

Supporting Information

Mixed Composition Microribbon Hydrogels Induce Rapid and Synergistic Cartilage Regeneration by MSCs in 3D via Paracrine Signaling Exchange

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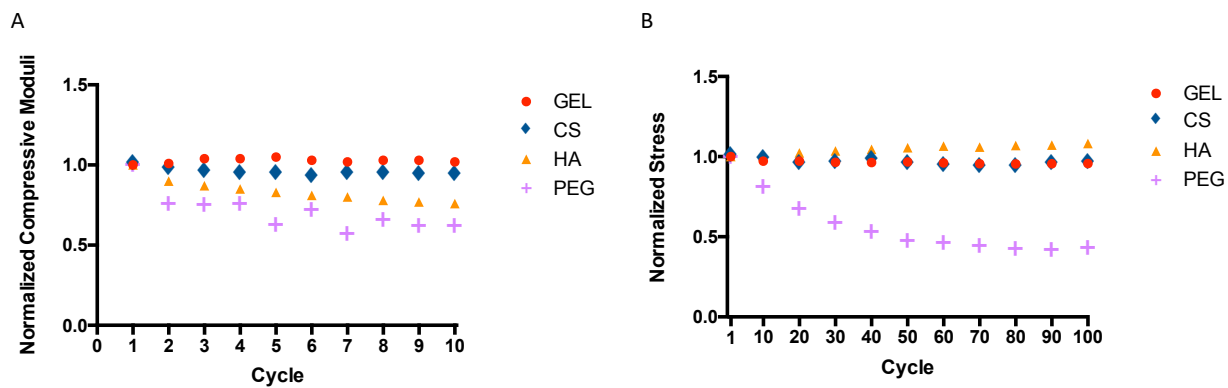


Figure S1. A) Normalized compressive moduli of μ RB scaffolds undergoing cyclic compression at 10-20% strain. B) Normalized maximum stress of scaffolds undergoing cyclic shear. Values are normalized to cycle 1 values.

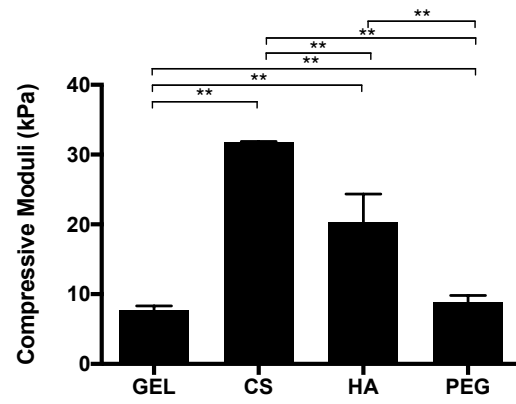


Figure S2. Bulk compressive moduli of Day 1 scaffolds as measured through unconfined compression testing. Data are mean \pm SD for n=3 samples. * $p < 0.05$, ** $p < .001$.

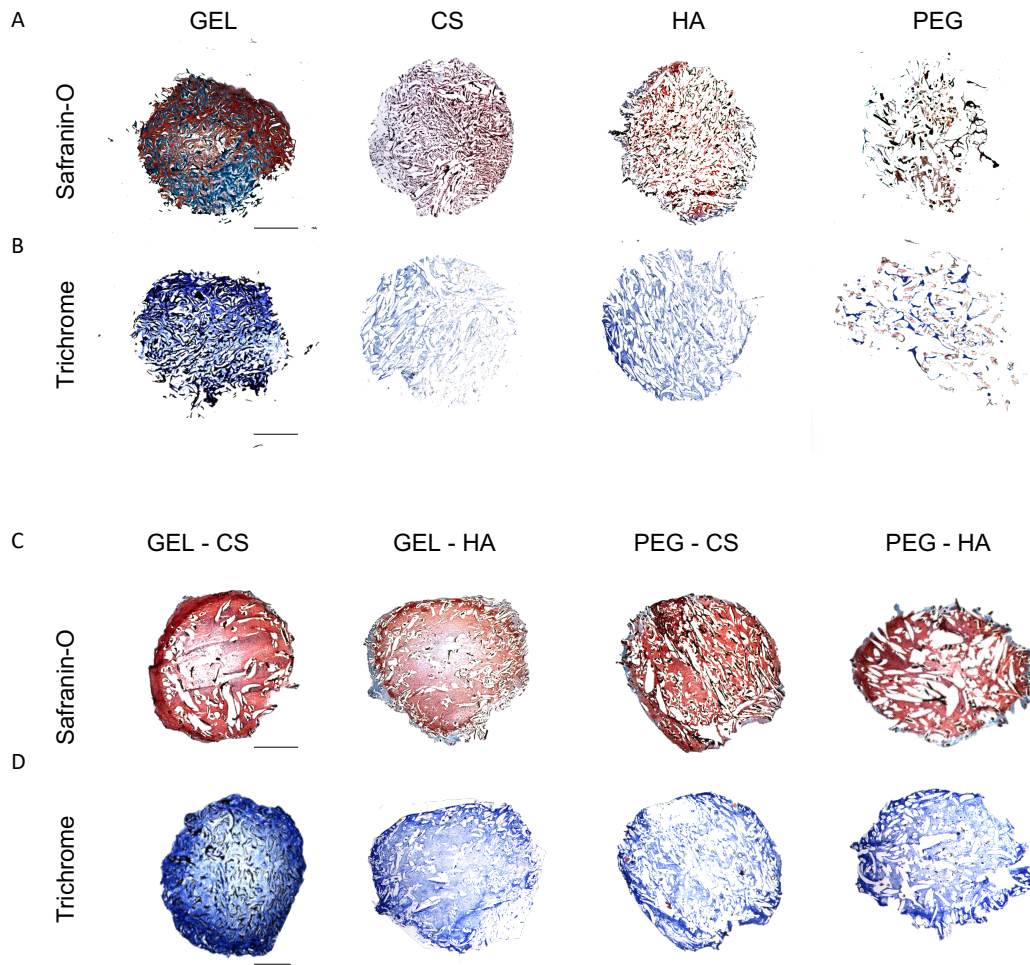


Figure S3. Low magnification histological images of μ RB scaffolds at day 21 showing macroscopic distribution of neocartilage matrix. A) Safranin-O staining for GAG and B) Trichrome staining for collagen distribution in individual compositions μ RB scaffolds. C) Safranin-O staining for GAG and D) Trichrome staining for collagen distribution in mixed compositions μ RB scaffolds. Scale bars: 1 mm. N = 2 samples sliced and imaged for histology.

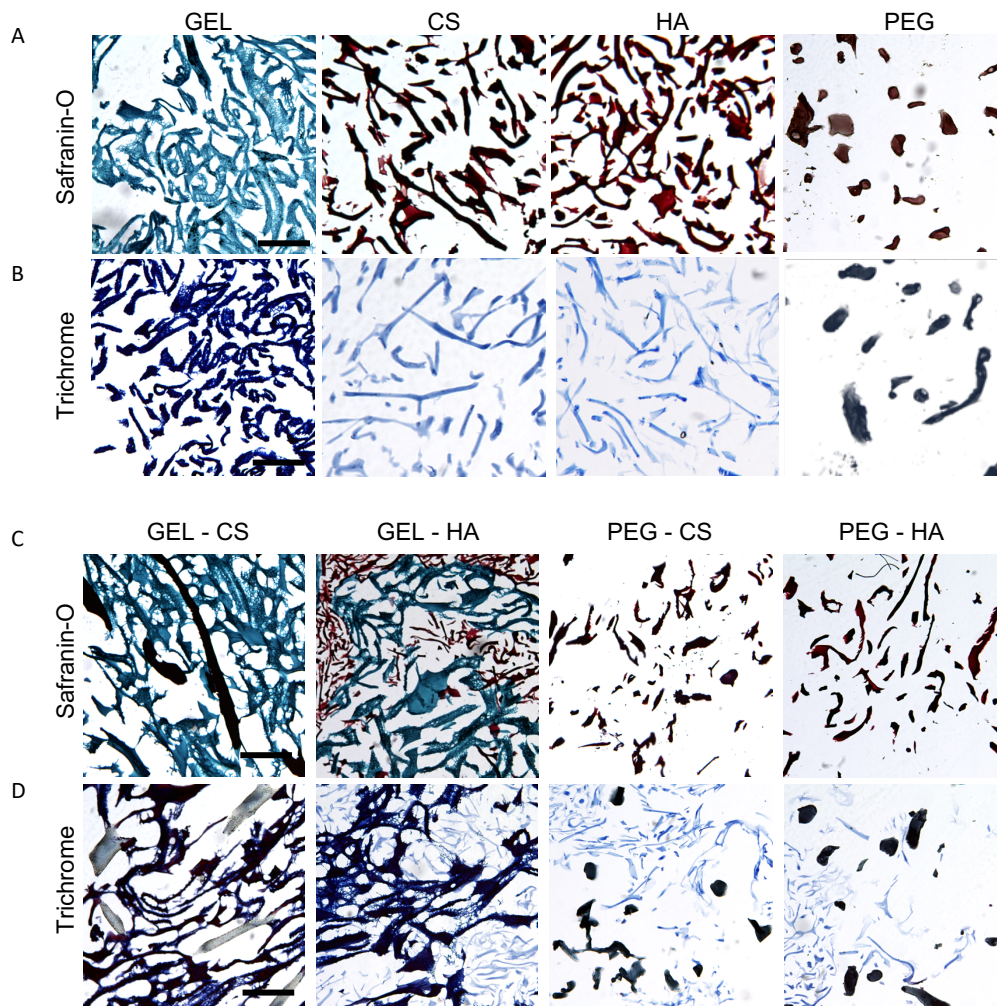


Figure S4. Histological analysis and of acellular μ RB scaffolds at day 21. A) Safranin-O staining for GAG and B) Trichrome staining for collagen distribution in individual compositions μ RB scaffolds. C) Safranin-O staining for GAG and D) Trichrome staining for collagen distribution in mixed compositions μ RB scaffolds. Scale bars: 200 μ m. N = 2 samples sliced and imaged for histology.

Efficiency of BSA release from μ RB scaffolds

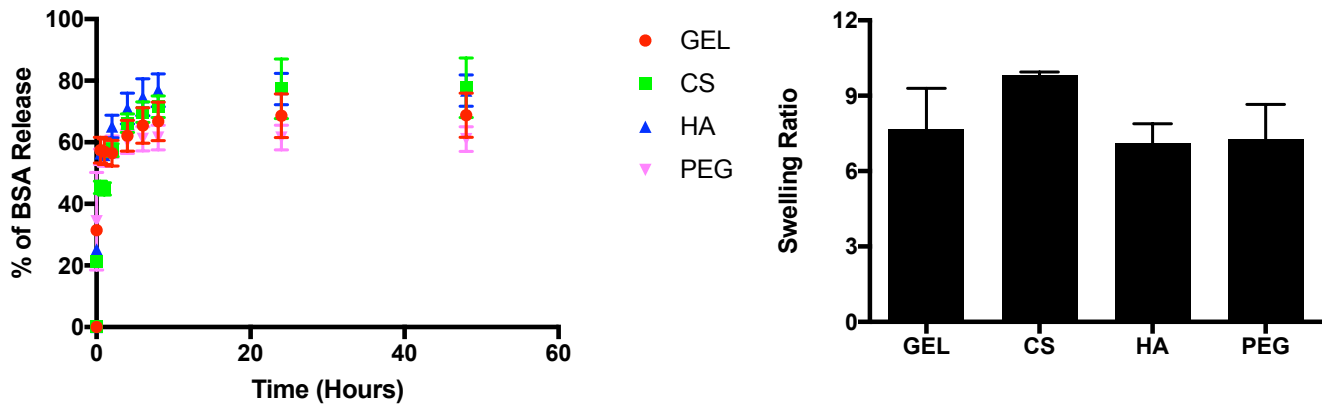


Figure S5. μ RB composition does not affect diffusion or swelling ratio of scaffolds. A) BSA release from scaffolds over 48 hours. B) Swelling ratio of scaffolds after 21 days in chondrogenic medium. Data are mean \pm SD for n = 3 samples. No statistical significance was observed.

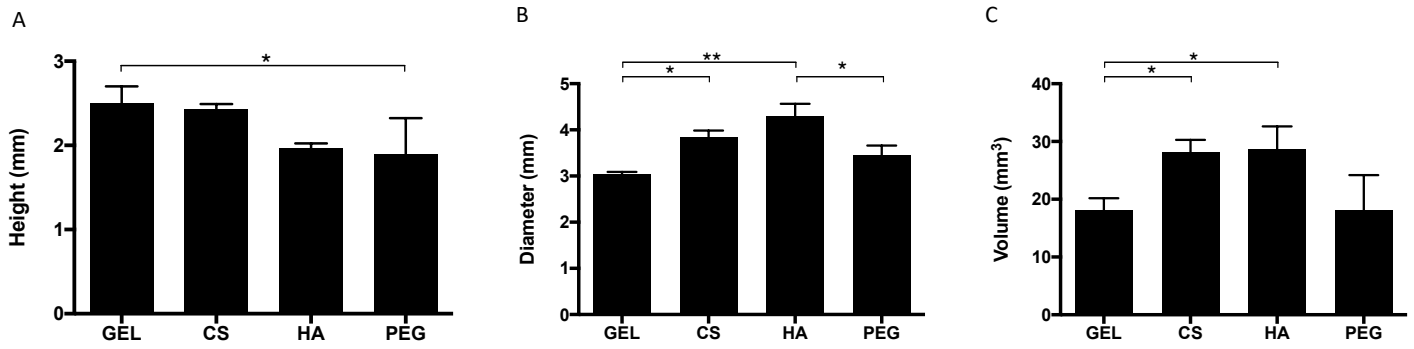


Figure S6. μ RB composition minimally affects dimensions of the scaffolds. A) Height, B) diameter, and C) volume of the scaffolds after 21 days in chondrogenic culture. Data are mean \pm SD for n=3 samples. * p < 0.05, ** p < .001.

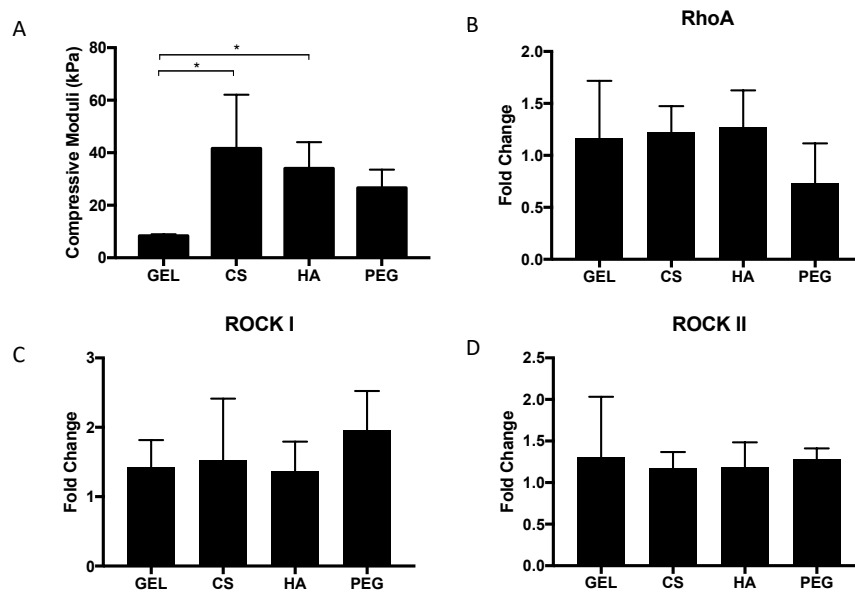


Figure S7. Mechanical properties of individual μ RBs and mechanosensing gene expression of encapsulated cells. A) Compressive moduli of individual μ RBs as measured with AFM. Mechanosensing gene expression after 7 days in culture, normalized to MSCs from Day 1 on TCP for C) RhoA, D) ROCK I, and E) ROCK II. Data are mean \pm SD for n=3 samples. * $p < 0.05$, ** $p < .001$.

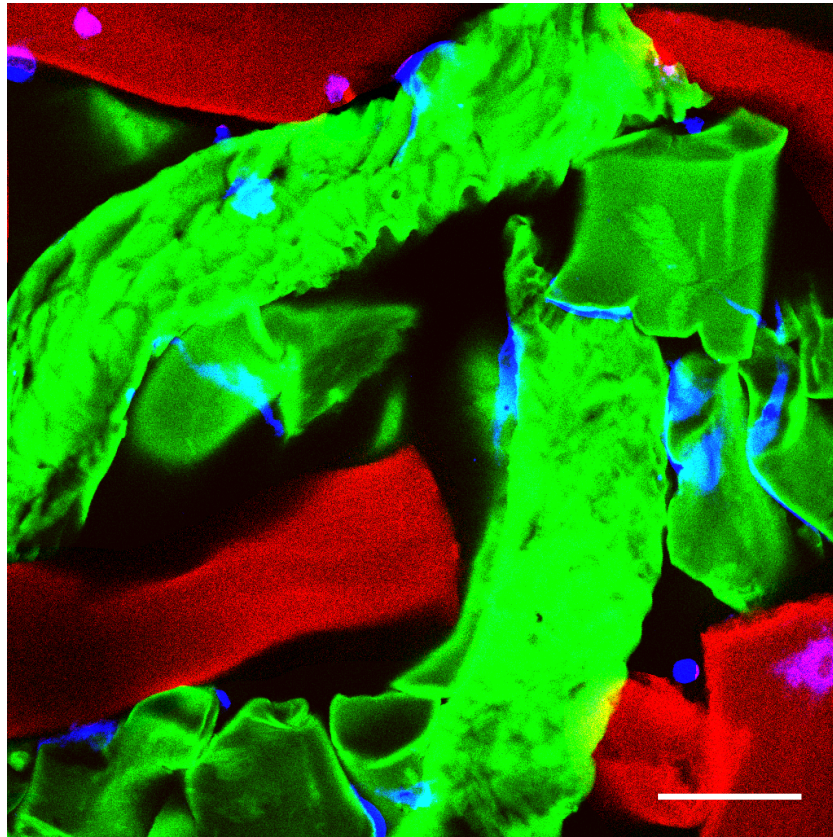


Figure S8. Confocal imaging of MSCs encapsulated within mixed μ RB scaffold. Individual MSCs within a mixed μ RB scaffold generally attach to only one μ RB. Red = GEL μ RB, green = CS μ RB, blue = MSC. Scale bar: 100 μ m.

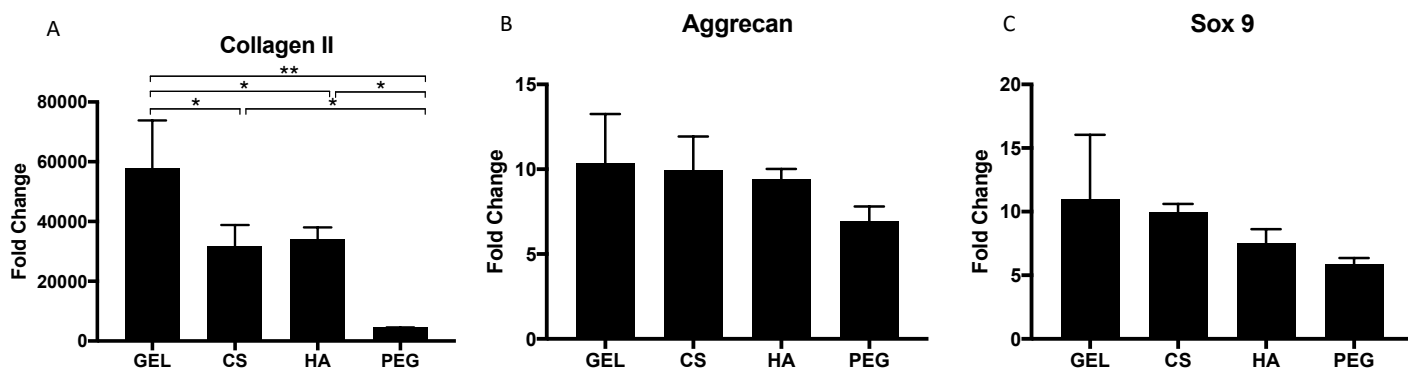


Figure S9. μ RB composition affects chondrogenic gene expression of MSCs in individual μ RB scaffolds at Day 7. Gene expression for A) Collagen II, B) Aggrecan, and C) Sox 9 normalized to MSCs from Day 1 on TCP. Data are mean \pm SD for n=3 samples. * p < 0.05, ** p < .001.

Supplementary Table 1. Primers used for RT-PCR.

Gene Name	Primer Sequence
GAPDH	F: CGCTCTCTGCTCCTCCTGTT R: CCATGGTGTCTGAGCGATGT
RhoA	F: GGAAAGCAGGTAGAGTTGGCT R: GGCTGTCGATGGAAAAACACAT
ROCK I	F: GGTGGTCGGTTGGGGTATTTT R: CGCCCTAACCTCACTTCCC
ROCK II	F: TCAGAGGTCTACAGATGAAGGC R: CCAGGGGCTATTGGCAAAGG
Sox9	F: AGCGAACGCACATCAAGAC R: CTGTAGGCGATCTGTTGGGG
Agg	F: TGAGGAGGGCTGGAACAAGTACC R: GGAGGTGGTAATTGCAGGGAACA
Col II	F: TCACGTACACTGCCCTGAAG R: TTGCAACGGATTGTGTTGTT