Supporting Information

Enzymatic Formation of an Injectable Hydrogel from a Glycopeptide as a Biomimetic Scaffold for Vascularization

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Contents

1) Materials and methods

2-chlorotrityl chloride resin, Fmoc-Tyr(H_2PO_3)-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Asp-OtBu, and Fmoc-Phe-OH were obtained from Shanghai GL Biochem (Shanghai, China). 2-amino-D-glucose and proteinase K were provided by Sigma-Aldrich. 2-(naphthalen-6-yl) acetic acid was obtained from Aladdin (Shanghai, China). Alkaline phosphatase was purchased from Thermo Fisher Scientific. Desferrioxamine (DFO) was obtained from MedChem Express (Shanghai, China). 4% Paraformaldehyde, 0.1% Triton X-100, 1% BSA, fluorescein-phalloidin and hoechest were obtained from YEASEN (Shanghai, China). Staining dyes for live/dead cell assays and CCK8 were provided by Dojindo Laboratories (Shanghai, China). Anti-alpha smooth muscle Actin antibody was purchased from Abcam (China). All the other raw materials were purchased from Sigma and Enox Chemicals and used without further purification. ^{1}H and ^{31}P NMR spectra were recorded on a Unity Inova 400 by using DMSO-*d*6 as a solvent. CD spectroscopy study was carried out on a JASCO J-810 spectrometer. Liquid chromatography–mass spectrometry (LC–MS) analysis was performed on an Agilent 6120 Quadrupole LC/MS system with electrospray ionization (ESI) source. HPLC purification and analysis were conducted on a Waters 600E Multi-solvent Delivery System using CH_3CN (0.1% of TFA) and H_2O (0.1% of TFA) as eluents. Fluorescence microscopy images were obtained from an OlympusIX71 fluorescence microscope and a Leica TCS SP5 confocal fluorescence microscope.

2) Synthesis of Fmoc-Asp (Glc)-OH

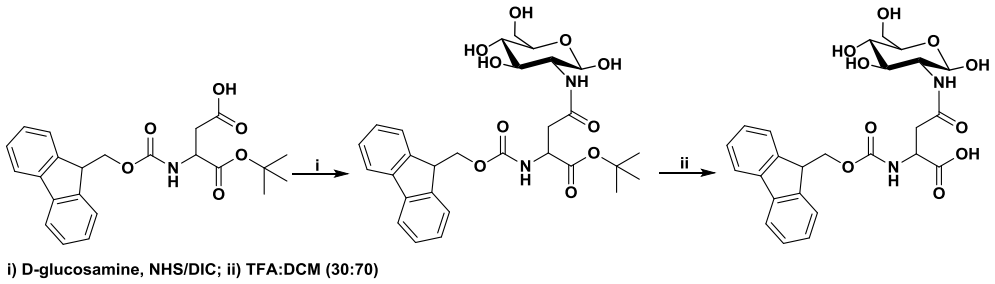

Figure S1. A synthetic route for the preparation of Fmoc-Asp (Glc)-OH.

Synthesis of Fmoc-Asn(Glc)-OH: Fmoc-Asp-OtBu (4.11 g, 10 mmol), NHS (1.51 g, 12 mmol) and DIC (1.38 g, 12 mmol) were dissolved in 80 mL DMF and stirred at room temperature for 8 h. D-Glucosamine (2.15 g, 12 mmol) dissolved in water was added， and the solution was stirred and react at room temperature for 24 hours. After removal of the solvent, the residue was re-dissolved in water and the pH was adjusted to 2-3 with HCl. The white precipitate was filtered off and purified by silica gel column chromatography to get Fmoc-Asn(Glc)-OtBu. The deprotection reaction was carried out in a mixed solution of TFA and DCM at a ratio of 1: 1 which afforded the final product of Fmoc-Asn(Glc)-OH (yield: 76%). ^{1}H NMR (400 MHz, DMSO-d_6): δ12.54 (s, 2H), 7.95-7.87 (d, 2H), 7.77-7.66 (m, 3H), 7.52-7.46 (d, 1H), 7.46-7.38 (t, 2H), 7.38-7.30 (t, 2H), 6.41-6.37 (d, 1H), 4.95-4.87 (m, 2H), 4.67-4.17 (m, 6H), 3.83-3.41 (m, 5H), 3.20-3.01 (m, 1H), 2.77-2.58 (m, 2H). MS: calcd M^+=516.17, obsd $(M + H)^+$ =517.18.

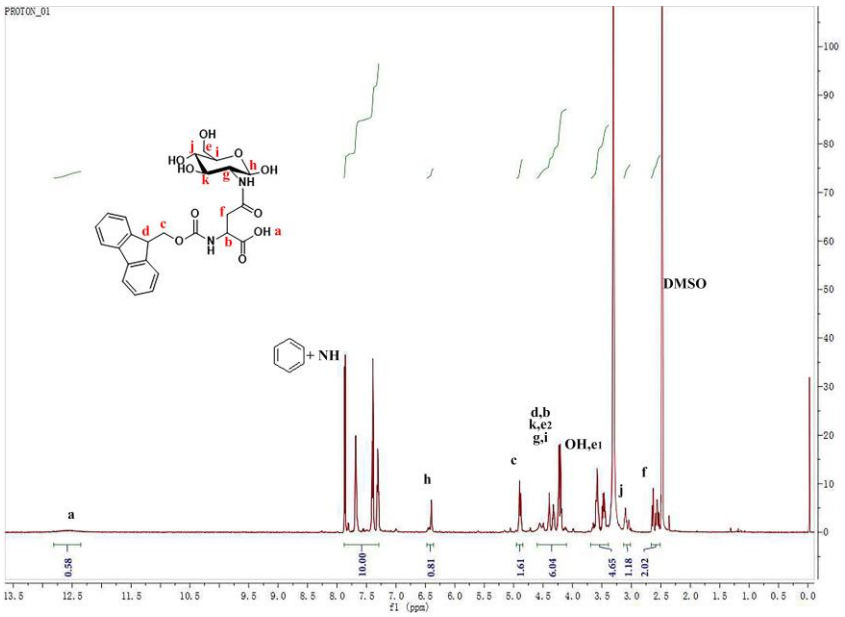

Figure S2. ^{1}H NMR of Fmoc-Asn(Glc)-OH in DMSO-*d6*.

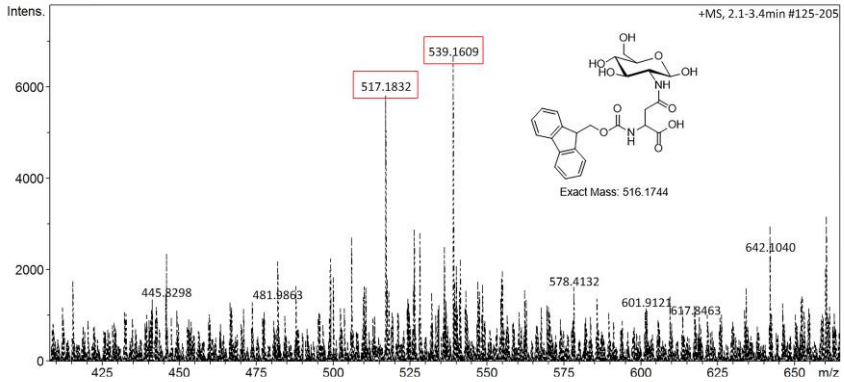

Figure S3. LC-MS spectrum of Fmoc-Asn(Glc)-OH.

3) NMR and LC-MS analysis of gelator precursor 1

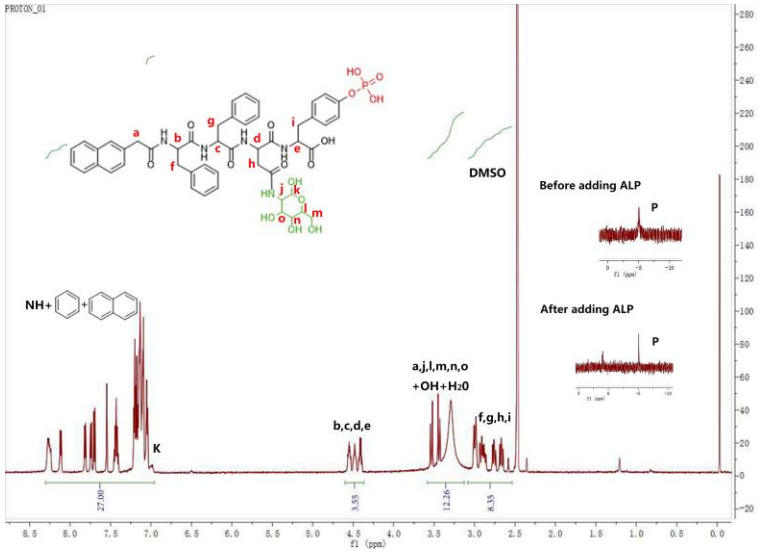

Figure S4. ^{1}H and ^{31}P NMR of gelator precursor **1** in DMSO-*d6*.

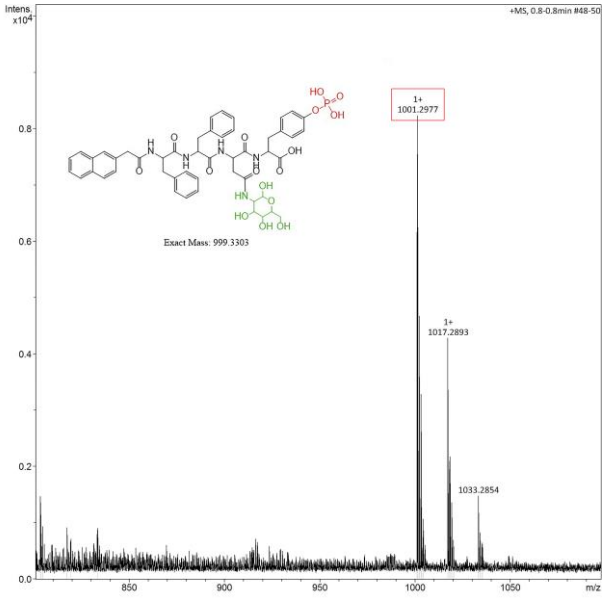

Figure S5. LC-MS spectrum of gelator precursor **1**.

4) Rheological measurements of Gp gel

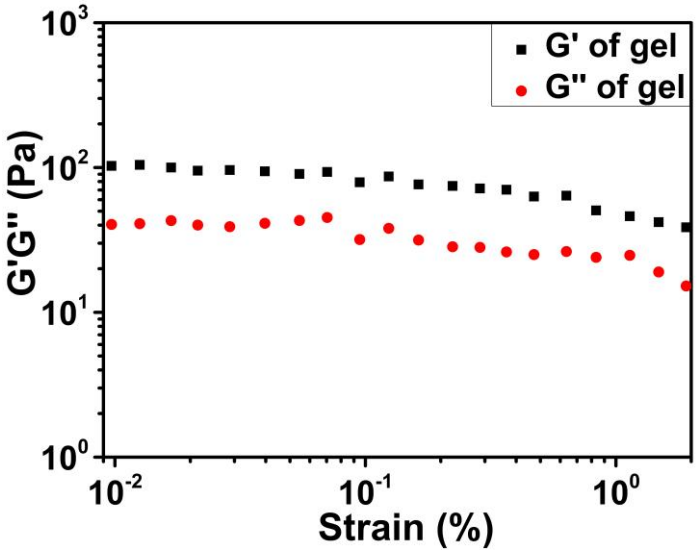

Figure S6. Strain dependence of the dynamic storage moduli (*G'*) and the loss moduli (*G''*) of Gp gel (0.6 wt %).

5) 24 h and 72 h cell viability tests of gelator precursor 1

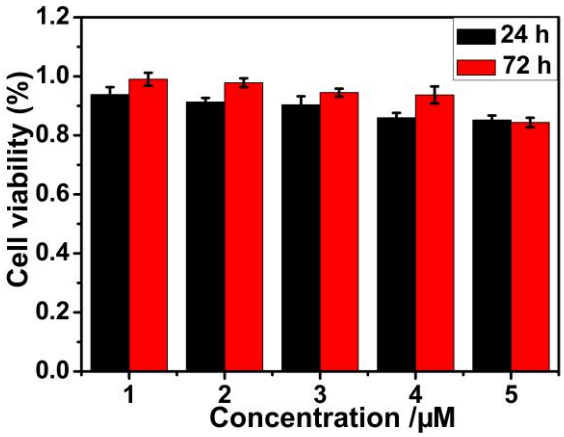

Figure S7. 24 h and 72 h cell viability tests of gelator precursor **1** on HUVECs.

6) Gel stability assays in vitro

In order to verify the stability of Gp gel in cell culture medium, 200 μL of DMEM was added to the surface of Gp gel (0.6 wt %). After incubation for 7 days, we observed that DMEM could permeate through the whole hydrogel, and the hydrogel was still stable.

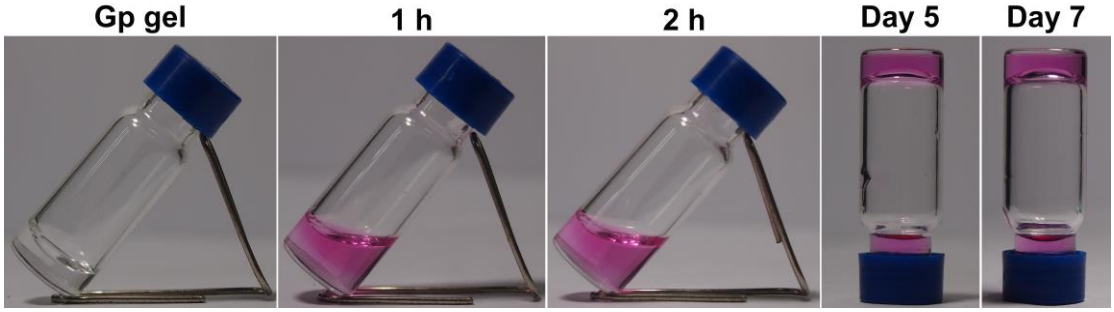

Figure S8. The stability of Gp gel (0.6 wt %) in cell culture media over the course of 7 days.

7) Cell adhesion and proliferation on tissue culture plates

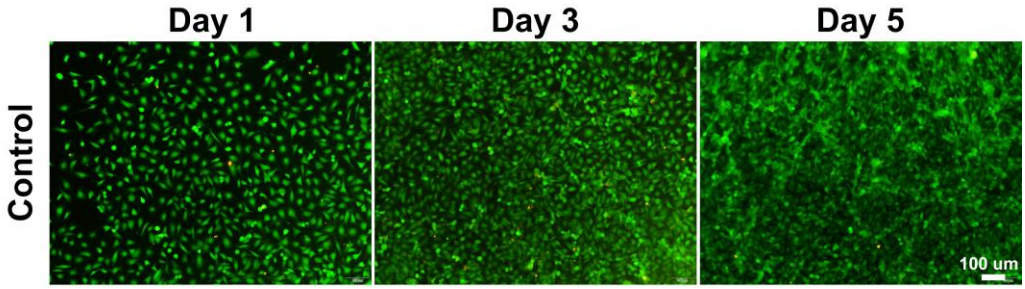

Figure S9. Fluorescence images of live/dead assays of HUVECs cultured on 48-well plate over the course of 5 days.

8) Sustained release of DFO from Gp gel

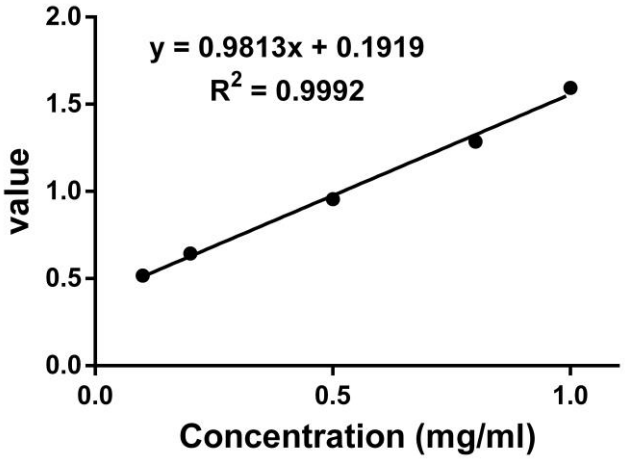

Figure S10. Calibration curve between the optical absorptions of DFO at 485 nm and DFO concentrations in solutions.

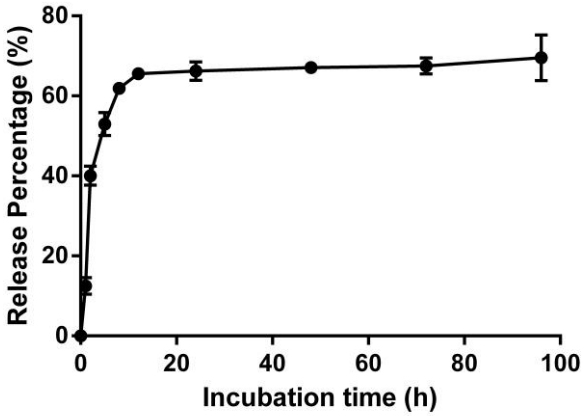

Figure S11. Cumulative release of DFO from Gp gel in PBS buffer (10 mM, pH=7.4, and 0.1% BSA) at room temperature.

9) Rheological measurements of Gp gel encapsulating DFO

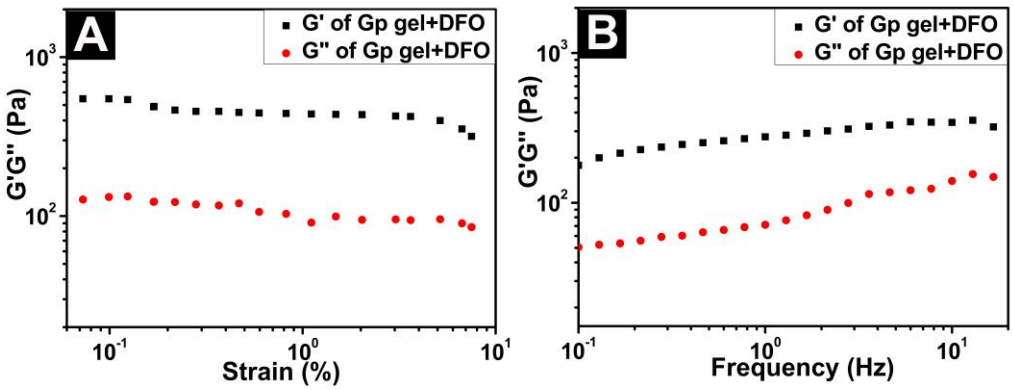

Figure S12. (A) Strain and (B) frequency dependence of the dynamic storage moduli (G') and the loss moduli (G'') of Gp gel (0.6 wt %) encapsulating DFO (1 mg/mL).

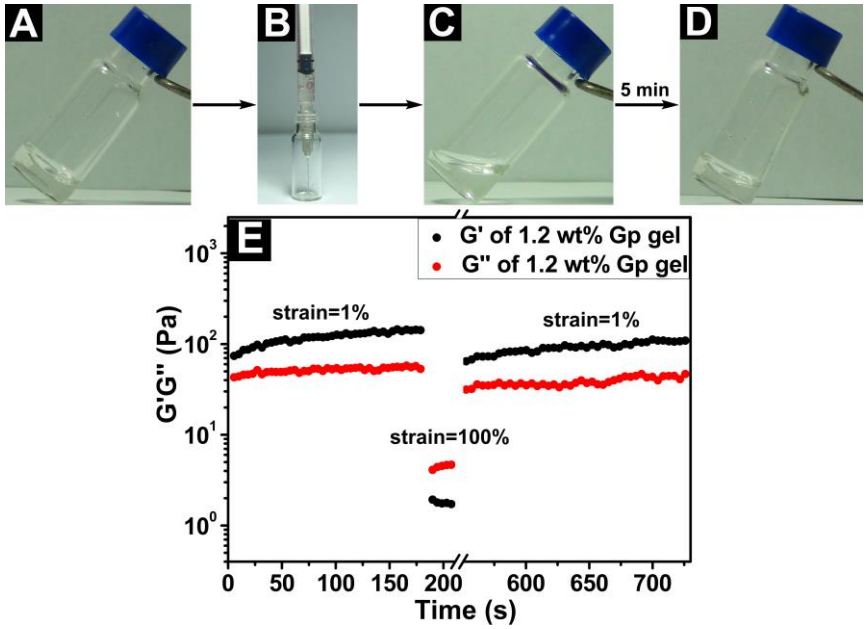

Figure S13. Optical images of (A) Gp gel (1.2 wt %), (B) the process of extruding Gp gel through a needle with a diameter of 0.4 mm, (C) the state of sample collected in the process shown in Figure S7B, (D) the recovered Gp gel from the sample shown in Figure S7C, (E) time-dependent of repetitive cycles of the step-strain analysis of Gp gel (1.2 wt %).

10) Gel biostability tests in vivo.

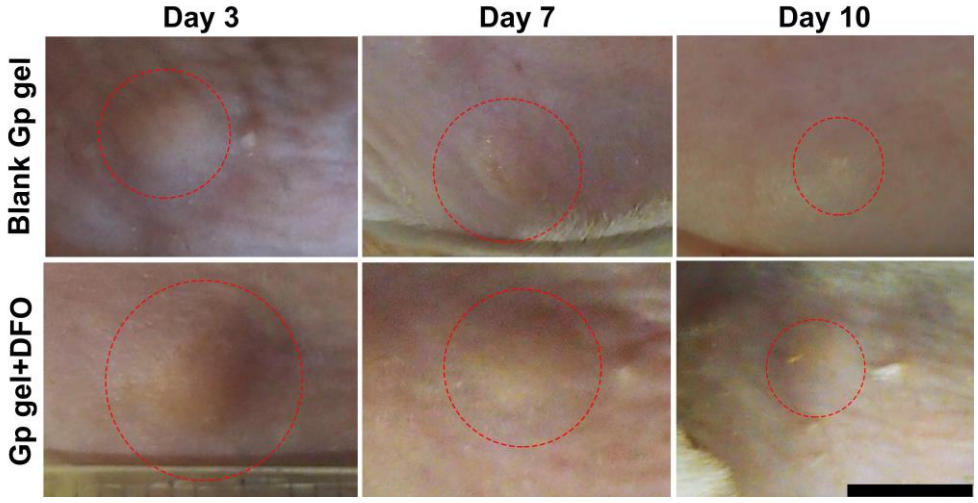

Figure S14. Gross images of the implanted Gp gel with/without loading DFO at day 3， day 7 and day 10 after subcutaneous injection on the dorsal side of mice. Scale bar=5 mm.

11) Histological images of Gp gel constructs over the course of 10-day culture

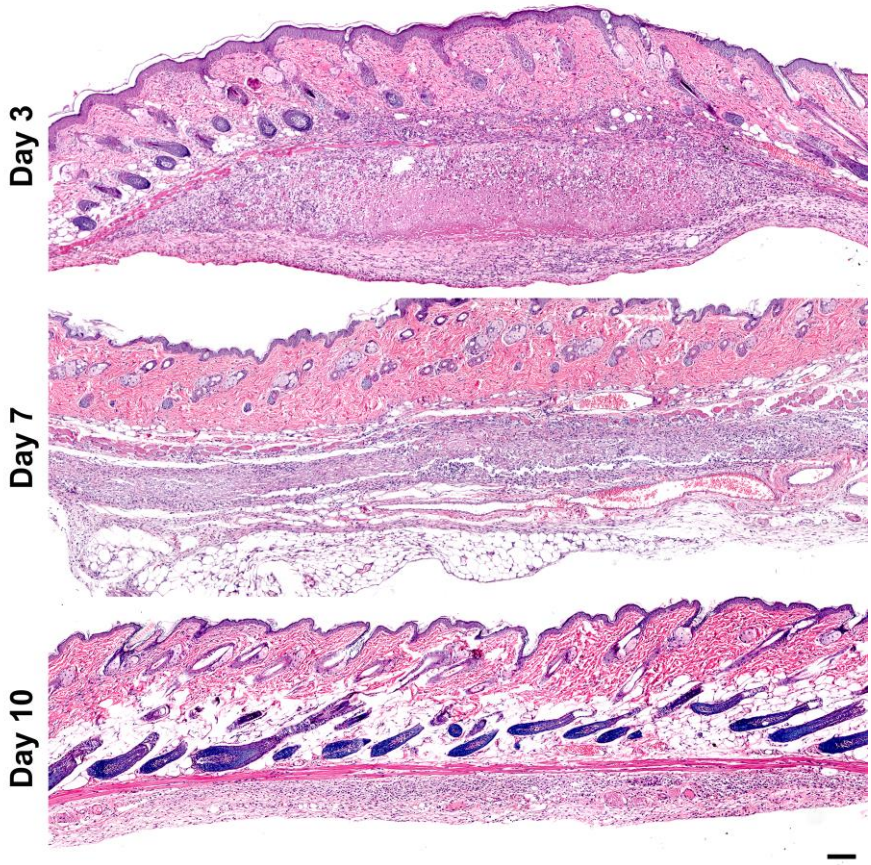

Figure S15. H&E staining images of Gp gel constructs over the course of 10-day implantation. Scale bar=100 μm.

12) Quantitative analysis of blood vessel areas in connective tissues via CD31 staining

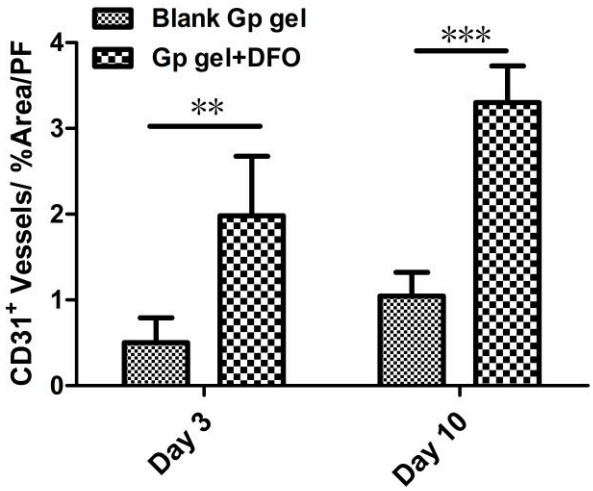

Figure S16. The average blood vessel areas in connective tissues after subcutaneous injection of blank Gp gel and Gp gel loaded with DFO within the course of 10-day implantation. The error bars indicates the SD.