Supporting information

rADSC-loaded tubular units composed of multilayer electrospun membranes promoted bone regeneration of critical-sized skull defects

Huamin Jiang [1,2 #], Zhaoyi Lin[1,2 #], Jinze Li[1,2], Ting Song[1,2], Hongyun Zang[1,2], Pengwen Li[1,2], Jiarun Li[3], Wenyi Hou[4], Jianhua Zhou[1,2], and Yan Li[1,2,*]

[1] School of Biomedical Engineering, Shenzhen Campus of Sun Yat-sen University, Shenzhen 518107, China

[2] Guangdong Provincial Key Laboratory of Sensor Technology and Biomedical Instrument, Sun Yat-sen University, Guangzhou 510006, China

[3] Hospital of Stomatology, Guangdong Provincial Key Laboratory of Stomatology, Institute of Stomatological Research, Guanghua School of Stomatology, Sun Yat-sen University, Guangzhou 510062, China

[4] The Third Affiliated Hospital of Sun Yat-sen University, Guangzhou 510630, China

#Both authors contributed equally

*Correspondence: liyan99@mail.sysu.edu.cn

Table S1 Volumes of electrospinning solutions for single-layer fiber membranes (10 × 10 cm).

Number	Sample name	Type of spinning solution	Volume (μL)
1	SPLG80	PLG80	50
2	SPLG85	PLG85	50
3	SPLG80-H	PLG80-H	50
4	SPLG85-H	PLG85-H	50

Table S2 Specific parameters of different BLCMs.

Number	Proportion of PLG80-H/PLG85-H	Total volume of electrospun solution (μL)	Total thickness of BLCM (μm)
1	1/1	50	2.5
2	1/2	50	2.5
3	1/3	50	2.5
4	1/4	50	2.5
5	1/2	30	1.5
6	1/2	100	5
7	1/2	200	10

Table S3 Isosceles triangle specifications of BLCM units after laser ablation.

Number	Waist length (mm)	Vertex angle (°)
1	1.5	30
2	1.5	45
3	1.5	120
4	2.0	30
5	2.0	45
6	2.0	120
7	2.5	30
8	2.5	45
9	2.5	120

Table S4 Detailed sample names.

Sample name	Membrane type	HAp	Waist length (mm)	Vertex angle (°)	rADSC seeding surface
SPLG85-H(3.5)	SL	+	3.5	45	PLG85-H
BPLG80-H(3.5)	BL	+	3.5	45	PLG80-H
BPLG85-H(3.5)	BL	+	3.5	45	PLG85-H
BPLG85(3.5)	BL	-	3.5	45	PLG85
BPLG80-H(1.5)	BL	+	1.5	30	PLG80-H
BPLG85-H(1.5)	BL	+	1.5	30	PLG85-H

Table S5 Particle size and Zeta potential of HAp precipitated at different reactant concentrations after 0.5 h of reaction at a maintained temperature of 25℃.

Number	$CaCl_2$ (mM)	Na_2HPO_4 (mM)	Casein (mg/mL）	Average particle size (nm)	Zeta potential (mV)
1	50	30	8	255.9	-30.3
2	100	60	8	362.7	-20.6
3	150	90	8	669.1	-20.9
4	200	120	8	6053.0	-17.1

Table S6 Particle size and Zeta potential of HAp precipitated at different casein concentrations after 0.5 h of reaction at a maintained temperature of 25℃.

Number	$CaCl_2$ (mM)	Na_2HPO_4 (mM)	Casein (mg/mL）	Average particle size (nm)	Zeta potential (mV)
1	50	30	2	356.0	-19.6
2	50	30	4	270.6	-21.0
3	50	30	6	255.9	-30.3
4	50	30	8	255.3	-30.4

Table S7 Particle size of HAp synthesized at different temperatures with reactant concentrations of 50 mM $CaCl_2$, 30 mM Na_2HPO_4, and 8 mg/mL casein (the reaction was allowed for 0.5 h).

Number	Temperature (℃)	Average particle size (nm)
1	25	242.8
2	60	368.2
3	90	465.5

Table S8 The crystallinity of SL membrane based on DSC data.

Sample name	Melting enthalpy (J/g)	Crystallinity relative to PCL amount in membrane (%)	Crystallinity relative to membrane (%)
As-spun SPLG85-H	1.31	6.25	0.94
Aft-shrink SPLG85-H	6.72	32.12	4.82
As-spun SPLG80-H	1.94	3.96	1.39
Aft-shrink SPLG80-H	6.58	23.58	4.72

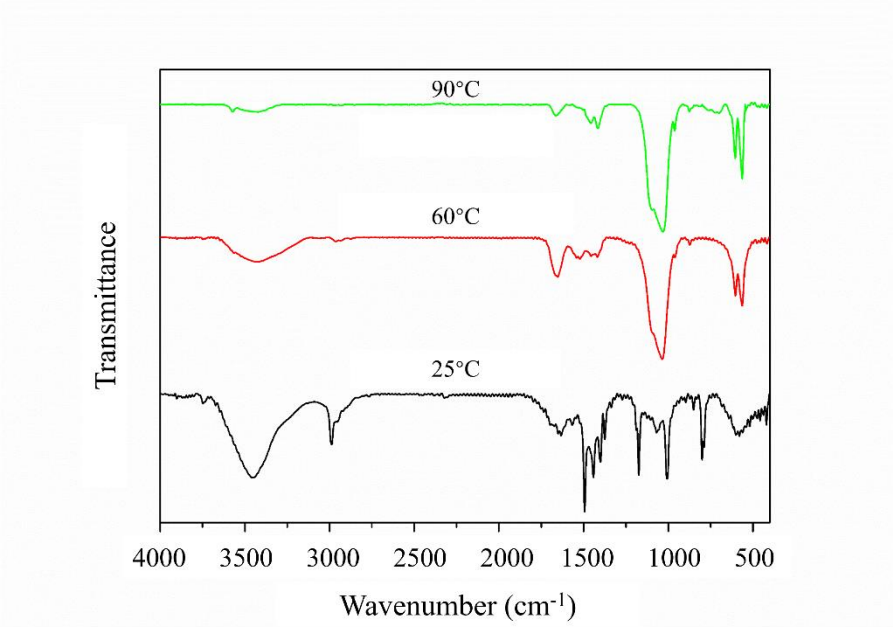

Fig. S1 FT-IR spectra of HAp synthesized at different reaction temperatures. The one at 90°C showed the characteristic absorption bands for HAp.

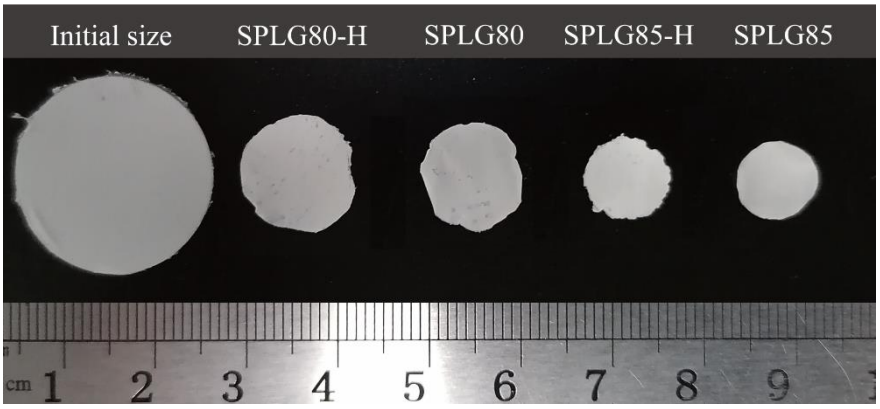

Fig. S2 Shrinkage properties of SL composite nanofiber membranes. From left to right, the membrane before shrinkage, SPLG80-H, SPLG80, SPLG85-H and SPLG85 after shrinkage are shown in sequence.

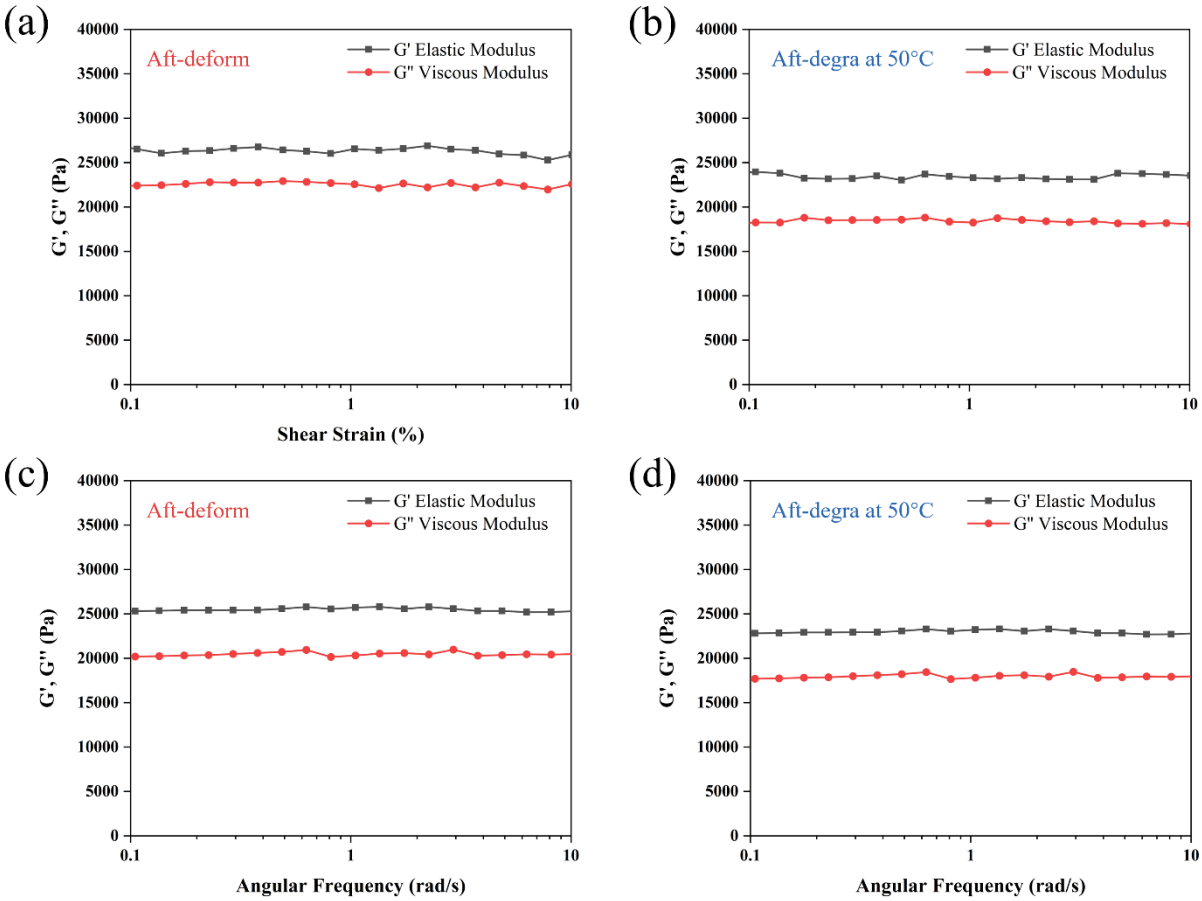

Fig. S3 Mechanical properties of BPLG-H(3.5) evaluated using a rheometer. An 8mm diameter plate was used, with a testing gap set to 0.5mm. Frequency sweep tests and amplitude sweep tests were conducted. The variation in the elastic modulus (G') and viscous modulus (G'') with strain when the frequency was fixed at 1 Hz for the sample (a) after deformation (aft-deform) and (b) after degradation (aft-degra) at 50°C for 7 days. The variation in the elastic modulus (G') and viscous modulus (G'') with angular frequency when the strain was fixed at 1% for the sample (c) aft-deform and (d) aft-degra at 50°C for 7 days. All samples were rehydrated.

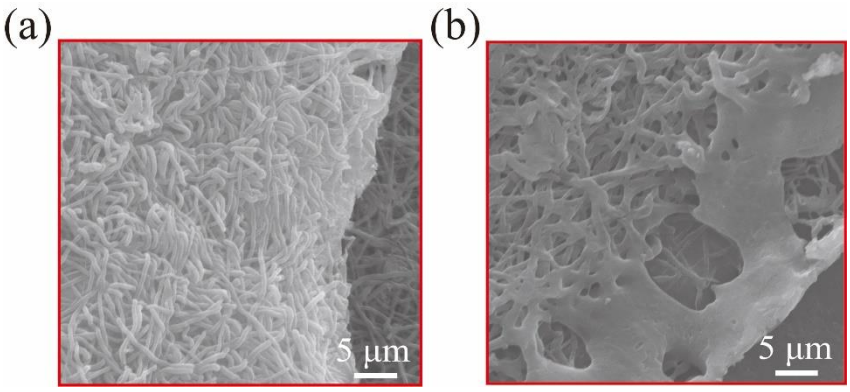

Fig. S4 SEM images of the membrane edges after (a) knife cutting and (b) laser ablation.

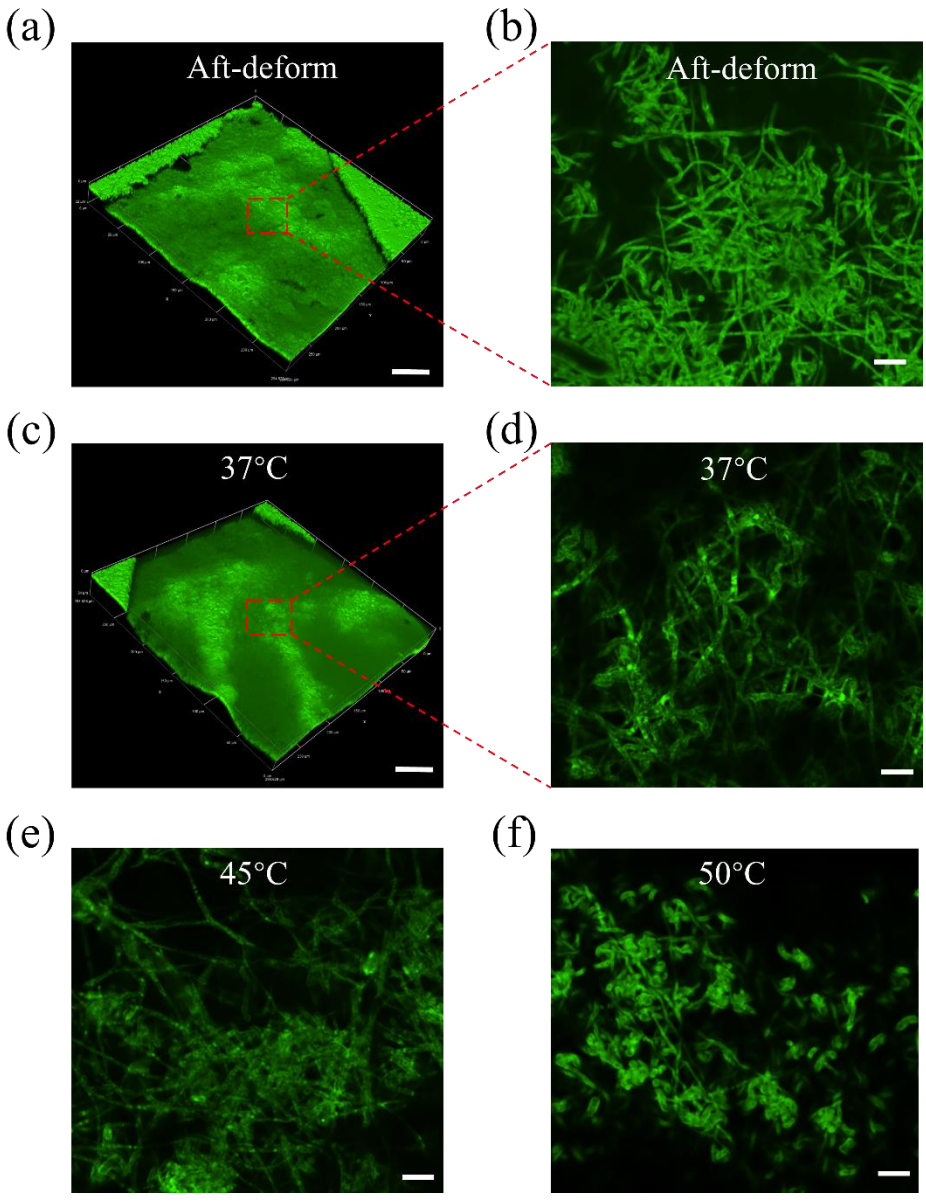

Fig. S5 CLSM images of BPLG-H(3.5) (a)(b) after deformation (aft-deform) and after

7 days of degradation at (c)(d) 37°C, (e) 45°C, and (f)50°C. Scale bars in (a)(c) represent 50 μm while the others represent 5 μm.

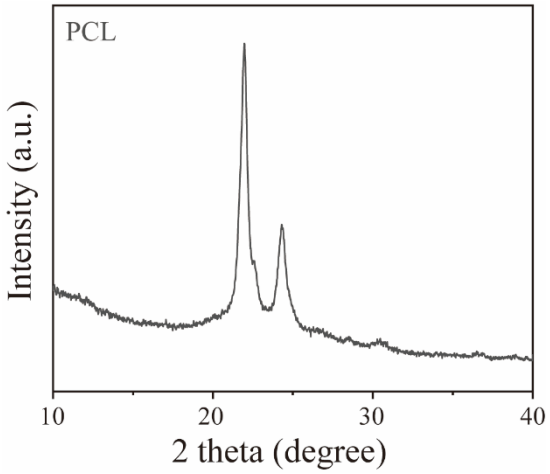

Fig. S6 XRD pattern of pure PCL fiber membrane.

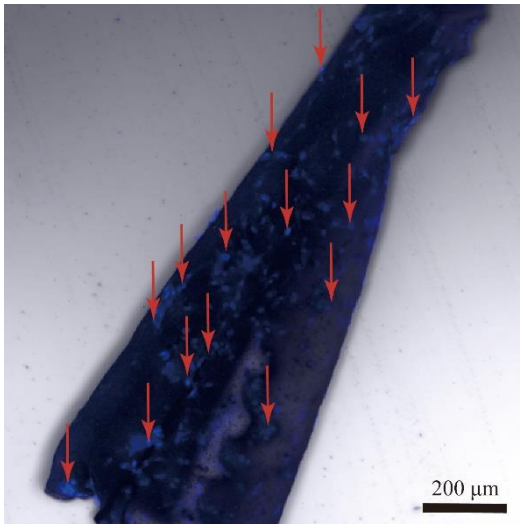

Fig. S7 3D reconstructed CLSM image for BPLG85-H(3.5) seeded with cells. Red arrows pointed to rADSCs.

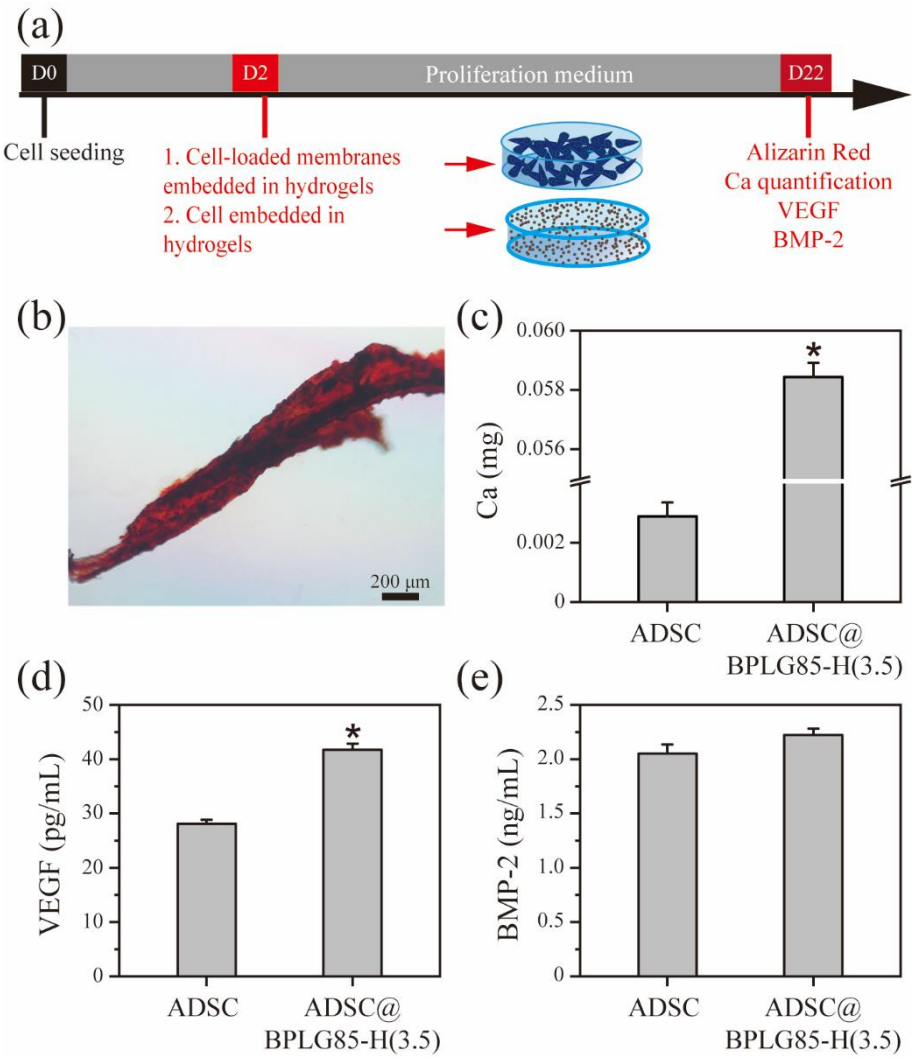

Fig. S8 In vitro comparison of osteogenic differentiation properties for rADSCs in gelatin hydrogel. (a) The timeline for embedding rADSC-seeded BPLG85-H(3.5) units in hydrogel (ADSC@BPLG85-H(3.5) group) or directly embedding rADSCs in hydrogel (ADSC group), culturing in proliferation for 20 d, and then evaluation of osteogenic differentiation properties. (b) Light microscope image of the membrane "ADSC@BPLG85-H(3.5)" after Alizarin Red staining. (c) Quantitative results of calcium contents in ADSC@BPLG85-H(3.5) and ADSC group. Concentrations of (d) VEGF and (e) BMP-2 in culture medium of samples on day 22 which were quantified using ELISA. (Data = mean ± SD; n = 3. * $p < 0.05$ compared with ADSC group)

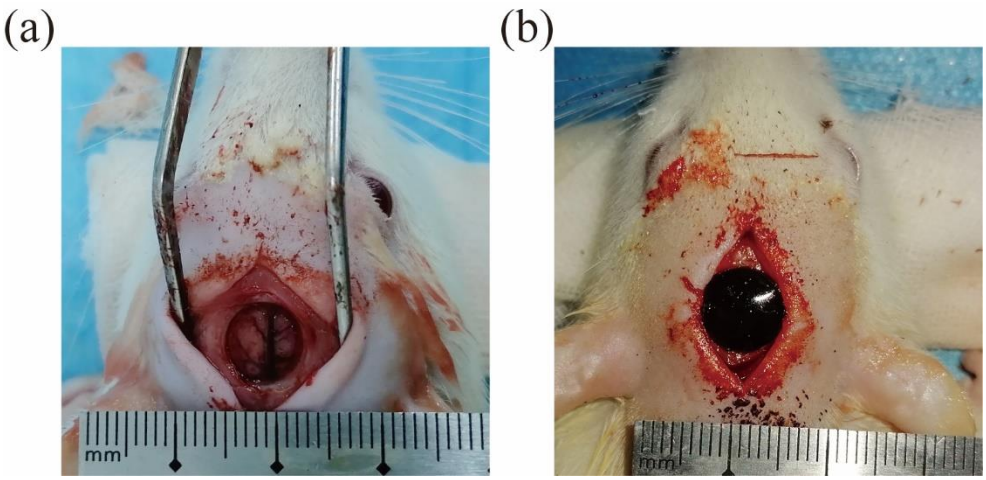

Fig. S9 (a) and (b) are digital photos of Blank group and ADSC@BHM group on day 0 in animal experiment, respectively. The ADSC@BHM scaffold was dark purple as gelatin hydrogel was crosslinked with genipin.

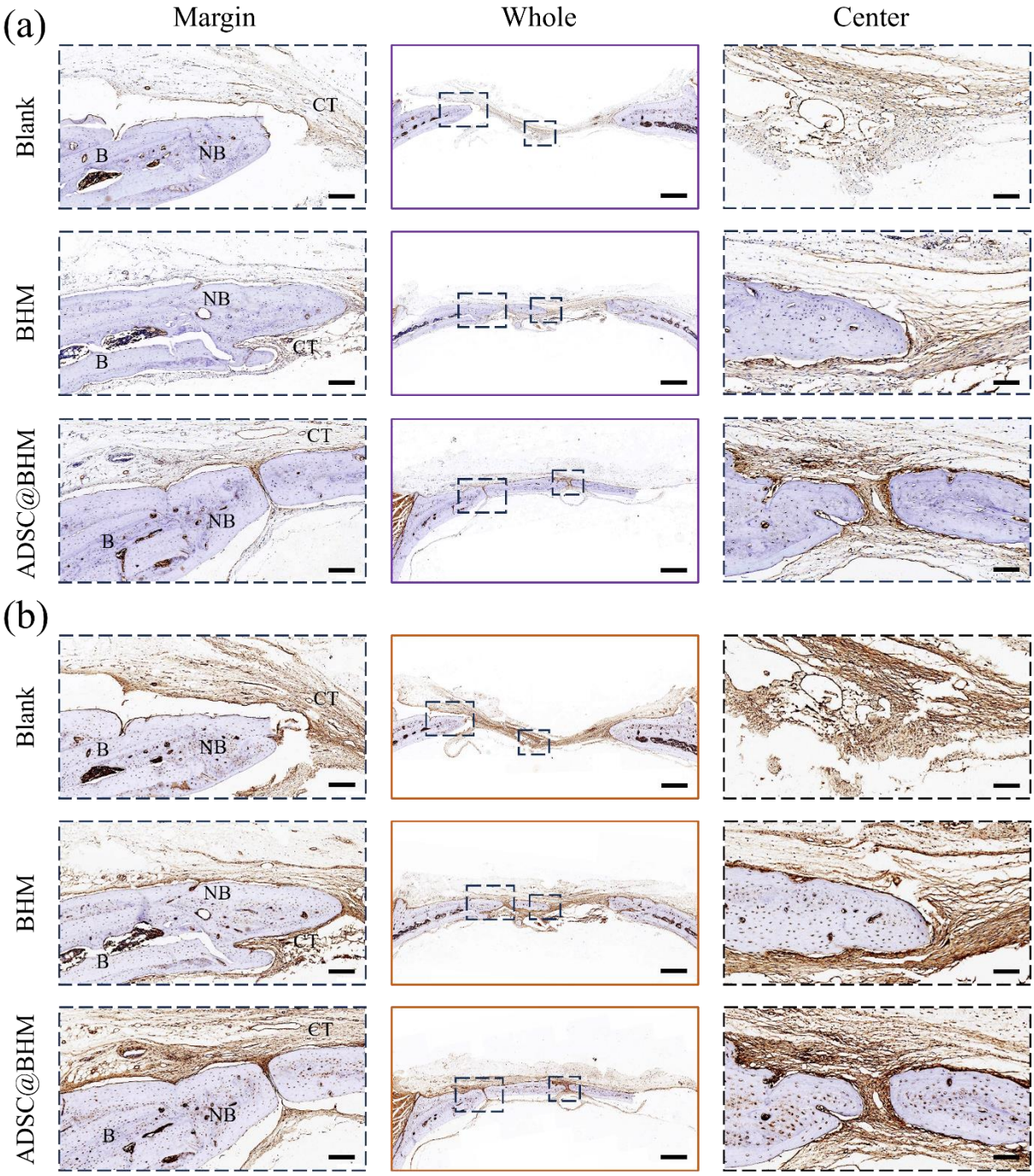

Fig. S10 Representative immunohistochemical staining images of bone tissues for the Blank, BHM, and ADSC@BHM groups at the 12th week to detect the expressions of (a) iNOS and (b) CD206 (NB, new bone; CT, connective tissue; B, host bone). Scale bars in the "Margin" column represent 200 μm, scale bars in the "Whole" column represent 1 mm, and scale bars in the "Center" column represent 100 μm.

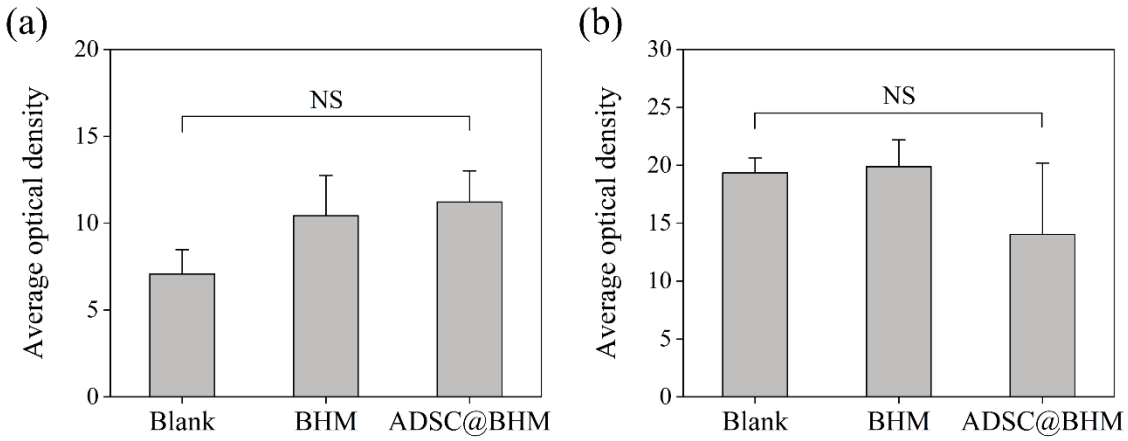

Fig. S11 The semi-quantitative analysis of immunohistochemical staining images of bone tissue sections for the Blank, BHM, and ADSC@BHM groups at the 12th week to detect the expressions of (a) iNOS and (b) CD206. (Data = mean ± SD; n = 3; NS represents no significance)