Force Generation and Cytoskeletal Structure of Single Platelets

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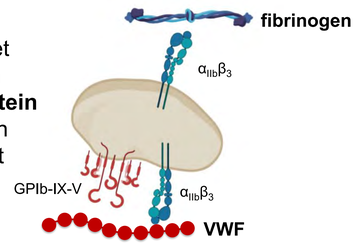
Introduction

Fibrinogen and **von Willebrand Factor** are key blood proteins that mediate platelet adhesion. These proteins both contain **RGD**, an adhesive ligand that binds **integrin $\alpha_{IIb}\beta_3$**, and VWF additionally contains the **A1 domain that binds platelet glycoprotein Ib (GPIb)**. This difference in adhesive ligands on fibrinogen versus VWF provides an opportunity to investigate how GPIb mediates changes in the actin cytoskeleton that drives platelet shape, spreading, and contraction.



Results

Platelet F-actin morphology differs on fibrinogen and VWF and is detectable via machine learning

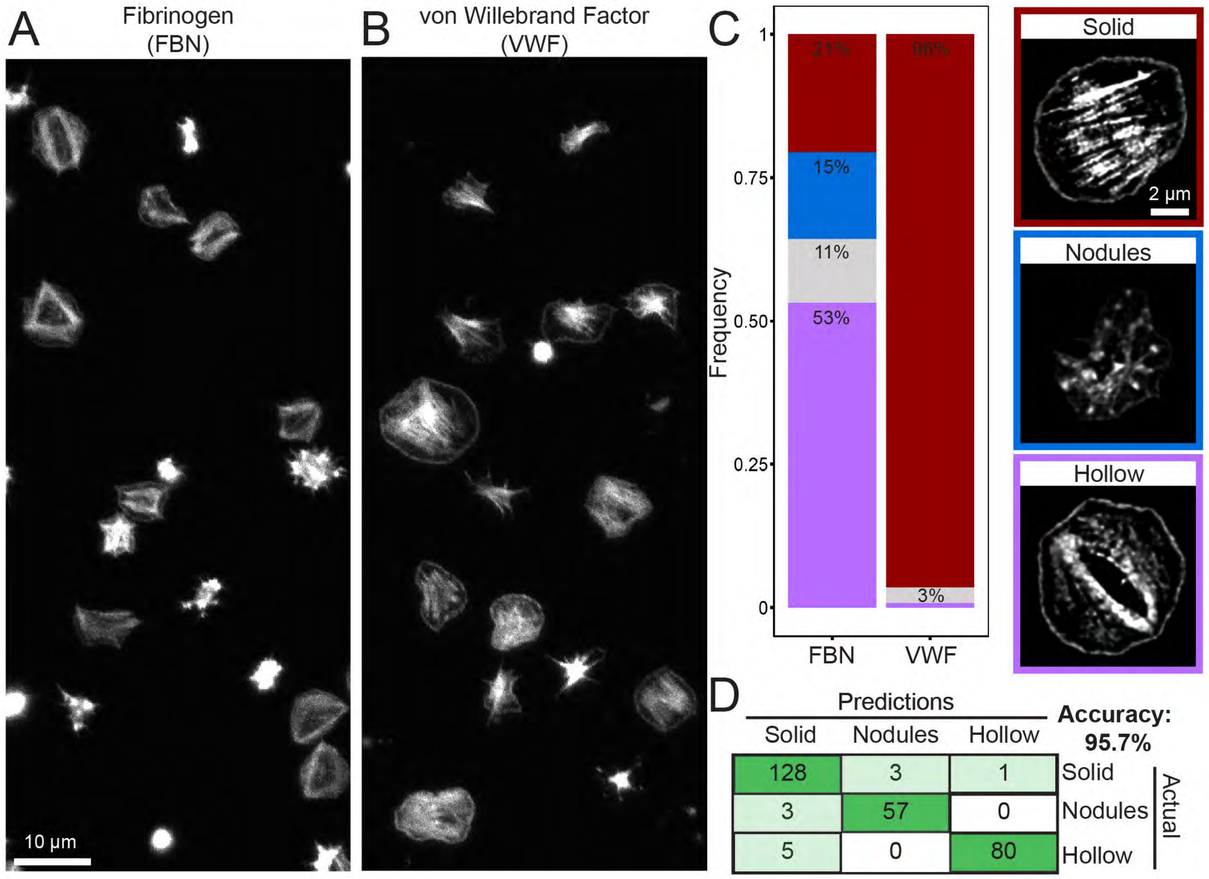


Figure 1. (A) F-actin of platelets on a fibrinogen (FBN)-coated coverslip show platelets with nodules or hollow structure while **(B)** platelets on von Willebrand Factor do not. **(C)** Frequencies of manually classified F-actin morphologies are significantly different ($p < 0.0001$, Pearson's Chi-squared test) and superresolution microscopy was used to observe these morphologies in higher resolution. **(D)** To reduce user bias and increase yield, the manual classifications were used to train a machine learning model, which predicts the F-actin morphology with 95.7% accuracy.

Black dots to co-measure single platelet forces and cell markers

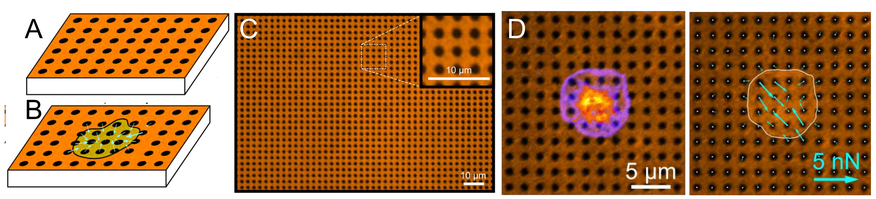


Figure 2. **(A)** Black dots is a fluorescent pattern on a flexible polydimethylsiloxane (PDMS) surface. Without cells, the pattern is undisturbed. **(B)** With cells, pattern is displaced by cell forces. **(C)** The pattern is fabricated by microcontact printing fluorescent-bovine serum albumin. **(D)** Black dots is compatible with fixing and staining, making it possible to co-measure F-actin morphology (left) and traction forces (blue arrows on right).

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Results

Platelets on VWF generate more force and more centrally localized force than platelets on fibrinogen

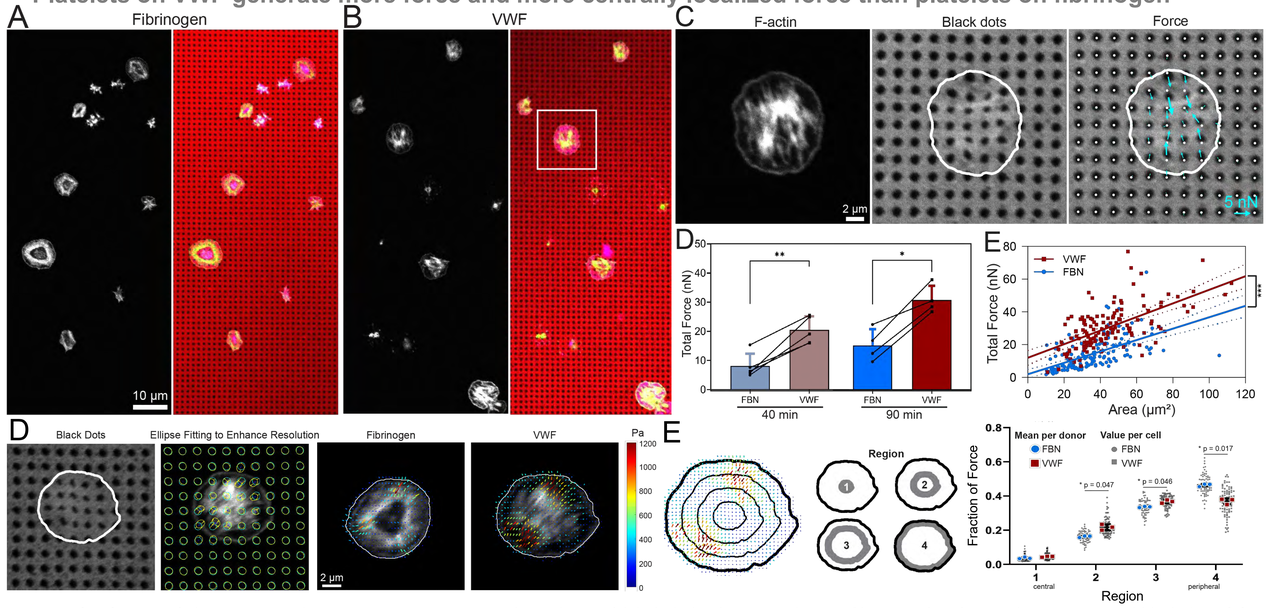

Figure 3. (A-B) F-actin (white on monochromatic image, green on colorized image)-labeled platelets spreading on fibrinogen(FBN)-coated or VWF-coated black dots. **(C)** Inset white box from B. The dots underneath a platelet are deformed by forces generated by the platelet. Forces (blue arrows) are calculated from the displacement of the dots from their undeformed centroids (white dot). **(D)** Average total platelet force per donor is significantly higher on VWF than FBN at 40 minutes ($p < 0.01$, paired t-test) and 90 mins ($p < 0.05$). Lines between dots indicate the same donor. Error bars are standard deviation. **(E)** Platelets on VWF produce more force per area than platelets on FBN ($p < 0.001$) when tested with an analysis of covariance. **(D)** Calculation of the deformations was improved upon by fitting ellipses (yellow) to the deformed dots, resulting in four displacement vectors per black dot (yellow arrows) instead of one displacement vector at the dot centroid (blue arrows). Higher resolution force maps show high forces at the ends of F-actin fibers. **(E)** Forces on VWF are significantly more central and forces on FBN are significantly more peripheral.

Next steps

Preliminary results examining whether these changes are due to GPIb mechanotransduction

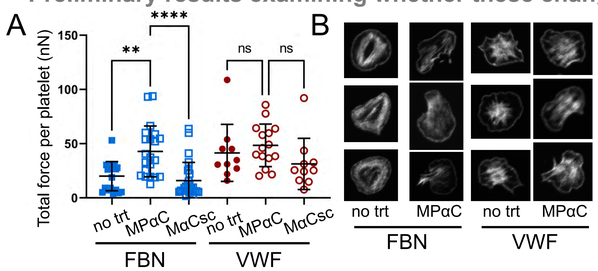

Figure 4. (A-B) MPαC[1], an inhibitory peptide that binds to the GPIb cytoplasmic domain containing the binding site for 14-3-3ζ, was added to platelets. Interestingly, MPαC appears to increase force (A) and alter the F-actin morphology (B) of platelets on fibrinogen, but not on VWF. No significant differences were observed with the scramble control MαCsc. 1 - generously provided by Xiaoping Du.

Next steps

1) Create surfaces with controlled adhesive domains (only A1, only RGD, A1 + RGD)
2) Measure F-actin morphology and force generation in the presence of other inhibitors of GPIb binding and/or mechanotransduction

Conclusions

- Platelet **F-actin morphology is significantly** different on surfaces treated with fibrinogen versus VWF and these morphologies are identifiable via machine learning
- **Platelets on VWF produce significantly more force**, more force per area, and more centrally localized force
- **Black dots enable** co-measurement of F-actin structure and high-resolution single-platelet forces in a single image without restraining cell spreading. These observations would not be possible in low-resolution methods, methods that restrict cell spread size and shape, and/or methods that are incompatible with fixing and staining
- We hypothesize that these differences in F-actin morphology are GPIb-mediated; this hypothesis is under investigation

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