Pairwise agonist screening and microfluidics reveal platelet function phenotype
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In this study we consider the relationship between two high-throughput approaches designed to investigate platelet activation as it pertains to calcium mobilization and aggregation. In Chatterjee et al. (2010) the authors demonstrate that calcium responses to a variety of platelet agonists in various combinations can serve as a platelet function phenotype in an assay called Pairwise Agonist Scanning (PAS). We believe that this technique represents a powerful diagnostic tool and we have supported that claim through the use of a microfluidic focal adhesion model. Using PAS data collected for 3 healthy donors we have successfully predicted the relative response to the anti-platelet agent Indomethacin, a non-selective COX inhibitor.

Whole blood, anticoagulated with PPACK (100 μM), was treated with Indomethacin (140 μM), Iloprost (a stable prostacyclin analog that globally inhibits platelet activation, 1 μM), both agents together, or was untreated (control). Each condition was loaded in duplicate into a separate inlet of an 8 channel microfluidic device with a single outlet. The samples were perfused over a fibrillar collagen surface at a shear rate of 200 s^{-1} for 8 min. Platelet accumulation at the collagen strip was measured in each channel simultaneously in 15 sec intervals. To perform the PAS study platelet rich plasma from the same blood draw was loaded with a calcium sensitive dye and Indomethacin (to prevent autocrine activation via TXA_2). Samples were treated with all pairwise combinations of 4 concentrations (zero, low, medium, high) of the platelet agonists convulxin (a collagen mimetic), ADP, and U46619 (a stable TXA_2 mimetic), in the presence or absence of Iloprost. Platelet calcium mobilization was measured via fluorescence for 200 sec.

The platelet collagen response was recorded for 4 trials per donor on two separate days, with corresponding PAS data. While Iloprost was effective for all donors (mean aggregation inhibition of 70%), Indomethacin showed effectiveness only for donors 1 and 2 (35% and 20%, respectively), and caused a significant increase in platelet accumulation for donor 3 (20%). Platelet calcium mobilization in response to U46619 alone illustrated a reduced sensitivity to the TXA_2 analog for donor 2 as compared to donor 1 (~44% reduction in Ca^{2+} mobilization) and for donor 3 as compared to donor 2 (~80% reduction). In order to confirm an activating role for high doses of Indomethacin, the study was repeated at 14 μM Indomethacin. At this concentration donor 1 showed a 50% reduction in platelet adhesion, donor 2 showed a 34% reduction, while donor 3 showed at 13% reduction. PAS data was not affected by the change in Indomethacin concentration. Furthermore, we have observed a 2-fold increase in platelet aggregation for donor 1 as compared to donors 2 and 3 in the control condition. We believe this result is reflective of a universal increase in Ca^{2+} mobilization as measured under all conditions in the PAS output.

These data reveal that donor sensitivity to anti-platelet agents can be predicted using the PAS technique and confirmed in a microfluidic model of platelet adhesion. The combination of these methods holds promise as a novel diagnostic tool and a method for evaluating anti-platelet therapy ex vivo.

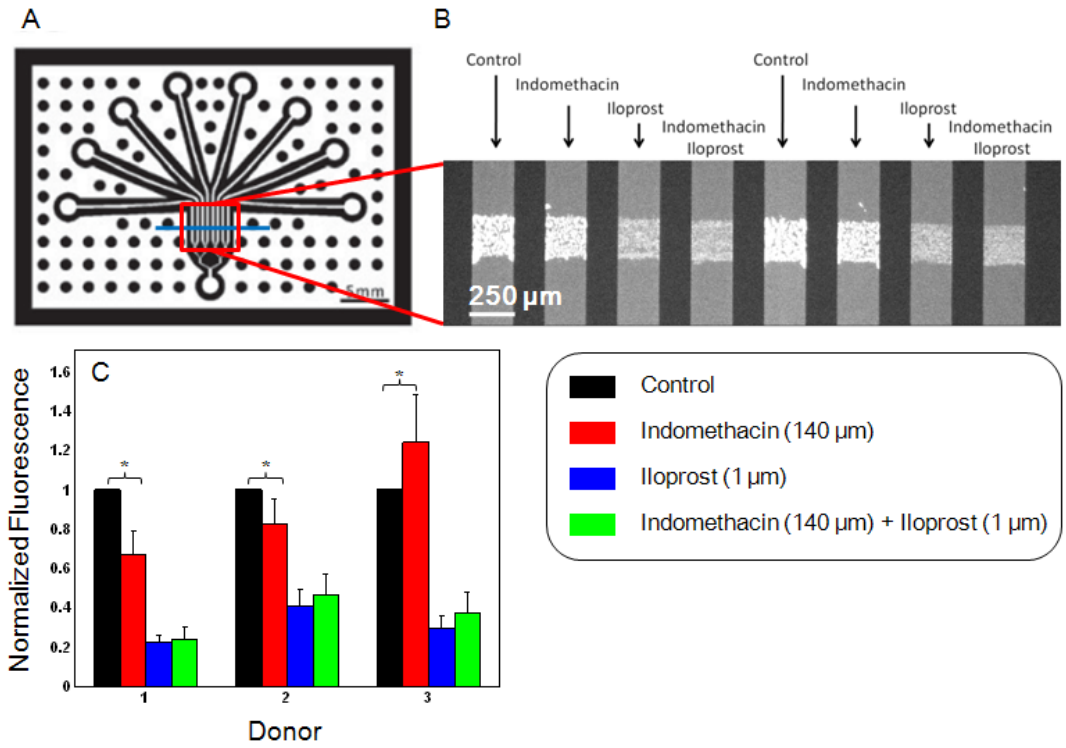

Figure 1 (A) Schematic of the 8 channel microfluidic device. 8 separate channels converge on one patterned collagen strip (blue) that can be imaged in a 2x field of view. **(B)** Whole blood (PPACK 100 µM), treated with the indicated anti-platelet agents, is perfused at 200 s^{-1} and platelet accumulation on the collagen surface is measured in 15 sec intervals. **(C)** Normalized platelet accumulation for three separate donors (n=16). Iloprost shows effectiveness for all 3 donors while Indomethacin shows effectiveness for donors 1 and 2 and an activating effect for donor 3. (* $p < .01$)