Hybrid protein-glycosaminoglycan hydrogels promote chondrogenic stem cell differentiation

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Supporting Information

S.1 Mechanical properties

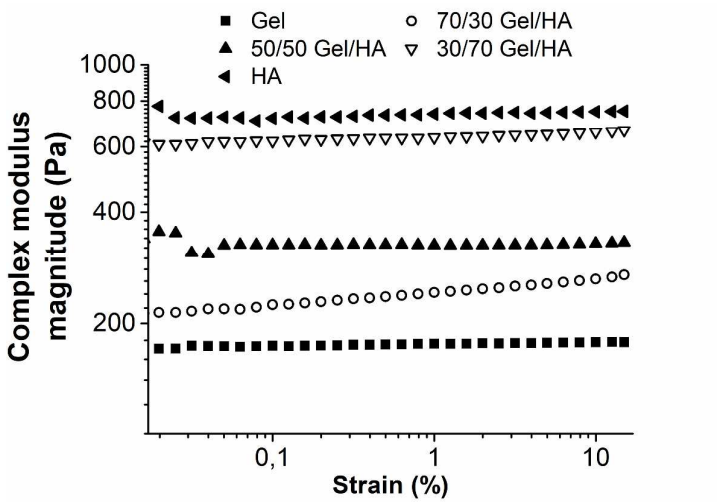

Figure S1. Rheometric complex modulus magnitude (|G*|) of crosslinked hydrogels as a function of the strain at a frequency of 1 Hz at 37°C. Each curve corresponds to the average of three different samples.

S.2 Live/Dead

The viability of the cells in the injectable Gel/HA hydrogels was evaluated in a culture using the Live/Dead kit for mammalian cells. After 14 days the samples were washed with DPBS and incubated for 15 min at 37 ºC in DPBS with 1 μM of calcein AM and 2 μM ethidium homodimer-1 (EthD-1). An in vivo analysis of live (stained in green with calcein AM) and dead cells (stained in red with EthD-1) was then performed with a Zeiss Observer Z1_AX10 fluorescence microscope. Several images were taken from two different replicates of each sample. The resulting images shown in Figure S2 were representative of the whole sample.

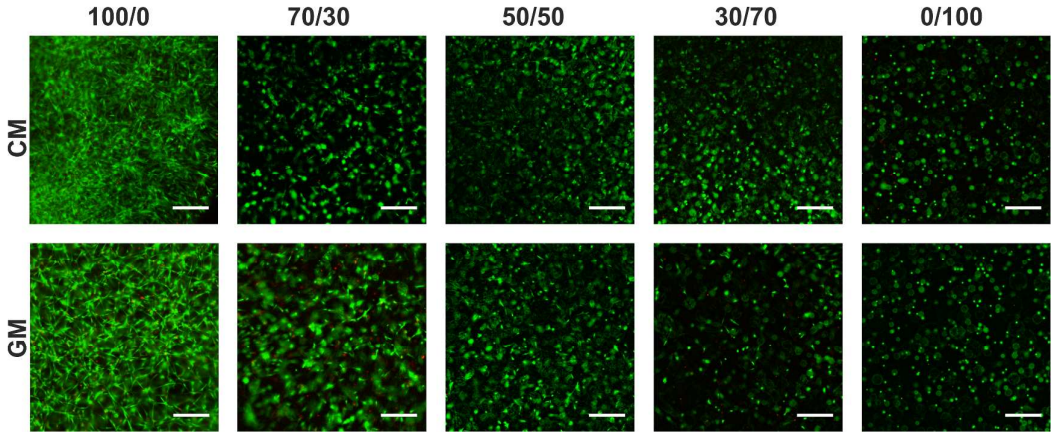

Figure S2. Live/dead images of BM-hMSCs cells cultured within Gel/HA hydrogels in GM and CM for 14 days. Scale bar 300 μm.

S.3 Optical microscopy observation of cells encapsulated into the hydrogels

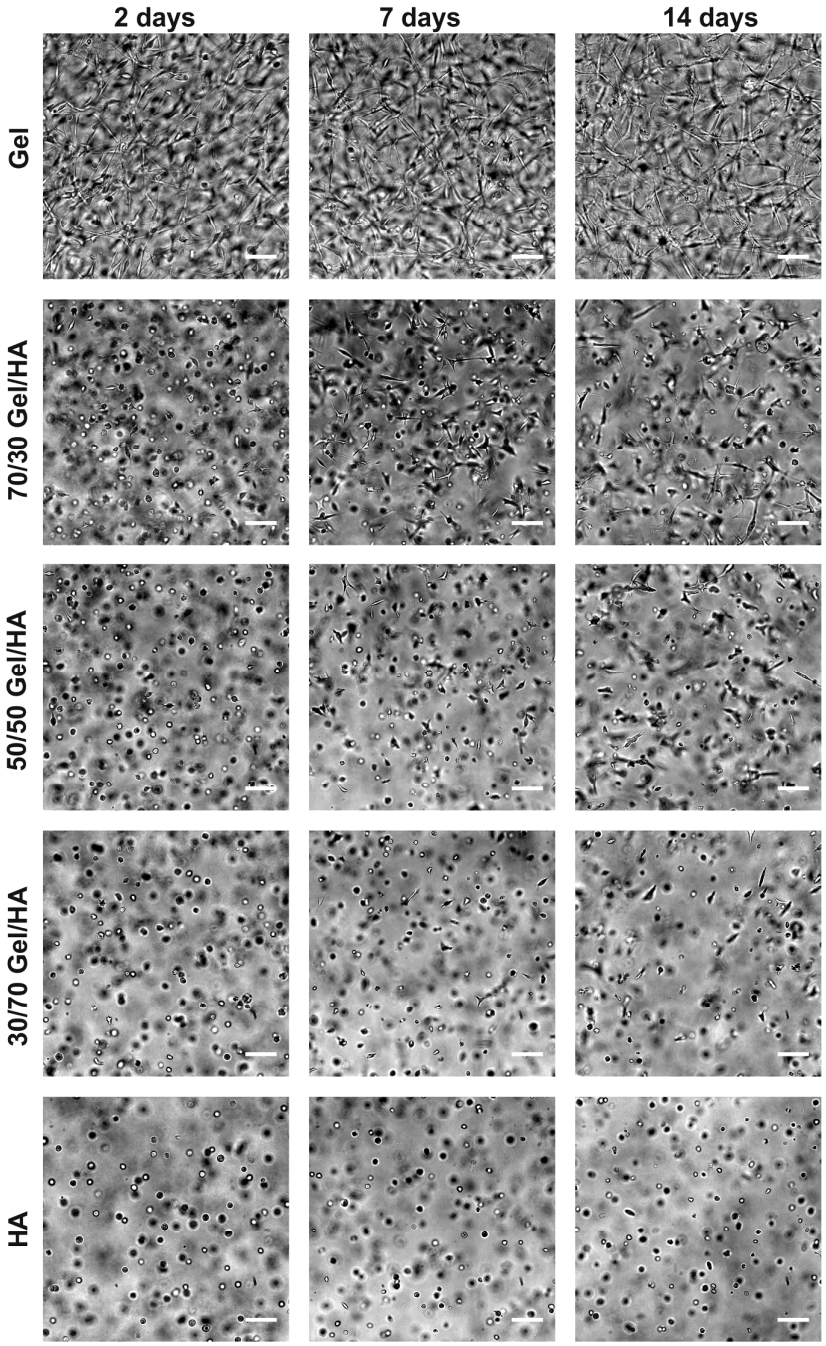

Figure S3.1. Phase contrast images of Gel/HA hydrogels cultured in GM for 2, 7 and 14 days. Scale bar 100 μm.

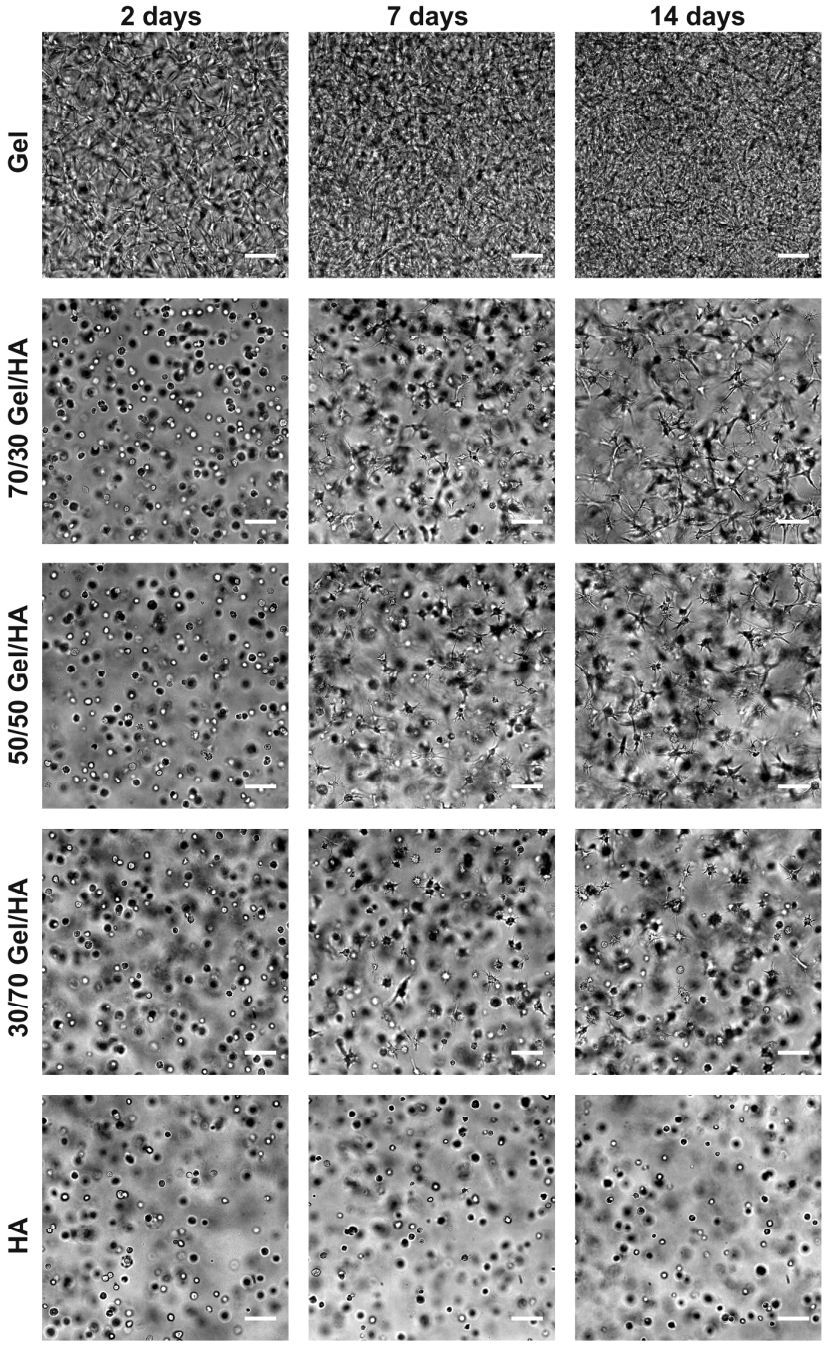

Figure S3.2. Phase contrast images of Gel/HA hydrogels cultured in CM for 2, 7 and 14 days. Scale bar 100 μm.

S.4 Immunofluorescense study

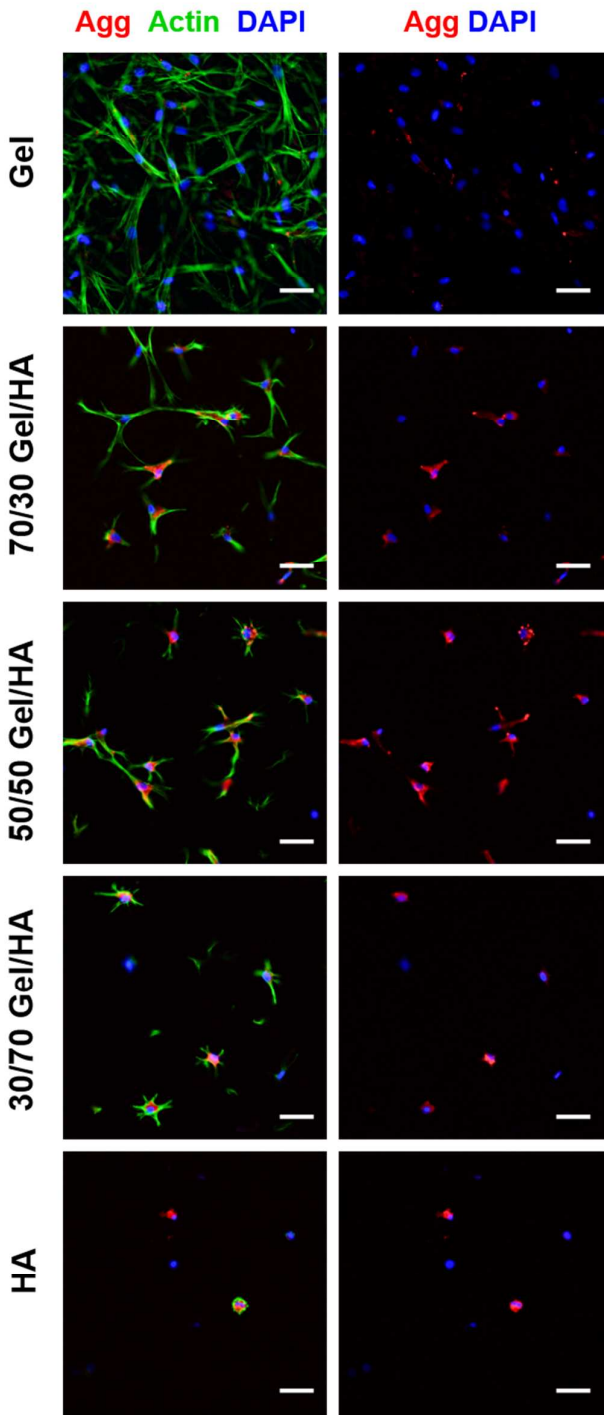

Figure S4.1. Immunofluorescence images for Aggrecan of BM-hMSCs cultured in Gel/HA hydrogels and in CM for 14 days. Nuclei are stained with DAPI, cytoskeleton is stained in green and Aggrecan is stained in red. Scale bar 50 μm.

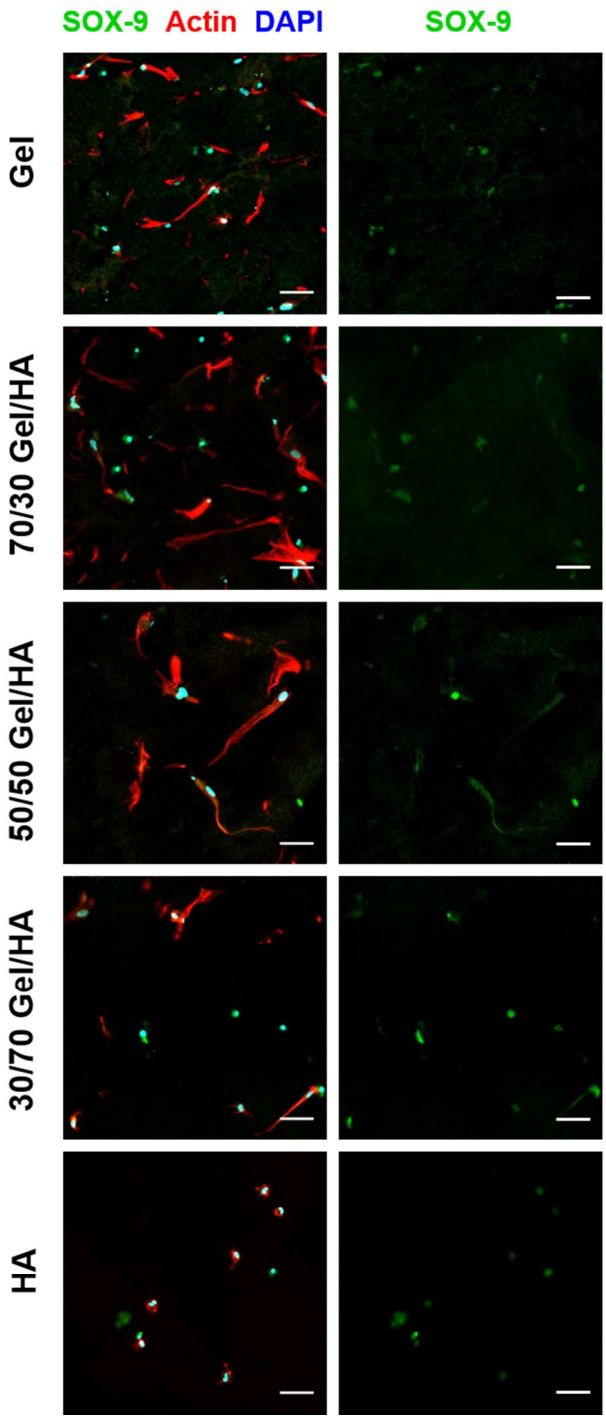

Figure S4.2. Immunofluorescence images for SOX-9 of BM-hMSCs cultured in Gel/HA hydrogels and in CM for 14 days. Nuclei are stained with DAPI, cytoskeleton is stained in red and SOX-9 is stained in green. Scale bar 50 μm.

S.5 Cell distribution and Blue Level/Area from Alcian Blue histologies

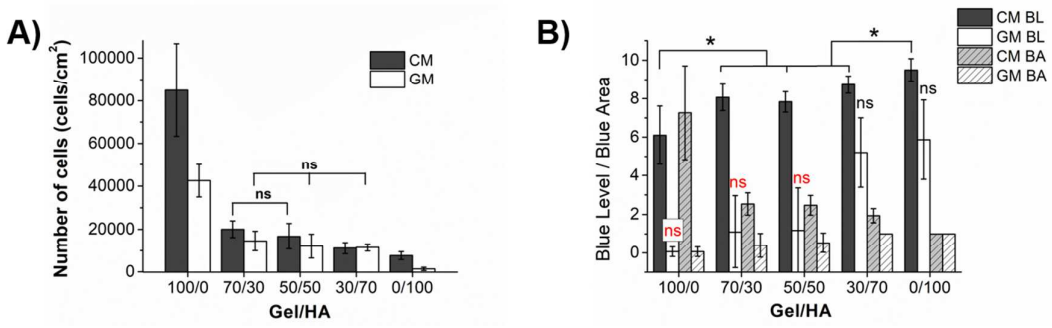

Figure S5. Quantification of the cell cultures of BM-hMSCs encapsulated in the Gel/HA hydrogels and cultured in growth medium (GM) and chondrogenic medium (CM) for 14 days. **A)** Total number of cells/cm^2 obtained from the immunofluorescence images of aggrecan. Mann-Whitney-Wilconson test demonstrated that groups within a type of culture medium show statistically significant differences between each other, except those marked with "ns". **B)** dark blue level (BL) and blue area (BA) around the cells in alcian blue images. Mann-Whitney-Wilconson test was performed to find statistical differences, * for $p<0.05$ and "ns" for not significant differences.