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Regular Article

The effect of physiologically relevant dynamic shear stress on platelet and endothelial cell activation

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A B S T R A C T

Introduction: Blood flow induced shear stress plays an important role in platelet and endothelial cell functions. The goal of this study was to investigate the effect of physiologically relevant dynamic shear stress on platelet and endothelial cells.

Materials and Methods: Pulsatile shear stress waveforms mimicking the flow in a normal left coronary artery (0.1-1 Pa), at a 60% stenosis (0.2 - 6 Pa) and in the recirculation zone (0.01 - 0.5 Pa) behind a stenosis were used to stimulate platelets and endothelial cells in a cone and plate shearing device. Platelet activation was measured by CD62P expression and thrombogenicity. Meanwhile, endothelial cell activation and damage was measured by cell surface ICAM-1 and tissue factor expression using fluorescence microscopy. Endothelial tissue factor activity was measured using a commercial kit.

Results: Results showed that for platelets, a short exposure to elevated shear stress at the stenosis throat did not induce significant increase in platelet activation or thrombogenicity. While the low pulsatile shear stress had a potential for enhanced thrombosis. Both low and high pulsatile shear stress led to a significant increase in ICAM-1 expression on endothelial cell surface, but only low shear stress caused tissue factor over expression and enhanced tissue factor activity.

Conclusion: These results suggest that low pulsatile shear stress may be more atherogenic, compared to elevated shear stress induced by stenosis.

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1. Introduction

Both vascular endothelial cells (EC) and circulating blood platelets play important roles in thrombosis and atherosclerosis. They both are constantly exposed to dynamic shear stresses induced by blood flow. For endothelial cell functions and activities, it has been reported that unidirectional laminar shear stresses are atheroprotective, while low oscillatory shear stresses can be pro-atherogenic[1–3]. Some *in vitro* studies have shown that shear stress between 1.0 and 1.5 Pa promotes atheroprotective endothelial gene expression, while shear stress below 0.4 Pa leads to an atherogenic phenotype[4,5]. It is also reported that enhanced shear stress can up-regulate intercellular adhesion molecule-1 (ICAM-1), the endothelial activation marker, which assists leukocyte adhesion to the vascular wall[6]. Activated endothelial cells also synthesize tissue factor (TF), a major initiator of blood coagulation, under complex shear stress waveforms[7]. For platelets, both shear stress magnitude and shear exposure time play critical roles in platelet activation[8]. Even though elevated shear stress is more likely to cause acute damage to platelets, elongated exposure to shear stress at any level can lead to platelet activation[9–11]. Many *in vitro* models have been developed to study the effect of altered shear stress induced by disturbed blood flow on endothelial cells and platelets. One common disease model is stenosis, which is often achieved by rigid-walled tubes with constrictions[12,13]. Cone and plate viscometers have also been widely used to establish high shear stress observed in stenosed blood vessels. However, in these models, platelets were usually exposed to elevated shear stress for elongated periods (from a few seconds to a few minutes), which are significantly longer than the time it takes platelets to travel through a stenosed area *in vivo*. For example, it takes platelets approximately 90 seconds to circulate through the whole cardiovascular system, less than 10 seconds to travel in the entire coronary circulation[14], and no more than 0.1 second to pass through a stenosis throat[15]. As platelets pass through a stenosis, it is possible that they can be exposed to shear stress as high as 200 dynes/cm^2 [16]. However, shear stress condition in the stenosed area is not simply high. Due to the pulsatile nature of the blood flow, and the recirculation zone that usually develops past a stenosis, platelets may experience low shear stress. Also, regardless of the shear stress level, the shear conditions are dynamic (or pulsatile), with the pulsatility especially profound in large to medium sized arteries. In the same manner, vascular endothelial cells are continuously exposed to dynamic shear stresses. Dai and Blackman have been working on endothelial cell responses

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under normal shear stress (distal internal carotid artery) and oscillatory shear stress waveforms (carotid sinus) [3,17]. However, effects of physiologically relevant shear stress waveforms on platelet and endothelial cell functional changes have not been thoroughly studied. To investigate the roles of physiologically relevant dynamic shear stress in platelet and endothelial activities will help us to better understand the mechanisms behind thrombosis and atherosclerosis and may provide new therapeutic solutions to these disease conditions.

2. Materials and methods

2.1. Platelets

Fresh platelet rich plasma was purchased from Oklahoma Blood Institute. Washed platelets (250,000/µl) were prepared in HEPES buffered modified Tyrode's solution (HBMT, pH 7.4.) from fresh platelet rich plasma as described before[18].

2.2. Endothelial cells

Human umbilical vein endothelial cells (HUVEC) were purchased from ScienCell Research Laboratories (Carlsbad, CA). HUVEC were grown on 1% gelatin (Sigma-Aldrich) and were maintained in endothelial cell medium (ScienCell), supplemented with 5% fetal bovine serum (FBS), endothelial cell growth factors and 1:100 Penicillin/Streptomycin. HUVEC were used between passages 2 and 5. Human bone marrow microvascular endothelial cells (BMEC) were obtained from Dr. Barbette Weksler at the Department of Hematology and Oncology, Weill Medical College of Cornell University (New York, NY), and cultured in Dulbecco's Modified Eagle's Media (DMEM), supplemented with 5% fetal bovine serum (FBS), 10 mM HEPES, and 1:100 Penicillin/Streptomycin. BMEC were grown on 0.2% gelatin in 6-well culture plates and used between passage 14 and 28[19].

2.3. Antibodies

FITC conjugated monoclonal murine anti-human P-selectin antibody (1:50 dilution in HBMT, Ancell Corporation, Bayport, MN) was used to measure platelet surface P-selectin (CD62P) expression. Endothelial cell surface ICAM-1 (intercellular adhesion molecular 1) was measured using a FITC conjugated monoclonal murine anti-human ICAM-1 antibody (1 µg/ml, Ancell Corporation). Endothelial cell surface tissue factor (TF) expression was measured using a FITC conjugated monoclonal murine anti-human TF antibody (10 µg/ml, Abcam Inc., Cambridge, MA). FITC conjugated MOPC31C antibody (10 µg/ml) (Ancell Corporation) was used as a negative control for all experiments with fluorescence antibodies.

2.4. Shear stress application to platelets and endothelial cells

Three physiologically relevant shear stress waveforms (Fig. 1) were applied to platelets and endothelial cells *in vitro* through a dynamic cone and plate shearing device[18]. The shear conditions mimic that in normal and stenosed left coronary arteries, based on blood flow velocity and local blood vessel geometry, using the results obtained from a computational fluid dynamics method[15]. For platelets (Fig. 1A), under normal conditions, platelets are exposed to pulsatile normal shear stress varying between 0.05 and 1 Pa (1 Pa = 10 dyne/cm^2). With a medium sized stenosis (~60%) present, as platelets go through the stenosis throat, they can be exposed to elevated shear stress as high as 6 Pa for a very short duration of time (< 0.1 sec). As soon as they have passed the narrowed area, platelet shear stress level goes back to normal (0.05 and 1 Pa). The cone and plate shearing device was programmed to generate a narrow shear stress peak at 6 Pa once every 90 seconds, assuming platelets go through the same stenosis throat every pass through the cardiovascular system. For platelets that are trapped in the recirculation zone past the stenosis, they are exposed to low amplitude pulsatile shear stress varying between 0 and 0.4 Pa at all time. The direction of shear stress applied to platelets is always against platelet velocity direction. For vascular wall endothelial cells (Fig. 1B), they are exposed to pulsatile shear stress continuously. Depending on their locations, shear stresses along the cells may vary significantly. Under normal conditions, vascular wall shear stress in coronary arteries varies between 0.05 and 1 Pa; around the stenosis throat cells can be exposed to shear stress varying between 0.1 and 6 Pa. Under these two conditions, endothelial cells are exposed to forward flow, therefore shear stress applied to them is against the major blood flow direction. In the recirculation zone, wall shear stress maintains at a very low level (0 and 0.4 Pa) at all time. Due to the reversed flow condition, shear stress applied to endothelial cells in the recirculation zone is following the major blood flow direction (against the recirculation flow direction, presented as negative shear in the figure).

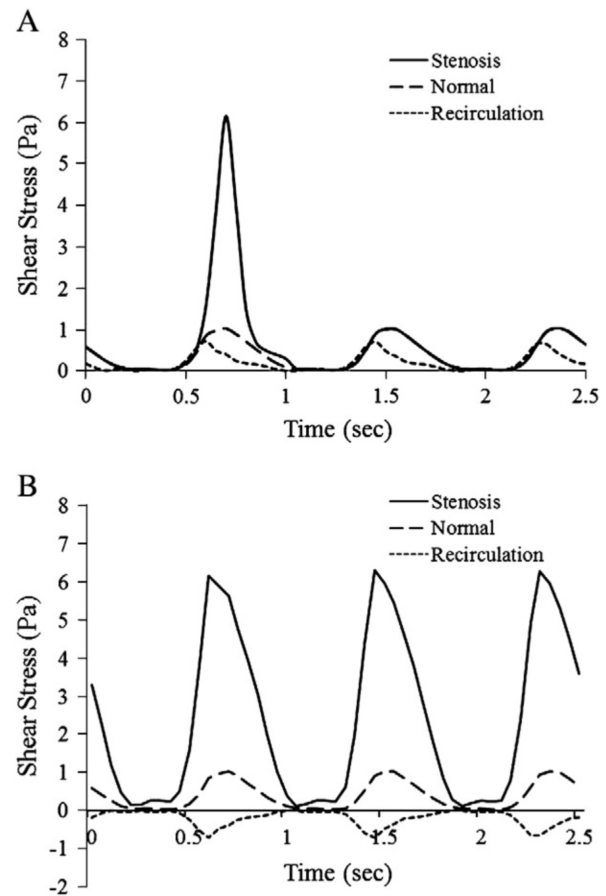

Fig. 1. Shear stress waveforms applied to platelets and endothelial cells *in vitro*, through a dynamic cone and plate shearing device. (A) Shear waveforms that were applied to platelets, representing that under normal, stenosis and recirculation zone conditions. (B) Wall shear stress waveforms applied to endothelial cells, representing that for normal, stenosis and recirculation zone conditions. Note: 1 Pa = 10 dyne/cm^2.

To measure platelet activation induced by shear stress, platelets were exposed to shear stress in the cone and plate shearing device for 30 minutes. Timed samples were taken out every 10 min for thrombogenicity measurement. Platelet surface P-selectin measurement was conducted at the end of each experiment. To assess endothelial cell activation and tissue factor expression induced by shear stress, confluent bone marrow microvascular endothelial cell (BMEC) or HUVEC monolayers were exposed to various shear waveforms in the cone and plate shearing device at 37 °C for 30 minutes. All endothelial cell experiments were conducted immediately after shear exposure.

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