SUPPORTING INFORMATION

TITLE

Chondrogenic and chondroprotective response of composite collagen I/II-hyaluronic acid scaffolds within an inflammatory osteoarthritic environment

AUTHORS

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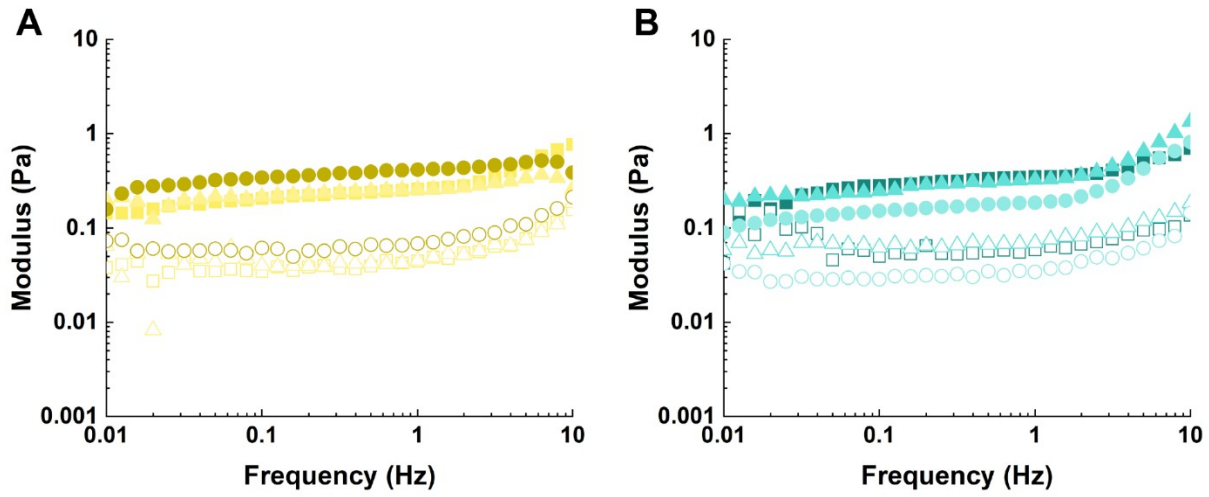

Figure S1. Raw frequency sweep data for (A) *-HA* in yellow and (B) *+HA* in blue. Each replicate (n = 3) is shown in a different shade of yellow or blue. For the same shade of yellow or blue, closed symbols represent storage modulus and open symbols represent loss modulus for the same replicate sweep.

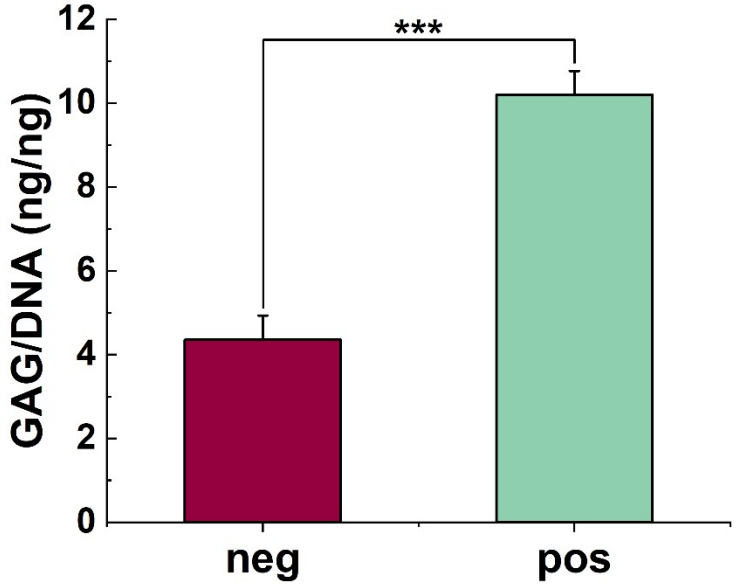

Figure S2. Pellet controls confirmed chondrogenic differentiation potential of cells during matrix quantification experiments. "neg" is negative control pellet without TGF-β3 in chondrogenic medium, and "pos" is positive control pellet with TGF-β3 in medium. Statistical significance is denoted as *** $p < 0.001$.

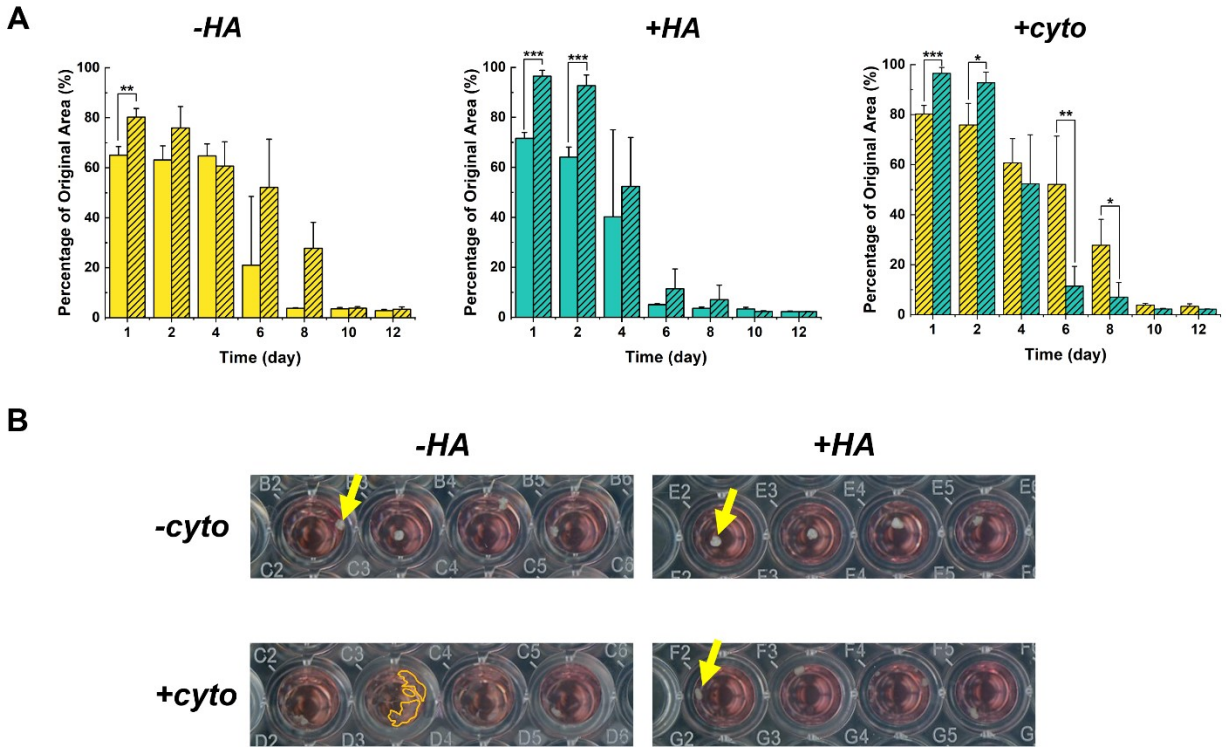

Figure S3. Plate scans show that hydrogels contracted faster for *-cyto* groups compared to *+cyto* groups. *-HA +cyto* after 8 days did not form one homogenous hydrogel but, rather, smaller fragments. **(A)** Quantification of plate scans were performed by calculating the percentage of the original gel area (%) over time for the *-HA*, *+HA*, and *+cyto* treatments. *-HA* treatments are in yellow and *+HA* are in blue. Bars with stripes represent cytokine media treatment (*+cyto*). Statistical significance is denoted as * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. The *+cyto* graph replots the striped bars in the previous two graphs (*-HA* and *+HA* graphs) to facilitate comparison of the effect of *+HA* in the presence of cytokines. **(B)** Plate scan after 8 days in culture. Treatments without cytokines (*-cyto*) contracted more homogenously than *+cyto* treatments. Arrows point to the contracted pellets in the wells, and gel fragments are outlined in the *-HA +cyto* group.

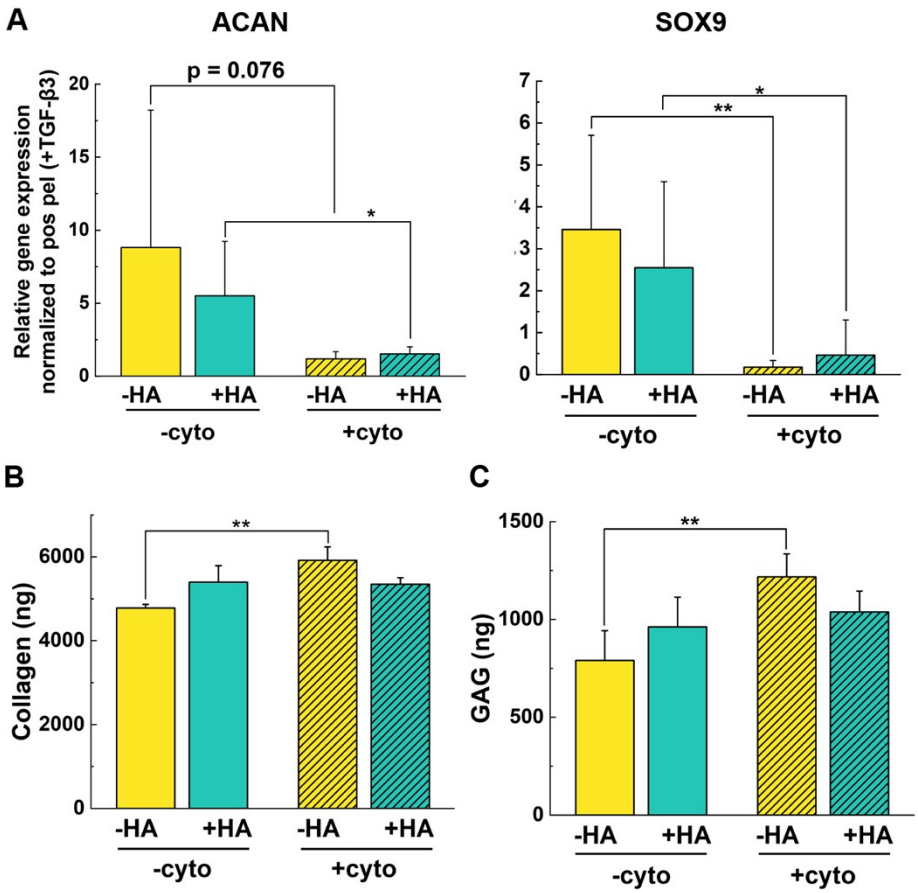

Figure S4. Cartilage gene expression and matrix levels. (A) Cartilage-related genes, aggrecan (ACAN) and SOX9, were higher for *-cyto* treatments compared to *+cyto*. Gene expression at 7 days was normalized to GAPDH and analyzed relative to a positive pellet control. **(B)** Col and **(C)** sulfated GAG were statistically higher for *-HA +cyto* compared to *-HA -cyto* and were similar for *+HA* without and with cytokines. Hydrogels were cultured for 28 days. *-HA* treatments are in yellow and *+HA* are in blue. Bars with stripes represent cytokine media treatment (*+cyto*), and statistical significance is denoted as * $p < 0.05$ and ** $p < 0.01$.

Table S1. Linear model for cytokine production. Specific constants and p-values for linear model.

Experimental Conditions Explanatory Variables	Dependent Variable					
	IL-4		IL-6		IL-8	
	β	p-value	β	p-value	β	p-value
TNF-α treatment	-0.637	**2.8e-5**	-0.583	**1.8e-4**	0.187	0.333
HA and cyto treatment	1.322	0.489	-0.533	0.799	-3.234	0.178
HA treatment	-1.073	0.423	1.034	0.482	3.079	**0.071**
cyto treatment	7.376	**7.7e-5**	8.651	**4e-5**	3.910	0.117
p-value model	6.7 e-5		1.2 e-4		0.011	

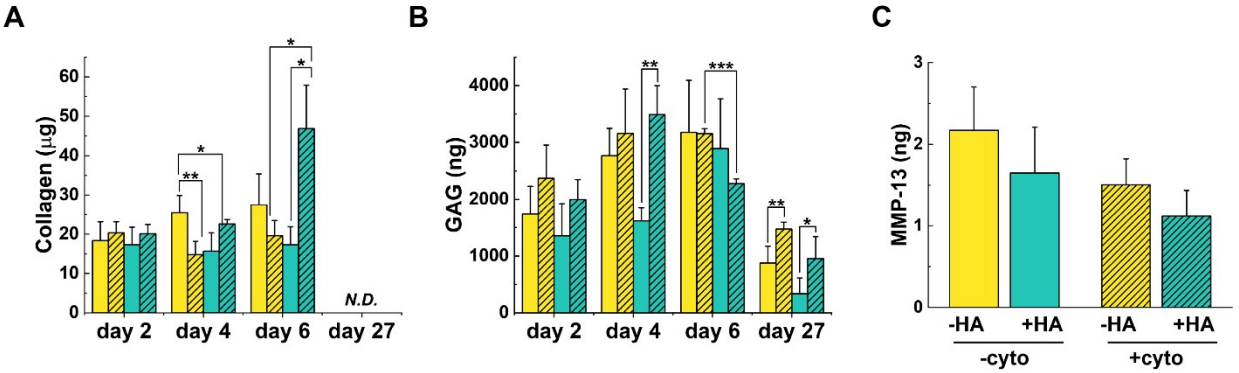

Figure S5. Collagen and GAG released into the medium from all hydrogel treatments. (A) Col in media on days 2, 4, and 6. No col was detectable in the medium on day 27 (denoted as N.D.). **(B)** GAG in media on days 2, 4, and 6. By day 27, +*cyto* treatments released more GAG into the medium than -*cyto* treatments**. (C)** MMP-13 release to media on day 14 showed no statistical difference between groups (α = 0.05) and was lower than on day 6 and day 8 (Figure 5B and 5C). -*HA* treatments are in yellow and +*HA* are in blue. Bars with stripes represent cytokine media treatment (+*cyto*) and statistical significance is denoted as * $p < 0.05$, ** $p < 0.01$, *and* *** $p < 0.001$.