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Authors

Rahman, Shekh
Hlady, Vladimir
Pumford, Elizabeth

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Effect of Upstream Priming on Transient Downstream Platelet-Substrate Interactions

Elizabeth Anne Pumford[a], **Shekh Mojibur Rahman**[b], **Vladimir Hlady**[a,*]

[a]Department of Biomedical Engineering, University of Utah, Salt Lake City, UT 84112, USA

[b]Department of Chemical Engineering, University of Utah, Salt Lake City, UT 84112, USA

Abstract

Upstream exposure of platelets to activating proteins 'primes' platelets for increased downstream adhesion, though the mechanics of platelet translocation before permanently arresting are not well understood. To investigate platelet translocation on platelet-binding proteins, primed platelets' transient contacts with immobilized proteins were recorded and analyzed. Using a microfluidic channel, representative of a vascular graft, platelet-activating proteins were covalently attached to the upstream priming, center, and downstream capture positions. Image sequences of platelet interactions with the center protein were captured as platelet-rich plasma (PRP) was perfused through the channel. There was an increase in both platelet pause events and net platelet adhesion on von Willebrand factor, collagen, or fibrinogen following upstream exposure to the same protein. Upstream priming also caused a decrease in average platelet velocity. The duration of transient platelet arrests on the protein-coated surface and the distance that platelets travel between pause events depended on the protein with which they were interacting. The most significant increase in platelet pause events frequency and decrease in average velocity occurred on immobilized von Willebrand factor, compared to the control with no upstream priming. These results demonstrate that platelet priming increases downstream platelet-protein interactions prior to permanent adhesion.

Graphical Abstract

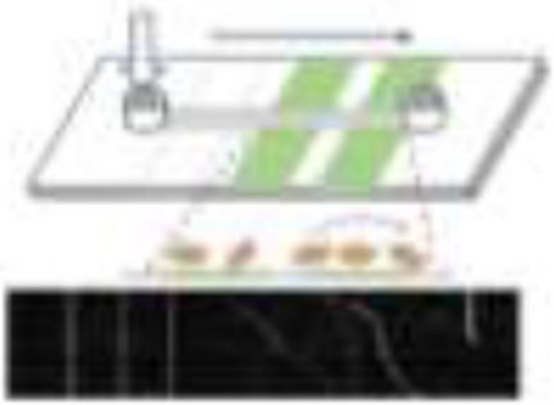
*Corresponding Author: Vladimir Hlady, D.Sc.Mailing address: 20 S. 2030 E., Rm. 506B, Salt Lake City, UT 84112, USA, vladimir.hlady@utah.edu, Phone: 801-581-5042.

Author contributions

E. Pumford conducted the research, analyzed the data, and prepared the drafts of the manuscript. S. Rahman contributed to the planning the experiments, writing of the manuscript, and data interpretation. V. Hlady designed the research, performed the data analysis and interpretation, and critically revised the manuscript.

Conflict of Interest

The authors declare no conflict of interest.



Keywords

Platelet priming; Platelet flipping; Microfluidics; Microcontact printing

1. Introduction

Patients who receive implanted vascular devices, even those that are bioresorbable, are at increased risk of thrombosis [1,2]. Vascular injury is sustained at the anastomotic regions of vascular grafts during implantation, exposing the subendothelium, which contains a number of platelet-adhesive proteins [3]. Soluble plasma proteins—primarily fibrinogen —may also adsorb to the synthetic material surface. Transient platelet interactions with platelet-binding proteins have been shown to serve as the main “priming” events, in which platelets are pre-activated for increased downstream adhesion and activation [4,5]. Exposure to high wall shear strain rates has also been shown to prime platelets for higher downstream adhesion and activation [4, 6–8]. The primed platelets adhere at the downstream anastomotic region, leading to thrombosis and vascular smooth muscle cell proliferation [9]. Thrombosis significantly reduces blood flow and may lead to a thromboembolism, resulting in heart attack or stroke [10]. To advance the development of blood-contacting biomaterials, understanding the mechanics of transient platelet contacts with exposed subendothelial proteins and surface adsorbed proteins prior to final adhesion and the influence of upstream priming on these events is critical.

When a vascular graft is implanted, the blood vessel is damaged and the extracellular matrix, composed largely of collagen, is exposed at the anastomotic sites [11]. Subendothelial collagen exposure allows for the attachment of platelets via GPVI receptors on the platelet surface, which in turn activates GPIa/IIa [12,13]. Plasma von Willebrand factor (vWF) protein then binds to exposed collagen where the graft meets the native vessel [14]. The collagen-bound vWF interacts with platelets through a catch-bond with the platelet GPIb-IX-V complex [15]. vWF binding to GPIb-IX-V is the initial platelet adhesion step and is reversible due to the high association and dissociation rates [14,16]. Finally, following the implantation of vascular device, plasma fibrinogen may adsorb to the biomaterial surface.

The adsorbed fibrinogen interacts with platelet GPIIb/IIIa, a key step in initiating platelet aggregation [17,18].

The initial phase of platelet adhesion occurs as platelets form a tether bond to exposed platelet-activating molecules, and translocate by flipping [19]. Platelets often exhibit stop-start behavior as they reversibly adhere, detach and translocate, and eventually form a stable adhesion to the surface once activated [20,21]. Jeffery described the motion of ellipsoidal particles in viscous solution, which has since been utilized to model platelet translocation [22,23]. Jeffrey's model emphasized that ellipsoidal particles flip as they translocate, and do not simply roll as spherical particles such as leukocytes do. An improved model examining individual platelet flipping events assessed the dynamics of each instance in which the platelet attaches to the vWF-coated surface at a single anchorage point and the platelet flips about that point [24]. While exposure to upstream platelet-activating proteins has been shown to prime platelets for downstream adhesion, little is known about primed platelets translocation and surface interactions before their permanent arrests [5].

In this study, we aimed to characterize translocation of primed platelets using platelet-rich plasma (PRP) to better understand the role of transient adhesive protein-platelet interactions that occur before final downstream adhesion. We hypothesize that exposure to upstream platelet-activating proteins pre-activates platelets and increases their propensity to adhere on downstream protein-coated surfaces and extends the duration of these reversible arrest events. To investigate the effect of upstream priming, microfluidics experiments were designed to mimic the physiological conditions present within a large venous vascular graft. Vascular injury sustained at the anastomotic regions during device implantation was represented in experiments with covalently attached platelet-activating proteins in an upstream and a downstream position. A platelet-activating protein was also immobilized on a region between these upstream and downstream positions. This center protein region of the flow channel was the focus of this study, and transient platelet contacts with such substrates were recorded to characterize platelet transient interactions with three immobilized proteins. Microscopy videos were analyzed to quantify differences in platelet translocation events between experimental groups. The experimental groups tested had a center region of immobilized collagen, or fibrinogen, or vWF, with an upstream priming region containing the same protein. Controls for each group had these proteins in the center regions, but an upstream priming region contained immobilized platelet-inert human serum albumin (HSA).

2. Methods

2.1. μ-Contact Printing

Polydimethylsiloxane stamps (PDMS, Sylgard 184, Dow Corning, Midland, MI, USA) were prepared by combining the silicone elastomer with the curing agent in a ratio of 10:1. The PDMS was cast on a large polystyrene Petri dish to create a flat surface, and cured at approximately 23°C for at least 24 hours. The PDMS was degassed while curing by placing the Petri dish in a vacuum for 30 min to remove any air bubbles. Once the PDMS had fully cured, the material was removed from its mold and cut into stamps (7 mm × 20 mm). Fresh PDMS stamps were sonicated in dilute Alconox for 10 min, rinsed thoroughly with DI water, and dried with N_2.

Three stamps were prepared per experiment with an upstream platelet-activating protein—one for priming, one for center, and one for the capture region—using collagen (abbr. “C”, type I, Ibidi, Fitchburg, WI, USA), fibrinogen (abbr. “F”, EMD Millipore, Burlington, MA, USA), or vWF (abbr. “V”, Haematologic Technologies, Essex Junction, VT, USA). Human serum albumin (abbr. “H”, HSA, 1 mg/mL in PBS, Sigma Aldrich, St. Louis, MO, USA) was used upstream as a negative priming control. Each experimental group was labeled with three letters, the first of which corresponds to the protein in the priming region, the second corresponds to the protein in the center region, and the third corresponds to the protein in the capture region (Table 1). Eighty μL of the selected protein (fibrinogen 1 mg/mL, collagen 3 mg/mL, or vWF 10 μg/mL)was incubated on a PDMS stamp for 20 min while covered with a coverslip to prevent evaporation and ensure even coverage. Following protein incubation, the stamps were rinsed with DI water and dried with N_2. Nexterion-H slides (Schott, Tempe, AZ, USA) were μ-contact printed with the stamps to obtain 100% monolayer coverage as previously described [25]. Nexterion-H slides have a polyethylene glycol coating that is functionalized with *N*-hydroxysuccinimide esters, allowing proteins to be covalently attached by their amine groups [26]. The priming region was μ-contact printed 18 mm from the uppermost edge of the slide, the center region 3 mm below the bottom of the priming region, and the capture region 17.5 mm from the lower edge of the slide (Figure 1). Protein stamps were left in contact with the Nexterion-H slide for 1 hour before the stamps were removed.

2.2. Blood Collection and Preparation

Whole human blood was drawn from healthy donors that had not taken any anti-inflammatory or antiplatelet drugs for two weeks prior to donation. Blood was collected via venipuncture following protocols (protocol IRB_00051506) consistent with University of Utah Institutional Review Board regulations. Blood was drawn into a syringe pre-filled with 3.2% (0.105 M) sodium citrate, which acted as an anticoagulant by chelating Ca^{2+} in blood. Within 3 min of blood collection, the sample was further anticoagulated with thrombin-inhibitor Phe-Pro-Arg-chloromethylketone (PPACK, 80 μM, Haematologic Technologies, Essex Junction, VT, USA) to inhibit thrombin-induced coagulation. Blood was stored in a 37°C water bath until use, within 60 min of procurement.

2.3. Platelet Rich Plasma Preparation

PRP was prepared by centrifuging 15 mL of anticoagulated whole blood at 1,500 RPM for 10 min. The uppermost layer containing plasma, platelets, and white blood cells was transferred into a sterile collection tube and centrifuged for 15 min at 2100 RPM to obtain a platelet pellet. The top 2/3 of supernatant following the second centrifugation, platelet poor plasma, was discarded. The platelet pellet was gently re-suspended with the bottom 1/3 of plasma [27]. Using a hemocytometer, PRP platelet concentration was measured and the PRP was diluted to a concentration of 2.5×10^7 platelets/mL using Tyrode-HEPES buffer. In doing so, the plasma blood components were diluted by a factor ranging from 5 to 9, depending on the donor’s original platelet concentration. Platelets were not fixed prior to perfusion.

2.4. Flow Channel Assembly and Perfusion

A perfusion channel was prepared using the μ-contact printed Nexterion slide. A sticky-Slide I 0.1 Luer (ibidi, Martinsried, Germany) was adhered to the Nexterion slide, and uniform pressure was applied to create the perfusion channel with a complete seal. The assembled flow channel is shown in Figure 1, with the priming region 4.5 mm downstream from the channel inlet. The channel, luers, and connecting tubing were then passivated by adsorption with HSA (1 mg/mL in PBS) by incubating for 1 hour. Samples with a priming region of HSA and a center region of fibrinogen, collagen, and vWF acted as negative priming controls in experiments. Using a syringe pump (Genie Plus, Kent Scientific, Torrington, CT, USA) with a flow rate of 0.05 mL/min, phosphate buffered saline (PBS, pH 7.4, 37°C) was drawn through the channel for 2 min to remove HSA that had not adsorbed to the surface. Next, PRP was perfused at a constant wall shear strain rate of 100 s^{-1} through the channel for 5 min while microscope videos of the center region were recorded using cooled CCD (ORCA-ER, Hamamatsu, Shizouka, Japan) running on μManager software (ImageJ, LOCI, University of Wisconsin-Madison). Both phase contrast and fluorescence microscope image sequences were recorded using an exposure time of 10 ms, with 200 frames captured per trial. There was a time gap of approximately 80 ms between frames, accounted for by using elapsed time obtained from CCD time stamps for each frame when analyzing data. Finally, the channel was rinsed with PBS at a flow rate of 0.05 mL/min for an additional 2 min to remove non-adhered platelets from the channel, and the capture region was photographed immediately following.

2.5. Microscope Video Analysis

Microscope image sequences were imported into ImageJ, the background was subtracted, and contrast was increased by 3%. A stage micrometer was used to calibrate the images, converting the size of pixels in the recorded area to μm. Platelets were manually tracked using MTrackJ, an ImageJ plug-in, along their entire trajectory. The time stamp of each image frame—obtained from image metadata files—was used in each MTrackJ output to obtain complete platelet trajectories over time. An example MTrackJ track and corresponding platelet trajectory are shown in Figure 2.

The distance that each tracked platelet traveled in the flow direction over time was used to calculate instantaneous and average velocities. Average velocity was calculated along the entire trajectory of each platelet that paused, from the first frame in which the platelet is visible until it left the recording region, or the video terminates. The time in which a platelet remains arrested, with a velocity of 0 μm/s, was used to determine the platelet dwell time, and the distance each platelet traveled between pause events was defined as platelet step size. Trajectory step sizes greater than 20 μm were excluded from data analysis because, though such step sizes were rare, they dramatically skewed data without offering useful insight into platelet flipping on agonist surfaces.

The number of platelets that stopped temporarily was used to calculate the number of pause events per second. Platelets were excluded from pause event calculations if they were arrested on the surface at the beginning or the end of the recording and did not exhibit any additional pause events. Instead, these platelets provided information related to the dynamic

behavior of platelets and were used in calculating net platelet adhesion. Net platelet adhesion per mm^2 following a 5-min perfusion was calculated by taking the difference of the number of platelets initially arrested at the start of the recording and the number of platelets that remained paused at the end of the recording period. Particle analysis of images of the capture region was used to measure the surface area of each platelet adhered to the capture protein, which was then summed up and normalized to the field of view to determine the platelet surface coverage of each sample's capture region.

3. Results

3.1. Platelet Pause Events and Net Adhesion on the center region

Figure 3a shows platelet pause events per second on the center region for each experimental group. For all three proteins tested, the pause event frequency was greater for experiments when the same protein was in the upstream priming region than with upstream HSA. Over the course of a 20 sec video, the average number of platelet pause events was in the range of 4 to 62. When the pause events were normalized to one second, the values became markedly smaller. There was a slight, but not significant, increase in pause events per second on the center region of CCC compared to HCC. The difference in platelet pause event occurrence on the center region was statistically significant between HFF and FFF—increasing from 0.2 ± 0.4 to 1.8 ± 1.3 pause events per second—and between HVV and VVV—increasing from 0.2 ± 0.2 to 3.1 ± 0.5 pause events per second. Net platelet adhesion per mm^2 following a 5-min perfusion of PRP showed the same general trend (Figure 3b). Net adhesion on the center protein region was greater for experiments with the same protein in the priming region than with HSA in the upstream priming region, though differences were not significant. It is possible for net platelet adhesion to be negative, as with HVV, when more arrested platelets dislodged from the substrate than interacting platelets arrest on the same substrate.

Capture region surface coverage was greater for all proteins when the same protein, compared to HSA, was in the upstream priming region (Figure 4). Average surface coverage on the three capture proteins increased by at least 150% when primed by the upstream protein. It is important to note that even unprimed samples experienced a pre-activation effect, as platelets were exposed to the protein in the center region prior to downstream capture. In other words, there were no "HHX" experiments (where X stands for C, F, or V). No differences were significant due to the large standard deviations within each experimental group, with p-values ranging from 0.24 to 0.63.

3.2. Platelet Trajectory Analysis

Mean platelet dwell times (533 ± 347 ms) on center fibrinogen following fibrinogen priming were significantly shorter than on center collagen after collagen priming (2,896 ± 2,770 ms) or on center vWF after vWF priming (3,562 ± 4,418 ms) (Figure 5a). For each group a cumulative distribution of dwell times (Figure 5a) was also fit to an exponential function to obtain characteristic dwell times. Characteristic dwell times based on the exponential fit were 625 ms on fibrinogen, 1,863 ms on collagen, and 1,974 on vWF. The step sizes between pause events were much smaller on collagen than on either fibrinogen or vWF (Figure 5b). This difference was only significant between collagen and fibrinogen. It was

also found that platelet pause events following HSA priming were very infrequent. For experiments with HSA priming, too few platelets exhibited stop-start behavior on the center region for meaningful analysis, regardless of which protein was immobilized there. As such, step size and dwell times could not be compared between CCC, FFF, and VVV experiments and their HSA-primed controls- HCC, HFF, and HVV.

Average velocities of platelets that interacted with the substrate are shown in Figure 5c. Average velocity on a given protein decreased when the same protein was in the upstream priming region, compared to a control priming by HSA. The difference in average platelet velocity was only significant between HVV and VVV, with respective mean velocities of 49.30 ± 19.68 μm/s and 11.46± 10.55 μm/s.

To illustrate the difference between trajectories, the trajectories of platelets that paused in CCC and VVV experiments are shown in Figure 6. In comparing the experiments, platelet pause event occurrence, dwell times, and step sizes had different distributions along individual trajectories. While the dwell times and step sizes were not significantly different between the two groups (Figures 5ab), the number of platelet pause events per second was far greater on vWF than on collagen (3.1 and 0.9 pause events per second, respectively) (Figure 3a). On collagen, individual platelets stopped more times along a single trajectory, while on vWF, many platelets paused fewer times. This contributed to longer effective travel lengths of platelets on collagen, while platelets on vWF traveled a shorter cumulative distance before joining the bulk plasma flow. Additionally, platelets interacting with a collagen center region exhibited the longest dwell times at the end of their trajectories after traveling a greater distance on the substrate. Platelets interacting with vWF, on the other hand, traversed a shorter distance and tended to pause for longer durations in the middle of the trajectory.

4. Discussion

Collagen, fibrinogen, and vWF were chosen as the proteins of interest, because they are all known to bind platelets. Nexterion-H slides were selected for the substrate, because these three proteins could be covalently attached to its surface by their amine groups, preventing desorption during perfusion. Further, a fibrinogen μ-contact printed Nexterion-H surface has previously been characterized using lateral force microscopy and fluorescence, showing highly controllable platelet surface coverage [25]. For perfusion of the channels, PRP was used instead of whole blood to eliminate platelet margination and dislodgement due to red blood cells. Finally, a wall shear strain rate of 100 s^{-1} was used in these experiments, as this is a representative shear strain rate of large veins. Thus, our results are primarily applicable to synthetic vascular grafts used to replace large veins.

4.1. Platelet Pause Events and Net Adhesion in the Center Region

Compared to the HSA-primed controls, platelet pause events per second and net platelet adhesion increased on collagen, fibrinogen, and vWF when the same protein was in the upstream priming region, as seen in Figures 3ab. This trend is likely caused by platelet pre-activation by the upstream protein, increasing tethering events between platelet receptors and the center protein. As these tether bonds form, the platelet slows and pauses on the protein

surface. The wall shear force acting on the bonds eventually causes them to break, and the platelet dislodges from the surface to join freely flowing PRP components. The most drastic increase in platelet pause events per second was seen between HVV and VVV, followed by HFF compared to FFF (Figure 3a). The large increases indicate that upstream priming by vWF and fibrinogen significantly pre-activates platelets and increases their propensity to arrest on downstream platelet-activating surfaces. This hypothesis appears consistent with existing knowledge of vWF and fibrinogen interactions with platelet receptor GPIIb/IIIa, which has an increased ligand affinity following platelet activation [28]. It also is expected that vWF would exhibit a high number of platelet pause events per second, as bonds between GPIb-IX-V and vWF-A1 are reversible, with high association and dissociation rates [24]. These characteristics would increase the likelihood of these bonds to both form and break. That was supported by experimental results, as platelet arrest events along the vWF surface increased following upstream vWF priming.

Net platelet adhesion increased most between HCC and CCC (Figure 3b). Platelets that have been primed by collagen were less likely to dislodge during plasma perfusion. The increase in net adhesion indicated that tether bonds between GPVI and GPIa/IIa receptors and collagen are strengthened by upstream platelet exposure to collagen. It is possible, however, that vWF present in the plasma has bound to the covalently attached collagen, and the increase in net adhesion was caused by predominantly by platelet GPIb-IX-V complex. vWF adsorption offers a plausible explanation, as GPIb-IX-V activation is required for stable adhesion on collagen [29].

Platelet surface coverage of the downstream capture region increased on all proteins when primed by the same protein (Figure 4). Increased platelet adhesion following protein priming is consistent with recent literature, that upstream activation—whether by shear or platelet-activating protein exposure—increases downstream adhesion on collagen, fibrinogen, and vWF [6,30,31]. The surface coverage averages displayed values that were comparable to the majority of reported values, which suggested that the three proteins were all viable and had been successfully covalently attached to the Nexterion-H slides [29]. One important consideration, however, is that platelets experience an additional priming effect as they encounter the adhesive protein in the center region. This likely contributed to platelet adhesion to the downstream capture protein. Even in experiments with a platelet-inert HSA in the priming region, platelets still undergo pre-activation as they are exposed to the protein in the center region, increasing their propensity to bind to downstream proteins.

Experimentally obtained surface coverages were slightly lower than other previously reported values in some instances, however. For example, a study by Rahman et al. examining downstream adhesion as a function of shear showed downstream surface coverage of up to 15% [6]. The difference in platelet adhesion may be due to a use of a higher shear strain rate or may also be explained by the use of whole blood. PRP used in experiments presented in this study lacked red blood cells and the resultant platelet margination that increases downstream adhesion [32]. It is likely that platelet margination could account for differences in downstream surface coverage. Despite experimental differences, it could be inferred from these data that surfaces coverages obtained in the present study have reasonable values.

4.2. Platelet Trajectory Analysis

Platelet step sizes and dwell times were only presented for experiments with the same protein in the upstream priming region (Figures 5ab). Experimental groups with upstream HSA priming had too few platelets arresting on the center protein for thorough analysis. The lack of data for these groups in itself is useful since it emphasized how drastic the increase in platelet pause events is following upstream priming with platelet-activating proteins. As seen in Figure 5a, platelet dwell times were longest on the center region of CCC and VVV. Following upstream collagen or vWF priming, platelets paused for a longer time on the identical protein in the center region, compared to on fibrinogen with upstream fibrinogen priming. Longer platelet dwell times on collagen are likely because in vivo, this is the first platelet-protein interaction with exposed subendothelial protein, and the first step in initiating hemostasis. Further, it has been shown that at low shear, platelet GPIb-IX-V complex can be activated by transient exposure to vWF which increases platelet adhesion, and may contribute to long platelet dwell times on vWF when primed by vWF [33]. The fibrinogen results are intriguing, because fibrinogen-GPIIb/IIIa interactions are required for stable platelet adhesion and aggregation. Intuitively, one would expect pre-activated platelets perfusing over a fibrinogen surface to exhibit the longest dwell times.

Platelet step size, the distance that platelets travel between pause events, was smallest on collagen following upstream collagen priming (Figure 5b). Small step sizes on collagen offer further evidence that platelet receptors have a strong affinity for collagen, and platelets travel shorter distances on collagen after pausing before additional tether bonds form. It is possible that the long dwell times and small step sizes on collagen are secondary to vWF-A3 binding to collagen. If this were the case, the results would instead represent GPIbα-vWF interactions. However, this phenomenon has only been reported at wall shear strain rates greater than 500 s^{-1} [29]. The larger step sizes on fibrinogen may be attributed to the localization of platelet GPIIb/IIIa following platelet activation [34]. In this study, however, it can be assumed that there is an excess of ligands, due to the dense protein surface coverage obtained through μ-contact printing. This resulted in a surplus of adhesive ligands, so that the probability of a platelet interacting with them should not have been a limiting factor. Particularly for GPIIb/IIIa, which is the most abundant platelet receptor, there should always have been a sufficient number of ligands for platelet adhesion [35]. Finally, the larger step sizes on vWF could likely be attributed to the low shear strain rates used in experiments. At lower shear strain rates, vWF-A1 has a decreased propensity to bind to platelet receptor GPIbα [36]. Reduced vWF binding to the GPIb-IX-V complex would subsequently also reduce GPIIb/IIIa affinity for both vWF and fibrinogen [37].

Figure 5c shows average platelet velocities along the entire trajectory of each platelet that pauses on the protein surface. The averages include velocities as the platelets freely flowed before and after arresting. For all proteins tested, average velocities decreased following priming by the same protein. The decrease in velocities was likely due to the increase in platelet pause events on proteins following upstream priming (Figure 3a). A platelet that paused on the surface reached a zero velocity for any duration of time, which in turn decreases platelet velocity over its trajectory. The large decrease in average velocities

between HVV and VVV could be explained by the significant increase in pause events, as well as the long dwell time of platelets, on vWF following vWF priming.

5. Conclusions

Perfusion channels used in this study were designed to mimic the physiological conditions present within a large venous vascular graft, with upstream and downstream anastomotic regions, and a center region of exposed endothelium or adsorbed proteins. By using PRP instead of whole blood, platelet margination and dislodgement due to red blood cells was mitigated, and the effect of priming on transient platelet-protein interactions could be quantified with fewer confounding factors. The results indicated that platelets primed by exposure to an upstream adhesive priming protein exhibited a greater number of pause events and net platelet adhesion on a center protein.

The increase in pause events was reflected in a decrease in average trajectory velocity of platelets that had been exposed to an upstream adhesive protein in the priming region, compared to the HSA controls. Additionally, the duration of platelet pause events and the distance traveled between pausing was highly dependent on the protein with which the platelets were interacting. Using a biomimetic experimental design, the results of the present study using PRP offer more insight into transient platelet-protein interactions, and may serve as a platform for additional work aimed to minimize the presence and priming effects of proteins at the upstream anastomotic region of vascular devices.

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Data availability

The authors confirm that the raw/processed data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Highlights

- Upstream platelet priming affected the platelet translocation on protein-coated surfaces.
- Platelet trajectories were analyzed for frequency and duration of the pause event.
- Upstream priming by adhesive proteins increased frequency of the pause events.
- Upstream vWF caused the most significant increase in platelet pause events frequency.
- The net platelet adhesion increased most on downstream collagen after priming.

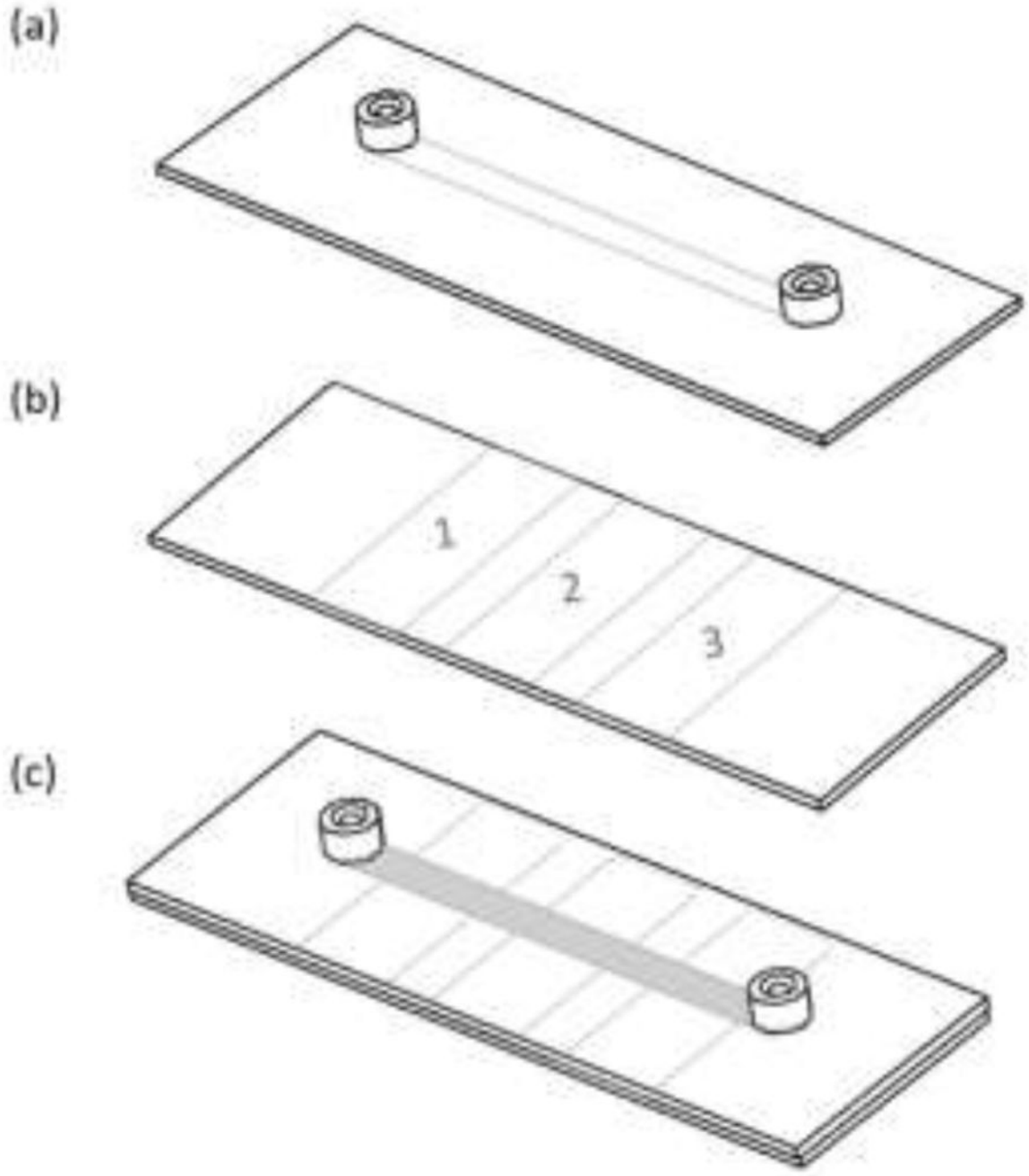

Figure 1.
Schematic illustration of flow chamber. (a) Sticky-Slide Luer 0.1; (b) μ-contact printed Nexterion-H slide with three regions: 1- priming protein, 2- center protein, 3- capture protein; (c) Assembled perfusion channel with sticky-Slide Luer adhered on top of Nexterion-H slide.

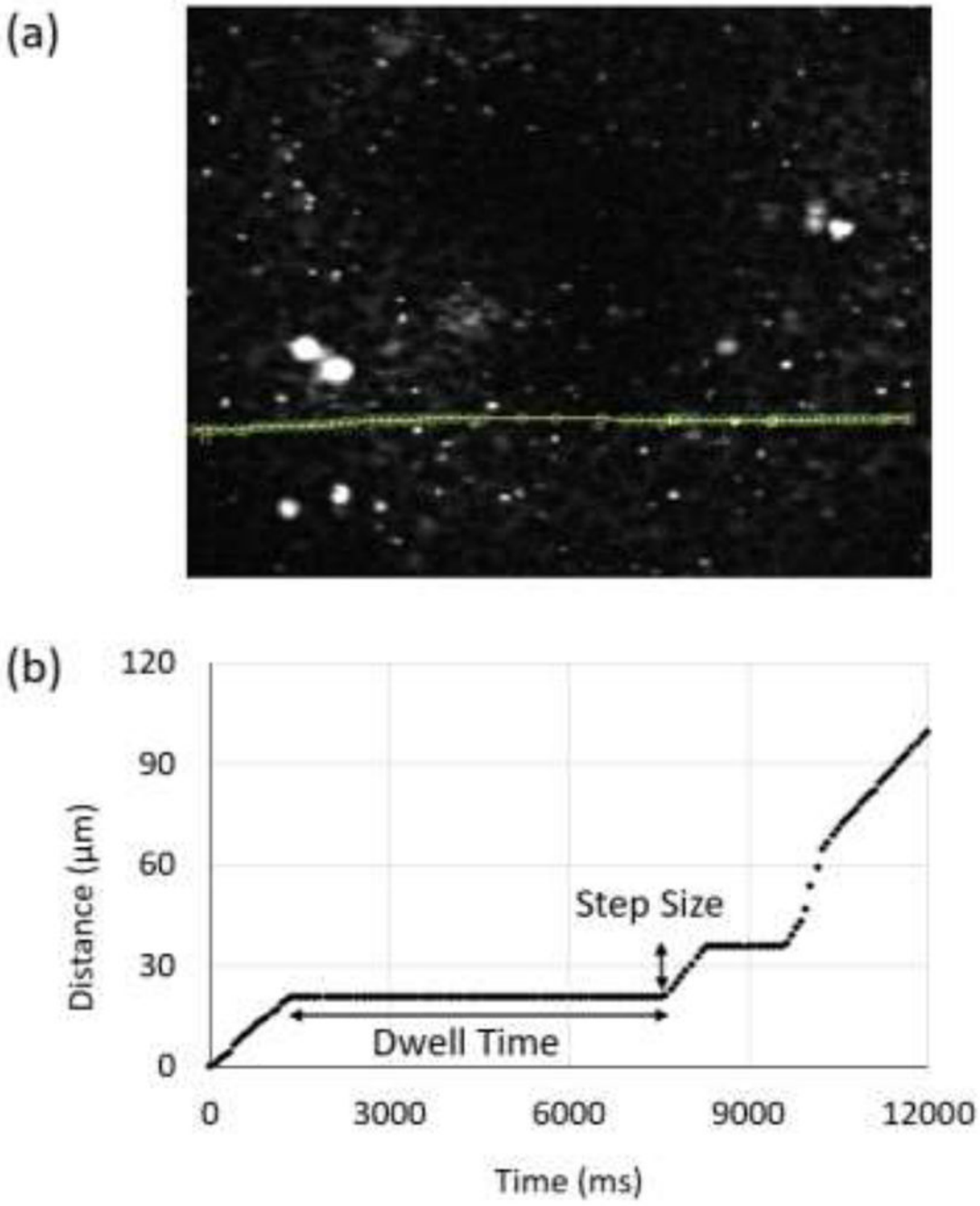

Figure 2.

Platelet tracking and trajectory mapping in real time using MTrackJ. (a) A single platelet tracked along its entire trajectory; (b) Platelet distance traveled over time, showing dwell time—the time in which a platelet arrests on the protein surface, and step size—the distance the platelet travels between pause events.

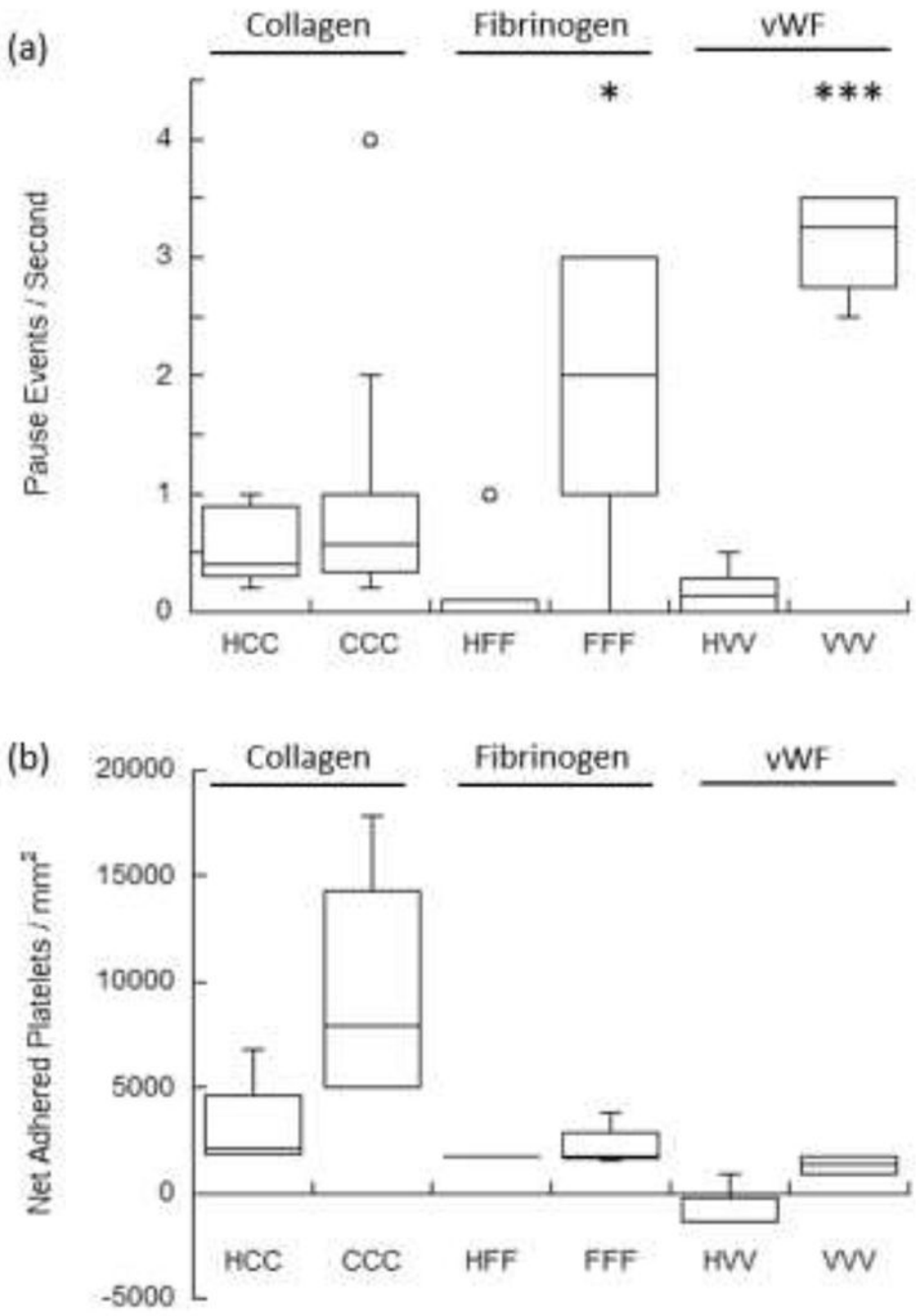

Figure 3.

Platelet pause events and net adhesion on the substrate using PRP. (a) Platelet pause events per second (n = 6); (b) Net platelet adhesion per mm^2 following five min perfusion (n = 4, except HFF n = 1). Both platelet pause events and net adhesion increase when the priming protein is identical to that in the center region. * $p < 0.05$; ** p 0.005; *** $p < 0.0005$, compared to respective HSA-primed controls.

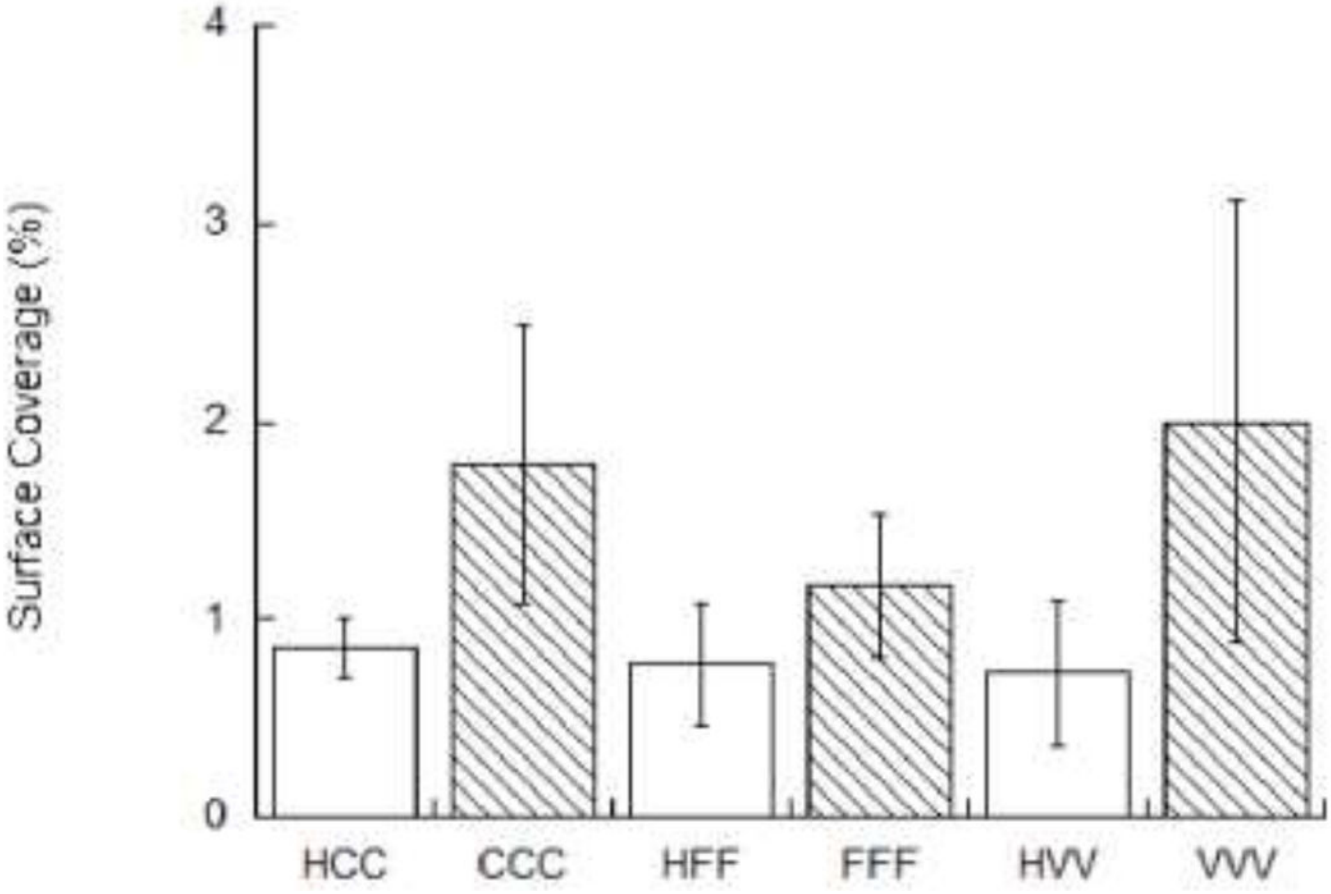

Figure 4.

Surface coverage of the downstream capture regions. Images were captured and analyzed following a 5-min perfusion of PRP and a 2-min rinse using PBS. Each value represents the average surface coverage of all trials within that experimental group (n = 4). Downstream surface coverage increases when the priming protein is the same as the protein in the center region.

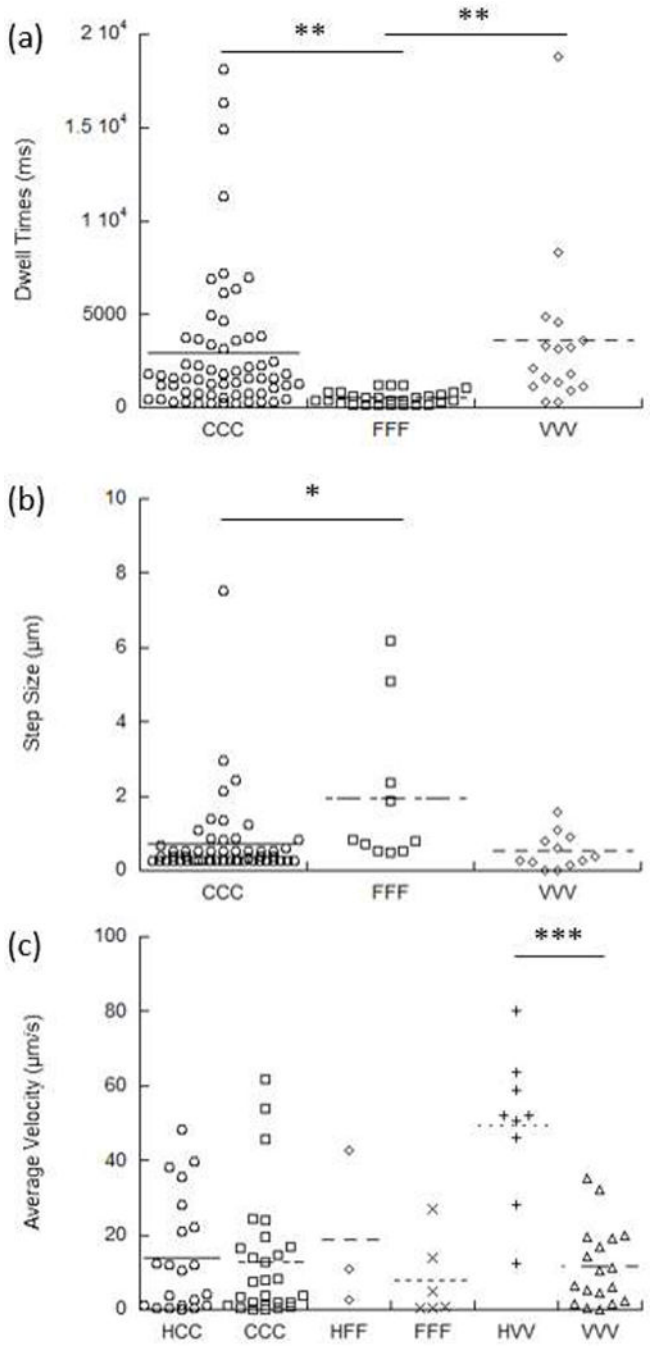

Figure 5.

Trajectory analysis of platelets that pause on the substrate using PRP. (a) Dwell times; (b) Step sizes ; (c) Average velocity along entire platelet trajectory, each point corresponds to the average velocity of a single platelet (n = 17-60). * $p < 0.05$; ** $p < 0.005$; *** $p < 0.0005$.

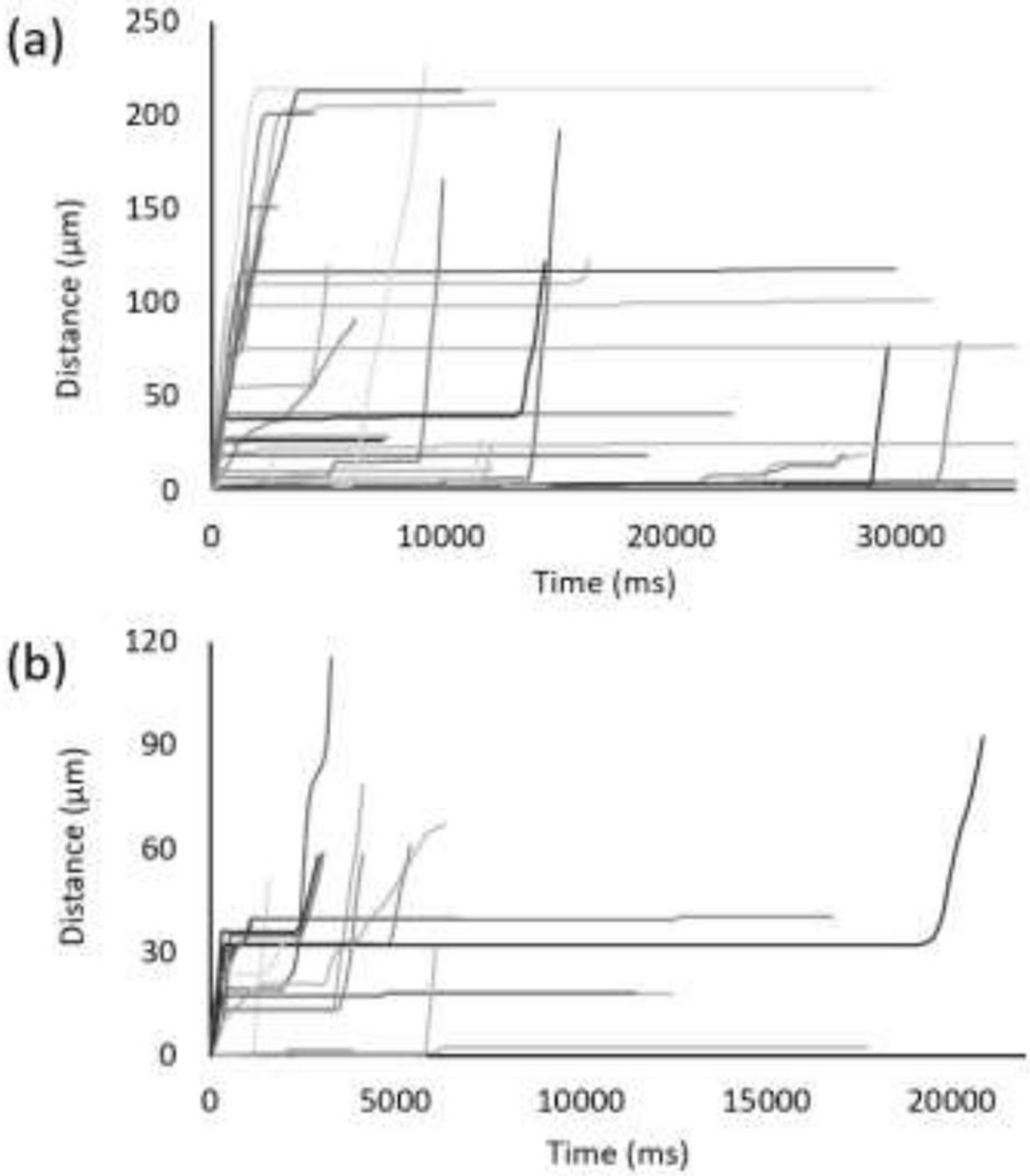

Figure 6.
Example trajectories of platelets that pause on the substrate following upstream priming by the same protein. (a) Platelet trajectories on collagen (n = 37), (b) Platelet trajectories on vWF (n = 18).

Table 1.

Naming conventions describing experiments in this study. The priming, center, and capture regions refer to platelet binding protein location on the μ-contact printed Nexterion-H slide. H-HSA; C- collagen; F-fibrinogen; V- vWF.

Priming	Rolling	Capture
H	C	C
C	C	C
H	F	F
F	F	F
H	V	V
V	V	V