Supporting Information

Injectable Thioketal-Containing Hydrogel Dressing Accelerates Skin Would Healing with Incorporation of Reactive Oxygen Species Scavenging and Growth Factor Releasing

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1. Materials and methods

Materials

Cysteamine, triethylamine, ethyl trifluoroacetate, 5-Norbornene-2-carboxylic acid, N,N'-dicyclohexylcarbodiimide (DCC), N,N-diisopropylethylamine (DIPEA), benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP), *p*-toluenesulfonic acid monohydrate (PTSA) and 1'-diphenyl-2-picrylhydrazyl (DPPH) were purchased from TCI organic Chemicals. Tetrazine-amine HCl was purchased from Chengdu Biocone Biological Technology Co., Ltd. 4-arm poly(ethylene glycol) amine (4-arm PEG-NH_2, 20 kDa) and 4-arm poly(ethylene glycol) carboxylic acid (4-arm PEG-CM, 20 kDa) were purchased by Xiamen Sinopeg Biotech Co., Ltd. BSA-FITC was purchased from Beijing Solarbio Science & Technology Co., Ltd. 2,2-dimethoxypropane, *p*-toluenesulfonic acid monohydrate (PTSA), pentachlorophenol (PFP), cobaltous chloride ($CoCl_2$) and terephthalic acid (TA) were supplied by Adamas. Epidermal growth factor (EGF) was purchased from PeproTech (China). RPMI 1640 medium, fetal bovine serum (FBS), trypsin-EDTA solution and penicillin-streptomycin solution (100 x) were purchased from Gibco, ThermoFisher Scientific. Hoechst 33342 was bought from Sigma-Aldrich (Shanghai, China). WST-1 cell proliferation and cytotoxicity assay kit, Calcein AM, Propidium Iodide (PI), 2',7'-Dichlorofluorescin diacetate (DCFH-DA) and dihydroethidium (DHE) and were purchased from Beyotime Biotechnology Co., Ltd. All other chemical reagents were commercially available and used as received.

Synthesis of acetone-[bis-(2-amino-ethyl)-dithioacetal]

Acetone-[bis-(2-amino-ethyl)-dithioacetal] was synthesized using a reported procedure with slight modifications.[1] As the synthesis route shown in Figure S1, compound 1 was prepared as follows. Cysteamine (7.7 g, 100.0 mmol, 1 eq), triethylamine (15.2 g,150.0 mmol, 1.5 eq) and ethyl trifluoroacetate (17.0 g, 120.0 mmol, 1.2 eq) were dissolved in 100 mL methanol, and the mixture was stirred at room temperature overnight. After this, DI water was added to stop the reaction. The product was extracted with ethyl acetate for 3 times and dried over anhydrous sodium sulfate. The concentrated product was further purified by silica gel column chromatography with hexane/ethyl acetate (V/V = 3:1). The chemical structure of compound 1 was confirmed by the ^{1}H NMR spectrum (Figure S2).

After that, compound 2 was synthesized Compound 1 (5.19 g, 30 .0 mmol, 3 eq), 2,2-dimethoxypropane (1.04 g, 10.0 mmol, 1eq) and PTSA (0.06 g, 0.3 mmol, 0.03 eq) were dissolved in 30 mL of dichloromethane (DCM). The mixture was refluxed for 4 h at 40 °C. After that, the concentrated solution was precipitated three times in hexane. The resulting white crude product was dissolved in DCM and purified by silica gel column chromatography with hexane/ethyl acetate (V/V = 1:1). The chemical structure of compound 2 was verified by the ^{1}H NMR spectrum (Figure S3).

Finally, acetone-[bis-(2-amino-ethyl)-dithioacetal] (compound 3) was synthesized through deprotecting the trifluoroacetate groups of compound 2. To be specific, compound 2 (0.95 g, 2.5 mmol) was dissolved in 7.5 mL of aqueous 6 M NaOH. After 4 h of stirring, the crude product was extracted with DCM for 3 times. The obtained

final product was an amber-colored oil. The chemical structure of acetone-[bis-(2-amino-ethyl)-dithioacetal] was confirmed by the ^{1}H NMR spectrum (Figure S4).

Synthesis of Nb-PFP

5-Norbornene-2-carboxylic acid (1.382 g, 10 mmol, 1 eq), PFP (2.208 g, 10 mmol, 1 eq) and DCC (2.06 g, 10 mmol, 1 eq) were dissolved in 50 mL dry 1,4-dioxane, and the mixture was vigorously stirred under a nitrogen atmosphere at room temperature overnight. After the reaction ended, the residue was removed by filtration and the filtrate was concentrated. Finally, the final oily product was obtained by column purification using a silica column with hexane/ethyl acetate (V/V = 5 :1). The chemical structure of Nb-PFP was verified by the ^{1}H NMR spectrum (Figure S5).

2. Results

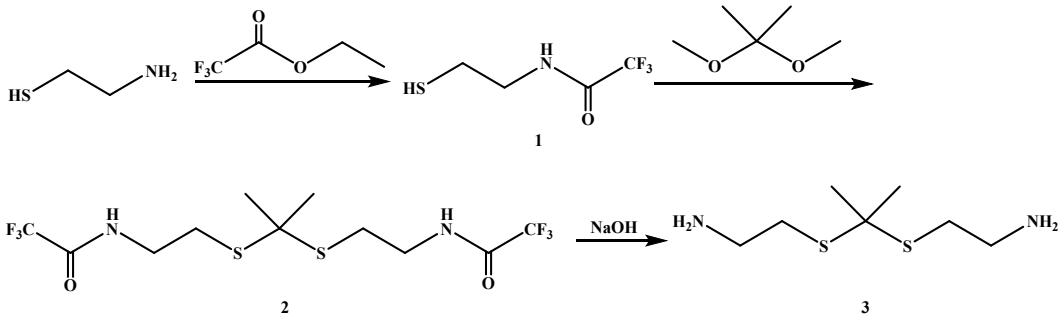

Figure S1. Synthetic route of acetone-[bis-(2-amino-ethyl)-dithioacetal].

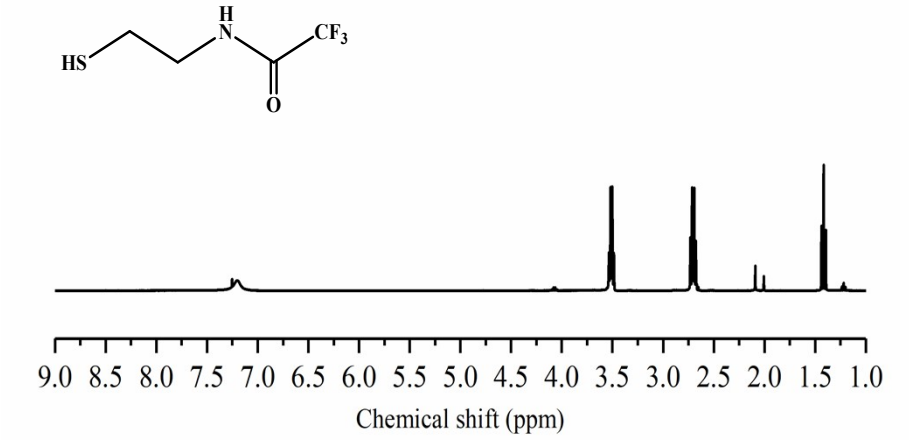

Figure S2. The structure and ^{1}H NMR spectrum of compound 1 (recorded in $CDCl_3$). δ 3.518 (q, 2H, $CF_3CONHC\underline{H_2}CH_2SH$), δ 2.698 (q, 2H, $CF_3CONHCH_2C\underline{H_2}SH$), δ 1.416 (t, 1H, $CF_3CONHCH_2CH_2S\underline{H}$).

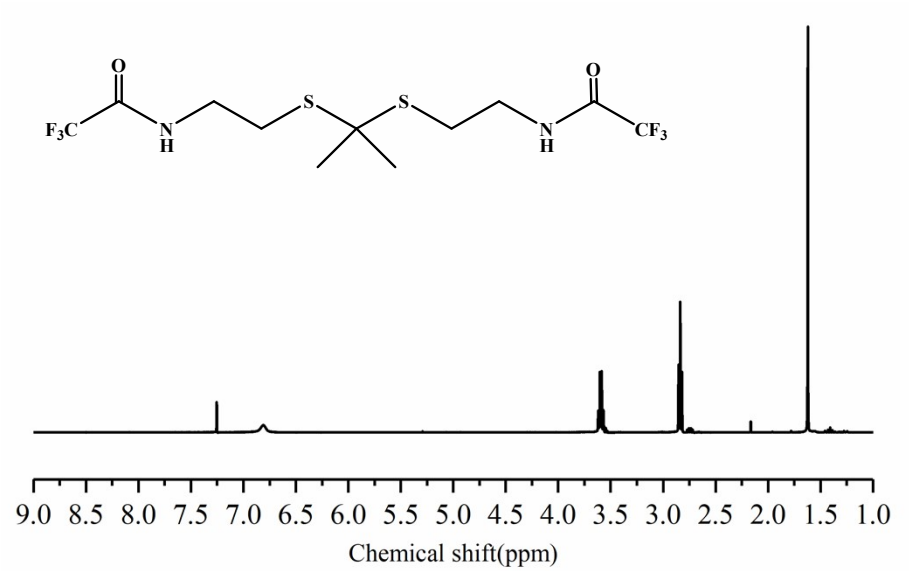

Figure S3. The structure and ^{1}H NMR spectrum of compound 2 (recorded in $CDCl_3$). δ 6.808(s, 2H, $CF_3CON\underline{H}CH_2CH_2SC(CH_3)_2SCH_2CH_2N\underline{H}COCF_3$), δ 3.830 (q, 4H, $CF_3CONHC\underline{H_2}CH_2SC(CH_3)_2SCH_2C\underline{H_2}NHCOCF_3$), δ 3.580 (q, 4H, $CF_3CONHCH_2C\underline{H_2}SC(CH_3)_2SC\underline{H_2}CH_2NHCOCF_3$), δ 1.621 (s, 6H, $CF_3CONHCH_2CH_2SC(C\underline{H_3})_2SCH_2CH_2NHCOCF_3$).

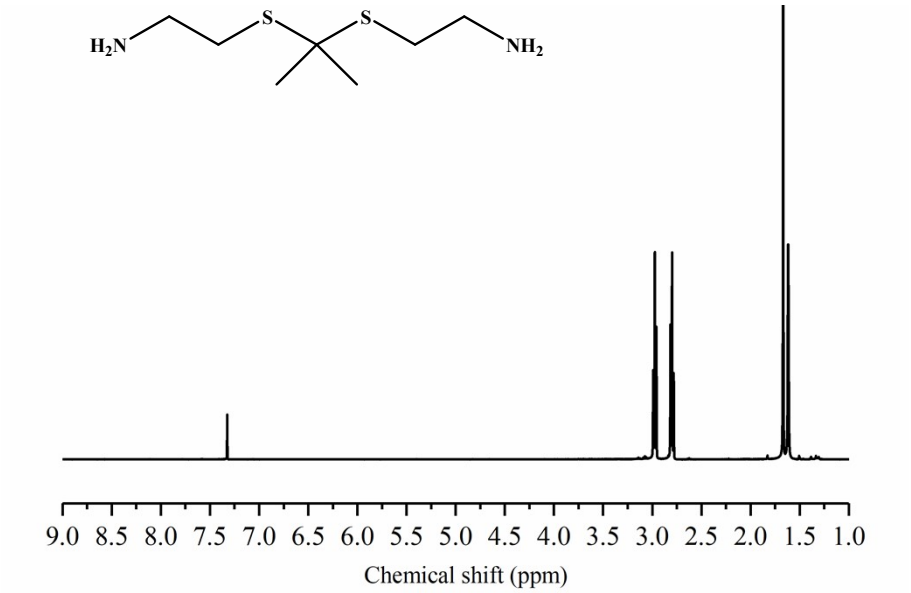

Figure S4. The structure and ^{1}H NMR spectrum of acetone-[bis-(2-amino-ethyl)-dithioacetal] (compound 3) (recorded in $CDCl_3$). δ 2.929 (t, 4H, $NH_2C\underline{H_2}CH_2SC(CH_3)_2SCH_2C\underline{H_2}NH_2$), δ 2.737 (t, 4H, $NH_2CH_2CH_2SC(CH_3)_2SC\underline{H_2}CH_2NH_2$), δ 1.624(s, 6H, CH_3), δ 1.572 (s, 4H, $N\underline{H_2}CH_2CH_2SC(CH_3)_2SCH_2CH_2N\underline{H_2}$).

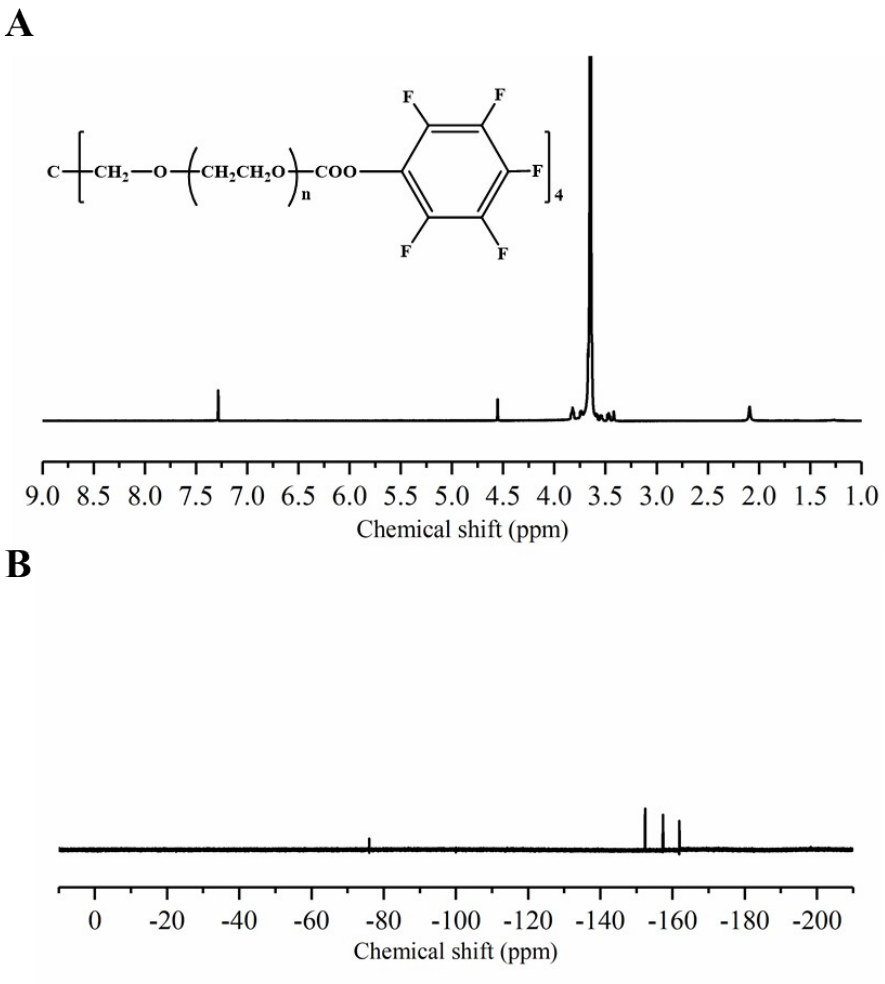

Figure S5. (A) The structure and ^{1}H NMR, and (B) ^{19}F NMR spectrum of 4-arm-PEG-PFP (recorded in $CDCl_3$). After PFP was modified on the terminal of 4-arm-PEG, the chemical shift of hydrogen atoms adjacent to PFP increased because of the electron absorption effect of PFP. Meanwhile, the ^{19}F NMR spectrum also demonstrated the successful synthesis of 4-arm-PEG-PFP.

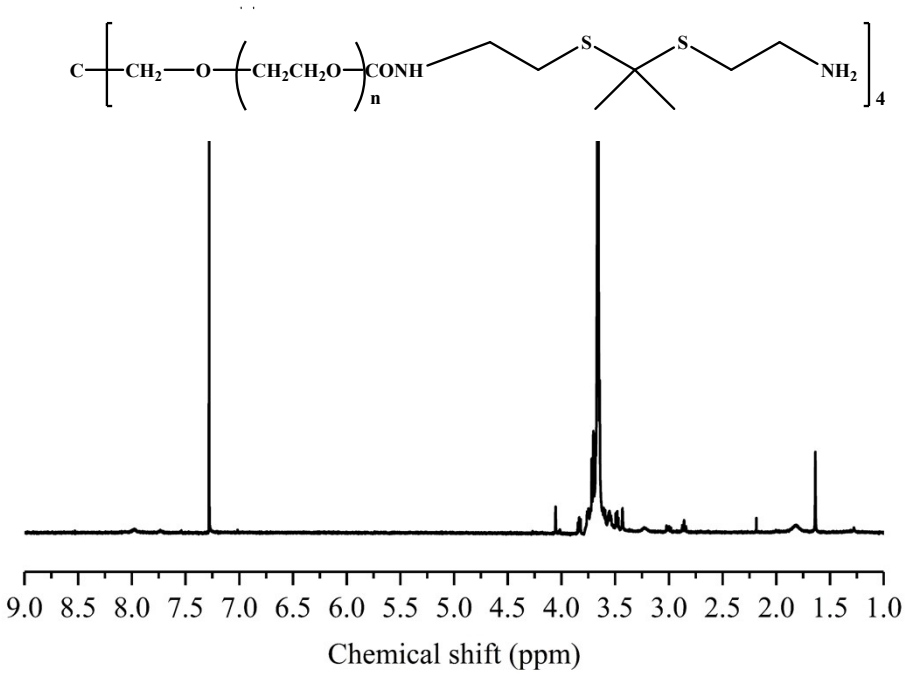

Figure S6. The structure and ^{1}H NMR spectrum of 4-arm-PEG-TK (recorded in $CDCl_3$).

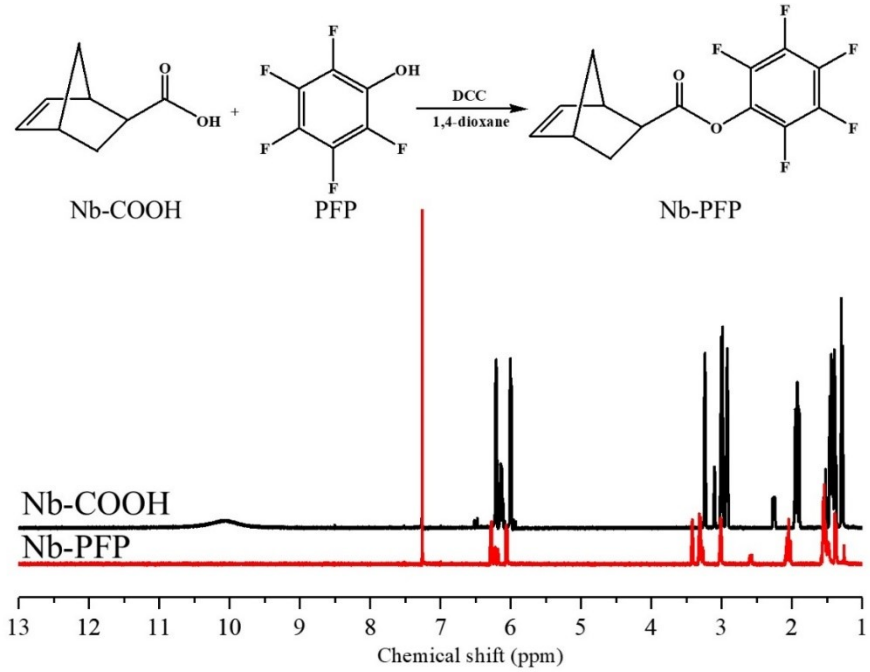

Figure S7. Synthetic route of Nb-PF and ^{1}H NMR spectra of Nb-COOH (black) and Nb-PFP (red) (recorded in $CDCl_3$). After the carboxyl of Nb was activated by PFP, the characterized peak of carboxyl at δ10 ppm disappeared. Meanwhile, the chemical shifts of all hydrogen atoms of Nb also increased due to the electron absorption effect of PFP.

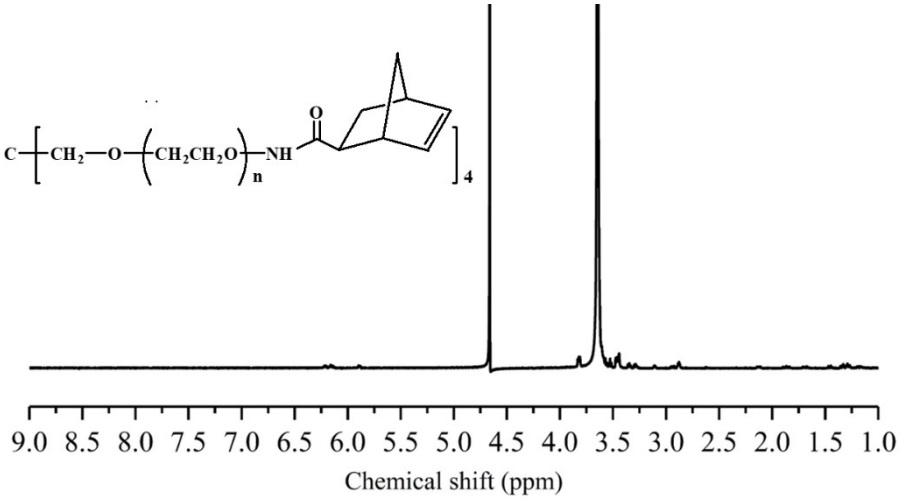

Figure S8. The structure and ^{1}H NMR spectrum of 4-arm-PEG-Nb (recorded in D_2O).

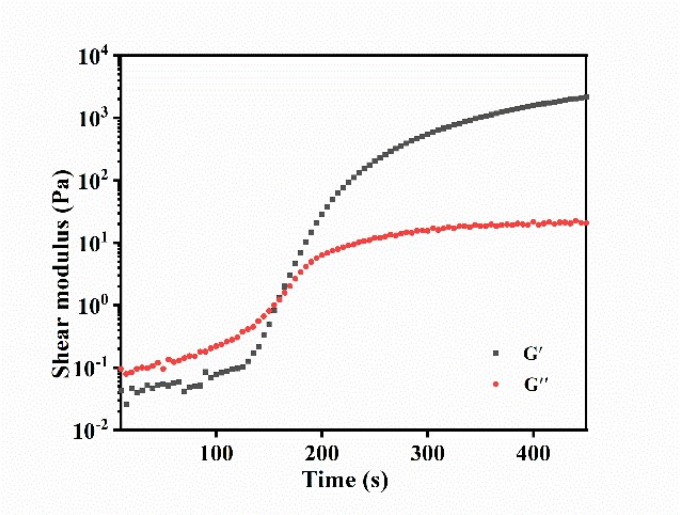

Figure S9. Rheological properties of the gelation process of the PEG hydrogel.

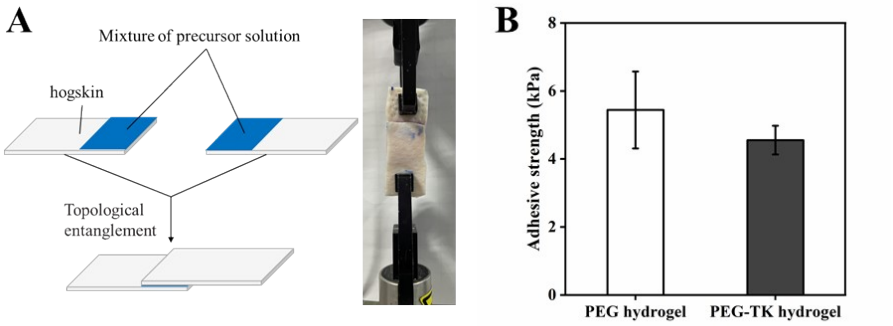

Figure S10. (A) Schematic presentation of the lap shear test. (B) Adhesive strength of different hydrogels on porcine skin.

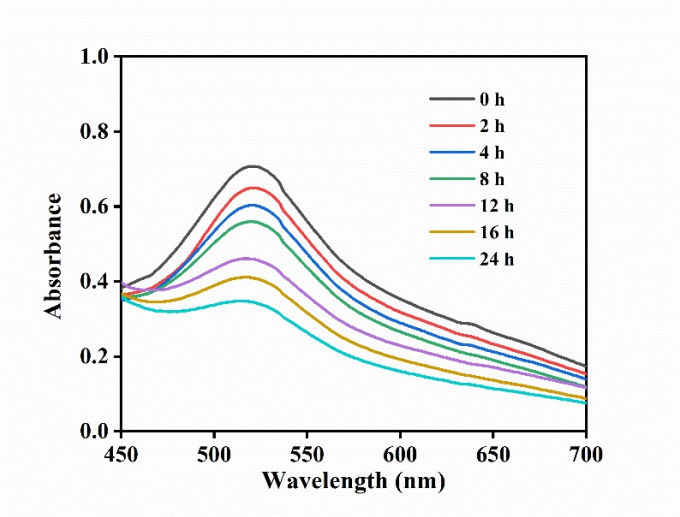

Figure S11. UV-vis spectra of DPPH/4-arm-PEG-TK-Nb solution within 24 h.

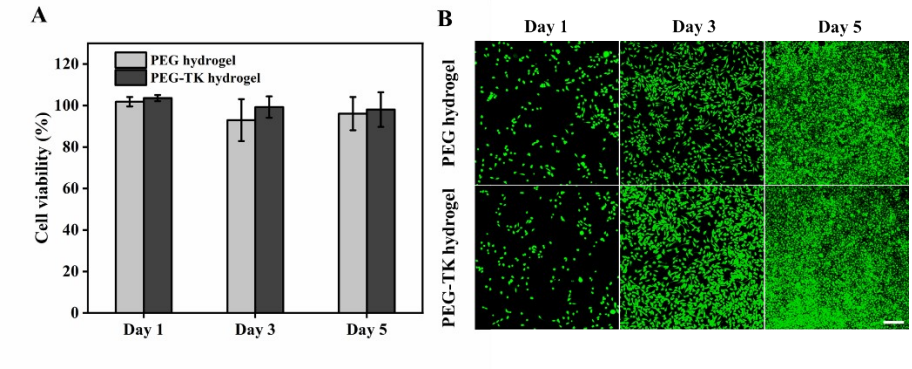

Figure S12. (A) The cell viabilities of L929 cells seeded in PEG-TK hydrogel or PEG hydrogel. (B) Live/dead staining of L929 cells on day 1, day 3 and day 5 for PEG-TK and PEG hydrogel, respectively. Scale bar = 200 μm.

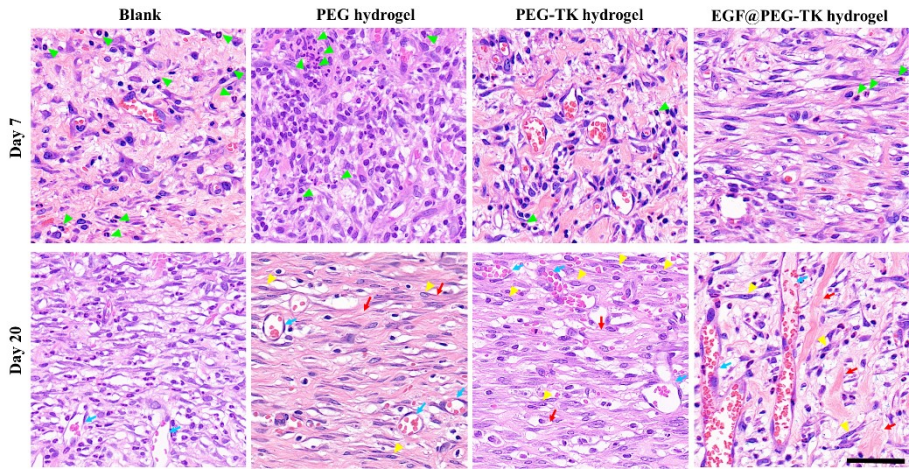

Figure S13. Magnified H&E staining histologic sections of the treated wounds on day 7 and day 20. The blue arrow indicated vascular, the red arrow indicated thin strand-like collagen bundles, the green arrowhead indicated neutrophil and the yellow arrowhead indicated fibroblast. Scale bar = 50 μm.

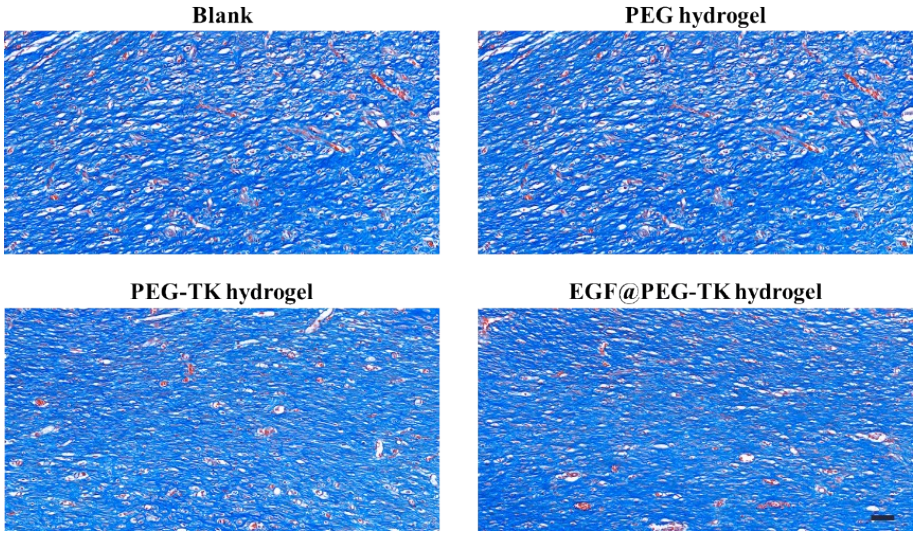

Figure S14. Representative Masson staining images of wound sections on day 20 post-surgery. Scale bar = 50 μm.

REFERENCE

1. M. S. Shim and Y. N. Xia, *Angew. Chem., Int. Ed.*, 2013, **52**, 6926-6929.