Supporting Information

Construction of a nanofiber network within 3D printed scaffolds for vascularized bone regeneration

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Supplementary experimental section

1. Synthesis of PGSLP

PGSLP was synthesized by using our previous method [1]. The specific experimental steps are as follows: Sebacic acid, LLA and PEG were added into a 250 mL single-necked round bottom flask, and the system was heated and stirred in an oil bath at 120°C under nitrogen protection for 2 h under the reaction pressure of 3000 Pa until the sebacic acid is completely dissolved and mixed uniformly, and then catalyst stannous octoate ($Sn(Oct)_2$) was added into the reaction system before the reaction temperature was increased slowly to 180°C for over 8 h. The reaction was continued for 8 h at the same pressure and temperature. And the pressure was reduced to 100 Pa to continue for 6 h to obtain the linear segment. 0.1 mol glycerol was added to the reaction system, stirred for 2 h under the protection of atmospheric and nitrogen protection keep the temperature constant, slowly reduced the reaction pressure to 3000 Pa and react for 6 h, then pressure again reduce to 100 Pa and continue to react for 2 h to obtain PGSLP.

2. DFO concentration screening

2.1. Tube formation assay

HUVECs on matrigel were stimulated with different concentrations of DFO to promote the formation of tubular structures. Briefly, the matrigel was coated onto 48-well plates at a volume of 100 μL per well, and gelation was formed at 37 °C for 30 minutes. HUVECs were dispersed by the serum-free media containing different concentrations of DFO (0, 5, 10, 25 and 50 μM) and cultured onto matrigel at 2×10^4

cells per well. The results of the tubular structure formation were photographed using a microscope (Olympus, Tokyo, Japan) after cultured for 6 hours, and the total length of tubules, number of junctions, number of meshes and total meshes area per high power field were calculated by ImageJ software.

2.2 Wound-healing assay

An in vitro wound-healing assay was performed to measure HUVECs migration [2]. HUVECs were seeded at 5×10^4 cells/well in 24-well plates and cultured in a cell incubator for 24 hours using complete medium. After the cells were attached to the plate, scratched a straight in the middle of every wells and washed off the scratched cells with PBS solution (pH = 7.4). Every well was photographed using a microscope, and the medium was replaced with various media containing different concentrations of DFO (0, 5, 10, 25 and 50 μM). The results of the wound-healing were photographed using a microscope (Olympus, Tokyo, Japan) after cultured for 24 and 48 h.

Supplementary figures

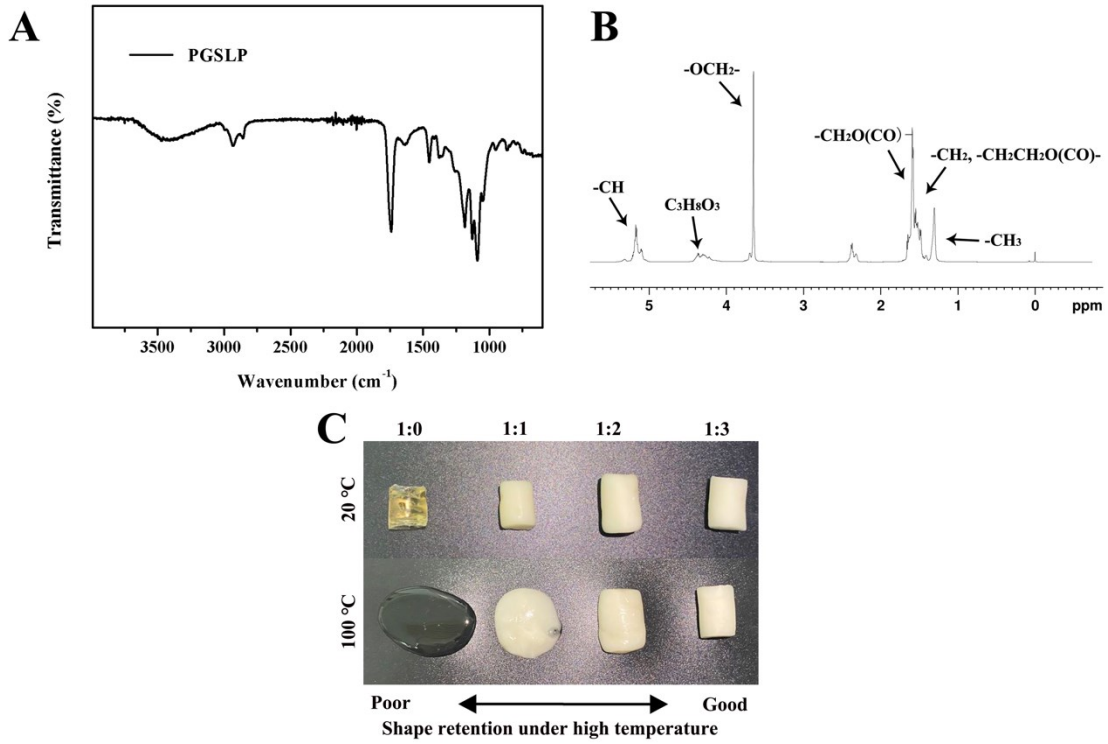

Figure S1. (A) FTIR spectrum of PGSLP prepolymer. (B) ^{1}H NMR spectrum of PGSLP prepolymer. (C) Shape retention of the uncrosslinked precursor constructs at high temperature and vacuum.

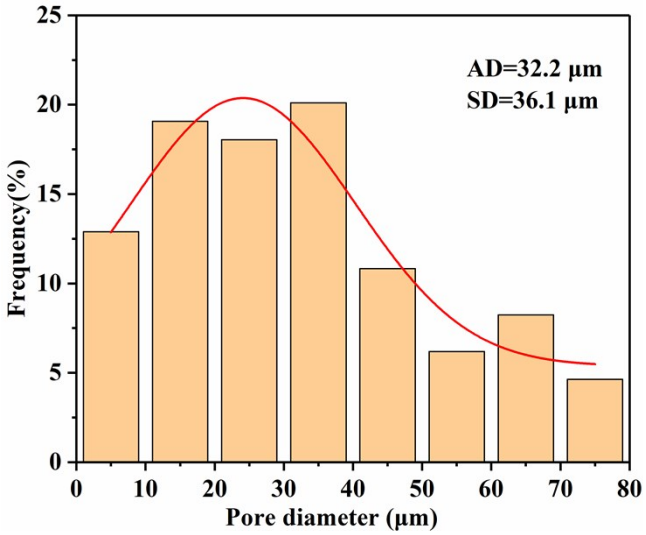

Figure S2. The pore diameter distribution of 3D printed PGSLP scaffold.

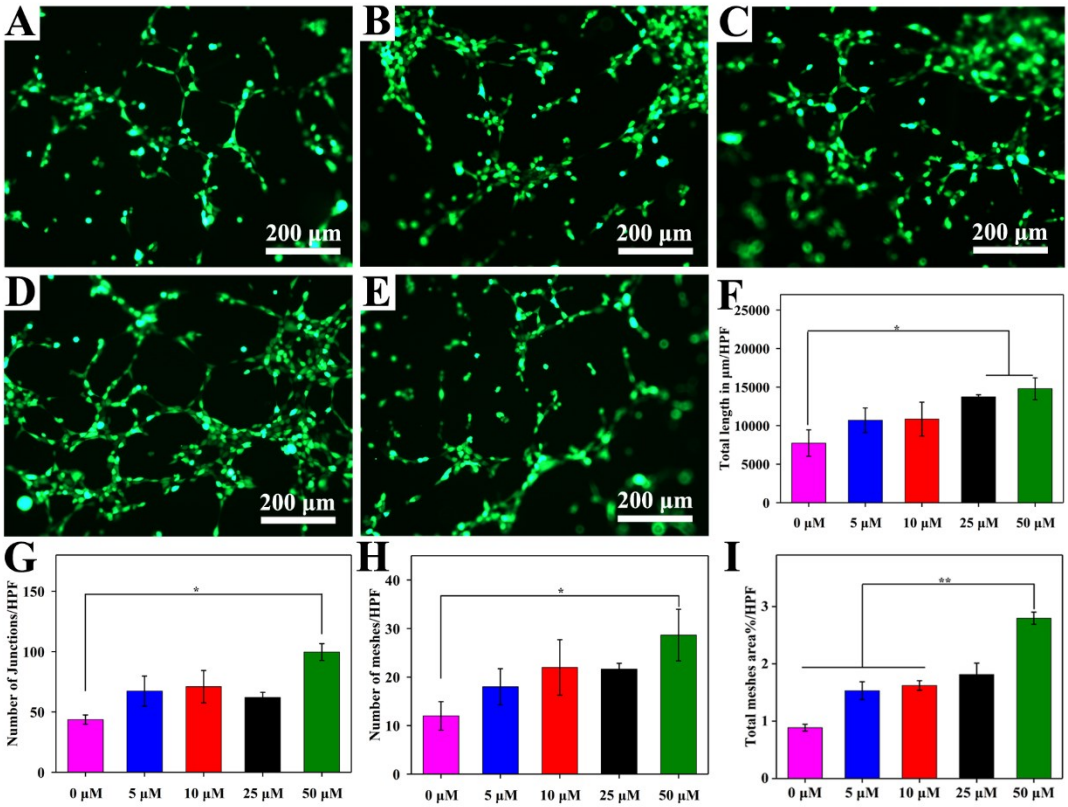

Figure S3. Fluorescent images of tubule networks after HUVECs cultured with different concentrations (A-E) of DFO on matrigel for 6 h,(A) 0 μM, (B) 5 μM, (C) 10 μM, (D) 25 μM, (E) 50 μM. (F-I) Summarized data showing the difference of (F) total length, (G) number of junctions, (H) number of meshes, (I) total meshes area per high power field (HPF) in HUVECs. *P < 0.05, **P < 0.01.

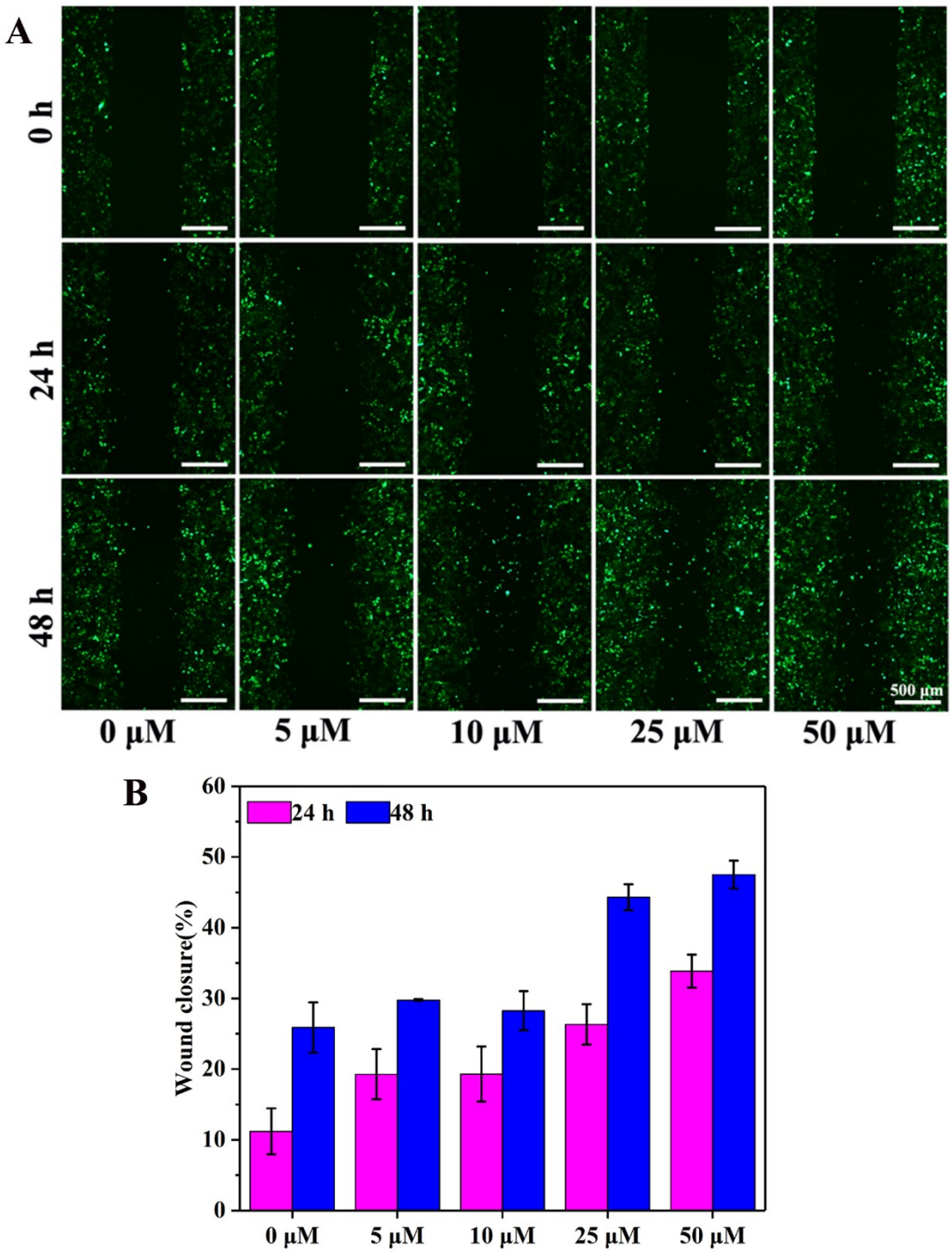

Figure S4. (A) Fluorescent images of the migration effects of HUVECs in wound-healing assay. (B) After inducing human wound and culture for 24 and 48 h, the migration rates of HUVECs in different concentrations of DFO. Wound closure was quantified by using ImageJ software analysis.

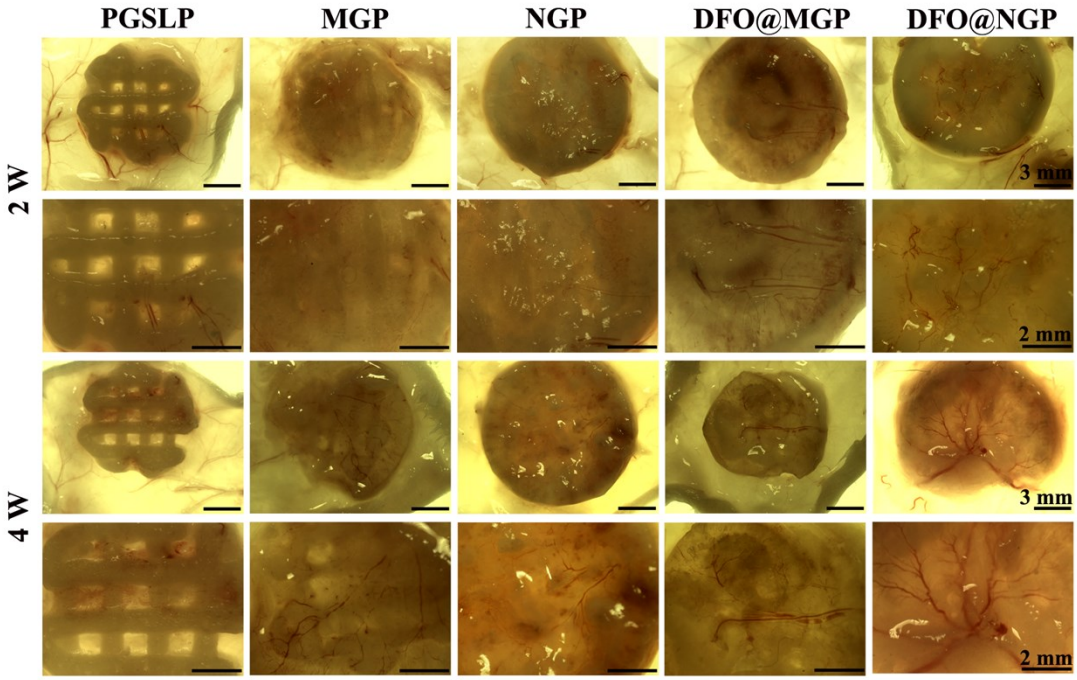

Figure S5. Images of various scaffolds after subcutaneous implantation in mice for 2 and 4 weeks.

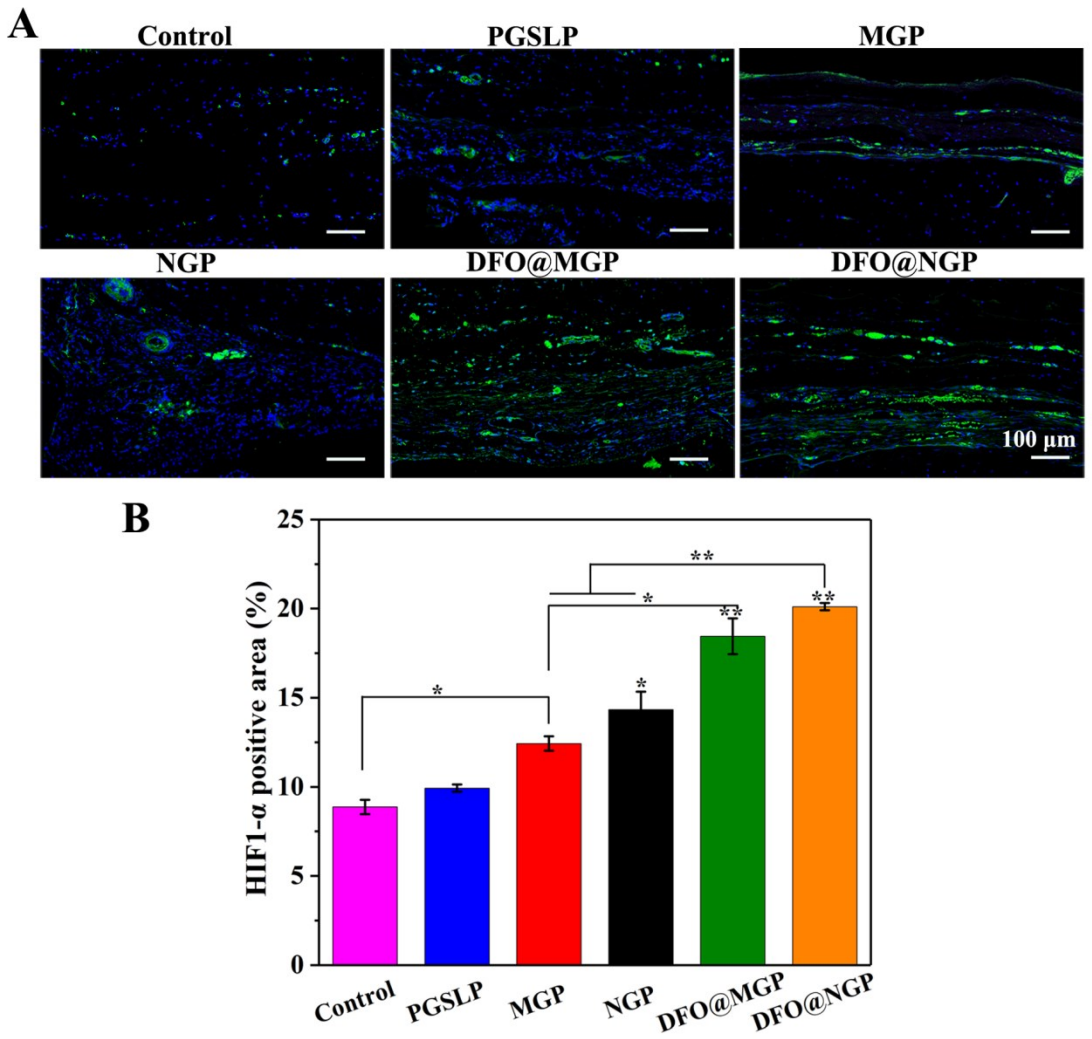

Figure S6. Immunofluorescence staining of HIF1-α (green) expression around in situ bone repair site in SD rats after 12 weeks. Cell nuclei were counterstained with DAPI (blue). *P < 0.05, **P < 0.01.

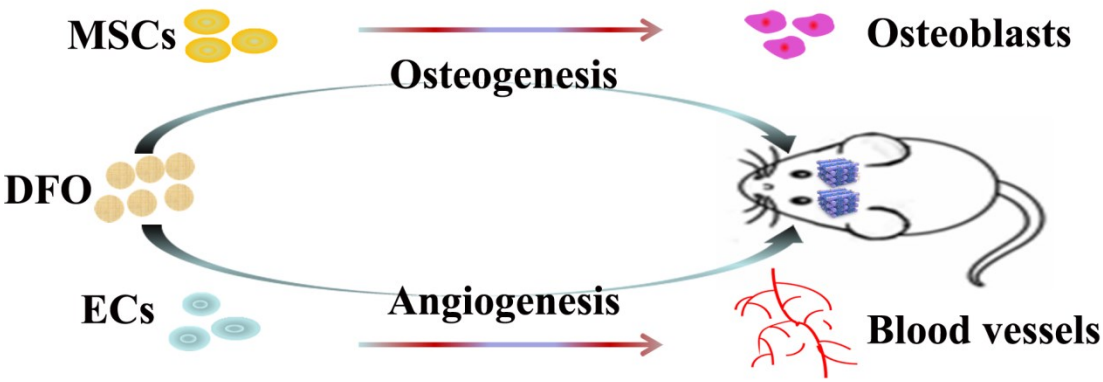

Figure S7. Schematic diagram of the DFO@NGP to promote osteogenesis and angiogenesis.

Table S1 Primers for RT-PCR analysis.

Gene	Primer sequences
OCN	Forward 5'- CAACCCCAATTGTGACGAGC -3'
	Reverse 5'- GGCAACACATGCCCTAAACG -3'
Runx-2	Forward 5'-CAGTATGAGAGTAGGTGTCCCGC-3'
	Reverse 5'-AAGAGGGGTAAGACTGGTCATAGG-3'
OPN	Forward 5'- GATGAACAGTATCCCGATGCCA -3'
	Reverse 5'- GTCTTCCCGTTGCTGTCCTGA -3'
Col1a	Forward 5'- GACGCATGGCCAAGAAGACAT -3'
	Reverse 5'- TCTTTGCATAGCACGCCATCG -3'
GAPDH	Forward 5'- CTGGAGAAACCTGCCAAGTATG -3'
	Reverse 5'- GGTGGAAGAATGGGAGTTGCT -3'

References

1. Y. T. Jia, W. Z. Wang, X. J. Zhou, W. Nie, L. Chen and C. L. He, *Polym Chem-Uk*, 2016, **7**, 2553-2564.
2. A. D. van der Meer, K. Vermeul, A. A. Poot, J. Feijen and I. Vermes, *Am J Physiol-Heart C*, 2010, **298**, H719-H725.