Extracellular DNA traps promote thrombosis

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Neutrophil extracellular traps (NETs) are part of the innate immune response to infections. NETs are a meshwork of DNA fibers comprising histones and antimicrobial proteins. Microbes are immobilized in NETs and encounter a locally high and lethal concentration of effector proteins. Recent studies show that NETs are formed inside the vasculature in infections and noninfectious diseases. Here we report that NETs provide a heretofore unrecognized scaffold and stimulus for thrombus formation. NETs perfused with blood caused platelet adhesion, activation, and aggregation. DNase or the anticoagulant heparin dismantled the NET scaffold and prevented thrombus formation. Stimulation of platelets with purified histones was sufficient for aggregation. NETs recruited red blood cells, promoted fibrin deposition, and induced a red thrombus, such as that found in veins. Markers of extracellular DNA traps were detected in a thrombus and plasma of baboons subjected to deep vein thrombosis, an example of inflammation-enhanced thrombosis. Our observations indicate that NETs are a previously unrecognized link between inflammation and thrombosis and may further explain the epidemiological association of infection with thrombosis.

Neutrophils | Platelets | Histones | Red blood cells | Chromatin

Thrombosis depends on the adhesion, activation, and aggregation of platelets (1). RBCs, whose function in thrombosis is not well defined, are especially abundant in venous thrombi. Final thrombus stability requires scaffolding provided by large polymers, such as fibrin and von Willebrand factor (VWF) (2, 3). Thrombus formation can be enhanced by inflammation and endothelial dysfunction (4). Deep vein thrombosis (DVT), which affects over 375,000 patients per year in the United States (5), is often linked to inflammation (6) and infections (7).

In sepsis, neutrophil extracellular traps (NETs) are formed within the vasculature (8). NETs are extracellular DNA fibers comprising histones and neutrophil antimicrobial proteins (9). They are known for their antimicrobial function and have been proven beneficial against infections (10). NETs are formed by a cell-death program, which proceeds from the dissolution of internal membranes followed by chromatin decondensation and cytolysis (11). In vitro, neutrophils, basophils, and mast cells release extracellular DNA traps (9, 12, 13) in response to microbial and inflammatory stimuli, like IL-8 and reactive oxygen species (12–14). Interestingly, extracellular DNA traps are also observed in inflammatory but noninfectious diseases, like pre-eclampsia (15) or small-vessel vasculitis (16).

Here, we show that extracellular DNA traps are a unique link between inflammation and thrombosis. Extracellular DNA traps provide a stimulus and scaffold for thrombus formation and markers of extracellular DNA traps are abundant in DVT.

Results

NETs Provide a Scaffold and Stimulus for Platelet Binding and Aggregation. We used extracellular traps released from neutrophils as a model to study their interaction with blood. We perfused NETs with platelets suspended in plasma and observed 3D NETs with avidly adherent platelets (Fig. L4). Electron micrographs showed platelet accumulation on a fibrous meshwork of NETs (Fig. 1B) and filopod formation indicated that platelets adherent on NETS were activated (Fig. 1C). Perfusion of NETs with anticoagulated blood at high shear rates (90/s) (Fig. 1D–K and Movie S1) or low, typically venous shear rates (20/s) (Movie S2) resulted in time-dependent platelet aggregation. Strings of NETs aligned (Fig. 1D and E) in the direction of flow and, importantly, NETs were not a static surface but moved in three dimensions (Movie S1). Within 1 min from onset of perfusion, small platelet aggregates appeared on NETs (Fig. 1D and I, arrows). Platelet adhesion and aggregation on NETs increased over the next 9 min (Fig. 1E and J). DNase treatment simultaneously removed NETs and platelets, indicating that platelets were indeed attached to NETs (Fig. 1F and K, and Movie S1). Quantification showed that areas covered by NETs were constant (Fig. 1G), whereas platelets adhered and aggregated in a time-dependent manner (Fig. 1H). Both platelet aggregates and NETs were removed by DNase (Fig. 1G and H). When blood was supplemented with DNase at the beginning of the perfusion, NETs were degraded rapidly (Fig. 1G) and platelet aggregates did not form (Fig. 1H). Thus, NETs were the only prothrombotic substrate in these experiments.

When we tested heparin, a common anticoagulant, on NET-induced platelet binding and aggregation, we observed that NETs were almost completely dismantled after perfusion with heparinized blood (Fig. 2A–C). In addition, heparin removed platelet aggregates from NETs (Fig. 2D) as efficiently as DNase. The effect of heparin was also observed in medium, indicating a direct interaction of heparin with the NETs (Movie S3). Heparin has high affinity for histones (17) and releases histones from chromatin (18). Consequently, incubation of NETs under static conditions with heparin or DNase alone released histones to the culture supernatant (Fig. 2E). This result indicates that heparin removes histones from the chromatin fibers that built the backbone of NETs and thus leads to the destabilization of NETs.

We tested whether platelets could interact directly with histones. Interestingly, histones were sufficient to induce platelet aggregation. Incubation of platelets with histones H3 and H4 stimulated aggregation (Fig. 2F and G), whereas histones H1, H2A, and H2B had no such effect (Fig. 2G). Thrombin served as a positive control in these experiments (Fig. 2F and G). Aggregation in response to histone H3 (Fig. 2F) and H4 (Fig. S1) was inhibited by EDTA, which excluded platelet agglutination caused by the positive charge of histones. Heparin completely abolished platelet response to these histones (Fig. 2F and Fig. S1). Dissociating NETs and inhibiting histones could add to the antithrombotic effects of heparin.

NETs Induce the Formation of a RBC-Rich Thrombus. We washed NETs after perfusion with blood and observed macroscopically a red
thrombus (Fig. 3A). DNA staining revealed a scaffold of DNA (Fig. 3B) and electron microscopy showed the presence of intact RBCs (Fig. 3C). Quantification of RBC hemoglobin in flow chambers coated with NETs or collagen showed that RBCs bound to NETs but not collagen (Fig. 3D), although platelets bound to both substrates (Fig. 3E). RBC adhesion to NETs was prevented when blood was supplemented with DNase, confirming that RBCs were attached to NETs. DNase had no effect on platelet adhesion to collagen-coated chambers, but prevented platelet adhesion to NETs (Fig. 3E). In summary, these data show that NETs provide a scaffold not only for platelets but also for RBC adhesion.

**NETs Bind Plasma Proteins Important for Thrombus Stability.** We questioned whether NETs can concentrate plasma proteins that promote and stabilize thrombi (19). Immunocytochemistry of NETs incubated with plasma showed that VWF and fibronectin, as well as fibrinogen, bound to NETs (Fig. S2). These findings are corroborated by previous reports that VWF and fibronectin interact with histones (20, 21) and that fibronectin bears a DNA-binding domain (22). We then studied whether the interaction of fibrinogen with NETs could promote fibrin deposition. NETs were perfused with calcified blood supplemented with fluorescent fibrinogen (Fig. S3A). We were able to detect fibrinogen along NET-DNA strings and the deposition drastically increased with perfusion time until the large fluorescent clot “embolized” together with the NETs. We inhibited thrombin in parallel samples to prevent fibrinogen conversion to fibrin and polymerization. Under these conditions, just traces of fibrinogen were found on NETs and NETs remained stable during the entire perfusion period (Fig. S3B). Taken together, these experiments show that NETs support platelet-adhesion molecule deposition and thrombin-dependent fibrin formation.

We compared the susceptibility of NETs and fibrin as scaffolds for blood clots to thrombolysis (Fig. S4). Therefore, we incubated neutrophils prestimulated with platelet-activating factor to release NETs with calcified blood under stirring conditions (23). A single clot in which DNA intercalated with fibrin formed under these conditions (Fig. S4D and G). Samples were treated with DNase to digest NETs or tissue plasminogen activator (tPA) for fibrin digestion. The tPA removed fibrin but did not prevent clot formation. In tPA-resistant clots, RBCs and platelets were held together by a DNA scaffold of NETs (Fig. S4F). Consequently, clot formation in the presence of activated neutrophils could be prevented only by simultaneous treatment with tPA and DNase. Thus, NETs may provide a clot scaffold independent from fibrin.

**Extracellular DNA Traps Are Present in Baboon DVT.** Red thrombi, as well as leukocyte recruitment, are characteristics of DVT (6). Thus, we investigated whether NETs are formed in experimental DVT in baboons. In brief, anesthetized juvenile male baboons underwent iliac vein thrombosis by temporary balloon catheter occlusion (6 h) as previously described (24). Plasma was col-

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**Fig. 1.** NETs provide a scaffold for platelet adhesion and aggregation. (A) Platelets (green) bound to NETs (blue, arrows). Neutrophils (blue, arrowheads) were out of focus and did not bind platelets. (Scale bar, 20 μm.) (B) Electron micrograph of platelets (Pts) attached to a fibrous meshwork of NETs. (Scale bar, 1 μm.) (C) Numerous filopods indicated that platelets (Pt) on NETs were activated. (Scale bar, 0.5 μm.) (D–K) Time-course of platelet adhesion and aggregation on NETs. (Scale bars, 100 μm.) NETs (D and E, green) were perfused with platelets (I and J, red) in whole blood. The flow direction was from left to right. Images showed NETs and platelets after 1 min (arrows in D and I) and 10 min (E, J) of perfusion. DNase added to blood after 10 min digested NETs (F) and removed platelets (K), indicating that platelets were attached to NETs. Quantiﬁcation of NETs (G) or platelets (H) in the presence (open circles) or absence of DNase (closed circles). DNase was added to untreated samples after 10 min (arrow). DNase removes NETs and inhibits platelet aggregation. A.U., arbitrary units. Data presented are representative of at least three independent experiments and are shown as mean ± SEM, n = 3.
mean blood before (−) and after (+) treatment with heparin. Data presented as mean ± SEM, n = 3; (Student's t test; *P < 0.05; **P < 0.01). (E) Heparin and DNase released histones from NETs. Immunodetection of histone H2B (arrow) in the culture supernatants of NETs treated with heparin or DNase (DN). A second band (arrowhead) may represent cross reactivity of the antibody with another protein.

Discussion

Inhibition of leukocyte infiltration in the baboon model of DVT produces unstable thrombi (24). One way leukocytes may promote thrombus stability is by producing NETs. Our results show that NETs colocalize with fibrin in vitro (Fig. S3 and Fig. S4), and it is conceivable that NETs interact closely with fibrin strands in the thrombus, thus potentially influencing thrombus organization and stability. Given the procoagulant activity of nucleic acids (28) and polyphosphates (29), future studies should address whether NETs promote coagulation, how NETs affect the mechanical properties of fibrin (2), and the susceptibility of clots to thrombolysis.

Histones in NETs or liberated after digestion of NET-DNA could also provide a stimulus for platelet aggregation. Our findings are corroborated by a recently published work showing microthrombi in lungs of mice infused with histones and a prominent role for histones in the pathology of sepsis (30). In humans, platelets and RBCs are the only blood cells lacking histones, which may help to prevent excessive thrombus propagation.

At present, the signaling mechanisms underlying induction of NETs in DVT are not understood. Ischemia results in the production of IL-8 (31) and reactive oxygen species (32). IL-8 is capable of inducing NETs (9) and is considered a risk factor for venous thrombosis (33). In vitro stimulation of neutrophils with exogenous reactive oxygen species is sufficient to induce NETs (11). Clinically, inflammation and infection are linked to thrombosis (7, 34) and it is conceivable that NETs contribute to this linkage. Mechanistically, NETs provide a scaffold for platelet and RBC adhesion and concentrate effector proteins involved in thrombosis. The interaction with NETs could be mediated in multiple ways. Platelet-binding to NETs could be accomplished via VWF, fibronectin, or fibrinogen immobilized on NETs. Alternatively, platelets could directly interact with DNA/histones in NETs. DNA has been detected on the cell surface of platelets from patients with systemic lupus erythematosus (35). Lupus patients are prone to develop venous thrombosis (6) and were recently described to have impaired NET degradation (36).

Adhesion of platelets and RBCs to NETs may be helped by electrostatic interaction of the negatively charged cells with positively charged histones in NETs (37). RBC adhesion to NETs could also play a role in sickle-cell disease, where a lethal crisis is often precipitated by infection (38).

In an infected wound, the synergy of antimicrobial and prothrombotic functions of NETs might be valuable to prevent sepsis and maintain hemostasis. In pathological leukocyte activation, targeting of the extracellular DNA and histones in NETs may prove beneficial to prevent thrombosis.

Materials and Methods

Isolation of Platelets and Neutrophils from Human Blood. The investigation conforms to the principles outlined in the Declaration of Helsinki and received approval from the Immune Disease Institute Institutional Review Board. After explaining the nature and possible consequences of the study, we obtained informed consent from all donors. Blood donors were healthy
and had not taken any medication for at least 10 d. Platelets and neutrophils were prepared from ACD-blood (39) or EDTA-blood (11), respectively.

**Perfusions of NETs.** Neutrophils were seeded into flow chambers (μ-Slide IV, IBIDI) at 0.5 to 1 × 10⁶ cells/mL. NET formation by the majority of cells was induced by phorbol 12-myristate 13-acetate (PMA, 50 nM, 4 h; Sigma Aldrich) or glucose oxidase (GO, 1 U/mL, 4 h; Worthington Biochem), as previously described (11). PMA-induced NETs were used for initial observations (Fig. 1A–C). Other experiments were done with GO-induced NETs. NETs were washed and blocked with 1% BSA (Calbiochem). NET-DNA was stained with Hoechst 33342 (1 μg/mL; Invitrogen) or SytoxGreen (1 μM, Invitrogen). Washed platelets were loaded with fluorescent Calcein-AM (2.5 μg/mL, 10 min, 37 °C; Invitrogen) or platelets in whole blood were labeled with Rhodamine 6G (5 μg/mL, 10 min, 37 °C; Sigma). NETs were perfused with ACD-anticoagulated blood supplemented with the irreversible thrombin inhibitor PPACK-Dihydrachloride (100 μM; Calbiochem) and recalcified by addition of 2 mM CaCl₂. Perfusions were carried out at a shear rate of 200 or 900 s⁻¹ and 37 °C using a peristaltic pump. If indicated, blood was supplemented with DNase1 (1 U/mL; Worthington Biochem) for 30 min. Culture supernatants were collected and centrifuged for 5 min at 10,000 × g. Aliquots were mixed with SDS reducing sample buffer. After 3 min at 95 °C, samples were subjected to 15% (wt/vol) SDS/PAGE, followed by an immunoblotting procedure as described (18). We used a polyclonal rabbit antibody against histone H2B (Abcam) as primary antibody and goat-anti-rabbit-IgG as secondary antibody. Detection was carried out with a Pierce ECL Western blotting substrate (Thermo Scientific).

**Electron Microscopy.** We induced NETs on glass coverslips and perfusion was performed using a parallel-plate flow chamber system (GlycoTech). NETs were washed and fixed with 2.5% glutaraldehyde and electron microscopy was performed as previously described (40).

**Quantification of RBCs and Platelets.** Cells firmly attached to collagen or NETs were lysed with 100 μL of 0.5% Triton × 100 in water. Hemoglobin content was measured using the method of Drabkin (41). To quantify platelets, Rhodamine-6G fluorescence of the sample was analyzed using a fluorometer.

**Plasma Coating of NETs.** Human NETs were induced on glass coverslips or in tissue-culture plates by stimulating cells with PMA (50 nM, 4 h). After the activation, NETs were incubated with 1% BSA for 1 h at 37 °C. NETs were washed and incubated with 50% plasma in PBS for 30 min at 37 °C. Next, NETs were washed and treated with DNase1 (100 U/mL, 10 min). After another wash, cells were fixed with paraformaldehyde (2%, 1 h at 37 °C) and unspecific binding sites were blocked with BSA (3%, 1 h at 37 °C). To visualize and quantify binding of VWF, fibrinogen, or fibronectin to NETs, samples were subjected to immunocytochemistry. Primary antibodies were used at 1 μg/mL in PBS supplemented with 1% BSA and 0.1% Triton X-100 (mouse-antifibrinogen, rabbit-antifibronectin (both Sigma); rabbit-anti-VWF, Chemicon). After incubation at 37 °C for 1 h, samples were washed with PBS and fluorescently conjugated secondary antibodies (Invitrogen) were applied at 10 μg/mL for 30 min at 37 °C. NET-DNA was stained with 1 μg/mL Hoechst 33258 (Invitrogen) for 15 min at 37 °C. Coverslips were mounted on slides and analyzed by fluorescence microscopy. DNA and Alexa 488 fluorescence was quantified for samples in tissue culture using a fluorescence microplate reader.

**Clot Lysis Studies.** Two-hundred-fifty microliters of 5 × 10⁶ neutrophils per milliliter in RPMI medium were activated by platelet activating factor (50 μM, Calbiochem) at 37 °C under static conditions to induce NET formation. Control samples were 250 μL medium alone, 250 μL of untreated neutrophils, or 250 μL of 50 μM PAF in medium. After 10 min, 250 μL of recalcified (40 mM CaCl₂) ACD anticoagulated blood was added. The mixture was incubated under stirring conditions (1,000 rpm) at 37 °C using an Eppendorf Thermomixer. Indicated samples were supplemented with tPA (25 μg/mL, Bacher) or DNase1 (100 U/mL, Worthington Biochem). After 20 min, the blood was passed through a 100-μm cell strainer to isolate the clot. Images were acquired and the clot was determined. Thereafter, 10-μm frozen sections of the clot were prepared and stained for fibrinogen (mouse-anti-human-fibrinogen, Sigma) and VWF (rabbit-anti-human-VWF, Chemicon). Isotype control antibodies were used to determine background staining and DNA was labeled fluorescently by Hoechst 33258 (Invitrogen).

**Baboon DVT model.** Juvenile male baboons were anesthetized and underwent iliac vein thrombosis by temporary balloon catheter occlusion (6 h) (24). Six days postthrombosis, the animal was humanely killed and both the thrombosed and nonthrombosed iliac veins were harvested. The iliac vein samples...
were then fixed and paraffin-embedded for immunohistochemical analysis. The animal research protocol was approved by the University of Michigan and its Committee on Use and Care of Animals.

**Baboon Plasma Analysis.** Blood was drawn from the left iliac vein using a 22-G vacutainer needle with a 4.5-mL sodium citrate vacutainer. After centrifugation at 3,350 × g for 15 min at room temperature, aliquots were flash-

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**Fig. 4.** Markers of NETs are abundant in baboon DVT. (A) Plasma DNA levels before (baseline: BL) or at indicated time points after initiation of DVT. Plasma DNA was elevated after 48 h (bar represents the mean value of groups; n = 3; Repeated measures ANOVA; **P < 0.01 compared with BL) and remained increased in baboon #1 at day 6. (B) Cross-section of thrombotic left iliac vein at day 6 stained for DNA. Figure is a composite of multiple micrographs of the thrombus. (Scale bar, = 500 μm.) (C–F) Immunostaining of the DNA core (arrow in B). (C) Staining for DNA shows a distinct dotted pattern indicating nuclei, as well as a diffuse pattern indicating extracellular DNA. (D) Positive staining for the DNA/H2A/H2B complex reveals that the extracellular DNA is of nuclear origin. (E) VWF strings are part of the DNA core. (F) Overlay of C, D, and E. (Scale bars C–F, 100 μm.) (G, H, and I) Immunostaining of area between DNA core and vessel wall (arrowhead in B). (G) DNA by SytoxGreen indicates nuclei (arrowhead) as well as extracellular DNA (arrow). (H) VWF in this area often colocalizes with extracellular DNA (arrow). (I) Overlay of G and H. (Scale bars G–I, 50 μm.) (J) Nuclei stained by SytoxGreen in an area free of extracellular DNA (arrowhead in B). (K) Extracellular histone H3 is abundantly present in this area although DNA is no longer detected. (L) Overlay of J and K. (Scale bars J–L, 20 μm.)
frozen in liquid nitrogen and stored in −80 °C. Time-points included: before (baseline), 6 h, 2 d, and 6 d postthrombus induction. We diluted plasma 10-fold in PBS and mixed diluted plasma with an equal volume of 1 μM of the fluorescent DNA dye SytoxGreen (Invitrogen) in PBS. Fluorescence was determined by a fluorescence microplate reader (Fluoroskan, Thermo Scientific). Samples were normalized to the mean of values obtained from plasma collected before induction of DVT (baseline).

**Immunohistochemistry.** We analyzed baboon DVT using a blood vessel staining kit (Millipore). We followed the manufacturer’s instructions, but replaced the antibodies. We used the following primary antibodies: mouse-antihistone H2A/H2B/DNA complex (42), rabbit-anti-VWF (Chemicon), and rabbit-antihistone H3 (Abcam). Primary and isotype control antibodies were employed at 1 μg/mL; fluorescently conjugated secondary antibodies (Invitrogen) at 10 μg/mL DNA was labeled with Hoechst 33342 (1 μg/mL, Invitrogen), or SytoxGreen (1 μM, Invitrogen).

**Statistical Evaluation.** Statistical analysis included mean ± SEM. ANOVA, Student’s t test, and repeated measures ANOVA. Results were considered significant at P < 0.05.

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Fuchs et al.

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Supporting Information
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Fig. S1. Aggregometry of platelets stimulated with human recombinant histone H4 (solid circles). EDTA (solid squares) and heparin (solid triangles) inhibited aggregation.

Fig. S2. von Willebrand factor (VWF), fibronectin, and fibrinogen bind to neutrophil extracellular traps (NETs). (A–C) Immunostaining of NETs. Human NETs were incubated with human plasma for 30 min, washed, and stained for VWF (A), fibronectin (B), or fibrinogen (C). A web-like staining indicates that these proteins bind to NETs. (Scale bars, 20 μm.) (D–F) Quantification showed that all three proteins were binding to NETs in a DNase-sensitive manner. Data are presented as mean ± SEM, n = 3; (Student’s t test); **P < 0.01; ***P < 0.001.
Fig. S3. NETs enable fibrin deposition. NETs shown in green were perfused with whole blood recalciﬁed with 20 mM CaCl₂ at the beginning of the experiment. To monitor fibrin formation, we added ﬂuorescent ﬁbrinogen (100 μg/mL). (A) Approximately 6 min after recalciﬁcation, we observed increased deposition of ﬁbrinogen along NET-DNA strings (arrows), which appeared to nucleate the formation of larger polymers (arrowheads). After 7 min, NETs did not withstand the increased shear forces and “embolized” together with the ﬁbrin. (B) NETs were perfused with recalciﬁed blood in the presence of the thrombin inhibitor PPACK (100 μM). Only traces of ﬁbrinogen could be detected and NETs did not embolize, even after 10 min of perfusion. This result indicates that NETs promote thrombin dependent ﬁbrin formation. (Scale bars, 100 μm.)
Fig. S4. NETs intercalate within a fibrin clot and generate a tissue plasminogen activator- (tPA) resistant scaffold. We induced fibrin polymerization in blood by recalcification and incubated it either in the absence (medium) or presence of neutrophils preactivated by PAF to induce NET formation (neutrophils + PAF). Samples were treated with DNase to digest extracellular traps or tPA for fibrin digestion. (A) Photographs of clots. In the absence of activated neutrophils, clots were rich in RBCs (#1), coincubation with DNase had no effect (#2), and tPA alone (#3), or together with DNase (#4) prevented clot formation. In the presence of activated neutrophils, blood clot appearance and size (#5) did not change even in the presence of DNase (#6). The tPA reduced clot size (#7) and prevented clot formation only in combination with DNase (#8). (Scale bar, 1 cm.) (B) Quantification of A by weighing the clots. The presence of PAF or unstimulated neutrophils alone did not produce a tPA-resistant clot. Data are presented as mean ± SEM, n = 4; (ANOVA; ***P < 0.001). (C–G) Immunohistological analysis showed that in the absence of tPA (C–E) all clots contained a fibrin meshwork (green). Nuclei of leukocytes (blue) and platelets labeled by VWF (red) were part of the clots. (D and G) NETs were incorporated into the fibrin network in clots containing preactivated neutrophils. (G) Higher magnification of the rectangle in D. Extracellular DNA fibers colocalized with fibrin and platelets distributed over the fibrin meshwork. (E) DNase removed NETs but not fibrin. (F) The tPA removed fibrin but NETs still provided a clot scaffold. (Scale bars: F, 50 μm; G, 20 μm.)
**Fig. S5.** The iliac vein not subjected to balloon catheter occlusion does not show indication of NETs. (A) Cross-section of the vessel stained for DNA. Without a thrombus, the vessel deflated and the lumen appeared empty. Figure is a composite of multiple micrographs of the vessel. (Scale bar, 500 μm.) (B) DNA staining indicating nuclei of the vessel wall. (C) Staining for the DNA/H2A/H2B complex labeled nuclei. (D) VWF staining demarcates the endothelium. (E) Overlay of B–D. (Scale bars, 50 μm.)

**Movie S1.** Time-lapse movie of platelets (red) in whole blood and perfused over NETs (green). The movie starts 30 s after the onset of perfusion. The time is displayed in the upper left corner (HH:MM:SS). After 10 min, DNase was added which degraded NETs and removed platelet aggregates. (Scale bar, 100 μm.)
Movie S2. Time-lapse movie of platelets (red) in whole blood and perfused over NETs (green) at venous shear (200/s). The movie starts 120 s after the onset of perfusion. The time is displayed in the upper left corner (HH:MM:SS). After 8 min, DNase was added which degraded NETs and removed platelet aggregates. (Scale bar, 100 μm.)

Movie S3. Time-lapse movie of NETs (green) perfused (shear rate 900/s) with RPMI medium. The time is displayed in the upper left corner (HH:MM:SS). After 5 min (at 00:00:00), 500 μg/mL heparin was added. The addition of heparin caused the destabilization of NETs. Ten minutes after the addition of heparin only traces of NETs were present. (Scale bar, 100 μm.)