Affinity for Human Dermal Fibroblast Cells to Adhere to Collagen-infused Cryogel Matrix

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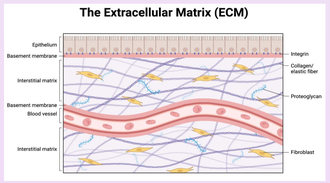
Background

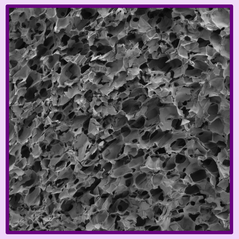
Orofacial Malformations: Cleft Palate and Cleft Lip
- Palate tissue is not properly connected before birth
- 1 in 700 live births just in the United States
- Invasive and complex surgical procedure
- Difficult because of the complex geometry of the structure
- Limited donor availability

Chitosan-Gelatin Scaffold Structure
- Increased pore size when hydrated
- Increased overall volume
- Made using lyophilization method to expel all of the liquid using freeze drying and vacuum
- Consistent size for common cleft palate malformations
- Can withstand high pressure like what would be experienced in the body
- Cylindrical shape is best for optimal pore sizes

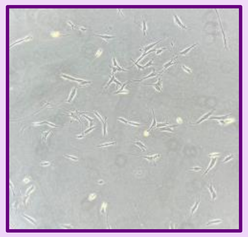
Collagen I
- Abundant in the body
- Makes up a component of the ECM, why it is used for this project
- Needed for best elasticity and tissue movement of extracellular matrix



Swelling Ratio
- Increases pore size when hydrated
- Increased volume
- Increased surface area for cell adhesion

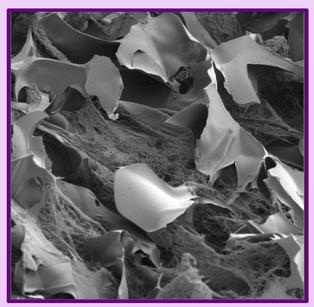


SEM image cryogel, hydrated in PBS 500X mag



Human Dermal fibroblast cells under 10X mag

SEM Imaging with Collagen Matrix

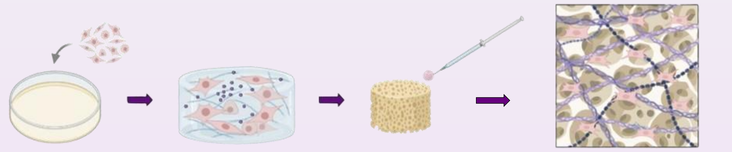


Cryogel with collagen gel, taken at 500X mag

Cryogel with collagen gel, taken at 2000X mag

Experimental Design

Human dermal fibroblast (HDF) cells were cultured and left to grow over a one-week period. The cells were then integrated into the 3D collagen gel before being injected into six different cryogels, which had been hydrated in PBS and had an average swelling ratio of 16.84. Once implemented in the gel, the cells from two cryogels were fixed on days one, three, and seven. After all six were fixed, the cryogels were ready to be stained with auto-fluorescent dyes that would illuminate cell growth in confocal images. Finally, the cryogel samples were washed with alcohol and prepped for scanning electron microscope (SEM) imaging.

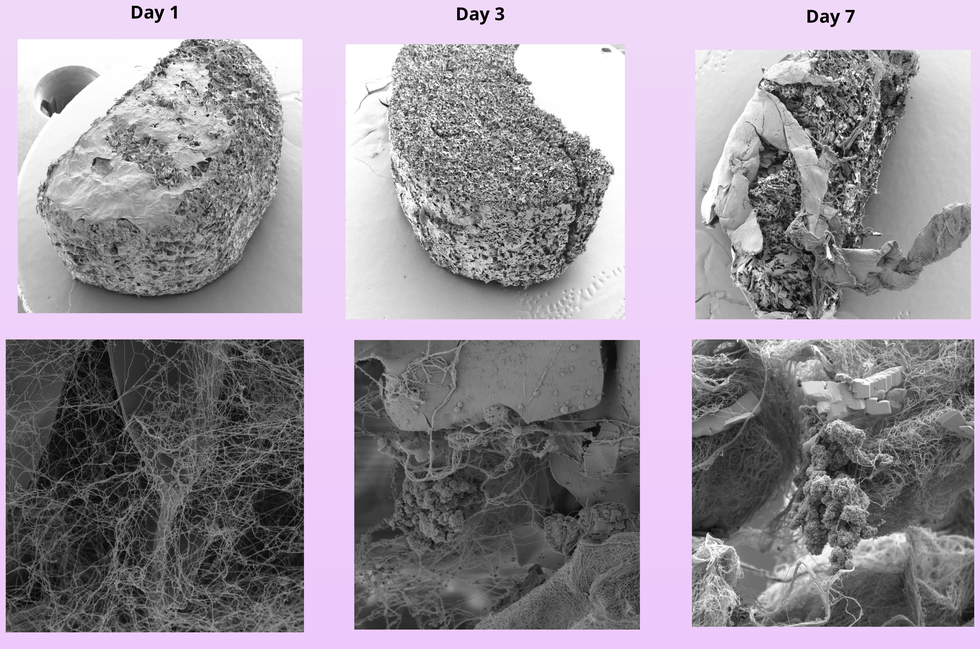


HDF Cell Culture | 3D Cell-Hydrogel | 3D Gel to Scaffold | Predicted SEM Final Imaging

Confocal Imaging of HDF Proliferation

Cryogels Stained with DAPI and Phalloidin to differentiate between the cells and the scaffold

SEM of Scaffolds with HDF Cells



Cryogel scaffold with HDF cells and Collagen Gel taken at 40X mag

Cryogel scaffold with HDF cells and Collagen gel taken at 5000X magnification

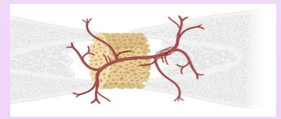
Results

The confocal imaging is overall inconclusive. The SEM Imaging shows cell growth on collagen fibers within the scaffold. Cells did not adhere to cryogel, but collagen did attach to cryogel. The cells and collagen gel did not penetrate the cryogel very effectively, resulting in more of the gel being on the plate outside or around the scaffold. Overall, more trials and samples need to be done to find an average trend in data to understand the actual affinity of the Collagen-HDF cell matrix.

Future Work

Experimental Design Enhancement
- Do additional replicate trials of experiment
- Try different methods for syringing collagen into the cryogel (such as using smaller gauge needles)
- Lower the gel to cell ratio to increase concentration of cells relative to the cyrogel
- Analyze the layers of the gel deeper than surface-level for cell growth and proliferation
- Thoroughly wash ionic buffer solution off gels before imaging to ensure salts are thoroughly removed from matrix (to make results clearer)
- Quantitively analyze the number of each cells growing on the gel relative to time

Further Applications of Cryogel
- Apply gel to live patient cells specific to the palate
- Apply in imitation of human mouth with cleft palate and bone integration
- Medically insert cryogel scaffold into cleft palate of a patient in an experimental medical procedure
- Replace current procedures to treat cleft palate fully with methods using cryogel scaffold



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