Endothelial cells with low fibronectin leucine rich transmembrane protein 2 (FLRT2) expression exhibit enhanced retention within vascular grafts

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Introduction

There is a strong clinical need for a small-diameter (<6 mm) vascular graft that remains patent

- Severe coronary artery disease often requires surgical intervention with autologous bypass grafting
- While medium- and large-diameter vascular grafts perform well clinically, there are no small-diameter (<6 mm) synthetic graft options due to unacceptable patency rates with reports of ~60% and ~14% patency after 1 and 3 years of implantation, respectively[1]
- Loss of patency occurs in the absence of an endothelial cell (EC) monolayer to prevent platelet activation, thrombosis, and neointimal hyperplasia

Seeded ECs are rapidly lost from synthetic vascular grafts *in vivo* upon exposure to circulating blood

- Most seeded ECs are lost from the surface of synthetic grafts with reports of 40% of cells detaching 1 hr and over 80% after 24 hrs of implantation[2]

The mechanism allowing some ECs to remain adherent when exposed to fluid shear stress (SS) from flowing blood is unclear

- Our lab previously identified a subpopulation of adherent ECs that resists detachment when exposed to fluid SS *in vitro,* which provides evidence that EC detachment is not an entirely random process

Hypothesis & Objectives

- *We hypothesize that differential gene expression allows some ECs to adhere more strongly compared to others and resist detachment when exposed to fluid shear stress*
- The goal of this work is to use RNA-sequencing (RNA-seq) to examine the gene expression of adherent ECs compared to the whole population and to develop a method to select for the highly adherent ECs to seed on a small-diameter vascular graft

Materials & Methods

- Primary human umbilical vein endothelial cells (HUVECs) were exposed to 0 (whole population), 5, 10, or 30 min (adherent subpopulations) of 15 dyn/cm^2 fluid SS using a parallel plate flow chamber
- RNA from the adherent ECs at each time point was extracted for RNA-seq and transcribed into cDNA for RT-qPCR
- Transcript abundances from the RNA-seq data were estimated with StringTie and fragments per kilobase of transcript per million mapped reads (FPKM) values for expressed genes & transcripts were filtered with Ballgown R package
- Fibronectin leucine rich transmembrane protein 2 (FLRT2) was examined as a target based on its significant downregulation in the adherent subpopulation and existing literature on its role in adhesion[3]
- HUVECs were sorted using fluorescence activated cell sorting (FACS) into low, intermediate, and high FLRT2 expression groups
- Si-FLRT2 HAECs were seded onto a commercially available goretex graft and stained with DAPI. Western blot analysis were performed to analyse the expression of VCAM-1 and ICAM-1 post FLRT2 silencing.

Materials & Methods

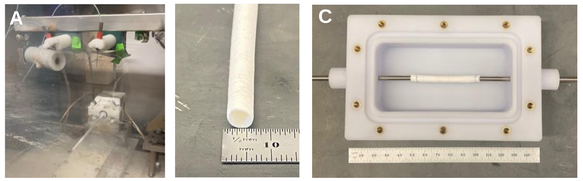

Figure 1. Electrospun DP15 grafts (A) Electrospinning nanofibers on a rotating mandrel to create vascular graft samples **(B)** 4mm inner diameter vascular graft **(C)** Graft mounted in the custom perfusion chamber for testing

Results

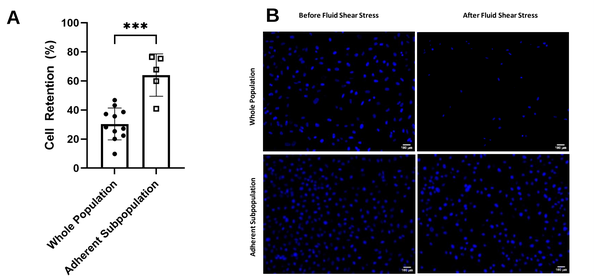

Figure 2. The adherent subpopulation resists detachment upon exposure to fluid SS (A) Adherent subpopulation isolated after 30 min of 15 dyn/cm^2 fluid SS shows augmented retention when re-exposed to SS. Retention of the whole population was 30.4±3.3% compared to 64.1±6.5% for the adherent subpopulation. T-test, ***p-value <0.001 represented in **(B).**

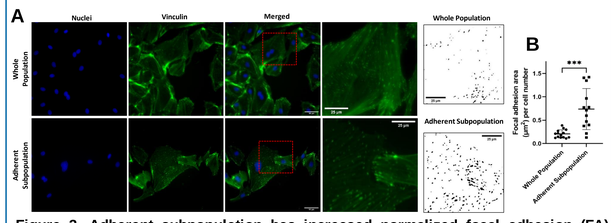

Figure 3. Adherent subpopulation has increased normalized focal adhesion (FA) area. Whole population and adherent subpopulation representative images for FA analysis using nuclei (blue) and vinculin (green) staining **(A)** .FA area per cell number was significantly greater for the adherent subpopulation **(B)**. Unpaired t-test, ***p<0.001.

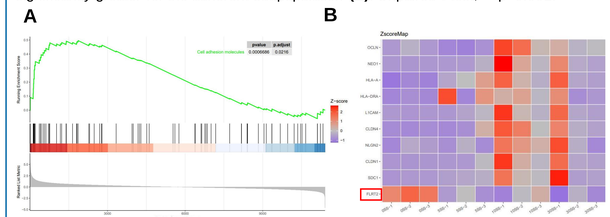

Figure 4. RNA-sequencing identifies enrichment of the "cell adhesion molecules" pathway in the adherent subpopulation compared to the whole population (A&B) Gene set enrichment analysis (GSEA) plot for cell adhesion molecules in the adherent subpopulation **(A)** Z-score heatmap from the cell adhesion molecules pathway **(B)**

Results

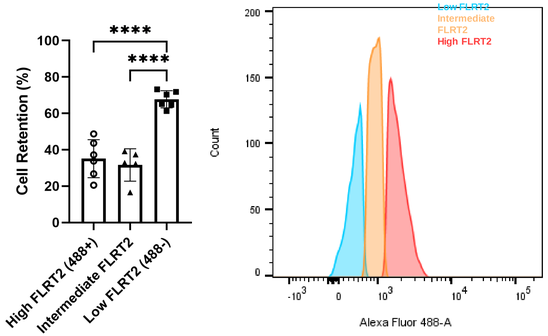

Figure 5. HUVECs with low FLRT2 expression show increased retention when exposed to fluid SS Quantification of flow cytometry shows significantly lower surface expression of FLRT2 in the adherent subpopulation **(A)** Histogram of gating into low (blue), intermediate (orange), and high (red) FLRT2 expression groups with FACS **(B).** ANOVA with Tukey's analysis** p<0.001, **** p<0.0001.

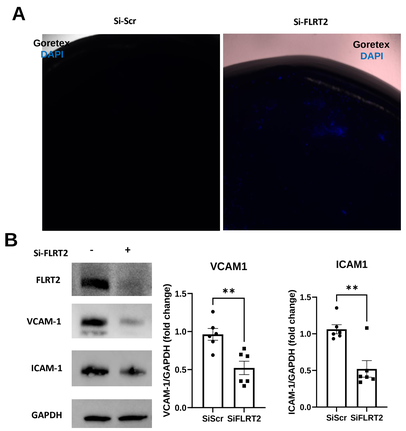

Figure 6. HAECs with low FLRT2 expression show increased retention on Goretex graft and decreased endothelial cell activation. Retention of HAECs with decreased FLRT2 expression show greater adhesion on commercially used goretex graft **(A)** additionally, FLRT2 silencing show decreased VCAM-1 and ICAM-1 densitometries normalized to GAPDH **(B)** Values are means + SEM. of > 4 independent experiments., Unpaired t-test, ***p<0.001

Conclusions & Discussion

- We demonstrate that we can select for the highly adherent ECs in a population. Using FACS, we sort ECs based on the expression of FLRT2 and show that the ECs expressing low levels of FLRT2 have increased retention when exposed to fluid SS
- To our knowledge, this is the first study to successfully predict which ECs within a population are more likely to remain adherent within a vascular graft and provides proof-of-concept for a novel approach to seed a small-diameter vascular graft with only the highly adherent ECs in a population
- The mechanism by which downregulation of FLRT2 allows for enhanced EC retention under exposure to fluid SS is still largely unknown. Our study showed that we can selectively sort for the more adherent ECs in a population based on low FLRT2 expression, which was the goal of our study
- Silencing FLRT2 expression increased cell retention in commercially available vascular grafts as well as decreased endothelial cell activation.

Future Directions

- Ongoing studies are investigating the mechanism that allows ECs with lower FLRT2 expression to resist detachment
- The biological response including thrombosis and neointimal hyperplasia will need to be studied in a pre-clinical animal model in the future

References

1. Chard, R. B., Johnson, D. C., Nunn, G. R., & Cartmill, T. B. (1987). Aorta-coronary bypass grafting with polytetrafluoroethylene conduits: early and late outcome in eight patients. *The Journal of thoracic and cardiovascular surgery*, *94*(1), 132-134.
2. Rosenman, J. E., Kempczinski, R., Pearce, W. H., & Silberstein, E. B. (1985). Kinetics of endothelial cell seeding. *Journal of vascular surgery*, *2*(6), 778-784.
3. Camillo, C., Facchinello, N., Villari, G., Mana, G., Gioelli, N., Sandri, C., ... & Serini, G. (2021). LPHN2 inhibits vascular permeability by differential control of endothelial cell adhesion. *Journal of Cell Biology*, *220*(11), e202006033.

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