Supporting Information

High mobility group box 1-immobilized nanofibrous scaffold enhances vascularization, osteogenesis and stem cells recruitment

Yonggang Lv*[ab] and Chongwen Lin[ab]

[a] Key Laboratory of Biorheological Science and Technology (Chongqing University), Ministry of Education, Bioengineering College, Chongqing University, Chongqing 400044, P. R. China. *E-mail: yglv@cqu.edu.cn
[b] Mechanobiology and Regenerative Medicine Laboratory, Bioengineering College, Chongqing University, Chongqing 400044, P. R. China.

Characterization of the morphology of PLLA and PLLA/PCL electrospun nanofibers

After the nanofibrous scaffolds were covered by a sputtered gold coating, their morphology was analyzed by field-emission scanning electron microscope (FESEM) (Zeiss Auriga crossbeam system, Germany) with an accelerating voltage of 10 kV. The diameter of the fibers was measured based on these images by using image analysis software (Image J, National Institutes of Health, USA). The average diameter of the fibers was determined by performing measurements on 100-150 randomly selected fibers.

Uniaxial mechanical testing of PLLA and PLLA/PCL electrospun nanofibers

The scaffolds were cut into rectangles with dimensions of 60 mm × 20 mm (length × width) along the fiber arrangement, and the approximate thickness of each sample was 200 μm. The mechanical properties of different scaffolds were analyzed with a tabletop uniaxial testing instrument (Instron 5567, USA). Ten millimeters of the long sides of the samples were fixed on mechanical loading grips. A 50 N load cell under a crosshead speed of 10 mm/min was applied under ambient condition. From the stress-strain curve, Young's modulus, tensile strength, and elongation at break were obtained.

MSCs isolation and culture

After anesthesia, SD rats (body weight 150-180 g) (Animal Center, Daping Hospital, Third Military Medical University, Chongqing, China) were used. Animal care was provided in accordance with the Guiding Principles of the Institute of Care and Use of Animals in Chongqing University. The Animal Care and Use Committee of our institute approved all procedures. The rats were sacrificed by cervical dislocation in a sterile environment. Their femora and tibiae were carefully removed, and the bone marrow was flushed out using the Dulbecco Modified Eagle's Medium (DMEM) (Gibco Life Technology, USA) containing 10% fetal bovine serum (FBS) (Gibco Life Technology, USA). Cell suspension was percussed repeatedly and 3 mL suspension was seeded in 25 cm^2 cell culture flask at 3×10^6 cells per flask. MSCs adherent culture was developed in 3 mL DMEM containing 10% FBS with antibiotics (100 units/mL penicillin G and 100 mg/mL streptomycin). After seeding for 72 h, free-floating cells were removed, and the medium was replaced with fresh medium. Thereafter, the medium was replaced every other day. When cell confluence reached nearly 80%, adherent cells were trypsinized and passaged.

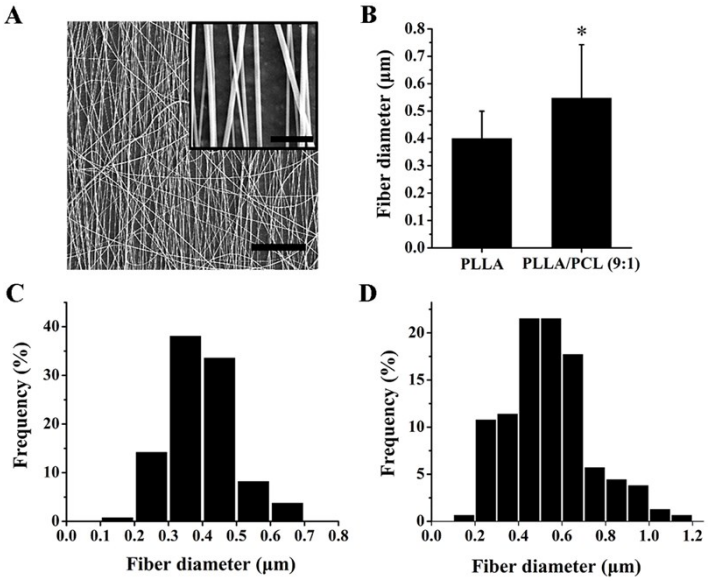

Fig. S1 Characterizations of electrospun nanofibers. (A) SEM images of aligned PLLA/PCL (9:1) nanofibers (The scale bar = 50 μm). The top-left corner diaplays an amplification image (The scale bar = 5 μm). (B) Average fiber diameters of PLLA and PLLA/PCL (9:1) nanofibers. (C) Fiber diameter distribution frequency of PLLA nanofibers. (D) Fiber diameter distribution frequency of PLLA/PCL (9:1) nanofibers. Data were presented as the mean ± SD. 100-150 randomly selected fibers in each group were calculated. * represents a significant difference with the Control group ($p < 0.05$).

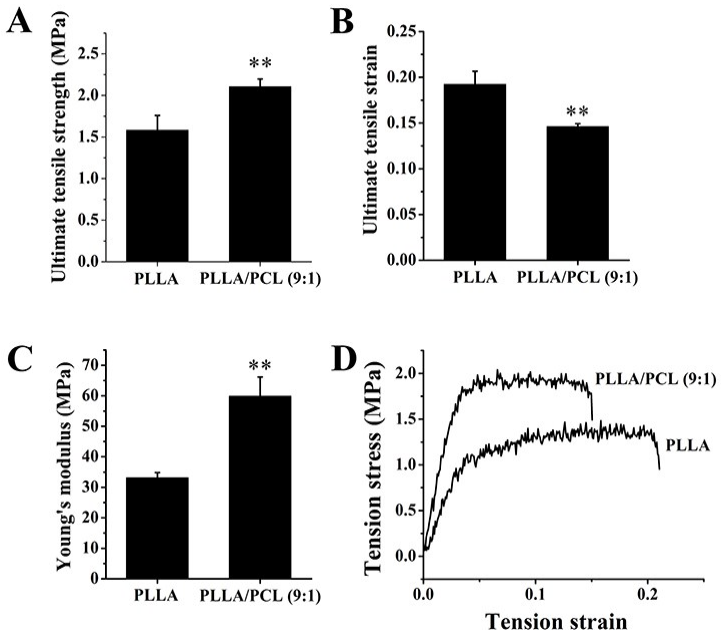

Fig. S2 The ulitimate tensile strength (A), ulitimate tensile strain (B), Young's modulus (C), and representative strain-stress curves (D) of PLLA and PLLA/PCL (9:1) nanofibrous scaffolds. Data were presented as the mean ± SD (n = 4). ** represents a significant difference with the PLLA nanofibers ($p < 0.01$).

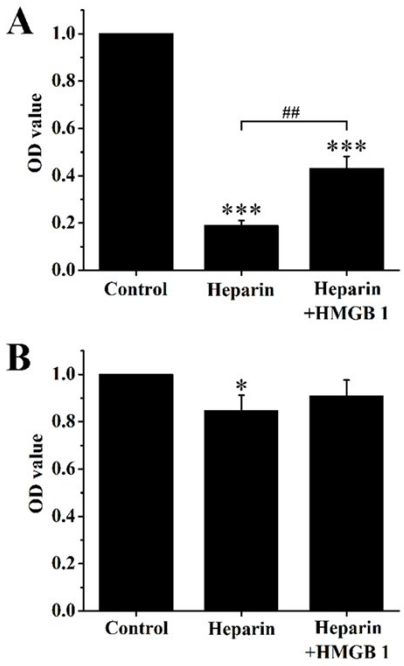

Fig. S3 MTS assay for MSCs proliferation on PLLA/PCL nanofibrous scaffold (Control), heparin-functionalized nanofibrous scaffold (Heparin) and HMGB 1-immobilized nanofibrous scaffold (Heparin+HMGB 1) after 3 days (A) and 7 days (B). Data were presented as the mean ± SD (n = 5). * represents a significant difference with the Control group ($p < 0.05$), *** represents a significant difference with the Control group ($p < 0.001$), and ## represents a significant difference with the Heparin group ($p < 0.01$).

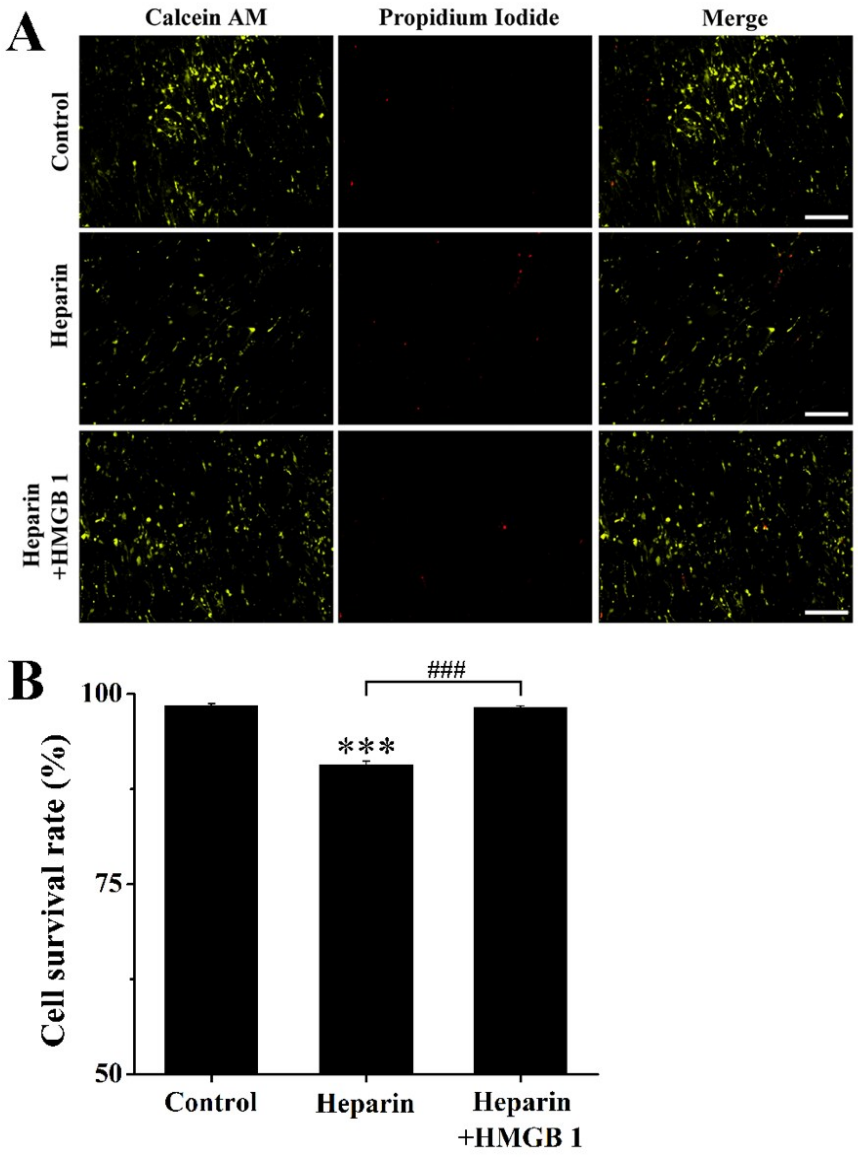

Fig. S4 Live/dead assay for MSCs cultivated on the PLLA/PCL nanofibrous scaffold (Control), heparin-functionalized nanofibrous scaffold (Heparin) and HMGB 1-immobilized nanofibrous scaffold (Heparin+HMGB 1) for 14 days. (A) Immunostaining for live/dead cells. Calcein-AM (green) indicates live cells while propidium iodide (red) indicates dead cells. The scale bar = 100 μm. (B) Cell survival rates in different scaffolds after culture for 14 days. Data were presented as the mean ± SD (n = 5). *** represents a significant difference with the Control group ($p < 0.001$), ### represents a significant difference with the Heparin group ($p < 0.001$).

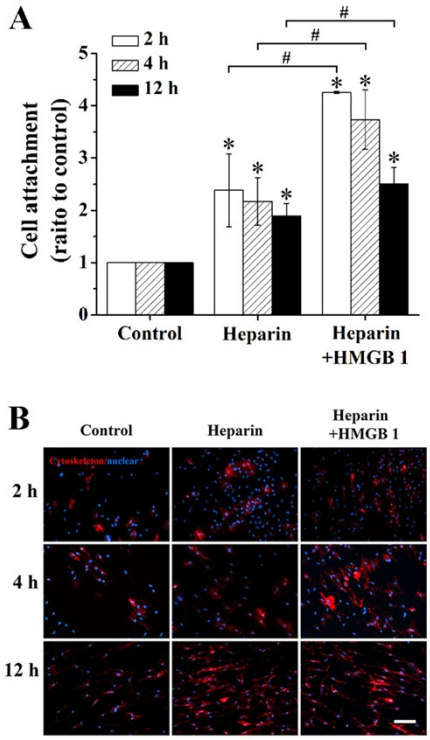

Fig. S5 Evaluation of MSCs attachment on the PLLA/PCL nanofibrous scaffold (Control), heparin-functionalized nanofibrous scaffold (Heparin) and HMGB 1-immobilized nanofibrous scaffold (Heparin+HMGB 1). (A) Relative attached cell number of different scaffolds after culture for 2, 4, and 12 h. Data are presented as the mean ± SD (n = 3). * represents a significant difference with the Control group at the same time point ($p < 0.05$), # represents a significant difference with the Heparin group at the same time point ($p < 0.05$). (B) Immunofluorescent staining of cytoskeleton (red) and nuclear (blue) of MSCs attached on different scaffolds after culture for 2, 4, and 12 h. The scale bar = 100 μm.

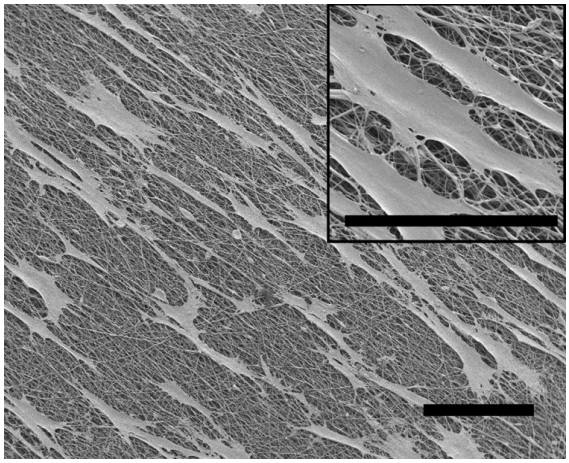

Fig. S6 SEM image of MSCs attached on the PLLA/PCL (9:1) nanofibrous scaffold. The top-left corner diaplays an amplification image. The scale bare indicates 100 μm.

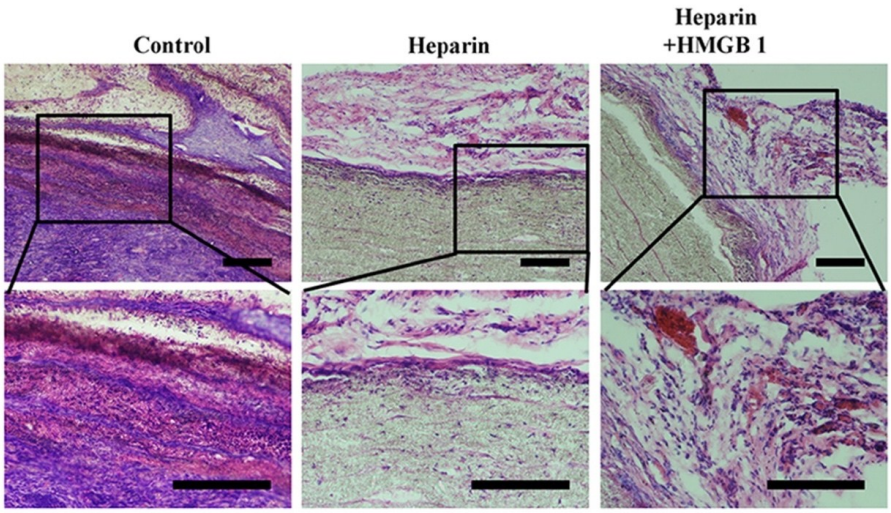

Fig. S7 H-E staining of the PLLA/PCL nanofibrous scaffold (Control), heparin-functionalized nanofibrous scaffold (Heparin) and HMGB 1-immobilized nanofibrous scaffold (Heparin+HMGB 1) after subcutaneous implantation for 4 weeks. The scale bar indicates 200 μm.

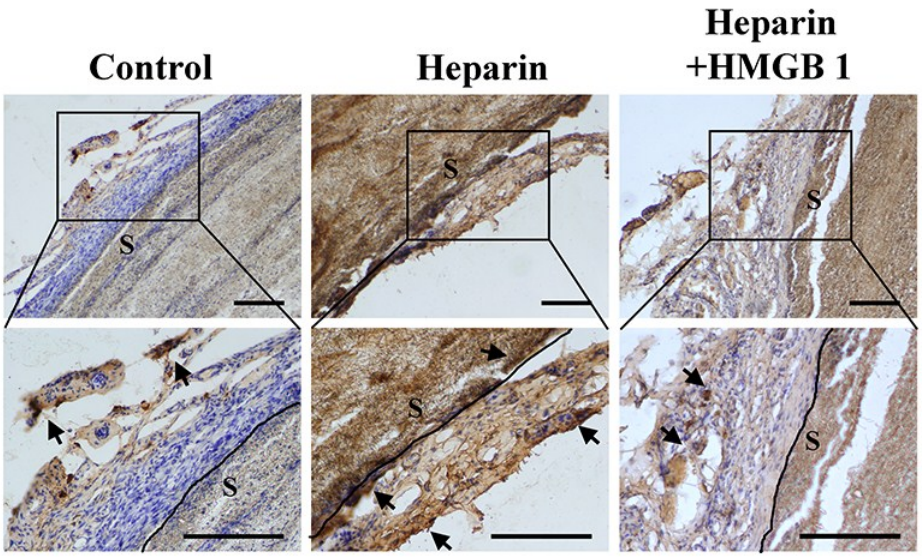

Fig. S8 Representative images of immunohistochemistry staining for CD68 in the PLLA/PCL nanofibrous scaffold (Control), heparin-functionalized nanofibrous scaffold (Heparin) and HMGB 1-immobilized nanofibrous scaffold (Heparin+HMGB 1) after subcutaneous implantation for 4 weeks. Arrows indicate positive CD68 staining and S indicates the scaffold. The scale bar indicates 200 μm.

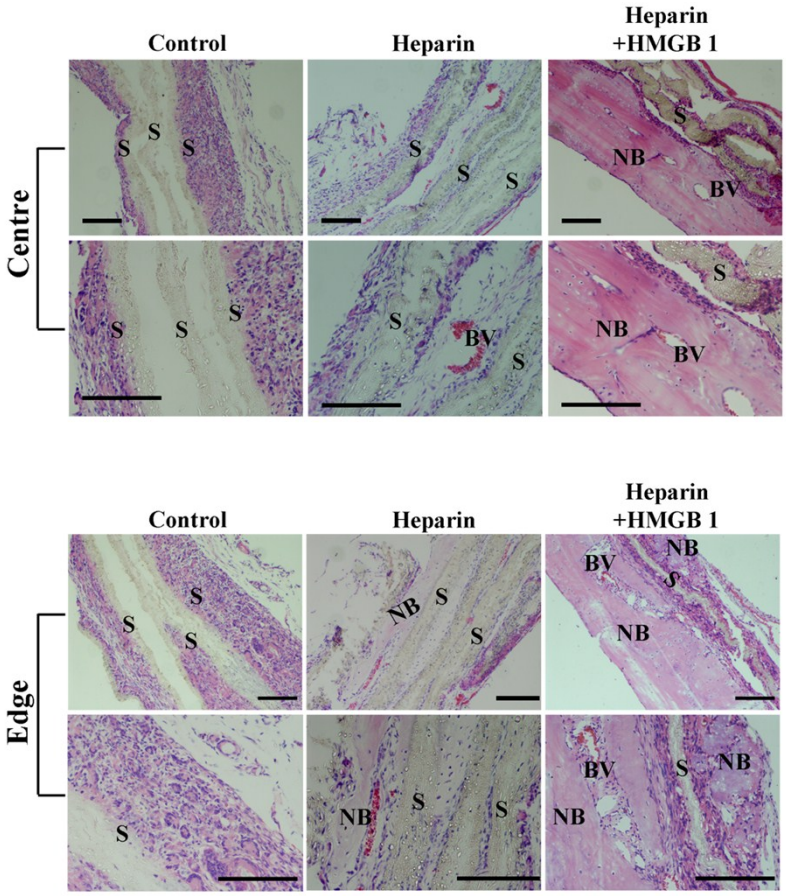

Fig. S9 Representative images of H-E staining for the center and edge areas of cross-sections in the PLLA/PCL nanofibrous scaffold (Control), heparin-functionalized nanofibrous scaffold (Heparin) and HMGB 1-immobilized nanofibrous scaffold (Heparin+HMGB 1) after calvarial defects implantation for 6 weeks. The scale bar indicates 200 μm. S indicates the scaffold, NB denotes new bone formation and BV indicates new blood vessel.

Table S1 Real-time RT-PCR primer sequences.

Gene	Forward	Reverse	Product size(bp)
Runx 2	CACTGGCGCTGCAACAAGA	CATTCCGGAGCTCAGCAGAATAA	127
ALP	GGACCATTCCCACGTCTTCAC	CCTTGTAGCCAGGCCCATTG	137
OCN	CCCAGGCGCTACCTGTATCAA	GGTCAGCCAACTCGTCACAGTC	112
OPN	ACACATATGATGGCCGAGGTGA	TGTGAGGTGATGTCCTCGTCTGTAG	115
GAPDH	GCAAGTTCAACGGCACAG	CGCCAGTAGACTCCACGAC	114