Development of a Method to Culture Endothelial Cells in the Presence of Cyclic Waveforms

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ABSTRACT

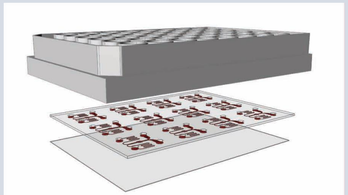
The development of *in vitro* models that allow simulation of *in vivo* conditions is now recognized to be important to studying many disease paradigms, such as hepatic cell biology, cancer biology, and atherosclerosis. In some cases, growing cells *ex vivo* in a 3D culture model is sufficient to maintain tumor markers or other biological markers seen *in vivo*. However, in the case of endothelial cell biology, the ever-present shear force exerted by blood flow is a major factor in gene expression and subsequent pathologies. Here, we describe a method of specialized endothelial cell culture using controlled shear flow to generate cyclic waveforms to mimic athero-prone and athero-protected sites in the vasculature (Dai, G. et al 2004). We grew endothelial cells under different flow regimens for a period of 24 hours. We then evaluated temporal changes in protein expression and morphology during the application of programmed shear and found significant differences in actin localization, cell density, and connexin 37 and 43 expression. This method is extensible to atherosclerosis modeling as well as investigation of other aspects of endothelial cell biology.

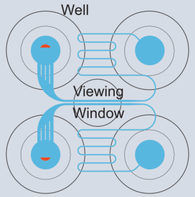
INTRODUCTION

- Atherosclerosis is a leading cause of death worldwide and high-throughput, pulsatile shear devices are required for feasible drug discovery and development
- Compared to static cell culture, endothelial cells display different phenotypes and behaviors when exposed to shear stress
- Current shear devices do not support physiologically relevant pulsatile flow and are low-throughput

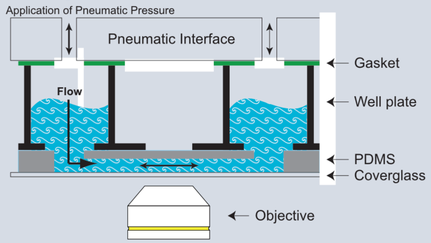
METHODS

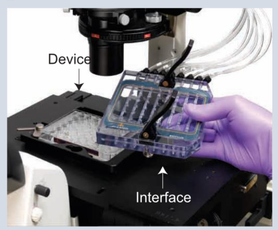
Microfluidic Device for Pulsatile Flow





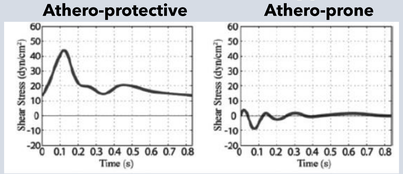
Operation of Microfluidic Device using Pneumatic Pressure

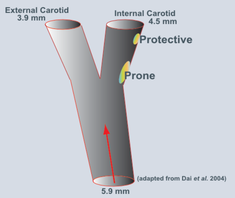


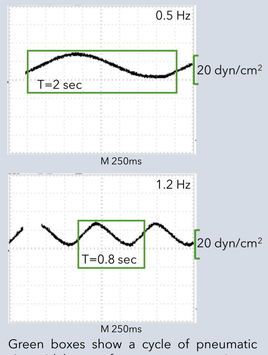


Archetypal Carotid Waveforms

Athero-protective **Athero-prone**







Green boxes show a cycle of pneumatic sinusoidal waveforms at a maximum amplitude of 20 dyn/cm².

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- Mimic *in vivo* conditions
- Enhance the physiological relevance of *in vitro* cell cultures
- Increase cell imaging throughput

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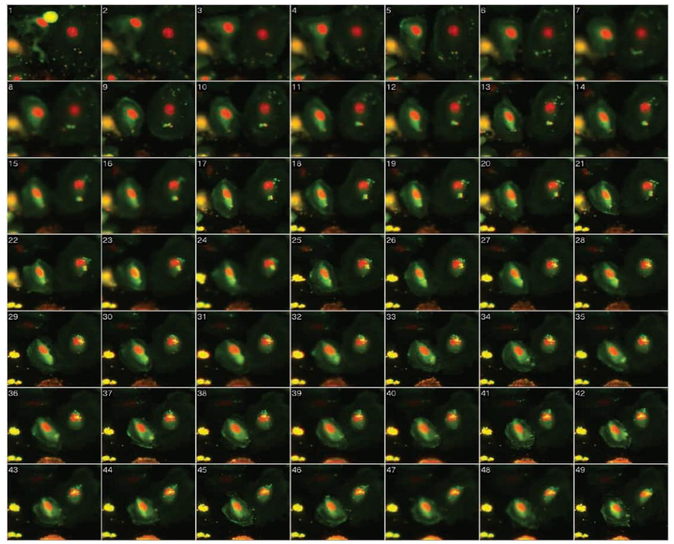
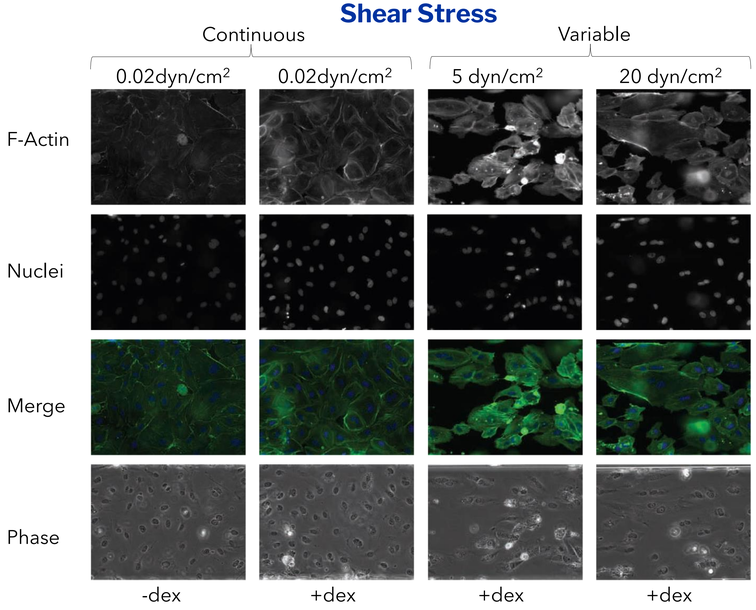
RESULTS

Variable shear changes cell polarity and cytoskeleton

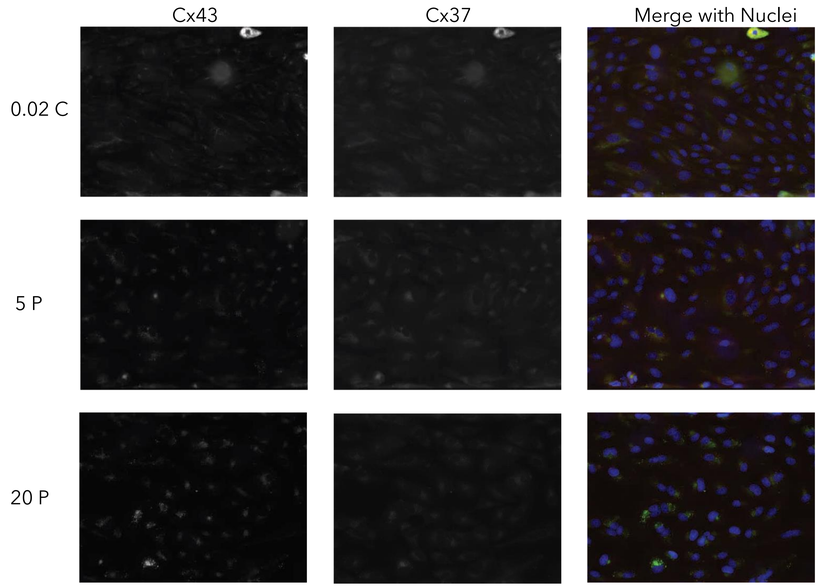


Time-lapse imaging of HUVECs expressing actin-GFP (Green) and NLS-RFP (red) during pulsatile flow (5 dyn/cm2 max amplitude) for 1.2 Hz. Cells began reorientation 1 hour after beginning flow. Pulsatile shear was stopped after 24 hours and cells were stained with phalloidin-Alexa 488. A marked difference was observed in actin polarity and monolayer density. To extend the duration of experiments, high molecular weight dextran was added to channels with high flow magnitudes. Scale bar is 100 microns.

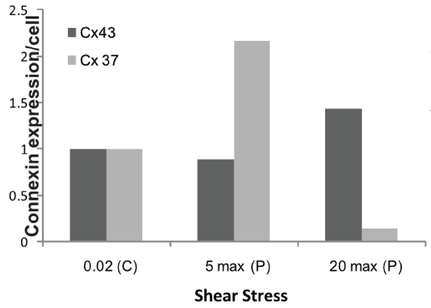
Shear Stress



Connexin expression is altered by variable shear



HUVEC monolayers were subjected to either, continuous shear (0.02 dyn/cm²) (0.02 C), intermittent low pulsatile shear (5 – 0 dyn/cm²) (5 P), or intermittent high pulsatile shear (max amplitude = 20 dyn/cm²) at 1.2 Hz for 24 hours (20 P).



Shear was stopped, cells were fixed, and stained by indirect immunofluorescence for connexin (Cx) 37 and 43 expression. Expression of Cx 43 was upregulated in 20 P and localization was distinctly perinuclear. In contrast, Cx 37 was upregulated in 5 P compared to both gravity flow control and 20 P. This contrasts with the change in Cx expression frequently reported in literature. Raw fluorescence intensity was measured at a set threshold and expressed as a function of total cell number. Intensity was normalized to 0.02 C. Scale bar is 100 microns.

CONCLUSION

- Endothelial cell orientation is altered by shear flow
- Actin polarity and monolayer density of endothelial cells are altered by pulsatile flow
- Continuous and pulsatile shear have a differential impact on endothelial organization
- Connexin expression is upregulated in an intensity-dependent manner in response to shear
- The physiological relevance of many cell cultures can be improved by adding in-vivo like shear flow