Electronic Supplementary Information for

Polyurethane-Gelatin Methacryloyl Hybrid Ink for 3D Printing of Biocompatible and Elastic Vascular Networks

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General procedures

Poly(ethylene glycol) 2000 (PEG, J&K Chemicals), poly(L-lactic acid) diol 2000 (PLA, Huateng Pharm., Changsha, China), lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP, TCI), and other commercially available chemicals (Innochem) were purchased and used as received. Gelatin methacryloyl (GelMA, ~75% methacryloyl substitution) was synthesized according to literature procedures.[1]

Proton nuclear magnetic resonance (^{1}H-NMR) spectra were obtained on a Bruker Fourier 300 instrument operated at 300 MHz at room temperature. Gel permeation chromatography (GPC) results were obtained on a Waters 1515 system and calibrated with polystyrene standards in DMF (with 0.05 M LiBr). Infrared (IR) spectra were obtained on a Bruker Equinox 55 spectrometer with an attenuated total reflection (ATR) module and the background signals of air were subtracted. Thermogravimetric analysis (TGA) was performed on a PerkinElmer Pyris 1 thermogravimetric analyzer. For scanning electron microscope (SEM) images, cross-linked hydrogels were soaked in PBS before being frozen in liquid nitrogen. The frozen gel was cut to expose the internal bulk part and freeze-dried to remove water. The exposed surface was treated by Au sputtering before imaging on a JEOL JSM 6700F microscope with an accelerating voltage of 3.0 kV. Bright-field microscope images were captured on an Olympus IX71 microscope. Confocal laser scanning microscope (CLSM) images were captured on an Olympus FV 1200 microscope. Rheological properties were tested on a Discovery HR-1 hybrid rheometer (TA Instruments). Tensile strength and elongation of crosslinked hybrid hydrogels were measured on a Mark-10 ESM303 extensometer.

Cells were cultured in a humidified atmosphere of 5% CO_2 at 37°C. The culture medium for HUVEC was DMEM formulated with 10% FBS and 1% streptomycin-penicillin, and the medium was refreshed daily.

Synthesis of the water-soluble PU

A mixture of poly(ethylene glycol) (PEG-2000, 8 g) and poly(L-lactic acid) diol (PLA-2000, 2 g) was heated to 100°C under argon. When the entire mixture melted, a solution containing isophorone diisocyanate (IPDI, 3.73 mL) and stannous octoate (0.81 μL) was added under mechanical stirring (250 rpm), and the mixture was heated at 100°C for 3 h. Then, 2,2-bis(hydroxymethyl)propionic acid (DMPA, 0.67 g) was added to the reaction mixture. The temperature was maintained at 100°C for another 1 h before being adjusted to 80°C. 2-Hydroxyethyl methacrylate (HEMA, 460 μL) was added and the mixture was stirred at 80°C for 1 h before being cooled to 50°C. After adding triethylamine (Et_3N, 697 μL), the mixture was stirred at 50°C for another 30 min. Finally, ethylenediamine (EDA) aqueous solution (334 μL EDA dissolved in 37 mL H_2O) was added to the reaction mixture under vigorous mechanical stirring (800 rpm). The pale yellow to white aqueous dispersion was dialyzed against water using a dialysis membrane with a molecular weight cut-off of 3.5 kDa. After freeze-drying, the PU was obtained as a white powder for characterization purposes. After freeze-drying, the solid content was 30% (w/w) as calculated by the weight change. The PU powder was re-dissolved in DMF for GPC measurements. The number-average molecular weight (M_n) of the PU was 23.8 kg/mol with a polydispersity index (PDI) of 1.22.

Preparation of the hybrid ink

PU (30% in water), HEMA (10% in water), and GelMA (20% in PBS) were mixed in equal volumes and heated to 40°C under vigorous magnetic stirring. Once the mixture became uniform, LAP was added and the mixture was vigorously stirred again at 40°C. The warm uniform mixture was transferred into a syringe compatible with the extrusion printer. If necessary, the ink was centrifuged to remove the bubbles. When stored in the dark at 4°C, the obtained hybrid ink can be stable for at least two weeks.

Measurements of tensile strength

Plastic molds for casting the dog-bone-shaped strips were placed together in parallel inside a Petri dish. The hybrid ink containing PU, GelMA, and HEMA was injected into the molds and irradiated for 2 min under a UV lamp (365 nm, 10 mW/cm^2). After irradiation, PBS was poured into the Petri dish to immerse the entire mold for 5 min. Before tensile strength measurements, the sample strips were de-molded and soaked in fresh PBS.

Measurements of rheological properties

Thermo-responsive and shear-thinning behaviors of the hybrid ink were measured before photo-crosslinking. The sample was placed in the fridge at 4ºC before measurements and was transferred to the Peltier plate temperature control and pressed by a 20 mm parallel-plate geometry base. The temperature was raised from 4ºC to 40ºC by 1ºC/min, and the storage and loss moduli were measured under 1% strain at 10 rad/s. The shear-thinning test was performed by increasing the shear rate from 0.1 to 100 s^{-1} at 20ºC. Shear moduli of the crosslinked hydrogel were measured at 25ºC at an angular frequency of 10 rad/s.

Hemolysis rate of the crosslinked PU-GelMA

The hemolysis rate of the crosslinked PU-GelMA was compared with GelMA and PDMS, while the PBS and ultrapure water were used as the negative and the positive controls, respectively (N = 3 in each group). Whole blood was diluted with saline in a ratio of 4:5 (v/v) and treated separately. In individual centrifuge tubes, 10 mL of saline was added to PU-GelMA, GelMA, or PDMS, while 10 mL PBS was added to negative control and 10 mL water was added to positive control. The samples were kept at 37 ºC for 30 min. 200 µL of diluted whole blood was added to each centrifuge tube and the samples were kept at 37 ºC for another 2 h. After centrifuging at 1200 rpm for 5 min, the optical density (at 545 nm) of the supernatant was

obtained for each sample. The hemolysis rate (HR) of the sample was calculated as:

$$HR = [OD\ (sample) - OD\ (PBS)] / [OD\ (water) - OD\ (PBS)] \times 100\%$$

Extrusion printing and crosslinking

3D printing was performed on a CPD1 3D printer (SUNP Biotech) with temperature control. The print platform temperature was set to 4ºC, and the syringe holder temperature was adjusted for individual formulations (typically 10-25ºC). Inside the syringe, the holder was a 10 mL BD syringe loaded with ~3 mL hybrid ink, equipped with a 250 μm nozzle. The selected 3D structure was printed on a Petri dish placed on the print platform. Once the printing had been completed, the as-printed 3D construct was placed under a 365 nm UV lamp for photo-curing. After two minutes of irradiation (10 mW/cm^2), the cured construct was removed from the lamp and immediately soaked with PBS at room temperature. The crosslinked object was detached spontaneously or carefully removed from the platform.

Evaluation of Printability

A grid network of 20×20 mm (unit square cell of 2×2 mm) was printed and photographed. The digital image of the grid was analyzed by ImageJ software ("analyze particle") that exports the perimeter and area of each recognized square cell. The printability (*Pr*) was defined as $Pr = L^2 / 16A$, where L is the perimeter and A is the area of each square cell between adjacent printed lines.

Construction of branched channels using sacrificial F127

1 mL of PU-GelMA mixture (15% PU, 10% GelMA, 5% HEMA, 0.5% LAP) was placed in a 35 mm Petri dish and cooled to 18°C. On top of this uncrosslinked PU-GelMA hydrogel, the branched pattern was printed with F127 (30% w/w, colored with eosin Y). Another 1 mL

of the same PU-GelMA mixture was gently added to cover the entire printed object, cooled to 18°C, and the entire sample was photo-crosslinked for 2 min (365 nm, 10 mW/cm^2). The crosslinked gel was trimmed with a blade to remove surplus materials and both ends of the printed structure. To remove the F127, the trimmed gel was immersed in DI water and cooled to 4°C. The gel was gently pushed between two pieces of tissue paper to remove the trapped liquid, and immersed in fresh DI water again at 4°C.

Adhesion and proliferation of HUVEC on PU-GelMA

Before incubation with cells, the crosslinked PU-GelMA hydrogel was soaked in PBS, 75% EtOH, and PBS again (at least 1 h in each solution), and placed in a small Petri dish. HUVEC (1×10^6 cells/mL) were suspended in the culture medium and seeded onto the top surface of PU-GelMA. After incubation at 37°C for 4 h, more culture medium was added to the Petri dish so that the entire gel was immersed. The culture medium was refreshed daily. Optical microscope images (10× magnification) were taken with the top surface of PU-GelMA in focus. Cell density was obtained by counting the number of cells in the entire image, and normalized relative to the first day of cell adhesion.

Live-dead staining of HUVEC on PU-GelMA

HUVEC were seeded on PU-GelMA scaffolds as described above. To assess the viability cells were stained with 1 μL/mL calcein-AM in culture medium for 25 min at 37°C. After removing the calcein-AM solution, 2 μL/mL PI in culture medium was added to stain the cells for another 2 min at 37°C. The cells were washed with PBS 3 times and fluorescence images were captured using the CLSM microscope. (Calcein AM: excitation 488 nm, emission 505-525 nm; PI: excitation 559 nm, emission 600-630 nm.)

Supplementary Figures

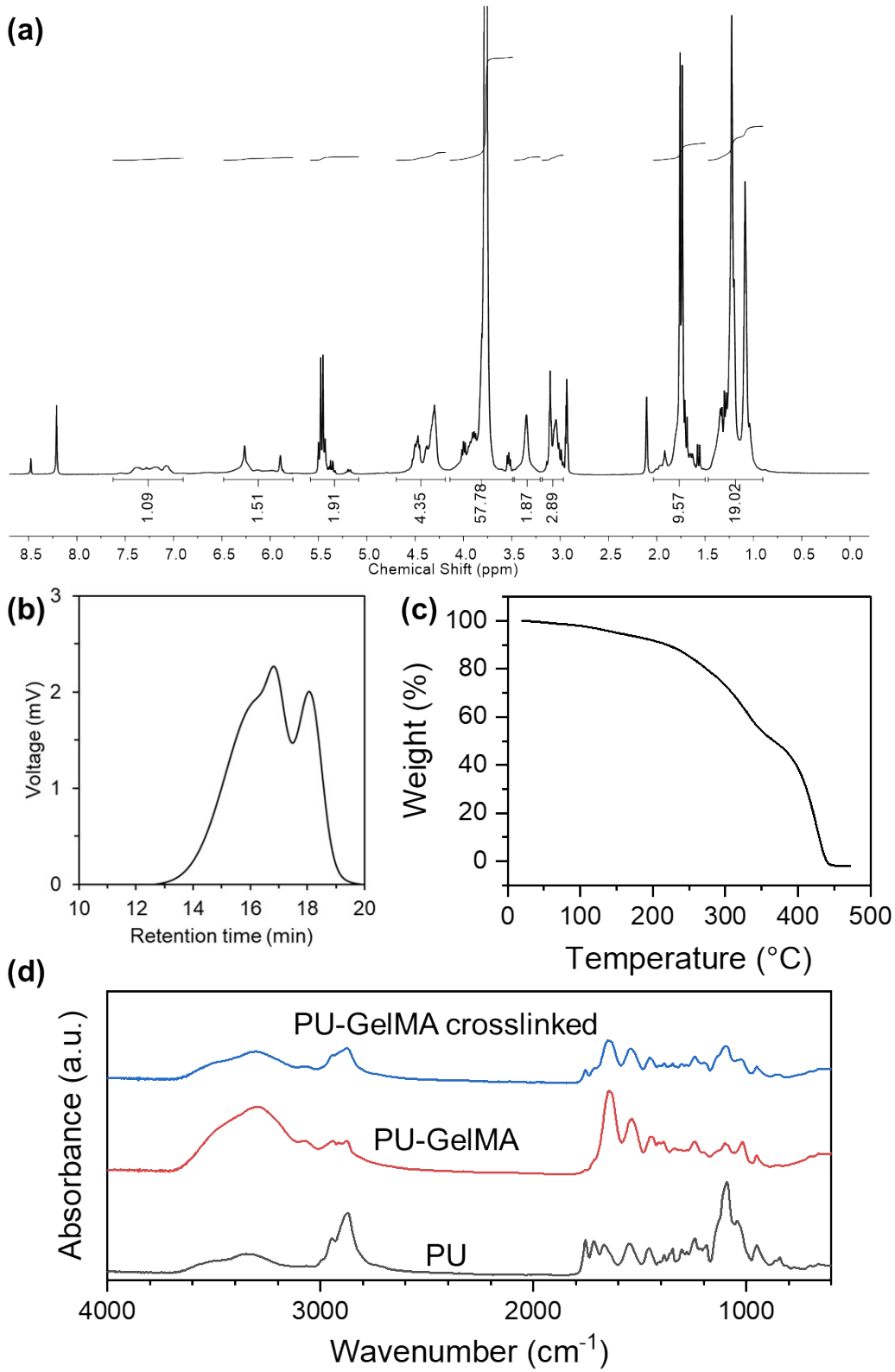
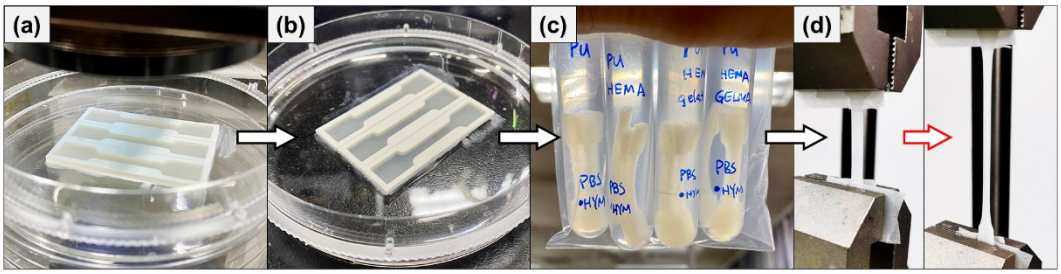


Figure S1. (a) ^{1}H-NMR spectrum (in d_7-DMF), (b) GPC and (c) TGA profiles of the crosslinkable PU. (d) Infrared spectra of the crosslinkable PU and GelMA.



(e) Calculation of tensile strength

Unloaded dimension (mm)	W = 3.16, D = 2.13, L_0 = 17.50
Cross-section area (mm^2)	$A = WD$ = 6.73
Elongation at break (%)	$\boldsymbol{\lambda = \Delta L / L_0}$
Ultimate tensile strength (kPa)	$\mathbf{UTS = F / A}$

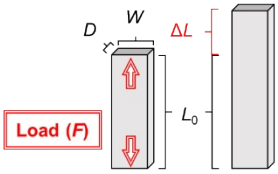


Figure S2. Measurements of elongation and tensile strength. (a) Photo-crosslinking of tensile specimens under a UV lamp. (b) Specimens soaked in PBS before demolding. (c) Demolded specimens in PBS. (d) Tensile strength measurements on an extensometer. (e) Calculation of elongation and ultimate tensile strength.

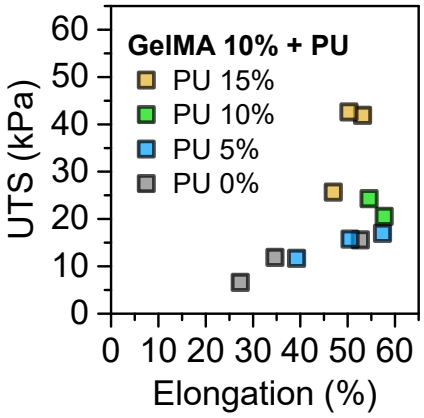


Figure S3. Tensile strength of PU-GelMA under variable PU content.

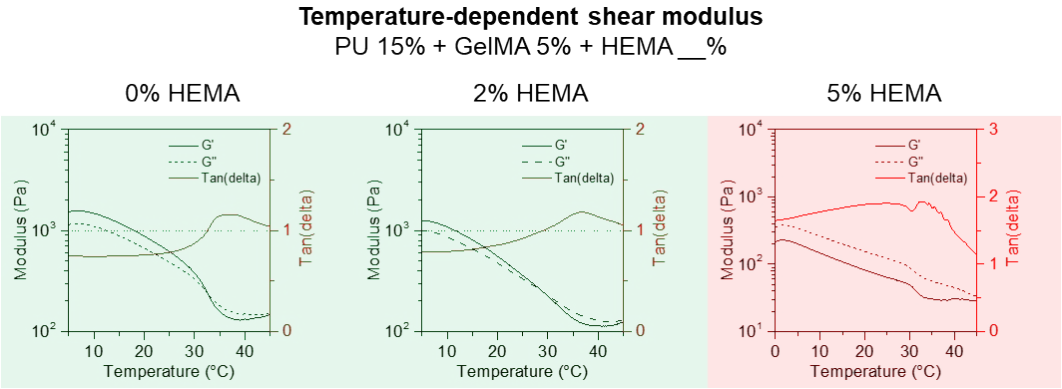


Figure S4. Storage and loss moduli (G' and G'') were measured for indicated inks under variable temperature. The inks were categorized as "printable" (green) or "non-printable" (red).

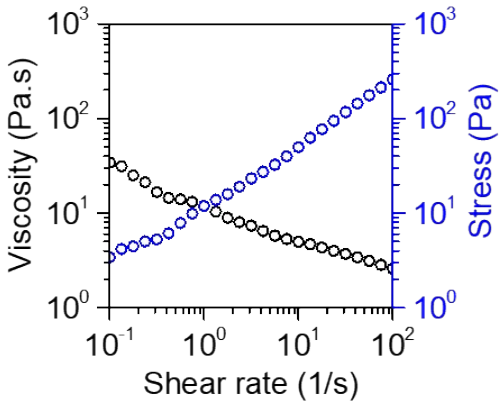


Figure S5. Shear-thinning profile of the PU-GelMA ink with 10% HEMA monomer.

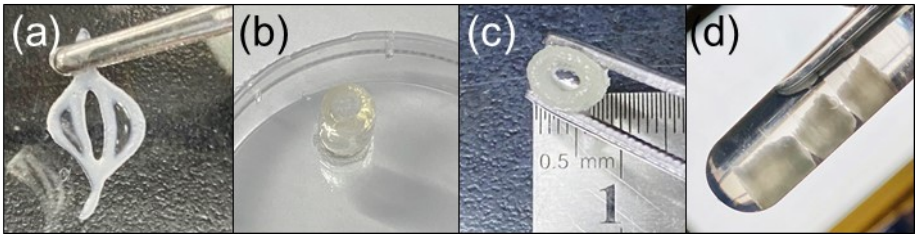


Figure S6. (a) Branched connector can be printed and cured by photo-crosslinking. (b) The tubular structure with a void in the center can be directly printed along the vertical direction, and (c) photo-crosslinked to yield an elastic tube. (d) The crosslinked tubes did not swell severely in PBS, water, or 75% ethanol. The photo showed the soaking of mini-tubes in ethanol

in a 5 mL centrifuge tube.

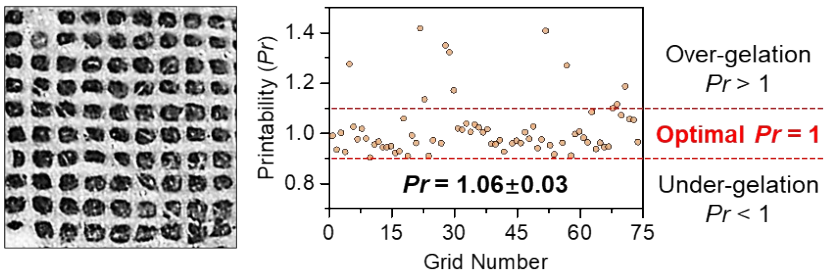


Figure S7. Grid network printed with PU-GelMA hybrid ink (10% GelMA, 15% PU, 5% HEMA) was analyzed for the evaluation of printability index (Pr).

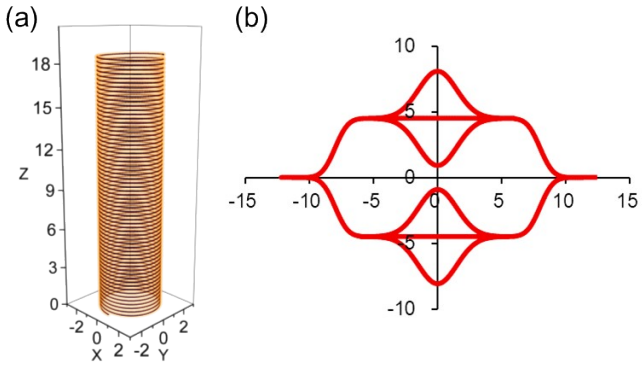


Figure S8. Programmed nozzle trajectories (in mm) in extrusion printing of (a) the vertical tube in Figure 3a and (b) the sacrificial branched network in Figure 3c.

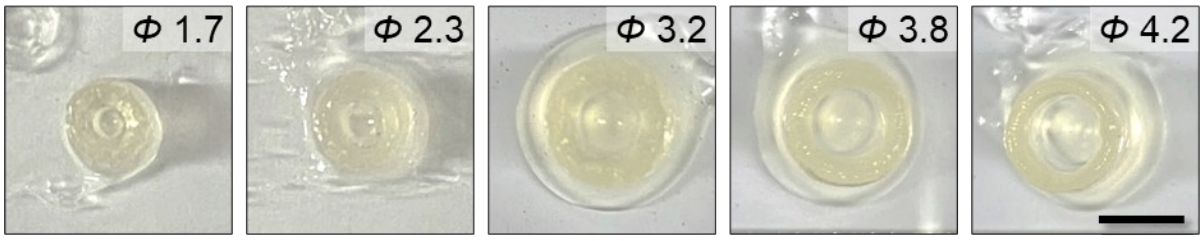


Figure S9. Tubes were printed with inner diameters (in mm) varied from 1.7 mm to 4.2 mm. Scale bar, 5 mm.

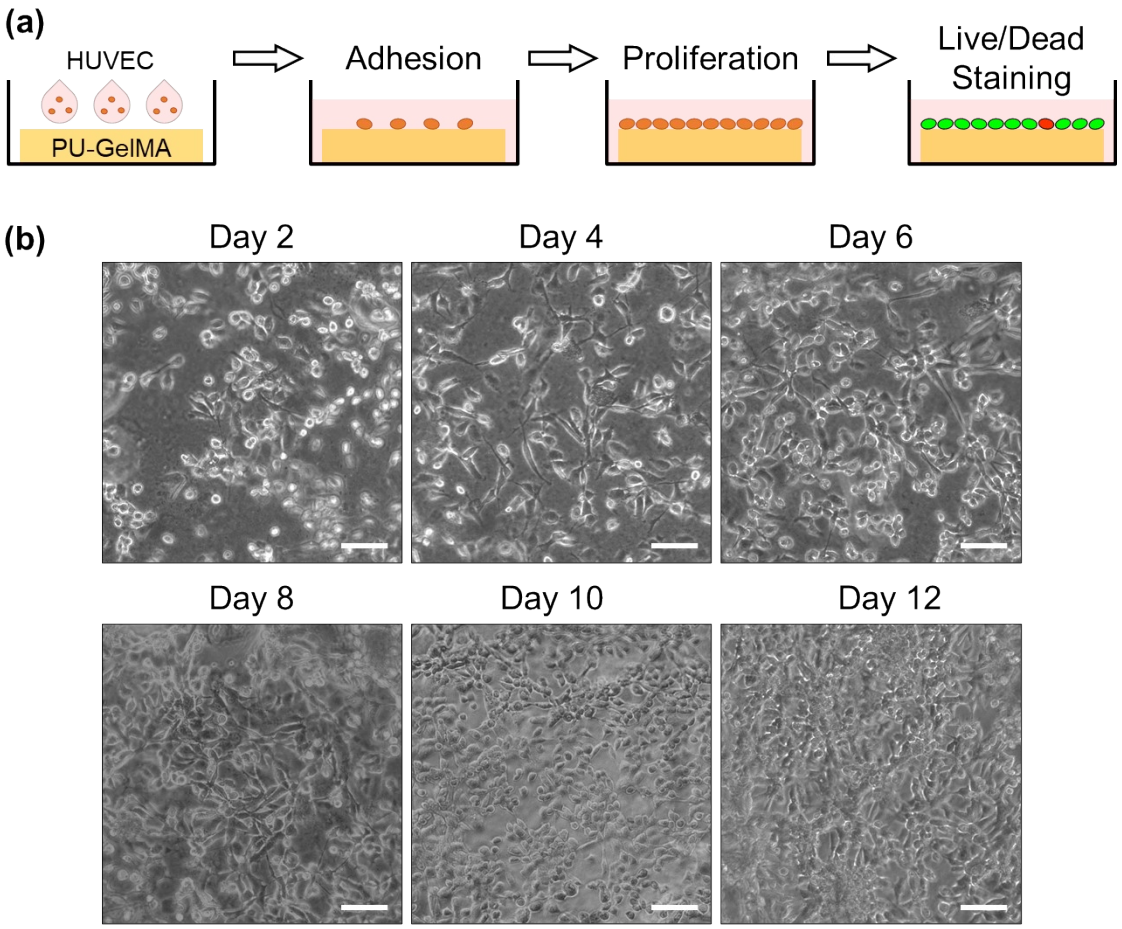


Figure S10. Biocompatibility evaluation of the PU-GelMA scaffold. (a) Schematic illustration of evaluating HUVEC proliferation and viability on PU-GelMA. (b) Optical microscope images of HUVEC adhesion and spreading on the crosslinked PU-GelMA surface. Scale bars, 100 μm.

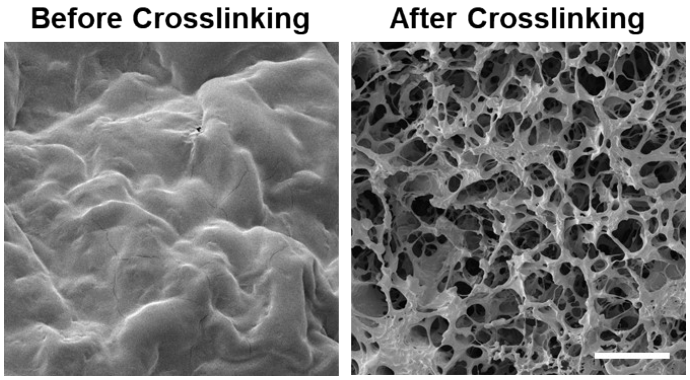
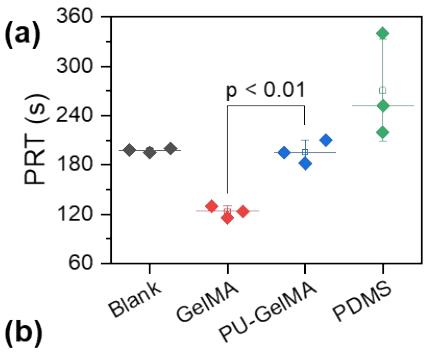
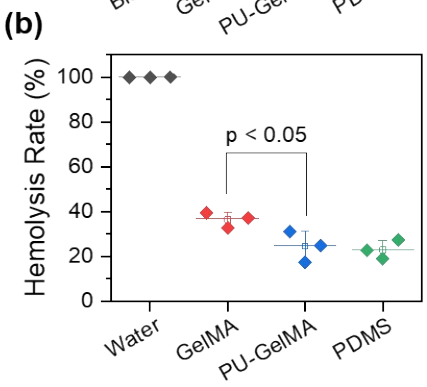


Figure S11. SEM images of the PU-GelMA hydrogel. Scale bar = 200 μm.



Group	PRT (s)
Blank	198±3
GelMA	123±7
PU-GelMA	196±14
PDMS	271±62



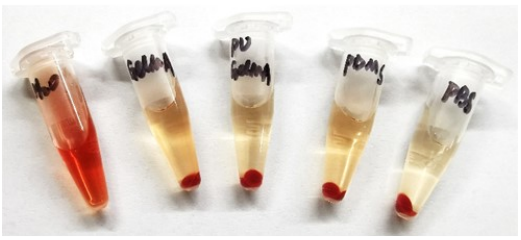


Figure S12. Blood compatibility of the crosslinked PU-GelMA scaffold. (a) Plasma recalcification time (PRT) and (b) hemolysis rate of PU-GelMA compared to the crosslinked GelMA alone. Commercially available PDMS was used as a benchmark reference.

References

1. Shirahama, H.; Lee, B.H.; Tan, L.P.; Cho, N.-J., Precise tuning of facile one-pot gelatin methacryloyl (GelMA) synthesis. *Sci. Rep.* **2016**, *6*, 31036.