Differential Fluid Flow, Oscillating Hydrostatic Pressure, and TGF-β3 Synergistically Promote Spatial Chondrogenic mRNA Expression in Engineered Articular Cartilage

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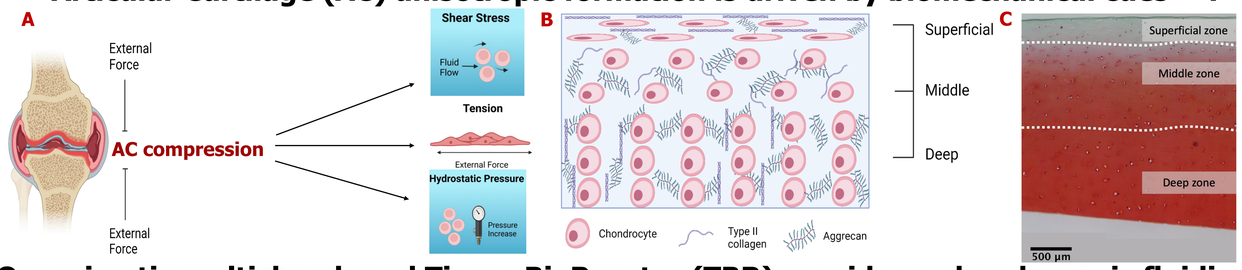
Background

Articular Cartilage (AC) anisotropic formation is driven by biomechanical cues [1,2].



Our mimetic multichambered Tissue BioReactor (TBR) provides a chondrogenic fluidic environment with shear stress gradients and oscillating hydrostatic pressures.

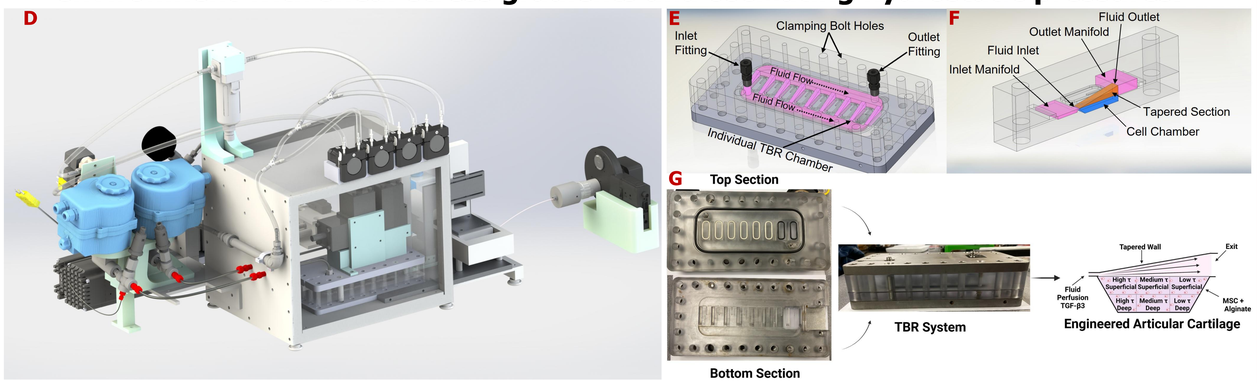


Figure 1: (A-G) **TBR mechanically representative manufacturing strategy of striated AC.** (A) Biomechanical forces experienced by native AC which contribute towards zonally organized tissue regions [3] (B) further supported by a histological stained image of native AC (C). (D) Our bioprocess system includes a Tissue BioReactor (E) with a tapered channel that support maintenance of gradated fluid shears (F). (G) TBR culture of bone marrow derived mesenchymal stromal cells (MSC) encapsulated in 1.5% alginate constructs provides an anisotropic engineered tissue product that can be spatially assessed with molecular biology and biomechanical techniques to validate manufacturing processes.

MSCs & Varied Fluid Shears

Gradated fluid shears spatially direct mesenchymal stromal cell (MSC) synthesis of AC which is supported by expressed chondrogenic mRNAs.

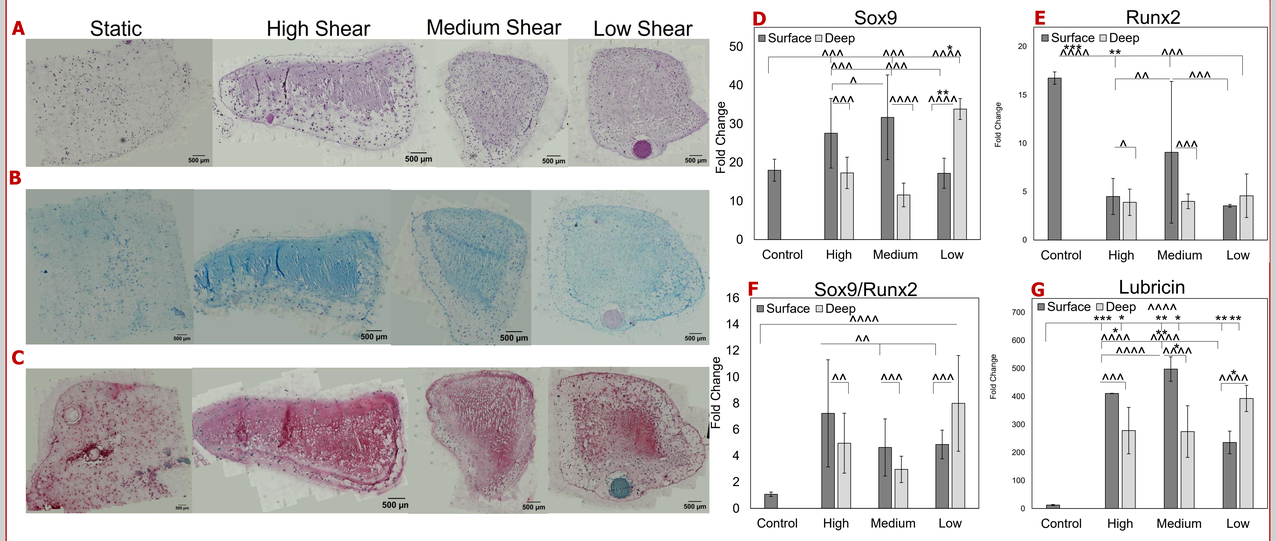


Figure 2: (A-G) **14-Day MSC Perfusion study.** (A) H&E, (B) alcian blue, (C) and safranin O histological stains illustrate tissue organization for non-perfused and perfused constructs. Images for perfused constructs highlight dense chondrogenic tissue formation compared to static samples which is further supported by mRNA expressions mapping (D) Sox9, (E) Runx2, (F) Sox9/Runx2, and (G) Lubricin genes. More interestingly, we see that respective alcian blue and safranin o histological stains for high fluid shears provide concentrated localization of sulfated glycosaminoglycan (GAG) and cartilage-specific proteoglycans compared to tissue regions stimulated by medium and low shears. Lubricin mRNA expression, a type of proteoglycan specifically expressed by superficial AC, indicates increased gene upregulation for high and medium surface shears compared to low fluid shears marked by significant fold changes of 1.7 and 2.1, respectively, which correlate to our tissue stains. Furthermore, stains for medium and low shears illustrate bold bands near the tissue surface which agree with similar mRNA findings showing elevated chondrogenic expression at the superficial layer.

MSCs, OHP & Varied Fluid Shears

Combined oscillating hydrostatic pressure (OHP) and differential fluid shears synergistically enhance striated tissue regions.

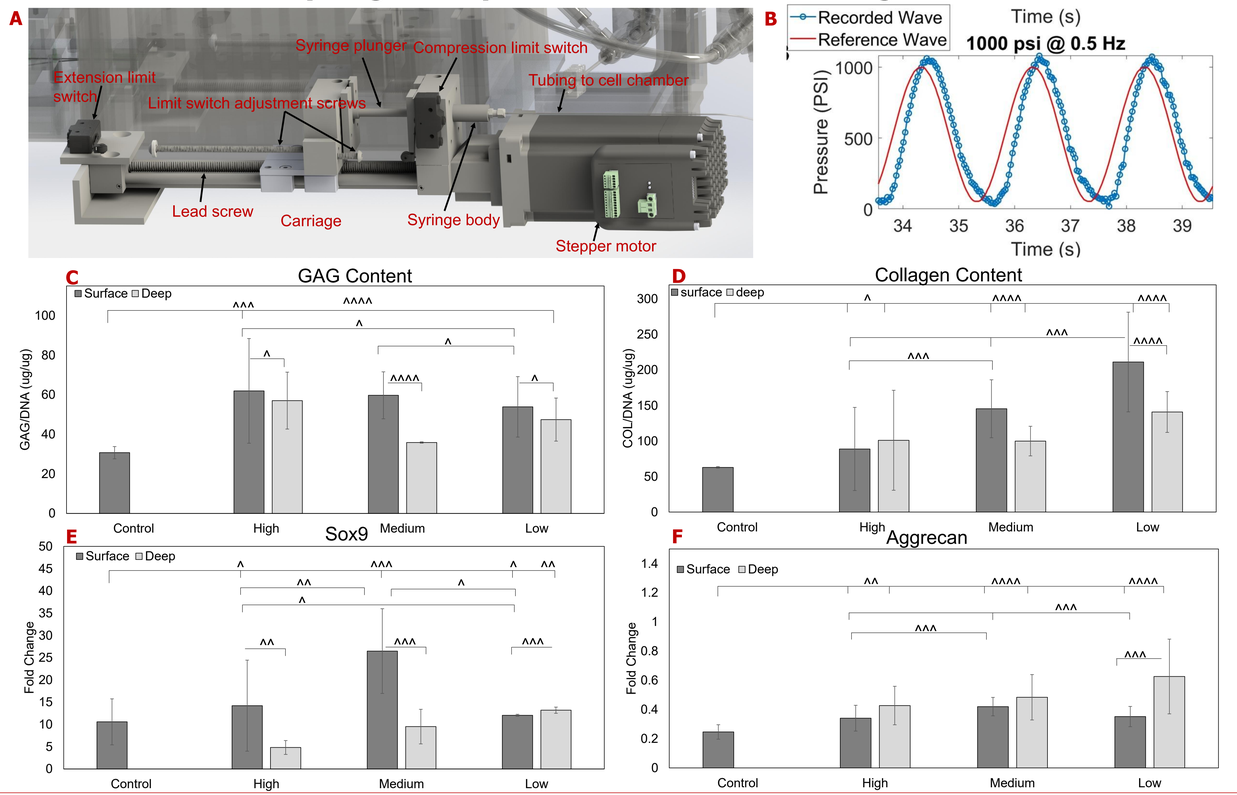


Figure 3: (A-F) **4-Day MSC Perfusion and 2-hr/day (4-hr total) 5 MPa OHP at 0.5 Hz.** (A) Our custom-build OHP mechanism consisting of a controllable syringe pump can deliver various pressure magnitudes at different frequencies (B). In a recent 4-day study, mechanically stimulated cells display early indications of increased total GAG (C) and collagen (D) deposition compared to static controls marked by respective fold change ranges of 1.2 – 2.0 and 1.4 – 3.4. Similarly, chondrogenic Sox9 (E) and aggrecan (F) mRNA indicates higher expression levels for reactor samples compared to static conditions which is supported by respective fold change ranges of 1.1 – 2.5 and 1.4 – 2.5. Most importantly, varied fluid shear and OHP combinations demonstrate definitive spatial improvement of manufacturing anisotropic AC which is shown by varied protein deposition and mRNA expression with respect to fluid shear magnitude as well as superficial vs. deep tissue regions.

Conclusion

- Our novel TBR development furthers our ability to perform robust bioprocess engineering studies mimicking native AC biomechanical conditions.
- Gradated fluid shear differentially impacts cell expression of chondrogenic markers shown by increased mRNA expression, sulfated GAG and proteoglycan stains for engineered tissue regions exposed to high fluid shears compared to other fluid shear magnitudes.
- OHP and fluid shear stimulation show enhanced biochemical deposition and chondrogenic mRNA expression that vary regionally throughout the engineered construct.
- We expect future studies with increased culture duration with OHP will definitively improve spatial cell chondrogenesis and further our production of spatially organized tissue.

Acknowledgements

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References

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