Rotational Seeding of Porcine Aortic Sinus Adventitia

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INTRODUCTION

Valvular Heart Disease (VHD) effects more than 5 million adults in the US. [1] It is characterized by the stenosis and calcification of heart valves that causes abnormal blood flow usually due to stiffened leaflets and inefficient closing/opening of the valve.
The aortic sinus is composed of three wall layers (intima, media, adventitia). The adventitia is the outer, relatively thin layer made up of collagen, elastin and fibroblast cells.[2] The adventitia provides structural integrity to the valve and contains its own network of small blood vessel [3]

AIM: To successfully reseed the adventitial layer of the aortic sinus using a fibrin gel cell delivery method and rotational seeder design

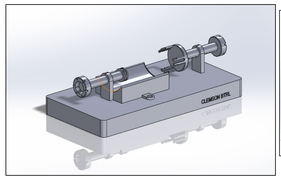
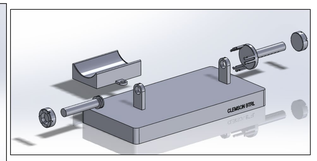
Rationale: We hope to contribute this adventitial focused research to current tissue engineering efforts of the aortic valve so that they can be synergistically combined to produce the first fully tissue engineered aortic valve

METHODS

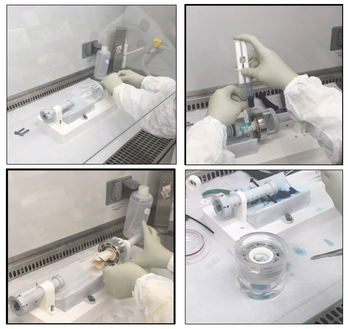
Porcine Valve Dissection and Decellularization
- Porcine hearts were dissected to isolate the aortic valve (AV)
- Coronaries were ligated shut
- Valves were decellularized with immersion decellularization protocol for 14 days

Cell Culture
- Human Adventitial Aortic Fibroblasts (hAAFs) were grown in DMEM (10% FBS and 1% AbAm)
- 7 million cells grown to seed one valve

SolidWorks Design
- Designed for no touch seeding system
- Design compatibility for decellularization, bioreactor, and rotator hardware

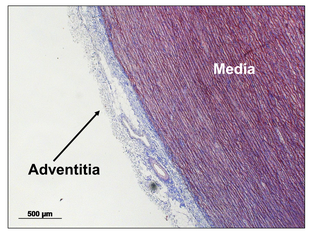



Cell Seeding and Rotator
- Valve mounted and placed onto the rotational seeder
- Fibrin components and cells mixed via double barrel syringe and applied linearly across length of the valve
- 30 seconds of wait time after seeding each section and rotation
- Placed seeded valve in rotator for 36 hours with DMEM Media 10% FBS 1% AbAm

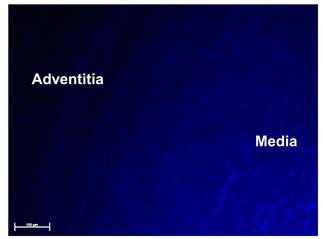


RESULTS

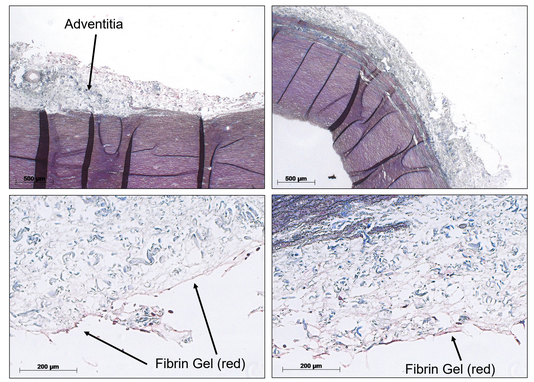
Target Tissue : Adventitia of the Aortic Valve Sinus



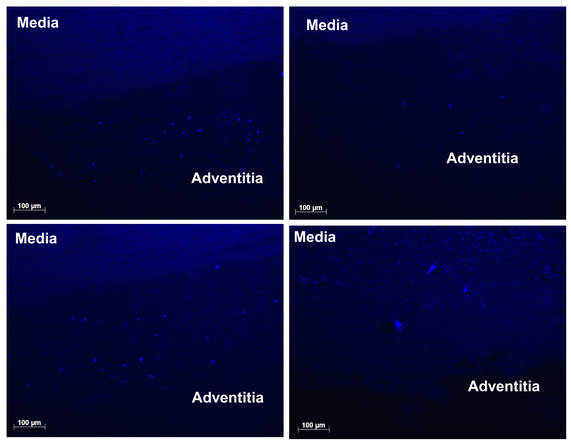
Immersion Decellularization Analysis



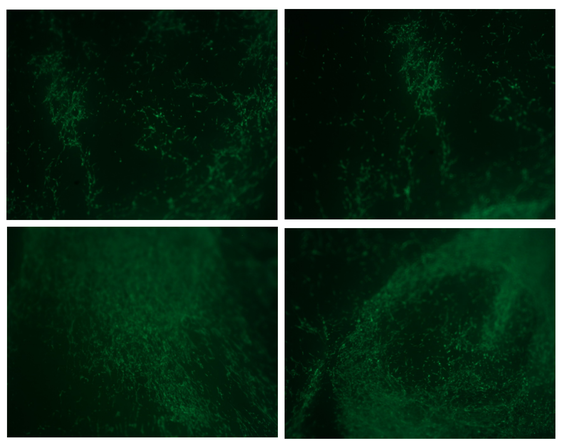
Trichrome – Fibrin Gel Coverage



DAPI – Cell Viability (Nuclei) in adventitia after 36 hours



Live/Dead Fluorescence – Cell Viability after 36 hours



CONCLUSIONS

- Immersion decellularization is acceptable for removal of cells from adventitial layer of aortic sinus
- Fibrin gel deposits were found on the aortic sinus adventitial surface after 36 hours
- Coverage of fibrin gel on adventitial surface was uneven and spotty
  - Initial uneven coverage of fibrin gel during seeding
  - Fibrin gel loss during rotation time in the incubator
- Fibroblast nuclei were observed in adventitial region of valve sinus after 36 hours (DAPI)
- Great cell viability was observed using live/dead fluorescence of adventitial surface after 36 hours

FUTURE WORKS

- Decellularization of valves using perfusion system for more complete removal of cells
- Streamline rotational seeding device design
  - Remove screw securement points
  - Multiple plug arms with different diameters
  - Lengthen both plug arm and ring arm
- Observation of cell viability at longer timeline, preferably at 72 hours
- Use of stromal media with hAAFs for more efficient cell growth

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