

RESEARCH ARTICLE

Chondrocyte spheroid-laden microporous hydrogel-based 3D bioprinting for cartilage regeneration

Supplementary File

Table S1. Parameters for bioprinting lattice-shaped constructs

Project	Parameter
Printing Type 1	Chondrocyte spheroid-laden GelMA/PEO bioink
Printing Type 2	Cell-laden GelMA/PEO bioink
Printing model	Lattice shape
Cube dimension	7.0 × 7.0 × 2.4 mm
Pattern filing	Cross mesh
Hatch type	Line
Strand spacing	1.1 mm
Layer height	400 μm
Number of layers	6
Deposition angle	90°
Printing nozzle	Low-temperature nozzle (22G)
Temperature	19°C
Printing speed	6.0–8.0 mm/s
Extrusion pressure	0.6–1.0 bar
Platform temperature	20°C
Crosslinking	Blue light (405 nm, 20 mW/cm ²)

Table S2. Forward and reverse primer sequences used for RT-PCR

Gene name	Forward primer	Reverse primer
<i>GAPDH</i>	5'- CAAGAAGGTGGTGAAGCAGG -3'	5'- CACTGTTGAAGTCGCAG -3'
<i>COL2A1</i>	5'- CACGCTCAAGTCCCTCAACA -3'	5'- TCTATCCAGTAGTCACCGCTCT -3'
<i>ACAN</i>	5'- GGAGGAGCAGGAGTTTGTC AA -3'	5'- TGTCCATCCGACCAGCGAAA -3'
<i>SOX 9</i>	5'- GCGGAGGAAGTCGGTGAAGAAT -3'	5'- AAGATGGCGTTGGGCGAGAT -3'
<i>ELN</i>	5'- GGGCCTTGGAGGTGTGTCT -3'	5'- TCCTGGGAATGGTCTGGTGC -3'
<i>PCNA</i>	5'- ACTCAAGGATCTCATCAACGAG -3'	5'- TTTGGTGCTCAAATACTAGCG -3'

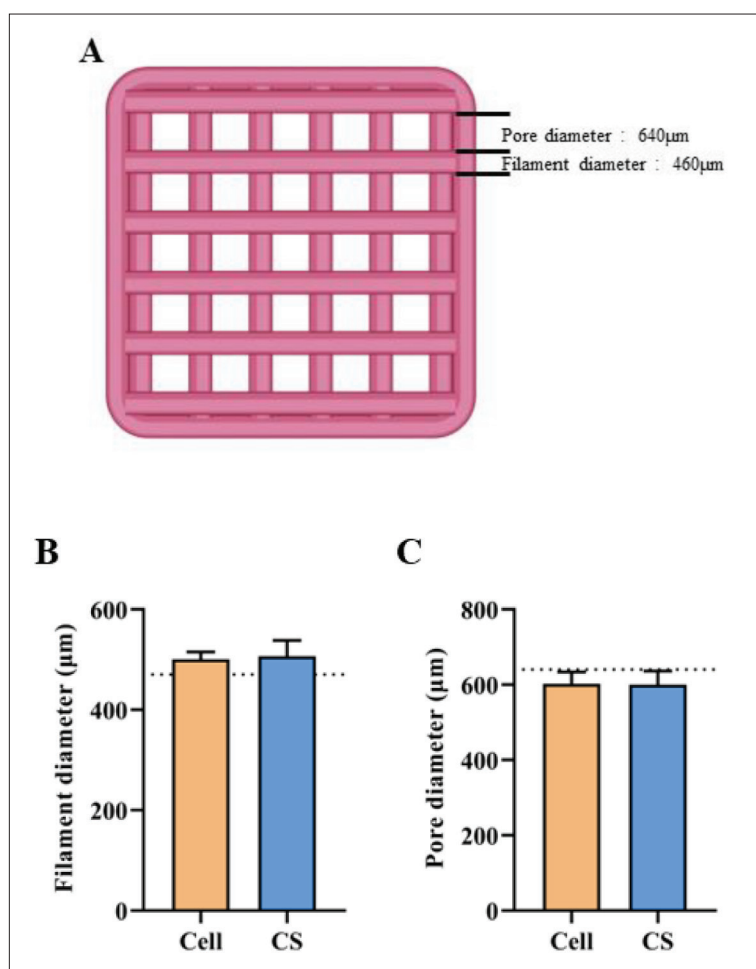


Figure S1. Comparison of designed and printed filament and pore diameters. (A) Schematic diagram of the designed lattice-like constructs. (B) Comparison of filament diameter of the two groups of printed constructs. (C) Comparison of pore diameter of the two groups of printed constructs. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

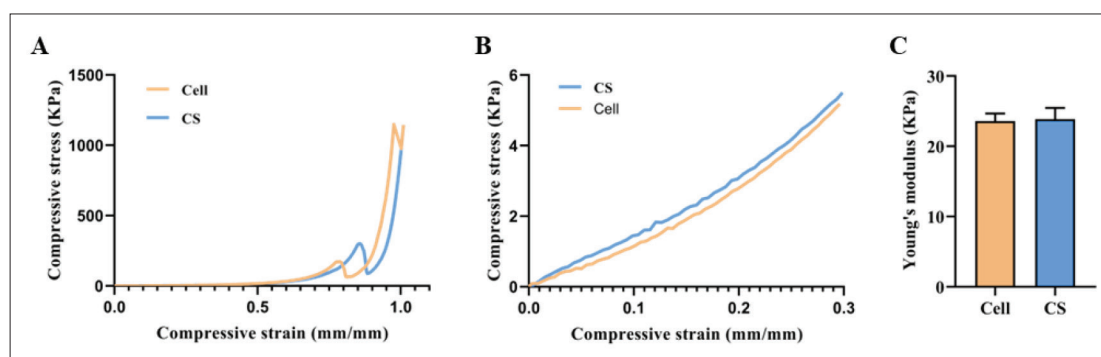


Figure S2. Mechanical properties of the printed constructs. (A) Stress-strain curve. (B) Stress-strain curve (0%–30% strain). (C) Young's modulus. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

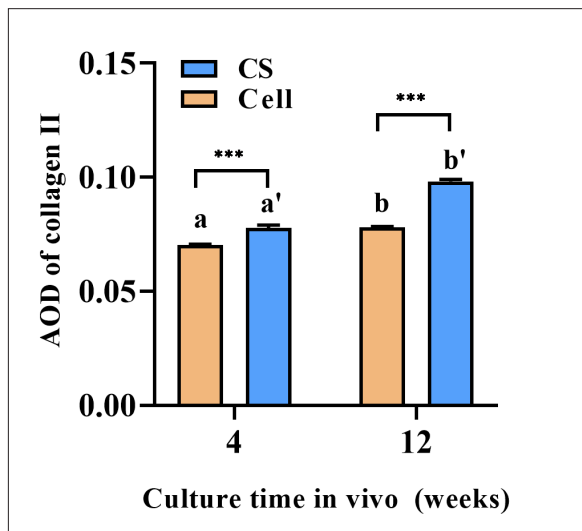


Figure S3. The average optical density (AOD) of type II collagen of two groups of constructs after 4 and 12 weeks of culture *in vivo*. Statistical analysis of the same group at different culture time is indicated by letters, and indication with different letters represents $p < 0.05$. Statistical analysis between different groups is indicated by asterisks: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

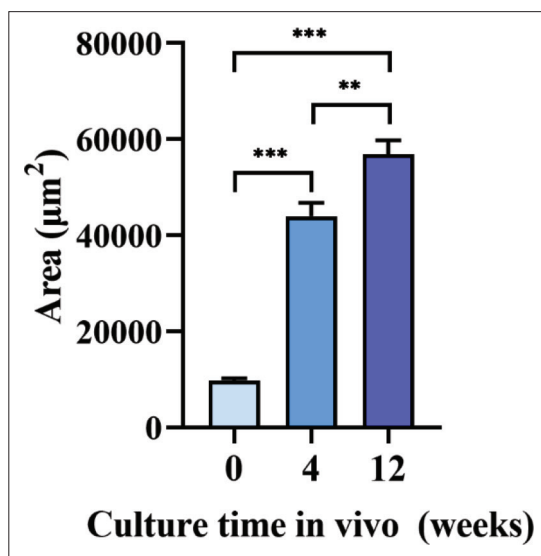


Figure S4. Area of CSs before implantation and after 4 and 12 weeks of *in vivo* culture. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

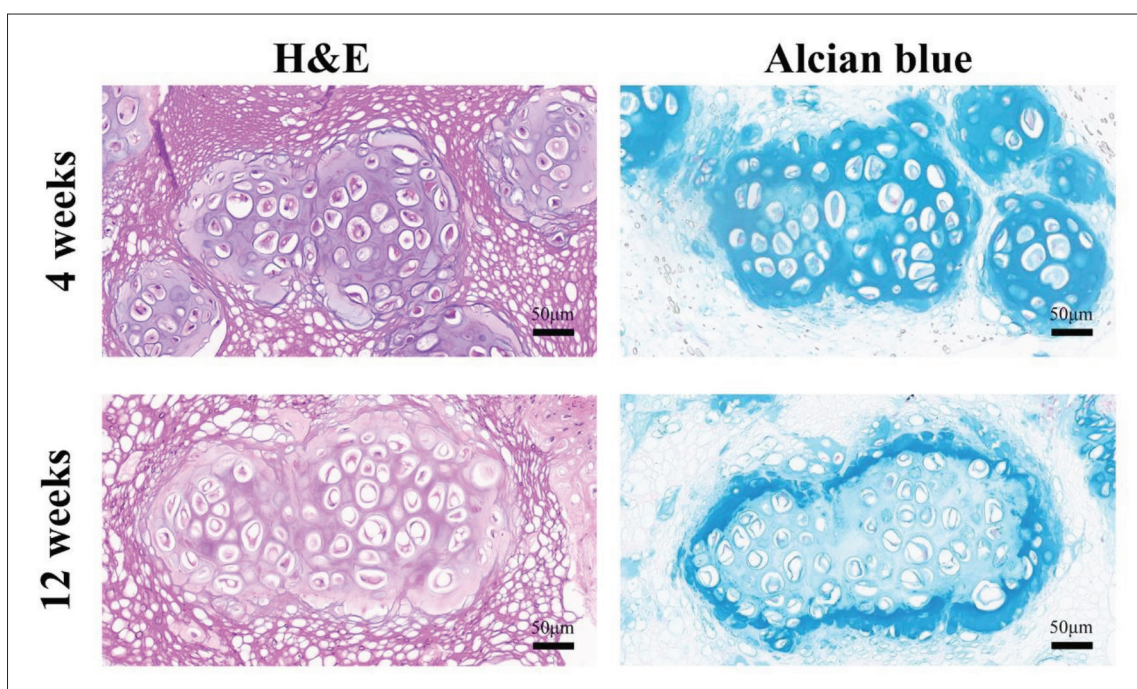


Figure S5. H&E and Alcian blue staining of the CS-laden constructs at 4 and 12 weeks of implantation *in vivo*, with black arrows pointing to the fusion between the adjacent CSs.

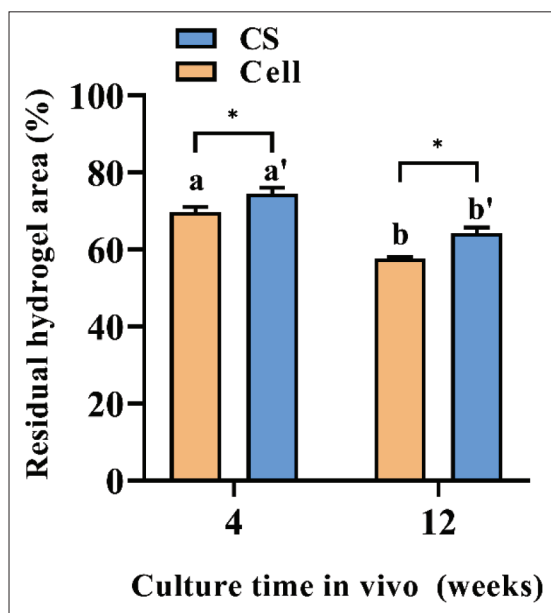


Figure S6. The percentage of the residual hydrogel within the two groups of constructs after 4 and 12 weeks of culture *in vivo*. Statistical analysis of the same group at different culture time is indicated by letters, and indication with different letters represents $p < 0.05$. Statistical analysis between different groups is indicated by asterisks: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.