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Patterned Alginate Hydrogels to Induce Chondrocyte Alignment

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Introduction

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.

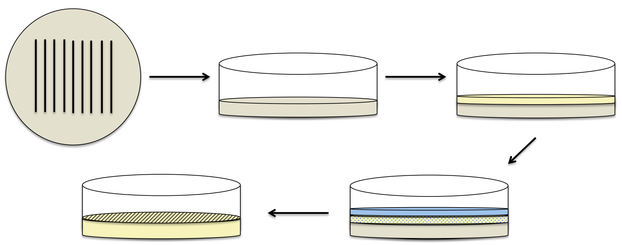

Figure 1: Patterned alginate fabrication method 1, via photolithography.

Method 2: Polystyrene Mesh

Mesh with a 200μm 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.

Methods, cont.

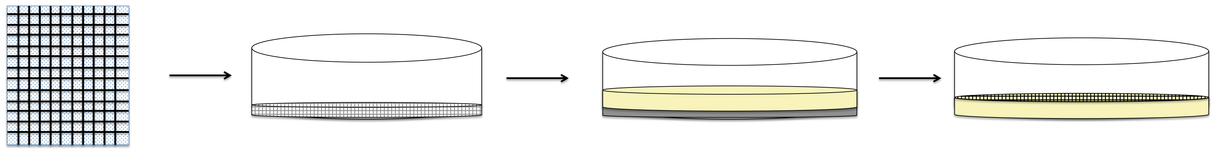

Figure 2: Patterned alginate fabrication method 2, via polystyrene mesh.

Cell Seeding

RCS cells were seeded onto the patterned alginate disks with variable seeding density. The disk and cell system was allowed to incubate in 8.0% CO_2 for 48 hours, imaging at 24 and 48 hours.

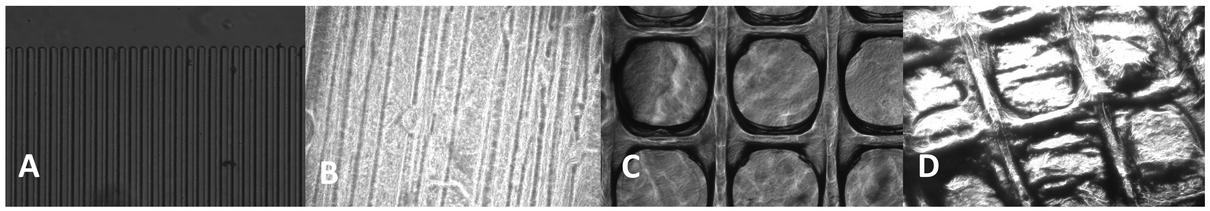

Figure 3: (A) Mold developed through photolithography (B) Alginate disk pattern using (A) (C) Mesh and alginate system (D) Patterned alginate disk after mesh was removed

Results

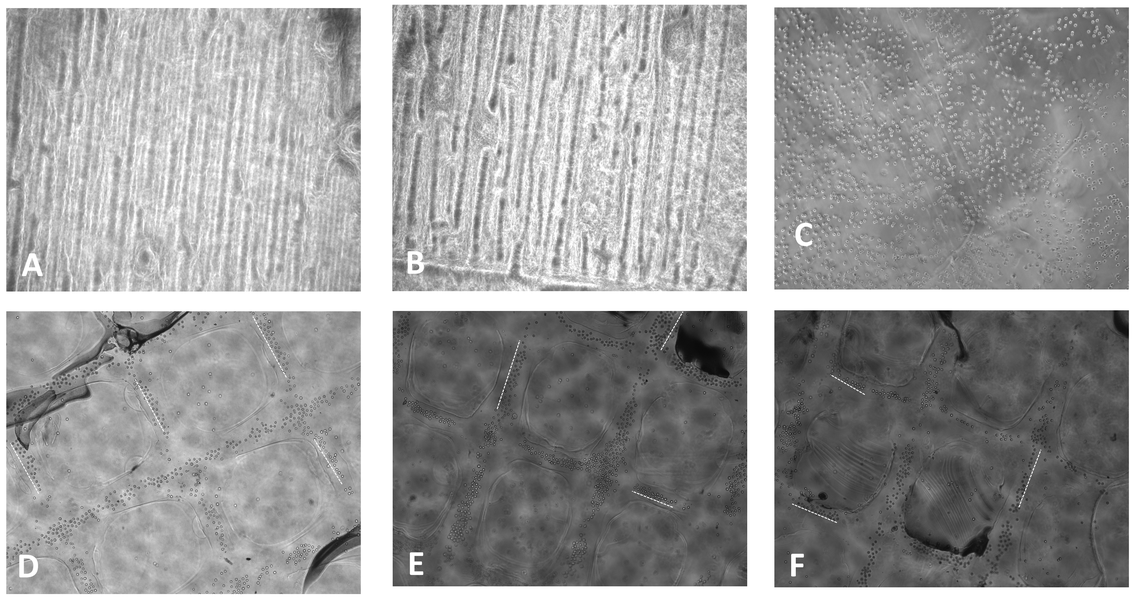

Figure 4: (A,B) Patterned alginate using photolithography mold (C) Cell seeding of 90,000 cells/well using alginate patterned with photolithography mold (D, E, F) Cell seeding at 90,000 cells/well using alginate molded via mesh

Results, cont.

- 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

- Alginate 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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