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Perfusion Cell Seeding and Expansion in Dual Mechanical Stimulation Bioreactor for In Vitro Tissue Development

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A Bioreactor for Perfusion Seeding and Development of Engineered Tissue Vascular Grafts in Dynamic Conditions

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ETM3 Engineered Tissue Multiscale Mechanics & Modeling

INTRODUCTION

Small-caliber engineered tissue vascular grafts (ETVGs) for coronary artery bypass surgery have yet to make significant progress towards clinical trials.

1951	1958	1970	1986	1993	1999	2003	2012	2016
Autologous saphenous vein used for lower extremity bypass.	DeBakey uses dacron in aortic reconstruction.	Emergence of PTFE and ePTFE as vascular conduits.	Weinberg and Bell create the first ETVG.	L'Heureux creates first ETVG without permanent synthetic components.	Niklason fashions ETVG under radial strain.	Dahl utilizes decellularization techniques for "of the shelf" access.	Wu reports acellular graft techniques for rapid remodeling in vivo.	Huang reports biaxial stretch of cellularized grafts in vitro leads to better mechanical properties

The implantation of an ETVG initiates a host response known as the foreign body reaction (FBR). Previous works have found that unsuitable ETVG mechanical properties leads to maladaptive FBR which ultimately results in graft failure.

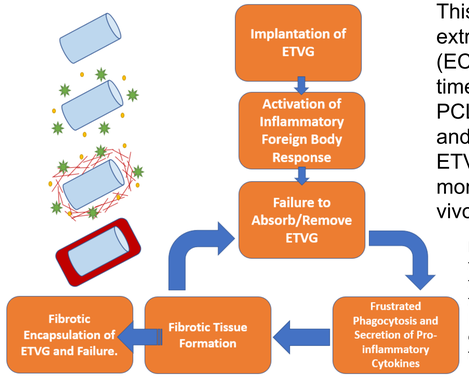

Figure 1. Diagram of the maladaptive cycle of the inflammatory FBR to the implantation of ETVGs leading to full encapsulation and graft failure.

This work examines extracellular matrix (ECM) accretion over time onto electrospun PCL scaffolds in vitro and its effects on ETVG composition, morphology and in vivo FBR.

DESIGN

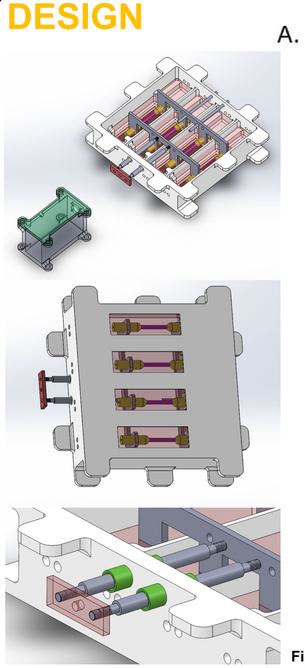

Figure 2. Images from the Solidworks assembly of the dual axial stimulation bioreactor.

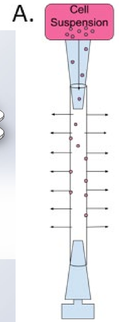

Figure 3. A diagram of the seeding technique. In A, the distal end of the graft is closed forcing the cell suspension to perfuse through the pores of the electrospun scaffold and become lodged along the interior of the scaffold. In B, after a short period of no flow to allow for initial cell attachment the distal end is unblocked, and cell medium is allow to flow freely..

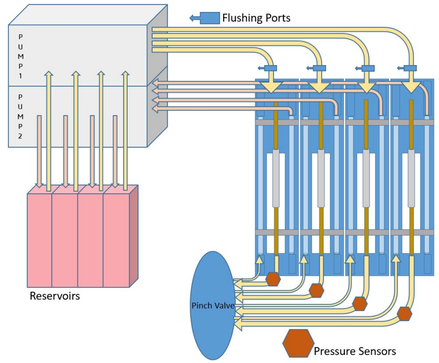

Figure 4. A schematic of the bioreactor flow system. Each ETVG has its own flow loop and reservoir feeding it, eliminating signaling between the cells propagating on each graft. The pinch valve is essential for the seeding mode of the Bioreactor system and will also provide the mechanism for the pressurization of the scaffolds for stimulation. Flushing ports provide easy access to the flow loop to clear possible blockages.

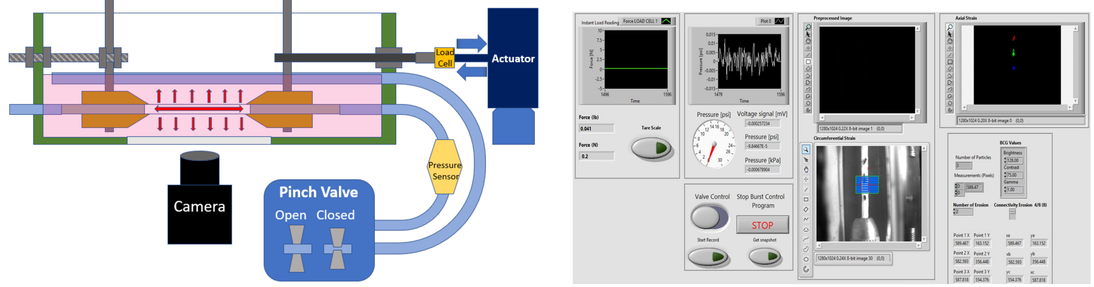

Figure 5. A schematic of the apparatus involved in the dual axial mechanical stimulation of the ETVGs. A linear actuator provides the tensile stress to the ETVG through a linear bearing, which provides a watertight seal without restricting motion. A load cell between the actuator and frictionless shaft will read the tensile force being exerted on the ETVG. The pressurization the ETVG will be facilitated by the pinch valve. As it pinches closed, pressure builds in the ETVG which is read and reported by the pressor sensor situated between the pinch valve and the end of the ETVG. The pressure and force measurements are compared against physical changes in the ETVG recorded by the camera. An example of our lab view program for controlling and reading these processes is seen on the right.

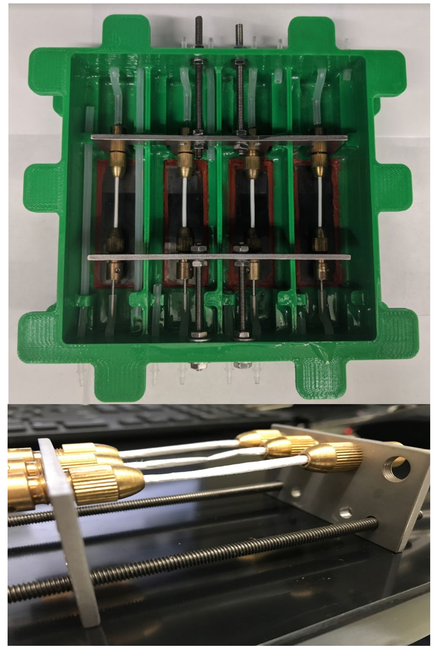

Figure 6. Final assembly of the ETVG chambers. Notice the brackets hold all four of the grafts in place. The actuator will provide motion for all four grafts via the one bracket.

RESULTS

The driving idea behind dual axial stimulation for ETVG culture is that by recreating the conditions of native vasculature, cells will be encouraged to organize in a similar way which will increase strength without sacrificing compliance. So great care had to be taken with the flow calculations.

Native vasculature experiences a shear stress along its walls of 3 dynes/cm^2. Using basic flow equations:

$$Q = \rho u_{avg} A$$

$$A = \frac{\pi}{4} D^2$$

$$u_{\max} = 2u_{avg}$$

$$\tau = \frac{4\mu u_{avg}}{R}$$

We measure average flow rates from our pump to see if we could generate the required stress.

Figure 7. Flow rate from each trough by pump speed. The pump provides a linear increase in flow rate as the speed of the pump increases up to ~40 RPMs. After that, the flow rates vary in a seemingly uncontrollable manner.

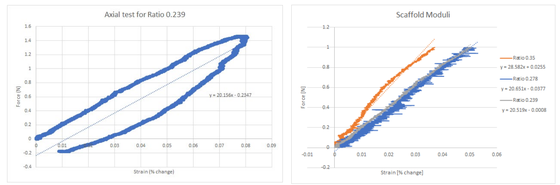

Figure 8. Initial testing of the dual axial stimulation systems. The tensile strain measuring set up provides an accurate cyclic stretch profile when tested with virgin scaffolds. Scaffolds of similar structure (mass/length ratios) consistently produced comparable results. The pressurizing system worked well also, however technical difficulties with data recording kept us from including a figure.

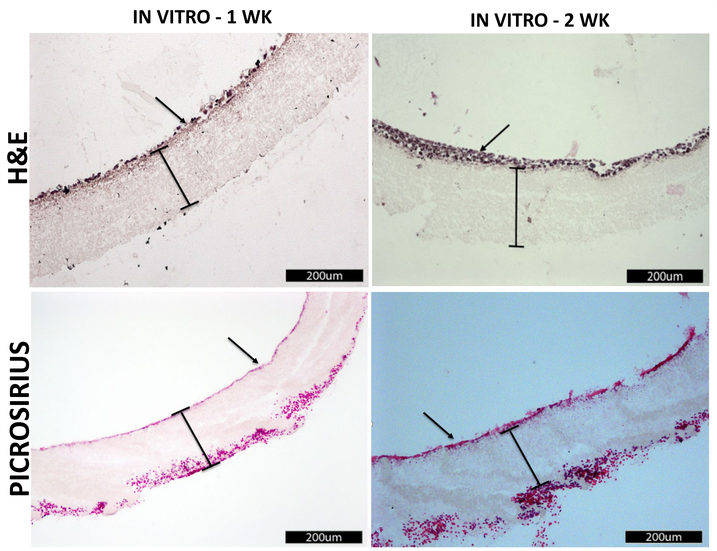

Figure 9. In our previous static bioreactor we successfully seeded and cultured cells for up to 2 weeks, These cells underwent no stimulation through out their culture periods. The cells grew in random layers (H&E) and deposited collagen in an unorganized matter (Picrosirius). The stimulation provided by the new bioreactor should enhance the development of organized tissue structure and increase strength..

FUTURE DIRECTIONS

The next step in our experiments will be to compare scaffolds seeded and cultured statically in our new bioreactor to those from the previous system. And confirm our seeding method does not lose efficiency between systems.

After this is confirmed we will utilize dynamic training during in vitro incubation to modify the structure and organization of the ECM in the ETVGs before implantation as seen in Figure 10. After confirming we can manipulate the graft properties in a significant way we will move to in vivo trials. as seen in Figure 11 .

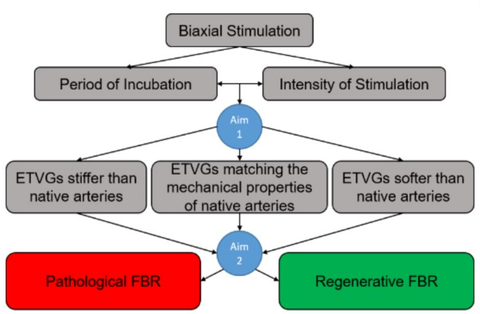

Figure 10. Experimental design for future works.

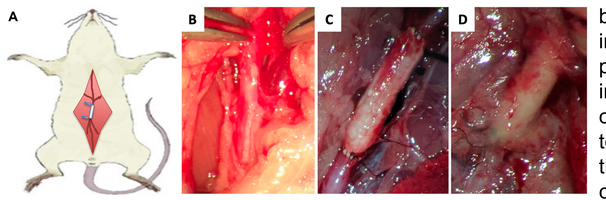

Figure 11. A: schematic of the surgical procedure of the deployment of ETVG in as an infra-renal aortic interposition graft; **B:** native aorta; **C:** ETVG after implantation sutured to the native aorta; **D:** the same ETVG at explant after 2 weeks in vivo.

We will further test the foreign body reaction (FBR) after implantation with cytokine panels, western blotting, and immunoassays to map the change from inflammatory FBR to wound healing and evaluate the level of manipulation we can exert through mechanical stimulation.