Dual Effects of Shear Rates and Platelet Therapy Dosage on Thrombosis in a Microfluidic System
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Introduction: Thrombosis is the pathological formation of platelet aggregates that occlude blood flow causing strokes and heart attacks—leading causes of death in developed nations. Dosing platelet therapies for thrombosis prevention is a persistent problem—studies estimate up to 45% of patients still undergo adverse cardiovascular events for unknown causes. One proposed cause for altered efficacy is local flow conditions including increased shear rate and vascular constriction (stenosis) [1]. However, there is presently no instrumentation able to simultaneously examine therapeutic efficacy at a wide range of fluid shear rates from physiological (500-1500 s⁻¹) to pathological levels typical in vascular stenoses (>4000 s⁻¹). Thus, in previous work, we developed a microfluidic system for simultaneous measurement of platelet aggregation at multiple initial wall shear rates within multiple stenotic channels in label-free whole blood [2]. In this work, we apply the same microfluidic system to simultaneously evaluate effects of both shear rates and two different types of platelet therapy (eptifibatide and aspirin) on platelet thrombosis.

Materials and Methods: Blood samples were collected from healthy subjects in accordance with Georgia Tech’s Institutional Review Board and anti-coagulated with 3.5 Units/L heparin. Platelet therapies eptifibatide (Integrilin ®) or aspirin were added at dosage concentrations of 0 to 2.4 μM, or 0 to 2 mM, respectively, to separate aliquots of each subject’s sample. We then tested each aliquot with the microfluidic system to simultaneously evaluate thrombosis at initial wall shear rates of 500, 1500, 4000, 10000 s⁻¹. Platelet thrombosis to occlusion was measured by flow rates Q(t). Occlusion time, t_{occlusion}, was defined by the time (in seconds) when Q(t) = 0, and channels which did not occlude within 1 hour of observation were considered “non-occluded”. Results were analyzed for significance using a Cox survival model in SAS.

Results and Discussion: Results are depicted as 1/t_{occlusion}, whose value decreases as occlusion times are prolonged (indicating decreasing platelet activity) until reaching zero (“non-occluded” trial). Data pooled from N=5 subjects (bars indicate standard error) was plotted as dose-response curves for each tested shear rate (Fig. 1). Results from both eptifibatide and aspirin showed that all shear rate dose-response curves were significantly different (p<0.01) from eachother, save for the low shear (500 vs. 1500 s⁻¹) comparison. Increasing eptifibatide prolonged occlusion until its elimination at 1.2 μM (0.5X clinical dosage) and above. In contrast, aspirin dosages of 0.36 mM and greater eliminated occlusion at only low shear (500, 1500 s⁻¹), but not at high shear rates (4000, 10000 s⁻¹), even at very high concentrations (20X clinical dosage). Our results are consistent with clinical reports of reduced benefit or no benefit of aspirin therapy in subjects with high degrees of arterial stenosis [3].

![Dose-response curves at varying shear rates treated with eptifibatide (a) or aspirin (b). In both drug models, dose-response curves were significantly (p<0.01) different from eachother at all shear rates save for the 500 vs 1500 s⁻¹ comparison. Eptifibatide dosages (a) eliminates thrombosis at 1.2μM, while aspirin dosages (b) do not eliminate thrombosis at high shear rates (4000, 10000 s⁻¹).](image)

Conclusions: Measurements of thrombosis at multiple shear rates and platelet therapies using a microfluidic system show that physiological vs. pathological shear rates have significant effects on dose-response efficacy of eptifibatide and aspirin. While eptifibatide eliminated thrombosis at clinical dosages for all shear rates, aspirin was unable to eliminate high shear thrombosis even at very high dosages.

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References: