Differential Effects of Fluid Shear Stress on Mesenchymal Stromal Cell Differentiation

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Introduction

- Articular cartilage (AC) complex biomechanical environment drives anisotropic tissue formation illustrated by regionally diverse cell functionality.[1]
- Our bioreactor design and development serves to mimic the AC cell niche with flexibility to regulate biochemical and mechanical cues.
- Fluid shear stress is one of many native AC mechanical factors which arises from tissue compression.
- Perfusion bioreactor regulation of fluid shear stress shows improved chondrogenic properties supported by enhanced extracellular matrix (ECM) synthesis and robust tensile modulus for engineered tissue.[2]
- **Hypothesis:** correlating surface shear levels to cell differentiation as a function of location within a construct is crucial for manufacturing striated tissue resembling AC.

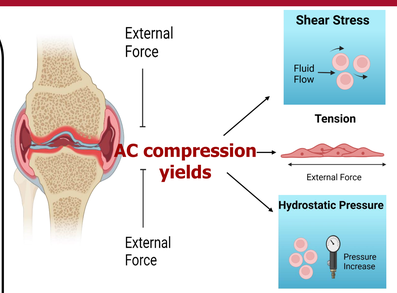

Fig. 1: Mechanical loading types experienced by AC.

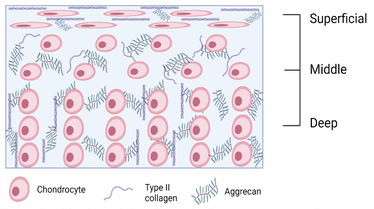

Fig. 2: Native AC with distinct zonal regions.

Objective

- Construct a multi-chambered Tissue BioReactor (TBR) to assess gradated fluid shear influence on cell chondrogenesis and correlate surface strain impacts on cell behavior as a function of depth within a scaffold.

Methods

- Mesenchymal stromal cells (MSCs) were encapsulated in an alginate matrix molded into individual TBR chambers.
- Non-perfused samples with similar geometry served as a control for bioreactor comparison
- TGF-β3 containing medium was either perfused at 20 mL/min for TBR samples or supplemented for control samples with 3-day medium changes for 7 days.
- Samples were tested for secreted extracellular matrix proteins (ECM) and mRNA expression.

Native AC

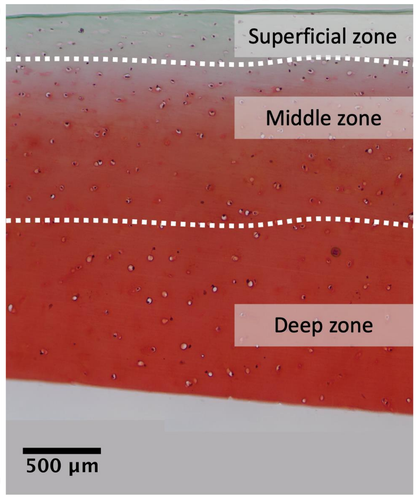

Fig. 3: Safranin O staining of human AC.

Our Strategy

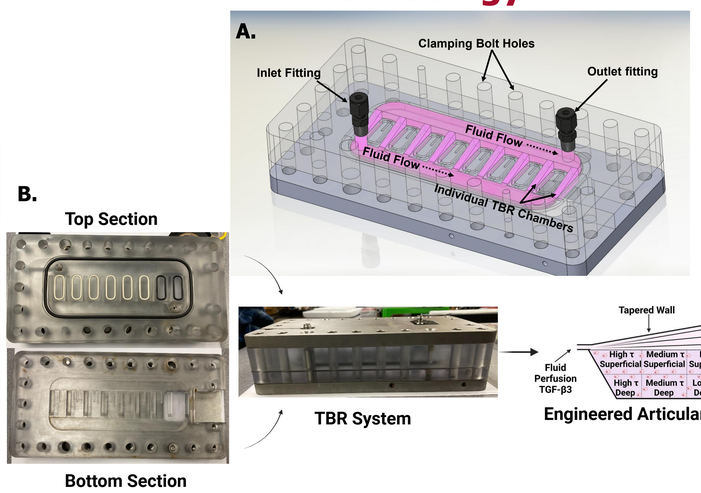

Fig. 4: TBR design (**A.**) and process schematic for tri-layered AC production in our TBR (**B.**)

Results

Extracellular Matrix Content

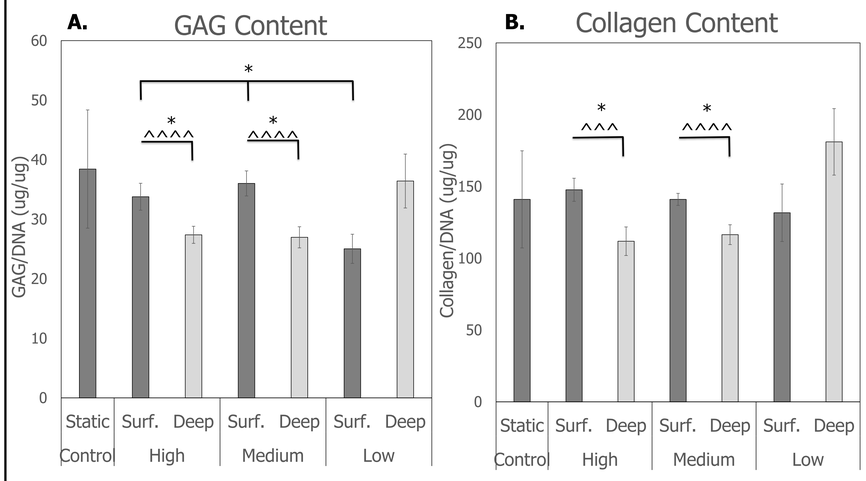

Fig. 5: Secreted biochemical levels for total collagen (**A.**) and glycosaminoglycan (**B.**)

- MSCs exposed to High and Medium surface fluid shear stress yield increased protein deposition compared to cells deep in the construct.
- Collagen production is proportionally related to fluid shear stress magnitudes.

Chondrogenic Markers

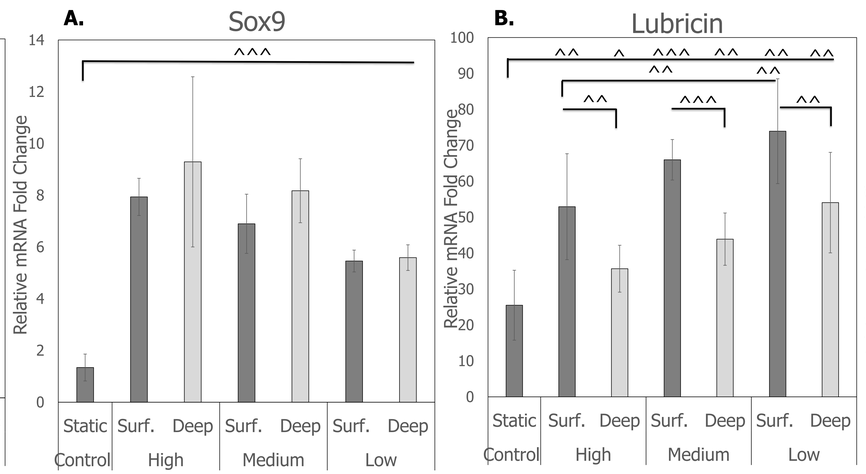

Fig. 6: mRNA expression profiles for Sox9, master transcription factor, (**A.**) and lubricin (**B.**)

- Sox9 mRNA expression, chondrogenic transcription factor, is highly upregulated for TBR samples subjected to fluid perfusion.
- TBR hydrodynamic environment creates localized lubricin expression at engineered tissue surface like native AC.

Non-chondrogenic Markers

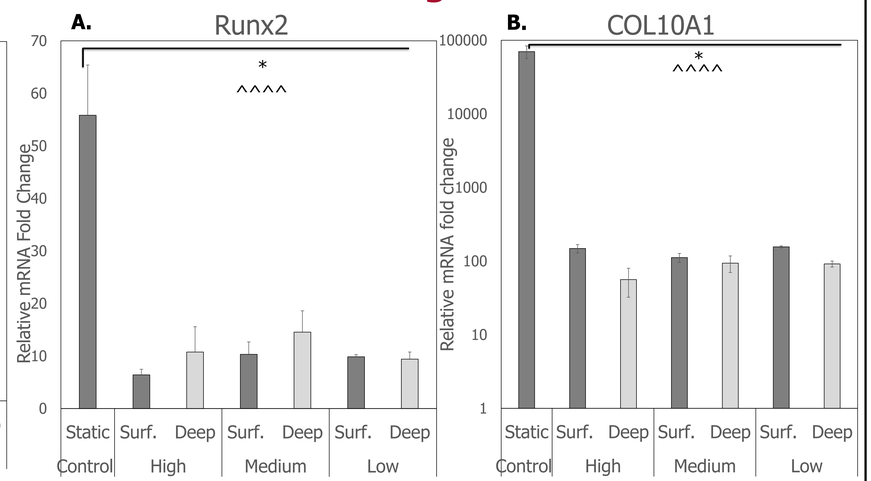

Fig. 7: mRNA expression profiles for Runx2, osteogenic transcription factor, (**A.**) and col10a1 (**B**).

- Non-chondrogenic mRNA expression markers show an alternative profile that reveals increased hypertrophic characteristics for control samples.
- Runx2 and Col10a1, osteogenic markers, are significantly suppressed in a continuous fluid shear environment.

Conclusions

- Perfused MSC-laden constructs show deposited ECM proteins along with improved chondrogenic characteristics marked by an average 4-fold Sox9 mRNA upregulation, **Fig. 6A**, and Runx2 reduction to 0.16-fold, **Fig. 7A**.
- Spatial mapping of total collagen, GAG, and lubricin reveals an average 1.2-, 1.3-, and 1.5-fold increase, respectively, for perfused surface MSCs compared to ones present deep in the construct
- Engineered AC developed in our TBR show varied effects where total collagen content at tissue surface increases with elevating mechanical loading magnitudes while lubricin expression heightens with decreasing loading magnitudes.

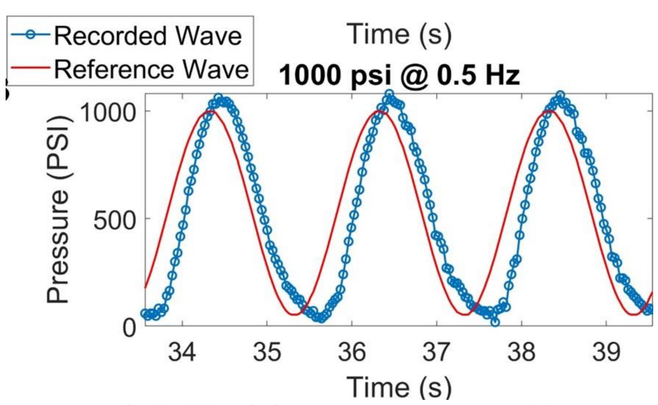

Fig. 8: 1000 psi hydrostatic pressure oscillations at 0.5 Hz.

Future Work

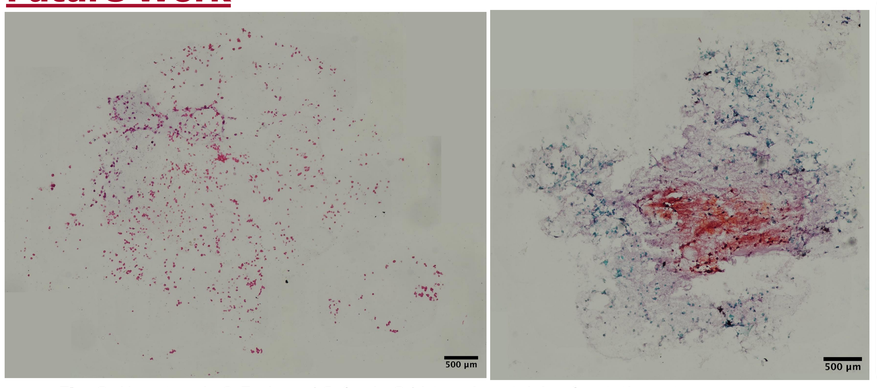

Fig. 9: Hematoxylin & Eosin and Safranin O histological staining for static control samples

Acknowledgements

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