

Introduction

- 610,000 people die of heart disease every year (CDC 2015)
- Over 1 million people each year undergo coronary interventions.
- Risks of coronary intervention include: thrombosis, embolization, infection, and re-narrowing of the artery.
- Improving the adhesion capabilities of endothelial cells may significantly reduce the associated risks of coronary interventions.

Objectives

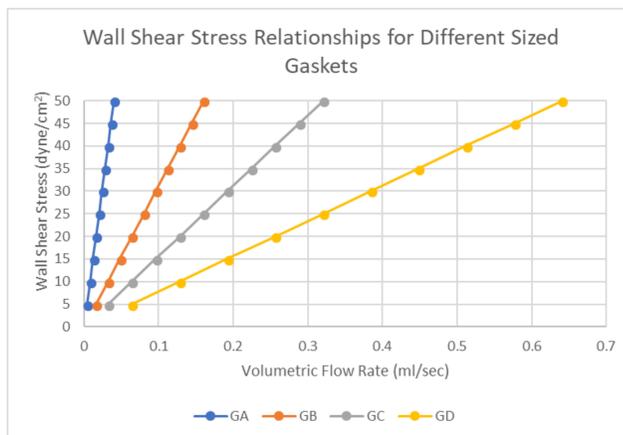
- Validate a parallel plate flow chamber (PPFC) apparatus that will be used to test treatments that promote endothelial cell adhesion.
- Develop and test treatments that may improve endothelial cell adhesion.

Methods

Validation of PPFC Apparatus

- Preliminary calculations were conducted to determine PPFC conditions required to achieve a shear stress equivalent to that which endothelial cells are exposed within blood vessels (15 dyne/cm²).

$$\tau_w = \mu \dot{\gamma} = 6\mu Q/h^2 w$$



- Two main characteristics were tested: laminar flow presence and flow rate accuracy.
- Laminar flow presence was determined through observation of dyed water flowing through the apparatus.

- Flow rate accuracy was tested by inputting known flow rates, syringe diameters, and syringe volumes into the pump system, followed by recording the amount of time it took for a certain flow rate to dispense a particular amount of fluid.
- The amount of time taken to dispense a certain volume of fluid was compared to the calculated expected time.



Optimization of Cell Adhesion

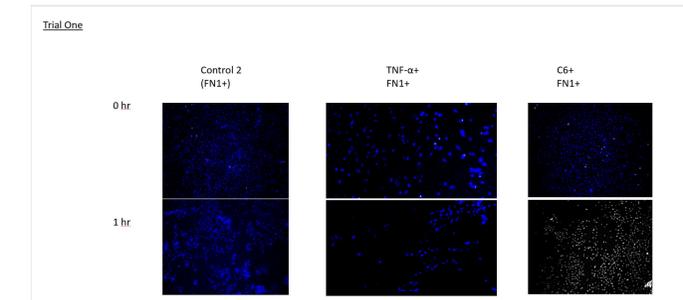
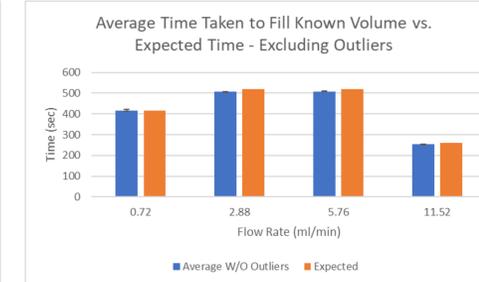
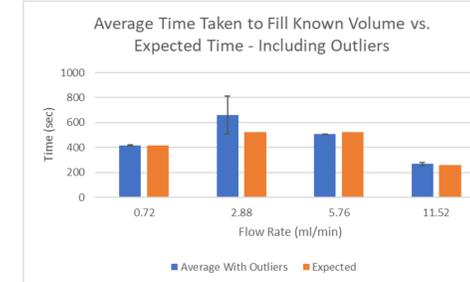
- Utilizing the Reactome database and previous literature research, four novel treatment options were designed that focused on improving cell-adhesion capabilities in endothelial cells. Two subsequent control treatments were also developed.

Control 1 (n=3)	Control 2 (n=3)	TNF- α + FN1 - (n=3)	C6 + FN1 - (n=3)	TNF- α + FN1 + (n=3)	C6 + FN1 + (n=3)
250 μ l Media	250 μ l Media	250 μ l Media	250 μ l Media	250 μ l Media	250 μ l Media
	76.8 μ g FN1	5 μ g TNF- α	5 μ g C6	5 μ g TNF- α	5 μ g C6
				76.8 μ g FN1	76.8 μ g FN1

- Porcine pulmonary artery endothelial cells were cultured and exposed to various treatment conditions.
- Groups were exposed to a shear stress of 15 dyne/cm² for 30 min.
- Analysis of images of groups taken before and after shear stress exposure was used to calculate the efficiency of the treatment in promoting cell adhesion.

Results

- Utilizing a 95% confidence interval (CI), it appears that the expected values for flow rates (ml/min) 0.72, 2.88, and 11.52 in the outlier containing data and 0.72 in the non-outlier data, fell within 95% CI of the experimental values.
- However, all other expected values for flow rate 5.76 in the outlier containing data and 2.88, 5.76, and 11.52 in the non-outlier data, did not fall within 95% CI of the experimental values.



Trial	Treatment	Cell # at 0 hr	Cell # 1 hr	Total Cell Loss (# cells)	Total Percentage of Cell Loss (% cells)
Trial One	Control + FN1	388	235	153	39.4
	TNF- α + FN1	134	99	35	26.1
	CEACAM 6 + FN1	565	440	125	22.1
Trial Two	Control + FN1	376	150	226	60.1
	TNF- α + FN1	359	326	33	9.2
	CEACAM 6 + FN1	251	157	94	37.5

	Control	TNF- α	C6
Average cell loss based on data from 2 trials	189.5	34	109.5
Average Percentage of cell loss based on data from 2 trials	49.8	17.7	29.8

- Statistical analysis of the cells lost and the percentage of cells lost (ANOVA and Tukey's posthoc test) indicated that the treatment groups demonstrated a trend towards higher percentage cell retention (or lower percentage cell loss), however, statistical significance could not be reached using n=2 and p<0.05.

Discussion and Conclusions

- The PPFC apparatus is capable of achieving relatively accurate flow rates necessary to output a shear stress of 15 dyne/cm² for a variety of gasket sizes. However, further research is needed to understand the underpinnings behind the PPFC's functionality at flow rates of 2.88 and 5.76 ml/min.
- Preliminary results of the TNF- α treatment is relatively promising, however, in the future an experiment with a larger sample size and a longer shear stress exposure time (e.g. 1 hour) is needed.

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