Contractile Force of Platelet Aggregates Formed Under Shear Flow Reflects VWF Concentration and ADAMTS13 Activity

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I. Introduction

- Disruptions to hemostasis, specifically related to the balance of von Willebrand Factor (VWF) and ADAMTS13, can cause life-threatening bleeding or thrombi.
- Platelet forces may be a useful metric for assessing platelet function and monitoring thrombotic or bleeding risk.
- We have developed a microfluidic device, with force sensors embedded in the channel, that induces the adhesion and aggregation of platelets under shear flow. [1]
- In preliminary studies, we have found that the formation of platelet-rich thrombi in our microfluidic device is highly sensitive to VWF and ADAMTS13.

II. Approach

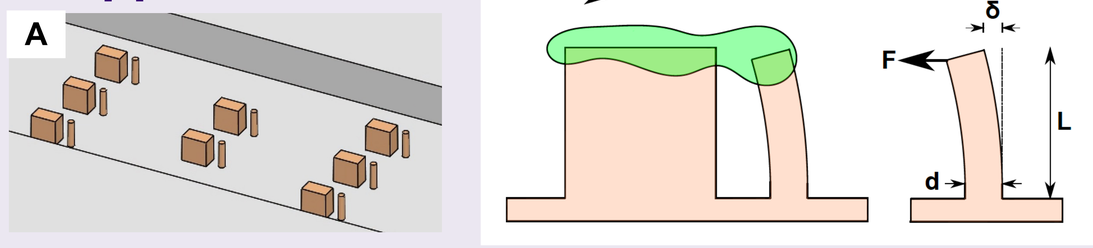

Figure 1. The microfluidic device. ***(A)*** *Each microchannel contains discrete force sensors comprised of a rigid block to create a high shear gradient to activate platelets and a flexible post that deflects in response to platelet (shown in green) forces.* ***(B)*** *Post deflection (δ) was measured to calculate platelet-plug contractile force (F) using Hooke's Law (F = kδ) where* $k = 3\pi E d^4/64L^3$ *, E is the modulus of elasticity, d is the diameter of the post, and L is the length of the post.[2]*

III. Results

Platelet-plug formation is dependent on VWF concentration in a microfluidic device.

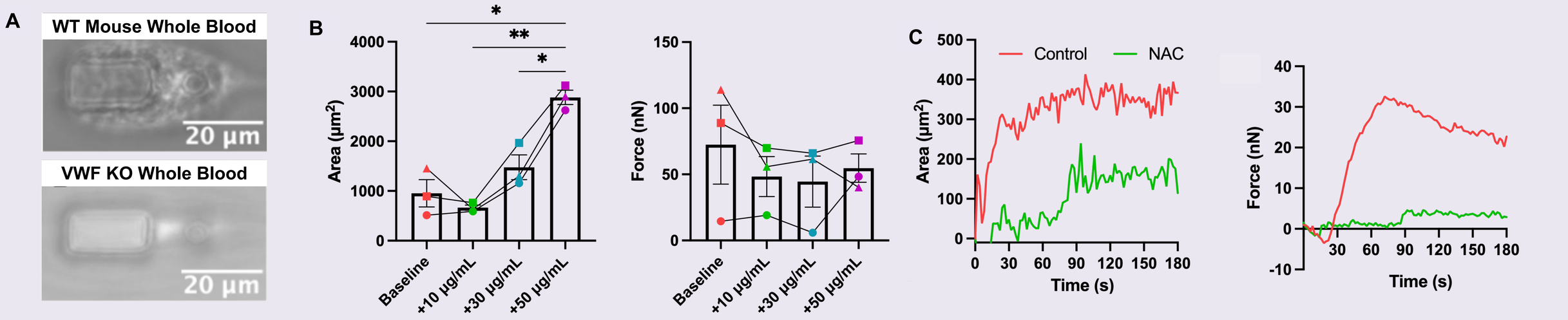

Figure 2. Platelet-plug formation is dependent on VWF concentration. ***(A)*** *In comparison to WT, blood from a VWF KO mouse was unable to form a plug.* ***(B)*** *For whole blood doped with VWF, plug area increased significantly at +50 µg/mL from baseline 120 seconds after blood entered the channel; however, there was no significant difference in contractile force. The asterisk denotes p < 0.05 and double-asterisk denotes p < 0.005, one-way ANOVA with post hoc.* ***(C)*** *N-acetylcysteine (NAC) reduces VWF multimers, which decreases platelet-plug area and contractile force.*

Platelet-plug formation is dependent on ADAMTS13 activity in a microfluidic device.

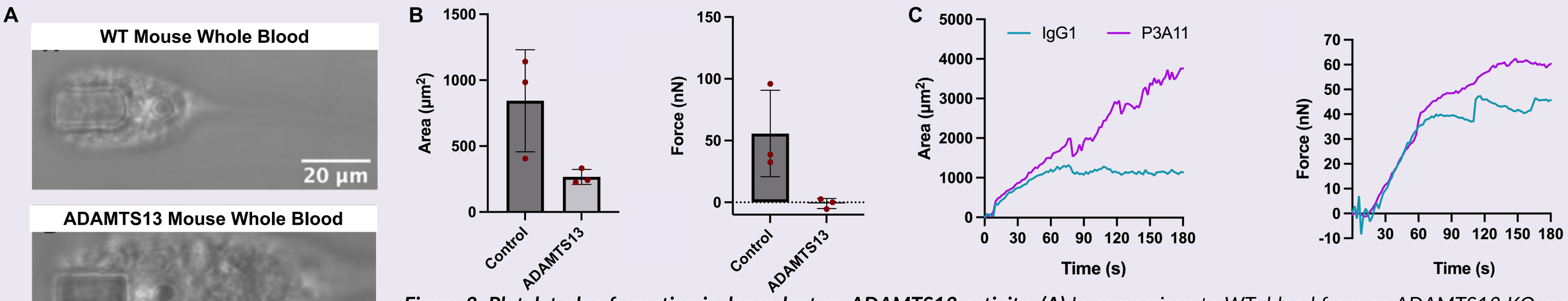

Figure 3. Platelet-plug formation is dependent on ADAMTS13 activity. ***(A)*** *In comparison to WT, blood from an ADAMTS13 KO mouse formed a large plug.* ***(B)*** *For whole blood doped with ADAMTS13, platelet-plug area and contractile force decreased from the healthy control 120 seconds after blood entered the channel.* ***(C)*** *Similarly, Adding an ADAMTS13 antibody (P3A11) into whole blood caused decreased platelet-plug area and contractile force.*

VWF counteracts the effect of antiplatelet drugs on platelet-plug contractile force.

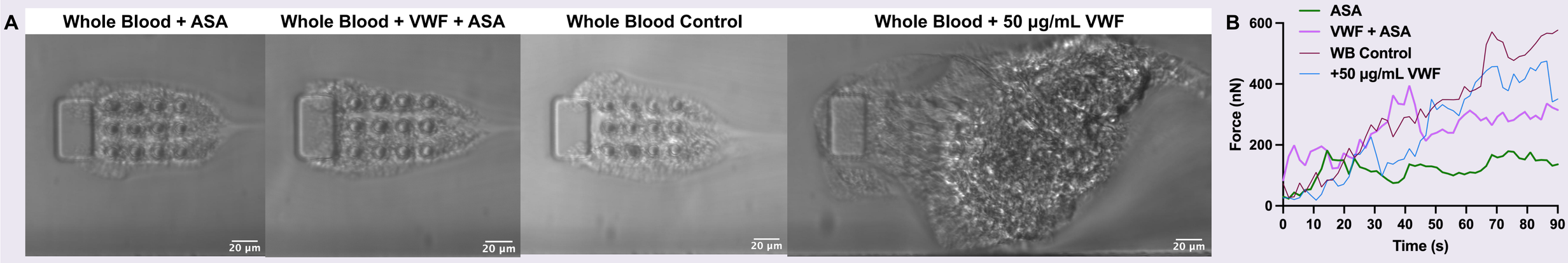

Figure 4. A thromboxane inhibitor, acetylsalicylic acid (ASA), reduces platelet-plug contractile force less significantly in VWF doped blood. ***(A)*** *Representative images of platelet-plugs 90 seconds after blood enters the channel for whole blood (WB) + ASA, WB + 50 µg/mL VWF + ASA, whole blood, WB +50 µg/mL VWF.* ***(B)*** *Platelet-plug contractile force vs. time.*

Future Work

- Evaluate platelet-plug forces for VWF doping and antiplatelet conditions for more donors.
- Assess plug stability by tracking the movement of individual platelets.
- Determine if platelet forces correlate with the degree of platelet activation using a p-selectin antibody and calcium imaging.

Reference / Bibliography

1. Ting et al., *Blood*, 2019
2. Miles et al., *Blood Advances*, 2021

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Funding

Research reported in this publication was supported by the National Heart, Lung, And Blood Institute of the National Institutes of Health under Award Number F31HL156697. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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