INADVERTENT VARIATIONS IN FLUID FLOW ACROSS A PARALLEL PLATE FLOW CHAMBER RESULTS IN NON-UNIFORM GENE EXPRESSION

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INTRODUCTION

Gene expression studies are important for understanding gene regulation, deducing cell signal cascades, *etc*. More broadly, these studies are often used to understand disease development, as specific genes are commonly up regulated in clinical pathologies. These studies can be paired with the application of a mechanical stimulus as well; for example, endothelial cells can be exposed to a defined fluid flow (physiological or pathological) in order to understand the possible mechanisms for atherosclerosis development.

Cyclooxygenase (COX)-2 and endothelial cell nitric oxide synthase (ecNOS) are genes with known responses to physiological fluid shear stress, and are important in the development of atherosclerosis. Specifically, COX-2 is involved in the production of prostacyclin (PGI_2), an inhibitor of platelet aggregation and smooth muscle cell growth and migration [1]. ecNOS catalyzes the reaction of arginine to citrulline along with nitric oxide (NO), which acts as a vasodilator and mimics PGI_2 in other ways, as a product [2].

Such flow-mediated cell responses are commonly studied with the parallel plate chamber, first developed by Frangos *et al*. [3]. In these studies, flow is assumed to be laminar and uniform over the length of the plate. Therefore, all cells in the chamber experience the same conditions regardless of their plate location. Following these flow experiments, cells are collected from the whole chamber; thus gene studies represent an average cell response over the entire plate.
More recently, many research groups have modified the parallel plate flow chamber. We were interested in determining whether experimental flow irregularities caused by slight machining variations from one modified flow chamber to the next would affect sensitive cell responses (such as gene expression) over the chamber area. Therefore, for the first time, this study combined µ-PIV flow measurements and gene expression studies to elucidate flow irregularities following exposure of endothelial cells to fluid flow in a modified parallel plate flow chamber.

MATERIALS AND METHODS

µ-PIV Studies

Velocity fields within the flow chamber were obtained using micro-Particle Image Velocimetry (µ-PIV). The µ-PIV system consists of a pulsed Nd:YAG laser, a fast interline transfer CCD camera, and an epifluorescent microscope which captures two consecutive images of a fluorescent particle seeded flow illuminated by the laser. A 0.00038% concentration of 1 µm diameter seed particles and a 20X microscope lens were used to image a 300x300 µm area within the flow cell. Water (20 °C) was pumped through the chamber by a gear pump at a flow rate of 37.3 ml/min, which corresponds to a shear stress of 10 dynes/cm^2. Five image pairs were taken at each measurement volume within the chamber and temporally averaged to obtain the velocity. *DaVis 6.0* software (developed by *LaVision*) was used to capture and process the image pairs and to average the vector fields. Additionally, the channel height was measured at each PIV measurement location.

Cell Culture

Rat aortic endothelial cells (RAEC) (VEC Technologies) were maintained in MCDB-131 Complete Medium (VEC Technologies). Cells were grown under standard cell culture conditions, that is, a humidified, 37°C, 5% CO_2, 95% air environment. All cells were used at population numbers five through twelve without further characterization.

Exposure of RAEC to Laminar Flow

Prior to fluid flow exposure, endothelial cells were seeded (35,242 cells/cm^2) onto etched glass cover slides previously coated with fibronectin (10 µg/mL; Sigma). Once confluent (approximately 1.5 days), endothelial cells were exposed to laminar fluid flow in a modified version of the parallel plate flow chamber [3] for 6 hours. The shear stress generated in this system was 10.4 dyne/cm^2.

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mRNA Expression by RAEC Exposed to Fluid Flow

Following exposure to fluid flow, sterile, silastic strips were used to divide the glass slides (experimental and control) into six equal regions (top left, top center, top right, bottom left, bottom center, and bottom right). mRNA from endothelial cells in each region was digested with Triazol (Gibco), extracted with chloroform, and precipitated with isopropanol. Reverse transcriptase-polymerase chain reaction was performed using sample RNA and the Ambio Retroscript Kit with primers (20 pmol/μL; Gibco) specific to glyceraldehyde 3-phosphate dehydrogenase (GAPDH), cyclooxygenase-2 (COX-2), or endothelial cell nitric oxide synthase (ecNOS). Electrophoresis of cDNA samples was performed in a gel casting system (Biorad) with 1X TAE buffer for 1.5 hours at 200V. The amount of cDNA in each lane was determined using a phospho imager (Biorad) and densitometry.

RESULTS AND CONCLUSIONS

μ-PIV studies revealed that flow characteristics within a modified parallel plate chamber were location and geometry dependent. Specifically, the region nearest the inlet experienced the overall highest average velocity and the two regions in the center of the plate experienced the overall lowest average velocities. Furthermore, the channel height was found to be the deepest in the regions with the lowest velocities. mRNA expression corresponded with the velocity profiles, providing evidence that cellular responses are not tolerant to these small geometry changes.

Based on results of this study, it is clear that different regions of fluid flow do exist across this modified version of the parallel plate flow chamber; similar variations in other modified flow chambers could affect cell studies where average cell responses are not reported, such as representative cell staining/imaging. We would therefore recommend that custom-built, modified, parallel plate flow chambers are analyzed for flow characteristics across the plate; such data would provide a more exact correlation between shear stress and cell responses.

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