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Tissue Factor Activity under Flow

Scott L. Diamond, PhD
Institute for Medicine and Engineering, Department of Chemical and Biomolecular Engineering, University of Pennsylvania, Philadelphia, PA 19104, (215)-573-5702 tel, (215)-573-7227 fax

Scott L. Diamond: sld@seas.upenn.edu

Abstract

Coagulation processes under flow conditions are fundamentally different when compared to whole blood clotting in a tube. Due to red blood cell migration toward the center of the vessel, platelet concentrations are elevated several-fold in the plasma layer near the wall or thrombus. Evaluation of platelet function, coagulation proteases, and pharmacological agents can utilize *closed systems* of constant volume that lack flow (eg. intracellular calcium measurement, automated calibrated thrombography) or include flow (eg. aggregometry or cone-and-plate viscometry). However, these laboratory approaches fail to recreate the fact that intravascular thrombosis is an *open system* where blood is continually flowing over a thrombotic site. In open systems, the rapid accumulation of platelets at a surface leads to platelet concentrations greatly exceeding those found in whole blood and the delivery/removal of species by convection may impact the efficacy of pharmacological agents. During a clotting event under flow, platelets can accumulate via adhesion receptors to concentrations that are 10 to 50-fold higher than that of platelet-rich plasma. Using controlled in vitro perfusions of whole blood, it is possible to determine the critical level of surface tissue factor needed to trigger full scale coagulation on collagen. Such in vitro perfusion systems also allow a determination of the potency of anti-platelet agents as a function of wall shear rate.

Keywords

thrombosis; platelet; shear rate; coagulation

Tissue factor at venous and arterial flows

To evaluate how surface signals (collagen, and tissue factor) control the growth of a thrombus under controlled flow conditions, we printed microarrays with collagen features containing various concentrations of lipidated tissue factor (TF) from 0 to 25 molecules per μm^2. These microarrays were then mounted on parallel-plate flow chambers and perfused with recalcified citrated whole blood with corn trypsin inhibitor (CTI to block biomaterial activation of Factor XII). During these clotting events under flow, platelets can accumulate via adhesion receptors to concentrations that are 10 to 50-fold higher than that of platelet rich plasma [1].

Without added surface TF, the amount of fibrin formed during a 5 minute perfusion at a wall shear rate of 100, 500, or 1000 s^{-1} was negligible, indicating that “blood borne” TF was not

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kinetically significant over this time frame. A critical threshold level of TF between 2 and 10 molecules/μm^2 was required to trigger robust thrombosis with fibrin formation. The calculated EC50 to cause 50 % maximal response increased modestly from 3.6 to 10.2 molecules-TF/μm^2 as the wall shear rate was increased 10-fold from venous levels (100 s^{-1}) to arterial levels (1000 s^{-1}). From these studies, the operative concentrations of surface TF required to proceed from negligible to maximal production of fibrin was constrained to a very narrow window from 2 to 10 molecules-TF/μm^2 suggesting a switch-like function, consistent with prior theoretical predictions [2]. The reported amount of TF underneath plaques is 33 pg-TF/cm2 (~ 6 molecules-TF/μm^2) [3]. In contrast to these results with flowing blood, the titration of TF into diluted whole blood under no-flow conditions enhances clotting speed over a wide range from ~100 fM to 100 pM.

To test how an intense coagulation response over a TF-laden collagen feature can propagate in time and space under flow conditions, we printed microarrays where only the center lane of collagen spots contained TF while adjacent lanes of spots presented only collagen. This experiment was designed to understand if production of soluble species (eg ADP, thromboxane, thrombin) can amplify clotting on nearby collagen features lacking TF. We found that fibrin formation remained highly localized over the collagen features presenting TF, while features only 250 microns away lacked any fibrin formation. This result was consistent with numerical simulations of thrombin release from the TF-containing features into a flow field where thrombin could diffuse only short distances normal to the surface or transverse to the flow direction. Thus, flow rapidly convects all thrombin downstream to prevent lateral growth of a thrombus away from a TF-rich zone.

To study the interaction of ultralow levels of circulating TF with surface presented TF, we supplemented whole blood with 100 fM of lipidated TF. Under no-flow conditions, this level of TF caused no detectable production of thrombin within 5 minutes of addition, but reduced whole blood clotting times (assayed by TAT ELISA or the thrombin substrate boc-VPR-MCA) from 60 min to between 20 and 40 minutes. When CTI-treated whole blood (± 100 fM TF) was perfused over TF-microarrays for 5 minutes, the presence of the added TF caused a 2.5-fold increase in fibrin deposition. The addition of 100 fM TF had no effect on fibrin production on the microarrays in the absence of flow, indicating that the amplification of coagulation by 100 fM circulating TF required the action of flow (possibly via delivery of platelets to the thrombus surface).

Microfluidic devices for the study of coagulation under flow

More recently, in an effort to conduct flow experiments with low volumes of blood and with small quantities of antibodies, we developed microfluidic devices to perfuse blood over a focal patch of collagen [4]. These devices are particularly useful for experiments using mouse blood where < 100 uL is available for clotting under venous or arterial flow conditions. These devices can now operate with 8 parallel channels for simultaneous measurement of 8 clotting events which allows the measurement of IC50s under flow conditions [5]. The $P2Y_1$ inhibitor MRS 2179 (IC_{50} = 0.233 ± 0.132 μM) and $P2Y_{12}$ inhibitor 2-MeSAMP (IC_{50} = 2.558 ± 0.799 μM) were potent blockers of secondary platelet accumulation under flow, while the $P2X_1$ inhibitor (NF 449) and apyrase failed to reduce platelet accumulation. MRS 2179 and 2-MeSAMP had undetectable effects on initial platelet adhesion to collagen. These results demonstrate that certain therapeutic approaches may show different efficacy whether conducted in closed versus open systems. For example, apyrase which is potent at 1 U/ml in a closed system with whole blood shows little efficacy under flow at 1 U/ml because of the dramatic build up of platelets and platelet release products at the thrombus site and the relatively slow kinetics by which ADP is degraded. In fact, apyrase was shown to enhance clotting under flow due to its conversion of released ATP to ADP [5].

It is clear that microfluidic devices have become an increasingly powerful tool for the study of blood function in the presence of thrombotic stimuli. Lipidated surfaces presenting TF but lacking collagen have been utilized [6] and novel flow system designs allow several shear rates to be tested in a single run [7].

Summary

Overall, we conclude that (1) a critical threshold level of TF above ~10 molecules/μm^2 will trigger robust and rapid clotting on collagen at physiological wall shear rates; (2) active blood borne TF present in healthy donors is likely < 100 fM in concentration and is not kinetically significant in rapid clotting events triggered by surface TF; (3) 100 fM circulating TF can amplify a clotting event triggered by surface TF in the presence of flow, indicating that disease states that result in circulating TF at levels as low as 100 fM may present a risk for elevated thrombotic response.

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