

INTERACTION BETWEEN POSITIVE AND NEGATIVE FEEDBACK IN PLATELET ADHESION ON AN IMPROVED MICRO CHANNEL PATTERNED WITH THROMBOGENIC AND NON-THROMBOGENIC REGIONS

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ABSTRACT

Models are being developed for platelet-mediated thrombogenesis, but these models consider the effects of platelet activators and inhibitors separately. The interactions between these two types of agents depend on the spatial and temporal relationships between inhibitors such as nitric oxide (NO) and activators such as adenosine diphosphate (ADP). The effects of positive and negative feedback signals acting at various locations are the main controlling factors of the final thrombus size. To analyze these effects, positive and negative feedback effects were examined simultaneously on regions in which thrombus formation is active next to regions in which thrombus is prevented. This configuration allows platelet derived activators and inhibitors in one region to influence platelets in a different region under varying shear conditions. The percent surface area coverage is used as a measure of thrombus formation.

To study this interaction, we have used a flow system in which the lower surfaces of microchannels are patterned with thrombogenic regions of fibrinogen surrounded by non-thrombogenic regions that are covered with bovine serum albumin. Platelets were extracted from bovine whole blood and labeled with carboxyfluorescein succinimidyl ester (CFSE). The CFSE-labeled platelet rich plasma was perfused through the microchannel under varying conditions. Microchannels were imaged using a florescence microscope and then processed with a MATLAB program to determine percent surface area coverage. This process was repeated under various experiments with L-arginine (L-A) and NG-Methyl-L-arginine acetate salt (LNMMA) added to the CFSE-labeled platelet rich plasma. L-arginine is a requirement for production of nitric oxide, and LNMMA competes with L-arginine, thus leading to less nitric oxide production.

Experiments were completed to investigate the effects of shear rates, and concentrations of L-A and LNMMA on the percent surface area coverage in the microchannel developed in our lab. At a shear rate of 500 s^{-1} the amount of adhesion with L-arginine was similar to adding no chemical, but the amount of adhesion was increased by a factor of ten or more when LNMMA was added. At a shear rate of 1500 s^{-1} the amount of adhesion with LNMMA was similar to adding no chemical. The effect of 80 μL added L-arginine was not statistically significant, but 40 μL added L-arginine was statistically significant and decreased adhesion by a factor of ten. With LNMMA, the upstream region exhibited more adhesion than the middle or downstream region. Differences between the three regions were not significant for the L-arginine case and the case of no chemicals added.

Keywords : CFSE, L-arginine, LNMMA, Nitric Oxide, Downstream, Upstream, Microchannel, Platelet rich plasma(PRP), Fibrinogen, Bovine whole blood, Platelet adhesion.