Supporting Information

Bioprinted Biomimetic Hydrogel Matrices Guiding Stem Cell Aggregates for Enhanced Chondrogenesis and Cartilage Regeneration

Yuetian Liu[1, 2#], Lijuan Du[2#], Hua Zhang[2, 4]*, Guanrong Li[1, 2], Yang Luo[1, 2], Zeming Hu[2], Rong Xu[1, 2], Jie Yao[1], Zheyuan Shi[1, 2], Yige Chen[2], Chi Zhang[1], Zhanping Jin[1], Caihua Zhang[1], Chanchan Xie[1], Jun Fu[3]*, Yabin Zhu[2]*, Yingchun Zhu[1]*

[1] The First Affiliated Hospital of Ningbo University, Ningbo, Zhejiang 315010, China

[2] Research Institute of Smart Medicine and Biological Engineering, Health Science Center, Ningbo University, Ningbo, Zhejiang 315211, China

[3] Key Laboratory of Polymeric Composite and Functional Materials of Ministry of Education, Guangdong Engineering Technology Research Centre for Functional Biomaterials, School of Materials Science and Engineering, Sun Yat-Sen University, Guangzhou, 510275 China

[4] State Key Laboratory of Molecular Engineering of Polymers (Fudan University), Shanghai 200438, China

***Corresponding Authors**: fyyzhuyingchun@nbu.edu.cn; zhanghua@nbu.edu.cn; zhuyabin@nbu.edu.cn; fujun8@mail.sysu.edu.cn

[#]These authors contributed equally to this work.

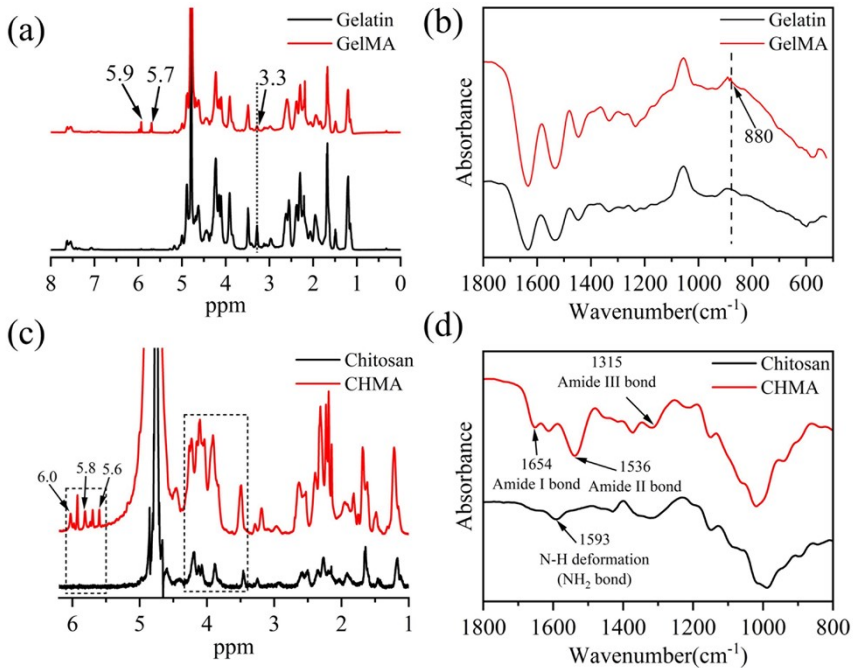

Figure S1. (a) ^{1}H-NMR and (b) FT-IR spectra and of gelatin and gelatin methacrylate (GelMA). (c) ^{1}H-NMR and (d) FT-IR spectra of chitosan and chitosan methacrylate (CHMA).

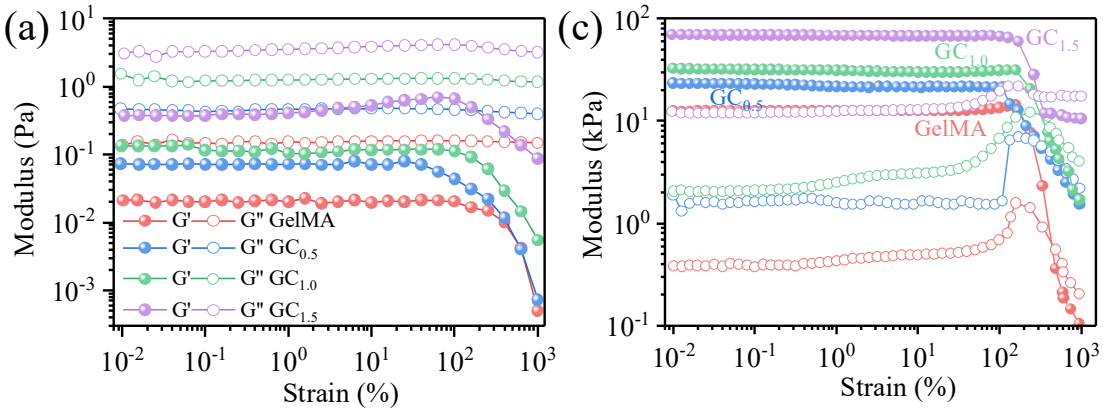

Figure S2. Amplitude sweep analysis of the GelMA and GC_m precursors (a) and their hydrogels (b) at 37 °C.

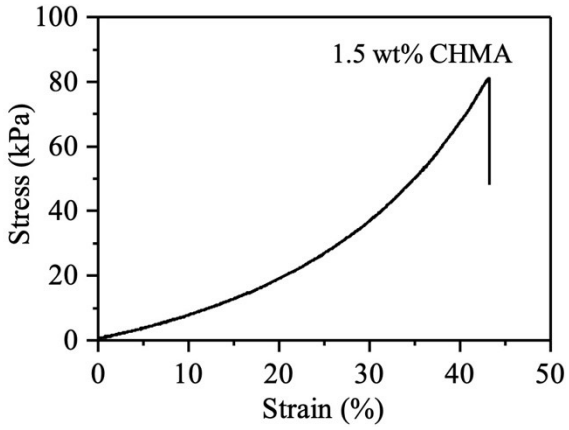

Figure S3. Compression stress-strain curve of the CHMA hydrogels.

Table S1. Polymer network pore sizes of hydrogels analyzed using rubber elastic theory.

Group	G (kPa)	V (nm^3)
10 wt% GelMA	12.66	338.1
$GC_{0.5}$	22.12	193.5
$GC_{0.5}$	32.59	131.3
$GC_{0.5}$	70.22	60.9

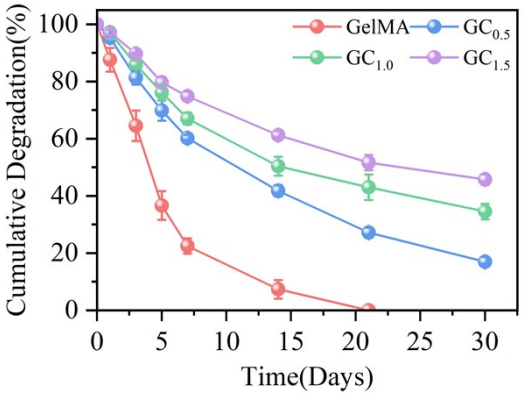

Figure S4. The *in-vitro* degradation behavior of GelMA and GC_m hydrogels.

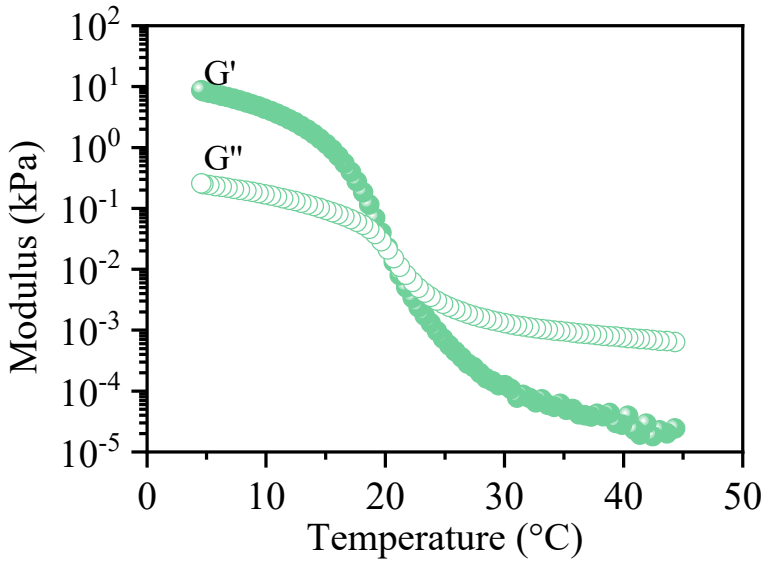

Figure S5. Thermo-sensitivity measurement via temperature sweep from 5 °C to 45 °C.

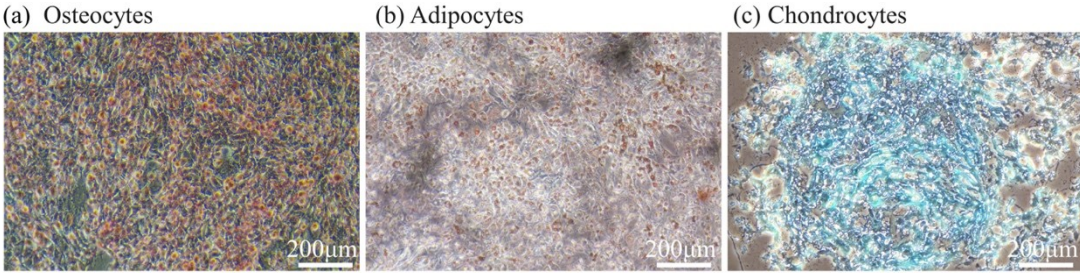

Figure S6. (a) Alizarin red staining, (b) Oil red O staining, (c) Alcian blue staining for osteogenically differentiated BMSCs, adipogenically differentiated BMSCs, and chondrogenically differentiated BMSCs, respectively.

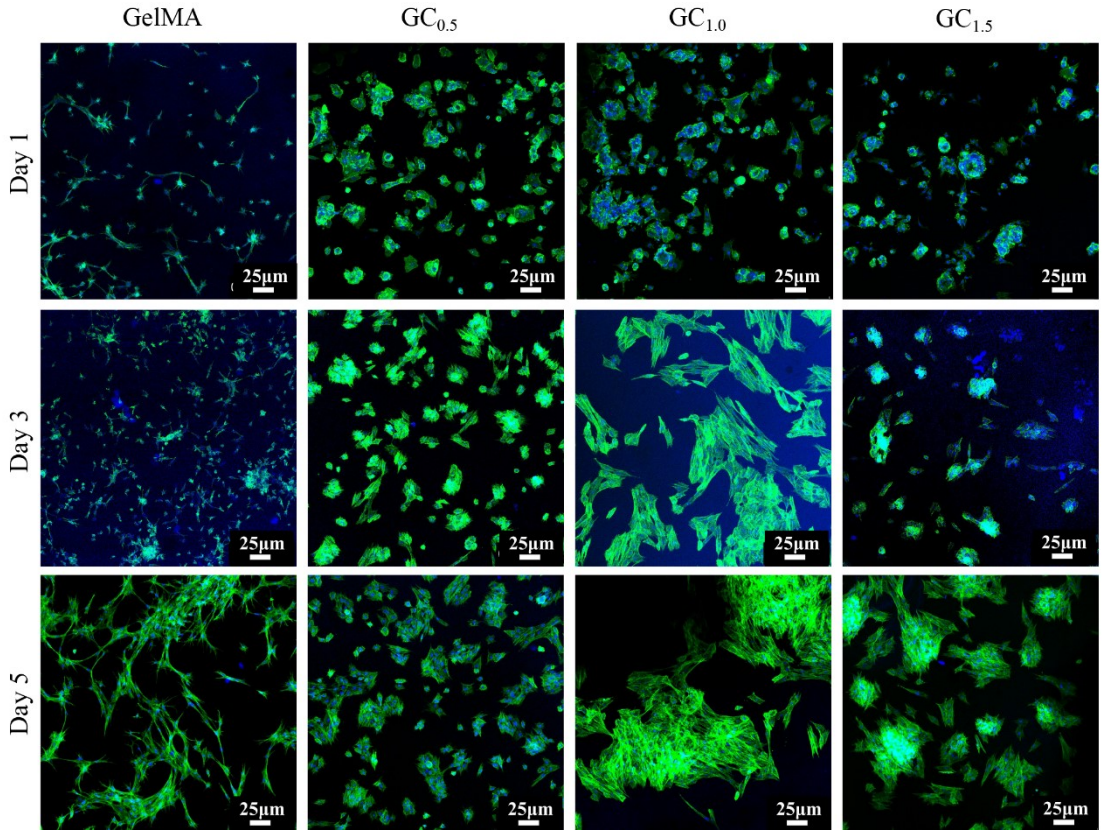

Figure S7. Fluorescence microscopy images of BMSCs micro-aggregate growth on the GelMA and GC_m hydrogel scaffolds (FITC-phalloidin for cytoskeleton, DAPI for nucleus) after 1, 3 and 5 days of culture, respectively.

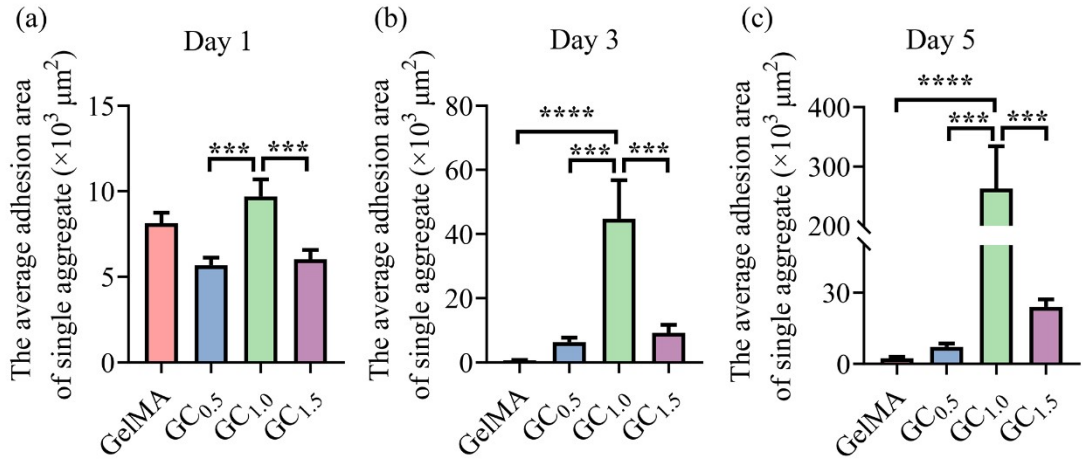

Figure S8. Adhesion area of each aggregate counted by the stained cellular

cytoskeleton at 1, 3 and 5 days.

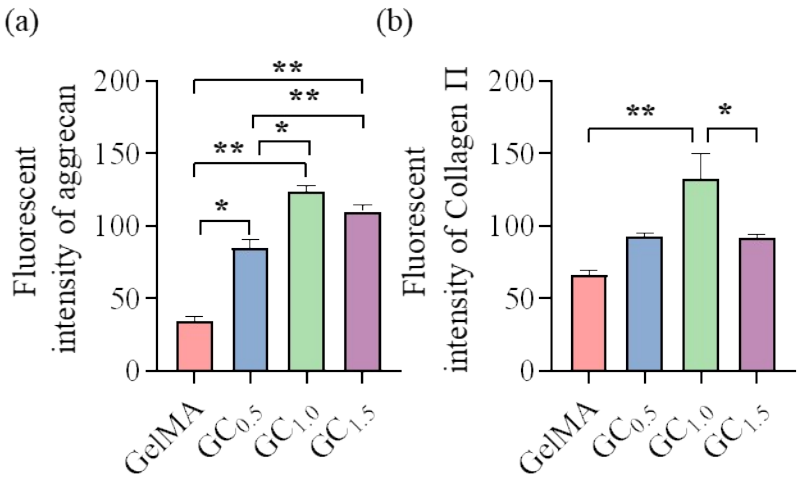

Figure S9. Fluorescent intensity analysis of (a) Aggrecan and (b) Collagen II protein expression.

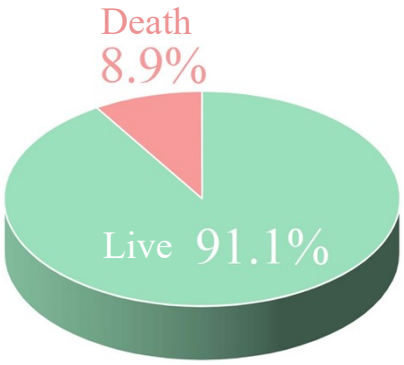

Figure S10. Survival rate of BMSCs cultured within 3D bioprinted hydrogel.

Table S2. (International Cartilage Repair Society) ICRS scoring system[1]

Cartilage repair evaluation	Points
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
0% repair of defect depth	0
Integration to border zone	
Complete integration with surrounding cartilage	4
Demarcating border <1 mm	3
3/4th of graft integrated, 1/4th with a notable border > 1 mm width	2
1/2 of graft integrated with surrounding cartilage, 1/2 with a notable border >1 mm	1
From no contact to 1/4th of graft integrated with surrounding cartilage	0
Macroscopic appearance	
Intact smooth surface	4
Fibrillated surface	3
Small, scattered fissures or cracs	2
Several, small or few but large fissures	1
Total degeneration of grafted area	0
Overall repair assessment	
Grade I: normal	12
Grade II: nearly normal	11-8
Grade III: abnormal	7-4
Grade IV: severely abnormal	3-1

Table S3. The modified O'Driscoll histologic score[2]

Characteristic	Score
I. Hyaline cartilage (%)	
80–100	8
60–80	6
40–60	4
20–40	2
0–20	0
II. Structural characteristics	
A. Surface irregularity	
Smooth and intact	2
Fissures	1
Severe disruption,fibrillation	0
B. Structural integrity	
Normal	2
Slight disruption, including cysts	1
Severe lack of integration	0
C. Thickness	
100% of normal adjacent cartilage	2
50% to 100% or thicker than normal	1
0–50%	0
D. Bonding to adjacent cartilage	
Bonded at both ends of graft	2
Bonded at one end/partially both ends	1
Not bonded	0
III. Freedom from degenerate changes in adjacent cartilage	
Normal cellularity, no clusters, normal staining	3
Normal cellularity, mild clusters, moderate staining	2
Mild or mod hypocellularity, slight staining	1
Severe hypocellularity, slight staining	0
IV. Reconstitution of subchondral bone	
Complete reconstitution	2
Greater than 50% recon	1
50% or less recon	0
V. Safrinin O staining	
>80% homogeneous positive stain	2
40%–80% homogeneous positive	1

	stain	
	<40% homogeneous positive stain	0
Total score		Max23

Reference

[1] M. P. J. Van den Borne, N. J. H. Raijmakers, J. Vanlauwe, J. Victor, S. N. De Jong, J. Bellemans, D. B. F. Saris. *Osteoarthritis and Cartilage*. **2007**. 15(12), 1397-1402.

[2] Orth Patrick, Madry Henning. *Histology and histopathologi*, **2015**, 30(8), 911-919.