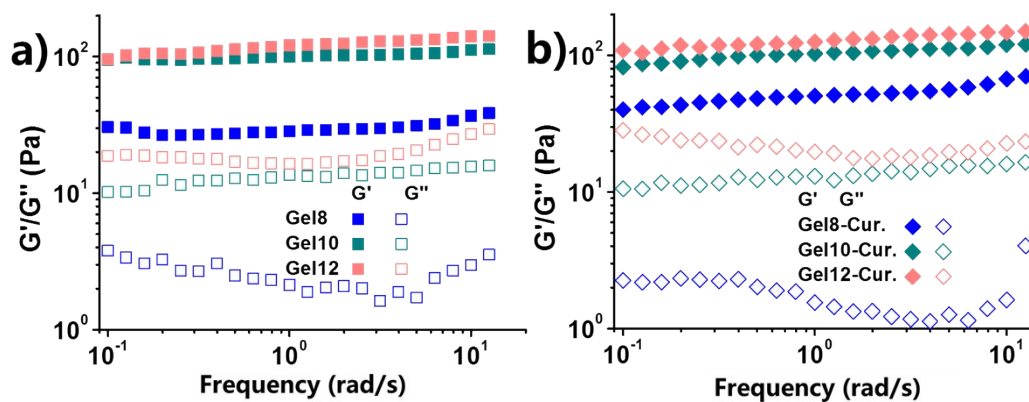


Figure S3. Moduli (G' & G'') of a) Gel12, Gel10, and Gel8; b) Gel12-Cur., Gel10-Cur., and Gel8-Cur., 1% strain, 25 °C.

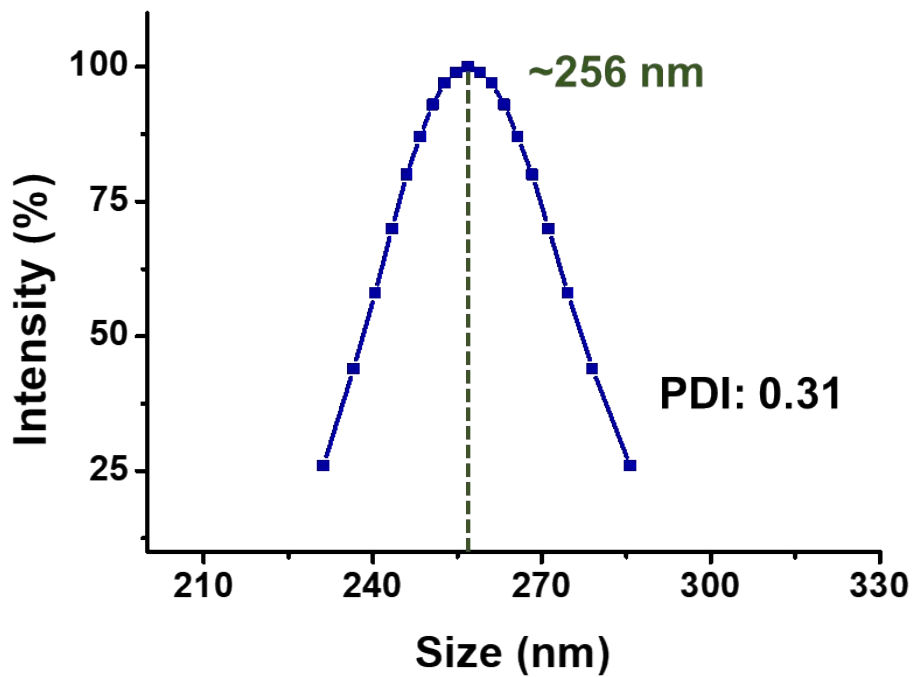


Figure S4. DLS result of the P1-curcumin (P1/curcumin: 12/1 mg/mL, ethanol: buffer = 5/95 (v/v), pH ~ 7.4).

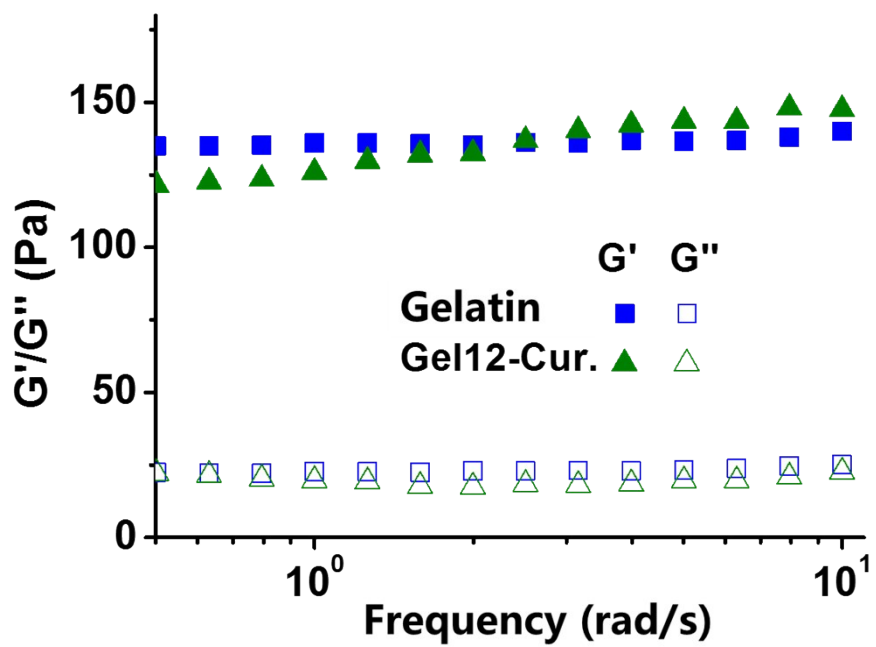


Figure S5. Shear storage moduli of the gelatin hydrogel (blue) and Gel12-Cur. hydrogel (orange) (0.1% strain and 6.3 rad/s).

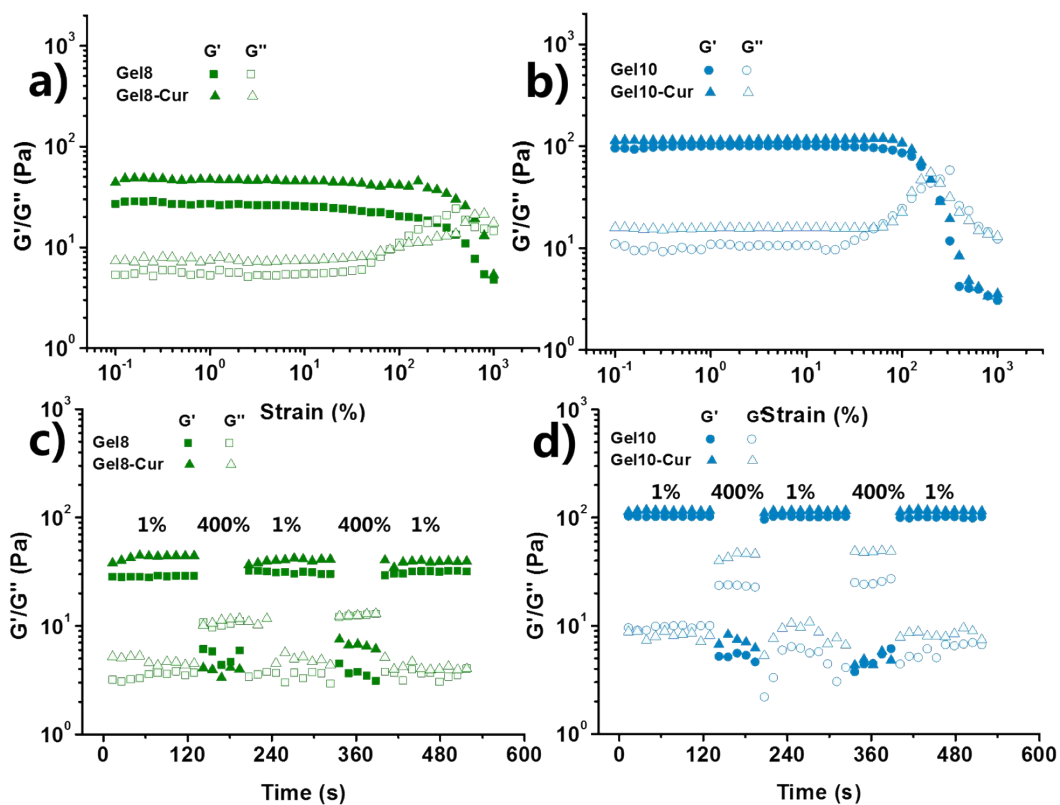


Figure S6. Moduli of a) Gel8-Cur. and b) Gel10-Cur. versus strain; moduli of c) Gel8-Cur. and d) Gel10-Cur. with alternating strains.

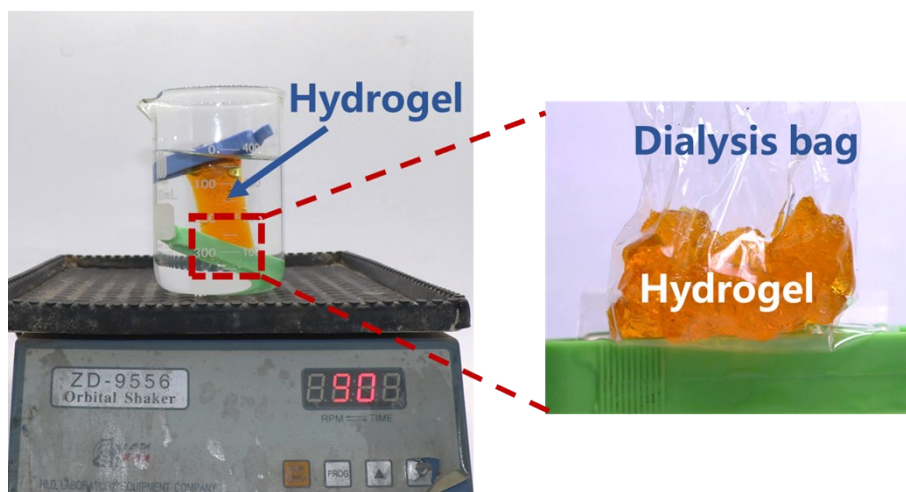


Figure S7. Experimental setup used to study curcumin release.

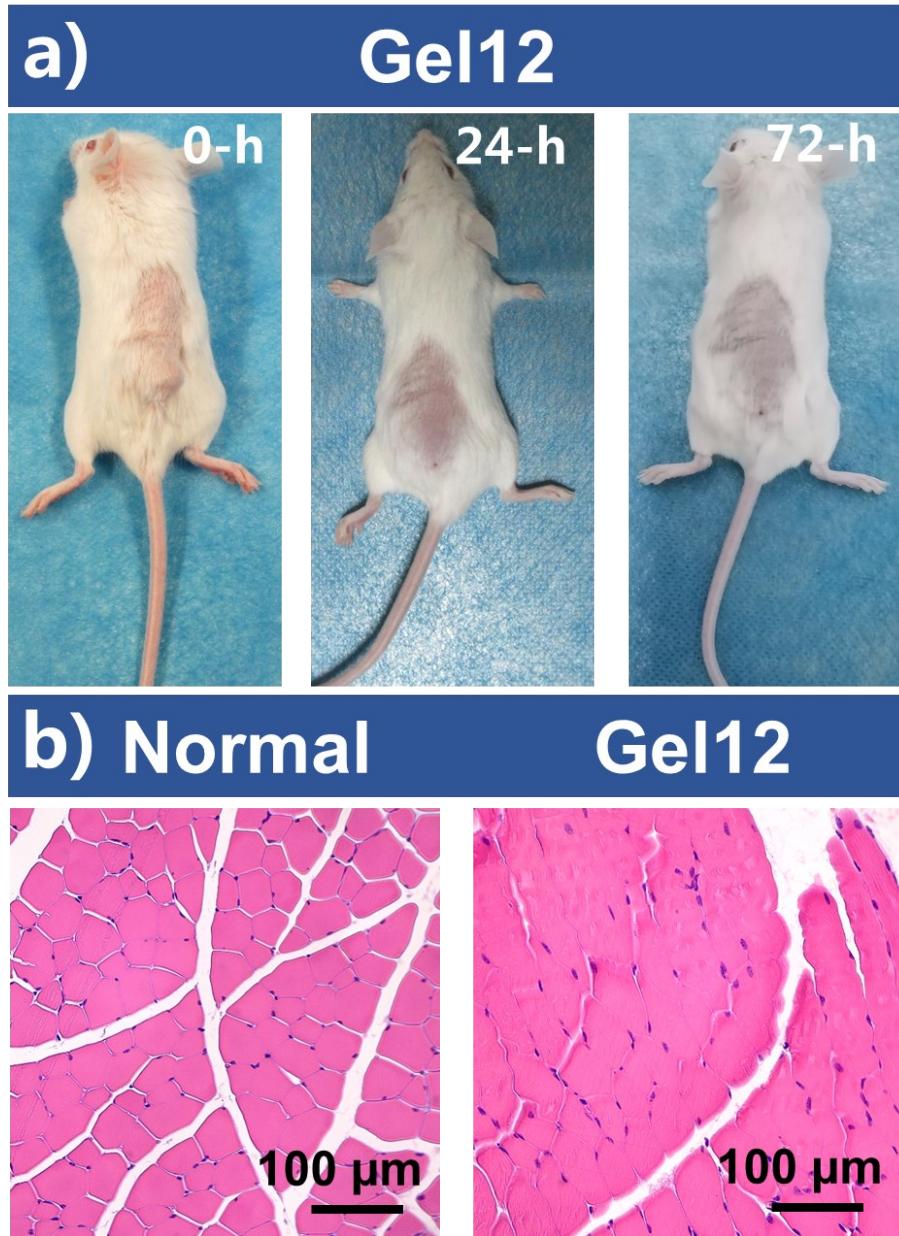


Figure S8. a) Images of mice after injection with Gel12. b) Images (H&E staining) of normal subcutaneous tissue and the subcutaneous tissue of the implanting site after 72 h.

Reference

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