Approaches in Heart Valve Tissue Engineering

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Introduction

- As the application of biomechanical stimuli to developing tissue has shown to be beneficial in terms of overall tissue properties, custom-built devices, termed bioreactors are designed so that they can provide appropriate mechanical conditioning to the engineered tissue.
- In heart valve tissue engineering applications, a bioreactor was successfully designed and used by Engelmayr et al [1]. This bioreactor subjected engineered tissue samples to flexure, flow and stretch (FSF) modes of mechanical stimuli [2].
- 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.

Methods: Stage 1

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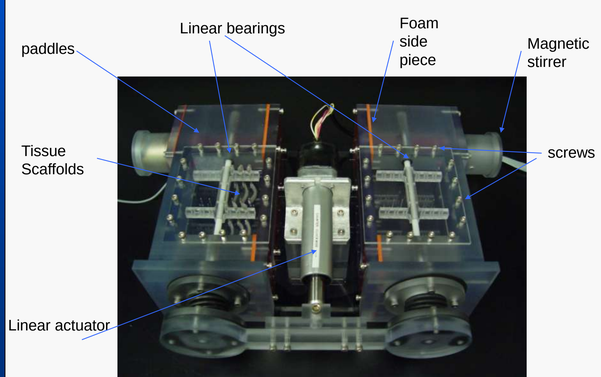

Figure 1:FSF Bioreactor [2]

Methods: Stage 2, Cell Culture and Tissue Engineering

- Bone marrow-derived mesenchymal stem cells (BMSCs) were grown in 500 cm^2 triple flasks using DMEM high glucose media with sodium pyruvate (Fig.2). Flasks were passaged at durations ranging from 7 to 10 days (Fig.6).
- 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 in Figs. 3 and 4 respectively.
- 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.
- 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.
- Computational Fluid Dynamics(CFD) software (Fluent Inc, New Hampshire) was used to create 3D laminar flow computational simulations of the FSF bioreactor. A density of 1009 kg/m3 and dynamic viscosity of 0.00076 kg/m-s was assumed for the media.

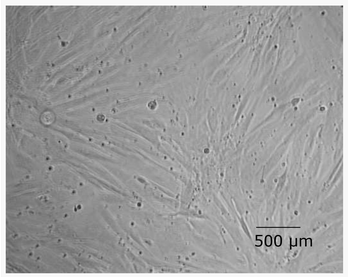

Figure 2: BMSC cells

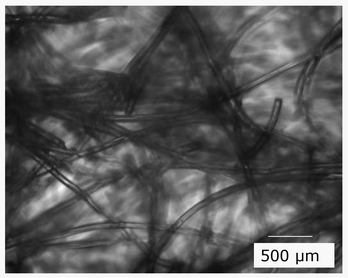

Figure 3: Empty scaffold

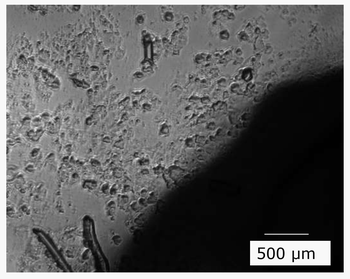

Figure 4: Scaffold after 5 days of static culture

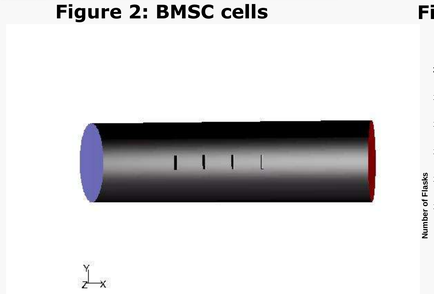

Figure 5: Hybridization Tube Schematic

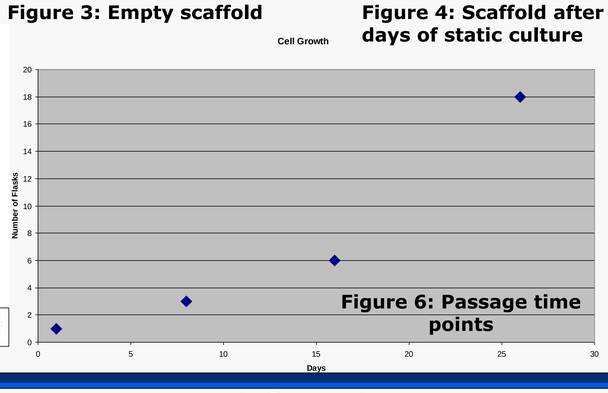

Figure 6: Passage time points

Results: Computational Simulations

- 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 both samples ($\leq 10^{-6}$ numerical error). Fully developed flow was obtained by using an entrance length before the inlet.

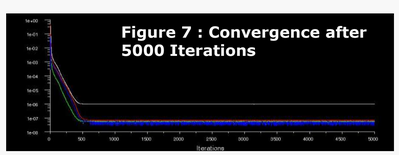

Figure 7 : Convergence after 5000 Iterations

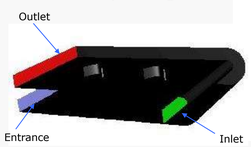

Figure 8: Bent sample mesh

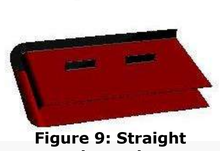

Figure 9: Straight sample mesh

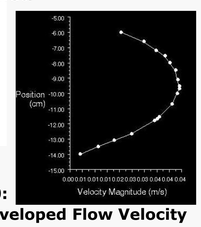

Figure 10: Fully Developed Flow Velocity

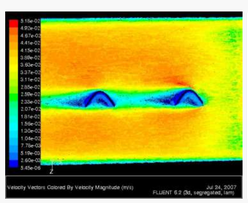

Figure 11: Velocity of fluid between bent samples

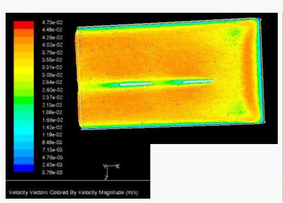

Figure 12: Velocity of fluid between straight samples

(Plane cuts axially through middle of samples)

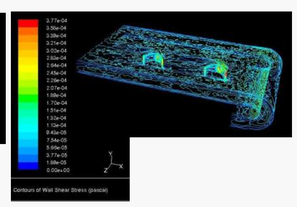

Figure 13: Fluid shear stress on bent samples

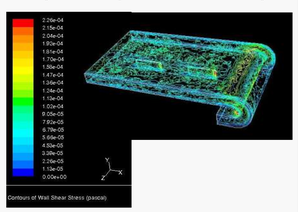

Figure 14: Fluid shear stress on straight samples

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.
- Static Culture Assays:
  Collagen Content: 818 µgrams/g wet weight
  DNA Content: 58.9 µgrams/g wet weight
  7.7 million cells / g wet weight

  Collagen Content per DNA:
  13.9 µgrams collagen per microgram of DNA
- 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.
- Mechanical stimuli increases tissue formation, and this effect will be seen if the experiment is carried further.

Acknowledgements

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- James Reber: *Astro Automation Inc.*
- Lorenzo Soletti: *University of Pittsburgh, Vascular Surgery & Vascular Biomechanics Research Laboratories, McGowan Institute for Regenerative Medicine*

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.