Quantifying microfluidics flow separation and comparative analysis of eNOS phosphorylation

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BACKGROUND

- **Atherosclerosis**: characterized by build-up of plaque in arteries and causes Cardiovascular Disease (CVD)
- **Endothelial cells (EC)**: line veins/arteries; sensitive to wall shear stress (WSS)
- Disturbed WSS occurs around curved/bifurcated segments, causes endothelial disfunction
- Laminar WSS occurs around straight segments.
  - Increased eNOS production = NO production = maintains homeostasis

INTRODUCTION

- **Knowns**
  - EC respond to mechanical loading
  - Increased heart rates leads to worsened Atherosclerosis symptoms
- **What we are looking into**
  - Are ECs sensitive to the frequency of loading
- **Objective**
  - Use Microfluidics system to quantify a flow gradient resulting from intercellular interaction between the top of the channel and the cellular layer on the bottom
    - Collagen Coat microfluidics chip to minimize flow gradient
  - Orbital Shaker Model (OSM)
    - Analyze P-eNOS under constant flow
    - Future: Compare to pulsatile flow of Microfluidics system

METHODS

- **Orbital Shaker Flow**
  - Human umbilical vein endothelial cells (HUVECs) are seeded at maximum confluence into a collagen coated six-well plate and subjected to orbital flow at 250 rpm for 24 hours
- **Microfluidics**
  - 10.00 µm Fluoresbrite YG Carboxylate Microspheres (Polysciences) at 1% concentration run through microfluidics system at moderate v. high stear stress velocities
  - Quantifying flow gradient of non-collagen coated v. collagen coated chips
  - Analysis with custom MATLAB code
- **Immunostaining & Image Analysis**
  - Cells stained with P-eNOS (phosphorylation)
  - Cell layers are imaged at center and periphery of each well with a confocal microscope (Zeiss LSM)
  - z-stack images are taken
  - P-eNOS z-stack images processed using custom ImageJ macro
  - Statistical analysis done in ImageJ and EXCEL

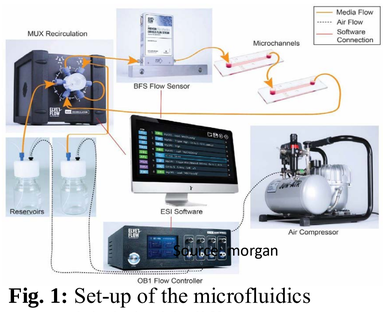

Fig. 1: Set-up of the microfluidics system labeled with different components

RESULTS

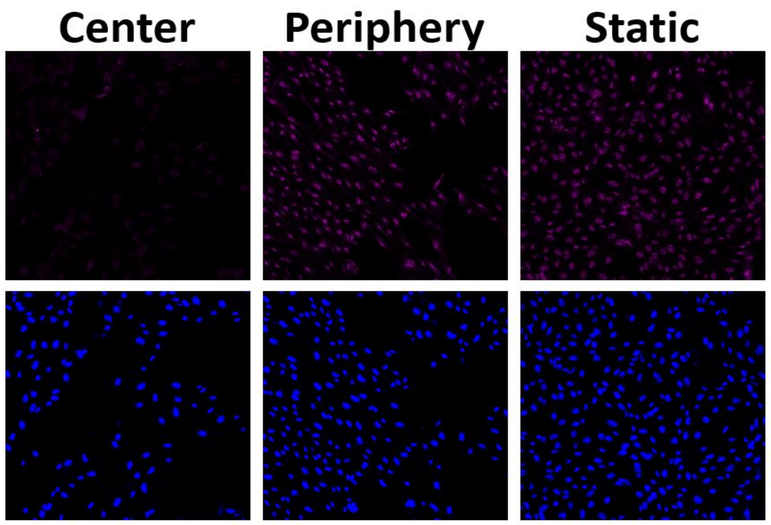

Fig. 2: DAPI (blue) and P-eNOS (pink) images for center, periphery, and static ECs

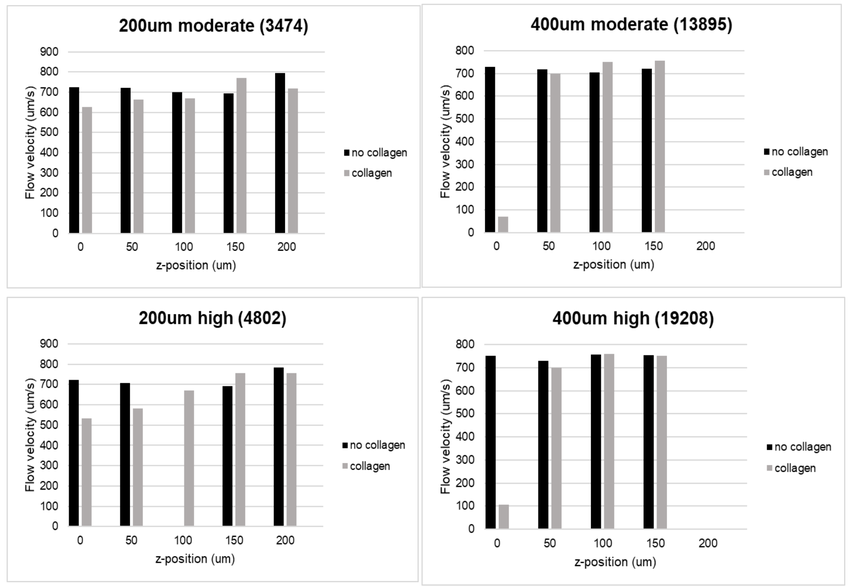

Fig. 3: Change in flow velocity of 1% concentration microspheres across a non-collagen coated chip v. a collagen-coated chip for moderate v. high shear stress

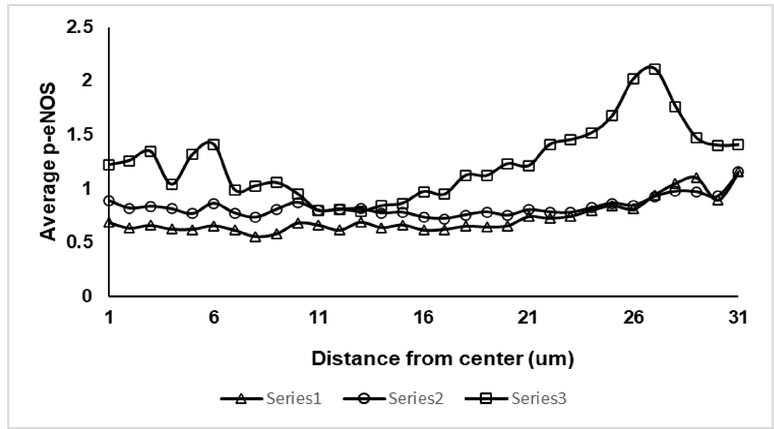

Fig. 4: Change in the P-eNOS averaged against cell density from the center of the well to the periphery after 24 hours of flow at 250 RPM

DISCUSSION

- **Key Findings**
  - Collagen-coating microfluidics channel had no significant quantifiable effect on the flow gradient in the microfluidics chip
    - The particle tracking software we used wasn't effective as a result of debris and other factors
  - Significant increase in P-eNOS brightness at the periphery of the well
    - Fairly constant from the center to right before the periphery
    - Consistent with previous finding of athero-protective markers
- **Future Directions:** Continue to try and minimize the flow gradient so that we can further explore pulsatile flow stimuli on ECs
  - Use new chip to start trials; lower the concentration of microspheres
  - Does pulsatile flow increase restorative effects of laminar flow for ECs?
    - Look at P-eNOS after 24-hr pulsatile flow
    - Develop more beneficial therapies more accurate to the in vivo environment

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