3D Printed Gelatin/Hydroxyapatite Scaffolds for Stem Cell Chondrogenic Differentiation and Articular Cartilage Repair

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Table S1. The score chart of International Cartilage Repair Society (ICRS) macroscopic evaluation of cartilage repair.

Categories	Scores
Degree of defect repair	
In level with surrounding cartilage	4
75% repair of defect depth	3
50% repair of defect depth	2
25% repair of defect depth	1
No repair of defect depth	0
Integration to border zone	
Complete integration with surrounding cartilage	4
Demarcating border-1 mm	3
Three-quarters of graft integrated, one-quarter with a notable border-1 mm in width	2
One-half of graft integrated with surrounding cartilage, one-half with a notable border-1 mm	1
From no contact to one-quarter of graft integrated with surrounding cartilage	0
Macroscopic appearance	
Intact smooth surface	4
Fibrillated surface	3
Small, scattered fissures or cracks	2
Several small or few large fissures	1
Total degeneration of grafted area	0
Overall repair assessment	
Grade I: normal	12
Grade II: nearly normal	8-11
Grade III: abnormal	4-7
Grade IV: severely abnormal	1-3

Table S2. The score chart of ICRS visual histological assessment scale.

Features	Scores
Surface	
Smooth/continuous	3
Discontinuities/irregularities	0
Matrix	
Hyaline	3
Mixture: hyaline/fibrocartilage	2
Fibrocartilage	1
Fibrous tissue	0
Cell distribution	
Columnar	3
Mixed/columnar-clusters	2
Clusters	1
Individual cells/disorganized	0
Cell population viability	
Predominantly viable	2
Partially viable	1
<10% viable	0
Subchondral bone	
Normal	3
Increased remodeling	2
Bone necrosis/granulation tissue	1
Detached/fracture/callus at base	0
Cartilage mineralization (calcified cartilage)	
Normal	2
Abnormal/inappropriate location	0
Toluidine blue stain	
Normal	4
Slight reduction	3
Moderate reduction	2
Severe reduction	1
No staining	0
Percent toluidine blue in defect	
75–100%	4
50–75%	3
25–50%	2
0–25%	1
No toluidine blue staining	0
	Max 24

Table S3. Primers used for RT-qPCR analysis.

Gene	Primer sequences
SOX9	qRT-f：5′-CGCAACGGCAACTTCCACC-3′
	qRT-r：5′-CAGGCCTCACTCTCGAAAGC-3′
COL1A1	qRT-f：5′-GTCACCCACCGACCAAGAAACC-3′
	qRT-r：5′-AAGTCCAGGCTGTCCAGGGATG-3′
COL2A1	qRT-f：5′-CCAACAA GCATGTCTGGTTAGGAG-3′
	qRT-r：5′-GCAATGCTGTTCTTGCAGTGGTA-3′
ACAN	qRT-f：5′-TCGAGGACAGCGAGGCC-3′
	qRT-r：5′-TCGAGGGTGTAGCGTGTAGAGA-3′
GAPDH	qRT-f：5′-TCCCTGAGCTGAACGGGAAG-3′
	qRT-r：5′-GGAGGAGTGGGTGTCGCTGT-3′

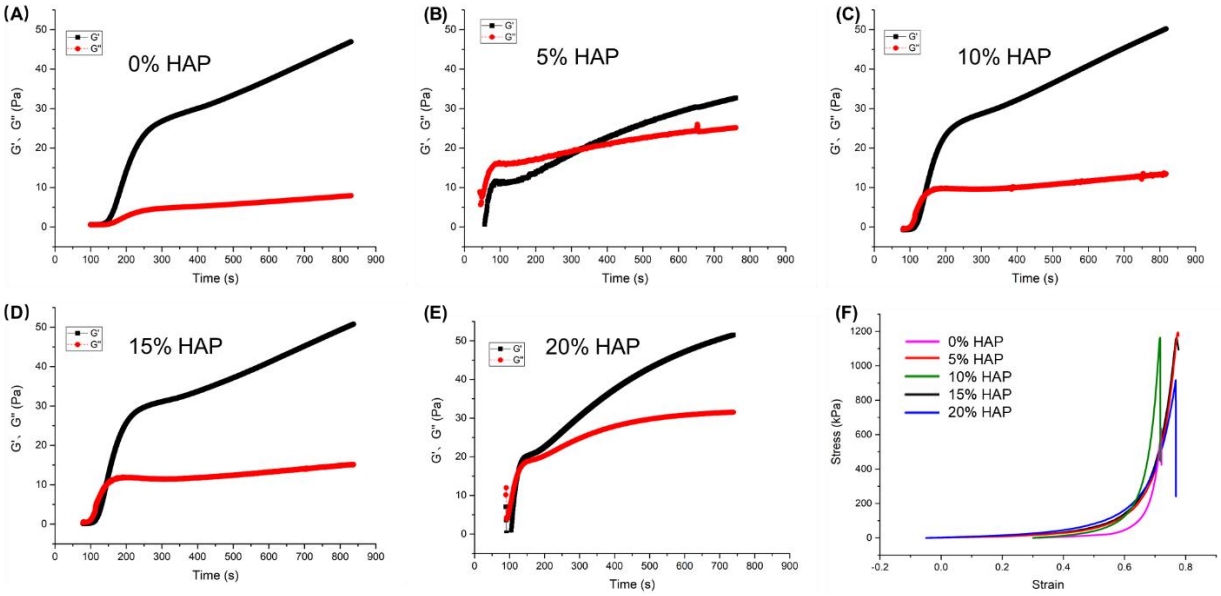

Figure S1. Rheological properties of the gelatin/HAP scaffolds with different composition of HAP. (A-E) The time sweeps. (F) Stress-strain curves.

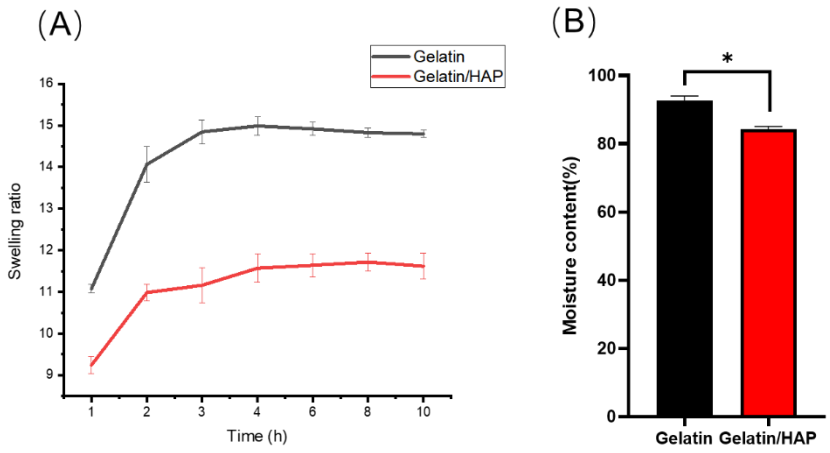

Figure S2. Physical properties of the scaffolds: swelling and moisture content. (A) The swelling property of the gelatin scaffold and gelatin/HAP scaffold. (B) The moisture content of the two scaffolds. *, $p<0.05$.

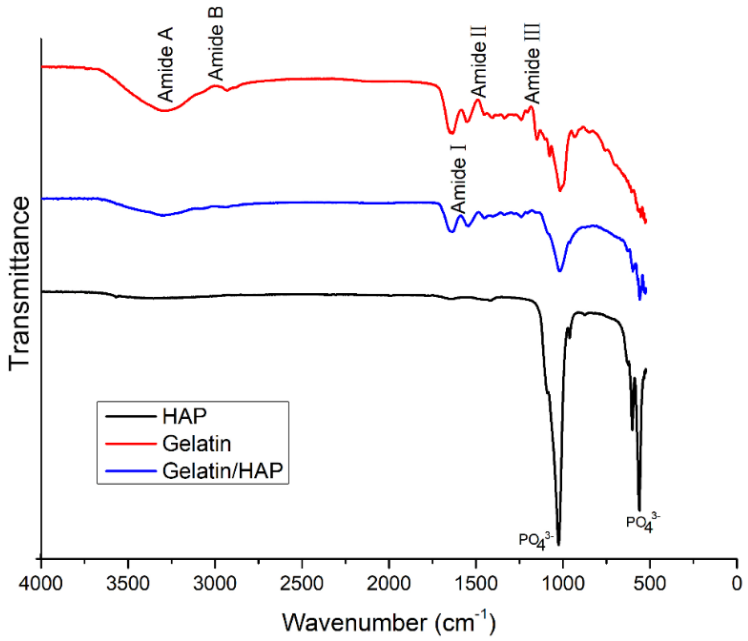

Figure S3. IR spectra of the gelatin scaffold, gelatin/HAP scaffold and HAP alone.

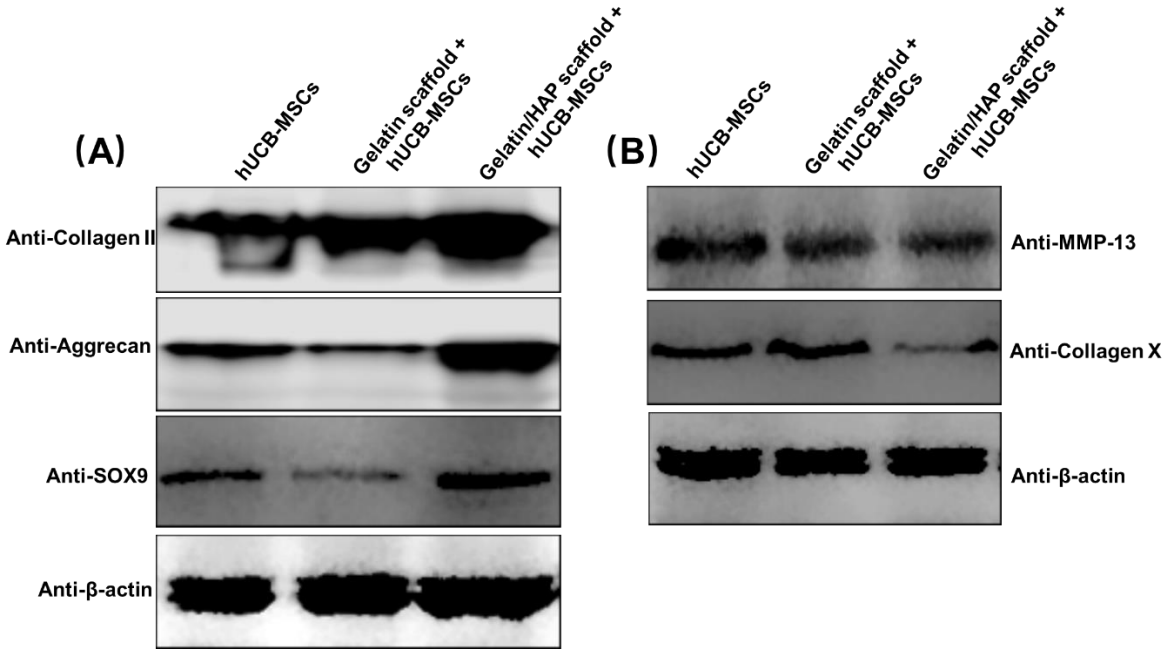

Figure S4. Western blot analysis of the protein expression level on different scaffolds. Briefly, hUCB-MSCs were cultured on gelatin or gelatin/HAP scaffolds for 21 days, before the protein levels were analyzed by western blots using anti-collagen II, anti-aggrecan and anti-SOX9 antibodies (A), and anti-MMP-13 and anti-collagen X antibodies (B).

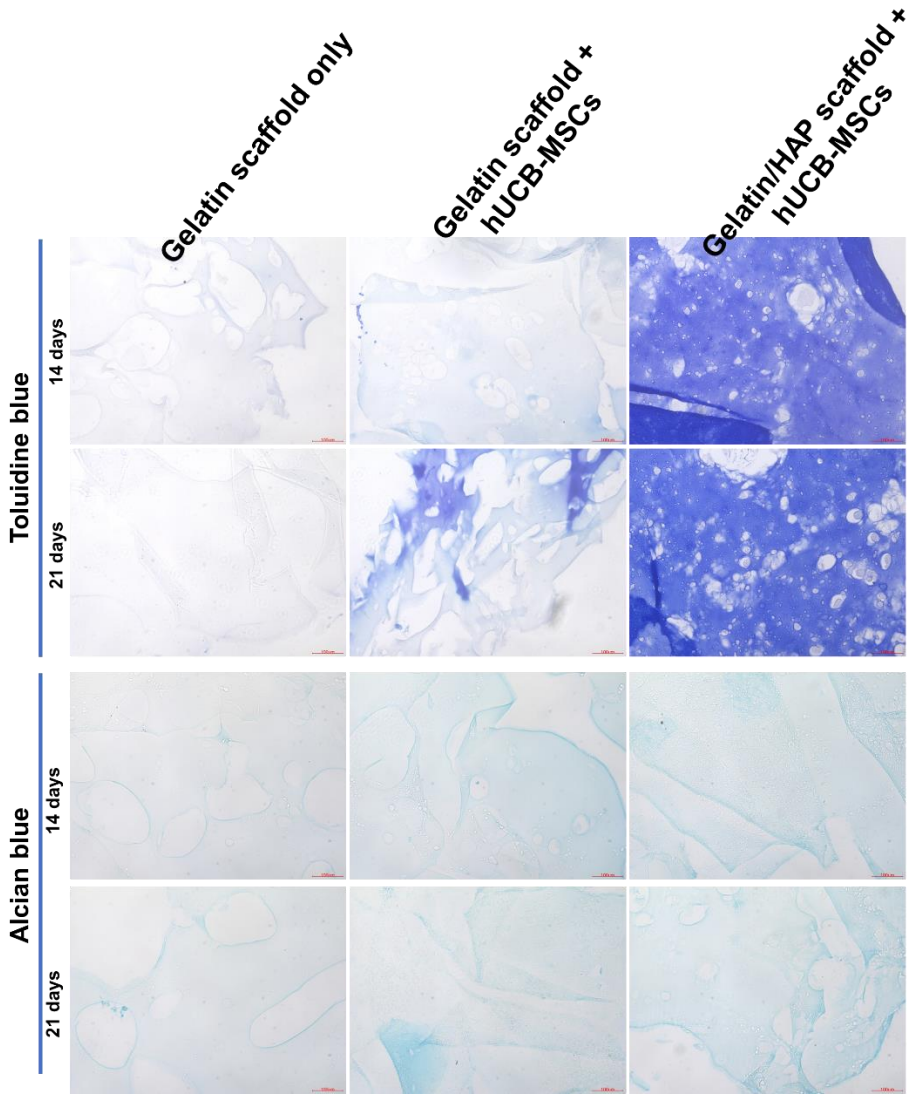

Figure S5. Histological analysis of the cell culture on different scaffolds *in vitro*. Briefly, stem cells were seeded on gelatin or gelatin/HAP scaffolds, and stained with Toluidine Blue or Alcian Blue dyes, and imaged. Scale bar, 100 μm.

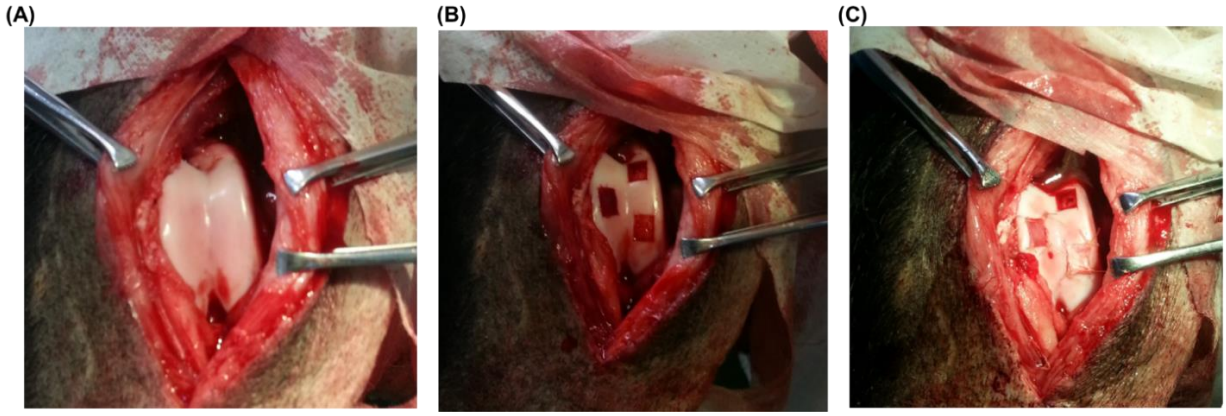

Figure S6. Surgical procedures of the cartilage damage and scaffold/MSC transplantation. (A) Exposure of the knee joint of the pig. (B) Cartilage damages after surgery. (C) Transplants into the damage sites.

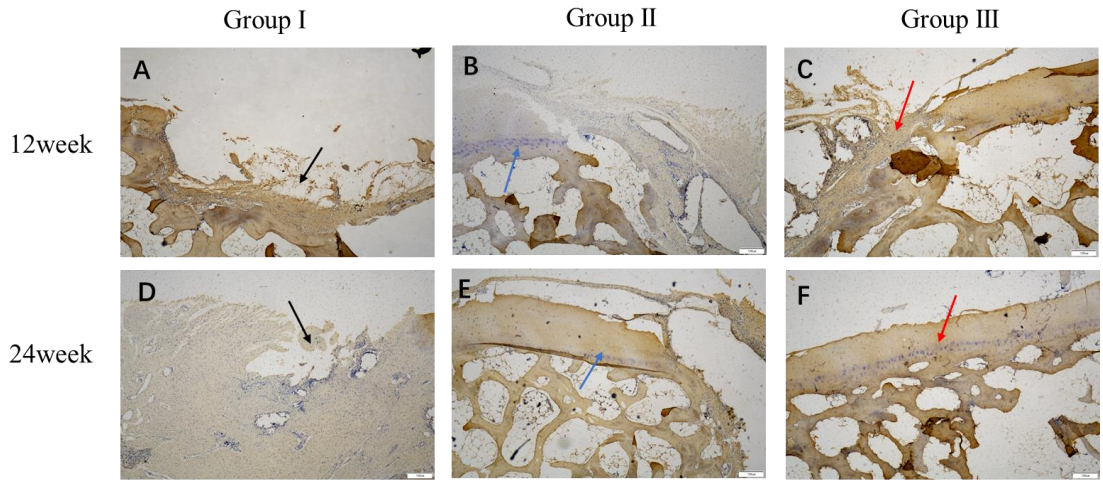

Figure S7. Immunohistochemical staining for collagen II. Black arrows indicate the formation of cavities, fibrous tissues or inflammatory tissues. Blue arrows indicate the partially regenerated cartilage tissues. Red arrows indicate fully regenerated cartilage tissues. Scale bar, 100 μm.

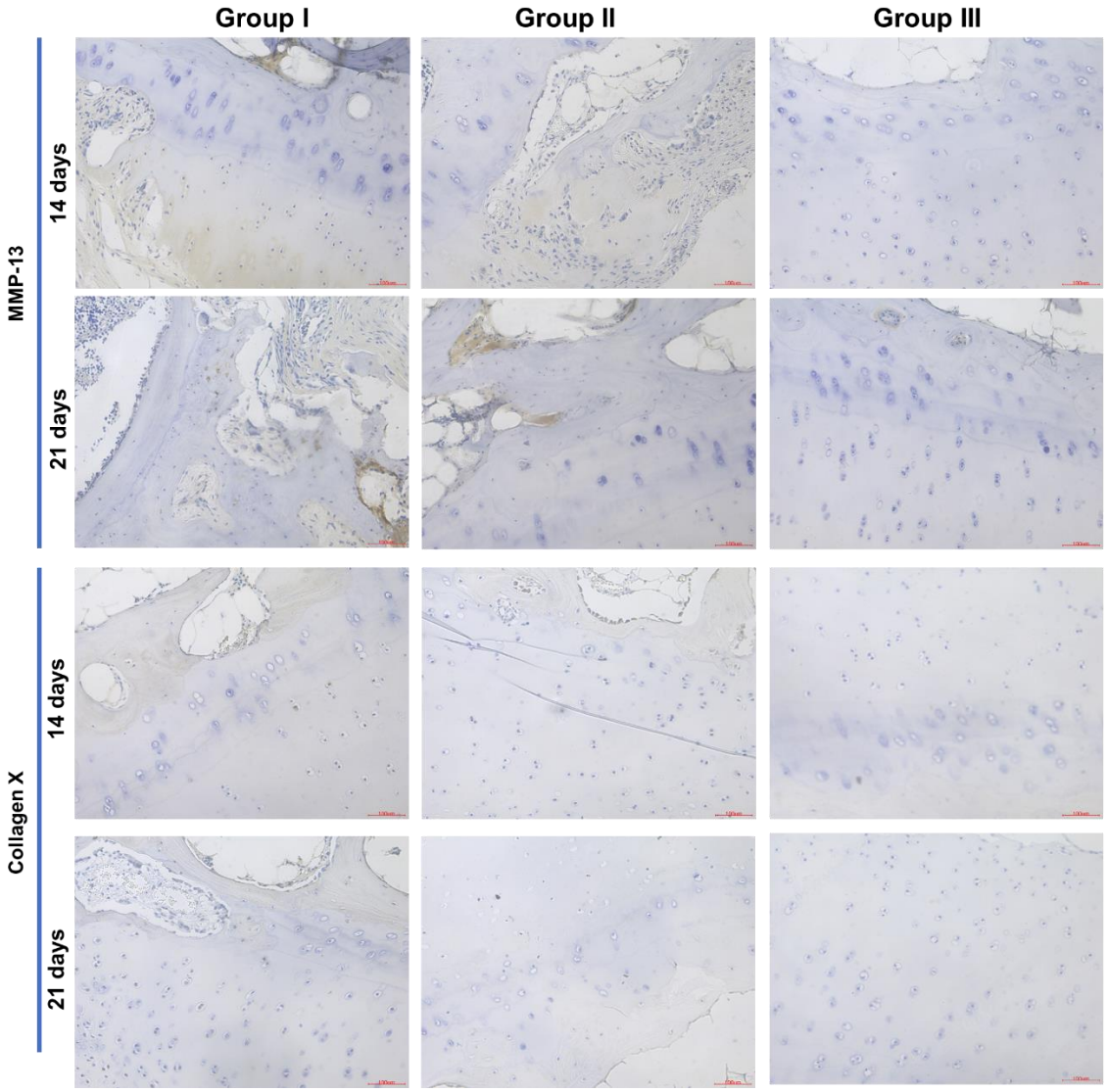

Figure S8. Immunohistochemical staining for collagen X and MMP-13 showing lower expression in Group III. Scale bar, 100 μm.