Method and Device Development for 3-Dimensional Chondrocyte Pellet Culture Studies

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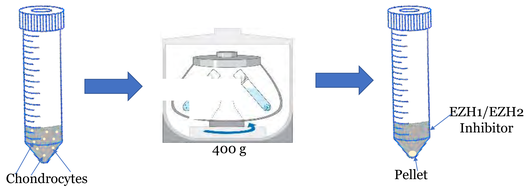
INTRODUCTION

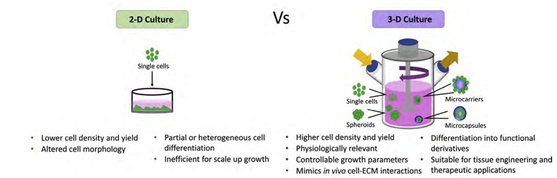
CHONDROCYTE 2D PELLET CULTURE STUDY

- 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



3D vs. 2D Pellet Culture

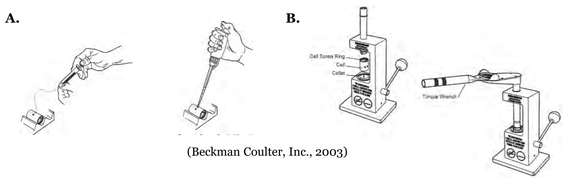
- Efficacy of chondrogenesis is better using a 3D culture system
- Chondrocytes cultured in 3D culture systems show less hypertrophic phenotypes



(McKee & Chaudhry, 2017)

Figure 2. Comparison of 2D vs. 3D culture experiments

REPURPOSING OF SAMPLE CELL USED IN ANALYTICAL CENTRIFUGATION



(Beckman Coulter, Inc., 2003)

Figure 3. Schematic of analytical ultracentrifugation sample cell preparation: A) Loading sample, and **B)** Assembly

- 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

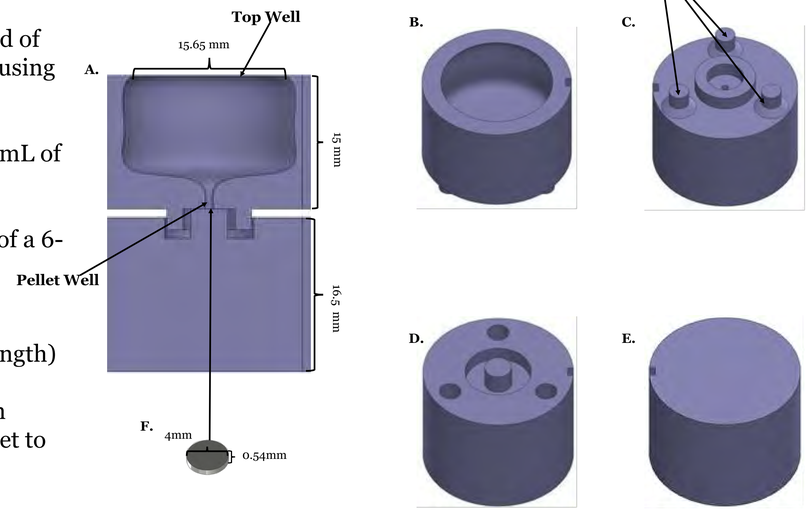


Figure 4. Images of the 3D printed insert parts: A) Assembled, **B)** Top view of top part, **C)** Bottom view of top part, **D)** Top view of bottom part, **E)** Bottom view of bottom part, and **F)** Teflon gasket

ASSEMBLY

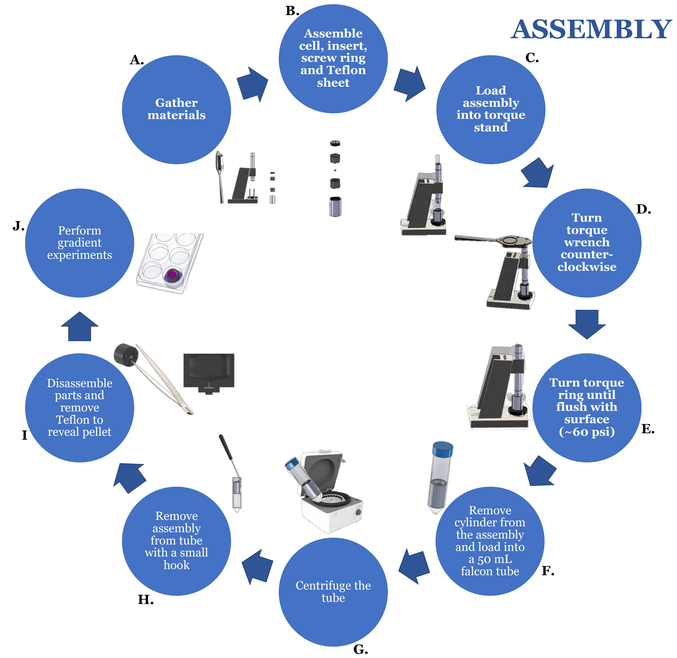


Figure 5. Assay: Insert assembly, centrifuge, pellet, and cell culture gradient experiment.

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.

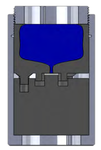


Figure 6. Schematic of 1G testing procedure

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

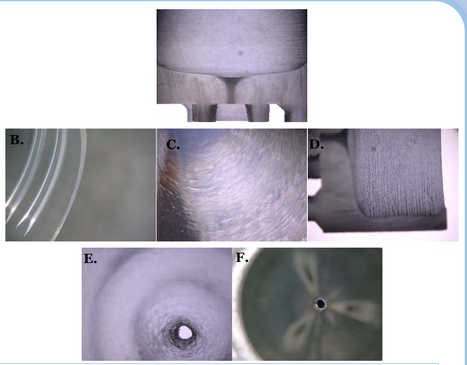


Figure 6. Images of 3D Printed Insert Under a Microscope: A) Image of the pellet well **B)** Falcon tube surface **C)** 3D printed insert, glossy finish **D)** 3D printed surface, matte finish **E)** Pellet well top view, matte finish **F)** Pellet well top view, glossy finish

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

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