

## INTRODUCTION

### CHONDROCYTE 2D PELLET CULTURE STUDY

- Combined loss of EZH1 and EZH2 in chondrocytes severely impaired skeletal growth in mice
- A 2D chondrocyte pellet culture study was used to understand chondrocyte hypertrophy.
- In the 2D pellet culture study, chondrocytes were pelleted in a falcon tube by centrifugation

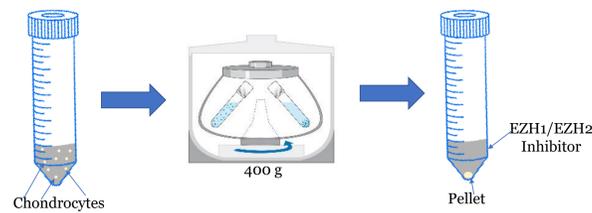


Figure 1. Schematic of the 2d pellet culture process used for original chondrocyte studies

### 3D vs. 2D Pellet Culture

- Efficacy of chondrogenesis is better using a 3D culture system
- Chondrocytes cultured in 3D culture systems show less hypertrophic phenotypes

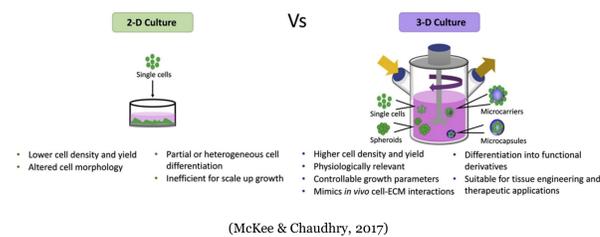


Figure 2. Comparison of 2D vs. 3D culture experiments (McKee & Chaudhry, 2017)

### REPURPOSING OF SAMPLE CELL USED IN ANALYTICAL CENTRIFUGATION

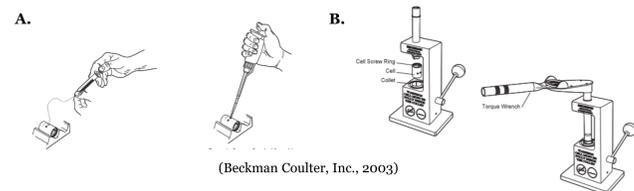


Figure 3. Schematic of analytical ultracentrifugation sample cell preparation: A) Loading sample, and B) Assembly (Beckman Coulter, Inc., 2003)

- Analytical centrifugation is a method used for the quantitative analysis of macromolecules in solution
- Assembly torque system controls clamping pressure to prevent leakage between two surfaces with Teflon gasket
- Analytical centrifugation sample cell fits into Falcon 50mL tube used for cell pellet centrifuge

## OBJECTIVE

Develop a method and device enabling centrifuge cell pellet capture for subsequent 6-well plate chondrocyte cell culture gradient experiments.

## MATERIALS

### 3D PRINTED INSERT TO BE PLACED IN SAMPLE CELL

- The insert is comprised of two parts, 3D printed using acrylic-like material
- Top well holds up to 2mL of chondrocyte solution
- Legs to elevate inside of a 6-well plate
- Pellet well is 1mm (diameter) X 2mm (length)
- A small circular Teflon sheet is used as a gasket to prevent leakage by clamping the top and bottom parts

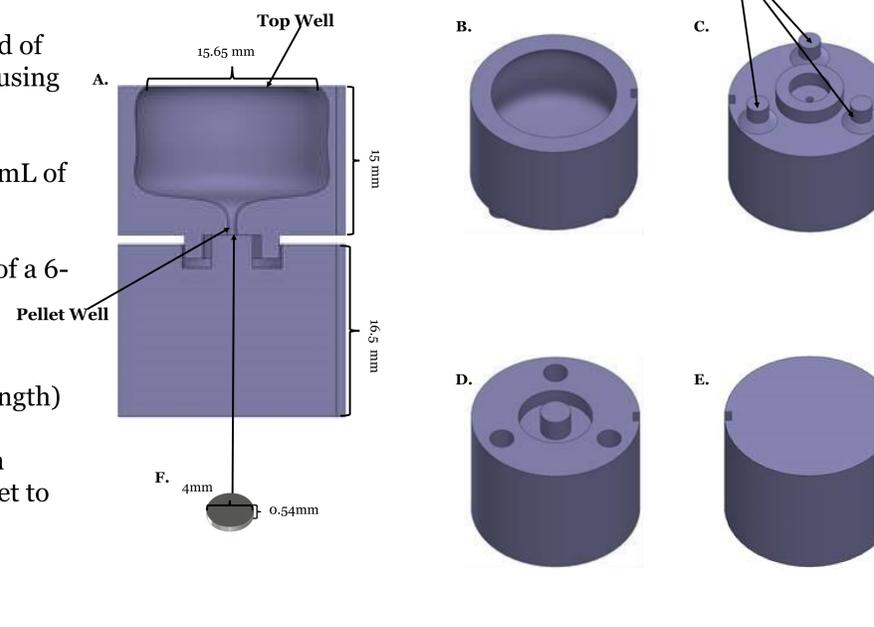


Figure 4. Images of the 3D printed insert parts: A) Assembled, B) Top view of top part, C) Bottom view of top part, D) Top view of bottom part, E) Bottom view of bottom part, and F) Teflon gasket

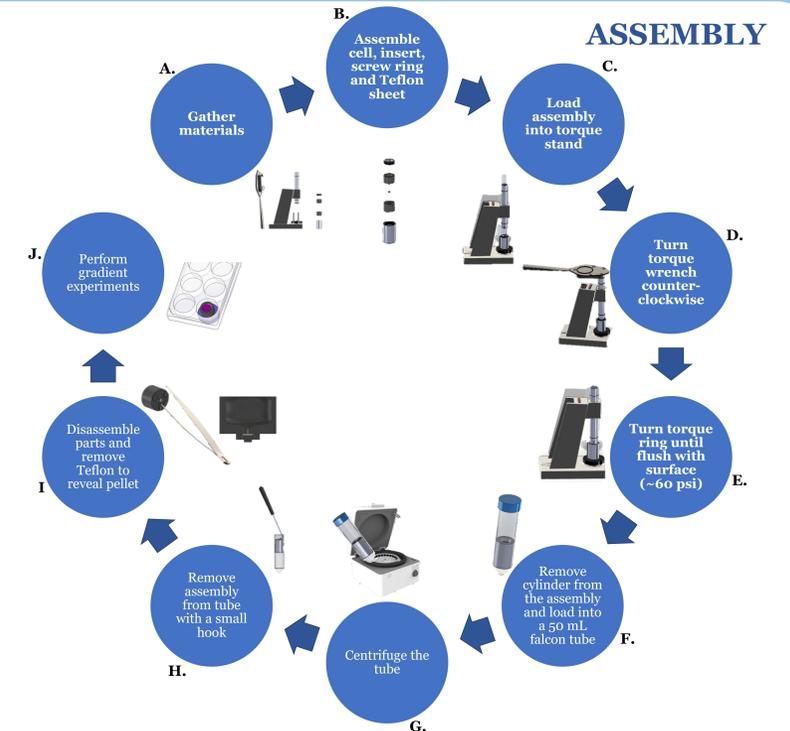


Figure 5. Assay: Insert assembly, centrifuge, pellet, and cell culture gradient experiment.

## TESTING

### Blue Dye Test

- 1G leakage test conducted overnight with blue liquid dye

### Centrifugation Test

- 1000G and 3000G leakage tests conducted for 5 minutes each with centrifuge
- **\*\*Cell pellet assay requires only 400G\*\***

### Centrifugation Test with Cells

- Chondrocytes were spun down in an Eppendorf tube, resuspend in 300uL fluid, spun in an outswing bucket centrifuge at 400g for 3mins.

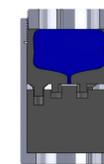


Figure 6. Schematic of 1G testing procedure

## RESULTS

- No leakage detected
- Cells congregated near the top of the pellet well and were unable to collect inside of the pellet well
- Protrusions sticking out of the surface due to the rough texture of the walls
- Pellet floats during cell gradient experiment
  - Pellet is not forming inside the hole
  - Hydrostatic pressure is different between the top well fluid and fluid in the six well plate

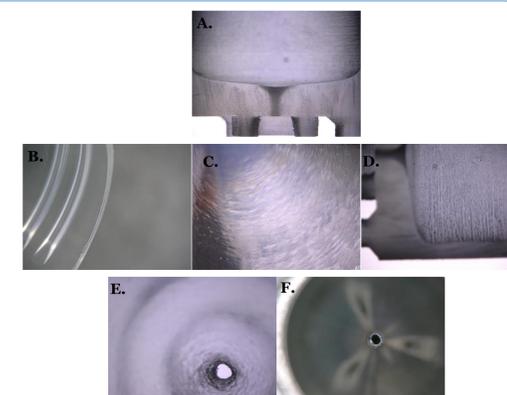


Figure 6. Images of 3D Printed Insert Under a Microscope: A) Image of the pellet well B) Falcon tube surface C) 3D printed insert, glossy finish D) 3D printed surface, matte finish E) Pellet well top view, matte finish F) Pellet well top view, glossy finish

## DISCUSSION

- Designed a method and device for cell pellet capture and subsequent culture media-gradient experiments
- Method will be used to further study chondrocyte hypertrophy

## FUTURE DIRECTIONS

- Modify the shape of the main well to improve the collection of the pellet in the pellet well
- Fabricate insert with a material that has a smoother surface texture

## REFERENCES

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