

Supporting Information

3D-Printed Biomimetic Scaffold Loaded with ADSCs and BMP-2 for Enhanced Rotator Cuff Repair

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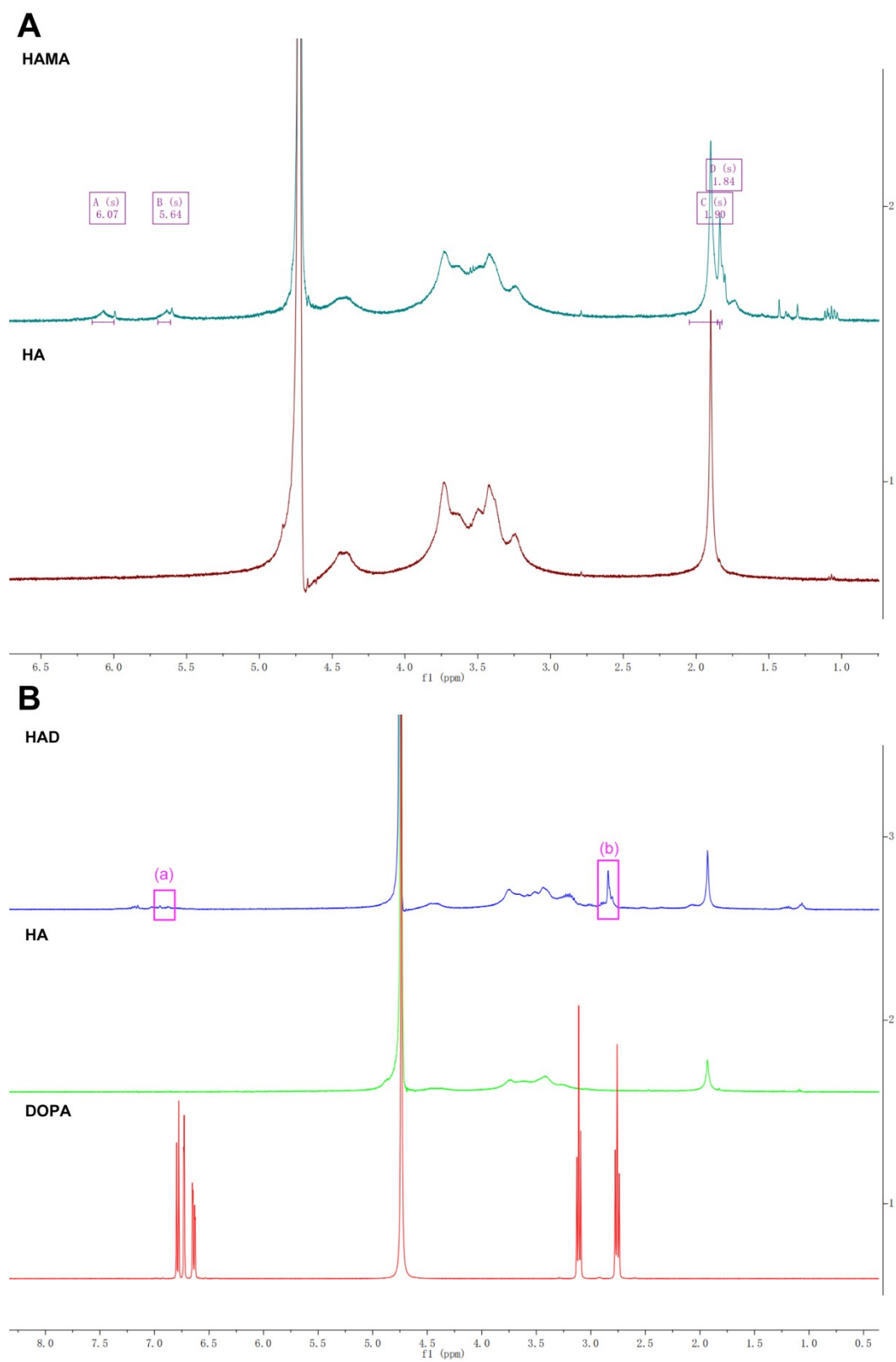


Fig. S1. (A) $^1\text{H-NMR}$ analysis of the HAMA. (B) $^1\text{H-NMR}$ analysis of the HAD.

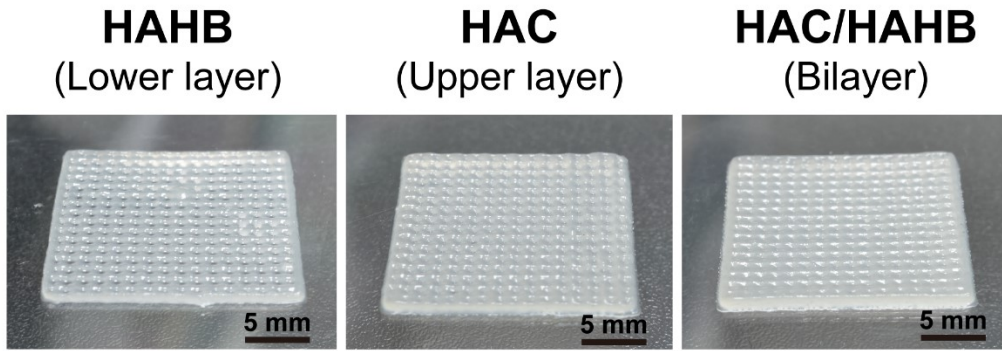


Fig. S2 The macrophotograph of the HAHB (lower layer), HAC (upper layer), and HAC/HAHB (bilayer) scaffold.

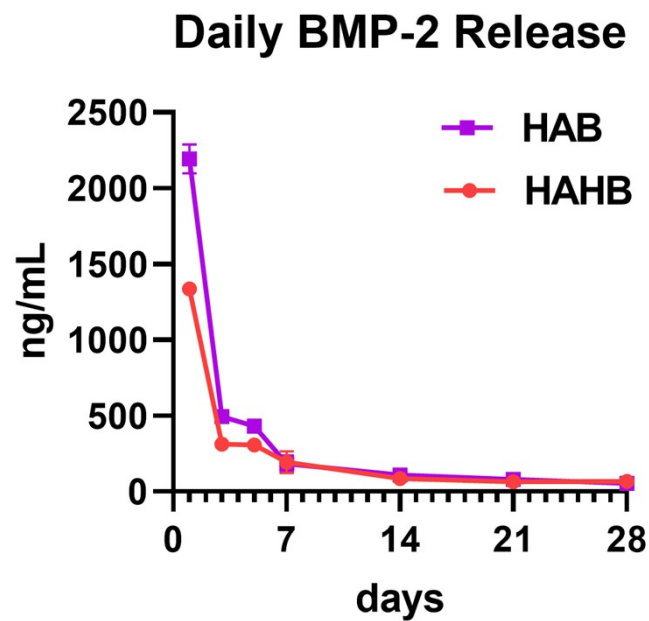


Fig. S3. BMP-2 daily release curves of hydrogel scaffolds within 28 days.

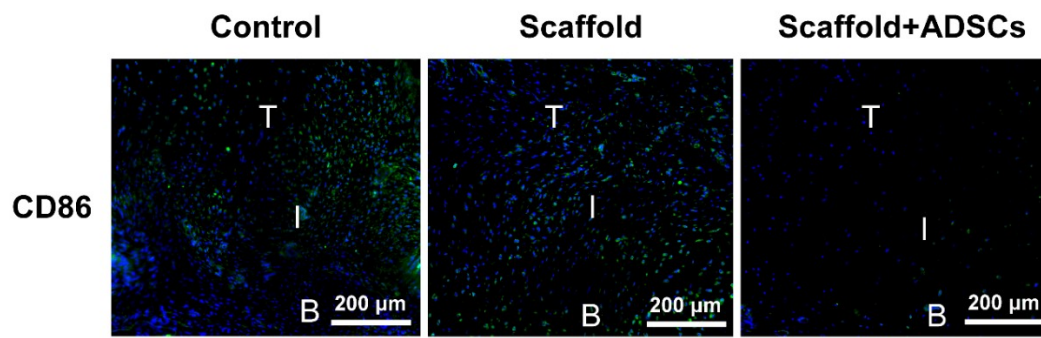


Fig.S4. Immunofluorescence staining of type 1 macrophage (CD 86 green) and DAPI (blue) was utilized to assess the inflammation phenomenon at 4 weeks.

Table S1. Primer sequences of genes.

Gene	Primers	
	Forward (5'-3')	Reverse (5'-3')
GAPDH	AAGAAACCCTGGACCACCCAGC	TGGTATTCGAGAGAAGGGAGGG
TNMD	CCATGCTGGATGAGAGAGGTTA C	CACAGACCCTGCGGCAGTA
SCX	AGAACACCCAGCCCAAACA	CGGTCTTTGCTCAACTTTCT
SOX9	CTCTCCTAACGCCATCTTCAAG	ACGTCTGTTTTGGGAGTGG
COL2A1	GACCTGCCGGTGAACAAG	GGTACCAGGTTCTCCATCTCT
OPN	TTGGCTTTGCAGTCTCTGCGG	AGGCAAGGCCGAACAGGCAAA
OCN	CAGTAAGGTGGTGAATAGACT	GGTGCCATAGATGCGCTTG

Table S2. The results of swelling, degradation, and mechanical properties.

Group	HA	HAC Upper layer	HAHB Lower layer	HAC/HAHB Bilayer
Swelling properties (24h)	416.0±17.5%	1090.3±13.4%	662.0±53.7%	757.0 ± 104.8%
mechanical properties	40.4±20.68 kPa	157.5±17.17 kPa	208.36±19.58 kPa	178.83±55.64 kPa
degradation properties (28 days)	40.62± 4.55%	32.62± 3.81%	38.00 ± 3.72%	31.02 ± 4.18%

Table S3. Histological examination scores.

Parameter	Scores			
	0	1	2	3
Cell morphology	Spindle	Slightly rounded	Moderately rounded	Severely rounded
Cellularity vascularity (%)	Normal <10	Slightly increased 10-20	Moderately increased 20-30	Severely increased >30

Table S4. Semi-quantitative data from histological staining and immunohistochemical staining.

Group	Control	Scaffold	Scaffold+ ADSCs
Histologic score (<i>HE</i>)	7.33 ± 0.58 points (4W)	5.67 ± 0.58 points (4W)	4.33 ± 0.58 points (4W)
	5.67 ± 0.58 points (8W)	3.33 ± 0.58 points (8W)	2.67 ± 0.58 points (8W)
Semi-quantification of metachromasia area <i>Safranin O-Fast Green</i> (<i>SO-FG</i>) staining	11638.3±223.9 μm ² (4W)	55998.3±4797.4 μm ² (4W)	69627.7±372.1 μm ² (4W)
	48466.3±821.9μm ² (8W)	87658.0±821.9μm ² (8W)	103572.7±4408.7μm ² (8W)
Semi-quantification of Collagen I content <i>Sirius Red (SR)</i> staining	2.5 ± 0.4% (4W)	15.1 ± 0.4% (4W)	16.2 ± 0.6% (4W)
	13.3 ± 0.7% (8W)	17.2 ± 0.8% (8W)	20.5 ± 1.30% (8W)
Semi-quantification of COL I expression	11643.7±445.4μm ² (4W)	18833.7±1180.8 μm ² (4W)	25874.7±567.5μm ² (4W)
Immunohistochemistry staining (IHC) COL I	20049.7±363.2μm ² (8W)	31191.0±1172.9 μm ² (8W)	35998.0±2678.5 μm ² (8W)
Semi-quantification of COL I expression <i>Immunohistochemistry</i> staining (IHC) <i>COL II</i>	7407.3±498.3μm ² (4W)	10884.7±1336.4μm ² (4W)	21565.7±1702.8μm ² (4W)
	18541.3±1142.8 μm ² (8W)	30905.0±1049.8 μm ² (8W)	36555.3±1131.0 μm ² (8W)