Integrating Cryogels and Fibrin Gels to Create a Scaffold for Bone Regenerative Medicine

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Introduction

- Current methods for treating cleft palates include bone scaffolding, which lacks specificity
- Children have less donor bone available, making bone scaffolds difficult to locate
- Cleft palate defects affect feeding, breathing, speaking, and smiling
- Cryogels are 3D printed macropourous sponge-like structures which have potential for being used as a scaffold for patient specific bone defects
- This marcoporous structure allows cell infiltration within 28 days
- They have excellent mechanical properties as they have a high swelling ratio and can be compressed without cracking
- Fibrin gels are composed of fibrinogen, an ECM protein, and is crosslinked using the blood clotting enzyme thrombin

Cryogel Composition

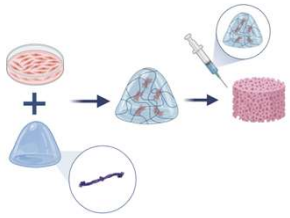
•The cryogel scaffolds are tissue-engineered products that are formed at sub-zero temperatures
•When heated, the scaffolds develop large pores that are suitable for cell culturing
•3D bioprinting can be used to create acellular scaffolds, and using 3D printed molds to create molds proves promising
•Cryogels are composed of 1% acetic acid and chitosan and the solution was then added to the 3D molds which was cooled at -20C for 30 hours
•After the 3D molds were cooled for 30 hours, they were thawed in lukewarm water

Methodology

- Created two tubes of 0.25 mL (one contained fibrin and PBS++ while the other contained thrombin (which solidified the gel) and PBS++)
- 0.5 mL of cells were added to the fibrin tube
- Combined contents of these tubes quickly and mixed well with pipette to ensure the gel could be injected
- Added media to feed the cells, wash away dead cells, and neutralize the freezing media
- Carefully removed the media after spinning to leave the cells behind
- Counted the cells using Epi and micro blue dye after adding more media to ensure we were adding enough living cells to the gel
- Allowed the cells to integrate and feed them regularly over a period of 2 weeks
- Conducted imaging to see if the cells had grown.

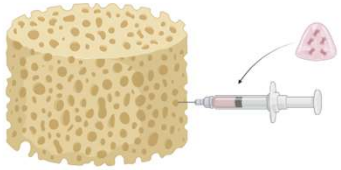
Integration of Cells and Scaffold

Culturing Cells in Cryogel



Mixing the fibrin and cell mixture with the thrombin crosslinker immediately prior to injection allowed for synthesis to occur within the structure.

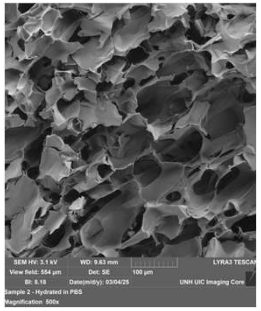
Hydrated Cryogel and Cells



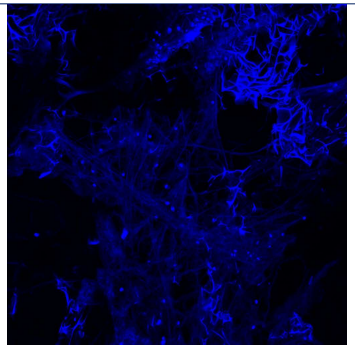
Injecting the gel directly into the structure allowed for maximum saturation throughout the scaffold.

Material Surface

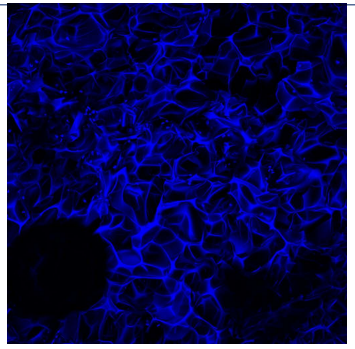
- Porosity is the amount of space/holes in between the material, which allows air and fluid to pass through.
- Is a very important component when considering scaffolds, as it is what enables cells to grow inside
- Investigated porosity of the cryogel by soaking it in PBS++ and analyzing the swelling ratio
- Swelling ratio provides insight on the material's potential to hold liquid and substances



Cell Migration in Scaffold



DAPI stained blue nuclei of HDFS



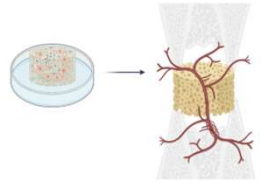
Autofluorescence of Scaffold presents issues for imaging cell proliferation and migration

Results

- HDFs in the fibrin gel were incapsulated in the cryogel matrix
- Some attachment and integration of HDFS was shown on scaffolds
- The majority of the gel flowed through the structure, pooling at the bottom of the dish along with the cells

Future Application

- Cryogels allows for personalization of scaffolds to fit patient need for bone regenerative medicine
- The cryogel scaffold helps to regenerate bone and speeds up the healing process, specifically when placed as the craniofacial defect site in cleft palate patients.
- A way to better encapsulate the gels and cells in the scaffold is needed to reduce pooling of the fibrin gels on the bottom of the dish
- A method for adhesion of the cryogel scaffold onto the petri dish could be improved
- Future studies seek to integrate vascularization into the cell gel scaffold matrix



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