

Platelet Adhesion

Platelet aggregation assays under controlled shear flow

Introduction

Platelet aggregation occurs in response to vascular injury where the extracellular matrix below the endothelium has been exposed. Platelets also play a central role in the formation of deadly clot formation, making them a desirable target for therapeutic intervention. Conventional screening techniques have typically focused on platelet aggregation in static fluid environments, such as well plates or aggregometer tubes. It is now widely recognized that platelets become activated in response to shear stress. Setting up a physiologically-relevant *in vitro* model should therefore incorporate the application of shear stress in the experimental protocol.

The BioFlux system (Figure 1) consists of a well-plate with an integrated array of microfluidic flow chambers (Figure 2) in which controlled-shear experiments may be performed simultaneously. Undiluted whole-blood assays can be run in a self-contained well-plate format, which obviates the need for pre-treatment and cleanup of tubing often associated with flow experiments. BioFlux plates are available in a variety of shear ranges to model appropriate conditions for aggregation. Here, we show platelet binding to two different ligands, collagen I and von Willebrand factor, and a dose-response assay to an anti-glycoprotein (GP) IIb/IIIa monoclonal antibody in the BioFlux microfluidic well-plate.

Methods

Whole blood treated with Sodium Citrate was obtained from a male donor free from aspirin and other anti-thrombotic medications as a reagent from Lampire Biological Laboratories (Pipersville, PA). Whole blood was labeled with Calcein AM (Invitrogen, Carlsbad, CA) at a 1/1000 v/v dilution (4 μ M final concentration) for 1 hour at room temperature. Calcein AM treatment does not affect platelet function (Kuwahara M, et al 2008). Human von Willebrand factor (Haematologic Technologies, LTD, Essex Junction, VT) and Collagen I (Invitrogen, Carlsbad, CA) were used to coat individual microfluidic channels at 100 and 200 μ g/mL in PBS respectively for 1 hour.

For dose response studies, anti-GPIIb/IIIa mouse monoclonal (Abcam, Cambridge, MA) was added to the whole blood with the Calcein. Channels were blocked with 0.5% v/v BSA for 10 minutes in PBS prior to the addition of the labeled blood to the wells. For vWF binding, whole blood was added to the waste wells and perfused at 10 then 20 dyn/cm² for 10 minutes at each shear. For collagen, whole blood was perfused from the waste wells at 10 dyn/cm² (significant clot formation precluded a second shear rate). Fluorescent micrographs were captured with the blood under flow using an inverted microscope (Nikon TS100), CCD camera (QICam, QImaging) and the BioFlux software. Intensity measurements were generated using the BioFlux software. Data were plotted using GraphPad Prism 5 (Graph Pad Software, LaJolla, CA).



Figure 1: The BioFlux System for live cell assays under controlled shear flow.

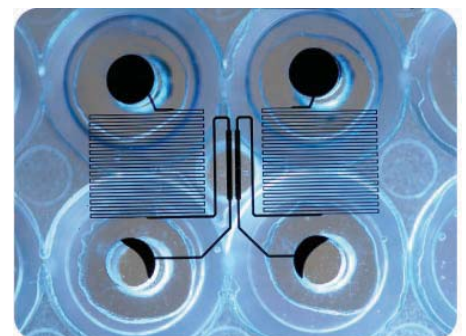


Figure 2: BioFlux Plate channels as viewed from beneath the well plate. Microfluidic flow cells are integrated into the bottom of an SBS-standard well plate. Each fluidic channel runs between pairs of wells and has a central viewing window for observation.

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Results

Platelets exposed to von Willebrand (vWF) factor were observed to roll and attach to the substrate under shear flow. The attachment was dependent upon shear, as platelets were observed to detach rapidly when shear was interrupted. Platelet aggregation was not observed at either 10 or 20 dyn/cm². No dose response at any concentration was observed (as expected) with the anti-GPIIb/IIIa monoclonal antibody. The GPIIb/IIIa glycoprotein is not involved in the interaction between platelets and vWF (Figure 3).

When whole blood was perfused over collagen, clot formation was observed within the first minute of perfusion (Figure 3). Clots were localized to the periphery of the channels. Inhibition of clot formation was observed with an IC₅₀ for this particular antibody at 17nM (Figure 4).

Conclusion

We demonstrated platelet adhesion and platelet aggregation in the BioFlux system. We found that platelets attached to vWF under shear but the attachment was reversible at the shear tested. Adhesion to collagen led to rapid thrombus formation which was inhibited in a dose-dependent manner with an anti-GPIIb/IIIa antibody.

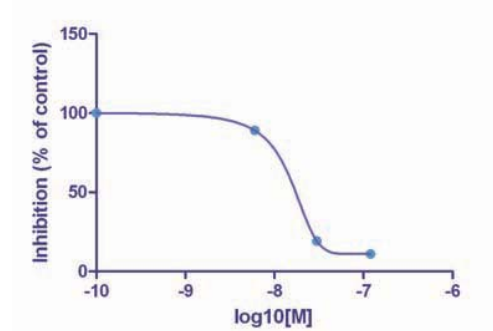


Figure 4: Inhibition of platelet aggregation with an anti-GPIIb/IIIa monoclonal antibody. Platelet aggregation was inhibited in a dose-dependent manner on collagen I at 10 dyn/cm². Inhibition was calculated from the total fluorescence intensity in pixels for each channel and expressed as a percentage over the intensity of the no antibody control. The IC₅₀ was 17nM.

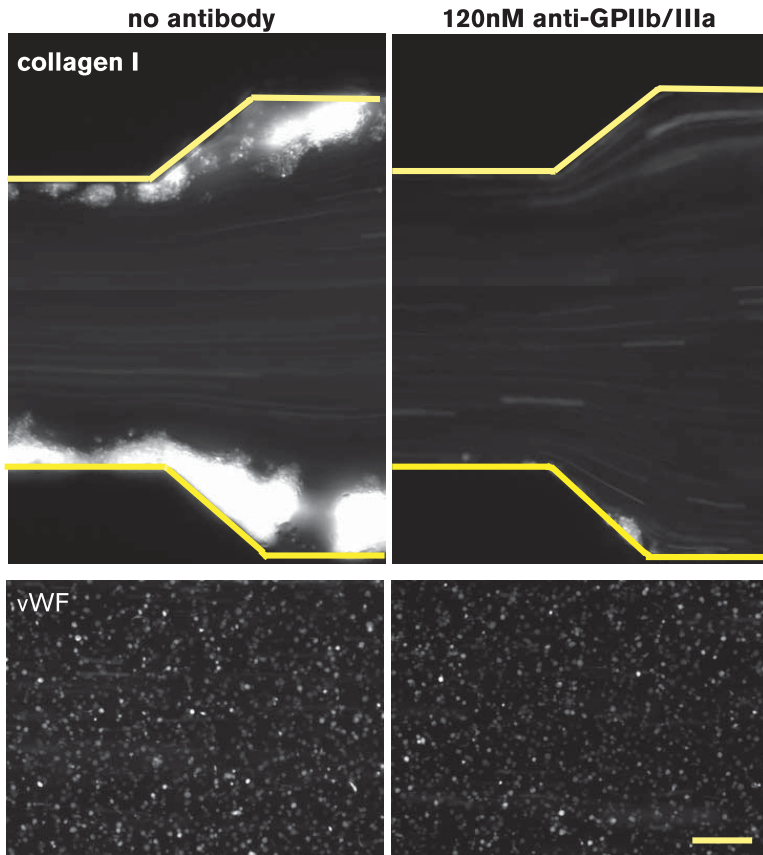


Figure 3. Platelet Adhesion and Aggregation in the BioFlux plate. Calcein AM labeled whole blood treated with anti-GPIIb/IIIa monoclonal antibody was perfused over either collagen I (top panels) or vWF (bottom panels) under shear flow. Platelet aggregation was observed with collagen but not with vWF under these conditions. Scale bars = 50 microns.



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