MSC Embedded Fibrin Loading of Poly(propylene) Fumarate Scaffolds for Bone Tissue Engineering: A SCID Mouse Model

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Introduction

Background:

3D-printed poly(propylene fumarate) (PPF) scaffolds have been developed for bone regeneration to provide structural support for tissue ingrowth and development. Fibrin gels have been shown to increase vascularization *in vivo*[1,2]. The vascularization of the newly formed bone is essential for tissue survival and function[1]. To promote and enhance this, we explored incorporating mesenchymal stem cells (MSCs) into the fibrin matrix.

Hypothesis:

We hypothesize that loading the PPF scaffold with a fibrin hydrogel as well as an MSC cell spheroid aggregate will promote tissue invasion and vascularization in an *in vivo* SCID mouse model.

Objective:

Develop a method to load the fibrin and cell aggregate into the scaffold such that these constructs contain equal volumes of the hydrogel as well as a viable aggregate at the time of surgical implantation in the rodent model. Confirm through histology this method worked.

Materials and methods:

Scaffold Preparation

- Scaffolds were printed and washed with acetone, 70% EtOH, and distilled water.
- Scaffolds were post-cured in a UV box for 8 hours and dried in an oven at 37^{O}C before loading.

Aggregate Formation

- Cell aggregates were created using P3 canine MSCs at 5000 cells per aggregate using a combination of methylcellulose and DMEM and left to incubate at least 24 hours prior to suspension in fibrin gel.

Preparation of Fibrin Gel

- Scaffolds were loaded in 3 layers. Layer 1: 10 uL of 10 mg/mL fibrinogen (Fg) + 0.5 uL 100 U/mL Thrombin (Tb). Layer 2: cell aggregate suspended in ~1 uL of PBS. Layer 3: 14 uL of 2 mg/mL Fg + 1 uL of 100 U/mL Tb.
- Each layer was allowed to incubate for 15-20 minutes in order for gel to form a firm but elastic layer.

Implantation of Loaded Constructs

- Loaded scaffolds were implanted dorsally on 5 male outbred ICR mice using subcutaneous pockets.
- Explants were retrieved post formalin perfusion after 1 week of growth and analyzed with H&E and Masson's Trichrome Staining.

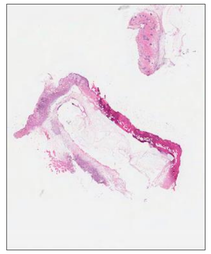
Results

Preliminary Imaging of Scaffolds Before Implantation

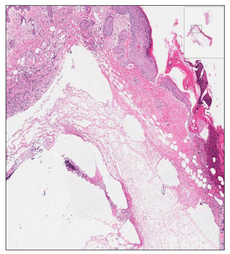
Empty Scaffold

Hope to have microscopy images of fibrin loaded scaffold here. I emailed Ruchi, hopefully she can get them to me since she never uploads anything to box. If I can't get them, maybe best to use pictures you uploaded last December? Either that or I can include solidworks pics for future directions.

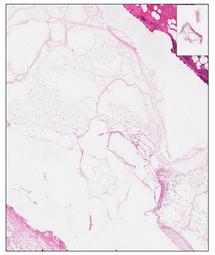
H&E Staining After 1 Week of Growth



Full Scaffold View



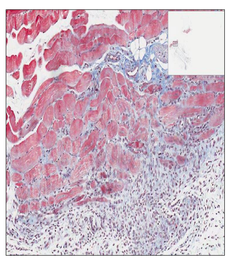
Surrounding Tissue



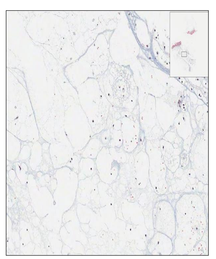
Scaffold Interior

Masson's Trichrome Staining After 1 Week of Growth

Full Scaffold View



Surrounding Tissue



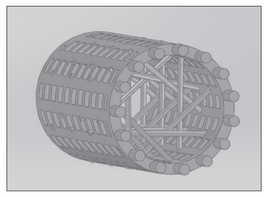
Scaffold Interior

Conclusions

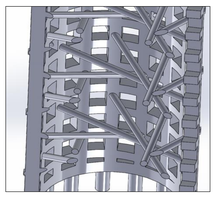
- The cell aggregates are present in about 50% of loaded scaffolds immediately after loading.
- Only about 50% of scaffolds that were loaded had the full amount of fibrin gel at at the time of imaging (pre-implant).
- Few if any scaffolds still contained both gel and aggregate at the time of implantation.
- The current loading method is not adequate for preparing implants.

Future Directions

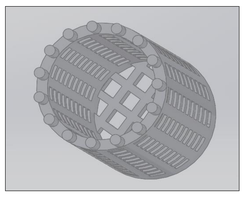
- Adjust the scaffold geometry to include more PPF surface area for gel to cling to (scaffold becomes more matrix-like, shown below)
- Adjust scaffold geometry to include a cap at one end, preventing gel from possibly leaking out prior to formation (shown below)
- Increase concentration of fibrinogen in mixture to create a firmer gel
- CD31, alpha-smooth muscle actin staining, and lectin imaging to analyze formation of vasculature
- 1 week SCID mouse model and 3 week SCID mouse model studies



Alternate Scaffold Geometry: Matrix



Alternate Scaffold Geometry: Matrix, Section View



Alternate Scaffold Geometry: Cap

References

[1]Jiang,B., Waller, T.M., Larson, J.C., Appel, A.A., Brey, E.M. Fibrin-Loaded Porous Poly(Ethylene Glycol) Hydrogels as Scaffold Materials for Vascularized Tissue Formation. Tissue Eng Part A, 224, 19, 2013.
[2]Brey, E.M., McIntire, L.V., Johnston, C.M., Reece, G.P., and Patrick, C.W., Jr. Three-dimensional, quantitative analysis of desmin and smooth muscle alpha actin expression during angiogenesis. Ann Biomed Eng 32, 1100, 2004.

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