Supporting Information

Nanofiber topographies enhance platelet-fibrinogen scaffold interactions

Martin Kenny, Stephani Stamboroski, Reem Taher, Dorothea Brüggemann, Ingmar Schoen**

Supplementary Figures

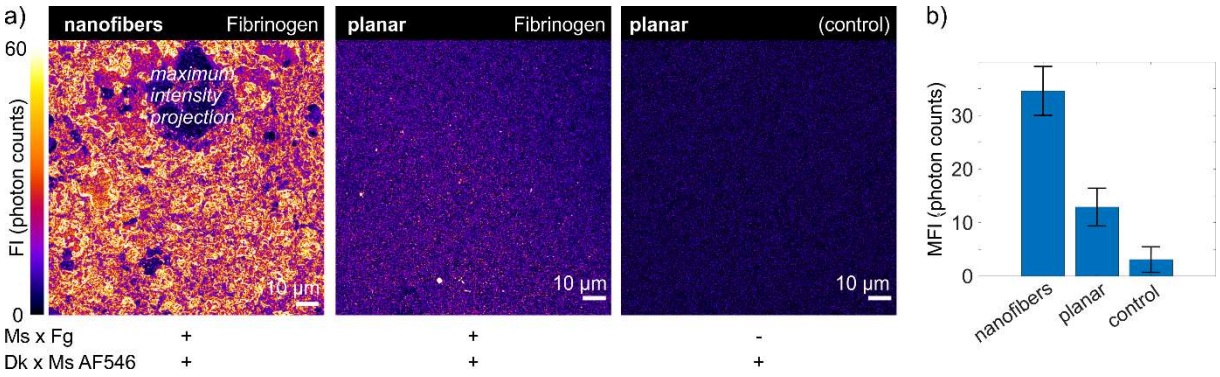

Figure S1. Immunostainings of fibrinogen scaffolds. Fibrinogen samples were prepared as described under materials and methods. To verify the presence of fibrinogen, samples were incubated with mouse anti-fibrinogen antibodies (Sigma F9902; 1:200 dilution in PBS containing 0.02% Tween-20) for 4 hours, washed 3x in PBS, incubated with donkey anti-mouse IgG (Alexa546 conjugate, Jackson ImmunoResearch, 1:100 dilution in PBS containing 0.02% Tween-20) for 2 hours, washed 3x in PBS, and mounted in MOWIOL. As a control, a planar substrate was processed without the addition of primary antibodies. Fluorescence images were obtained on a confocal microscope (Leica Stellaris) using a 100x oil immersion objective (NA 1.46) under identical settings (2% excitation at 488 nm, emission window 498-550 nm, HyD X detector in photon counting mode, 1x magnification, 4x line accumulation). (a) Representative images of fluorescence intensity (FI) of fibrinogen nanofibers (left) and planar fibrinogen films (middle and right) showed specific staining for fibrinogen. (b) Quantification of mean fluorescence intensity (MFI) from 5 images per condition showed ~2.5x stronger antibody stainings on nanofibers as compared to planar scaffolds, indicating a higher accessibility of fibrinogen epitopes on nanofibers.

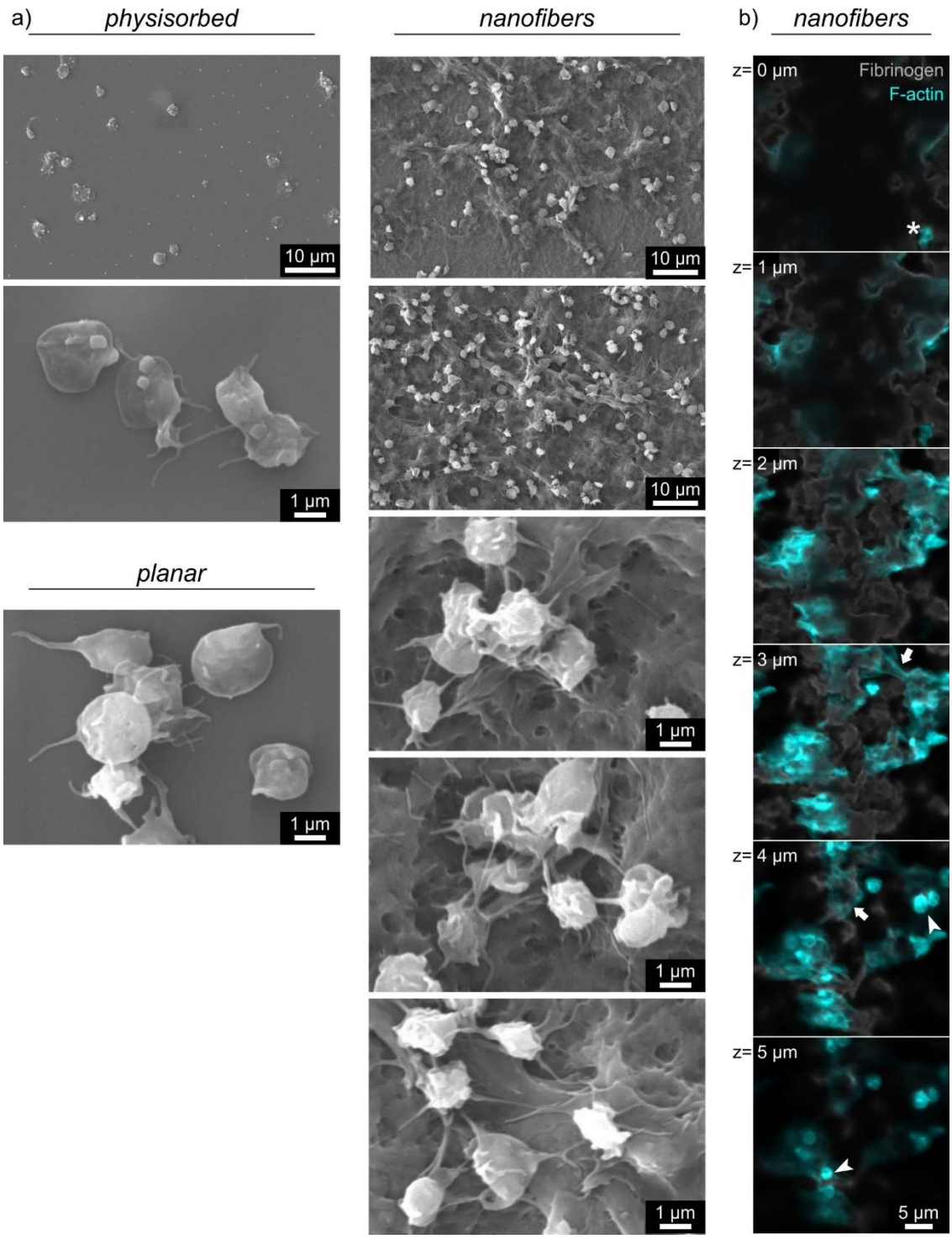

Figure S2. Interactions of platelet-rich plasma (PRP) with fibrinogen scaffolds. (a) Further examples of SEM images on physisorbed, planar, or nanofibrous fibrinogen. (b) Confocal z-stack from 0…6 µm of the F-actin cytoskeleton in platelets (cyan) on nanofibrous fibrinogen (gray). Symbols indicate: asterisk – single platelet spanning hole between two sides; arrow – stress fibers of platelets bridging a larger gap; arrowhead: rounded non-activated platelets on top of small platelet aggregates.

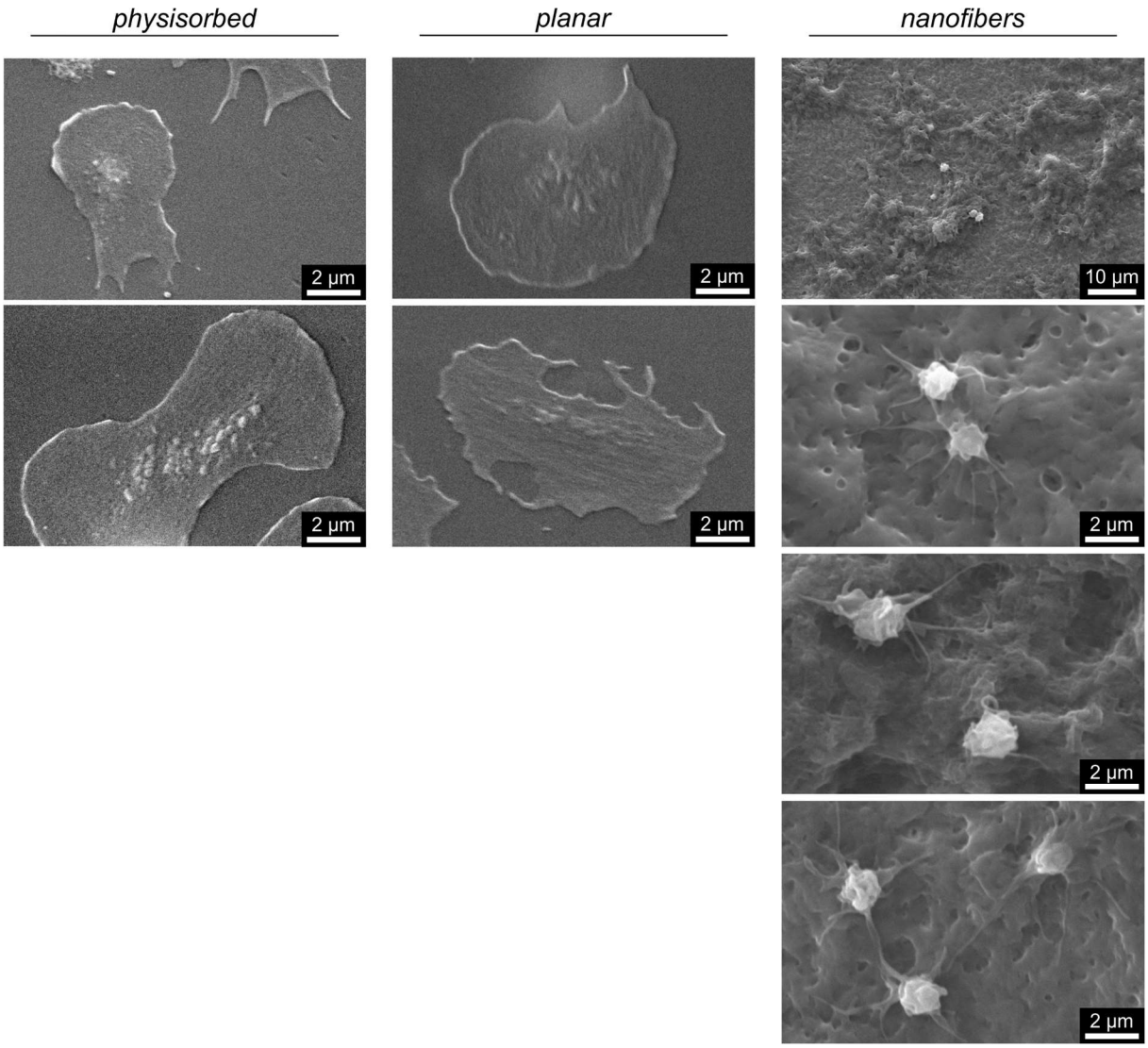

Figure S3. Interactions of washed platelets with fibrinogen scaffolds. Further examples of SEM images on physisorbed, planar, or nanofibrous fibrinogen.

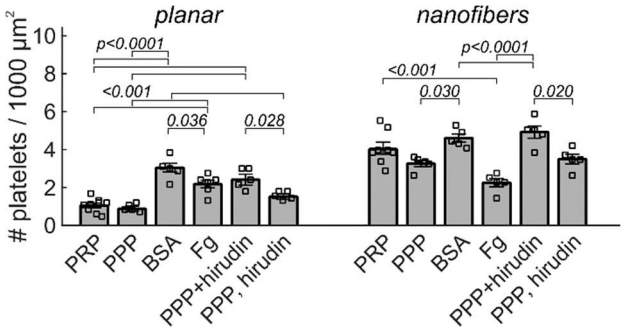

Figure S4. Modulation of platelet adhesion by plasma components. Experiments were performed as described in Figure 5. Bars and error bars show mean and s.e.m. of replicates, respectively. Differences between treatments on the same topography were assessed by one-way ANOVA with Tukey post-hoc multiple comparison. Only adjusted *p*-values smaller than 0.05 are displayed.

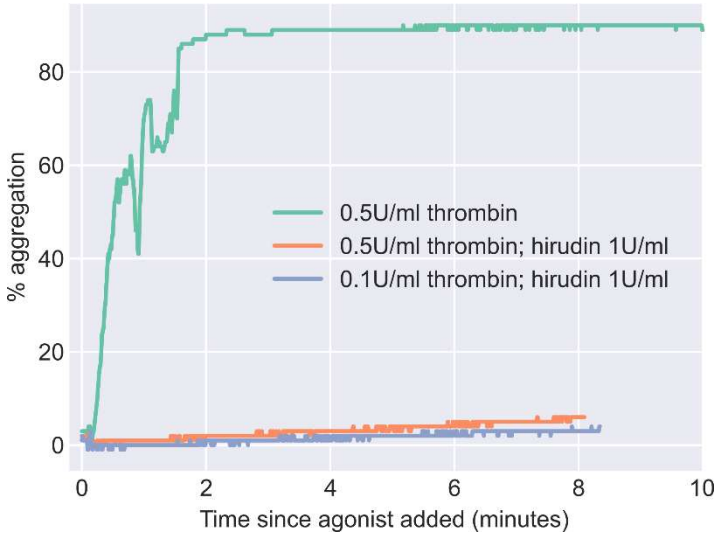

Figure S5. Inhibition of thrombin-induced platelet aggregation by hirudin. Light transmission aggregometry (LTA) was performed with washed platelets supplemented with 0.5 mg ml^{-1} fibrinogen. Platelet aggregation was induced by addition of thrombin (0.5 U ml^{-1}). In the presence of the thrombin inhibitor hirudin (1 U ml^{-1}), the aggregation response to thrombin was abolished.

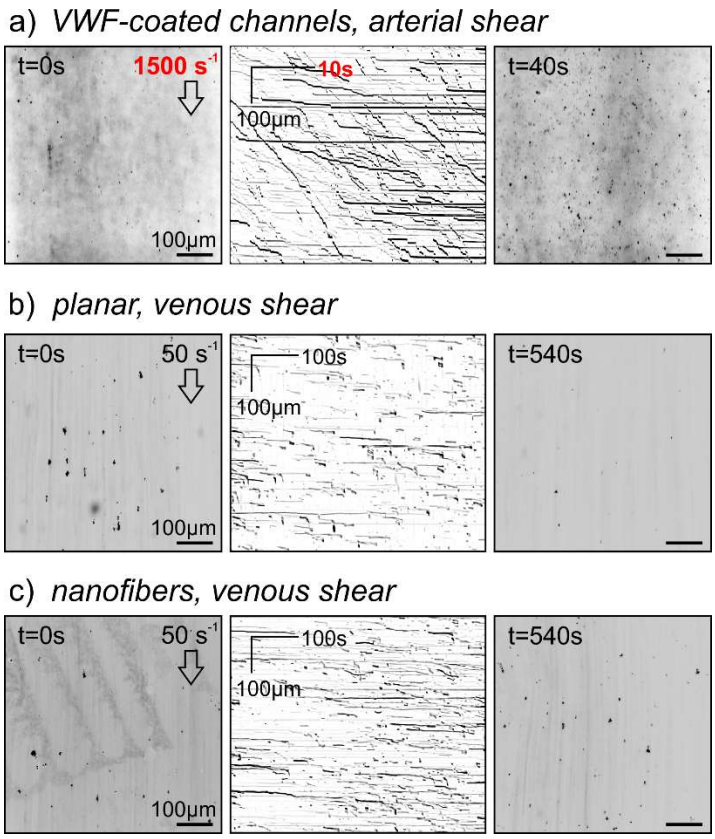

Figure S6. Interactions of platelets in whole blood with different surface. Whole blood was flown over (a) vWF-coated surface at a shear rate of 1500 s^{-1} or over (b) planar or (c) nanofiber scaffolds at a shear rate of 50 s^{-1}. Platelets were visualized by the fluorescent $DiOC_6$ membrane stain in epifluorescence at 25 frames per second (a) or 2 frames per second (b+c). Shown are kymographs (*center*) with start and end frames (*left & right*). Note the different time scaling on the kymographs between (a) and (b+c).