Supplemental Information:

Biochemical and Mechanical Gradients Synergize to Enhance Cartilage Zonal Organization in 3D

Danqing Zhu, Pavin Trinh, Elisa Liu, and Fan Yang, Ph.D.*

D. Zhu, E. Liu
Department of Bioengineering, Stanford University
Stanford, CA 94305 (USA)

P. Trinh
Department of Biology, Stanford University
Stanford, CA 94305 (USA)

Prof. Fan Yang
Department of Bioengineering, Stanford University
Department of Orthopaedic Surgery, Stanford University
Stanford, CA 94305 (USA)

Email: fanyang@stanford.edu

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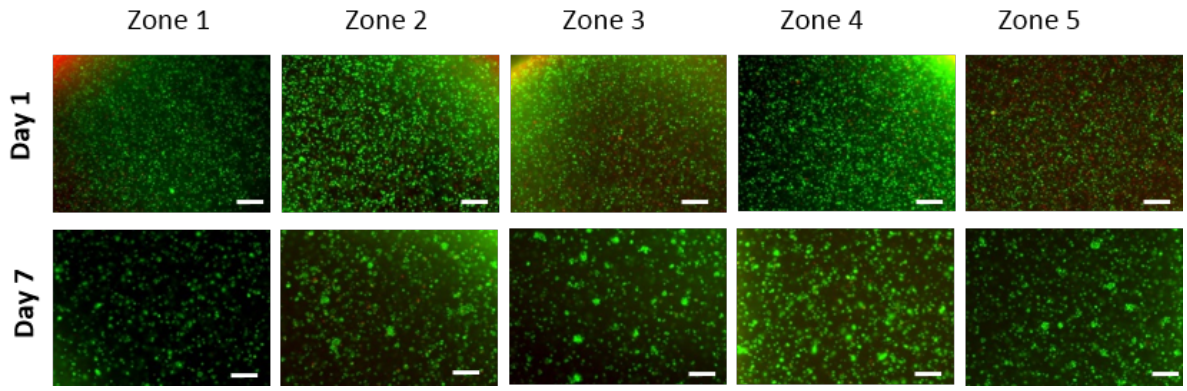

Figure S1: Chondrocytes viability within 3D dual-gradient hydrogels at Day 1 and Day 7 post-encapsulation. Live dead staining of chondrocytes encapsulated within dual-gradient hydrogels from zone 1 to zone 5. Scale bar = 200 µm.

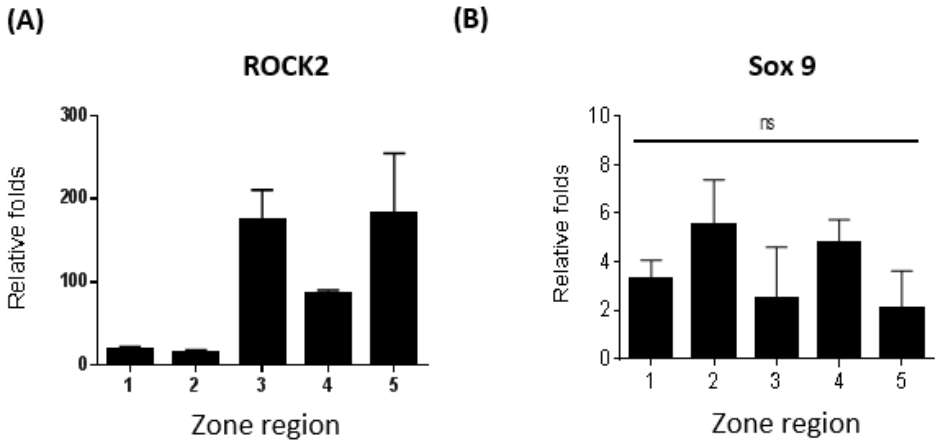

Figure S2: Additional quantitative gene expressions at Day 7 in 3D dual-gradient hydrogels. (A) ROCK 2; (B) Sox 9

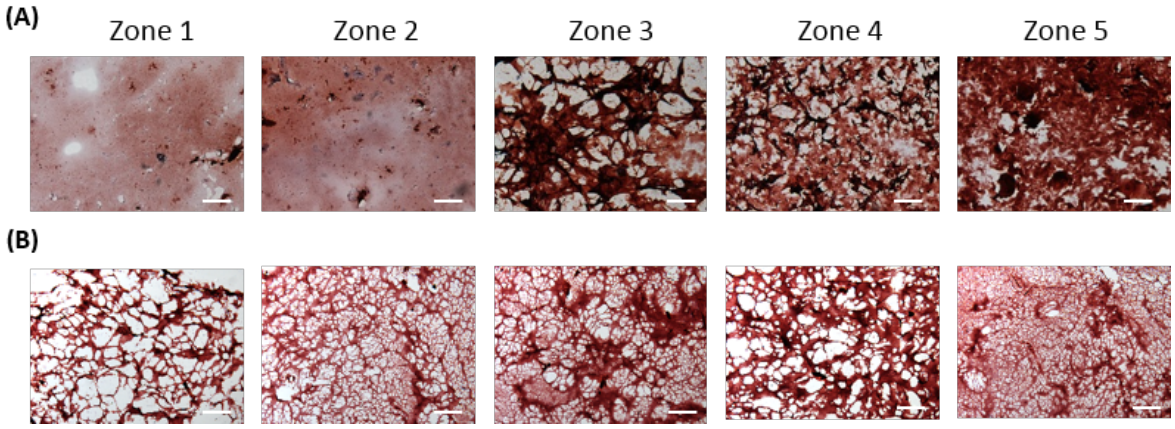

Figure S3: Safranin-O staining of sGAG deposition within dual-gradient hydrogels at Day 21 and acellular control at Day 1. (A) Cells in softer zones remained single with only marginal and diffusive sGAG deposition; cells reside in stiffer/high CS zones (i.e. zone 4/5) proliferated and formed large cell clusters with extensive sGAG staining. (B) Acellular control. Scale bar = 50 μm.

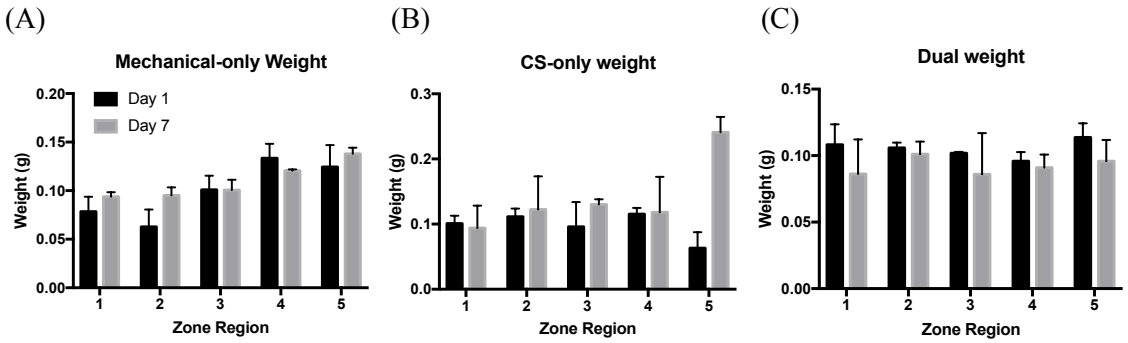

Figure S4: Total wet weights of (A) mechanical-only, (B) CS-only, and (C) dual-gradient hydrogels at day 1 and day 7.