Optimization of Endothelial Cell Seeding onto Engineered Tissue Valve

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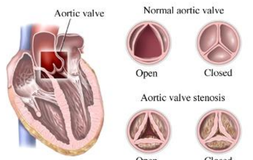
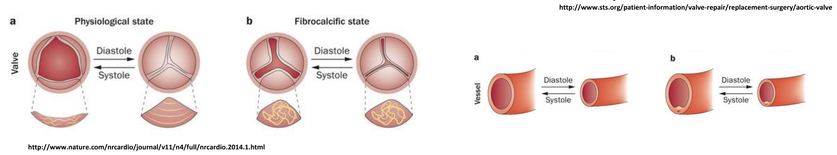
Introduction

Background

Advantages to Biological valves:
- Regeneration, remodeling, and growth possibilities
- Endothelialized tissue is non-thrombogenic



Tricuspid aortic heart valve
http://www.sts.org/patient-information/valve-repair/replacement-surgery/aortic-valve



http://www.nature.com/nrcardio/journal/v11/n4/full/nrcardio.2014.1.html

Objectives

1. Optimize protein pre-coating
2. Determine optimal seeding cellular density
3. Evaluate luminal versus abluminal cell adherence
4. Achieve endothelialized monolayer on both sides of valve

Methods

Experiment Design : Protein Coating

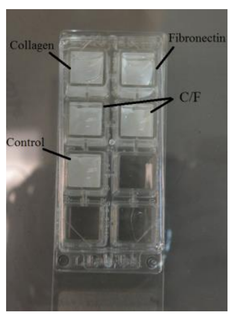
- Cut a previously decellularized engineered tissue in several pieces and placed in well plate (valves developed by remodeling of fibrin gel with seeded fibroblast)
- Coated each well with corresponding coating solution:

50 µg/ml of fibronectin

50 µg/ml of collagen

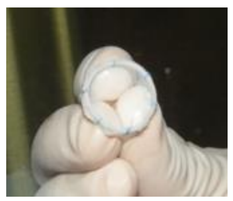
25 µg/ml of fibronectin + 25 µg/ml collagen

- After 24 hour incubation, seeded with hBOEC cells (100k/cm^2)
- Performed staining after 4 days

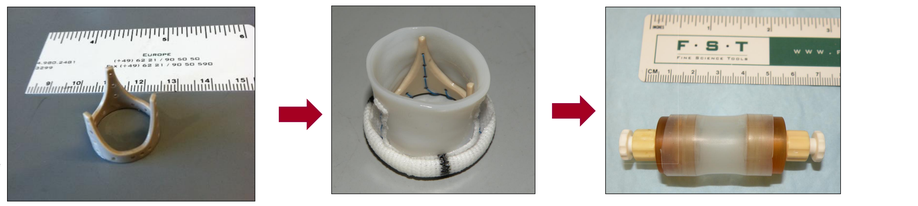


Experiment Design : Valve Seeding

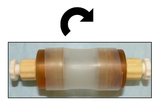
- Mounted the TEHV onto the frame using Prolene suture
- Coated valve with 50 µg/ml fibronectin – 24 hour incubation
- Seeded valve with 1.6 M/ml cell suspension in bioreactor Placed bioreactor in 50 ml conical vial and rotated at 1 RPM for 4 hours
- Valve maintenance involved changing hBOEC media every other day
- Histology was performed 4 days after seeding



Valve Seeding

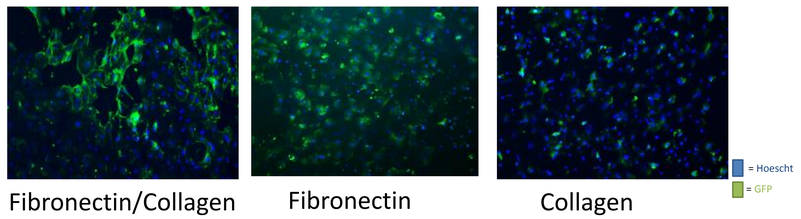


Mount graft in frame → **Place inside of bioreactor, add cell suspension** → **Rotate at 1 RPM**



Results

Protein Pre-Coating



Fibronectin/Collagen Fibronectin Collagen

Figures 1-3: 20x images of tissue pieces from the well plate stained for phalloidin. A confluent monolayer was present for each protein coating tested. After several trials fibronectin produced more similar results on a more consistent basis; overall low amount of variability shown between coatings.

Valve Seeding

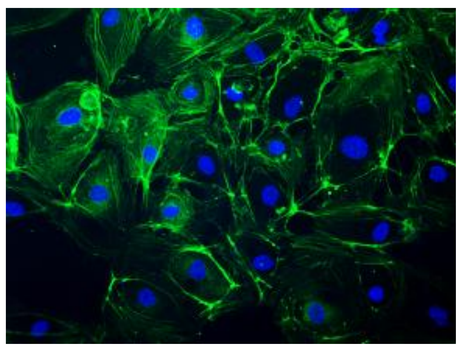


Figure 4

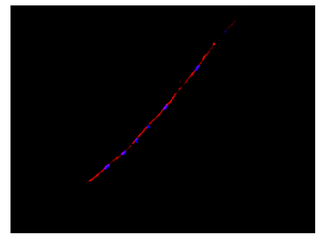
Figure 4: 10x image of valve tissue from luminal side of leaflet stained with phalloidin. Luminal side of all three leaflets displayed a confluent and fully formed monolayer of endothelial cells.

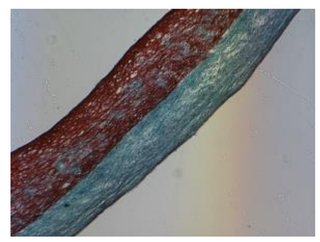
Optimal Seeding Density: 1.6 M/ml

The optimal seeding density has to be large enough to form a monolayer on the tissue, but also remain small enough to retain efficiency.

Results (cont)

Valve Seeding





Figures 5: 4x images of tissue staining for CD31 marker after 20 micron sectioning.

= CD31
= Hoescht

Figure 6: 4x Trichrome staining of valve leaflet after 20 micron sectioning. Fibrin within tissue degrades over time, but remains presence in more oxygenated layers near luminal side.

Conclusions

- Fibronectin protein pre-coating was chosen based on more consistent results over time
- Optimal seeding cell density: 1.6 M/ml in rotating chamber at 1 RPM for 4 hours
- Effective luminal coverage, however, abluminal side showed little to no cells

Future Studies

- Need to investigate methods in order to obtain a confluent monolayer on both sides of the valve.
- Test valve under physiological flow conditions to monitor adherence

References

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Acknowledgments

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