SUPPORTING INFORMATION

Gelatin-based Microribbon Hydrogels Support Robust MSC Osteogenesis across a Broad Range of Stiffness

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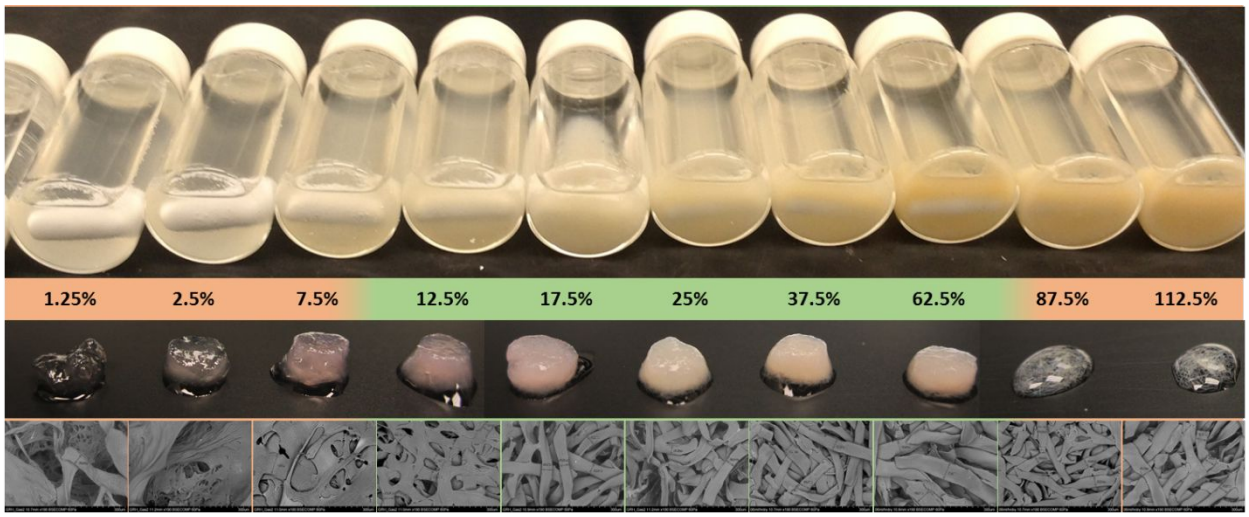


Figure S1. Determine the maximal stiffness range of μRBs that can be formed while still supporting intercrosslinking to form 3D macroporous scaffolds. Increasing crosslinker glutaraldehyde (GTA) concentration (1.25% - 112.5%) led to noticeable changes in color of the products. GTA concentration of 1.25% to 7.5% led to excessive swelling and form hydrogels rather than macroporous scaffolds. GTA concentration of 12.5% and above support μRB formation. GTA concentration of 87.5% and above led to μRBs with high stiffnesses with poor intercrosslinking efficiency and phase separation. Gross morphology and SEM of μRB scaffold showed maximal range of GTA concentration that support formation of 3D macroporous μRB scaffolds is between 12.5% to 62.5%.

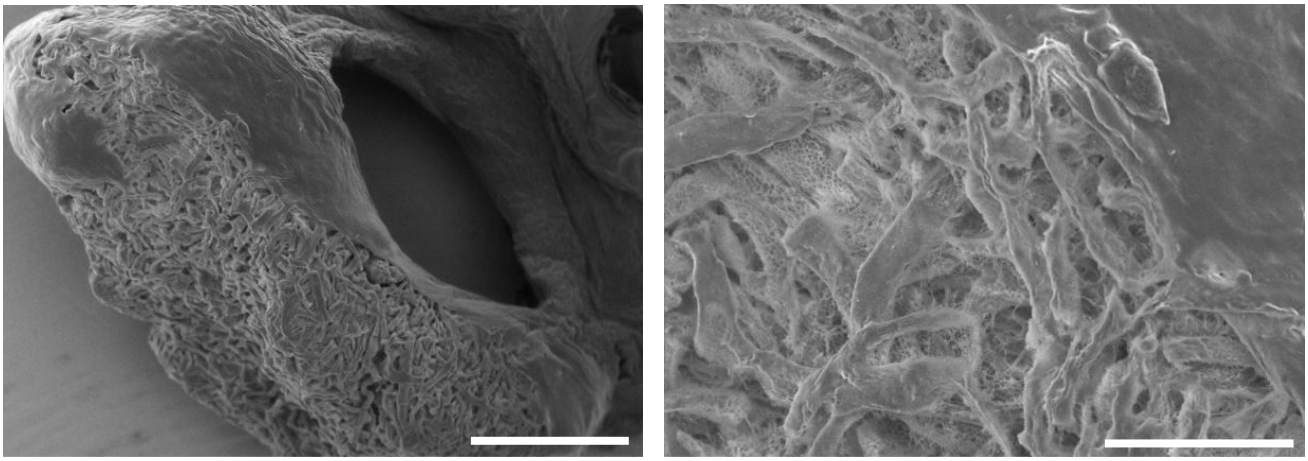


Figure S2. SEM image of cell-laden microribbon scaffolds on day 31 using microribbons with intermediate stiffness. Scale bar in low magnification image: 2 mm; Scale bar in high magnification image: 250 μm.

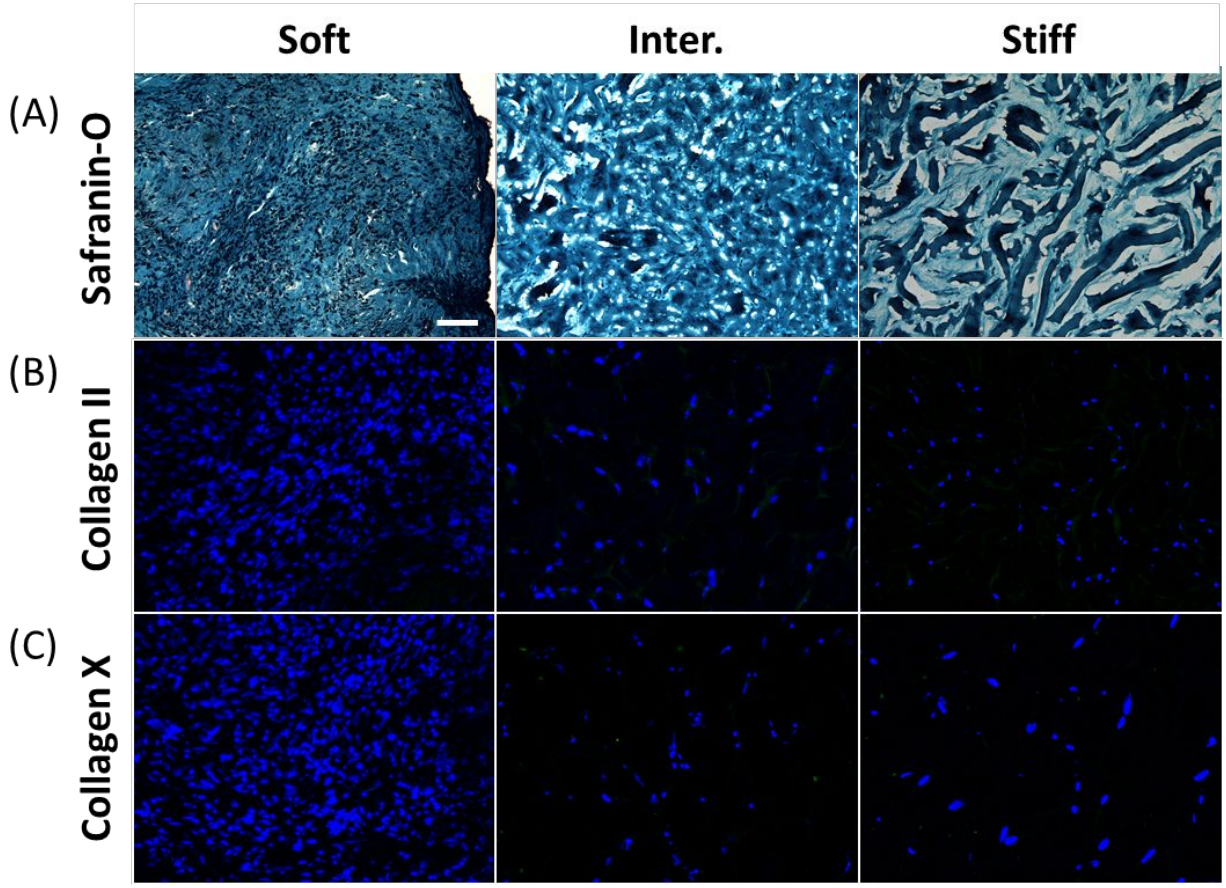
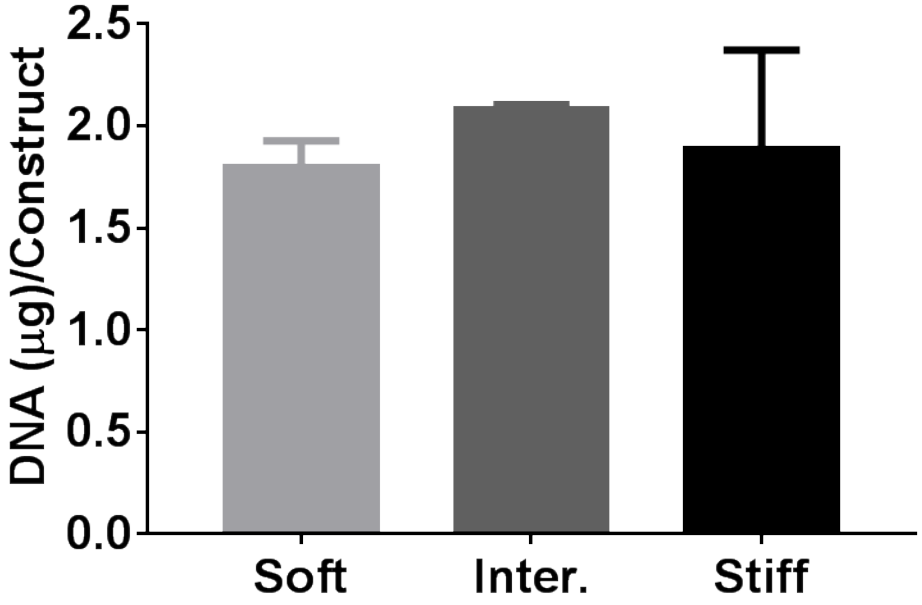

Soft
Inter.
Stiff
(A)
Safranin-O
(B)
Collagen II
(C)
Collagen X

Figure S3. Staining of glycosaminoglycans and collagens II and X showed absence of cartilage marker expression. These results suggest MSC encapsulated in microribbon scaffolds with all three stiffnesses underwent intramembranous ossification, not endochondral ossification. (A) Safranin-O, (B) type II collagen, and (C) type X collagen. Blue: cell nuclei; Positive immunostainings of collagen should be green, which is absent; Scale bar: 200 μm.

(A)


DNA (μg)/Construct
2.5
2.0
1.5
1.0
0.5
0.0
Soft
Inter.
Stiff

(B)

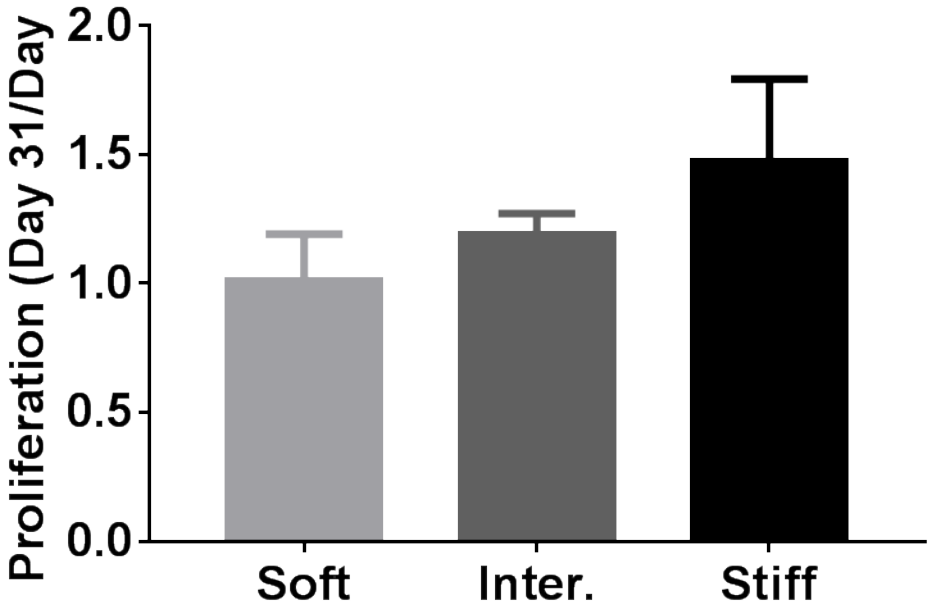

Proliferation (Day 31/Day 1)
2.0
1.5
1.0
0.5
0.0
Soft
Inter.
Stiff

Figure S4. μRBs supported high cell viability across all stiffnesses. (A) Gelatin μRBs scaffolds showed comparable numbers of cells across all three stiffnesses, (B) Fold of cell proliferation at day 31 measured by picogreen assay.

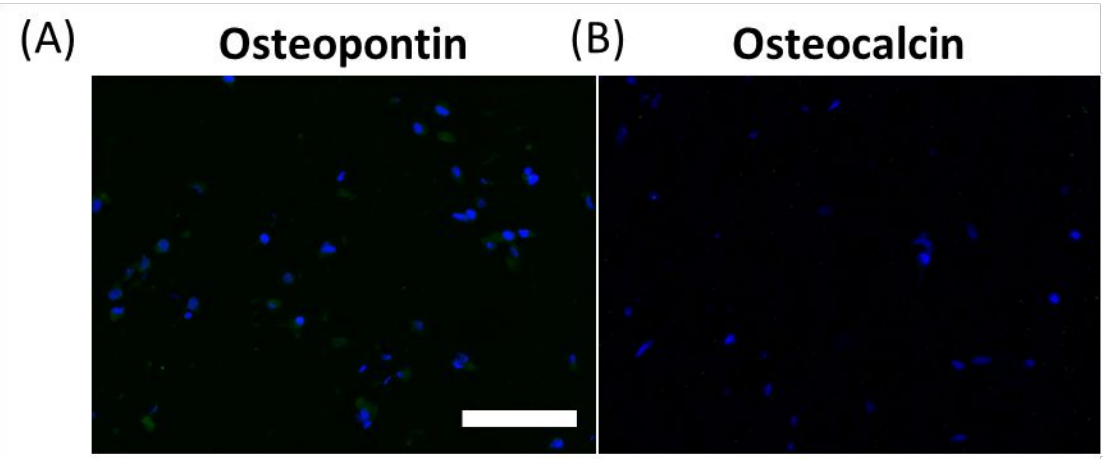


Figure S5. Immunostaining for (A) osteopontin and (B) osteocalcin for MSC seeded-μRB scaffolds with HA on day 1. No bone markers were observed at day 1. Green: osteopontin/osteocalcin, blue: nuclei; Scale bar: 100 μm.