Towards Efficient Blood Outgrowth Endothelial Cell Seeding of Small Diameter Gore-Tex Vascular Graft

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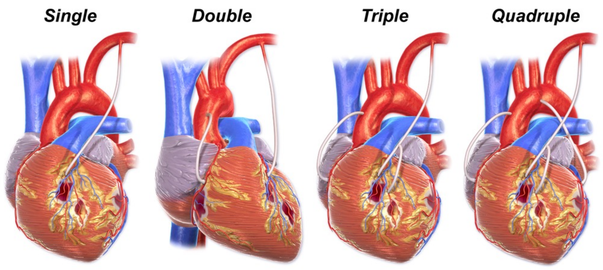
Background



Coronary Artery Bypass Graft (CABG)

- The Centers for Disease Control and Prevention (CDC) estimates that over 18 million adults in the US suffer from coronary artery disease (CAD) with holding 43% of causes of death due to Cardiovascular Disease.[1]
- Approximately 200,000 patients need bypass grafts annually to treat CAD.
- The gold standard of treatment, autologous vessel grafts, is limited by factors such as patient morbidity, inadequate donor sites, or a shortage of viable donor vessels.
- Individuals requiring bypass grafts but lacking autologous vessels must consider alternate forms of care.
- For medium and large diameter artery replacements, synthetic vascular grafts are a viable option.
- Methods to improve EC retention in small diameter (<6mm) synthetic vascular grafts
  - Biomaterial coatings
  - Surface modifications
  - Nanofiber topography
  - Shear stress preconditioning
  - Endothelial cell modifications
- Still no small diameter vascular grafts with an endothelial layer for clinical use because they cause patency loss from thrombosis and neointimal hyperplasia.

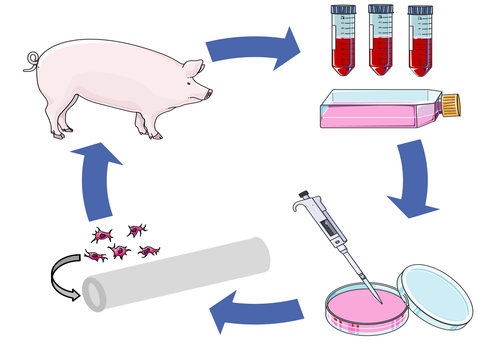
Objectives

✓ Establish a stable endothelium on the inner surface of a small diameter vascular graft through a molecular modulation approach.

✓ Identify molecular signaling pathways responsible for allowing a subpopulation of endothelial cells to remain adherent upon exposure to physiological shear stress.

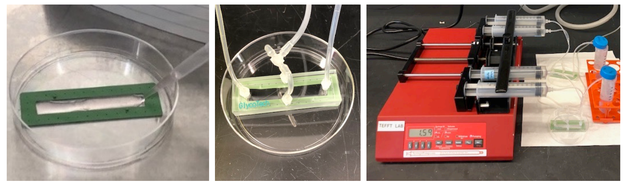
Material & Methods

Pig is a superior model due to similarities in anatomy and physiology to humans. Blood Outgrowth Endothelial Cells (BOECs) are isolated from the peripheral blood of pigs. BOECS Isolated from the mononuclear circulatory cells using density gradient separation Hence, match the cell type for future in vivo studies.[2]

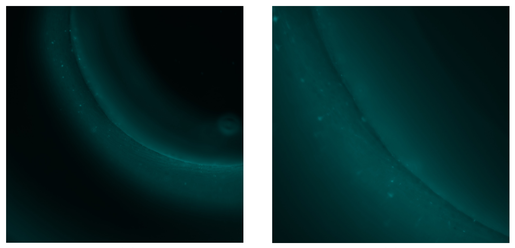


Methods

BOECS cultured on Tissue Culture plate exposed to 15 dyne/cm^2 Shear Stress with Parallel Plate Flow Chamber for 30 minutes, replated for an hour, imaged the nuclei, and then exposed to Shear Stress for 30 minutes more, imaged and measured the retention of endothelial cells.



In preparation for translation and the animal studies, a method has been developed for optimizing BOECS seeding on a clinically relevant known non-bioactive, hemocompatible material, e-PTFE. The experimentation method selected creating a rotational endothelial cell seeding approach utilizes encapsulation of BOECS in GORE-TEX® Vascular Graft and rotating at 15 rpm for 1.5 hours, followed by a 3-hour incubation period. This strategy for endothelial cell seeding is based on the cell adhesion of endothelial cells (200,000 cells/cm^2).



(A) (B)

Figure 1. Shows a diagonal view of the cross-section of a 4mm Diameter Gore-Tex seeded with BOECS at a density of 200,000 cells/cm^2, stained with Trypan blue. Panel (A) image at 4X magnification, Panel (B) shows the same view at 10X magnification

An approach based on Poiseuille's Law and using a pulsatile pump (Figure 2.)has been set up to introduce shear stress conditions that mimic physiological hemodynamics.
The 15 dynes/cm2 of fluid shear stress required in a tube calculated using stroke volume, tube size, and piston diameter. To convert stroke volume to flow rate, it divided by the time period and divided by mL/min. The average velocity of the fluid flowing through the tube calculated by dividing the flow rate by the cross-sectional area of the tube, ensuring the tube size is consistent. The shear stress calculated using the Hagen-Poiseuille equation, which relates shear stress to fluid viscosity, average velocity, and tube dimensions. The equation is expressed as:

$$(\tau) = (4 * \eta * V) / r$$

τ is the shear stress in dynes/cm^2.
η is the dynamic viscosity of the fluid in poise (dynes/cm^2).
V is the average velocity of the fluid in the tube in cm/s.
r is the radius of the cylindrical tube in cm.

This equation assumes fluid flowing through a cylindrical tube under laminar flow conditions.

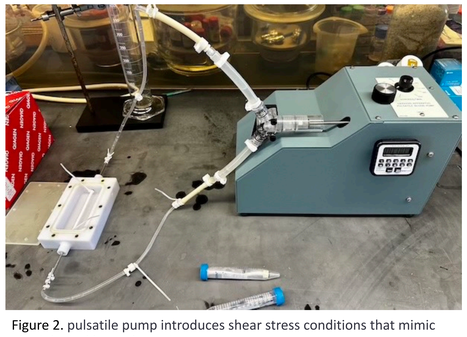


Figure 2. pulsatile pump introduces shear stress conditions that mimic physiological hemodynamics.

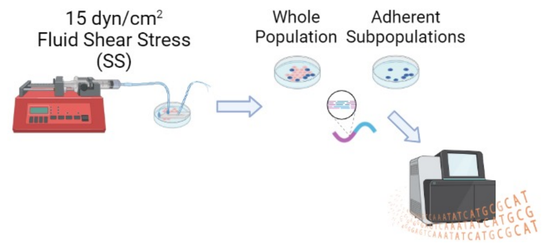


Figure3. Cultured endothelial cells exposed to fluid shear stress (SS) using a parallel plate flow chamber. The cultured cells are placed under vacuum, and the fluid rate can be controlled with a syringe pump to precisely expose cells to a desired fluid shear stress. The equation used to calculate this is provided below[3].

$$\tau_w = \frac{6\mu Q}{a^2 b}$$

τ_w = wall shear stress (dynes/cm^2)
μ = apparent viscosity of media (poise)
a = channel height (gasket thickness - cm)
b = channel width (gasket width - cm)
Q = volumetric flow rate, ml/sec

Results

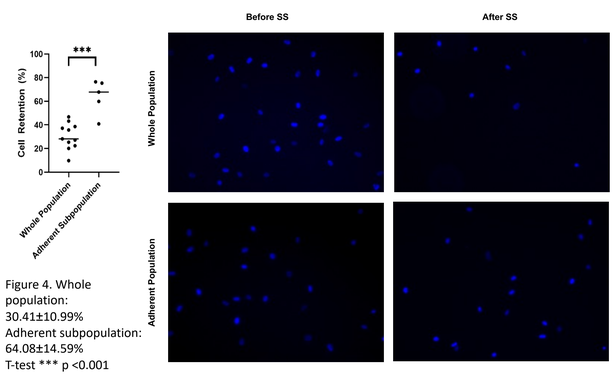


Figure 4. Whole population: 30.41±10.99%
Adherent subpopulation: 64.08±14.59%
T-test *** p <0.001

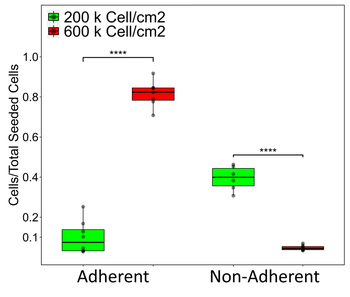


Figure 5.The chart demonstrate percentage of adherent and non adherent cells over total seeded cells in 4mm Diameter Gore-Tex. Control group, were seeded based on the cell adhesion of endothelial cells (200,000 cells/cm^2) which shows less than 10% of cells adhere to Gore-Tex. Cells in Enhanced group seeded with increase in number of cell density by factor of 3. data shows a dramatically increase in number of adherent cells almost 80%.

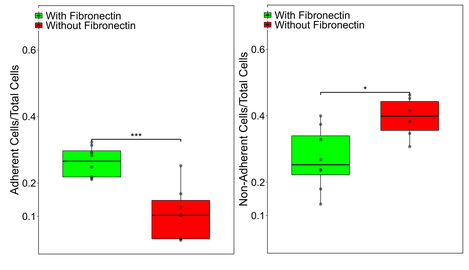


Figure 6The chart illustrates the impact of using Fibronectin to coat the inner surface of a Gore-Tex 4mm Diameter Vascular graft. The targeted cells(Adherents), essential for identifying gene transcriptome, show enhancement without an increase in cell count, solely through the utilization of Fibronectin.

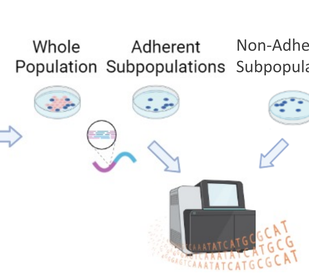
Discussion

The decision between using more cells or fibronectin in the stage of translating to more clinically becomes pivotal in optimizing BOCCS seeding to reduced hyperplasia risk. result using Fibronectin coating is promising and shows 49±10.99% better adherent rather untreated Gore-Tex with same number of cells. Disadvantage to use of biological glue in this technique is any region that is not endothelialized during seeding becomes even more thrombogenic upon implantation than the native graft material.

Also, as we are looking for genes that are responding to the shear stress and use them as a mechanism to sort out for the more adherent ECs, any other factor that has impact on signaling pathway will become a complication.

This achievement holds promise toward enhancing the biocompatibility and long-term functionality of various cardiovascular devices, including stents, heart valves, and pacemaker leads.

Future Work



The transcriptome of adherent and non-adherent cells intended to be examined by RNA-sequencing in comparison to the whole population, and each other to investigate the molecular mechanisms that allow some ECs to remain adherent.

The Goal is identifying patterns consistent among the groups and potentially trending. The response of ECs to fluid SS is not of interest; rather, SS is utilized solely as a sorting mechanism.

Develop sorting mechanism based on RNAseq results and seed them on Gore-tex followed by applying shear stress to confirm the genes are responsible for cell adherence.

Acknowledgements

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