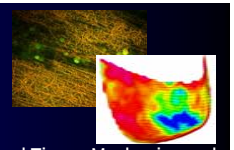

Approaches in Heart Valve Tissue Engineering

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• *Columbia University*

Mentors: Sharan Ramaswamy, Michael Sacks

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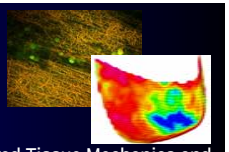
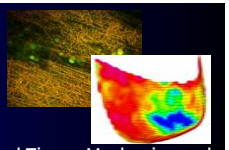
Introduction



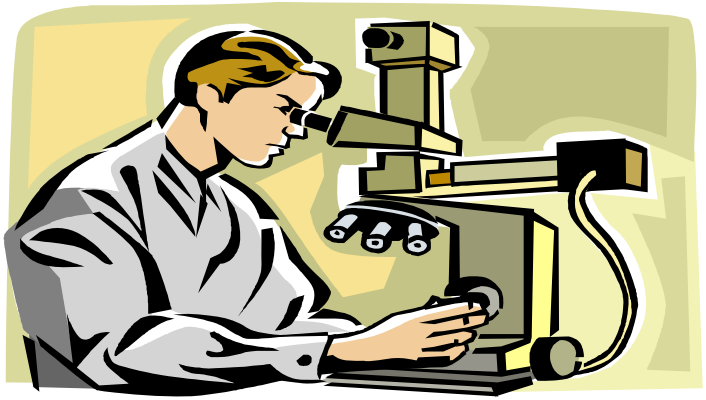
- In heart valve tissue engineering applications, a bioreactor was successfully designed and used by Engelmayr et al. [1]
- This bioreactor subjects engineered tissue samples to flexure, flow, and stretch modes of mechanical stimuli. The application of biomechanical stimuli has been shown to be beneficial to heart valve tissue growth, as the bioreactor simulates physiological conditions found in the heart.
- Here, we focus on relevant cell/tissue culture followed by engineered valvular tissue development. As a clinically viable cell source, we made use of ovine bone marrow mesenchymal stem cells. These cells were used to seed strips of nonwoven 50:50 blend poly(glycolic acid) (PGA) and poly(l-lactic acid) (PLLA) scaffolds.

Introduction

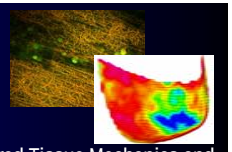


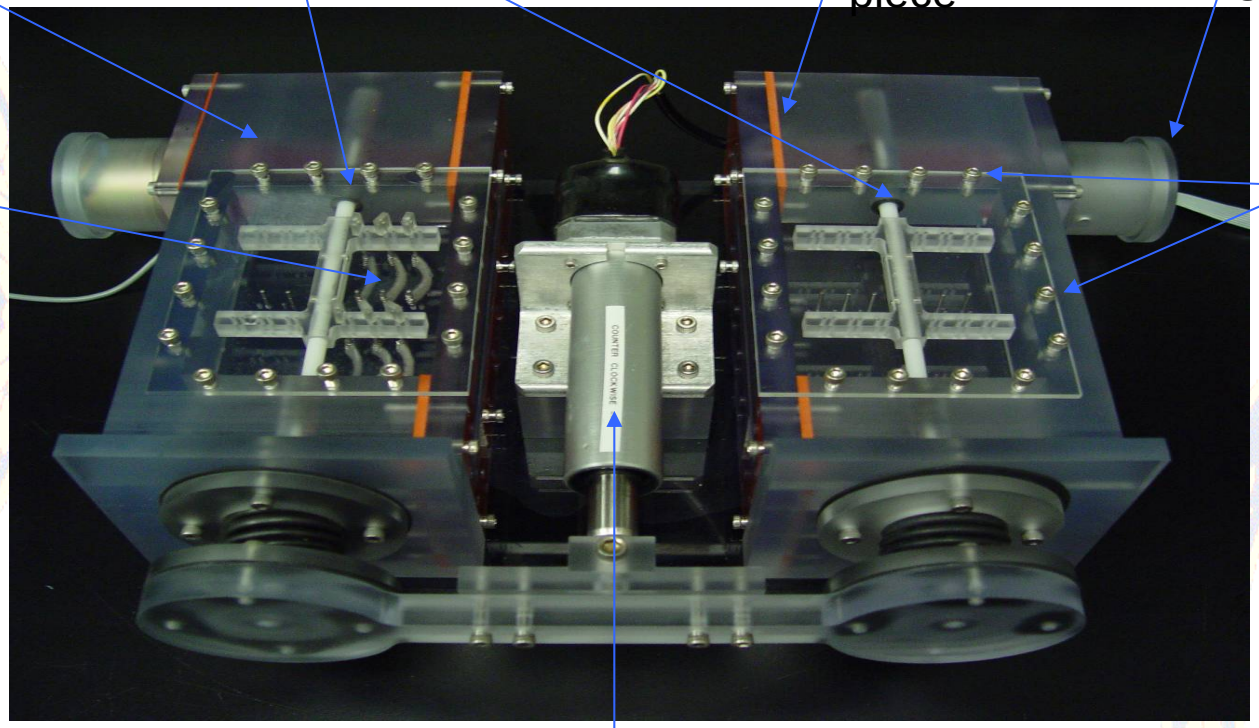
The Project consists of:

- Cell Culture Process
- Tissue Engineering and Computational Simulations



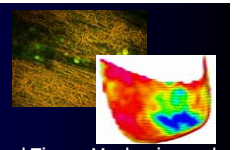
Introduction

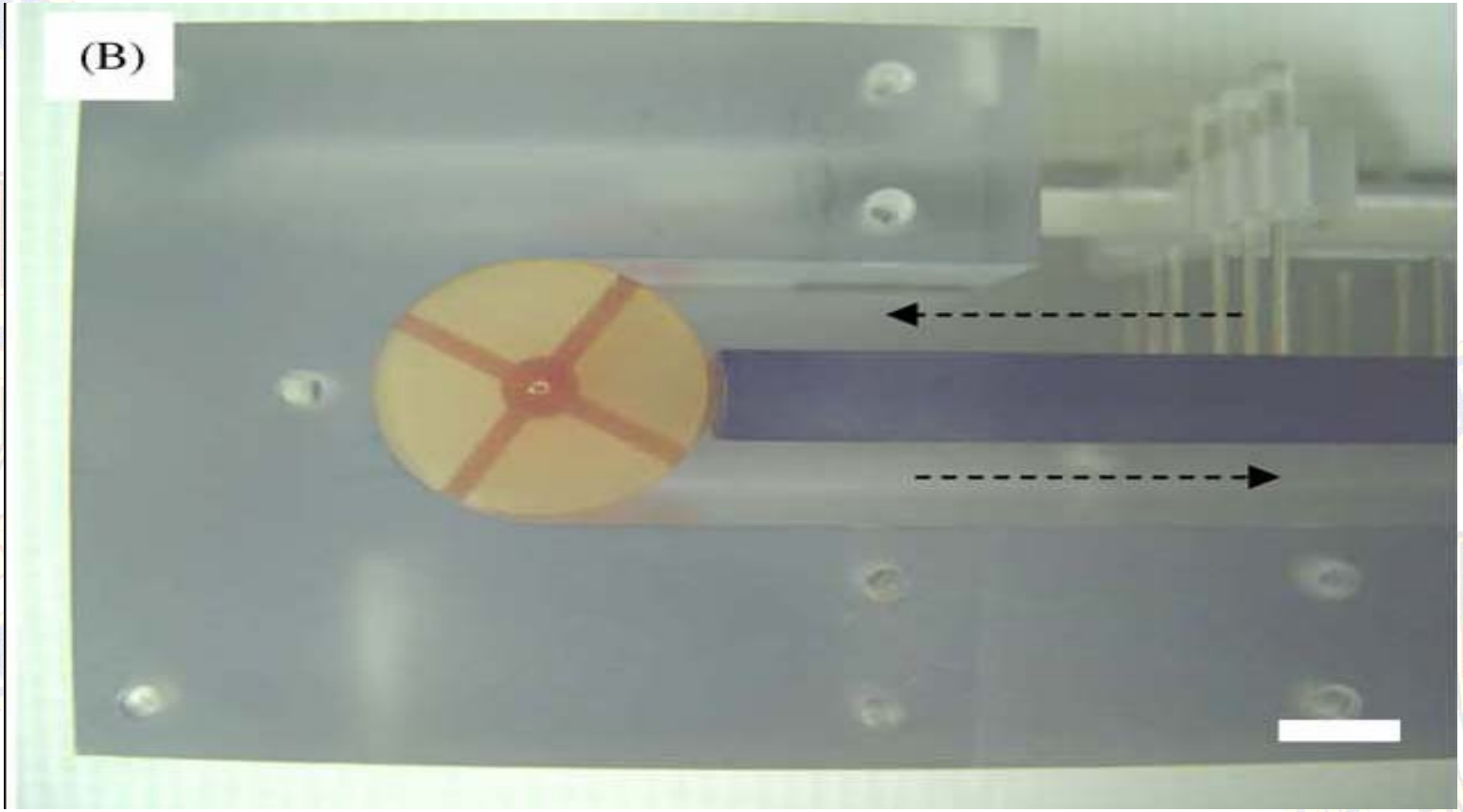




FSF Bioreactor [2]

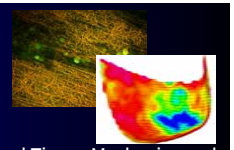
Introduction





FSF Bioreactor [2]

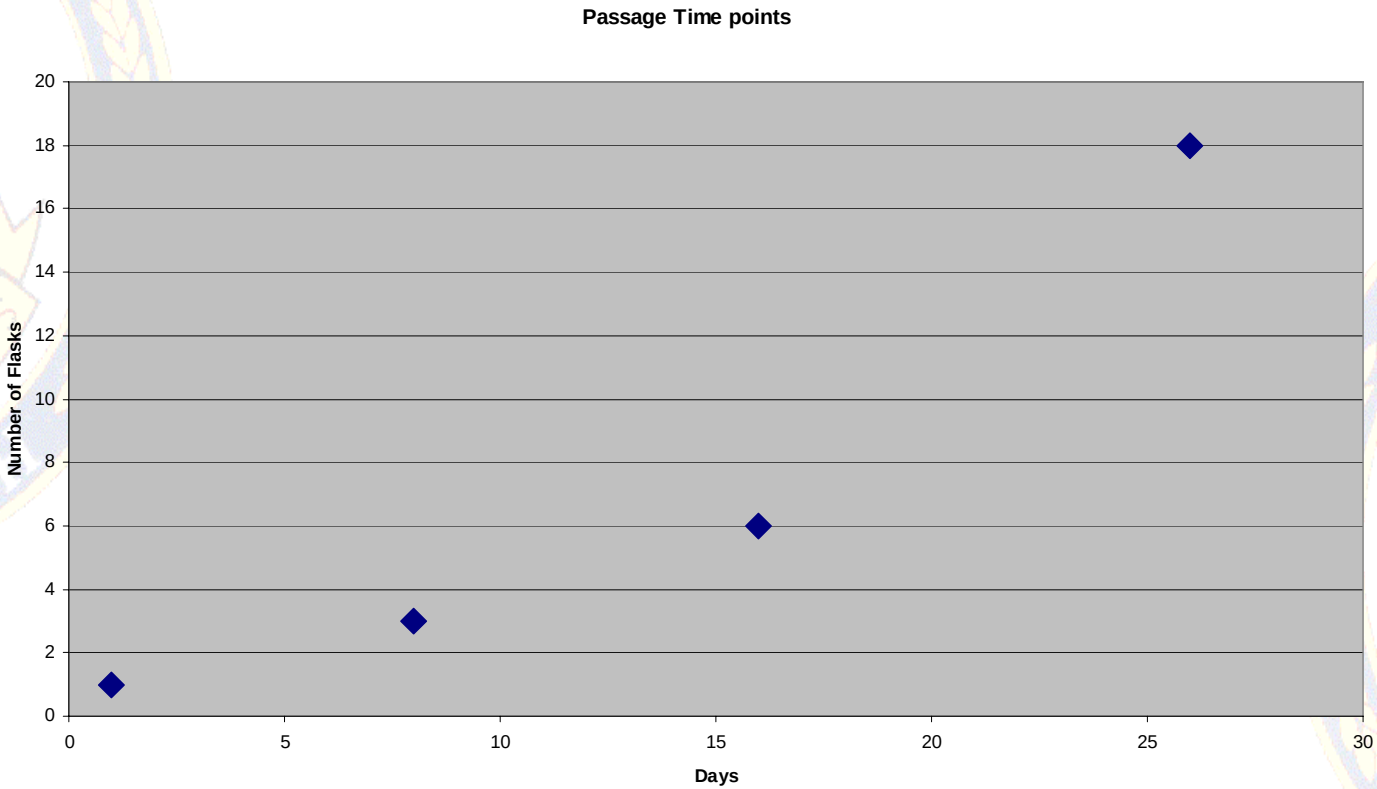
Methods: Stage 1



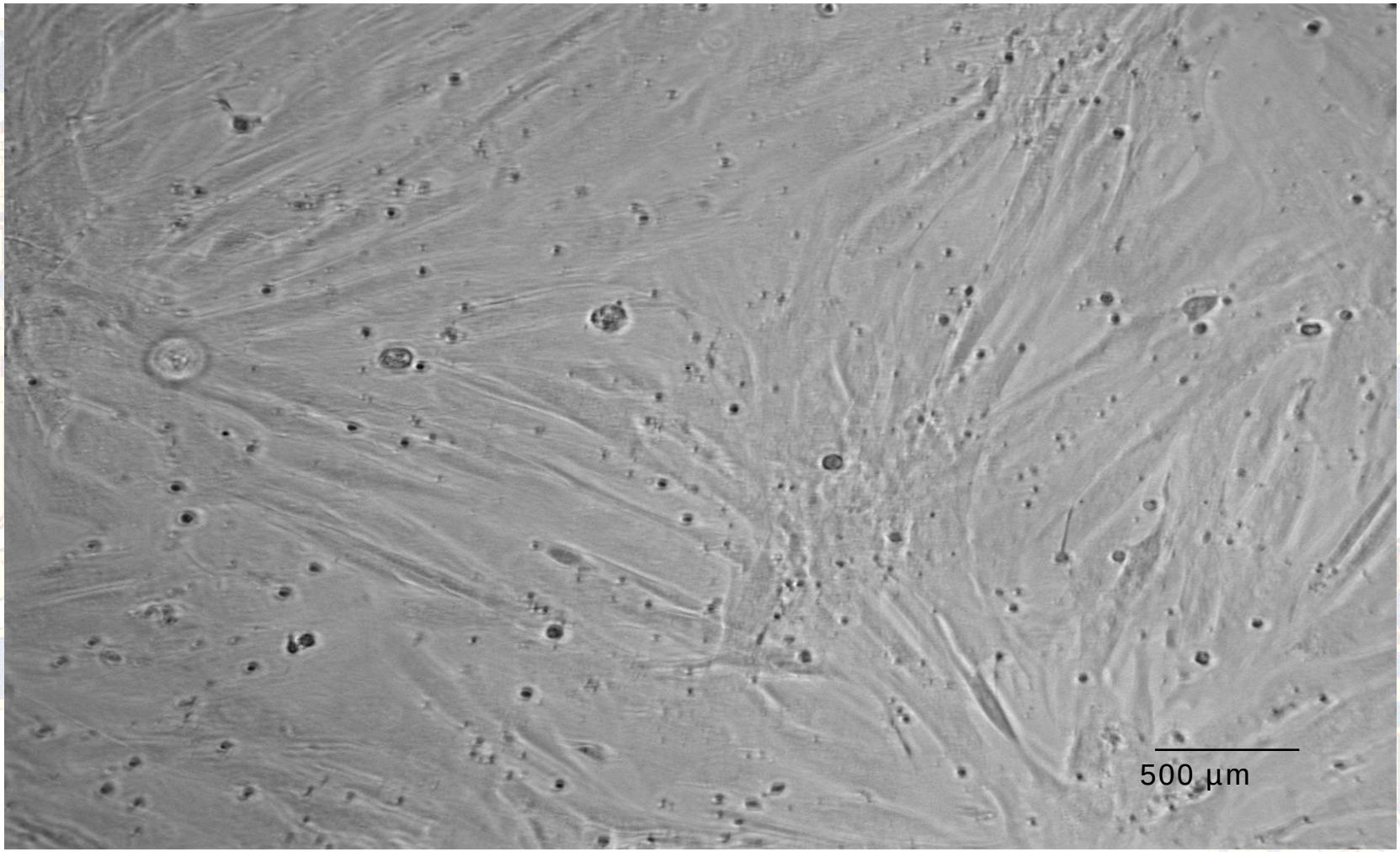
- Several slides were removed due to confidentiality issues.

Methods: Stage 2, Cell Culture Process

Bone marrow-derived mesenchymal stem cells (BMSCs) were grown in 500 cm^2 triple flasks using DMEM high glucose media with sodium pyruvate. Flasks were passaged at durations ranging from 7 to 10 days.



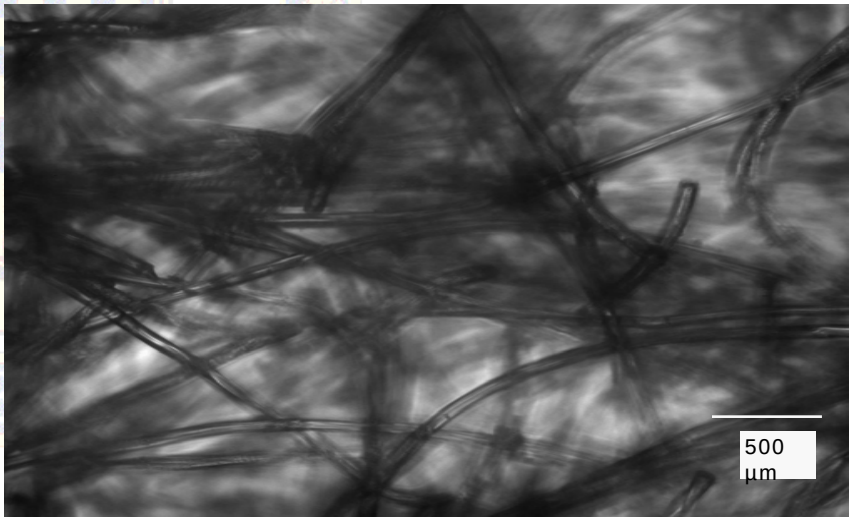
Methods: Stage 2, Cell Culture Process



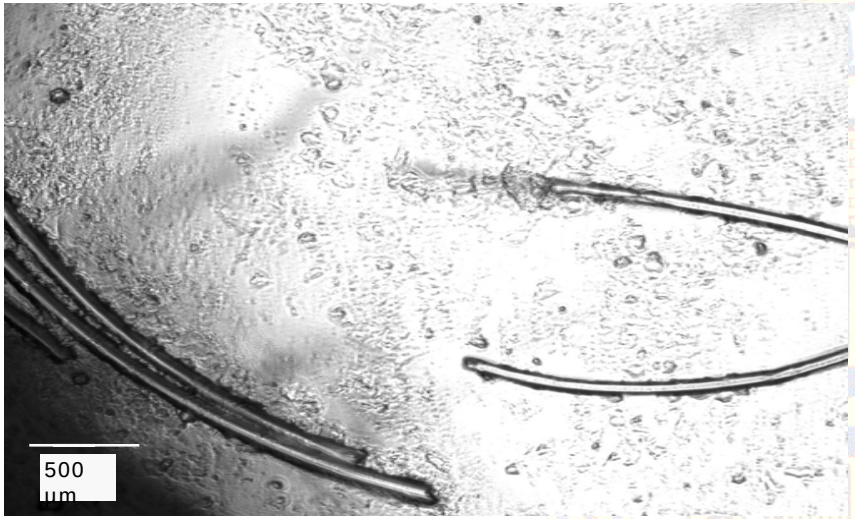
BMSC

Methods: Stage 3, Tissue Engineering Experiment

- After 25 days of cell growth, four 50:50 blend poly(glycolicacid) (PGA) and poly(L-lactic acid) (PLLA) scaffolds, to be used as a static control, were seeded with 85 million cells that came from 12 flasks. Empty and BMSC-cellular scaffold microstructure are shown below.



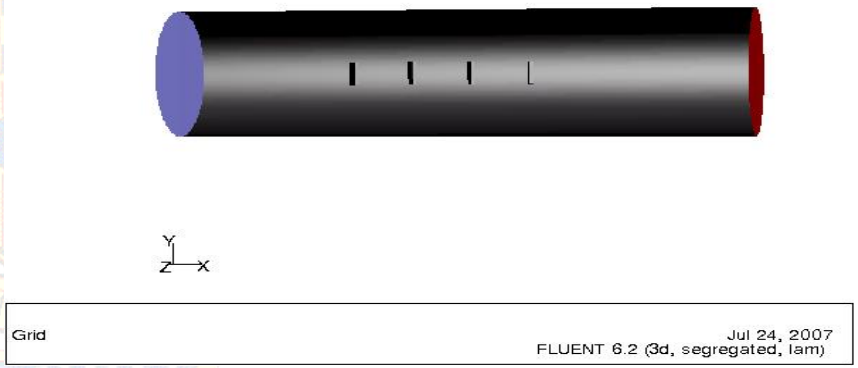
Empty Scaffold



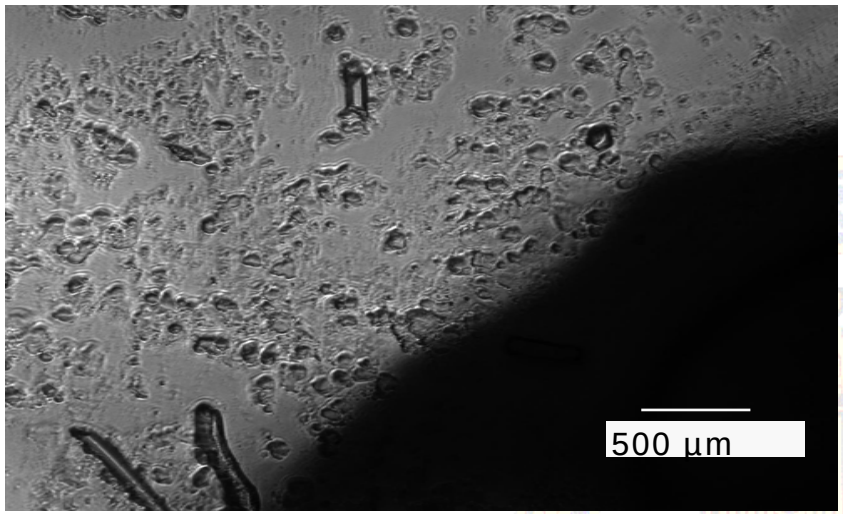
Scaffold after 5 days of static culture

Methods: Stage 3, Tissue Engineering Experiment

- Each flask held an average of 7 million cells, leading to a seeding density of 17 million cells per cm^2 of scaffold. The static culture remained in a hybridization tube rotating at 8 rpm for for 6 days, after which both collagen and DNA assays were performed.



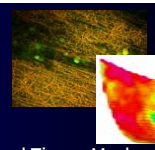
Hybridization tube schematic



Scaffolds after 5 days of static culture

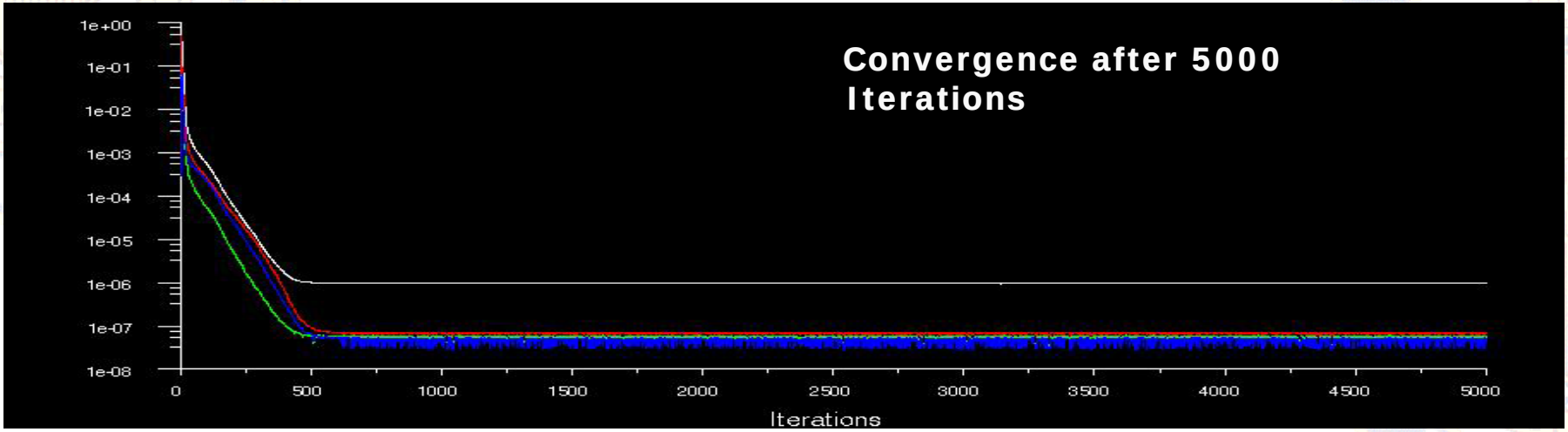
- After 35 days, scaffolds were seeded for the mechanical stimuli group. 185 million cells from 19 flasks were used to seed 2.5 scaffolds, which were larger (7.5 mm by 25 mm) than the previous scaffolds (7.5 by 7.5 mm). An average cell count per flask of 9.7 million cells was achieved. Due to time constraints, these scaffolds were never placed in the bioreactor.

Computational Simulation

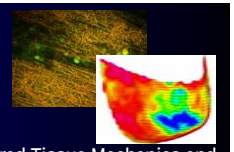


• Computational Fluid Dynamics (CFD) software (Fluent Inc, New Hampshire) was used to create 3D laminar flow computational simulations of the FSF bioreactor. A constant density of 1009 kg/m3 and dynamic viscosity of 0.00076 kg/m-s was assumed for the media.

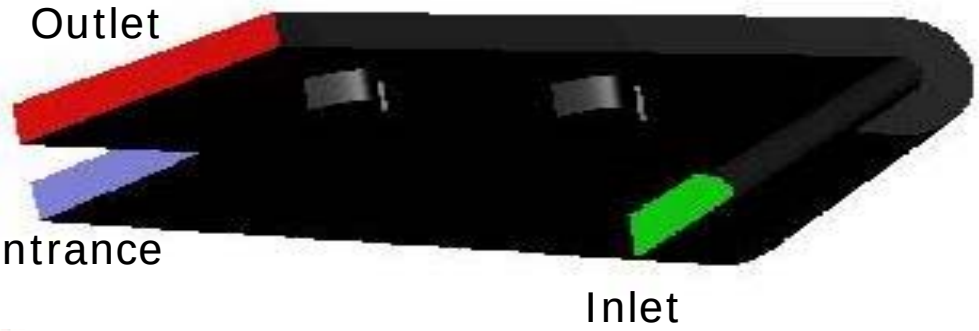
•Two simulations were run: one with bent samples and one with straight samples. Unstructured meshes were used with at least 180,000 grid points. Convergence of the CFD simulation was achieved in the bent samples (less than 10-6 numerical error).



Computational Simulation



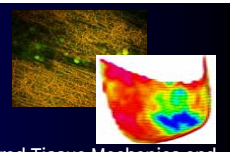
- A fluid velocity of 2.9 cm/s was used at the entrance. Everything, including the samples, were assumed to be rigid walls. For the outlet, an outflow boundary condition was prescribed and an interior boundary condition was used for the old inlet. Steady flow was used.



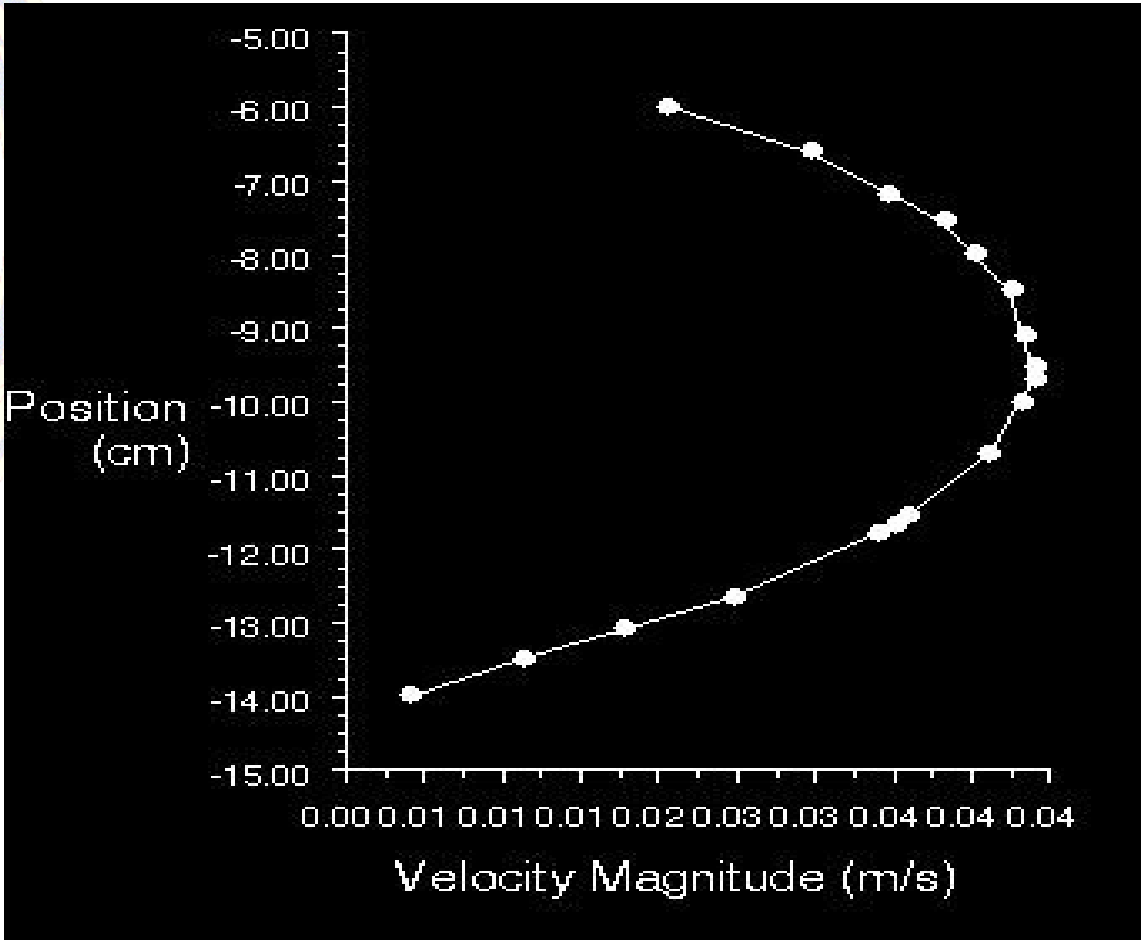
Straight sample mesh

Bent sample mesh

Results: Computational Simulation

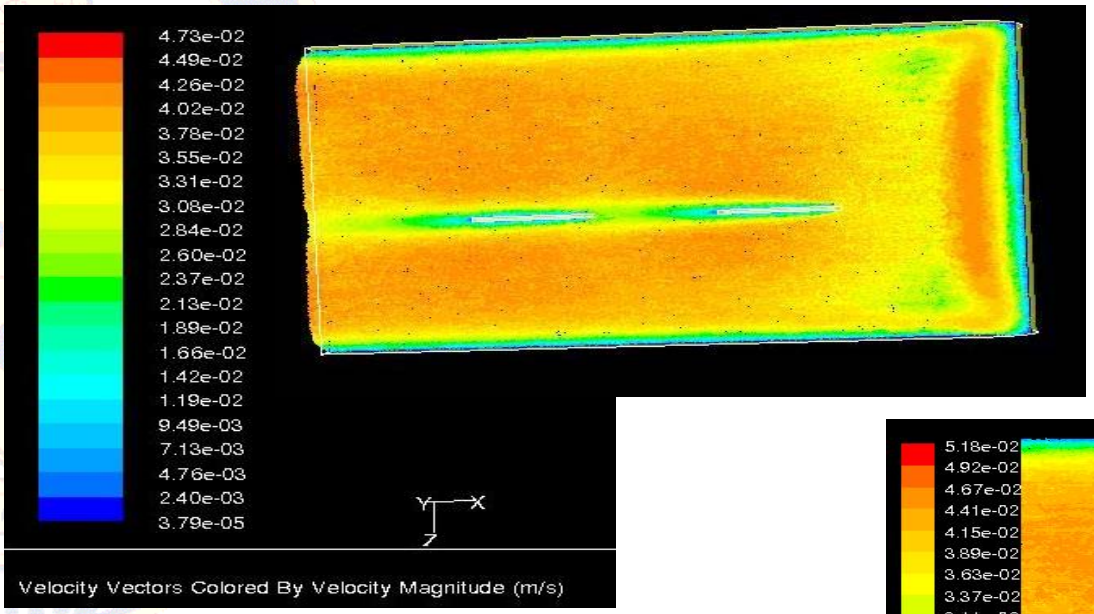


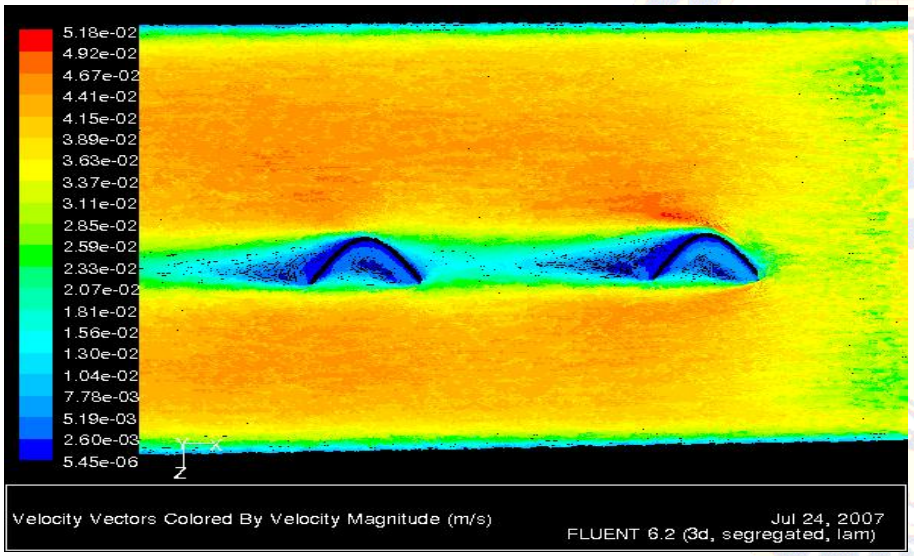
- The fluid velocity before the inlet develops fully, as seen by this parabolic graph, because of the entrance length. Fully developed flow means that viscous effects have spread throughout the fluid in the chamber.



Results: Computational Simulation

- The velocity of the fluid decreases between the samples, as the samples obstruct flow. The effect is greater in the bent samples.





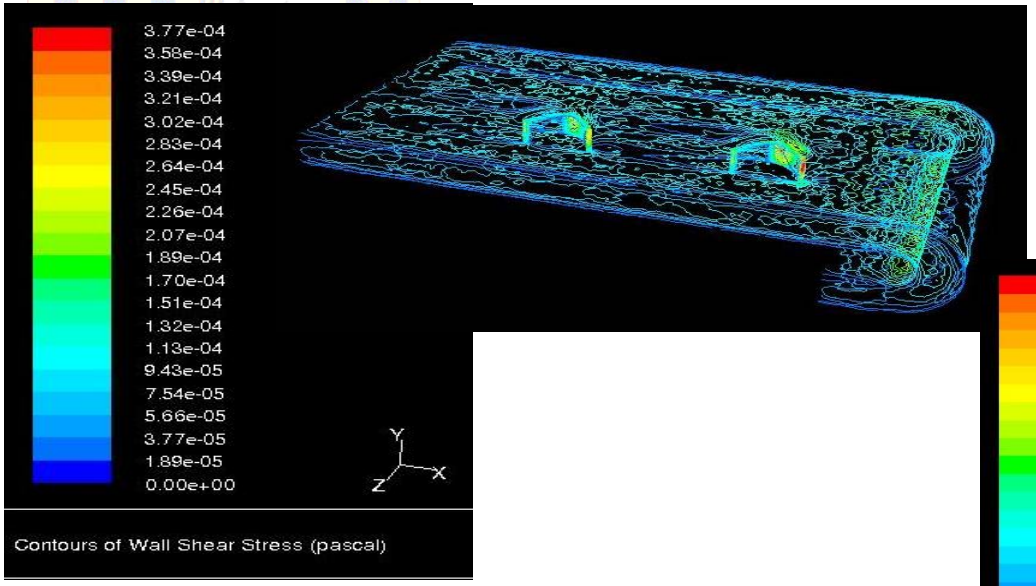
Results: Computational Simulation

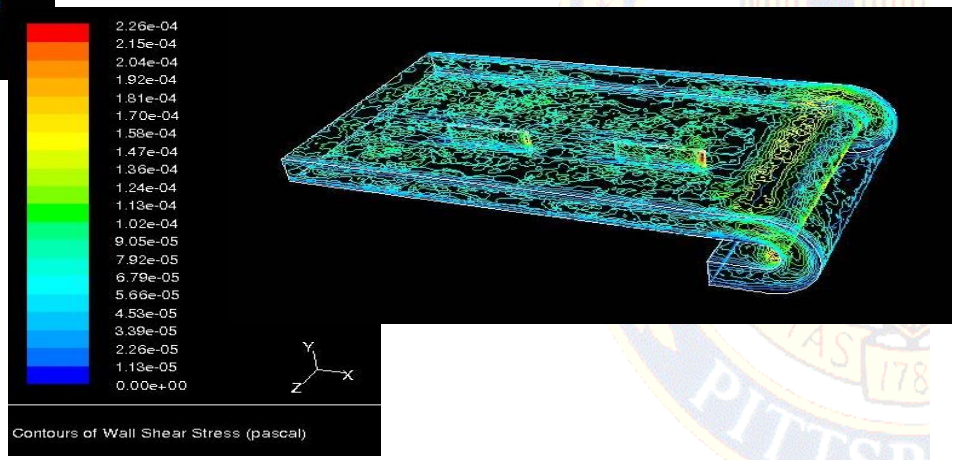
- First bent sample avg. shear stress: 7.64 * 10-5 Pa

  Second bent sample avg. shear stress: 5.39 *10-5 Pa

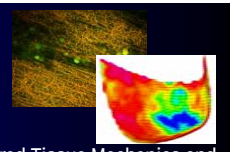
- In the bent sample, fluid shear stress was found to be greater for the first sample than for the second, meaning that number of samples, placement with respect to other samples, and position in the FSF bioreactor plays a role in the amount of shear stress that individual samples encounter.

- The shear stress of the bent samples was found to be greater than the straight samples through comparing the shear stress at the leading edges.





Results and Discussion

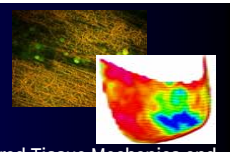


- One media change per week, as compared to two or more, was found to be sufficient.

- Media was changed everyday after seeding, and it was centrifuged to collect the cells that had not yet attached to the scaffold. It took 4 to 5 days for all cells to attach, as seen by the disappearance of the cell pellet.

- Cell flasks, when passaged, typically contained 20 to 30% of the expected number of cells. Cell growth was slower than expected, and the number of days to reach an apparent confluent state increased as time went on. Triple flasks made it difficult to observe cell growth, as only one layer can be seen under the microscope, so regular flasks or more frequent cell counts may offer a solution.

Results and Discussion



•Static Culture Assays:

Collagen Content: 818 +/- 202 µgrams/g wet weight

DNA Content: 58.9 µgrams/g wet weight (significant error)

7.7 +/- 2 million cells / g wet weight

Collagen Content per DNA:

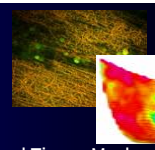
13.9 µgrams collagen per microgram of DNA

•Studies have shown that mechanical stimuli increases tissue formation, and this effect is expected to be seen if the experiment is carried further.

Department of Bioengineering

McGowan Institute for Regenerative Medicine

Acknowledgements



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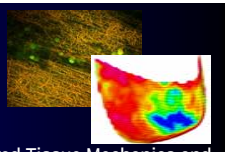
BBSI 2007 Program

References



[1] Engelmayr Jr George C., Sales Virna L., Mayer Jr John E., Sacks Michael S. Cyclic flexure and laminar flow synergistically accelerate mesenchymal stem cell-mediated engineered tissue formation: Implications for engineered heart valve tissues. Biomaterials 27 (2006): 6083–6095.

[2] Engelmayr Jr George C. , Soletti Lorenzo, Vigmostad Sarah, Budilarto Stephanus,

Federspiel William, Chandran Krishnan, Vorp David, Sacks Michael. Design and Qualification of a Novel Flex-Stretch-Flow Bioreactor for Engineering Heart Valve Tissues, Society of Heart Valve Disease, 4th Biennials meeting, June 15th-18th, New York, NY.