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Patterned Alginate Hydrogels to Induce Chondrocyte Alignment

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The growth plate has an intricate architecture, and this architecture is necessary for directional growth of bones. Specifically, the cells align in longitudinal columns. As the growth plate expands with this pattern, the bone elongates with the same alignment pattern.

The purpose of this research is to mimic this single celled, columnar alignment in vitro. In developing this alignment in vitro, this research will contribute to the overall study of growing growth for the development of improved therapeutic treatments and engineered tissues for transplants.

**Methods**

**Method 1: Photolithography**
A PDMS mold was created using a mask formed via photolithography. 1.5% (w/v) alginate crosslinked on top of this mold (Figure 1). The disk was cut away from the mold and the pattern was exposed for cell seeding.

**Method 2: Polystyrene Mesh**
Mesh with a 200 µm thread diameter was coated with calcium chloride through solution evaporation. Alginate was added on top of the coated mesh piece and allowed to crosslink for 24h (Figure 2). The mesh was removed from the disk, exposing the pattern for cell seeding.

**Results**

- Patterns were often damaged or ruined during removal from PDMS mold developed in Method 1 (Figures 3B, 4A,B)
- Cell alignment was unsuccessful when seeded on alginate disks from Method 1 (Figure 4C)
- Cell alignment was successful when seeded on alginate disks from Method 2 (Figure 4D,E,F)
- A seeding density of 90,000 cells/well optimized cell-to-cell interaction while reducing overcrowding, which are the factors necessary for alignment and proliferation
- Cells organized best when allowed to fall into a “trough”. This was observed using the dimensions from Method 2

**Conclusions/Future Work**

- Alginic patterning using photolithography is not successful on micro-scale
- Crosslinking from the bottom-up forms a smoother, more durable alginate disk
- Seeding Density of 90,000 cells/well optimizes proliferation while reducing overcrowding
- A thread diameter of 0.2 mm is too large for single cell alignment

**Future Work**

- Determine thread dimensions necessary for single cell alignment
- Engineer a template that promotes alignment and proliferation in longitudinal columns

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