Mechano-dependent Biosynthetic Response of Micro-integrated Cells in Elastomeric Scaffolds

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Problem

- Congenital heart valve failure is a common medical problem
  - Over 225,000 surgeries per year
  - AHA estimated 78,000 valve replacement surgeries annually
- Heart valve defects acquired from:
  - Age
  - Rhuematic fever
  - Endocarditis - bacterial infections of the heart valves and surrounding heart tissue

Current Replacement Solutions

Mechanical Valves

Ball and Cage

Tilting Disk

St. Jude Bileaflet

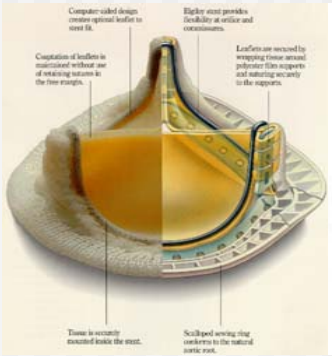
Pros:

- Durable design provides a long lifespan

Cons:

- Does not mimic native valve hemodynamics
- Requires anti-coagulants
- Sizing issues

Bioprosthetic Valves



Pericardial

Porcine

Xenograft artificial heart valves

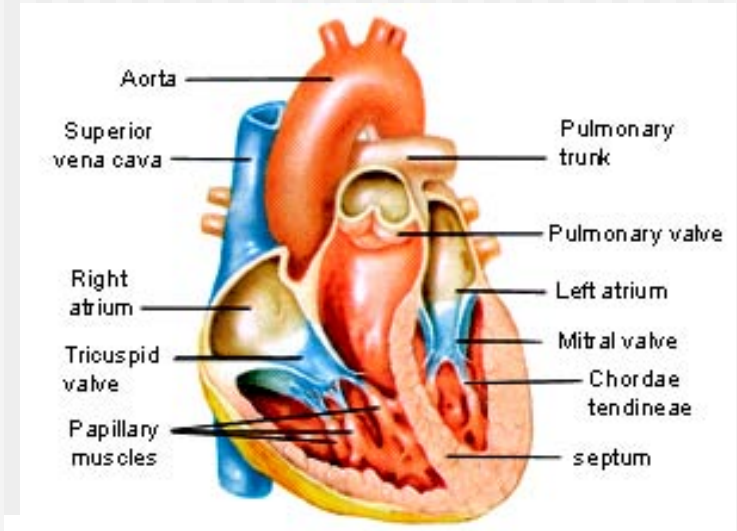
Pros:

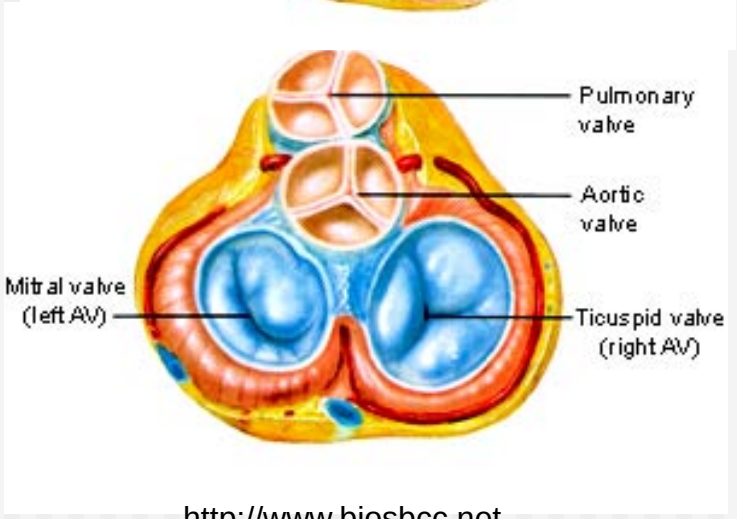
- Similar hemodynamics to native valves

Cons:

- Finite lifespan(10-15 yrs)
- Biologically inert tissue

Semi-Lunar Valves





http://www.biosbcc.net

- Anatomical similarities between AV and PV
  - Tri-leaflet valve with semi-lunar cusps
  - Small variations in fiber architecture do exist

Tissue Engineering and My Project

- The field of tissue engineering looks to provide one more solution to this problem
  - Combines biology and engineering in an effort to create biological substitutes that recapitulate the requisite mechanical and structural properties of healthy native tissues to restore, maintain, or improve tissue function

- My Project
  - Examines the biosynthetic effects of cyclic mechanical strain placed on a poly (ester urethane) urea (PEUU) scaffold densely integrated with rat vascular smooth muscle cells
  - Overall goal of developing an engineered tissue that mimics the load bearing capabilities of native tissue

PEUU Electrospun Materials

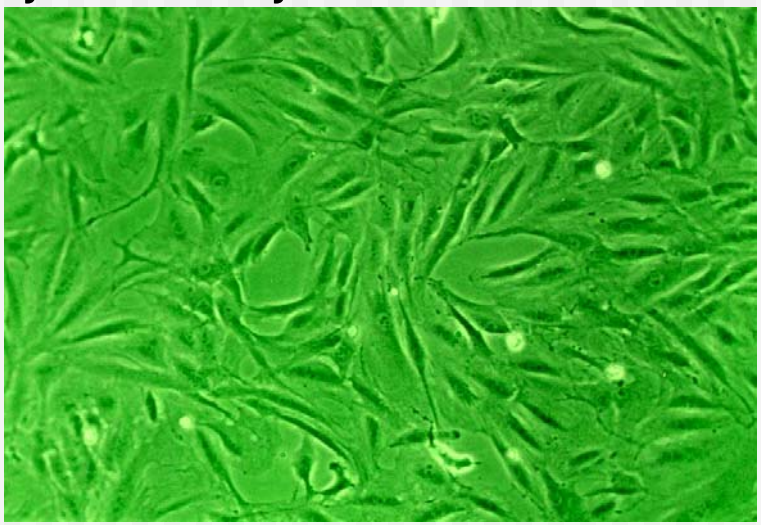
- **A solid foundation for scaffolds**
  - Versatile process
    - Controlled fiber morphology → mechanically dependent
  - Long fiber morphology provides adequate mechanical support
  - High surface area to volume ratio for enhanced cell attachment
  - Elastomeric – fully recoverable large deformations
    - No measurable creep
- **Allows us to study cell response to a mechanical stimulus in a controlled environment**

Methods

- Cell Expansion
- Specimen Preparation
- Mechanical Conditioning
- Quantification Assays

Methods: Cell Culture

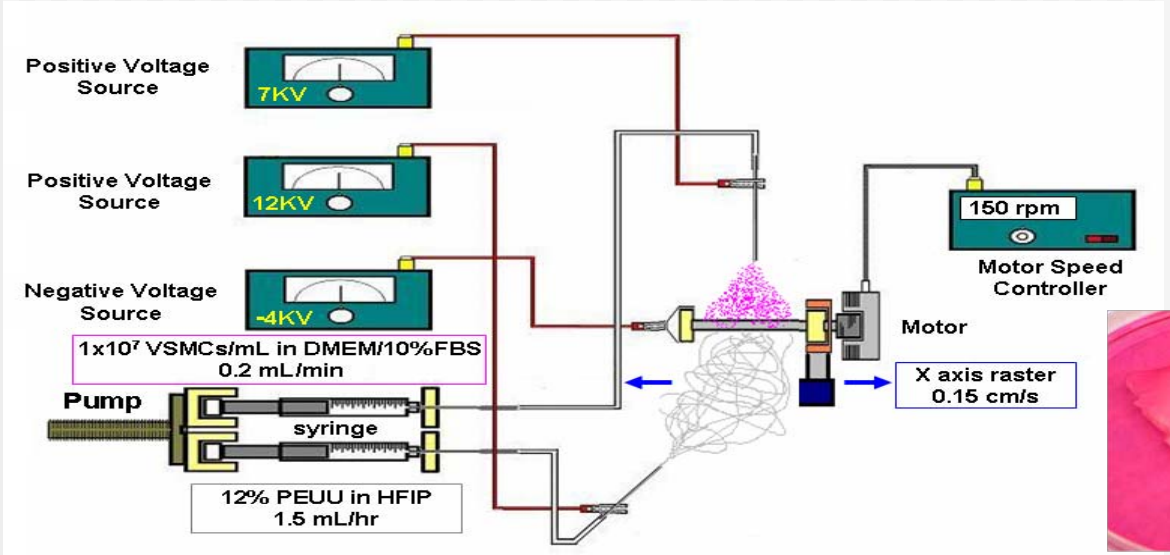
- Vascular smooth muscle cells (VSMC's) isolated from rat aorta were expanded onto tissue culture plates under Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum, 2% anti anti and 1% HEPES solution.
- After expansion, cells were incubated at 37 ^{o}C and 5% CO_2 for approx. 3-5 days until fully confluent.



www.genlantis.com/corp/images/RAOSMC%20cells.jpg

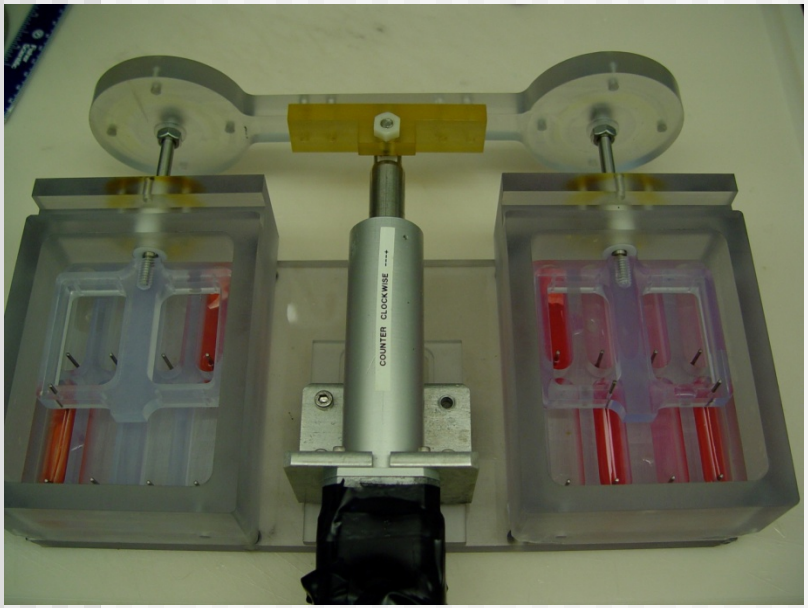
Methods: Specimen Preparation

- Concurrent electrospinning of the PEUU scaffold and electrospraying of the VSMC's
  - Process involves depositing a solubilized polymer across a voltage gradient onto a rotating mandrel
- Results in a continuous mat of tissue
  - Provides a suitable environment for cell proliferation and growth.



Methods: Mechanical Conditioning

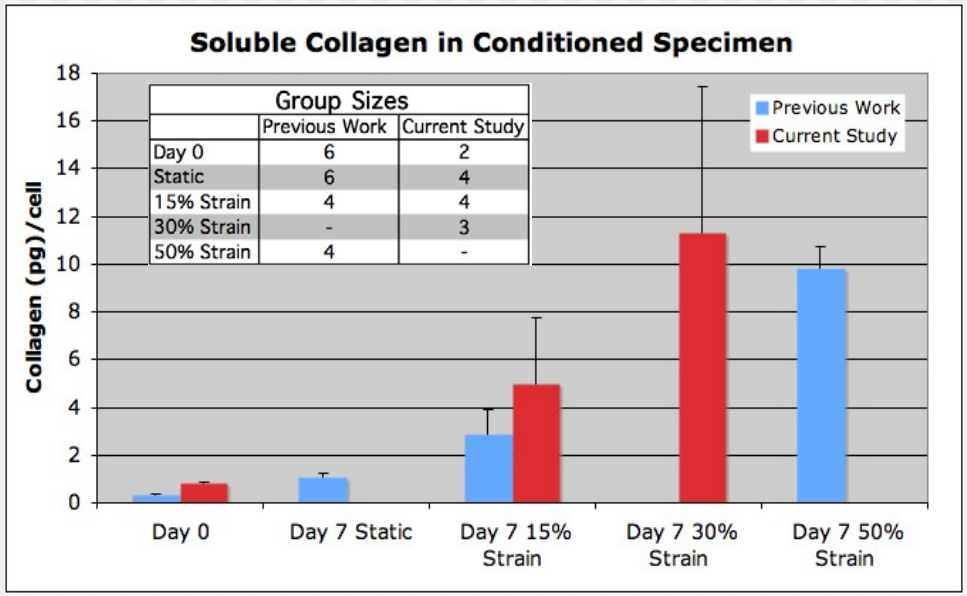
- Experimental Groups:
  - day 0 control (2 specimen)
  - day 7 static (4 specimen)
  - day 7 15% strain (4 specimen)
  - day 7 30% strain (3 specimen)



Methods: Quantification Assays

- Soluble collagen
  - Sircol™ Collagen Assay
- Proteoglycan DNA
  - PicoGreen dsDNA, Molecular Probes

Results



- Large strain was seen to induce significant increases in soluble collagen compared to Day 0 and static controls.

- Specimen conditioned at 30% strain exhibited a higher collagen production per cell than those conditioned at 50% strain possibly indicating an optimal strain for maximum collagen production.

Discussion

- Electrospinning PEUU fibers while concurrently electrospraying viable cells provides the opportunity to:
  - Characterize the mechanical behavior of the scaffold in response to dynamic conditioning
  - Evaluate the scaffold as a functional tissue

- Both the previous and current studies suggest that mechanical strain may induce an increased extracellular matrix production in artificial scaffolds.

Future Research

- Extend current study to 7 and 14 day endpoints.
- Assess the mechano-dependent biosynthetic response in a simulated physiological environment with a previously developed organ bioreactor.
- It is expected that the mechanical effects of the simulated pulmonary valve environment will cause an increase in extracellular matrix production.
- It is our hope that these studies will guide the emergence of new materials and processing methods to develop functional pulmonary valve (PV) tissue surrogates.

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