

Supporting Information

Heterogenous hydrogel mimicking the osteochondral ECM applicable to tissue regeneration

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MC3T3 cells	Group	AAm (wt.%)	VPA (wt.%)	Alginate (wt.%)	MBAA (wt.%)	Iragcure 2959 (wt.%)	Mineral ized
	pAAm	15	0	1	1	0.5	No
	pAAm-VPA	13.5	1.5	1	1	0.5	No
	pAAm-VPA @M	13.5	1.5	1	1	0.5	Yes
chondro cytes	Group	GelMA (wt.%)	AGA (wt.%)	PEGDA (wt.%)	CaCl ₂ (mM)	Iragcure 2959 (wt.%)	
	PEGDA	0	0	15	100	0.5	
	GelMA	15	0	0	100	0.5	
	GelMA-AGA	14	1	0	100	0.5	

Table S1. Formulas for *in vitro* cell study.

Gene	Primer sequences
GAP-Forward	TTCAACGGCACAGTCAAG
GAP-Reverse	TACTCAGCACCAGCATCA
OCN-Forward	GCAGTAAGGTGGTGAATAGA
OCN-Reverse	AACGGTGGTGCCATAGAT
COL1a1-Forward	CGAGTATGGAAGCGAAGG
COL1a1-Reverse	GCAGTGATAGGTGATGTTCT
Runx2-Forward	CTTCGTCAGCGTCCTATC
Runx2-Reverse	CTTCATCAGCGTCAACA
ACAN-Forward	CGAGTGAACAGCATCTACC
ACAN-Reverse	GAGTCATTGGAGCGAAGG
COL2a1-Forward	CAGCAAGAGCAAGGAGAA
COL2a1-Reverse	GGACAGTAGACGGAGGAA
SOX9-Forward	CCAGAGAACGCACATCAA
SOX9-Reverse	GTGGTCGGTGTAGTCATAC

Table S2. The sequences of primers used for real-time PCR

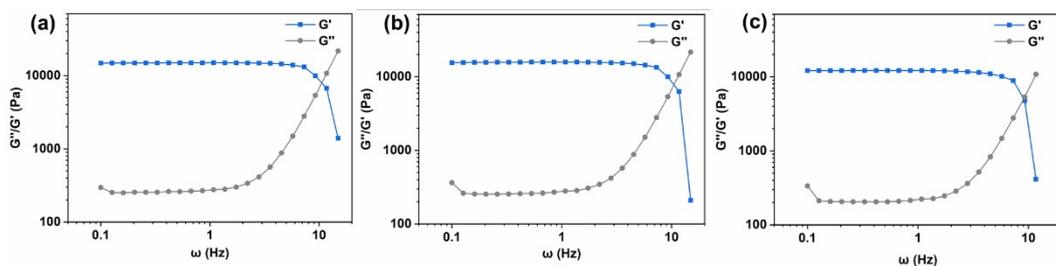


Figure S1. Frequency sweep for double layer hydrogel when the bottom layer was pre-irradiated for a) 3 min, b) 5 min and c) 7 min respectively

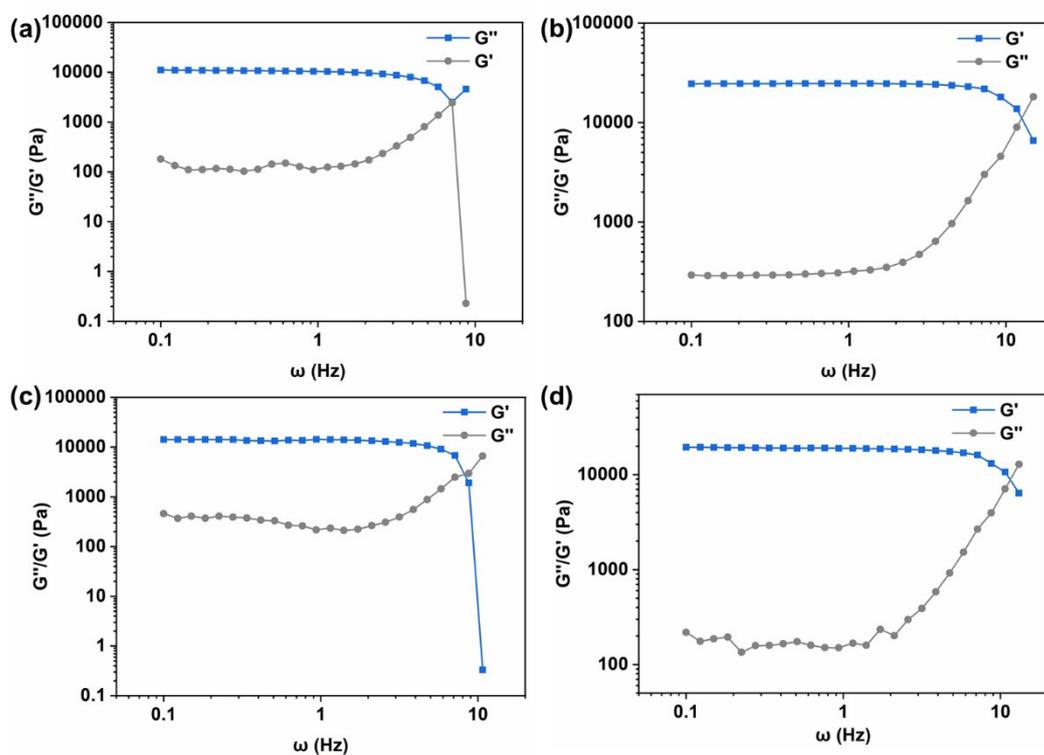


Figure S2. Frequency sweep for a) upper, b) bottom, c) double layer hydrogel without chelation, d) double layer hydrogel after mineralization respectively.

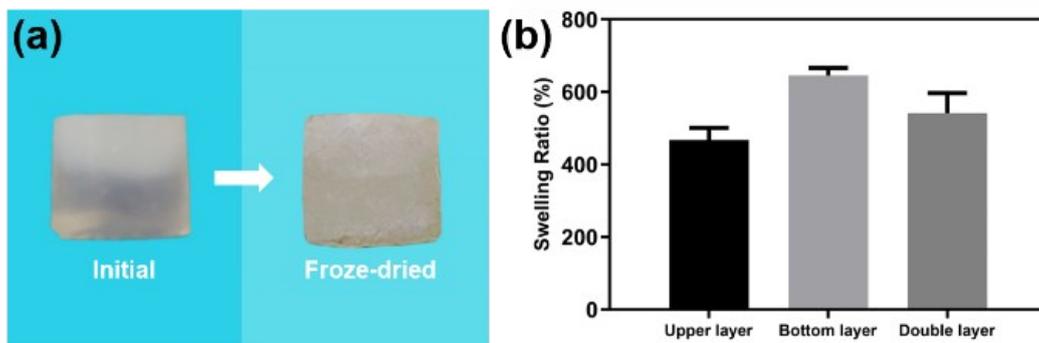


Figure S3. (a) Photographs of native bilayer hydrogel and lyophilized hydrogel. (b) Swelling ratio of single and double layer hydrogels (n=4).

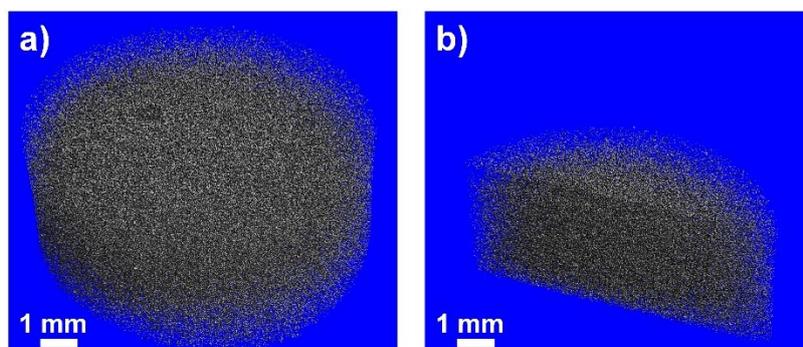


Figure S4. 3D reconstruction of hydrogel showing the pore interconnectivity.

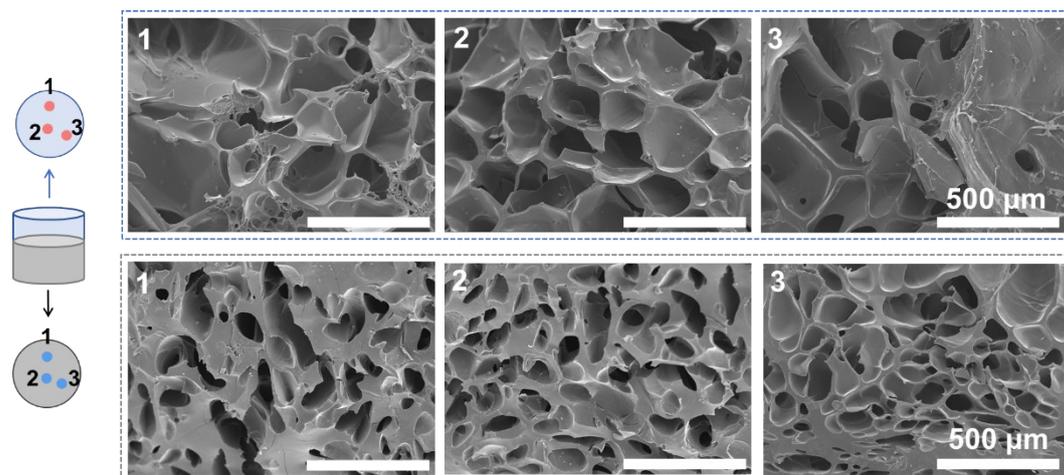


Figure S5. SEM images of double layer hydrogel of different sites, which illustrates the porosity of hydrogel.

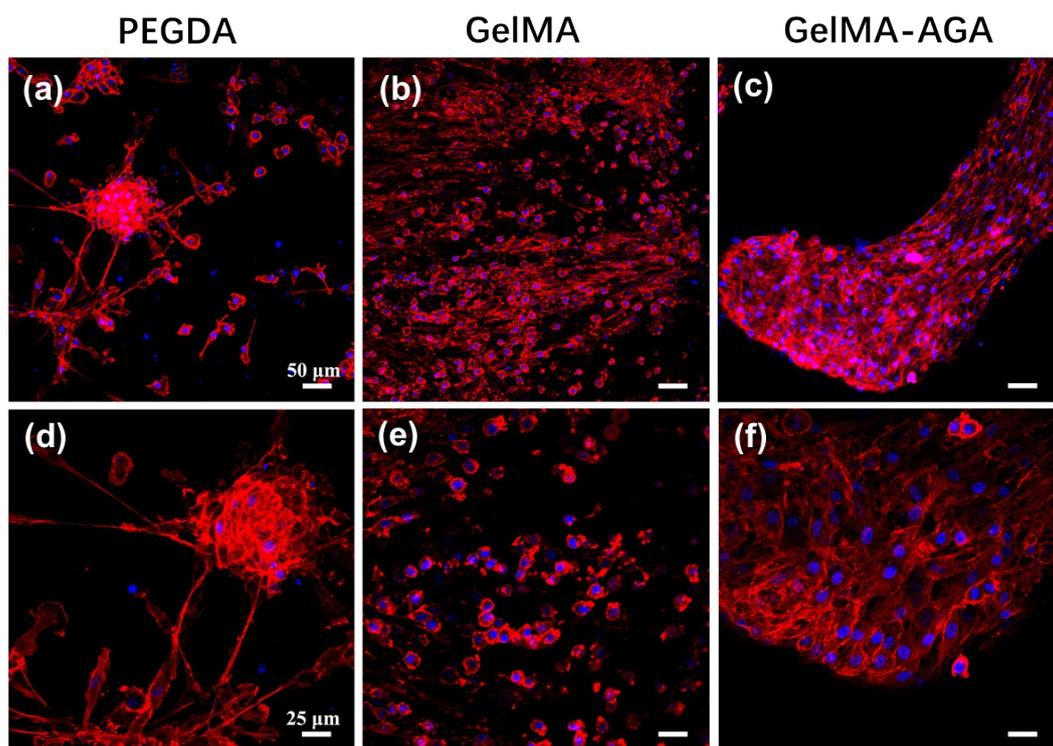


Figure S6. Fluorescence microscopic images of chondrocytes cultured on upper layer hydrogels for 7 days. Scale bar: 50 μm and 25 μm .

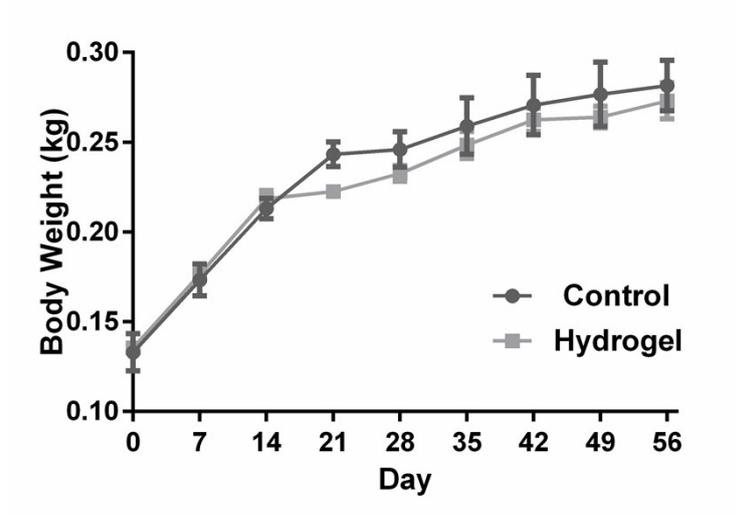


Figure S7. Body weight of SD rats in different groups post-surgery (n=3).

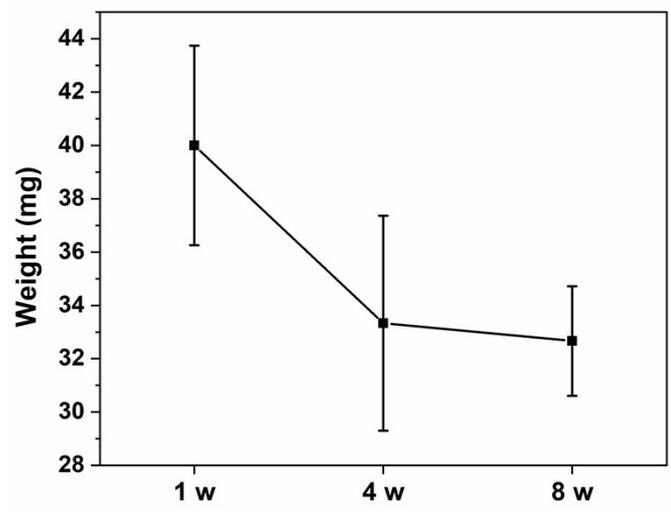


Figure S8. The weight of hydrogel at implantation of 1, 4, and 8 weeks

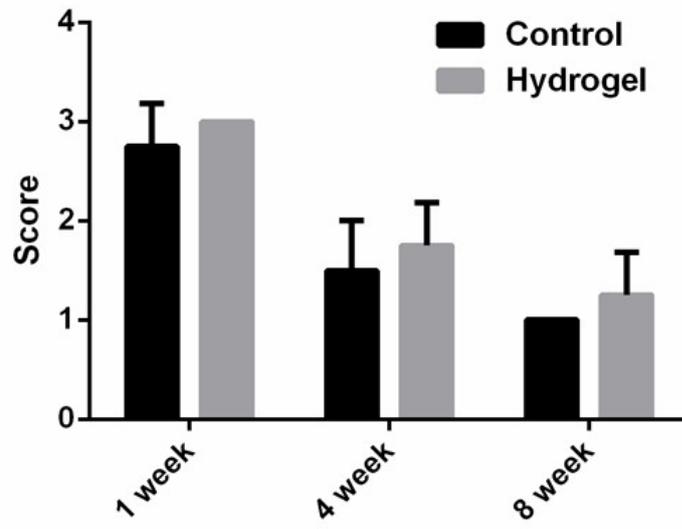


Figure S9. Score of HE stains in subcutaneous tissue (n=3).

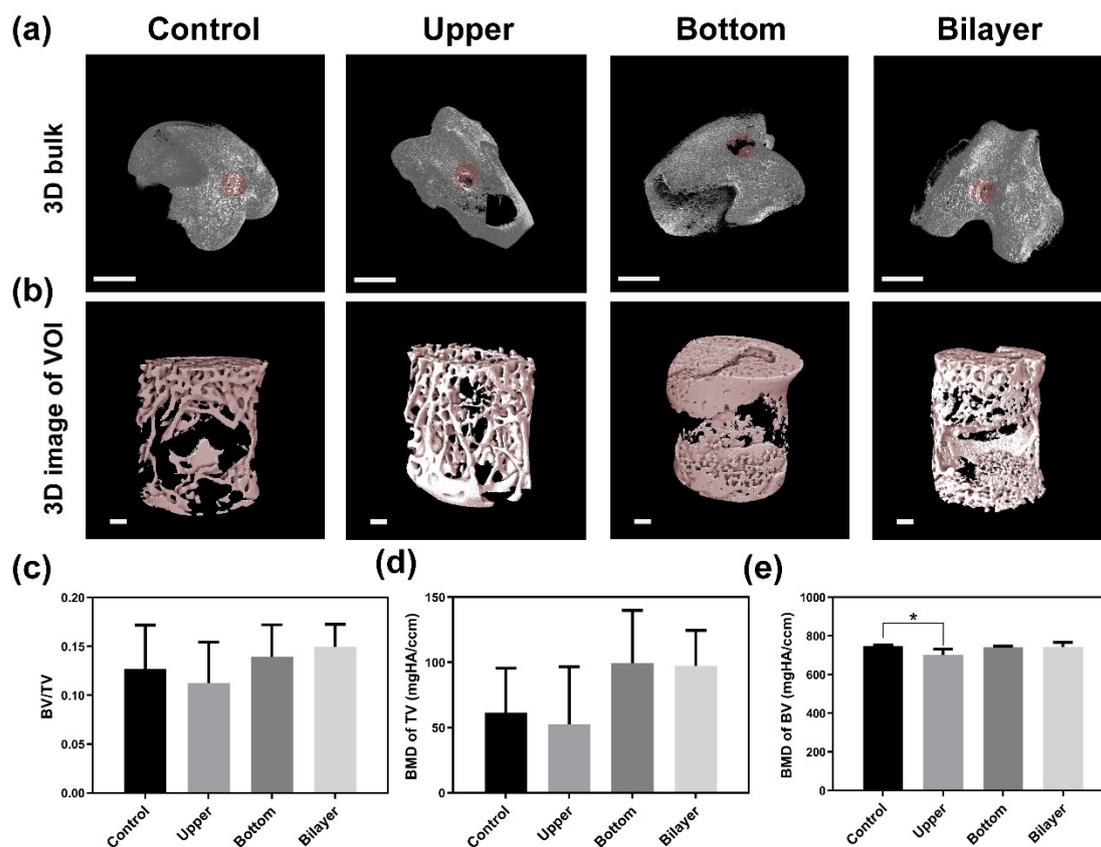


Figure S10. (a) 3D reconstruction of samples for different groups (implantation area is colored red, scale bar: 5 mm). (b) The reconstructed VOI images of defect area for different groups (scale bar: 500 μm). (c) Bone volume/total volume (BV/TV), (d) bone mineral density of total volume (BMD of TV) and (e) bone mineral density of bone volume (BMD of BV) calculated from Micro-CT results (n=4). Significances ($p < 0.5$, $p < 0.01$ and $p < 0.001$) were suggested as asterisks (*, ** and ***).

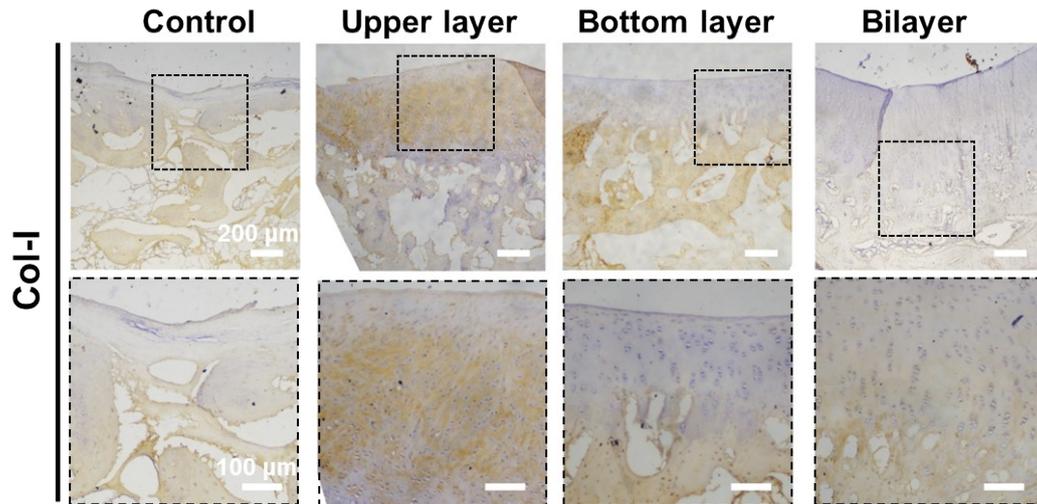


Figure S11. Col-I stain of collected knee joints after 6 weeks of implantation (scale bar: 200 μm and 100 μm).

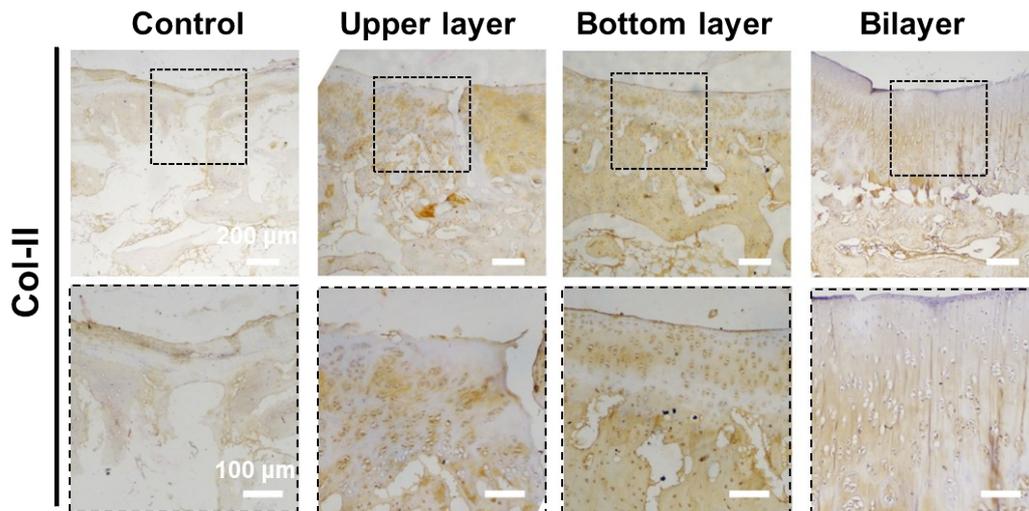


Figure S12. Col-II stain of collected knee joints after 6 weeks of implantation (scale bar: 200 μm and 100 μm).