The Mechanics of Human Tissue Engineered Constructs Seeded with Induced Pluripotent Stem Cells or Primary Chondrocytes

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Introduction

- Current tissue engineered cartilage techniques rely on procuring healthy cartilage to obtain autologous human chondrocytes, requiring 2 surgeries[1]
- Induced pluripotent stem cells (iPSCs) can differentiate into chondrocytes, produce a cartilage matrix, and have the potential to be an allogenic cell source[2,3]
- The mechanical properties of tissue engineered cartilage may be a better predictor of in vivo success than the biochemical properties alone
- The mechanical properties of cartilage constructs grown with iPSCs are unknown

Objective

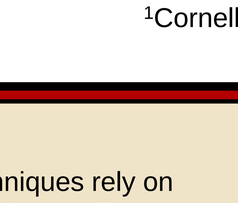
Compare the mechanical properties of constructs grown with iPSCs to constructs growth with human chondrocytes

Methods

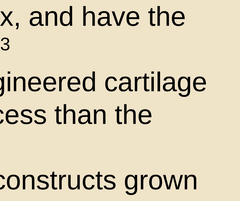
Tissue Engineered Construct Preparation

Using the following combination of cells and media

- Primary Chondrocytes (PC)
  Media: DMEM/F12 with 1% ITS, 10% FBS[4]
- iPSCs from Chondrocytes (Ch-iPSC)[5]
  Media: Chondrogenic supplied by Intrexon



Cells at a concentration of $5x10^6$ cell/ml were seeded into a collagen type I honeycomb scaffold
Constructs were incubated at 2% oxygen, 5% CO_2, and 37˚C for 5 weeks

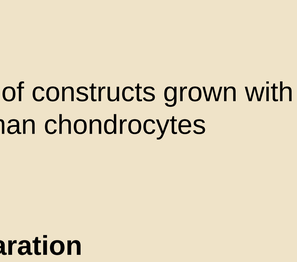


Biochemical Testing

- sGAG assay (DMMB)
- DNA assay (Hoechst)
- Saf-O staining (sGAG)

Mechanical Testing

- Confined compression
- Cartilage on glass tribology[6]
- Microscale compressive and shear mechanical testing[7]



Tribometer

Construct Preparation | Compressive Analysis | Shear Analysis

Digital image correlation (DIC) code analyzed movement and calculated strain[8]
Results were considered statistically significant based on a t test ($p < 0.05$)

References

1) Crawford+ 2012. 2) Kimura+ 2016. 3) Yamashita+ 2015.
4) Crawford+ 2009. 5) Yakubov+ 2010. 6) Gleghorn+ 2007.
7) Buckley+ 2008. 8) Blaber+ 2015. 9) Middendorf+ 2017a.
10) Bonnevie+ 2016. 11) Middendorf+ 2017b

Acknowledgments

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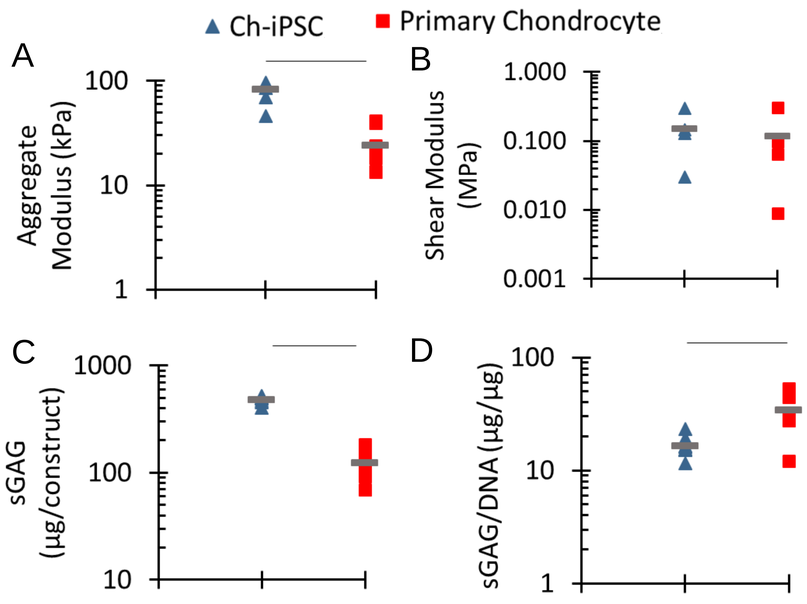
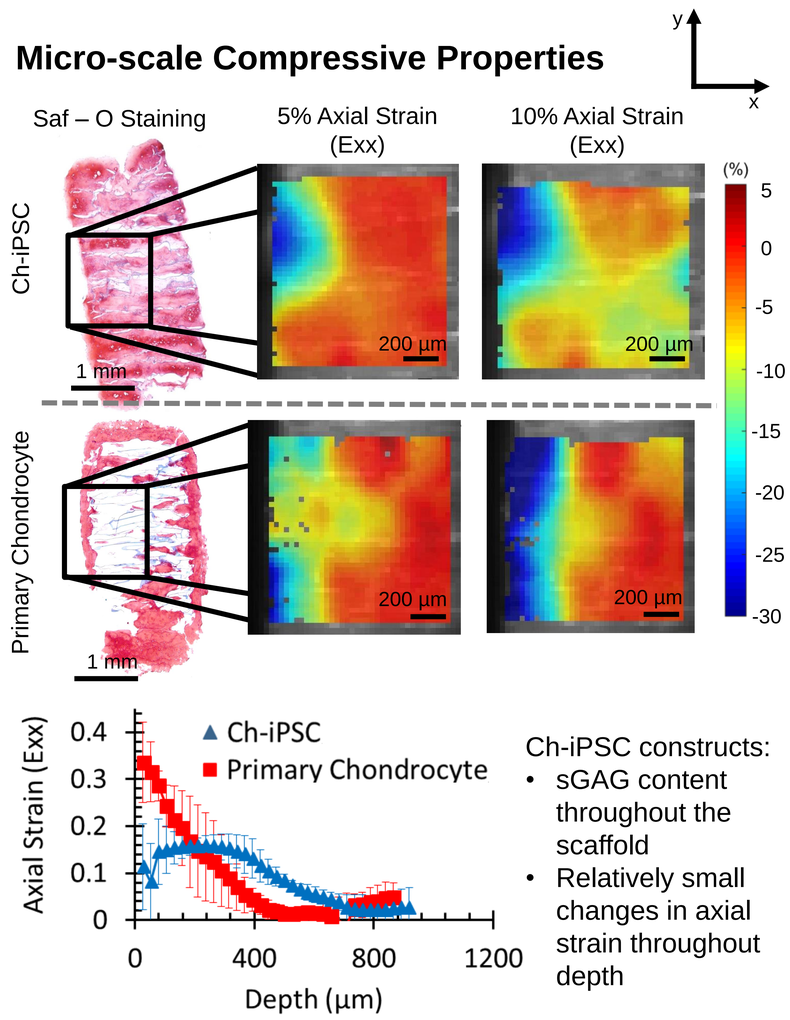
Results

Macro-scale Properties of iPSC form Chondrocytes and Primary Chondrocyte Constructs



A) Aggregate Modulus was not affected by cell type
B) Shear modulus was not affected by cell type
C)Total sGAG content was influenced by cell type
D) sGAG per DNA was affected by the cell type
E) Friction did not change based on cell type
(N = 8, bars indicate $p < 0.05$)

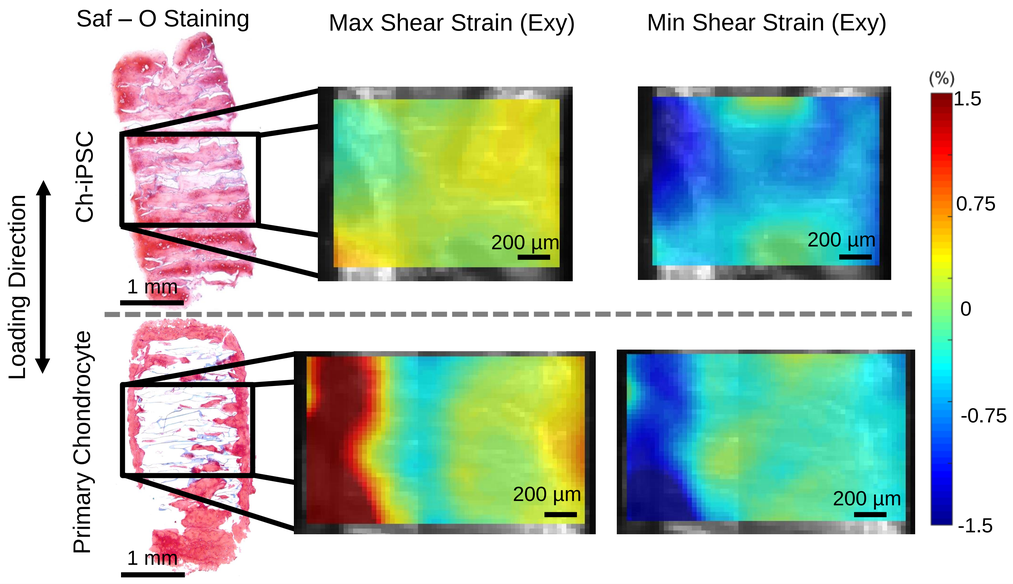
Micro-scale Compressive Properties

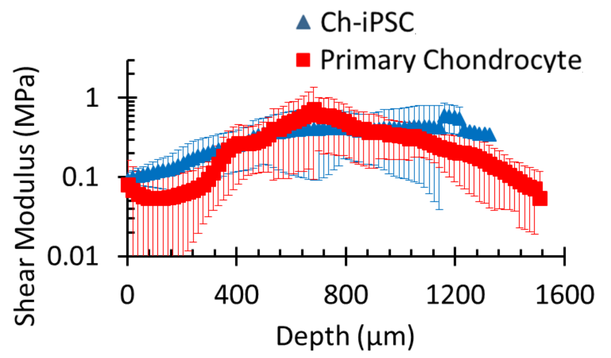


Ch-iPSC constructs:
- sGAG content throughout the scaffold
- Relatively small changes in axial strain throughout depth

Primary chondrocyte constructs:
- sGAG content concentrated on the edges of the scaffold with less sGAG content near middle of constructs
- High axial strain at construct surface (N = 4)

Micro-scale Shear Mechanical Properties





- The surface of the primary chondrocyte constructs was the most compliant under shear
- Ch-iPSC constructs had relatively small changes in shear modulus with changes in depth (N = 4)

Discussion & Conclusions

- Ch-iPSC produced more sGAG content and a higher aggregate modulus than primary chondrocytes after the same culture period
- Friction reached values similar to native tissue[9] and was not affected by cell type
- Shear modulus remained an order of magnitude below native tissue[9] and was not affected by cell type
- Differences in the depth dependent shear and compressive properties may have been caused by depth dependent sGAG content as seen in histology staining
- The role depth dependent mechanics has in the in vivo function of engineered cartilage is unknown. Both the low surface modulus of primary chondrocyte constructs[10] and the uniform matrix fill in the Ch-iPSC constructs[11] may benefit implant function.

Significance

Ch-iPSC produce a similar or more robust construct than primary chondrocytes based on the mechanical properties, indicating iPSCs could replace autologous human chondrocytes as a clinically viable cell source.