ACCSCIENCE PUBLISHING

International Journal of Bioprinting

RESEARCH ARTICLE

Collagen bioinks redefined: Optimizing ionic strength and growth factor delivery for cartilage tissue engineering

Supplementary File

Purity of the extract

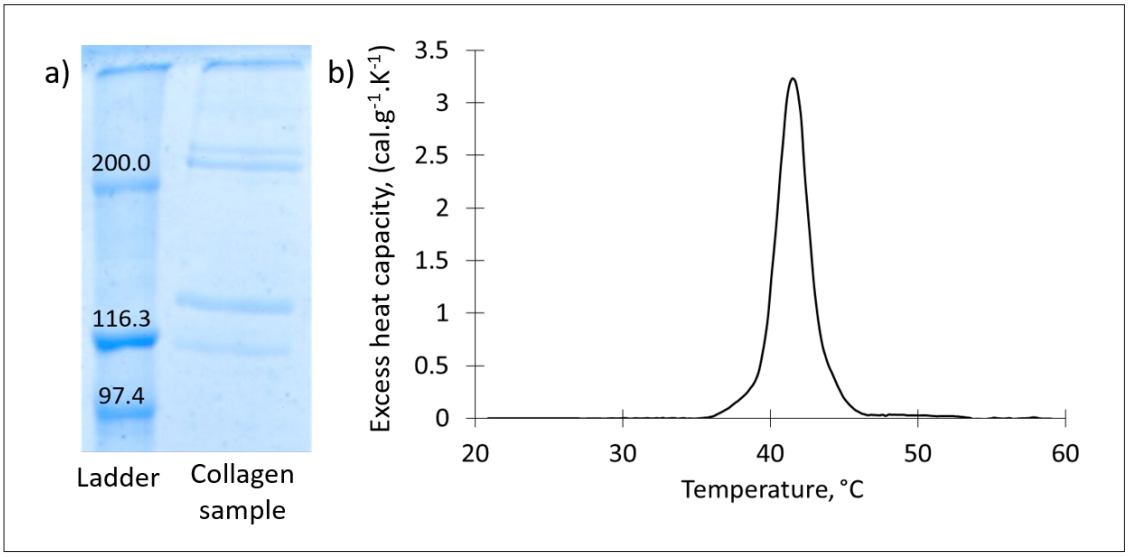

Figure S1. High quality of the extracted collagen was ascertained by (a) sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and (b) differential scanning calorimetry (DSC).

Rheology of collagen gelation

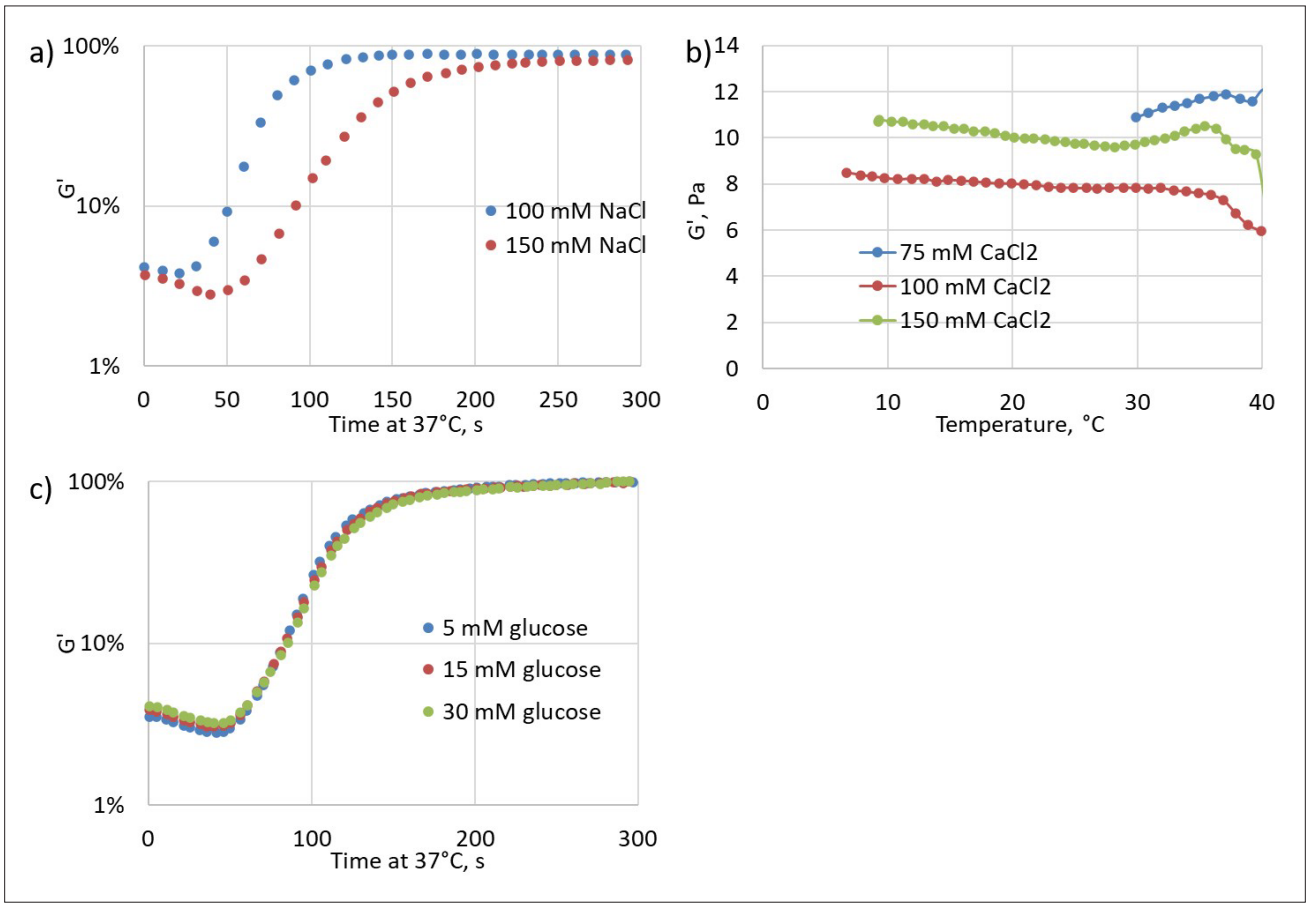

Figure S2. Results from rheological studies in oscillation mode, demonstrating the effect of (a) Cl^- ions, (b) Ca^{2+} ions, and (c) glucose in the presence of 1× phosphate-buffered saline (PBS) on storage modulus (G'), which corresponds to collagen gelation.

Proliferation of cells bioprinted in collagen inks with subphysiological ionic strength

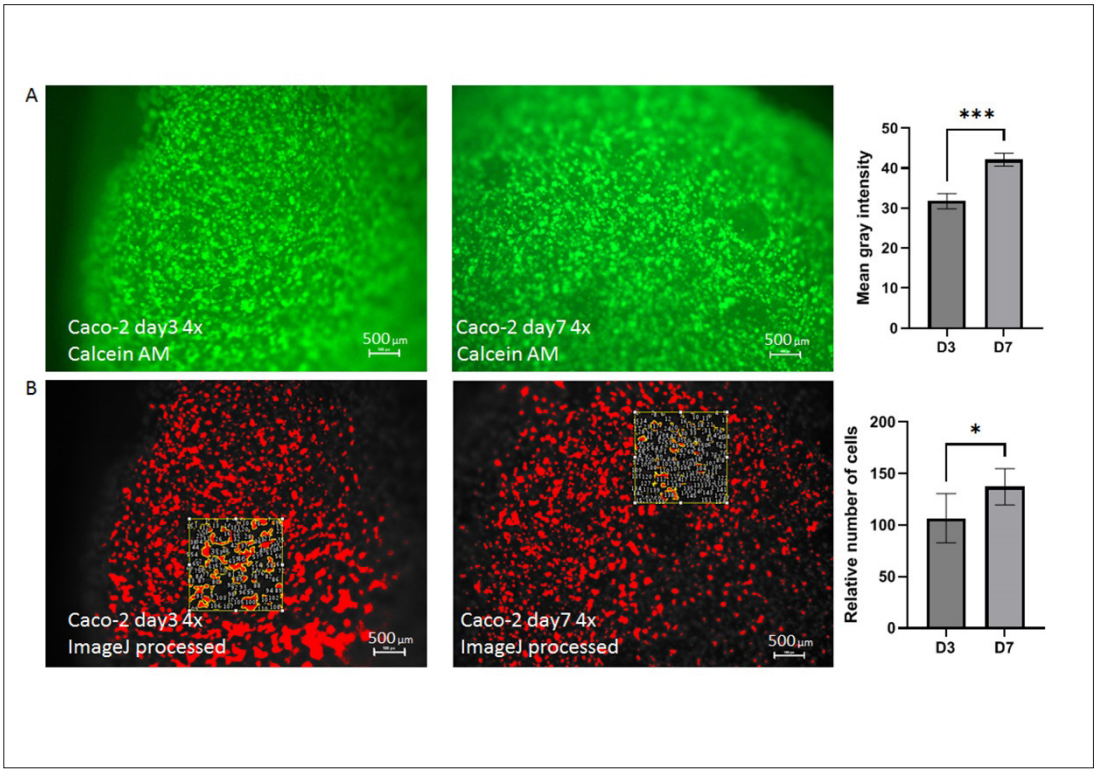

Figure S3. Assessment of proliferation of Caco-2 cells. (a) Representative images of calcein AM-stained Caco-2 cells on days 3 (left) and 7 (right) at 4× magnification. (b) Processing of the images in (a) with ImageJ for assessing the mean intensity and number of cells (particles) per ~2 mm^2. Images were processed in the same way (i.e., adjusting contrast and threshold values to the same parameters). Mean grey intensity and number of cells (particles) were estimated from at least two different regions of interest (~2 mm^2 each) from two images taken on day 3 and at least two different regions of interest from images taken on day 7. Scale bars:500 µm. Graphs represent the mean of the measurements with error bars (standard deviation). Student's t-test values of * denotes $p < 0.05$, and *** annotates $p < 0.0005$.

Collagen printability

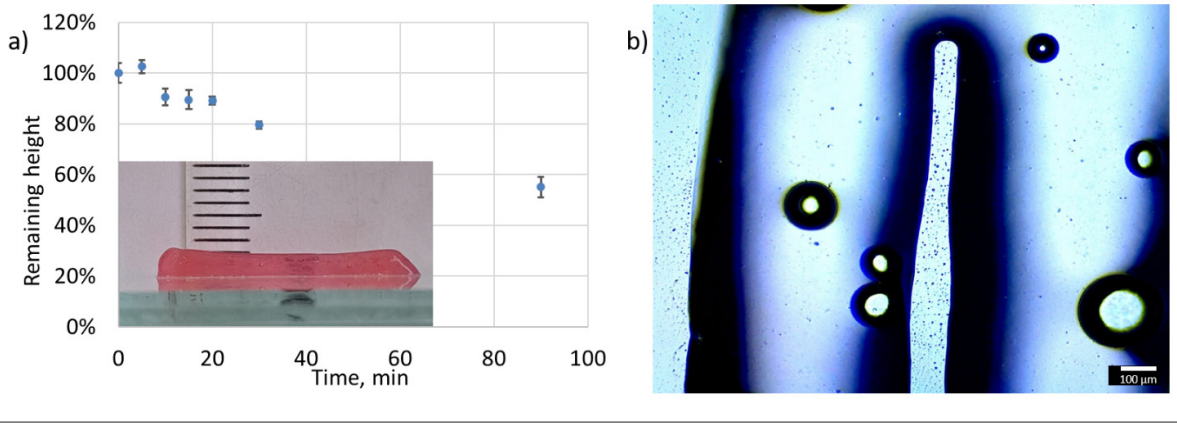

Figure S4. Collagen printability: (a) stability of a 10-layer "wall" at ambient conditions over 90 min (n = 5), and inset image displays a representative as-deposited 10-layer "wall"; and (b) collagen strands printed as close as 80 µm apart. Scale bar: 100 µm (b).

Comparison of KEGG pathways for glycosaminoglycan synthesis

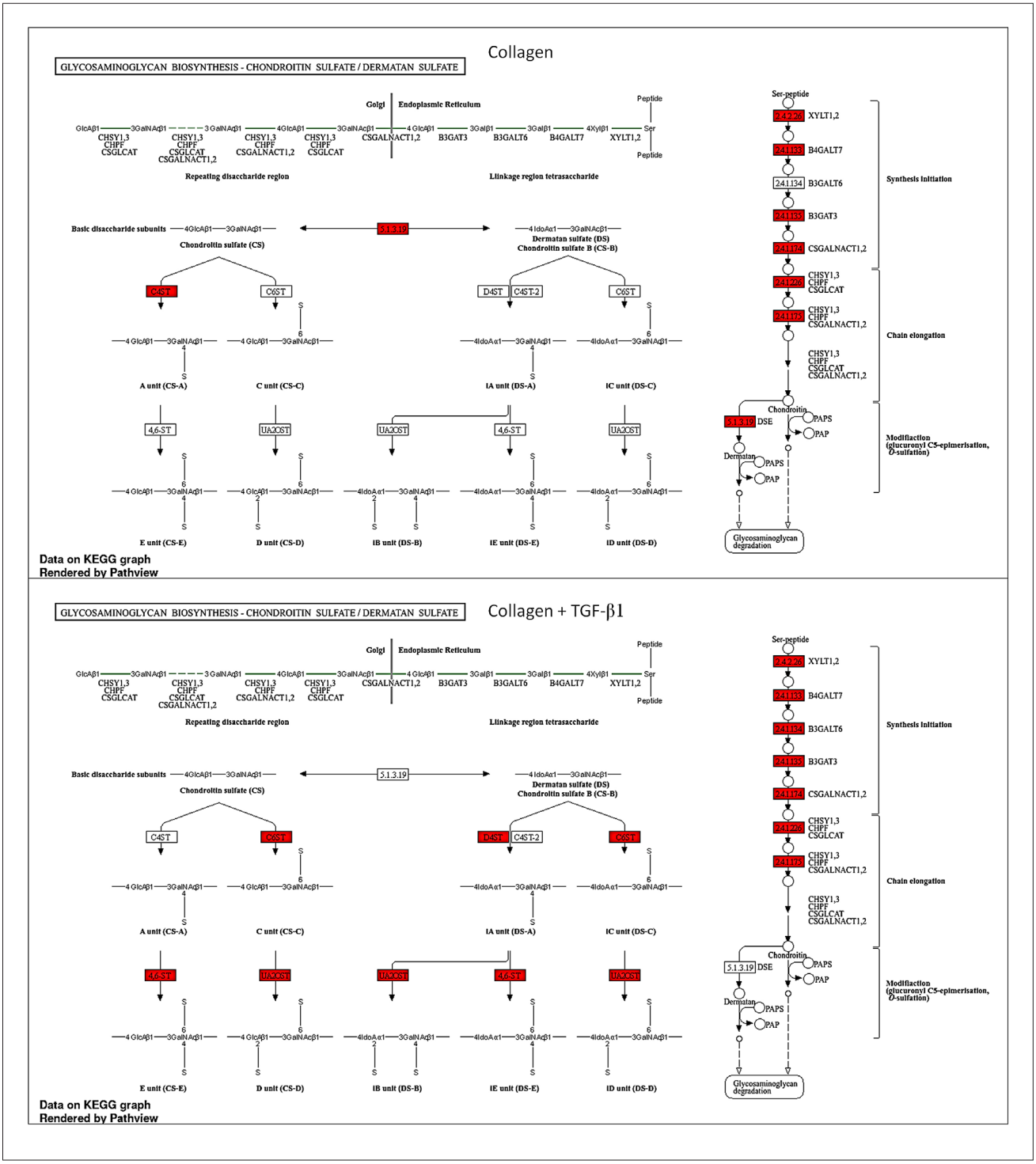

Figure S5. Comparison of upregulated genes in stromal vascular fraction (SVF) cells bioprinted in pure collagen (top) and biofunctionalized collagen (bottom) involved in cartilage extracellular matrix (ECM) biosynthesis.

Gene ontology analysis confirmation with StringDB

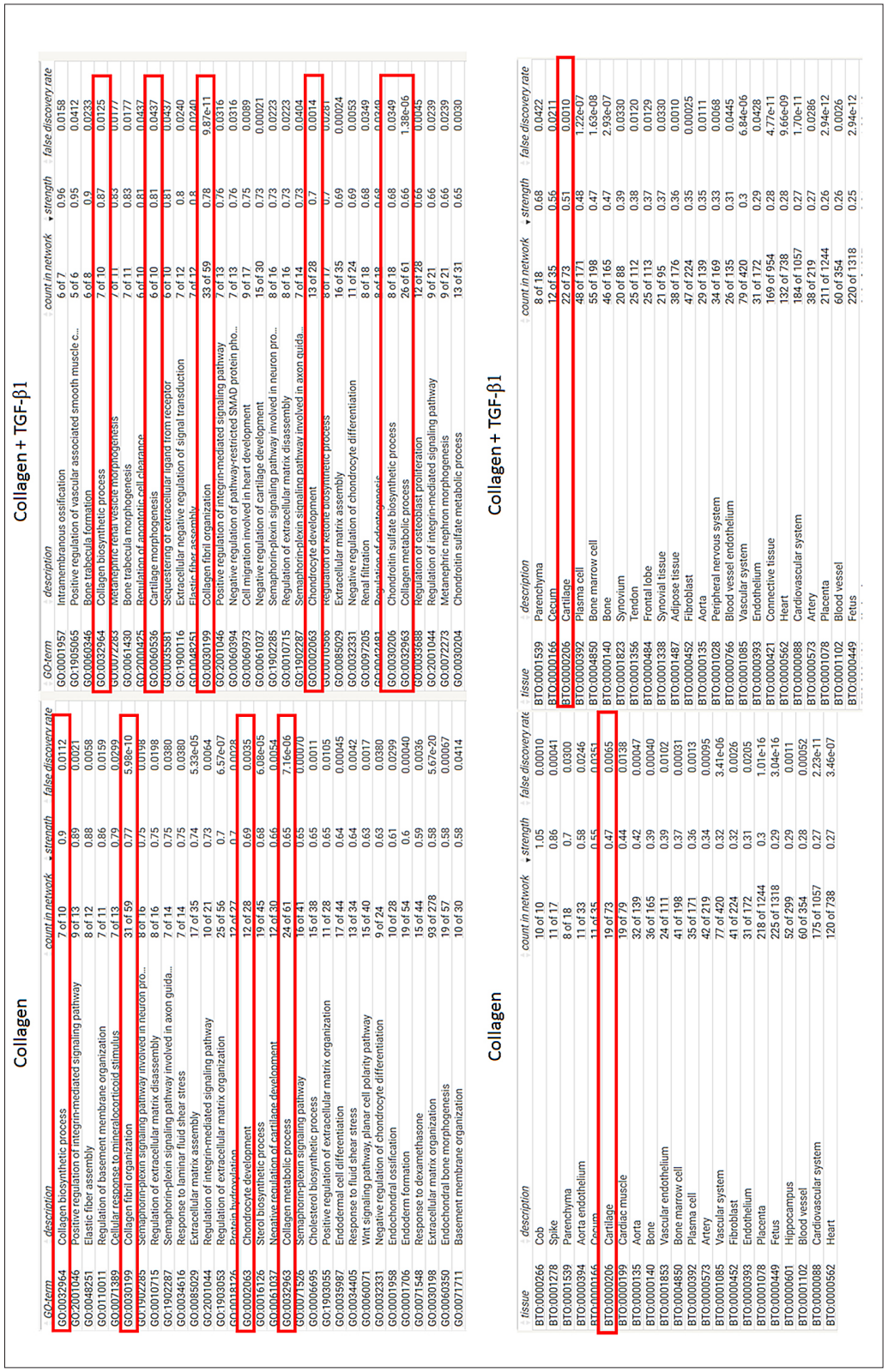

Figure S6. Gene ontology analysis with StringDB for biological processes and tissue expression. Red boxes highlight annotations of interest related to chondrogenesis.

Analysis of genes that are distinctly upregulated in 3D-bioprinted, biofunctionalized collagen

Biological Process (Gene Ontology)

GO-term	description	count in network	strength	false discovery rate
GO:2000096	Positive regulation of Wnt signaling pathway, planar cell pol...	5 of 9	1.06	0.0336
GO:0060536	Cartilage morphogenesis	5 of 10	1.01	0.0426
GO:2000052	Positive regulation of non-canonical Wnt signaling pathway	7 of 16	0.95	0.0102
GO:0060973	Cell migration involved in heart development	7 of 17	0.93	0.0128
GO:2000095	Regulation of Wnt signaling pathway, planar cell polarity pat...	6 of 16	0.88	0.0406
GO:0014829	Vascular associated smooth muscle contraction	6 of 17	0.86	0.0481
GO:2000050	Regulation of non-canonical Wnt signaling pathway	9 of 27	0.83	0.0069
GO:0035116	Embryonic hindlimb morphogenesis	8 of 28	0.77	0.0246
GO:0045668	Negative regulation of osteoblast differentiation	12 of 46	0.73	0.0033
GO:0035137	Hindlimb morphogenesis	9 of 35	0.72	0.0210
GO:0030501	Positive regulation of bone mineralization	11 of 44	0.71	0.0079
GO:0035115	Embryonic forelimb morphogenesis	8 of 32	0.71	0.0426
GO:0045778	Positive regulation of ossification	13 of 54	0.69	0.0031
GO:0010935	Regulation of macrophage cytokine production	9 of 38	0.69	0.0313
GO:0035136	Forelimb morphogenesis	9 of 39	0.67	0.0347
GO:0070169	Positive regulation of biomineral tissue development	12 of 54	0.66	0.0087
GO:0030282	Bone mineralization	12 of 57	0.63	0.0121
GO:0010862	Positive regulation of pathway-restricted SMAD protein pho...	10 of 49	0.62	0.0377
GO:0042733	Embryonic digit morphogenesis	11 of 57	0.6	0.0322
GO:0006939	Smooth muscle contraction	11 of 60	0.57	0.0416
GO:0045667	Regulation of osteoblast differentiation	22 of 125	0.56	0.00061
GO:0060393	Regulation of pathway-restricted SMAD protein phosphoryla...	11 of 62	0.56	0.0497
GO:0030278	Regulation of ossification	21 of 120	0.55	0.00096
GO:0030500	Regulation of bone mineralization	14 of 80	0.55	0.0165
GO:0030326	Embryonic limb morphogenesis	20 of 120	0.53	0.0024
GO:1903510	Mucopolysaccharide metabolic process	14 of 84	0.53	0.0224
GO:0030509	BMP signaling pathway	15 of 92	0.52	0.0184
GO:0006029	Proteoglycan metabolic process	13 of 80	0.52	0.0381
GO:0035108	Limb morphogenesis	23 of 144	0.51	0.0012
GO:0060070	Canonical Wnt signaling pathway	16 of 100	0.51	0.0148
GO:0006022	Aminoglycan metabolic process	19 of 126	0.49	0.0086

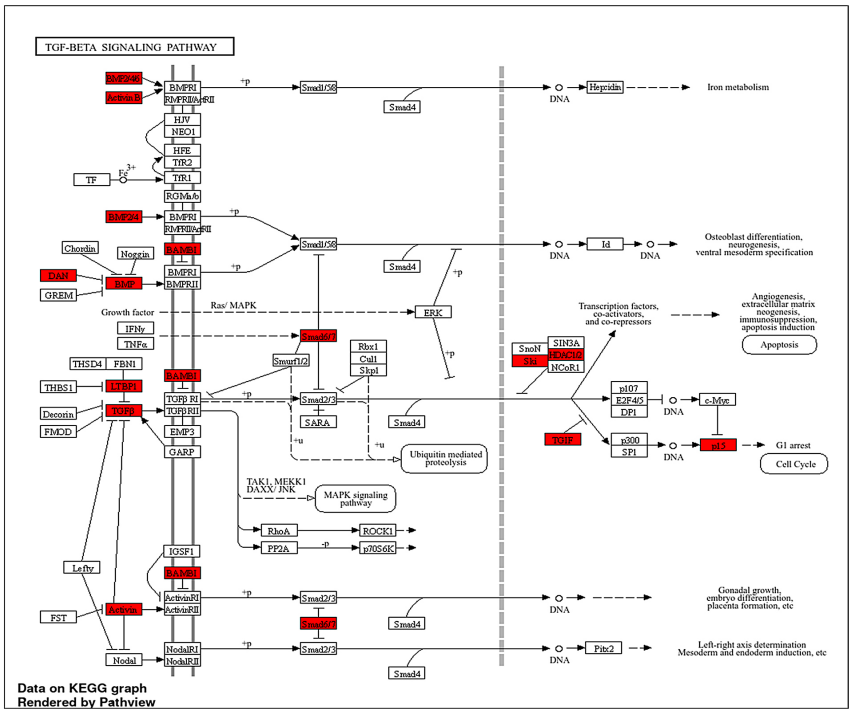

Figure S7. Gene ontology analysis with StringDB for biological processes and KEGG TGF pathway. Only genes that are exclusively upregulated in stromal vascular fraction (SVF) cells printed in collagen and TGF-β are considered in this analysis.

Video S1. Time lapse microscopy of 3D bioprinted CHON-001 cells one day after bioprinting. The video reveals the biocompatibility of the hydrogel with respect to cell viability and cell motility of chondrocytes within the bioprints.