The road to building a biological valve

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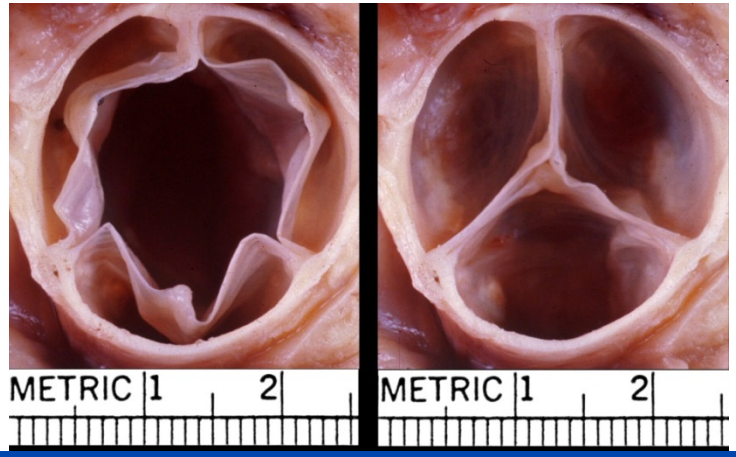
Unmet Needs in Valve Disease

- Adult
  - Number of patients requiring aortic and mitral valve replacement estimated to triple over the next 50 years
- Pediatric
  - Patients with congenital heart disease increased lifespan
  - Bioprosthetic failure rate as high as 20% in the first 6 years
  - No current valve will grow with the child
- Need for anticoagulation is a major issue
- Increasing use of TAVR

Would a tissue engineered heart valve address any of these concerns?

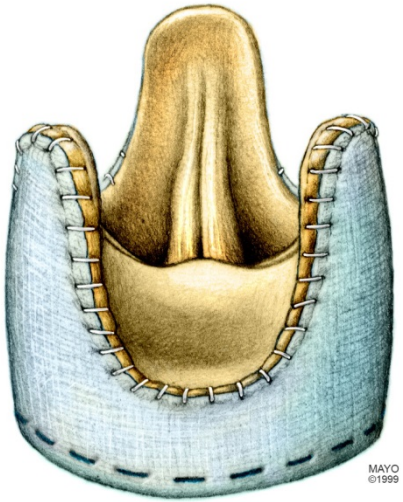
The Challenges in Creating Heart Valves

- Heart valves open and close 40 million times a year
- 3 billion times over a lifetime
- Heart valves:
  - Adapt
  - Unidirectional flow
  - Undergo structural and functional change throughout life
  - Maintain homeostasis
  - Resistance to infection and calcification



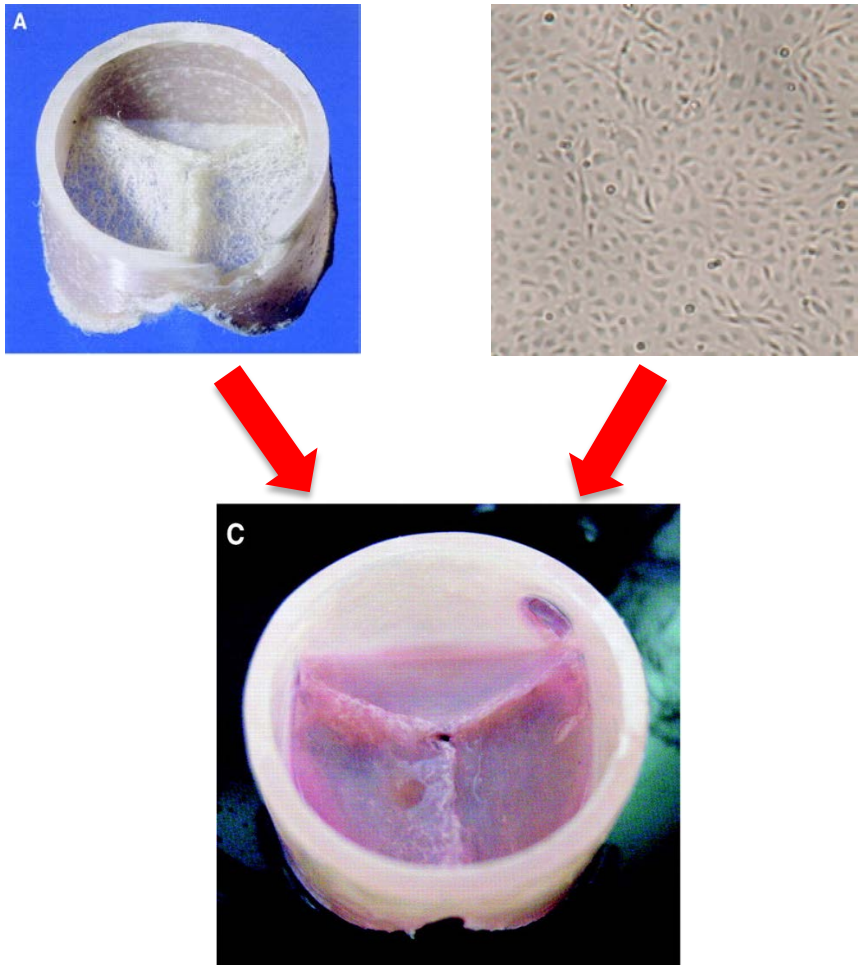
The Challenges : Prosthetic Heart Valves

- Mechanical valves have superior durability (20-30 yrs), but require anticoagulation therapy
  - Requires open chest procedure
- Bioprosthetic valves don't require anticoagulation therapy, but have inferior durability (10-15 yrs in patients over 65 yo; 8-12 yrs in patients under 65 yo)
- Neither type can grow with a pediatric patient



Tissue Engineered Valve

- A biological (not fixed) and/or synthetic scaffold seeded with living cells
- Living tissue valve capable of remodeling, repair, and regeneration
- Potential advantages
  - Superior durability due to resistance to wear, inflammation, and calcification
  - Anticoagulation therapy not required due to resistance to thromboembolism
  - Growth with a pediatric patient
  - Percutaneous



Circulation. 2006;114:I-152-I-158

Biological Scaffold

Synthetic Scaffold

Decell Porcine

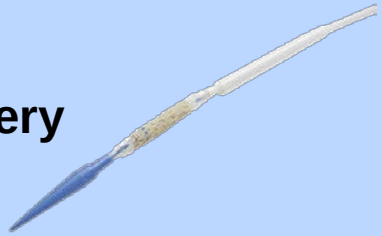
Electrospun Polyeurathane

Electrospun Trilayer

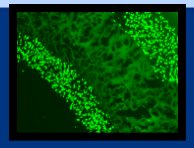
3D Printed

Animal studies

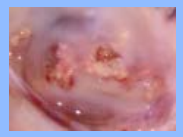
Percutaneous Delivery

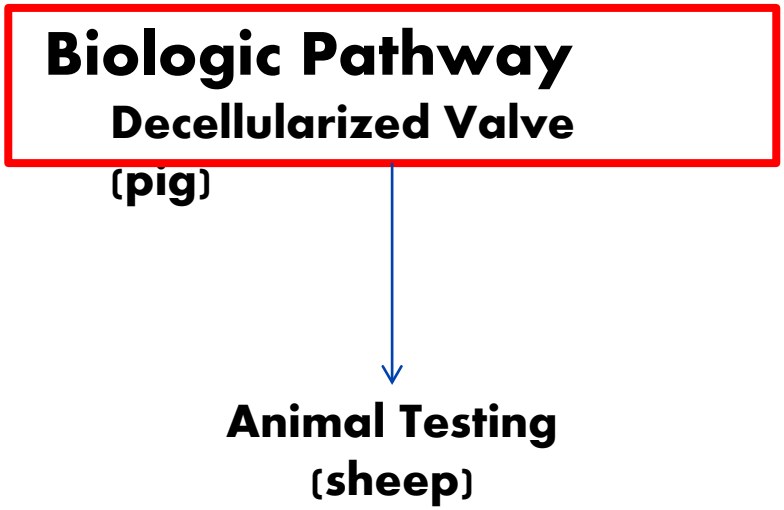


Recellularization



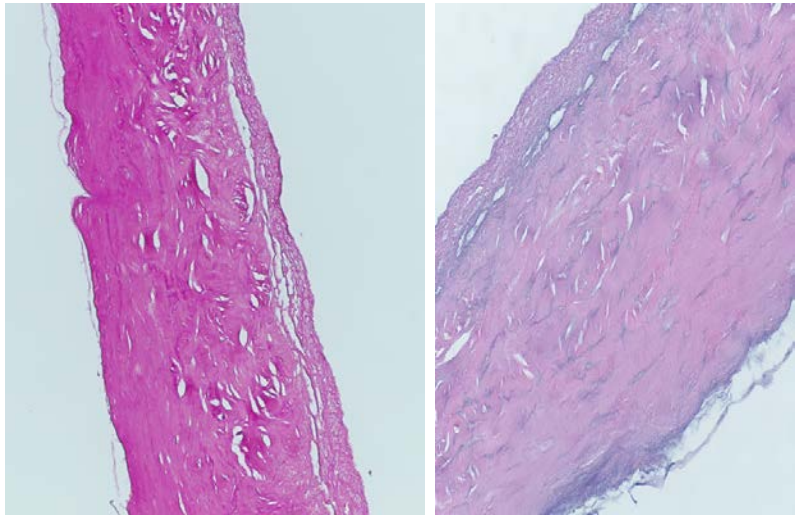
Anti-Calcification and calcification




Biologic Pathway
Decellularized Valve
(pig)
Animal Testing
(sheep)

Decell Porcine Valve Preparation

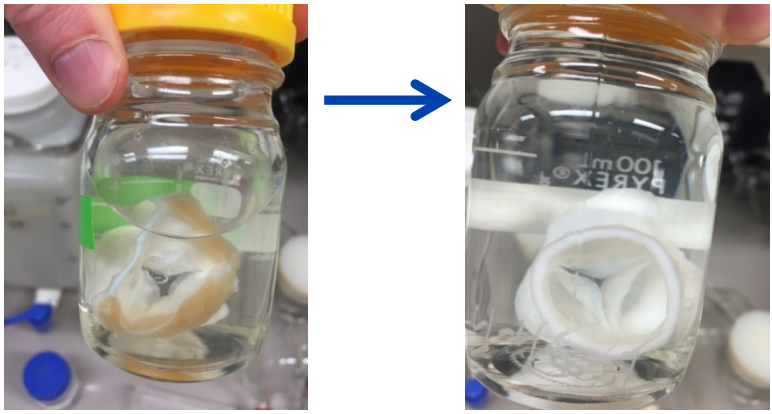
- Decellularization
  - Isolates the extracellular matrix (ECM) of a tissue from its inhabiting cells, leaving an ECM scaffold of the original tissue for tissue re-cellularization



Hemotoxalin, and Verhoeff staining

No cellular remnants no porcine DNA

Tissue soaked in 1% Sodium Dodecyl Sulfate (SDS)

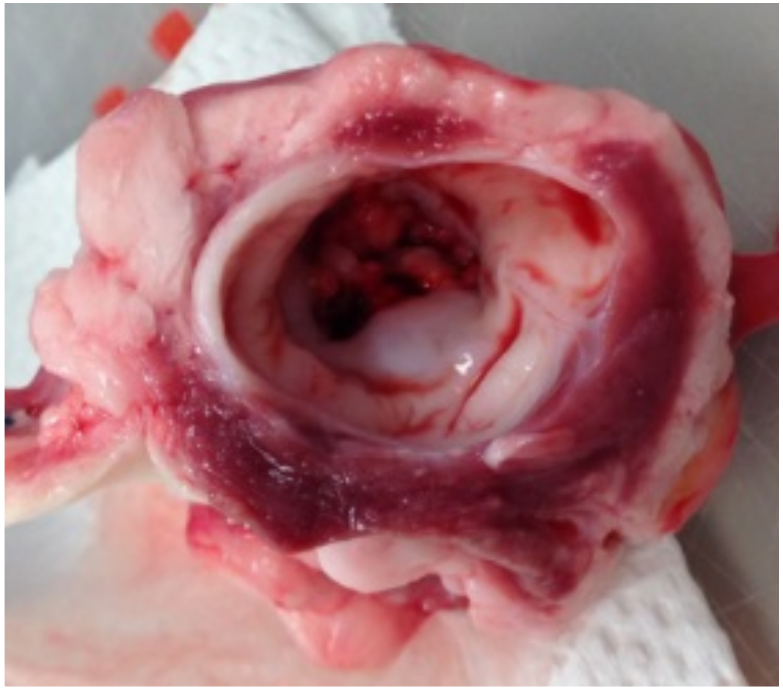


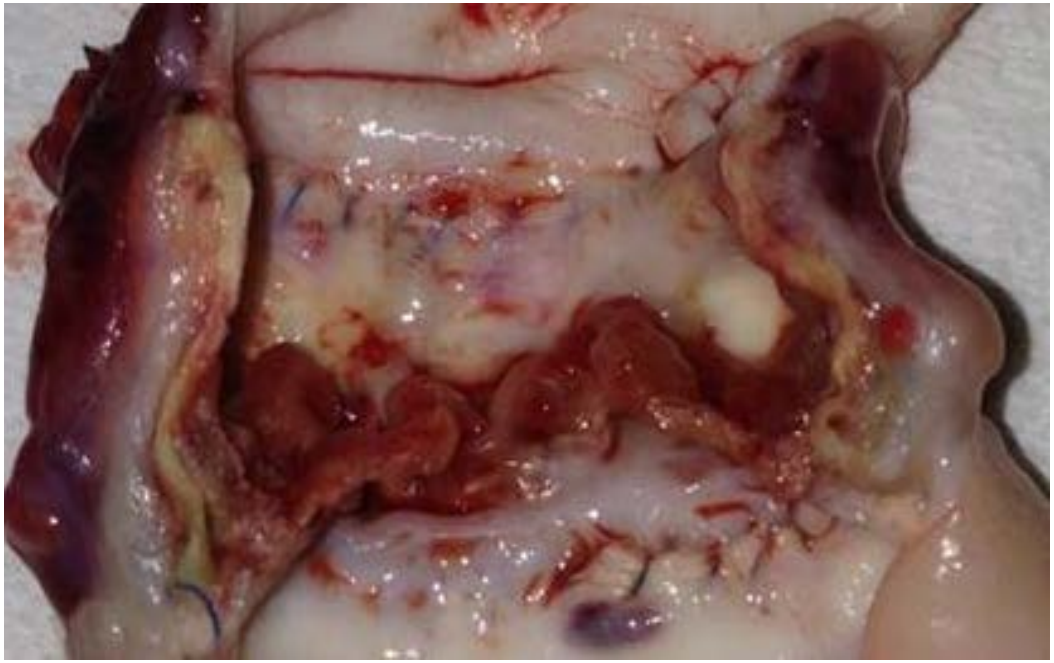
Preliminary Animal Studies

- Porcine valves
  - Decellularized with SDS & Sterilization with Gamma
  - Implanted pig tissue valve into the RVOT of 3 juvenile sheep
    - 2 month time point
    - Surgeries were successful
    - Valves functioned well at baseline
    - Increasing gradient seen on monthly ECHO through serial studies

Preliminary Animal Results

- All 3 sheep had cusps that were thickened, calcified, and structurally damaged
  - Working hypothesis: Gamma sterilization may degrade tissue too much





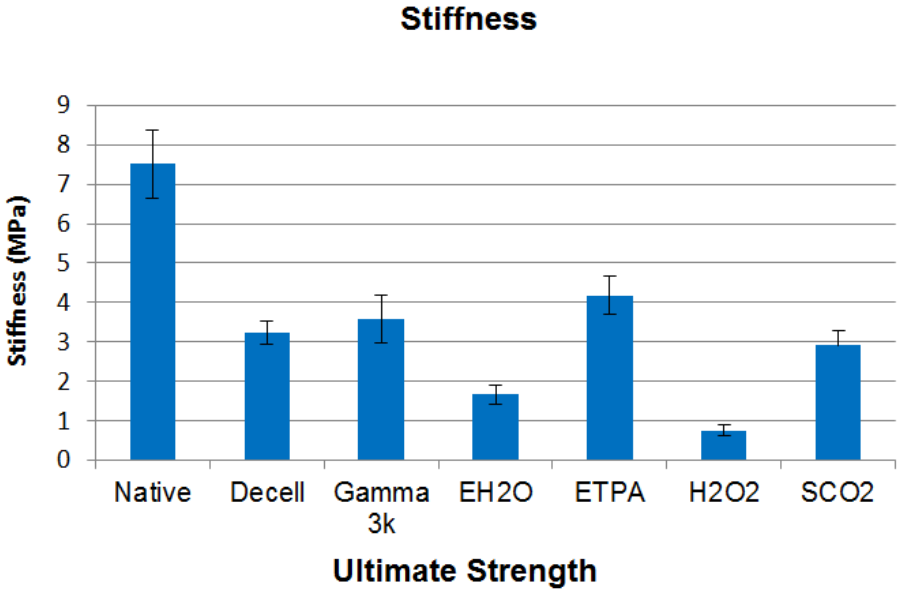
Affect of Sterilization Techniques on Strength

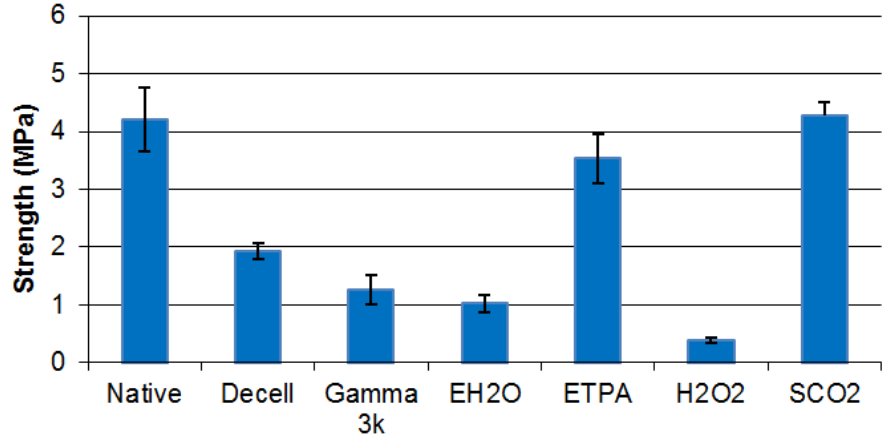
- Mechanical Testing Results

- The Decell process showed reduced strength compared to native cusps

- The SCO_2 and ETPA sterilization enhancement of the strength back to native tissue integrity

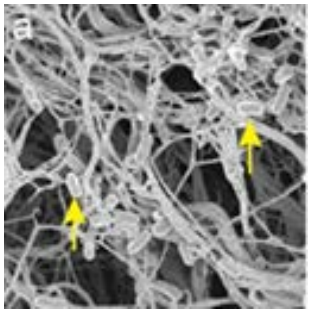
- ETPA fixes the tissue, therefore inhibits tissue recell and adaptation
  SCO_2



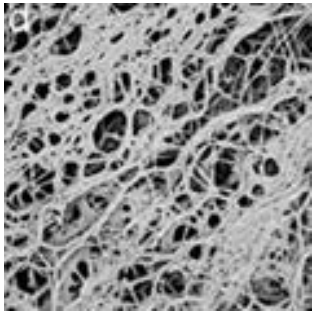


Sterility Confirmation

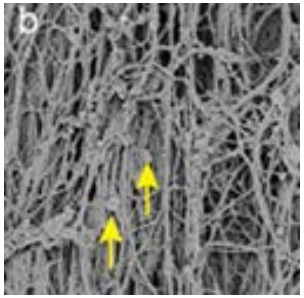
- SEM of sterilized Decell porcine valves
  - SEM performed near coronary sinus for each sterilization method
    - Bacterial and fungal infiltration
    - Tissue structure analysis
- Conclusions
  - Going forward new process will utilize Supercritical CO_2 sterilization selected for valves because of enhanced mechanical properties as well as maintain structure and sterility without fixation



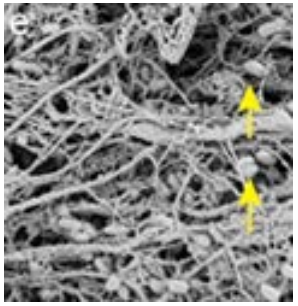
Decell



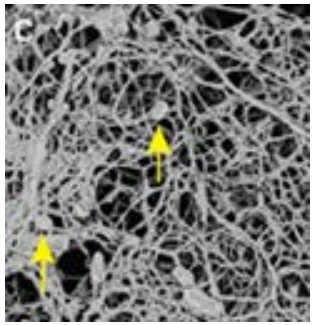
ETPA



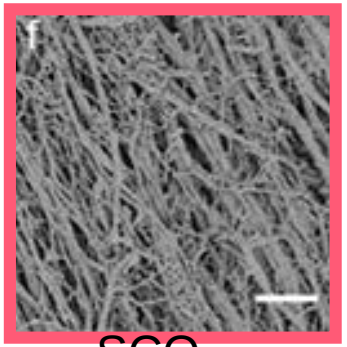
EH_20



H_2O_2



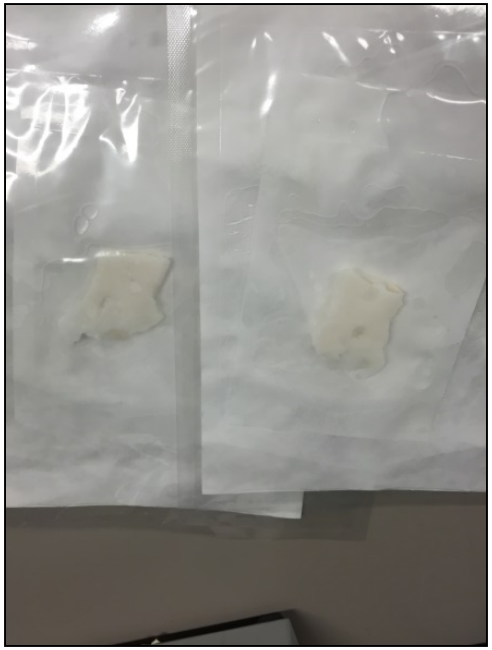
Gamma

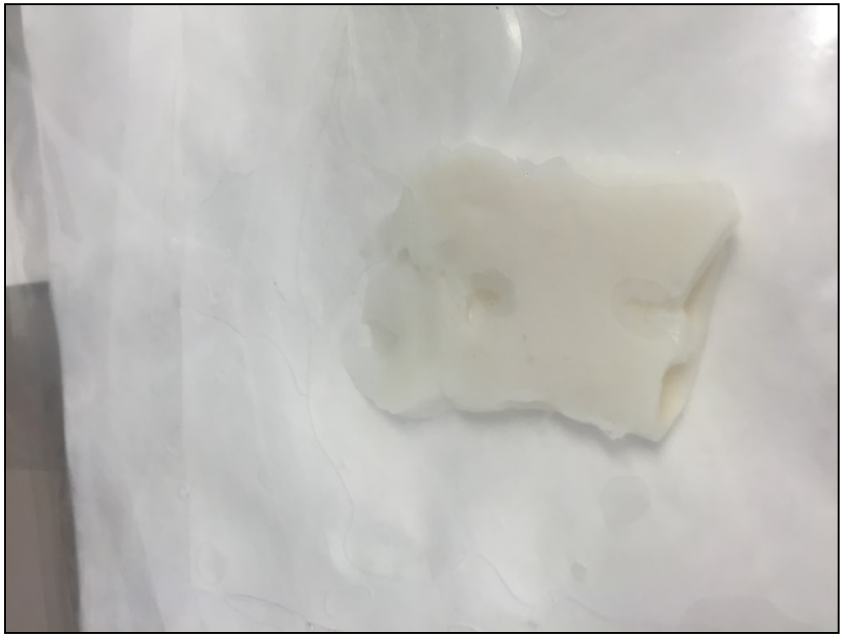


SCO_2

Novasterilis Collaboration

- Industry partner Novasterilis Inc. provides the supercritical CO_2 sterilization customized for our Xenograph tissue





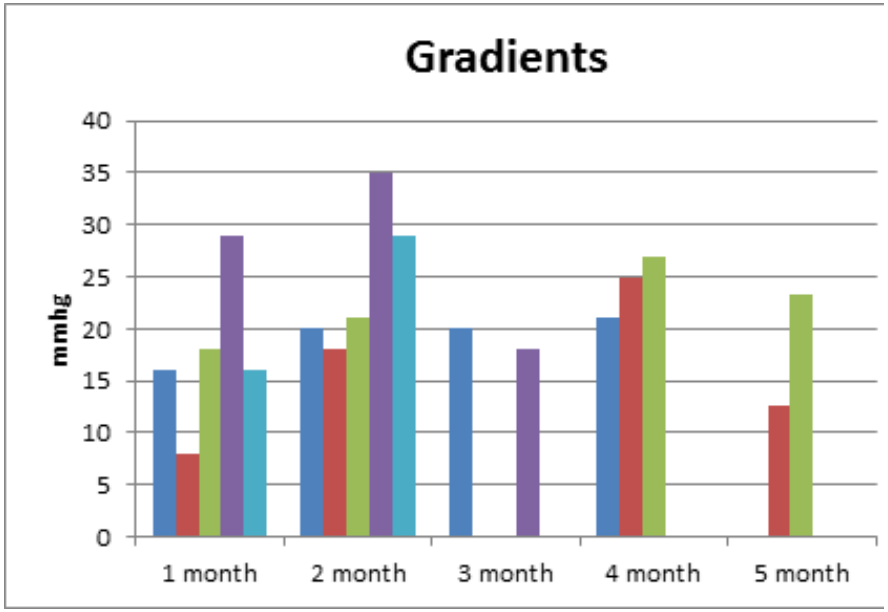
Decell Porcine Valve

- Animal Studies Round 2
  - Implanted Decell porcine tissue valve with improved new sterilization into the RVOT of 5 juvenile sheep
    - 5 month time point
    - Surgeries were successful without complication
    - Valves functioned well
    - Clinically no sign of rejection or infection
- Medication
  - Aspirin, antibiotic, heperin

Decell Porcine Valve Animal Study: ECHO and Swan Data

- ECHO gradients were taken at 1 month intervals for all sheep

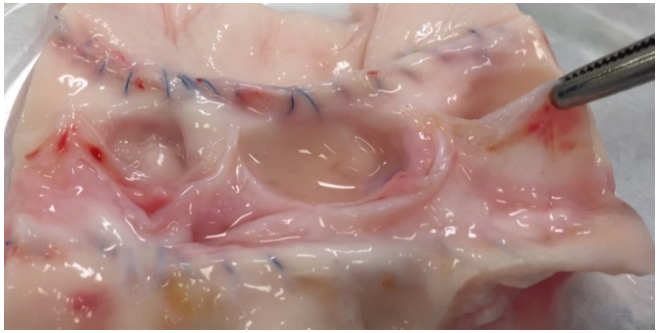
	S13	S14	S15
Pulmonary Artery Pressure (mmHg)	32/9	39/4	66/7
RV (mmHg)	40/0	53/0	62/3
Gradient (mmHg)	8	14	N/A

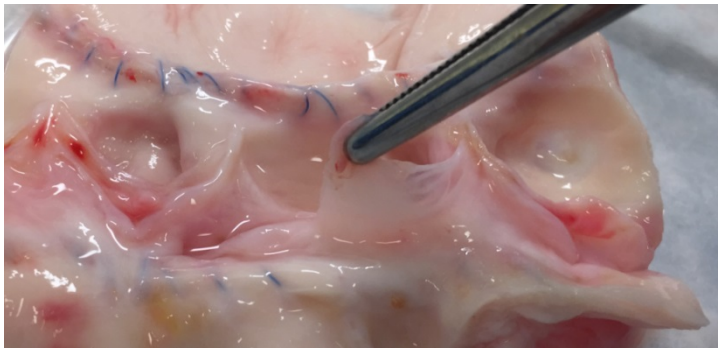


- Right heart catheterization confirmed valve performance
- Discrepancy in gradients between ECHO and right heart catheter method

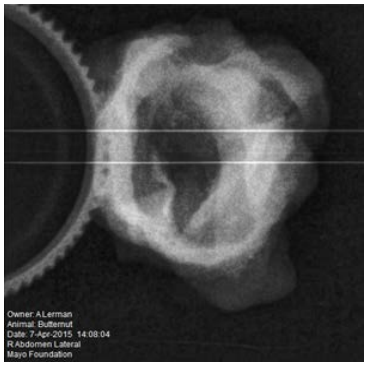
Decell Porcine Valve Animal Study Results

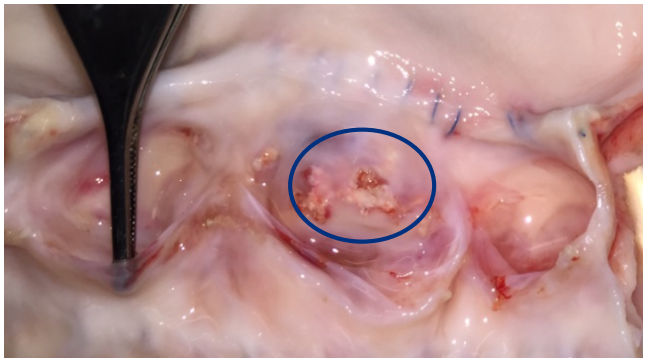
- Leaflets are intact





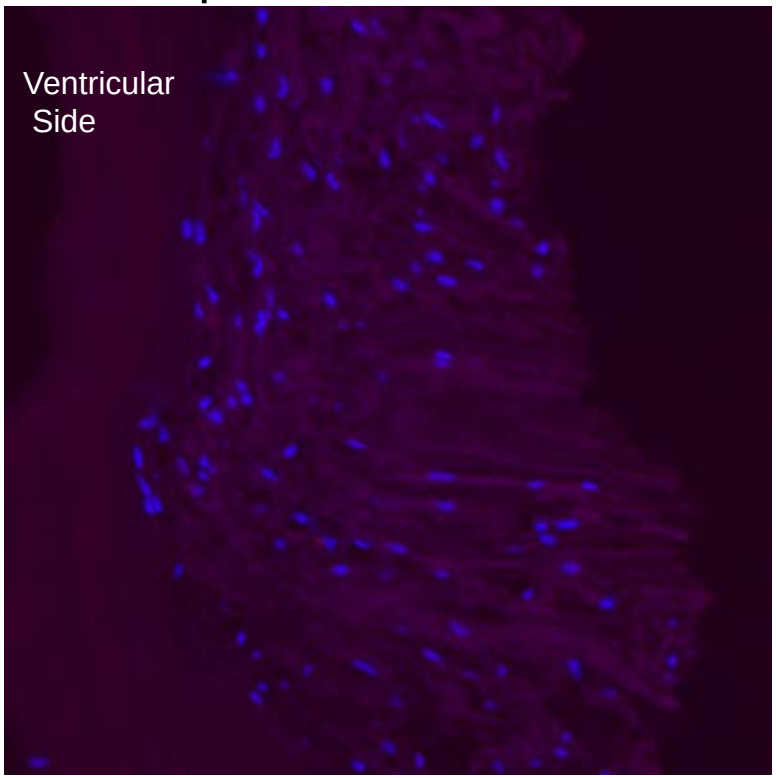
- Culture swab results showed no growth on valve, or at the anastomosis sites
- Monthly lab results were all in the normal range (CBC, White Count)
- Calcification nodules observed in one of the animals on the root, not leaflet calcification



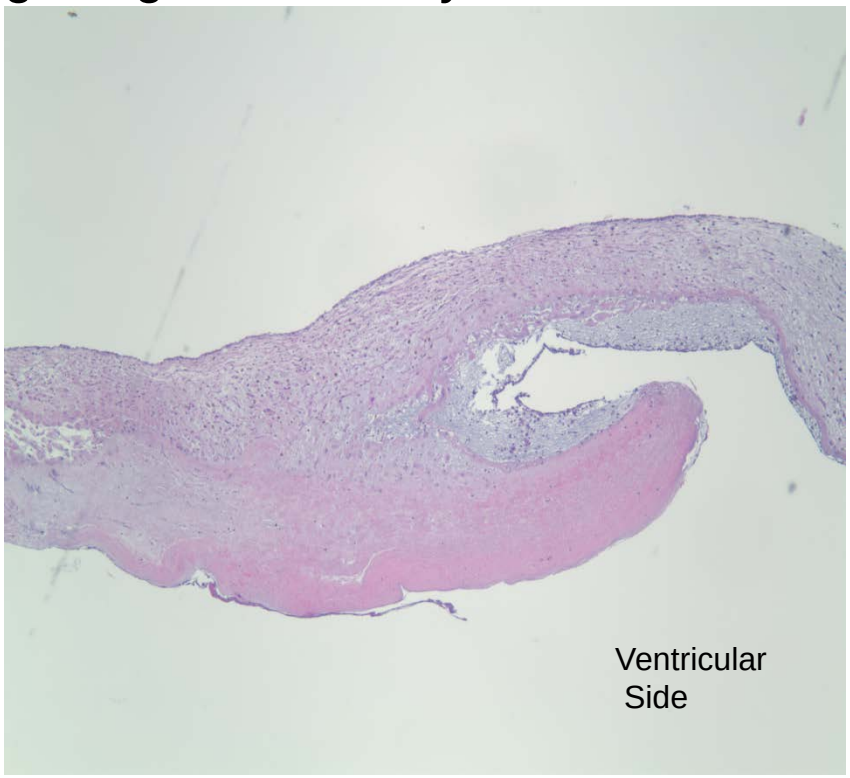


Tissue Cross section Analysis of the Explanted Leaflets

DAPI Nuclear fluorescent stain shows nuclei and confirms cellular presence



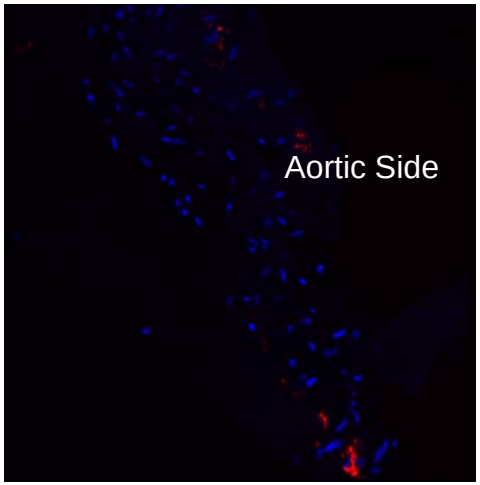
Hematoxalin and Eosin staining shows decelluarlized collagen getting infiltrated by fibroblast

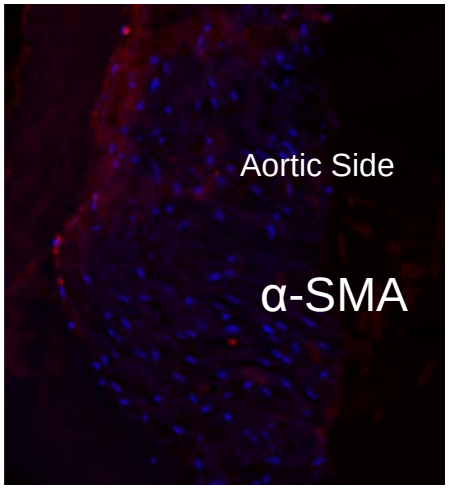


Tissue Analysis of the Explanted Leaflets

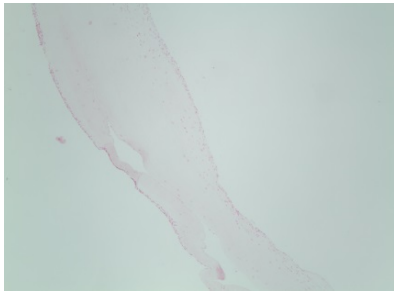
- Specimen cross section staining
  - α-SMA & Vimentin
    - Intracytoplasmic stain shows positive fibroblast like phenotype

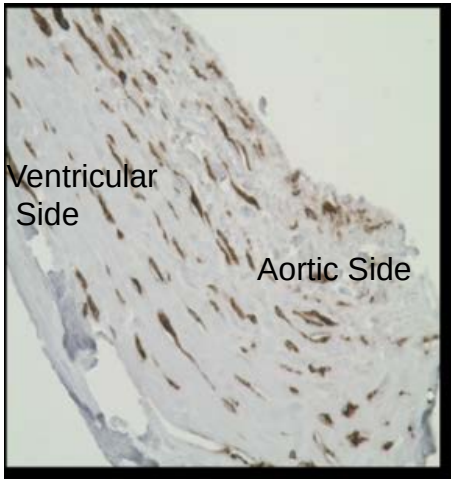
      (similar to VIC)

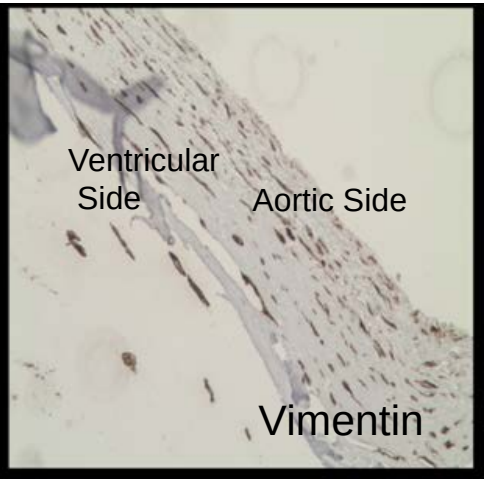




- Specimen cross section calcification staining
  - Von Kossa & Alizarin Red S stains
  - no calcification on cusps

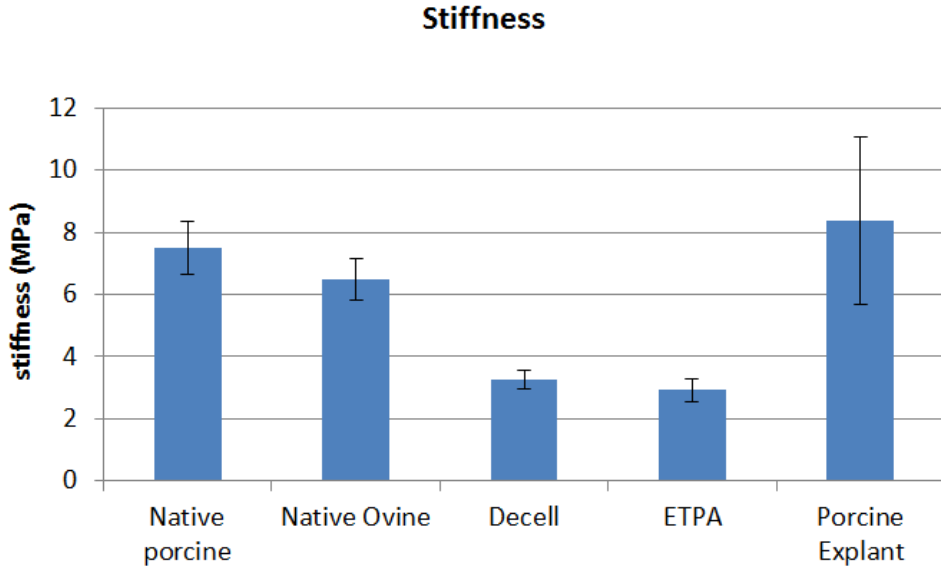


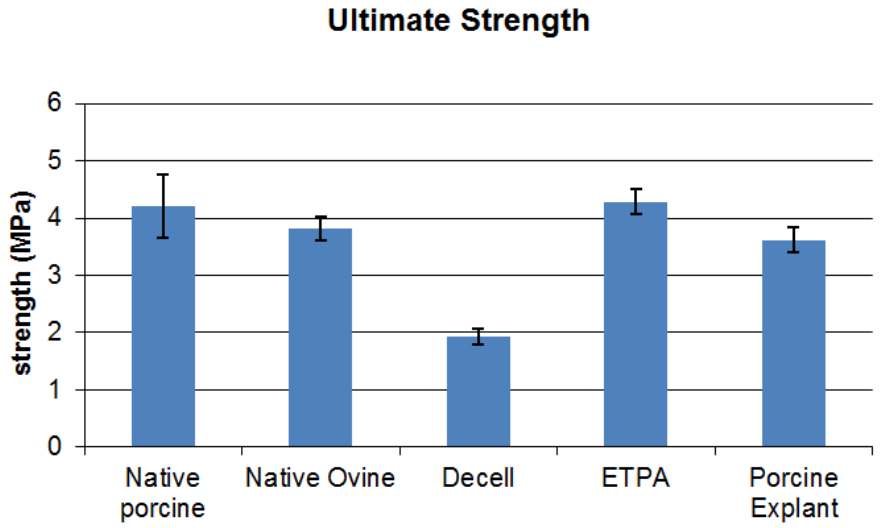


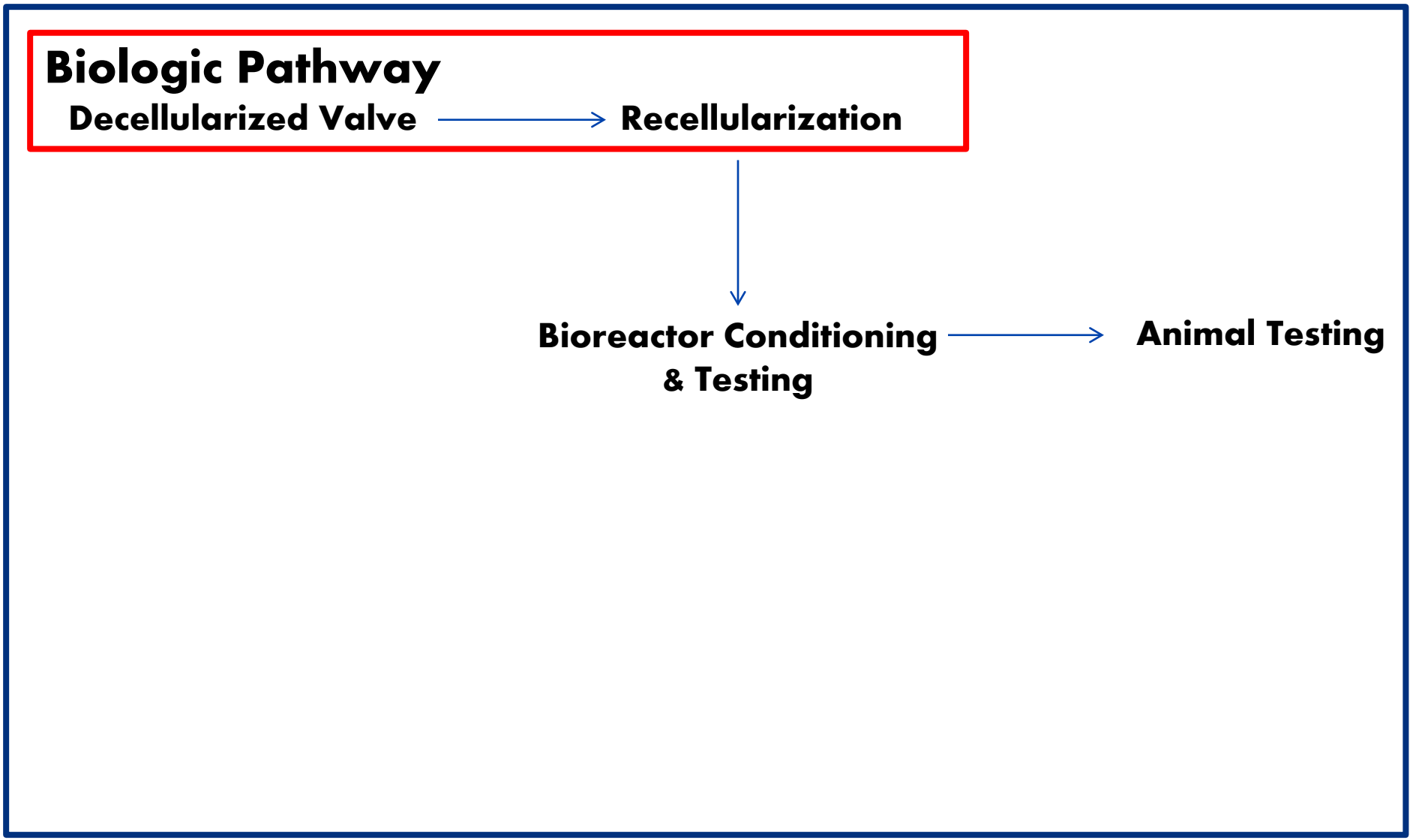


Mechanical Properties of Explanted Valves

- Explanted stiffness properties increased compared to implanted properties
  - Possibly due to cellular growth
  - Histology confirmed firbroblastlike cells inside leaflet cusps
- The strength was maintained from implant to explant
- Native porcine and native ovine had similar properties



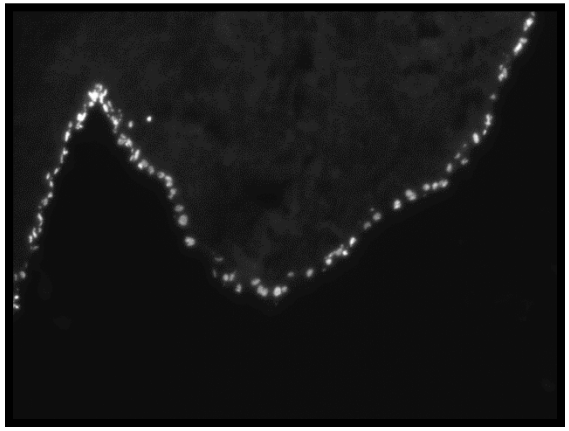



Biologic Pathway
Decellularized Valve
Recellularization
Bioreactor Conditioning & Testing
Animal Testing

Next Step: Pre Implant Re-cellularization of the Scaffold

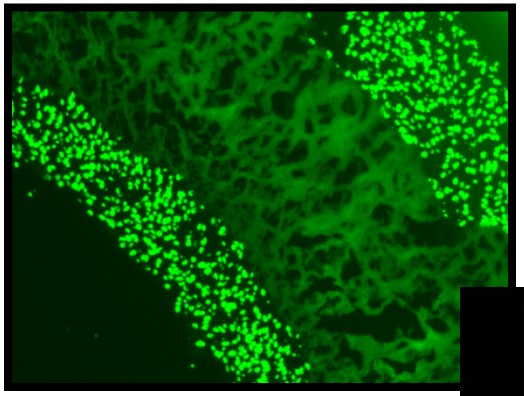
Endothelial cells were statically seeded on decell porcine cusps

- Successful monolayer established
- Sheep endothelial outgrowth cells grown on decellularized pigs valves statically for 5 days



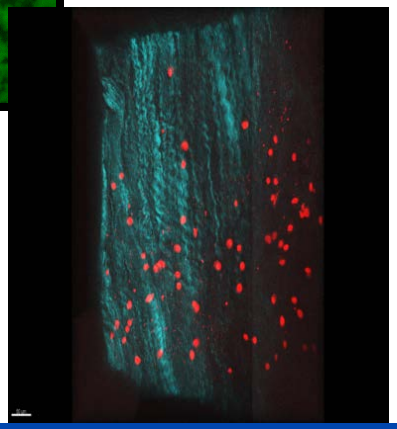
VICs were statically seeded on decell porcine cusps

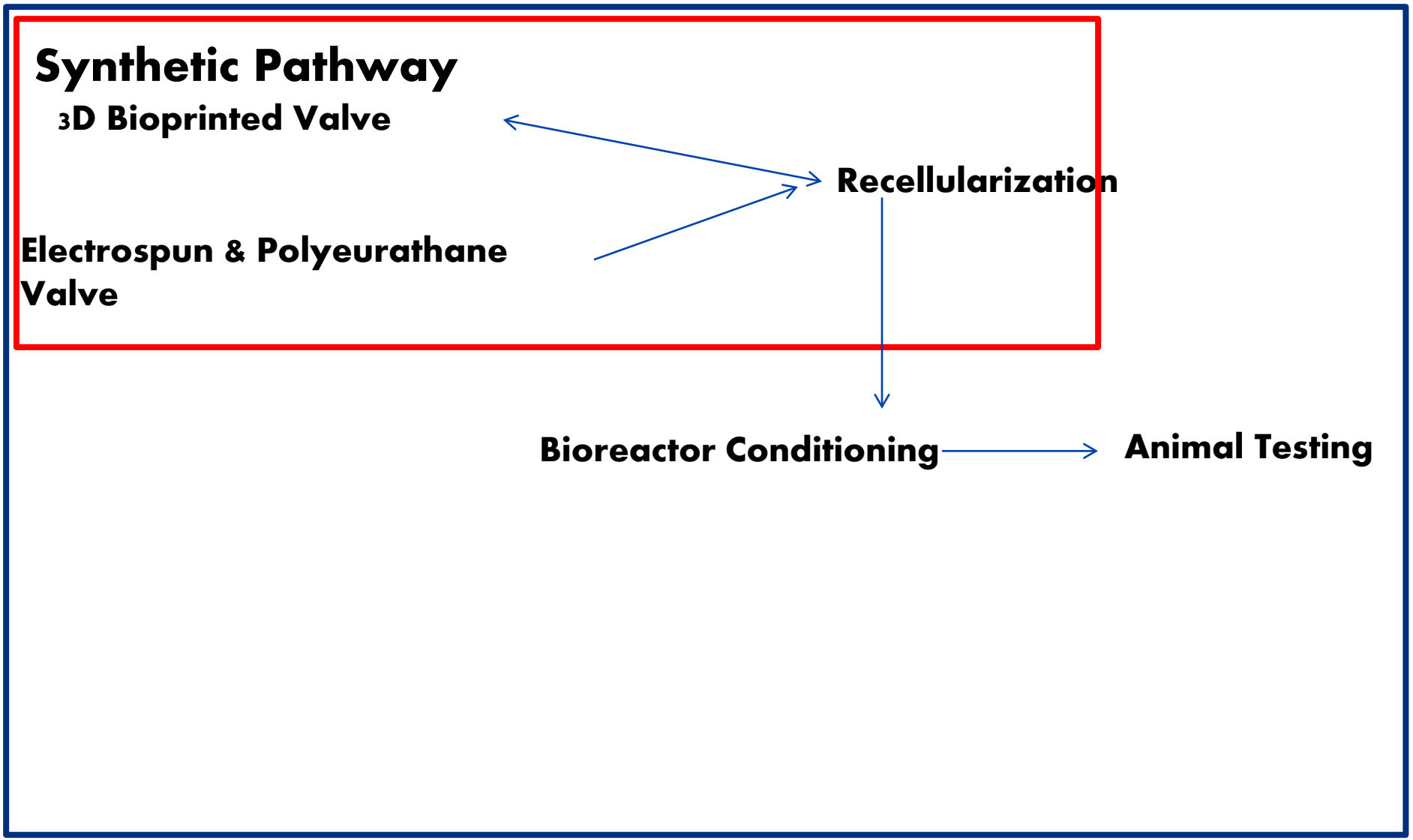
- No success of VIC migration into tissue
- VICs remained on surface



VICs were injection seeded into decell porcine cusps

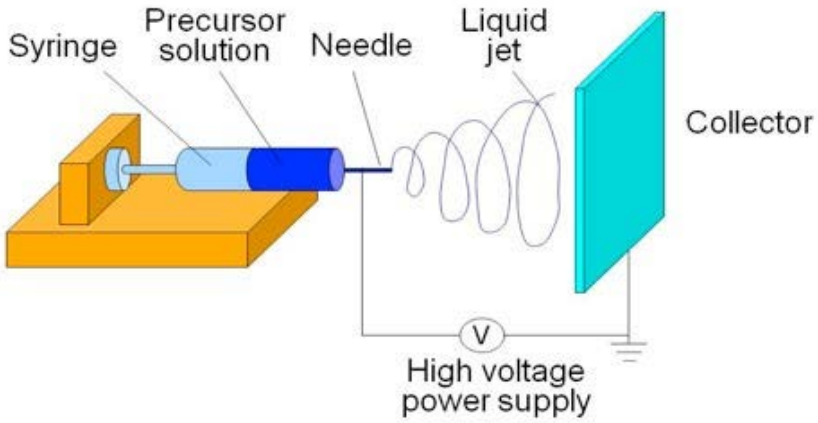
- Successful migration of VICs into tissue

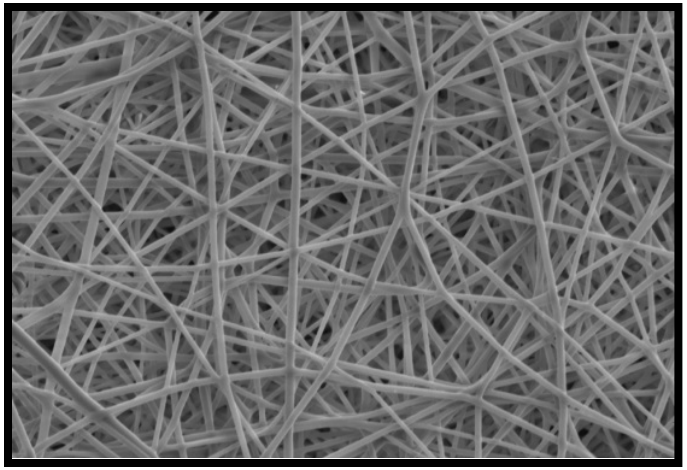



Synthetic Pathway
3D Bioprinted Valve
Electrospun & Polyeurathane Valve
Recellularization
Bioreactor Conditioning
Animal Testing

Electrospinning

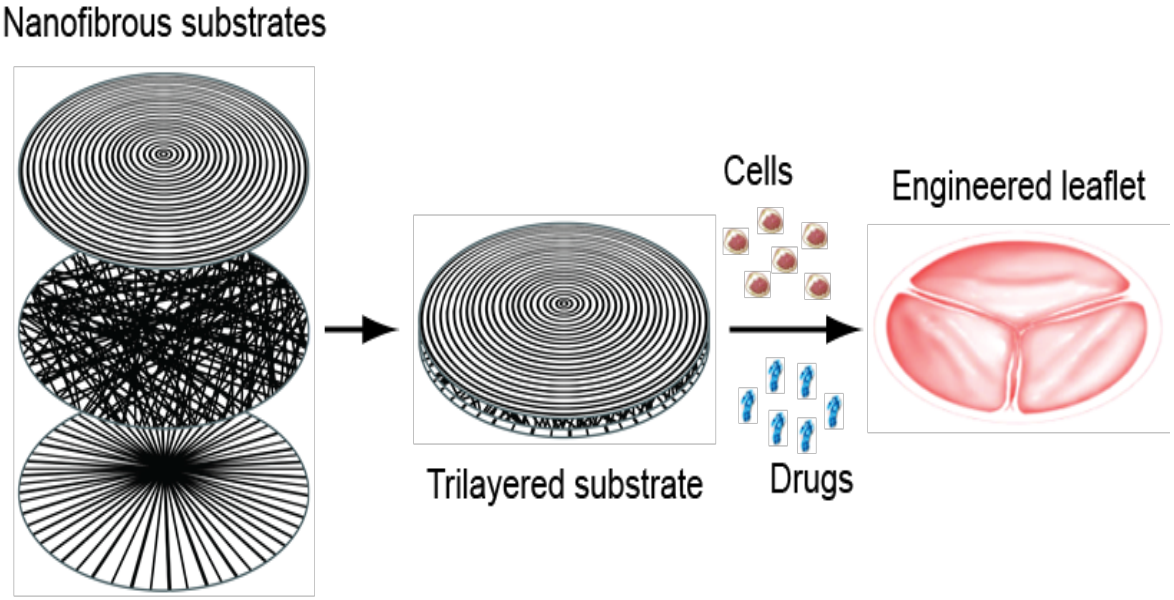
- Electrospinning process
  - Precursor solution is prepared: Polycaprolactone – an FDA approved non-toxic slowly biodegradable biomaterial.
  - Charged with high voltage in a syringe
  - Pump is used to control the flow rate
  - Electrostatic force causes the solution droplet to move toward the grounded collector
  - Solution droplet elongates
  - Polyelectrolyte solvent evaporates
  - Polymeric nanofiber forms and is deposited on the collector





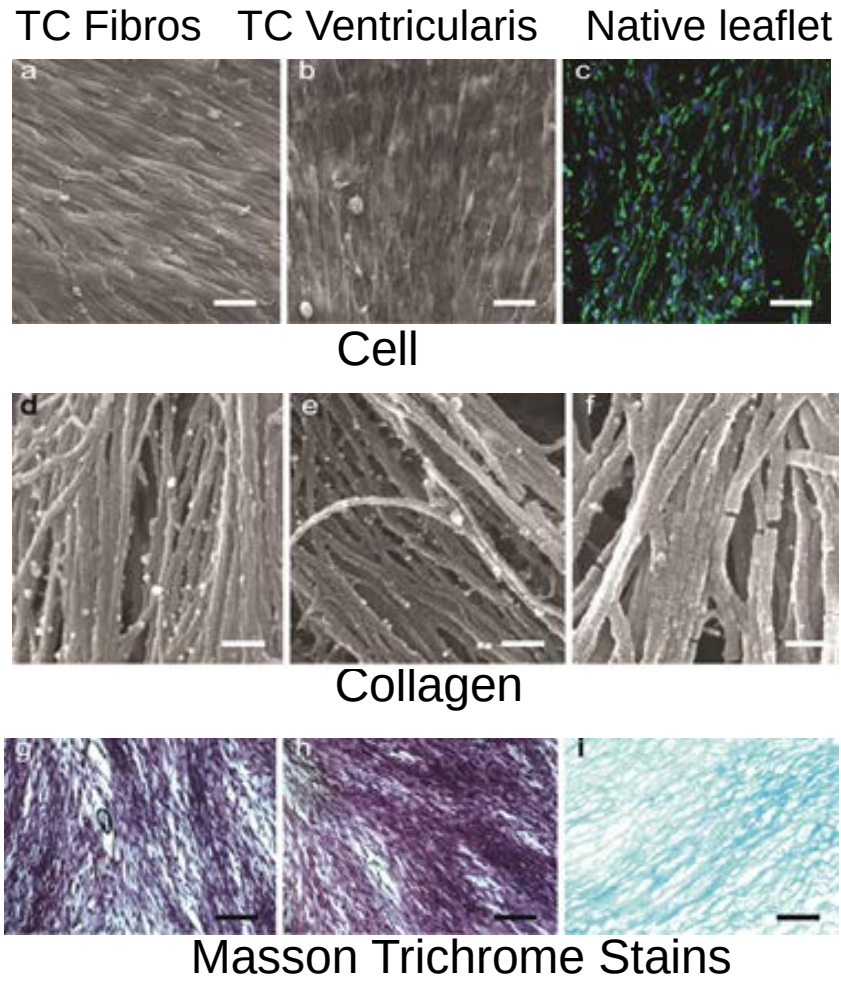
Electrospinning

- Trilayered biologic leaflet generation
  - 3 individual substrates are combined
  - Each substrate has different orientations
  - Porcine VIC cells are cultured in general media with ascorbic acid for 1 month
- Results
  - Engineered leaflet was obtained



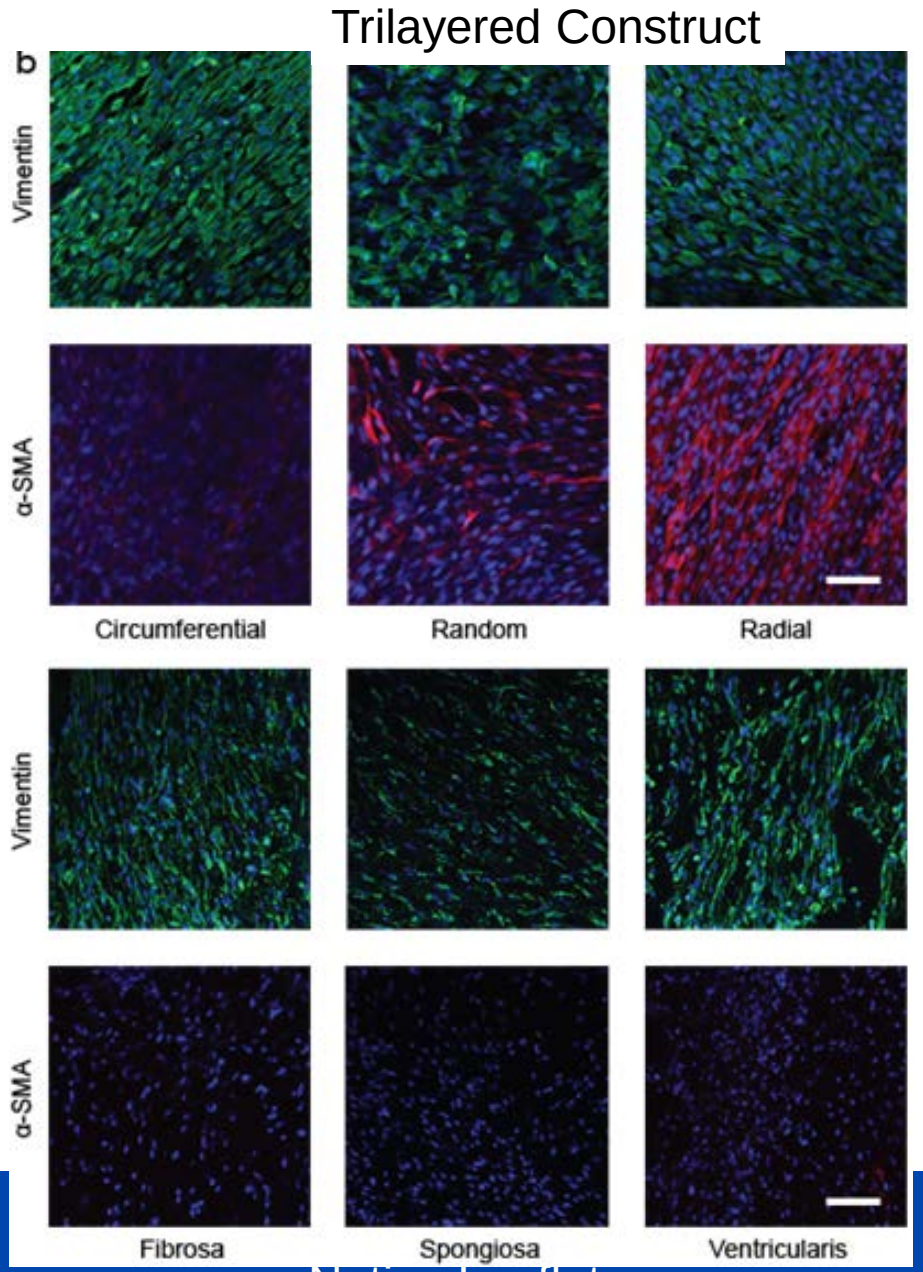
Cellularization of Electrospinning Scaffold

- Comparison of electrospun trilayered
  Trilayer nanofiber substrate was seeded with porcine VICs for 1 month
    - SEM & light microscope imaging
    - Analysis of cell and collagen orientation/structure
    - Masson Trichrome Staining performed
- Results
    - Cells in trilayered construct were suitably oriented
    - Trilayered construct collagen was oriented similar to native
    - Confirmed collagen Type I presence



Histology of Electrospun Trilayered Construct

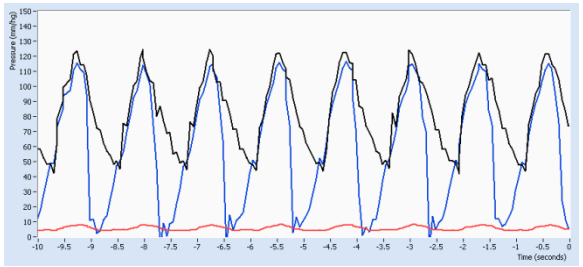
- Staining Results
  - Vimentin
    - Cells were observed in all layers
    - Cells showed fibroblast phenotype
  - α-SMA
    - Cells also showed smooth muscle cell expression
    - Confirmed construct is in growing state

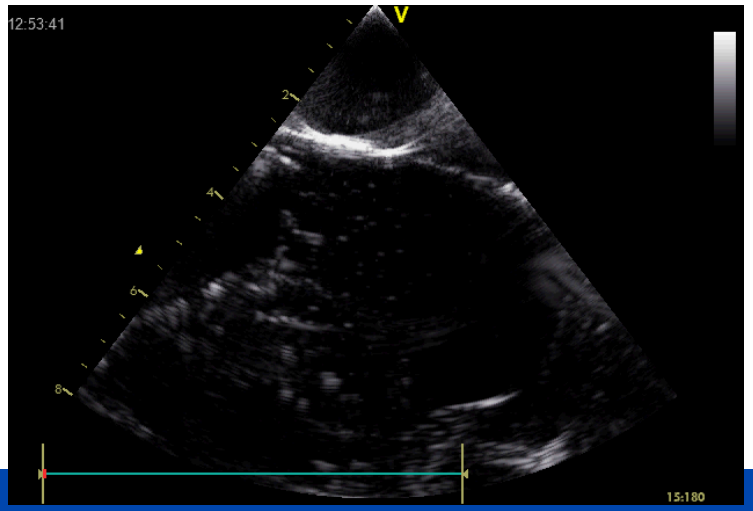


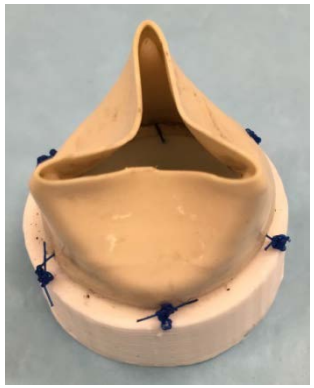
Electrospun PolyeurathaneValve

- Tube: Electrospun Polyurethane (PU) – 500 µm
- Crown: Bioplotted Polycaprolactone (PCL) with PCL coating

- Results: 30 days (2.6 million cycles)
  - Pressure waves indicate some regurgitation

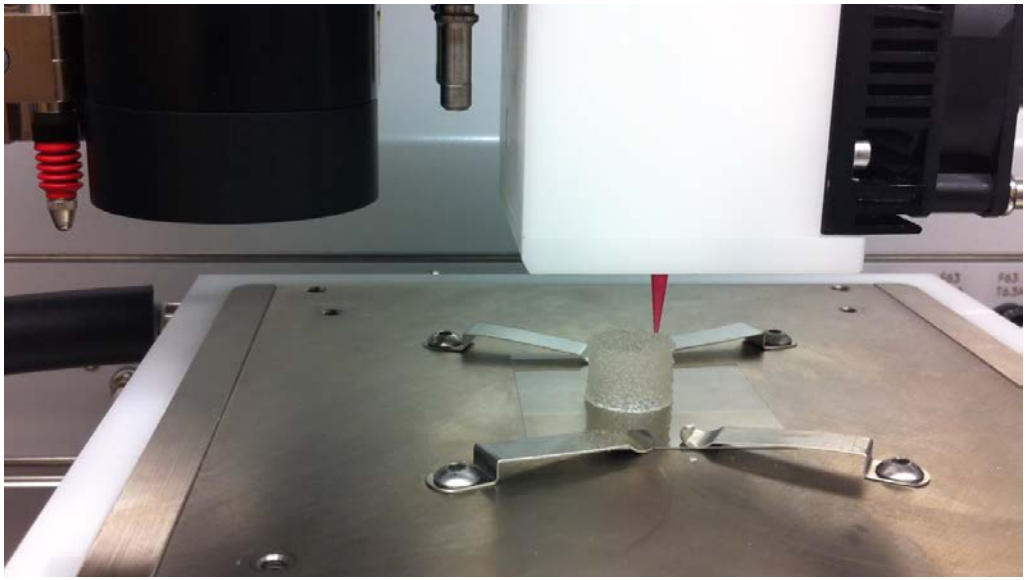


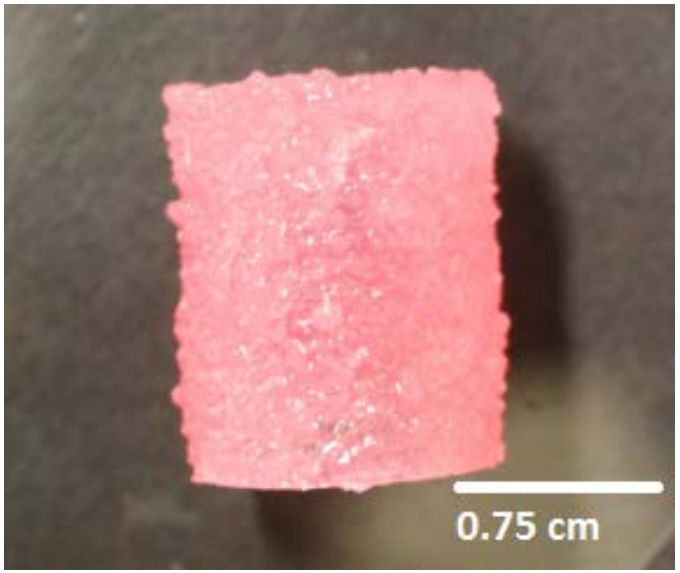




3D-Bioplotted Valve

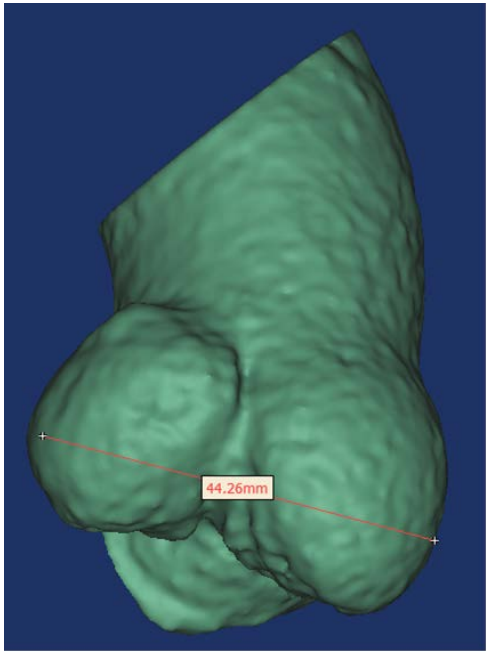
- Bioprinted synthetic valve capable of being integrated with cells
- Matrix materials include:
  - Hydrogels: **alginate**, collagen gel, fibrin gel, Matrigel, hyaluronic acid, gelatin, chitosan, polyethylene glycol (PEG), PEG-DA



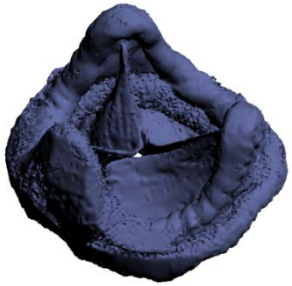


Alginate gel with VICs

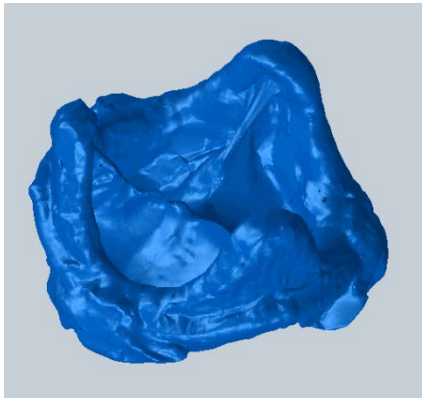
Imaging and Rendering of Patient Specific Aortic Valves



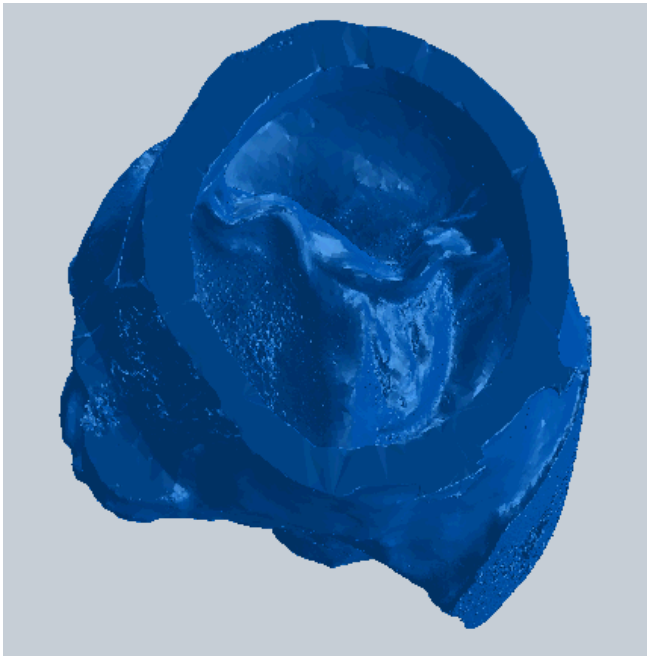
Patient Valve
CT Scan



Bioprosthetic Valve
NMR



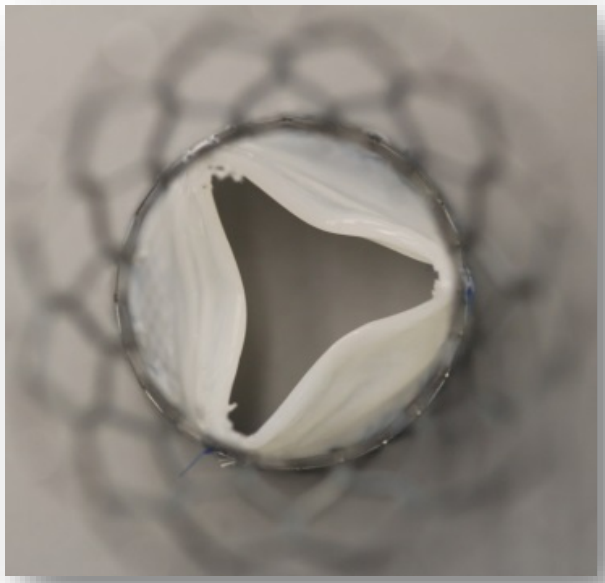
Bioprosthetic Valve
Coordinate Measuring Machine

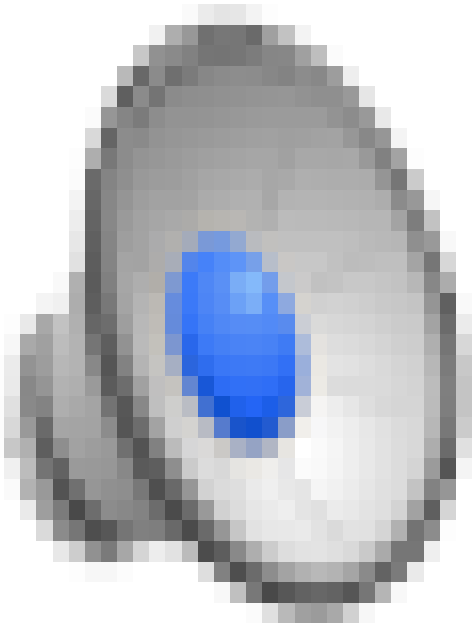


Porcine Valve
Micro-CT Scan

Percutaneous Valve Tissue Integration

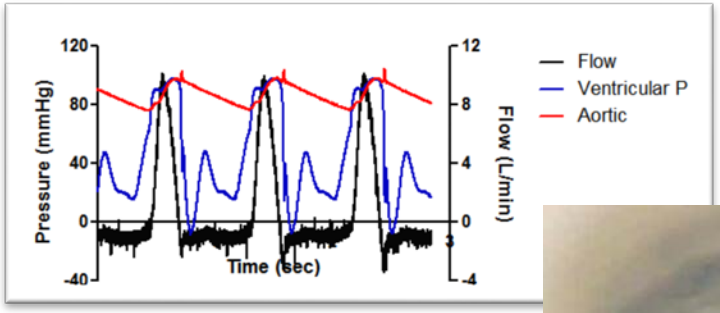
- TAVR Prototype
  - 20 mm decellularized collagen tube (0.4mm thick) sewn on 22mm (ID) stent

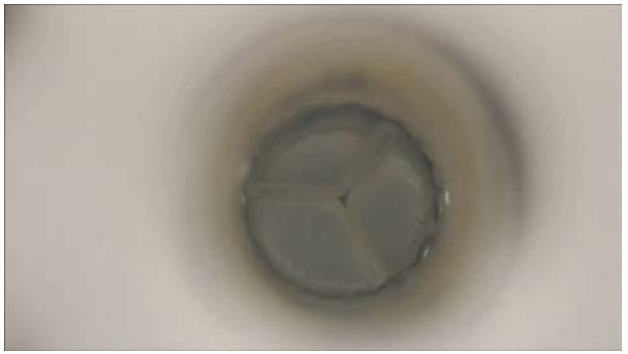




Percutaneous Valve

- Pulse duplicator testing results
  - Pulse Duplicator
    - Samll paravalvular leak observed
    - Small closing volume or systolic pressure drop





Pros and Cons

Biologic Scaffolds

- Native architecture maintained
- Potential for cellular cues
- Need to ensure removal of antigenicity
- Challenges in decellularized tissue
- Issues with TAVR

Synthetic Scaffolds

3D Printed

- No antigenicity
- Can print cells in matrix
- Control over material properties
- Lack of biologic cues
- Micro-scale
- Easy to sterilize

Electrospun

- No antigenicity
- Control properties of material (porosity)
- High mechanical integrity
- Lack of biologic cues
- Nano-scale
- Easy to sterilize

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Electrospun PolyeurathaneValve

- Tube: Electrospun Polyurethane (PU) – 500 µm
- Crown: Bioplotted Polycaprolactone (PCL) with PCL coating

- Results: 30 days (2.6 million cycles)
  - Pressure waves indicate some regurgitation