

Title: Stem cells catalyze cartilage formation by neonatal articular chondrocytes in 3D biomimetic hydrogels

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Supplementary Information

Figures S1-S7

Tables S1-S4

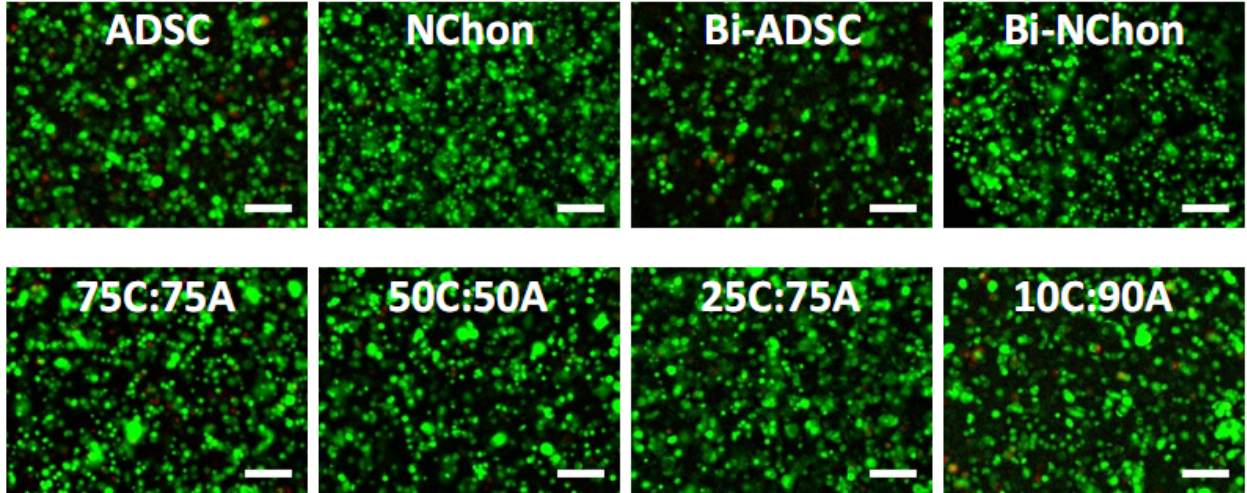


Figure S1. Live/dead staining of cell-hydrogel constructs 24 hours post-encapsulation showing >90% viability in all groups. Scarebars, 100 μ m.

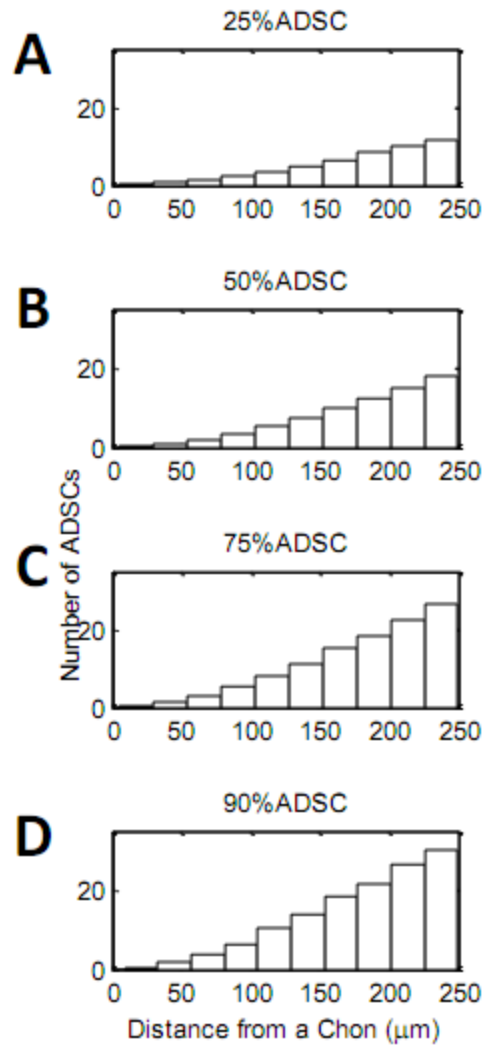


Figure S2. Histograms showing the distribution of intercellular distances between ADSCs and NChons that are within effective communication distance (250 μm) from an NChon in mixed cell culture with (A) 25% ADSC, (B) 50% ADSC, (C) 75% ADSC, and (D) 90% ADSC.

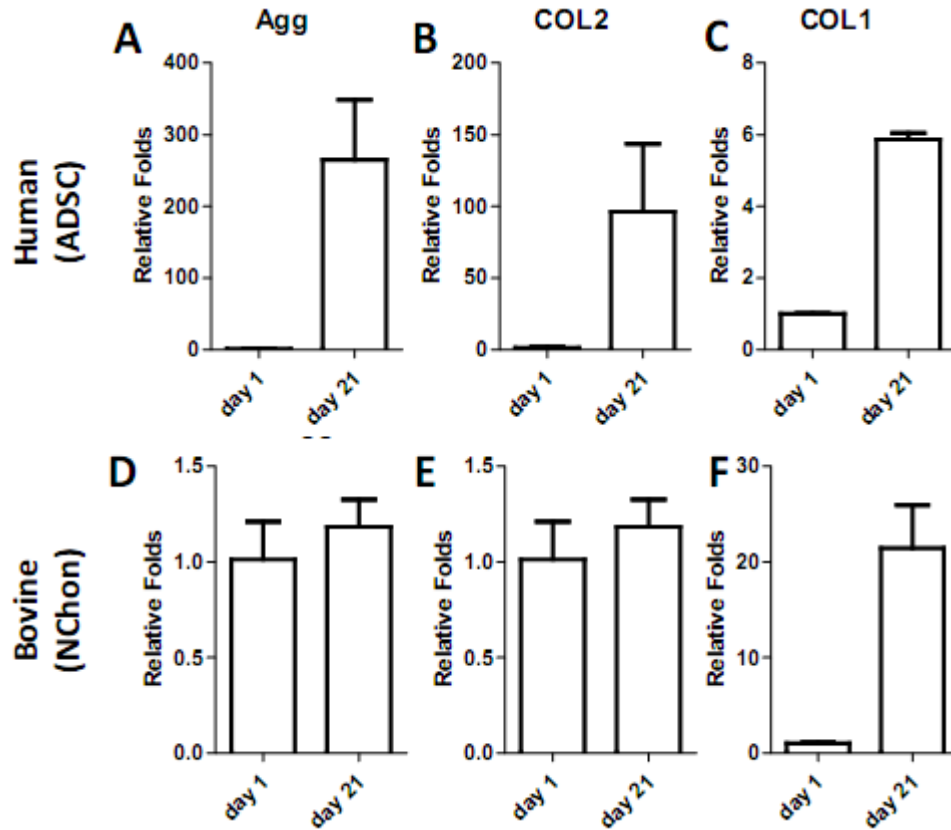


Figure S3. Gene expression of human adipose-derived stem cells and neonatal chondrocytes at day 1 and day 21 including (A, D) aggrecan (Agg), (B, E) type II collagen (COL II), and (C, F) type I collagen (COL I) of (A-C) ADSCs and (D-F) NChons after 21 days of culture in chondrogenic medium with TGF- β 3 supplementation. Fold change relative to day 1 ADSC (A-C) and NChon (D-F) controls. Data presented as mean \pm SD (n=3 samples/group).

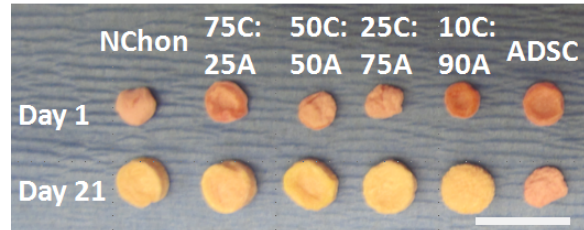


Figure S4. Gross appearance of the freeze-dried hydrogel constructs containing mixed cell-culture at day 1 and day 21. NChon control as well as all the mixed cell constructs increased in size at day 21 while ADSC control decreased in size. Scale bar, 10mm.

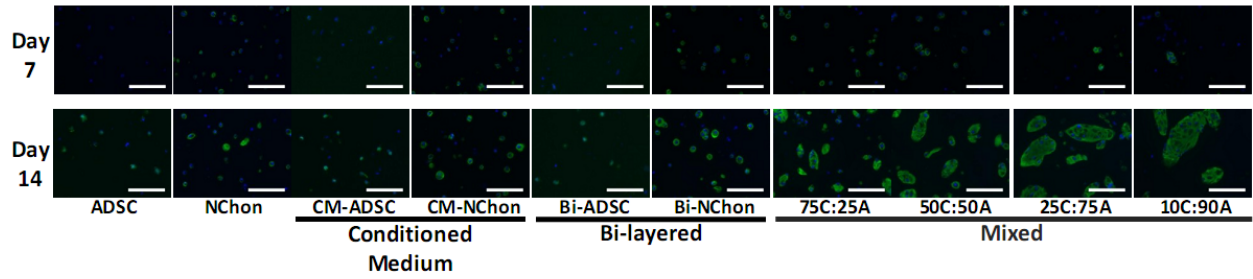


Figure S5. Immunostaining of type II collagen at day 7 (top row) and day 14 (bottom row) in various co-culture groups. Cells were evenly distributed in the hydrogel construct at day 7. At day 14, cell aggregates and cartilage nodules (type II collagen positive, green) started to emerge in all the mixed cell culture groups. Increasing ADSC ratio in the mixed co-culture led to an increase in the size of individual neocartilage nodules. Scale bars, 100 μ m.

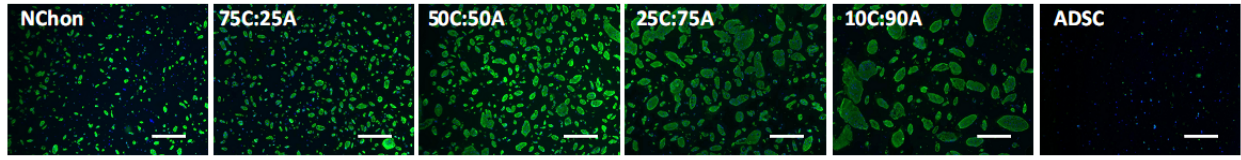


Figure S6. Immunostaining of type II collagen at day 21 at low magnification. Scale bars, 300 μm

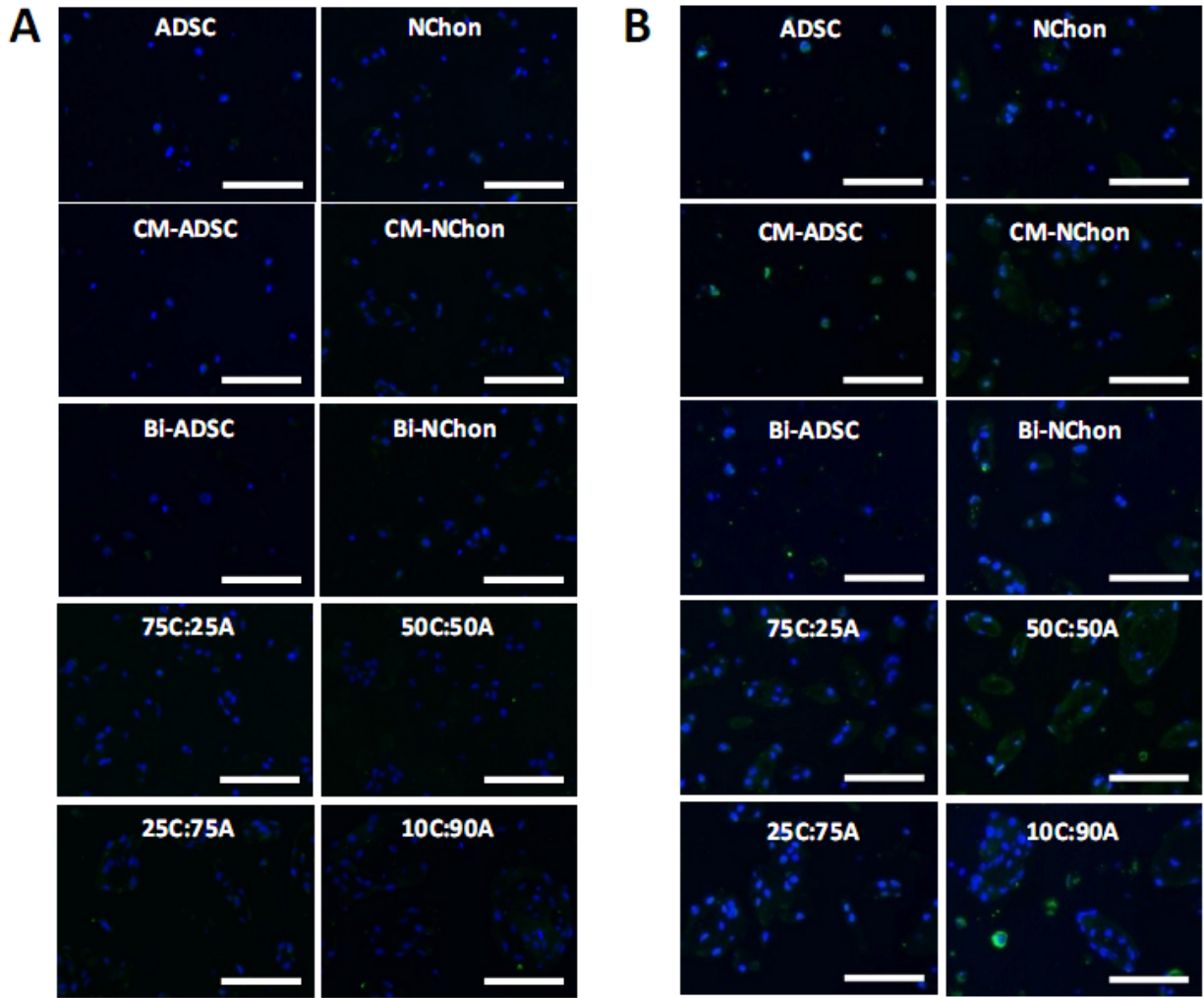


Figure. S7. Immunostaining of (A) type I collagen and (B) type X collagen at day 21 showing that type I and type X collagen were stained minimally. Scale bars, 100μm.

Table S1: DNA per wet weight on day 1.

Group	DNA/w.w. (ug/g)
	Average \pm standard deviation
NChon	53.46 \pm 6.48
75C:75A	50.76 \pm 3.0
50C:50A	53.02 \pm 2.51
25C:75A	53.04 \pm 3.65
10C:90A	52.37 \pm 2.07
ADSC	55.77 \pm 0.47

Table S2: Compressive moduli on day 1.

Group	Compressive moduli (kPa)
	Average \pm standard deviation
NChon	28.44 \pm 2.06
75C:75A	29.50 \pm 3.61
50C:50A	21.50 \pm 3.21
25C:75A	25.69 \pm 0.56
10C:90A	23.64 \pm 1.04
ADSC	31.89 \pm 3.61

Table S3: Interaction index for sGAG per DNA and collagen per DNA in mixed co-culture groups.

Group	Interaction index for sGAG/DNA	Interaction index for Collagen/DNA
	Average \pm standard deviation	Average \pm standard deviation
75C:75A	0.91 \pm 0.09	0.98 \pm 0.05
50C:50A	0.95 \pm 0.04	1.07 \pm 0.03
25C:75A	0.95 \pm 0.06	1.13 \pm 0.03
10C:90A	0.93 \pm 0.04	1.19 \pm 0.15

Table S4: List of species-specific primers used for real-time polymerase chain reaction.

Gene Name	Species	Primer Sequence	GenBank No.
GAPDH	Human	F: 5' CGCTCTCTGCTCCTCCTGTT 3' R: 5' CCATGGTGTCTGAGCGATGT 3'	NM_002046.3
	Bovine	F: 5' AGATGGTGAAGGTCGGAGTG R: 5' GATCTCGCTCCTGGAAGATG	NM_001034034.1
Aggrecan (Agg)	Human	F: 5' TGAGGAGGGCTGGAACAAGTACC 3' R: 5' GGAGGTGGTAATTGCAGGGAACA 3'	NM_001135.3
	Bovine	F: 5' CACCACAGCAGGTGAACTAGA 3' R: 5' GCTTGCTCCTCCACTAATGTC 3'	NM_173981.2
COL2A1 (COL2)	Human	F: 5' TCACGTACACTGCCCTGAAG 3' R: 5' TTGCAACGGATTGTGTTGTT 3'	NM_001844.4
	Bovine	F: 5' GTGGGGCAAGACTATGATCG 3' R: 5' TGCAATGGATTGTGTTGGTT 3'	NM_001113224.1
COL1A2 (COL1)	Human	F: 5' AGGGCAACAGCAGGTTCACTTACA 3' R: 5' AGCGGGGGAAGGAGTTAATGAAAC 3'	NM_000089.3
	Bovine	F: 5' ACATTGGCCCAGTCTGTTTC 3' R: 5' GGGAGGGGGAGTGAATTAAA 3'	NM_174520.2