FLUID FORCES MODULATE CYTOKINE STIMULI FOR PERIPHERAL BLOOD MONONUCLEAR CELL ADHESION TO ENDOTHELIAL CELLS

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ABSTRACT

We report using our innovative blood-vessel on chip (BVoC) to manipulate the inflammatory response of endothelial cells (ECs) subjected to simultaneous fluid force and cytokine stimulation. The BVoC presents a microfluidic branching blood vessel analog comprising an endothelial monolayer interfaced with an extracellular matrix (ECM). The BVoC incorporates physiologically valid effects of local fluid dynamics occurring in a blood vessel analog, capturing accurate stagnation pressures at the bifurcation point and shear stress at the branching vessel, in contrast to previous work.

KEYWORDS

Organ-on-chip; blood-vessel-on-chip, inflammation, infectious disease, permeability.

INTRODUCTION

Human immunodeficiency virus (HIV) infection is associated with increased atherosclerotic cardiovascular disease (CVD) risk with the chronic immune activation and inflammation being implicated as a major concern [1, 2]. In particular, plasma levels of interleukin (IL)-6, high-sensitivity C-reactive protein (hs-CRP), D-dimer, and soluble CD14 (sCD14) are independent predictors of mortality, including CVD-related deaths, in people with HIV (PWH). Many "drivers" activate innate immune pathways that result in production of cytokines (TNF, IL-6, IL-1β) that may also contribute to cellular activation and inflammation. These drivers of chronic inflammation likely contribute to altered adhesion molecule expression and migration capabilities of immune cell subsets; thereby promoting atherosclerosis.

Therefore, in this work we investigated using our blood-vessel on chip (BVoC) device to manipulate the inflammatory response of endothelial cells (ECs) subjected to simultaneous fluid force and cytokine stimulation. The BVoC presents a microfluidic branching blood vessel analog comprising an endothelial monolayer interfaced with an extracellular matrix (ECM). The BVoC also incorporates physiologically valid effects of local fluid dynamics occurring in a blood vessel analog, capturing accurate stagnation pressures at the bifurcation point and shear stress at the branching vessel.

To our knowledge, for human aortic endothelial cells (HAECs), this is the first report evaluating changes in peripheral blood mononuclear cell-endothelial cell (PBMC-EC) interaction and PBMC transendothelial migration (TEM) [3] when exposed to simultaneous mechanical and bio-chemical stimuli, critical to cardiovascular disease under a viral insult [4-7]. The adhesion of PBMCs to ECs is critical to the dysregulation of the EC-barrier functions, which directly impacts cardiovascular health. Pro-inflammatory cytokines, tumour necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) disrupt the endothelium and facilitate TEM of monocytes into the arterial intima and induce vascular inflammation [8]. We report an evaluation of the inflammatory response of ECs to physiologically relevant fluid flow and three distinct inflammatory cytokines TNF-α, IL-1β, and lipopolysaccharide (LPS) using our microfluidic device.

MATERIALS AND METHODS

The microfluidic device was fabricated with standard soft lithography of PDMS (polydimethylsiloxane) for a bifurcating channel geometry with a bifurcation point (BP) and two branching vessels (BVs) (Figs. 1A and 1B). Each BV was 500 µm wide x 50 µm deep, separated by a 400 µm wide chamber containing type I rat tail collagen forming the ECM (pH 7.4) [9, 10]. The microchannel was lined with HAECs forming an endothelial barrier (Fig. 1C). In a related study with venous ECs we have reported the protocol to measure endothelial permeability using fluorescent dyes [9]. The same protocol was used here but modified for use with migration of PBMCs. Briefly, HAECs were incubated with 100 ng/mL of LPS or 10 ng/ml of IL-1β or 10 ng/mL of TNF-α, concentrations we have used previously to elicit EC activation, for 24 hours before perfusing carboxyfluorescein succinimidyl ester (CFSE)-labelled PBMCs through the channel. PBMCs were obtained from the fresh blood of healthy male donors. For static control, PBMCs were flushed through the device and incubated for 1 hour at 37˚C. For flow conditions, endothelial growth medium (EGM) containing PBMCs was perfused at 10 µL/min (producing physiologically relevant 38 dyn/cm^2 stagnation pressure and 3 dyn/cm^2 laminar shear stress (LSS)) for 1 hour at 37˚C (Fig. 1D).

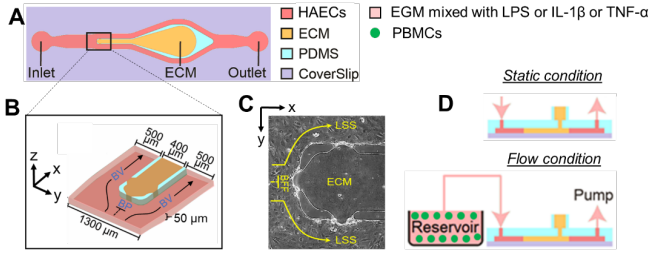

***Figure 1**: (**A**) Schematic of the microfluidic device, depicting the microchannels seeded with HAECs branching around the fibrous ECM. **(B)** Zoomed-in view of the bifurcation region illustrating the apertures at the bifurcation point (BP) and branched vessel (BV). (**C**) Phase-contrast image of HAEC monolayer (Scale bar: 100 µm). (D) Schematic of the experimental setup for "static condition" and "flow condition".*

RESULTS AND DISCUSSION

In a new finding, flow did not influence LPS or TNF-α induced PBMC binding but activated IL-1β for a 2-fold increase in PBMC-EC binding compared to no flow (Fig. 2). PBMC binding correlated to an increased adhesion protein (E-selectin) expression on the EC surface. Increased PBMC binding to ECs might have disrupted the endothelium barrier, initiating PBMC TEM for flow with IL-1β activation (Fig. 3). The binding of PBMCs was more at the BP than the BV for the flow with IL-1β activation suggesting a different mechanism of cell-binding mediated by stagnation pressure as opposed to fluid shear.

978-1-940470-04-7/HH2022/$25©2022TRF
DOI 10.31438/trf.hh2022.77

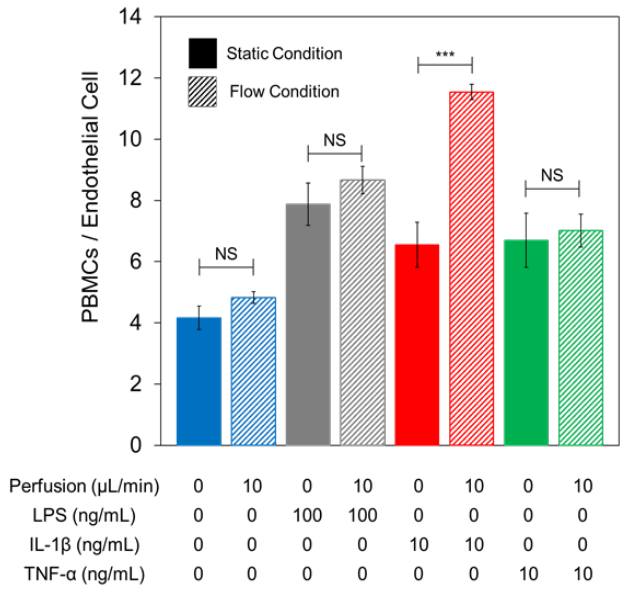

***Figure 2**: Quantitative representation of the number of PBMCs adhering to each endothelial cell for static and 10 µL/min perfusion (flow) condition, both in the absence and presence of LPS (100 ng/ml), IL1-β (10 ng/ml) or TNF-α (10 ng/ml). *** - p<0.001, NS – not significant. Error bars indicate ±SEM (Standard Error of Mean).*

The change in PBMC binding at the BP and BV did not occur for no flow with IL-1β activation. The difference in inflammatory response for IL-1β compared to TNF-α and LPS suggests that IL-1β, which plays a major role in regulating endothelial nitric oxide production, and is one of the major mediators of vasodilation in blood vessels and implicated in cardiovascular health.

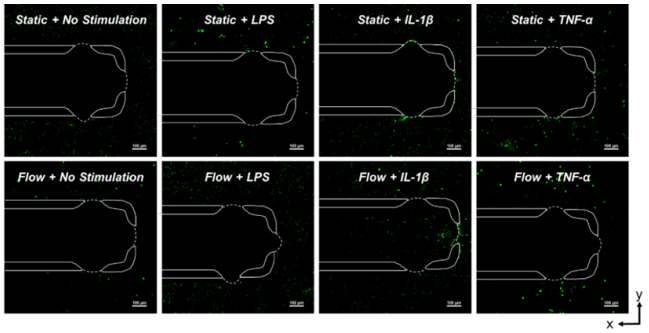

***Figure 3**: Confocal microscopy image showing PBMCs (CFSE tagged, green) transendothelial migration for both static and flow conditions, in the absence and presence of LPS, IL1-β and TNF-α. Scale bars are 100 µm.*

ACKNOWLEDGEMENTS

We acknowledge partial support for this work through the National Institutes of Health (Grant No. R01HL141941), US Army Research Office through grants numbered W911NF-16-0278 and W911-NF-1-0144. The authors acknowledge computational support and research license from the Ohio Supercomputer Center (OSC) and the OSU Nanotech West Cleanroom staff for assistance in fabrication. Images presented in this report were generated using the instruments and services at the Campus Microscopy and Imaging Facility (CMIF), The Ohio State University. This facility is supported in part by grant P30 CA016058, National Cancer Institute, Bethesda, MD. We also acknowledge support from the Ohio State College of Medicine and the Infectious Diseases Institute through their respective seed and bridge grant programs.

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