Experimental and Biological Investigation of Hemodynamics-induced Injuries for Cardiovascular Disorders

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Background

Abdominal aortic aneurysm (AAA) is a degenerative disease process of the abdominal aorta that leads to a focal dilation and irreversible remodeling of the arterial wall. In this condition, the aortic vessel diameter is dilated beyond 50% its its size.AAA might gradually expand until rupture If left untreated. Current surgical treatment options also are associated with high mortality rates. Therefore, for AAA, it is critically important to determine when the risk of rupture justifies repair. Current clinical practice is to surgically repair large AAAs with diameter > 5.5 cm. However, the incidence of rupture is independent of the diameter size. Currently there is no accepted technique to quantify the risk of rupture for individual AAAs. It is believed that, rupture locations are where peak wall stresses act. Hemodynamic forces by the flowing blood such as shear stress are also thought to contribute to the formation of aneurysm leading to rupture. Endothelial cells respond to disturbed flows in the aneurysm and initiate inflammation that are thought to be important in disease progression. However, little is known about the flow dynamics in AAA, and how it affects endothelial cell biology leading to AAA rupture. In this project, we will use different flow systems to induce shear stress over cell's monolayer. After inducing shear stress, gene expression for shear responsive genes and inflammatory markers will be assessed. Basically, we used the peristaltic pump to induce pulsatile flow over cell's monolayer, and laminar flow using our modified set up. We will compare gene expression data obtained from those two systems with data obtained from our Fluigent pressurized driven pump. Then, we will use another state-of-the-art system, namely a biological pulsed duplicator. Using the system, endothelial cells that are cultured within AAA shaped chambers will be exposed to physiological flows in order to reveal differential endothelial cell signals at potential rupture locations..

Aim

In this project we will culture endothelial cells in flow chambers to assess the gene expression of endothelial cells after exposure to physiological flows in order to reveal differential endothelial cell signals at potential rupture locations.

Method

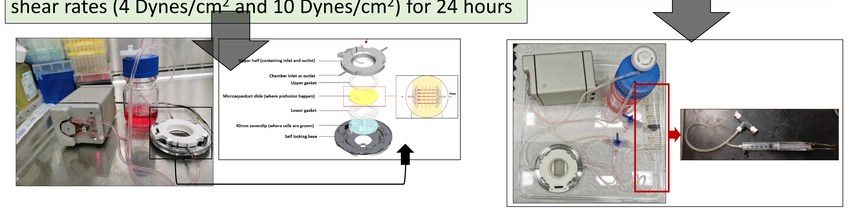
1.Cell culture

EA.hy926 (ATCC® CRL-2922™) endothelial cells were cultured following the vendor's instructions. 40 mm coverslips were collagen coated, and then 100,000 cells were seeded on each coverslip. After that, the coverslip where cells were seeded was assembled in the Bioptech's flow chamber and cells were exposed to either pulsatile or steady flow.

2. Generation of flow condition

To generate a pulsatile flow a micro-Perfusion peristaltic pump was used. Bioptechs FCS2 flow chamber and connected to the peristaltic pump. The pump withdraws the cell media from the media reservoir and infuse it to the flow chamber, back to the media reservoir in a closed-circuit manner. The cells were exposed to two different shear rates (4 Dynes/cm^2 and 10 Dynes/cm^2) for 24 hours

We created a pulse dampener to reduce pulsations and avoid water hammer effects. This system involves the use of an air-filled syringe



3. Flow pattern assessment

Stereo microscopy was used to assess the flow pattern for the pump we used, as well as to confirm the reduction of pulsations after using the air-filled syringe as a pulse dampener. This validation was achieved following our recently reported protocol

4. Cytoskeletal staining

The cell's cytoskeletal proteins influence the cell's shape and integrity. Cytoskeletal structure has been shown to change in morphology under fluid flow and shear stress due to the activation of flow sensing elements.

5. PCR and gene expression

qRT-PCR will be performed for the 17 genes using SYBR Green master mix (applied biosystems) as suggested by the manufacturer.

Results & discussion

Flow pattern assessment

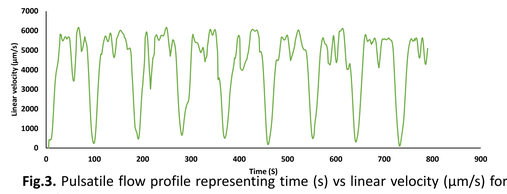


Fig.3. Pulsatile flow profile representing time (s) vs linear velocity (µm/s) for speed 20

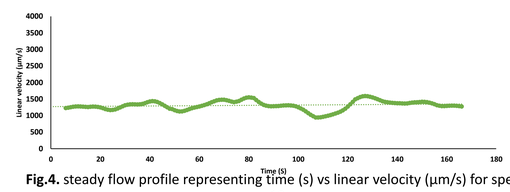


Fig.4. steady flow profile representing time (s) vs linear velocity (µm/s) for speed 10

Cell morphology & cytoskeletal staining

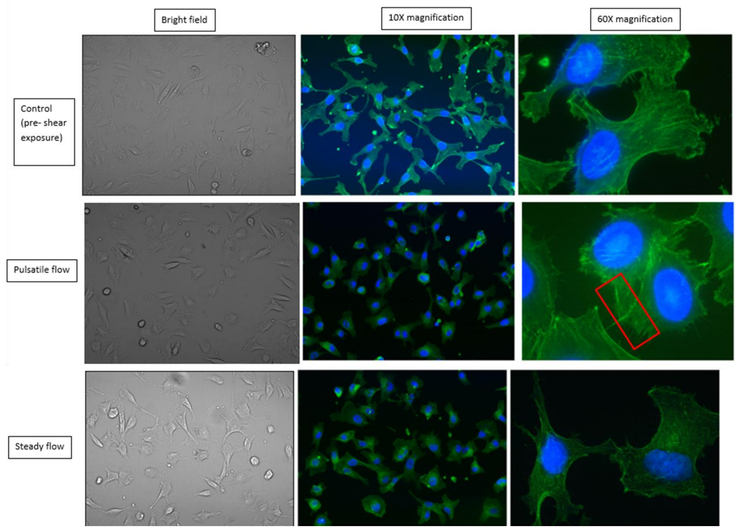


Fig.5. Cytoskeletal images for control, pulsatile and steady flow. Green is actin filaments and the blue represents the cell's nucleus.

PCR & gene expression

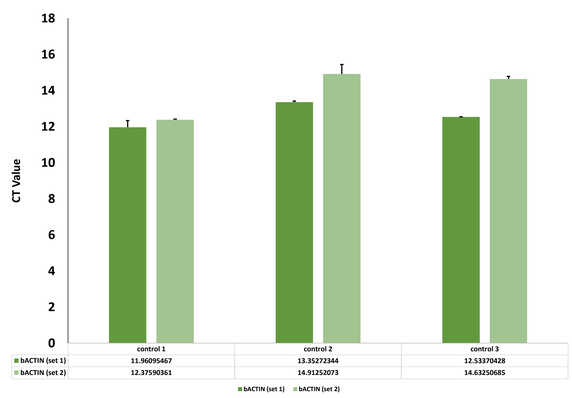


Fig.6: β ACTIN expression is stable among the 3 tested controls. Here, qPCR analysis was conducted on 3 control RNA extracts, each was tested in triplicates. 2 primer sets were used to detect the internal control β ACTIN. The data is presented as mean values of the triplicates, error bars show the SD.

Future Work

Conducting the experiment	✔
RNA extraction	✔
cDNA synthesis	✔
Primers functionality assessment	✔
PCR for flow exposed groups	...

Conclusion

In this project, pulsatile and steady flow were successfully induced and validated. Endothelial cells are mostly affected by mechanical signals, mostly shear stress. The cell's cytoskeleton is responsible for cell shape and integrity. Those proteins are affected by fluid flow. We expect to have a differences in the gene expression of different flow conditions. After we obtain the gene expression data, those will be compared to more precise flow set up (Fluigent pressurized driven pump)

References

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4. Bengtsson, H. and D. Bergqvist, *Ruptured abdominal aortic aneurysm: a population-based study.* J Vasc Surg, 1993. **18**(1): p. 74-80.