Synergy Between Tissue Factor and Exogenous Factor XIa in Initiating Coagulation

Karin Leiderman, William C. Chang, Mikhail Ovanesov, Aaron L. Fogelson

**Objective**—Recent evidence suggests involvement of coagulation factor XIa (FXIa) in thrombotic event development. This study was conducted to explore possible synergies between tissue factor (TF) and exogenous FXIa (E-FXIa) in thrombin generation.

**Approach and Results**—In thrombin generation assays, for increasing concentrations of E-FXIa with low, but not with high TF concentrations, peak thrombin significantly increased whereas lag time and time to peak significantly decreased. Similar dependencies of lag times and rates of thrombin generation were found in mathematical model simulations. In both in vitro and in silico experiments that included E-FXIa, thrombin bursts were seen for TF levels much lower than those required without E-FXIa. For in silico thrombin bursts initiated by the synergistic action of TF and E-FXIa, the mechanisms leading to the burst differed substantially from those for bursts initiated by high TF alone. For the synergistic case, sustained activation of platelet-bound FIX by E-FXIa, along with the feedback-enhanced activation of platelet-bound FVIIIa and FXa, was needed to elicit a thrombin burst. Furthermore, the initiation of thrombin bursts by high TF levels relied on different platelet FIX/FIXa binding sites than those involved in bursts initiated by low TF levels with E-FXIa.

**Conclusions**—Low concentrations of TF and exogenous FXIa, each too low to elicit a burst in thrombin production alone, act synergistically when in combination to cause substantial thrombin production. The observation about FIX/FIXa binding sites may have therapeutic implications. (*Arterioscler Thromb Vasc Biol. 2016;36:2334-2345. DOI: 10.1161/ATVBAHA.116.308186.)*

**Key Words:** blood coagulation ▪ blood platelets ▪ theoretical models ▪ thrombin ▪ thrombosis

Thrombin generation occurs through a series of overlapping initiation, amplification, and propagation stages that involve platelets and TF-bearing cells such as fibroblasts and monocytes.1–3 Based on in vitro experiments and in vivo observations, it is believed that in vivo thrombin generation in response to a vessel injury begins with the production of small amounts of coagulation factor IXa (FIXa) and FXa by the tissue factor (TF):FVIIa complex on a TF-bearing surface (subendothelium) at the site of injury. The FXa may activate its cofactor FV on the subendothelium, form prothrombinase, and generate small amounts of thrombin. If collagen in the subendothelium is also exposed, platelets that adhere may become directly activated by the collagen and provide an additional source of FV and FXa.4 The amplification stage includes the small amounts of thrombin activating more platelets, platelet-associated coagulation cofactors (also activated by small amounts of FXa), and FXI. Together, these lead to FIXa, FVIIa, and FXa becoming present on the activated platelets’ surfaces. Finally, in the propagation stage, tenase complexes (FVIIa:FIXa) form on platelet surfaces and begin to rapidly produce FXa. The locally made FXa binds to the platelet-bound FVa to make large quantities of prothrombinase (FXa:FVa) and an explosive burst of thrombin results.

Along with these biochemical reactions, biophysical mechanisms are important for thrombin generation in vivo. In particular, in addition to their procoagulant role in providing specific binding sites for the coagulation proteins,5–8 platelets play an anticoagulant role when they adhere to and pave over the TF-bearing cells, physically blocking the TF:FVIIa activity.9,10 Our group’s mathematical model predicted that thrombin generation exhibits a threshold dependence on exposed TF levels.11 Experiments directly followed that confirmed the threshold behavior in vitro with flow, but not without.11,12 Thus, explosive thrombin generation under flow may occur if there is sufficient TF exposure, but this is affected by the rate and extent of platelet coverage. The mathematical models further showed that the occurrence of a thrombin burst depends on the formation of a sufficiently high density of tenase (FVIIa:FIXa) on activated platelets before activity of TF:FVIIa is blocked because adherent platelets cover the subendothelium.13

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2334
**Nonstandard Abbreviations and Acronyms**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>E-FXIa</td>
<td>Exogenous FXIa</td>
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<tr>
<td>IGIV</td>
<td>Immunoglobulin intravenous</td>
</tr>
<tr>
<td>TF</td>
<td>Tissue factor</td>
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<tr>
<td>vWF</td>
<td>Von Willebrand factor</td>
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Because FXIa is one of the two components within the tenase complex, it is key to propagation of thrombin generation. FXI is activated by TF:FVIIa in the initiation stage and also by FXIa in the propagation stage. Although FXIa is not characterized as having an important role during initiation, it is essential (and distinct from FXa) in the propagation phase. This is because it binds to the platelet surfaces early, priming them for tenase formation once there is sufficient available FVIIIa. Although trace amounts of FXIa with no TF have been shown to induce a thrombin burst in static coagulation experiments, our previous simulations of intravascular coagulation under flow indicate that for low levels of exposed TF, the tiny amounts of FXIa produced by TF:FVIIa during initiation are insufficient to induce a thrombin burst. However, if there were another way to activate FIX during initiation, it might be possible to generate large amounts of thrombin even for low levels of exposed TF.

With this in mind, we consider FXIa. There is growing evidence that either FXI or FXIa is involved in the development of thrombotic events. For example, thrombotic events after treatments with immunoglobulin intravenous (IGIV) are known to be associated with FXIa contamination. Adverse thrombotic events associated with IGIV were first reported in 1986 and in 2003, black-box warnings for IGIV were requested by the Food and Drug Administration. Studies showed that some off-the-shelf IGIV products were able to generate thrombin, and that these same products were contaminated with FXIa. Another line of evidence is the thrombogenicity of FXI concentrates used to treat FXI-deficient patients. The addition of antithrombin or C1 inhibitor stabilized thrombogenic FXI concentrates and led to much lower elevation of coagulation markers in humans. Coagulation factors FIX, FX, and prothrombin were activated in baboons given FXIa. Finally, many studies have measured either circulating FXIa or FXIa-like activity in blood from patients with thrombotic events.

Circulating FXI and FXIa play different roles in thrombin generation and the clotting response. Zymogen FXI is activated (slowly) by thrombin during the amplification stage before it can take on a procoagulant role and activate FIX. Hence, the effects of thrombin-activated FXIa may occur after there has already been a significant thrombin response as reported by Panteleev et al. and von dem Borne et al. Previous simulations with our model support this possibility in that they showed that the threshold level of TF exposure was insensitive to the presence of thrombin-activated FXIa. In contrast, circulating FXIa would presumably activate FIX in the initiation stage and thus might have a powerful impact on thrombin generation, even for low levels of exposed TF. In this study, we use a mathematical model of coagulation under flow together with an in vitro thrombin generation assay to explore how varying levels of TF and exogenous FXIa (E-FXIa) affect thrombin generation. Using the in silico model, we elucidate a biochemical mechanism that explains the difference in lag times with high TF alone versus the combination of low TF and low E-FXIa.

**Materials and Methods**

The simulations performed in this article were conducted using the mathematical model described by Fogelson et al. Schematics of the reactions and of the physical setting in which the reactions are assumed to occur are given in Figure M-I in the online-only Data Supplement. A complete listing of the reactions included in the model and of the parameter values used in the simulations are given is Tables M-I through M-VII in the online-only Data Supplement. Listing of the model’s differential equations is present in the study by Fogelson et al. and further discussions of the model’s derivation and behavior are available in the study by Kuharsky and Fogelson. Details about the thrombin generation assays are available in the Materials and Methods in the online-only Data Supplement.

**Results**

**Computer Studies Show Threshold Behavior and Synergy**

In silico experiments involving platelet deposition and coagulation were conducted in a segment of a blood vessel. These simulations involved all of the reactions depicted in Figure M-IA in the online-only Data Supplement. The reactions were assumed to occur in a reaction zone (Figure M-IB in the online-only Data Supplement), which included the site of a small-vessel wall injury (10–20 μm long), represented by a collagen-exposing and TF-bearing lipid surface; liquid flowing blood that carries zymogens, unactivated platelets, and exogenous FXIa; and accumulated activated platelets that present binding sites for the coagulation proteins. We assume that each species is well mixed within the small reaction zone and represent it by its average concentration in the zone. The model consists of a system of ordinary differential equations that tracks separately the concentrations of each type of protein, for example, FXa, in the plasma and bound to the subendothelial or platelet surfaces. The inputs for individual simulations include zymogen concentrations, platelet count, and the flow shear rate. The outputs include the time course of the concentrations of all of the coagulation proteins, unactivated platelets, and accumulated wall-bound platelets. Below we discuss behaviors of key coagulation proteins. We note that, during a simulation, platelets accumulate in the reaction zone to concentrations of up to 100x their bulk concentration (250,000 per microliter) in the blood.

Figure 1A through 1C displays plasma thrombin concentrations at 10 and 20 minutes from simulations in which we specified a density (amount/area) of TF, [TF]a, initially exposed by injury and a concentration of exogenous FXIa, [E-FXIa], in the bulk plasma. Figure 1A shows that, without E-FXIa, thrombin has a threshold dependence on [TF]a. The threshold at 10 minutes (blue curve) is at $\approx 7$ to 9 fmol/cm²,
whereas at 20 minutes (red curve) a thrombin burst occurs for all \([\text{TF}]_d>3.2\) fmol/cm\(^2\). For \([\text{TF}]_d<1\) fmol/cm\(^2\), little thrombin is produced, consistent with results in our earlier studies.\(^{10,13}\) In the presence of E-FXIIa, threshold behavior persists, but shifts to much lower densities of TF as shown in Figure 1B and 1C. The heat-maps in these figures show the concentration of thrombin after 10 minutes (B) and 20 minutes (C) of clotting activity for various \([\text{TF}]_d\) and \([\text{E-FXIIa}]\) values. Substantial thrombin is produced by 10 minutes when \([\text{TF}]_d>0.14\) fmol/cm\(^2\) and \([\text{E-FXIIa}]>14\) pmol/L and by 20 minutes comparable amounts are produced for a much wider range of both TF and E-FXIIa \(([\text{TF}]_d>0.02\) fmol/cm\(^2\) and \([\text{E-FXIIa}]>4\) pmol/L). Thus, even small concentrations of E-FXIIa sharply increase the system’s response to exposed TF; there is a strong synergy the two stimuli. After a burst, thrombin plateaus at a concentration of 70 to 90 nmol/L for shear rate 100 per second (Figure 1C). For lower shear rates, synergy was also seen (Figure 1 in the online-only Data Supplement) but for slightly higher \([\text{TF}]_d\) and \([\text{E-FXIIa}]\) yielded higher thrombin plateaus. The in silico simulations normally include endogenous FXI and, during a simulation, thrombin activates some of this FXI to produce endogenous FXIIa. For simulations without endogenous FXI, the threshold for a thrombin burst was only slightly different (not shown). This indicates that endogenously produced FXIIa does not contribute to synergistic behavior in a meaningful way. The reason is that endogenous FXII activation occurs slowly and only after thrombin has formed. The thrombin concentration plateau was somewhat higher without endogenous FXI because no thrombin was sequestered in complex with FXI in that case.

**Dynamics of Intravascular Thrombin Production**

Figure 1D through 1F shows plasma thrombin concentrations from 3 sets of simulations; in each set, \([\text{TF}]_d\) was held fixed and \([\text{E-FXIIa}]\) was varied. For \([\text{TF}]_d=10\) fmol/cm\(^2\), a value that
is above the threshold without E-FXIa (Figure 1A); E-FXIa dose dependently decreased the lag time before a thrombin burst occurred but had little effect on the plateau thrombin concentration (≈93 nmol/L). (Note that the constant influx of prothrombin with the flow leads to a thrombin plateau, not the peak seen in static experiments as in Figure 2.) For all levels of [TF]₀, increasing the [E-FXIa] shortened the lag time in a dose-dependent manner with higher sensitivity to [E-FXIa] and longer lag times occurring at lower [TF]₀. Figure 1H shows that the thrombin velocity, that is, the maximum relative rate of thrombin concentration increase \( \frac{d[\text{Thrombin}]}{d t} \), was largely independent of [TF]₀ for each [E-FXIa], but that it increased with [E-FXIa] (Section 1.2, especially Figure II, in the online-only Data Supplement, for further discussion). Thus, in these simulations, [TF]₀ was important in determining whether a thrombin burst occurred and what the lag was before the burst, but it only weakly influenced how fast thrombin increased during the burst.

For bursts elicited by high [TF]₀ without E-FXIa, the story is different. In that situation, the thrombin velocity became progressively larger as [TF]₀ increased from 5 to 25 fmol/cm² and the lag time decreased (Figure III in the online-only Data Supplement). Figure II shows that for initiation by a combination of TF and E-FXIa, the thrombin plateau was only mildly sensitive to [TF]₀ and [E-FXIa]. These results indicate that the dynamics induced by high [TF]₀ alone are different than those triggered by a combination of low [TF]₀ and low [E-FXIa]. Below we compare the time courses for these 2 situations in more detail.

In Figure 2, we show results from in vitro thrombin generation experiments as described in the Materials and Methods in the online-only Data Supplement. These experiments were performed without flow, with corn tyrpsin inhibitor–treated normal pooled human plasma, supplemented with human platelets to a final platelet concentration of about 250,000 per microliter, and added TF-bearing vesicles. The top panels show the time course of the thrombin concentration for various TF and E-FXIa concentrations. For [TF]=5 pmol/L (Figure 2A); no E-FXIa was needed to cause a burst of thrombin production. For larger [E-FXIa], the lag time decreased slightly and the peak height increased, both in a dose-dependent way. For [TF]=0.06 or 0.02 pmol/L (Figure 2B and 2C), a thrombin burst occurred only for sufficiently high [E-FXIa]. For the higher doses of E-FXIa used, the peak heights were similar to those seen for higher [TF], but the lag times were progressively longer with smaller [TF]. For lower levels of [E-FXIa] that still produced a burst, the resulting lag times were longer with corresponding peak heights reduced. Figure 2D and 2E shows that the lag time and time to the peak both decreased with increasing [TF], for each [E-FXIa]; these changes in timing were more significant at lower [E-FXIa]. The peak heights always increased (Figure 2F) when [TF] or [E-FXIa] increased. For higher [E-FXIa], the peak heights were only weakly dependent on [TF], but
for lower [E-FXIa], they varied substantially with [TF]. For combinations of very low [TF] and very low [E-FXIa], no burst was seen. The dependence of lag times on [TF] and [E-FXIa] in the in vitro experiments is similar to that seen in the model simulations.

In both the mathematical simulations and in vitro experiments, we see that small doses of TF and E-FXIa, each far from sufficient to elicit a burst in thrombin production alone, act synergistically when in combination to cause substantial thrombin production. The thrombin concentration after 20 minutes in the in silico simulations for [TF] = 10 [TF] and no E-FXIa is very close to that seen with [TF] = 0.02 fmol/cm² with [E-FXIa] = 10 pmol/L. Similarly, in the in vitro experiments, [TF] = 0.5 pmol/L alone (Figure 2F, black curve) leads to a peak height similar to that seen with the much lower [TF] = 0.006 pmol/L with [E-FXIa] = 0.6 pmol/L (Figure 2F, dark blue curve). In the following, we focus on two of the simulations, the one with [TF] = 10 fmol/cm² and no E-FXIa, which we denote TF^pFXIa−, and the one with [TF] = 0.02 fmol/cm² and [E-FXIa] = 10 pmol/L, which we denote TF^sFXIa+. It is noteworthy that as shown in Figure 3A and 3B (red curves), there are significant amounts of platelet-bound FVIIIa (Plt-FIXa) available very early for both TF^pFXIa− and TF^sFXIa+, so what is needed for FVIIIa:FIXa formation is a sufficient concentration of Plt-FVIIia. In the simulations, we assume that Plt-FVIIia may form by activation of Plt-FVIII by either platelet-bound FXa or thrombin or by binding of thrombin-activated FIXa from the plasma.38,39 In Figure 3A, we see that for TF^pFXIa−, the Plt-FVIIia concentration rises sharply between 200 and 400 seconds (green curve), after an earlier rise in Plt-FXa (blue curve). A sharp rise in the concentration of FVIIIa:FIXa occurs shortly thereafter (Figure 3C, blue curve) and is closely followed by sharp rises in the prothrombinase and thrombin concentrations (not shown). For TF^sFXIa+, the sharp rises in Plt-FXa and then Plt-FVIIia occur much later (Figure 3B), and consequently, formation of FVIIIa:FIXa (Figure 3C, red curve), prothrombinase (not shown) and the thrombin burst (not shown) are substantially delayed. There is more to the story than just a delay; in the simulations, the dynamics of FVIIIa:FIXa formation for TF^sFXIa− are significantly different from those for TF^pFXIa−.

Figure 4A and 4B shows the rates of production of Plt-FVIIia by different mechanisms. For both TF^pFXIa− and TF^sFXIa+, FXa-mediated activation of FVIII on platelet surfaces occurs earliest and is the primary means of producing Plt-FVIIia for ≈200 seconds. Later, thrombin-mediated activation of FVIII on the platelet surface becomes the dominant way Plt-FVIIia is produced. Activation of plasma FVIII by thrombin followed by its binding to the platelet surface always contributes less. Although the lag time to the rise of Plt-FVIIia for TF^pFXIa− is much longer than for TF^sFXIa−, the rates of activation of Plt-FVIIia by Plt-FXa starting after the respective lag times are comparable in the 2 cases.

Figure 4C and 4D shows how Plt-FXa is created in these simulations. For TF^pFXIa−, TF^pFXIa produces substantial FXa and, although <1% of it binds to platelet surfaces because most is washed away by the flow, this is how most Plt-FXa is produced for the first 200 seconds (Figure 4C). This Plt-FXa

**Mechanisms Underlying Different Time Courses**

In the in silico simulations, a thrombin burst occurred for both TF^pFXIa− and TF^sFXIa+, but in the latter case, the burst happened much later. Similar behavior was seen in the in vitro experiments. In this section, we elucidate the biochemical and biophysical mechanisms behind the different response times in the simulations. In doing so, we make use of the fact that the simulations provide concentrations of all of the coagulation species shown in Figure M-IA in the online-only Data Supplement, as well as the rates at which the individual reactions shown there occur. Understanding the reason for these differences in the simulations may provide hints to what underlies the analogous differences in the experiments.

The critical event in determining whether and when a thrombin burst occurs is the formation of sufficient quantities of the platelet-bound FVIIia:FIXa complex.13 As shown in Figure 3A and 3B (red curves), there are significant amounts of platelet-bound FIXa (Plt-FIXa) available very early for both TF^pFXIa− and TF^sFXIa+, so what is needed for FVIIIa:FIXa formation is a sufficient concentration of Plt-FVIIia. In the simulations, we assume that Plt-FVIIia may form by activation of Plt-FVIII by either platelet-bound FXa or thrombin or by binding of thrombin-activated FIXa from the plasma.38,39 In Figure 3A, we see that for TF^pFXIa−, the Plt-FVIIia concentration rises sharply between 200 and 400 seconds (green curve), after an earlier rise in Plt-FXa (blue curve). A sharp rise in the concentration of FVIIIa:FIXa occurs shortly thereafter (Figure 3C, blue curve) and is closely followed by sharp rises in the prothrombinase and thrombin concentrations (not shown). For TF^sFXIa+, the sharp rises in Plt-FXa and then Plt-FVIIia occur much later (Figure 3B), and consequently, formation of FVIIIa:FIXa (Figure 3C, red curve), prothrombinase (not shown) and the thrombin burst (not shown) are substantially delayed. There is more to the story than just a delay; in the simulations, the dynamics of FVIIIa:FIXa formation for TF^sFXIa− are significantly different from those for TF^pFXIa−.

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**Figure 3.** Coagulation factor (FVIIia):FIXa formation and FXa production on platelets in in silico simulations. For A, TF^pFXIa− ([TF] = 10 fmol/cm² and exogenous FXa [E-FXa] = 0); and B, TF^sFXIa− ([TF] = 0.02 fmol/cm² and [E-FXa] = 10 pmol/L), time course of platelet-bound FIXa ( ). FVIIIa (g), and FXa (h). C, Time course of FVIIia:FIXa for TF^pFXIa− (b) and TF^sFXIa− (j). The onset of the thrombin burst is indicated by the dashed lines which indicate when the plasma thrombin concentration reached 1 and 30 nmol/L in the respective simulations. Platelet count 250,000 per microliter. Shear rate 100 per s. TF indicates tissue factor.
activates sufficient FVIII on the platelets (Figure 4E) so that a thrombin burst results by 400 seconds (Figure 1D, cyan curve). After the burst has started, FVIIIa:FIXa produces much more Plt-FXa. For TF LFXIa+, TF:FVIIa activates FXa \approx 1000\text{-}fold more slowly than for TF HFXIa−, and thus, much less plasma FXa binds to platelets and the Plt-FXa concentration only becomes significant around 600 seconds (Figure 4B) because of FVIIIa:FIXa (Figure 4B and 4D, red curves). This prompts the question: For TFLFXIa+, where does the Plt-FVIIIa come from to allow FVIIIa:FIXa to produce this additional platelet-bound FXa? The answer is that Plt-FVIIIa and Plt-FXa increase together by a bootstrap feedback process; a little Plt-FVIIIa allows some FVIIIa:FIXa formation that activates additional Plt-FXa that in turn activates more Plt-FVIIIa and allows further FVIIIa:FIXa formation. This story is suggested by the rate curves in Figure 4B and 4D and is confirmed by the simulation experiments shown in Figure 4E and 4F.

For simulations shown in Figure 4E and 4F, we slightly modified the model to allow us to track separately the FXa made by TF:FVIIa and the FXa made by FVIIIa:FIXa. Furthermore, we blocked the ability of FVIIIa:FIXa–activated Plt-FXa to activate Plt-FVIII, while allowing all of its other functions to occur as usual. Plt-FXa formed by the direct binding of TF:FVIIa–activated FXa retained its ability to activate Plt-FVIII. Figure 4E shows the concentrations of Plt-FXa containing FXa activated by TF:FVIIIa (solid blue curve) or by FVIIIa:FIXa (dashed blue curve), for TF6FXIIa+. Comparing the green and dashed blue curves in Figure 4E to the green and blue curves in Figure 3A, we can see that these model modifications did not change the time courses of Plt-FVIIIa and Plt-Xa concentrations, or the subsequent formation of prothrombinase and thrombin (not shown). For an analogous experiment for TF6FXIIa+ in which only TF:VIIa–activated FXa was allowed to activate FVIII on platelet surfaces, Plt-FVIIIa and Plt-FXa production were delayed and attenuated (Figure 4F). Comparing these results with those in Figure 3B, we see that without the bootstrap feedback process, the Plt-FXa and Plt-FVIIIa concentrations are at least 2 orders of magnitude lower. As a consequence, this simulation did not produce a thrombin burst, even after 20 minutes, showing that this feedback loop is essential for the production of thrombin in the TF6FXIIa+ situation.

Role of Platelet FIX/FIXa Binding Sites

For the simulations described above, each activated platelet was assumed to have equal numbers of 2 types of binding sites to which FIXa can bind.\textsuperscript{40–43} One is a shared binding site for which FIXa and FIX compete. We call these shared receptors

![Figure 4](http://atvb.ahajournals.org/)

**Figure 4.** Differences in platelet-bound coagulation factors FVIIIa and FXa formation in in silico experiments for (left) TF6FXIIa− ([TF]d = 10 fmol/cm\(^2\) and exogenous FXa [E-FXa]= 0) and (right) TF6FXIIa+ ([TF]d = 0.02 fmol/cm\(^2\) and [E-FXa]=10 pmol/L). A and B, Rates of formation of platelet-bound FVIIIa by activation of FVIII on platelet by FXa (r), by activation of FVII on platelet by thrombin (b) and by the binding of fluid-phase FVIIIa to platelet surfaces (g). C and D, Rates of production of FXa by TF:FVIIa (g) and by FVIIIa:FIXa (r) and rates of binding of fluid-phase FXa to the platelet surfaces (b). E and F, To test the importance of FXa produced on the platelet for platelet-bound FVIII activation and FVIIIa:FIXa formation, we blocked the ability to activate FVIII only for FXa produced on the platelets. The time courses shown in E for TF6FXIIa− are little different from those shown in Figure 3A, whereas those in F for TF6FXIIa+ are very much reduced compared with those in Figure 3B. The onset of the thrombin burst is indicated by the dashed lines, which show when the plasma thrombin concentration reached 1 and 30 nmol/L in the respective simulations. Platelet count 250,000 per microliter, shear rate 100 per s.
and denote them $\text{PFIX/FIXa}^{\text{shared}}$. The other is one to which FIXa, but not FIX, can bind, and we call these exclusive receptors and denote them $\text{PFIX/FIXa}^{\text{exclusive}}$. We refer to FIXa bound to the $\text{PFIX/FIXa}^{\text{shared}}$ and $\text{PFIX/FIXa}^{\text{exclusive}}$, respectively. Each of these can bind with Plt-FVIIIa to form the respective tenase complexes $\text{FVIIIa:FIXa}^{\text{shared}}$ and $\text{Plt-FIXa}^{\text{exclusive}}$, which we refer to collectively as the tenase complexes. The Plt-FIXa and FVIIIa:FIXa concentrations displayed in Figures 3 and 4 are the total concentrations for FIXa bound to either type of binding sites. We emphasize that the synergistic response to TF and E-FXIa stimulation shown earlier does not depend on the assumption that platelets have 2 types of FIXa binding sites. A synergistic response with approximately the same thresholds also occurs if platelets have either distinct sites for FIX and FIXa, (Figure S-IV, top, in the online-only Data Supplement), or only shared receptors (Figure S-IV, bottom, in the online-only Data Supplement). We showed that the primary effect of the exclusive binding sites is to allow TF-initiated thrombin bursts to occur at much lower TF levels than when only shared binding sites are present. The story is different for bursts initiated by low TF levels in conjunction with E-FXIa. When all 500 binding sites for FIX and FIXa on each activated platelet can bind FIX, there is a higher concentration of Plt-FIX to be activated by Plt-FXla. Hence, synergistic initiation of thrombin bursts happens at even lower $[\text{TF}]_d$ when each activated platelet has 500 shared FIX/FIXa binding sites. Compare Figure 1 with Figure S-IV, bottom, in the online-only Data Supplement.

Figure 3A and 3B shows that the total Plt-FIXa concentration rises early and rapidly for both the TF$^0$FIXa$^-$ and TF$^+$FIXa$^+$ simulations. For TF$^0$FIXa$^-$, it is overwhelmingly the Plt-FIXa$^{\text{exclusive}}$ concentration that is increasing during this period, while for TF$^+$FIXa$^+$, it is primarily the Plt-FIXa$^{\text{shared}}$ concentration that is rising (not shown). This can be inferred from Figure 5A and 5B where we show the rates of formation of platelet-bound FIXa by different routes. The rate of change of the Plt-FIXa concentration is affected by the rates at which FIXa in the plasma binds to the platelet $\text{PFIX/FIXa}^{\text{shared}}$ or $\text{PFIX/FIXa}^{\text{exclusive}}$ binding sites and by the rate at which Plt-FIX is activated by Plt-FXla. For TF$^0$FIXa$^-$, the early increase in Plt-FIXa is almost entirely because of the plasma FIXa binding to the exclusive sites (Figure 5A). During this time, there is little FXla and so TF:FVIIa is by far the dominant source of FIXa activation.

**Figure 5.** Production of platelet-bound coagulation factor FIXa and timing of FVIIIa:FIXa formation in in silico experiments for (left) TF$^+$. FIXa$^-$ ([TF]$=10$ fmol/cm$^2$ and exogenous FXla [E-FXla]$=0$) and (right) TF$^+$FIXa$^+$ ([TF]$=0.02$ fmol/cm$^2$ and [E-FXla]$=10$ pmol/L). The model includes 250 shared binding sites per activated platelet for which FIX and FIXa compete and 250 exclusive binding sites per activated platelet to which only FIXa can bind. A. For TF$^0$FIXa$^-$, the formation of platelet-bound FIXa for the first 400 s occurs almost exclusively by fluid-phase FIXa binding to the exclusive sites. FXla-mediated activation of platelet-bound FIX (red curve) contributes negligibly early in the simulation, but becomes dominant at later times. B. For TF$^+$FIXa$^+$, platelet-bound FIXa formation is dominated throughout the simulation by the activation of platelet-bound FIX (bound to the shared binding sites) by platelet-bound FXla (red curve). Little platelet-bound FIXa is formed by plasma FIXa binding to either type of platelet binding site (green and blue curves). C. For TF$^0$FIXa$^-$, platelet-bound FVIIIa becomes available early (Figures 3A and 4A) because of activation by platelet-bound FXa originally produced by TF:FVIIa, and so [FVIIIa:FIXa] also rises early and involves FIXa bound to the exclusive binding sites (green curve). Compared with these results, in D, we see that for TF$^+$FIXa$^+$, platelet-bound FVIIIa becomes available later (Figures 3C and 4B) because of the bootstrap feedback process, and so [FVIIIa:FIXa] rises much later and involves FIXa bound to the shared binding sites (red curve). The onset of the thrombin burst is indicated by the dashed lines, which show when the plasma thrombin concentration reached 1 and 30 nmol/L in the respective simulations. Platelet count 250,000 per microliter, shear rate 100 per s.
Because plasma FIXa competes with the more plentiful plasma FIX for PFIX/FIXa binding sites, the rate at which plasma FIXa binds these sites (blue curve) is much lower than that at which it binds the PFIX/FIXa sites (green curve). Only after ≈400 seconds when TF:FVIIa activation of FIX has slowed because platelets pave over the subendothelium and thrombin has activated FXI, does the FXIa-mediated production of Plt-FIXa become dominant (red curve), resulting in more FIXa bound to PFIX/FIXa sites than to PFIX/FIXa\textsuperscript{exclusive} sites. Figure 5B shows that for TF\textsuperscript{exclusive}FIXa, Plt-FIXa formation is always dominated by the activation of Plt-FIX by Plt-FXa (red curve), which is present from the beginning because of E-FXa in the plasma. The PFIX/FIXa\textsuperscript{exclusive} sites play a much smaller role in this case (green curve) because there are only small amounts of FIXa activated by TF:FVIIa or plasma FXIa, and most of this plasma FIXa is washed away by flow. For TF\textsuperscript{inclusive}FIXa, FVIIIa:FIXa formation primarily involves Plt-FXa\textsuperscript{exclusive} for the first 400 seconds (Figure 5C), and it is these FVIIIa:FIXa molecules that determine whether a thrombin burst occurs. For TF\textsuperscript{inclusive}FIXa, high concentrations of FVIIIa:FIXa are formed much later and mostly involve Plt-FXa\textsuperscript{exclusive} (Figure 5D).

The simulation results in Figure 5 revealed major differences in the relative importance of the exclusive FIXa platelet binding sites and the shared FIX/FIXa ones between TF\textsuperscript{exclusive}FIXa and TF\textsuperscript{inclusive}FIXa. These observations motivated an additional set of simulations (Figure 6A) in which all or some of the shared sites were removed. The thrombin bursts elicited by high [TF], were affected only marginally when 80% of the PFIX/FIXa\textsuperscript{exclusive} sites were removed (compare the red and blue curves). Even when all PFIX/FIXa\textsuperscript{exclusive} sites were removed (green curve), a thrombin burst occurred for almost the entire range of [TF]. Figure 6B shows the plasma thrombin concentration at 20 minutes for a range of [TF] and [E-FXa] values with the usual number of PFIX/FIXa\textsuperscript{exclusive} sites (these conditions are the same as for Figure 1 but include a wider range of [TF] values). Figure 6C and 6D shows the thrombin concentration when 80% or 100% of the PFIX/FIXa\textsuperscript{exclusive} sites were removed. For the 80% case, there is a much greater range of [TF] and [E-FXa] values for which a thrombin burst does not occur. For example, [E-FXa]=10 pmol/L, [TF]≈0.2 fmol/cm\textsuperscript{2} led to a thrombin plateau concentration of ≈62 nmol/L in the usual case, whereas [TF]>1.4 fmol/cm\textsuperscript{2} was required to elicit a comparable thrombin response in the 80% removed case. In the complete absence of the shared FIX/FIXa sites, combinations of relatively high [TF] and [E-FXa] were required for even a moderate thrombin response. Thus, preventing FIX/FIXa from binding to PFIX/FIXa\textsuperscript{exclusive} sites, substantially reduces the synergistic response to TF and E-FXa, but has little effect on thrombin production elicited by high [TF] values.

**Discussion**

Our mathematical model of platelet deposition and coagulation under flow in response to vessel injury has previously predicted that the occurrence of a thrombin burst depends on the outcome of a race between platelet coverage of subendothelial TF and establishment of a sufficiently high density of the tenase (FIXa:FVIIIa) complex on those platelets.\textsuperscript{13} In those earlier studies, done without E-FXa, TF:FVIIa produced FIXa and FXa only as long as part of the subendothelium remained exposed. Furthermore, it did so at a rate that was higher for a higher initial density of exposed TF and that progressively decreased as more and more of the subendothelium became covered by adherent platelets. Almost all of the FIXa and FXa made by TF:FVIIa was carried away by the flow but some of each became bound to the activated platelets. PF-FXa was the dominant enzyme-activating Plt-FVIII early in the coagulation process.

**Figure 6.** Regulation of thrombin generation by coagulation factor FIX/FIXa binding site concentrations. A, Plasma thrombin concentration at 20 min vs [TF] with no exogenous FXa with 250 (red), 50 (blue), 0 (green) PFIX/FIXa\textsuperscript{exclusive} sites per platelet. B–D, Plasma thrombin concentration at 20 min for various [TF] and exogenous FXa [E-FXa] with 250 (B), 50 (C), 0 (D) PFIX/FIXa\textsuperscript{exclusive} sites per platelet. Note different scale for D. Platelet count 250,000 per microliter. Shear rate 100 per s. TF indicates tissue factor.
response. For any positive concentration of Plt-FXa, the concentration of Plt-FVIIIa increased in time, but how rapidly it reached a particular value depended on how much TF:FVIIa–activated FXa had bound to the platelet surface. In contrast to Plt-FVIIIa, Plt-FIXa was produced at this stage of the coagulation response only by the binding of TF:FVIIa–activated FIXa to the platelets from the plasma. A thrombin burst occurred if the rise of Plt-FVIIIa was sufficiently rapid that it became plentiful, during the time that TF:FVIIa was still producing significant amounts of FIXa. In our simulations, this occurred for TF density of 3 to 4 fmol/cm² or more, the exact value depending on the near-wall speed of the blood flow (ie, whether we simulated venous or arterial conditions). For a below-threshold density of exposed TF, it was the insufficiency of FIXa, not that of Plt-FVIIIa, that accounted for insufficient tenase formation and consequently weak thrombin production.

Both the experiments and simulations reported in this article show that, with low concentrations of E-FXa, a thrombin burst can occur for much lower TF levels than required without E-FXa. That is, TF and E-FXIa act synergistically to elicit a thrombin burst. This is similar to the recent observation that rFXIIa and rFVIIa act synergistically.44

Qualitative Correspondence Between In Vitro and In Silico Models
Because our model and in silico experiments pertain to an open system in which flow brings new zymogens to and removes enzymes from the site of the coagulation reactions, and our in vitro experiments involve a closed static system without replenishment of zymogens, close quantitative agreement between their results is not expected. We found, however, wide areas of qualitative correspondence between the behaviors seen in the simulations and experiments. Most prominent, of course, was the existence of the synergy between TF and E-FXIa in triggering a thrombin burst. Other corresponding behaviors (Figures 1 and 2) include

For sufficiently high TF levels, no E-FXIa was needed to elicit a thrombin burst.
As the TF level dropped, the E-FXa level needed to trigger a thrombin burst increased. Thrombin bursts initiated by high TF levels occurred with a much shorter lag time than those initiated by low TF and E-FXa levels.
For TF and E-FXa levels that triggered a burst, increasing the TF or the E-FXa level shortened the lag time.
The lag time and the time to peak or plateau was quite sensitive to [E-FXa] for low TF levels and much less sensitive for high TF levels.
The lag time and the time to peak or plateau showed substantial or little variation with the TF level for low [E-FXa] and high [E-FXa], respectively.

For sufficiently high [E-FXa], the maximum rate at which thrombin increased in the simulations and the peak thrombin height attained in the experiments were both sensitive to variations in [E-FXa], but not to variations in the TF level.

To understand why this last correspondence is meaningful, note that for a system like that used for our in vitro experiments in which a fixed quantity of prothrombin is converted to thrombin while at the same time thrombin is inactivated by antithrombin, the peak thrombin concentration that is attained directly reflects the maximum rate of activation of prothrombin to thrombin. This wide-ranging qualitative similarity in the nature of the thrombin bursts, their timing, and their various sensitivities to changes in the TF and exogenous FXIa levels, gives confidence in the model’s value for exploring the mechanisms behind these behaviors.

Distinct Mechanisms to Tenase Formation for Different Initial Stimulus
To probe these mechanisms, we focused on 2 sets of initiating stimuli, TFFXIa+ with [TF]d=10 fmol/cm² and no E-FXIa and TFFXIa+ with [TF]d=0.02 fmol/cm² and [E-FXIa]=10 pmol/L. Both sets of stimuli led to a burst in thrombin production, but the thrombin dynamics are different for these 2 cases. For TFFXIa+, the plasma thrombin concentration first reaches 1 nmol/L at 410 seconds, the maximum relative rate of activation of prothrombin is

\[
\frac{d[\text{Thrombin}]}{dt} \bigg|_{\text{Thrombin}}
\]

at which the thrombin concentration increases following that time is 0.035 per second, and the thrombin concentration at 20 minutes is 993 nmol/L. For TFFXIa+, the lag time before the concentration of thrombin in the plasma reaches 1 nmol/L is longer at 812 seconds, the maximum relative rate at which the thrombin concentration increases, 0.092 per second, is much higher, and the concentration at 20 minutes, 94 nmol/L, is almost the same. These differences can be traced to the different dynamics by which the FVIIIa:FIXa complexes form and by the 3-fold difference in the concentrations of these complexes, 0.014 nmol/L for TFFXIa+ and 0.045 nmol/L for TFFXIa+, at the time the plasma thrombin concentration reaches 1 nmol/L in the respective cases. The formation of both Plt-FVIIIa and Plt-FXIa is necessary in order that the FVIIIa:FIXa complex form, and the dynamics of each of these is different for the 2 types of stimuli.

Positive Feedback Aids in the Formation of Platelet-Bound FVIIIa With Low TF
The in silico model incorporates our assumptions that FVIII can be activated in plasma by thrombin and on activated platelet surfaces by thrombin and FXa. There is solid evidence that FVIII binds to activated platelets and that these reactions occur.38,39 But the fact that FVIII circulates in the plasma, bound with von Willebrand factor (vWF), along with evidence that FVIII bound to vWF is protected from activation by FXa45,46 has led some to conclude that FXa-mediated activation of Plt-FVIII occurs to a minimal extent in vivo. Others note that the affinities of FVIII for vWF and for anionic phospholipids are similar47,48 and that FVIII bound to vWF distributes between vWF and the phospholipids when both are present.47,49 Because the larger vWF multimers to which most FVIII is bound readily bind to activated platelets, FVIII would come into close proximity to platelet surfaces, and thus be in position to bind to the platelet surface, be activated by Plt-FXa, and participate in the feedback loop involving FVIIIa:FIXa.39,45,50

The simulations show that the important paths to formation of Plt-FVIIIa involve activation of FVIII already bound...
to the platelet surface. Two paths involve activation of Plt-FVIII by Plt-FXa because we distinguish between activation by Plt-FXa that originated with FXa produced by subendothelial TF:FVIIa and activation by Plt-FXa formed by the platelet tenase complexes. The third path involves activation of Plt-FVIII by platelet-bound thrombin. Thrombin also activates FVIII in the plasma and some of the resulting FVIIIa binds to platelets, but this route was always less productive than 1 of the 3 already mentioned. For the TF$^+$FXIa$^-$ case, Plt-FXa produced by TF:FVIIa–activated FXa was the dominant activator of Plt-VIII for the first ≈200 seconds and the rate of Plt-VIII activation by FXa increased during this period in tandem with the increasing concentration of Plt-FXa (Figure 4A). After this initial period, platelet-bound thrombin became the dominant producer of Plt-FVIIIa. Blocking either of these pathways to producing Plt-VIIIa prevented a thrombin burst (results not shown). In contrast, the activation of Plt-VIII by Plt-FXa that was produced by platelet tenase played a small role in this case, as demonstrated when this reaction was blocked (Figure 4E). For TF$^-$FXIa$^+$, Plt-FVIIIa buildup proceeded more slowly than for TF$^+$FXIa$^-$.

It was initiated by FXa produced by TF:FVIIa, but this FXa contributed little beyond starting the process. Instead, most Plt-FXa–activated FVIIIa involved FXa that was itself activated on the platelet surface by the tenase complexes FVIIa:FIXa$^{\text{shared}}$ and FVIIa:FIXa$^{\text{exclusive}}$. Because these complexes were scarce until sufficient Plt-FVIIIa had been produced, this was a bootstrap process (illustrated in Figure 7) in which feedback activation of FVIIIa by platelet-tenase–activated FXa led to more platelet tenase production. If this feedback was blocked in the TF$^+$FXIa$^+$ case (Figure 4F), then platelet tenase and prothrombinase formation and thrombin production were substantially delayed. If the feedback functioned normally, but thrombin activation of FVIII was blocked, thrombin production was also reduced substantially (not shown).

![Figure 7. Schematic illustrating the different routes to tenase formation for (A) high densities of exposed tissue factor (TF) without exogenous FXIa (E-FXIa) and for (B) low densities of exposed TF together with a low concentration of E-FXIa. A, Sufficient FXa binds to the platelet surface to activate Plt-FVIII and thus allow tenase formation. B, The feedback loop in which small amounts of plasma FXa bind to the platelet surface and use bootstrapping to create more Plt-FXa. Note that all reactions shown take place on the platelet surface only. Schematic illustrating the initial formation of Plt-FIXa for (C) high densities of exposed TF without E-FXIa and for (D) low densities of exposed TF together with a low concentration of E-FXIa. C, With high density of TF, TF-activated FIXa initially binds both the FIX/FIXa shared and FIXa exclusive binding sites, but predominately the FIXa exclusive ones. In contrast, D shows that the FXa exclusive sites are, initially, mostly unbound and that FIX is activated on the platelet surface by Plt-FXia, whereas it is bound to the FIX/FXa shared sites.](http://ajh.ahajournals.org/content/suppl/2017/08/17/1521329X.1920.DC1.jpg)
Shared FIX/FIXa Binding Sites Important for Synergy

In the model, we assume that activated platelets display a population of binding sites shared by FIX and FIXa, and another population of binding sites exclusive to FIXa. Only the shared sites are important for synergy between TF and E-FIXa, but looking at the roles of both populations in the TFHFIXa− and TFHFIXa+ cases is instructive. Before doing so, we note that support for the existence of 2 populations of FIXa receptors comes from a series of studies,40–43 performed by the Walsh laboratory using a variety of equilibrium and kinetic approaches, in which they characterized the numbers and binding parameters of the receptors, and identified the portion of the FIX/FIXa Gla domain required for binding to the shared sites and the part of FIXa’s EGF2 domain required for its binding to the exclusive sites.

Even in the TFHFIXa+ case, the plasma concentration of FIXa was much lower than that of the zymogen FIX. Because of this, binding of FIXa to platelets from the plasma was overwhelmingly to the exclusive FIX binding sites rather than to the shared FIX/FIXa ones (Figure 5A). The presence of FIXa bound to the shared sites only became significant later in the process, after the initiation of the thrombin burst, when thrombin-activated Plt-FIXa activated Plt-FIX that was already bound to the shared sites. In contrast, for the TFHFIXa− case, plasma E-FIXa was available from the beginning, it bound rapidly to platelets as they became activated because of their interactions with subendothelial collagen, and the resulting Plt-FIXa activated Plt-FIX that was bound to the shared FIX/FIXa binding sites (Figure 5B). In this case, because the TF level was low, there was little production of FIXa by TF:FVIIa, and so little binding of FIXa from the plasma to the exclusive binding FIXa binding sites. Hence, the formation of Plt-FIXa in the 2 situations occurred by totally different routes and, importantly, involved 2 different types of platelet binding sites (Figure 7).

A potentially important prediction of the model is that inhibiting the binding of FIX to the platelet’s shared FIX/FIXa binding sites substantially reduces thrombin production induced by combinations of low-density TF exposure and circulating plasma FXa without substantially changing the rapid and robust production of thrombin in response to higher densities of exposed TF (Figure 6). This observation may have therapeutic utility in providing an avenue to reduce thrombosis without significantly affecting hemostasis in response to injury.

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Disclosures

None.

References

This study shows through in vitro experiments and mathematical simulations that low concentrations of tissue factor and exogenous FXa, each far from sufficient to elicit a burst in thrombin production on its own, act synergistically when in combination to cause substantial thrombin production. This may have bearing on thrombotic event occurrence associated with use of FXa-contaminated intravenous immune globulin treatments.

Using the mathematical model, we elucidate a biochemical mechanism that explains a large difference in lag times associated with high tissue factor alone versus the combination of low tissue factor and low exogenous FXa.

We suggest possible therapeutic interventions, via platelet-bound FXa, that block thrombin production under the synergistic conditions, but leave it largely unchanged in the case of initiation by high tissue factor levels alone.
Synergy Between Tissue Factor and Exogenous Factor XIa in Initiating Coagulation
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1 Materials and Methods

Title: Synergy Between Tissue Factor and Exogenous Factor XIa in Initiating Coagulation
Authors: Leiderman, Chang, Ovanesov, Fogelson

1.1 Experiments

Materials
Normal pooled plasma (NPP, VisuCon-F® Coag Screen N) and Factor XI affinity depleted plasma (FXI-DP) were obtained from Affinity Biologicals (Ancaster, Ontario, Canada). Apheresis platelets from normal donors collected using an MCS + LN 9000 (Haemonetics, Braintree, MA) in 1:10 of acid citrate dextrose (ACD) to blood ratio were obtained from the Department of Transfusion Medicine, National Institute of Health (Bethesda, Maryland, USA) as described in [1]. The fluorogenic substrate used in the thrombin generation test, Z-Gly-Gly-Arg-AMC, was from Bachem Americas (King of Prussia, Pennsylvania, USA). Phospholipid vesicles were obtained from Rossix (Molndal, Sweden). Lipidated human recombinant tissue factor (TF, Recombiplastin®) was from Instrumentation Laboratory (Bedford, Massachusetts, USA). Corn trypsin inhibitor (CTI) was from Haematologic Technologies (Essex Junction, Vermont, USA). Calcium chloride (CaCl₂) and prostaglandin E1 were from Sigma-Aldrich (St. Louis, USA). Sodium chloride was from Fisher Scientific (Fair Lawn, New Jersey, USA). Thrombin calibrator (Thrombinoscope brand) was from Stago US (Parsippany, New Jersey, USA). Activated Factor XI (FXIa) was from the National Institute for Biological Standards and Control (an international reference reagent for FXIa, NIBSC code 11/236, Potters Bar, United Kingdom).

Automated TG/FG assay
A dilution series of FXIa was prepared in Tris-BSA buffer using VIAFLO II electronic pipettes with the VIAFLO ASSIST robotic pipetting assistant (INTEGRA Biosciences, Hudson, NH, USA). The mixture of calcium chloride (10 mM final concentration), the fluorogenic substrate Z-Gly-Gly-Arg-AMC (800 μM final concentration), and Tris-BSA buffer (pH 7.4, Aniara, West Chester, Ohio, USA) was then added to FXIa wells, and these mixtures were then transferred onto a half-area 96-well flat bottom plate. Human plasma (50% vol/vol in the final reaction) was mixed with CTI (53-79 μg/ml final concentration). PGE1 (1 μM) was added to platelets to inhibit aggregation; these platelets were then centrifuged for 25 min at 1000 x g. The supernatant was pipetted off and the pellet was resuspended in an approximately equal volume (5 mL) of NPP + CTI as described above. This platelet-rich plasma (PRP) resuspension was shaken for at least 30 min at room temperature for the PGE1 inhibition to reverse, and platelets were counted by CELL-DYN 3700 (Abbott Laboratories, Abbott Park, Illinois, USA). NPP and PRP were mixed to give a platelet concentration of approximately 250,000 platelets per μL final concentration. Tris-BSA buffer or citrate-buffered saline (10 mM trisodium citrate, 150
mM sodium chloride, pH 7.4) was added to this plasma/platelet/CTI mixture as needed. In platelet-
free experiments, Tris-BSA buffer and phospholipid vesicles (4 µM final concentration) were added to
the plasma/CTI mixture. A dilution series of TF was prepared in Tris-BSA buffer using VIAFLO II
electronic pipettes with the VIAFLO ASSIST pipetting assistant. This mixture was transferred to a
96-well round bottom plate; Thrombinoscope thrombin calibrator was also added in place of TF in
two wells. The plasma/CTI/platelet or plasma/CTI/buffer/phospholipid mixture was then added to
these wells. Clotting was initiated by transferring the plasma mixture onto a half-area 96-well flat
bottom plate containing the FXIa/ calcium chloride/substrate mixture using a 96-channel pipettor
(Hydra Liquid Handling System, Thermo Scientific, Pittsburgh, USA or VIAFLO 96, INTEGRA Bio-
sciences) to ensure rapid and simultaneous recalcification in all wells of a microplate. The flat bottom
plate, containing the activated reaction mixture, was transferred to an Infinite F500 (Tecan, Mnndorf,
Switzerland) or Synergy H4 (BioTek Instruments, Winooski, Vermont, USA) microplate reader regu-
lated at 37°C. Fluorescence (360-380 nm excitation and 430-460 nm emission) and absorbance (499-492
nm) were recorded for at least two hours. To account for location-based microplate artifacts, each
FXIa and TF condition was tested in two wells positioned symmetrically with respect of center of the
microplate as described in [2].

**TG/FG curve processing software**

Data processing was performed using an OriginPro (OriginLab, Northampton, MA) software pack-
age described previously [3]; the package is available from us upon request. Thrombin activity was
calculated from the fluorogenic substrate conversion rates using a plot of fluorescence vs. change in
fluorescence (an internal calibrator approach) as described in [4]. Thrombin generation data in dupli-
cate wells were analyzed independently.

1.2 Mathematical Model

The simulations performed for this paper were carried out using the mathematical model described in
[5]. Here we summarize the model and its assumptions and a complete list of the reactions treated in
the model and the values of the kinetic parameters associated with these reactions are given in Tables
M-I - M-VIII below. More details on the model can be found in [5, 6, 7].

The mathematical model simulates the clotting response due to a small injury to a vessel wall. The
response is monitored in a small reaction zone above a region where tissue factor in the subendothelium
(SE) is exposed to flowing blood (see Fig. M-IB). Within the reaction zone, platelet and clotting
factor concentrations are assumed to change due to transport in and out of the zone and due to their
involvement in the coagulation reactions depicted in Fig. M-IC. Each species in the reaction zone
is assumed to be uniformly distributed (‘well-mixed’) and is described by its concentration, whose
dynamics are tracked through an ordinary differential equation. Adjacent to the reaction zone, in the
direction perpendicular to the flow, is an endothelial zone (Fig. M-IC) with height equal to that of
the reaction zone and width dependent on the flow shear rate and protein diffusion coefficients [7].
Thrombin can diffuse from the reaction zone into the endothelial zone, bind to thrombomodulin (TM), and produce activated protein C (APC), which may then diffuse into the reaction zone. Each species in the endothelial zone is also assumed to be well-mixed.

Platelets are either (i) unactivated, unattached, and so free to move with the fluid, or (ii) activated, bound to the SE or to other activated platelets, and therefore stationary. Platelet activation occurs by contact with the SE, by exposure to thrombin, or by contact with other APs. The last of these is used as a surrogate for activation by platelet-released ADP which we do not explicitly track in this model.

Protein species are characterized not only by their chemical identity but also by whether they are in the fluid, bound to the SE, or bound to an activated platelet surface (APS). Proteins bound to a surface are stationary whereas proteins in the plasma move with the fluid. During a transition from SE to APS, or vice versa, a protein is subjected to flow and thus might be carried downstream.

Our assumptions about protein interactions follow, and further discussion of them including citations to the literature can be found in [6]:

1. FVII and FVIIa can bind to TF in the SE. FXa can activate FVII in plasma and when FVII is bound to TF. FXa can bind to the TF:VII complex directly from plasma without having to first bind the SE.

2. FIX and FX can be activated by the TF:VIIa complex on the SE. They attach to TF:VIIa directly from plasma. FX can also be activated by the VIIIa:IXa (‘tenase’) complex on an APS.

3. FV and FVIII can be activated by thrombin in plasma and by thrombin and FXa on an APS.

4. FIX can be activated by FXIa in plasma and on an APS. FXI can be activated by thrombin in plasma and on an APS.

5. The chemical inhibitors that we include in the model are antithrombin (AT), APC, and TFPI. Since the concentration of AT is high in plasma, we assume it acts in a first order manner to inactivate plasma FIXa, FXa, FXIa, and thrombin. APC can bind to fluid-phase and platelet-bound FVa and FVIIIa to permanently inactivate them with second-order kinetics, but cannot bind to FVIIIa in a tenase complex or to FVa in a prothrombinase complex. APC is produced in the endothelial zone by a complex of thrombomodulin and thrombin. TFPI present in the plasma must first bind to FXa and then the complex TFPI::Xa must bind to the TF:VIIa complex to inhibit it.

6. The activity of the TF:VIIa complex decreases as platelet deposition on the injured tissue increases, i.e., we assume that a platelet that adheres to the SE physically blocks the activity of the TF:VIIa complexes on the patch of SE to which the platelet has adhered.

We wrote a FORTRAN program to set up the system of differential equations, to set parameter values, and to run the simulation. This program uses well-accepted methods, as implemented in the software package DLSODE [8], to solve this system of differential equations. Graphical processing of
simulation results was done with MATLAB. A complete listing of the model’s differential equations and of the base parameter values used in the simulations can be found in [5].

For each simulation, we specify the initial plasma concentrations of platelet and protein species, the rate constants for all reactions, the numbers of specific binding sites for coagulation factors on each APS, the dimensions of the injury, the flow velocity near the injured wall, the diffusion coefficients for all fluid-phase species, and the density of exposed TF. The outputs of the simulation are the concentration of every protein species in the reaction zone at each instant of time from initiation of the injury until the completion of the simulation, and the concentrations of platelets attached either directly to the SE or to other platelets.
Figure M-I: Schematic of (A) coagulation reactions included in our model. Dashed magenta arrows show cellular or chemical activation processes. Blue arrows show chemical transport in the fluid or on a surface. Green segments with two arrowheads depict binding and unbinding from a surface. Rectangular boxes denote surface-bound species. Solid black lines with open arrows show enzyme action in a forward direction, while dashed black lines with open arrows show feedback action of enzymes. Red disks show chemical inhibitors. Schematic of (B) reaction zone and (C) endothelial zone.
Kinetic and Physical Parameters:

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelets</td>
<td>$2.5 \times 10^{-7}$ cm$^2$/s</td>
<td>a</td>
</tr>
<tr>
<td>Proteins</td>
<td>$5 \times 10^{-7}$ cm$^2$/s</td>
<td>b</td>
</tr>
</tbody>
</table>

Table M-I: DIFFUSION COEFFICIENTS FOR PLATELETS AND FLUID-PHASE CHEMICAL SPECIES (a) From [9]. (b) From [10].

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Prothrombin</td>
<td>1.4 $\mu$M</td>
<td>a</td>
</tr>
<tr>
<td>Factor V</td>
<td>0.01 $\mu$M</td>
<td>b</td>
</tr>
<tr>
<td>Factor VII</td>
<td>0.01 $\mu$M</td>
<td>a</td>
</tr>
<tr>
<td>Factor VIIa</td>
<td>0.1 nM</td>
<td>c</td>
</tr>
<tr>
<td>Factor VIII</td>
<td>1.0 nM</td>
<td>a</td>
</tr>
<tr>
<td>Factor IX</td>
<td>0.09 $\mu$M</td>
<td>a</td>
</tr>
<tr>
<td>Factor X</td>
<td>0.17 $\mu$M</td>
<td>a</td>
</tr>
<tr>
<td>Factor XI</td>
<td>30.0 nM</td>
<td>a</td>
</tr>
<tr>
<td>TFPI</td>
<td>2.5 nM</td>
<td>d</td>
</tr>
<tr>
<td>Protein C</td>
<td>65 nM</td>
<td>e</td>
</tr>
<tr>
<td>Platelet count</td>
<td>$2.5(10)^5$/µl</td>
<td>f</td>
</tr>
<tr>
<td>$N_2$</td>
<td>1000/plt</td>
<td>g</td>
</tr>
<tr>
<td>$N_2^*$</td>
<td>1000/plt</td>
<td>g</td>
</tr>
<tr>
<td>$N_5$</td>
<td>3000/plt</td>
<td>h</td>
</tr>
<tr>
<td>$N_8$</td>
<td>450/plt</td>
<td>i</td>
</tr>
<tr>
<td>$N_5$</td>
<td>250/plt</td>
<td>j</td>
</tr>
<tr>
<td>$N_5^*$</td>
<td>250/plt</td>
<td>j</td>
</tr>
<tr>
<td>$N_{10}$</td>
<td>2700/plt</td>
<td>k</td>
</tr>
<tr>
<td>$N_{11}$</td>
<td>1500/plt</td>
<td>l</td>
</tr>
<tr>
<td>$N_{11}^*$</td>
<td>250/plt</td>
<td>l</td>
</tr>
<tr>
<td>$n_5$</td>
<td>3000/plt</td>
<td>m</td>
</tr>
<tr>
<td>$P_{PLAS}$</td>
<td>0.167 nM</td>
<td>n</td>
</tr>
</tbody>
</table>

Table M-II: NORMAL CONCENTRATIONS AND SURFACE BINDING SITE NUMBERS (a) From [11]. (b) From [12]. (c) [13] suggests that normal plasma concentration of fVIIa is about 1% of the normal fVII concentration. (d) From [14]. (e) (f) From [15]. (g) Estimated as described in the text of the Supplementary Information. (h) From [16]. (i) From [17]. (j) From [18]. (k) From [19]. (l) From [20, 21]. (m) Number of fV molecules released per activated platelet [22]. (n) Maximum concentration of platelets in a 2 µm high reaction zone assuming that 20 platelets can cover a 10µm-by-10µm injured surface [23].
For the following tables, the basic reaction nomenclature used is:

**Binding/Unbinding**

\[ S + B \xrightarrow{k_{on}} S:B \xrightarrow{k_{off}} B \]

**Enzymatic Activation**

\[ S + E \xrightarrow{k^+} S:E \xrightarrow{k^{cat}} P + E. \]
Table M-III: REACTIONS ON SUBENDOTHELIUM

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Activation Note</th>
<th>Binding Note</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(of -, by -)</td>
<td>(of -, with -)</td>
</tr>
<tr>
<td>(TF: FVII, FXa) k_{cat}^{m, e_10} = 5.0 \cdot 10^6</td>
<td>k_{cat}^{m, e_10} = 5.0 a</td>
<td></td>
</tr>
<tr>
<td>(TF: FVII, FIIa) k_{cat}^{m, e_10} = 3.92 \cdot 10^5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(FX, TF: FVIIa) k_{cat}^{m, e_10} = 5.0 \cdot 10^6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(FIX, TF: FVIIa) k_{cat}^{m, e_10} = 9.4 \cdot 10^6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(FVII, TF) k_{7}^{on} = 5.0 \cdot 10^7</td>
<td>k_{7}^{off} = 5.0 \cdot 10^{-3} e</td>
<td></td>
</tr>
<tr>
<td>(FVIIa, TF) k_{7}^{on} = 5.0 \cdot 10^7</td>
<td>k_{7}^{off} = 5.0 \cdot 10^{-3} e</td>
<td></td>
</tr>
</tbody>
</table>

(a) $k_{cat}^{m, e_10} = 5.0 \cdot 10^{-1}$ sec$^{-1}$ and $K_M = 1.2 \cdot 10^{-6}$ M [24]. (b) $k_{cat}^{m, e_2} = 6.1 \cdot 10^{-2}$ sec$^{-1}$ and $K_M = 2.7 \cdot 10^{-6}$ M [24]. (d) $k_{cat}^{m, e_10} = 1.15$ sec$^{-1}$ and $K_M = 4.5 \cdot 10^{-7}$ M [11]. (d) $k_{cat}^{m, e_2} = 1.15$ sec$^{-1}$ and $K_M = 2.4 \cdot 10^{-7}$ M [25]. (e) $K_d = 1.0 \cdot 10^{-10}$ M [26].
Table M-IV: REACTIONS IN THE PLASMA

<table>
<thead>
<tr>
<th>Reaction</th>
<th>$M^{-1} sec^{-1}$</th>
<th>$sec^{-1}$</th>
<th>$sec^{-1}$</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activation (of -, by -)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(FVII, FXa)</td>
<td>$k_{71}^{+} = 5 \cdot 10^6$</td>
<td>$k_{72}^{-} = 1.0$</td>
<td>$k_{92}^{cat} = 5.0$</td>
<td>a</td>
</tr>
<tr>
<td>(FVII, FIIa)</td>
<td>$k_{72}^{+} = 3.92 \cdot 10^5$</td>
<td>$k_{72}^{-} = 1.0$</td>
<td>$k_{92}^{cat} = 6.1 \cdot 10^{-2}$</td>
<td>b</td>
</tr>
<tr>
<td>(FV, FIIa)</td>
<td>$k_{25}^{-} = 1.73 \cdot 10^7$</td>
<td>$k_{25}^{+} = 1.0$</td>
<td>$k_{25}^{cat} = 0.23$</td>
<td>c</td>
</tr>
<tr>
<td>(FVIII, FIIa)</td>
<td>$k_{18}^{-} = 2.64 \cdot 10^7$</td>
<td>$k_{18}^{+} = 1.0$</td>
<td>$k_{18}^{cat} = 0.9$</td>
<td>d</td>
</tr>
<tr>
<td>(FXI-FXI, FIIa)</td>
<td>$k_{211}^{-} = 2.0 \cdot 10^7$</td>
<td>$k_{211}^{+} = 1.0$</td>
<td>$k_{211}^{cat} = 1.3 \cdot 10^{-4}$</td>
<td>e</td>
</tr>
<tr>
<td>(FIX, FIIa)</td>
<td>$k_{s0}^{-}^{h1} = 0.6 \cdot (10)^7$</td>
<td>$k_{s0}^{+}^{h1} = 1.0$</td>
<td>$k_{s0}^{cat}^{h1} = 0.21$</td>
<td>f</td>
</tr>
</tbody>
</table>

Rate constants apply also for thrombin-activation of FXIa-FXI. Rate constants apply also for activation of FIX by FXIa-FXIa.
Table M-V: BINDING TO PLATELET SURFACES

(a) For FIX binding to platelets, $K_d = 2.5 \cdot 10^{-9}$ M [18], and for FX binding to platelets, $K_d$ has approximately the same value [16]. For FX binding to PCPS vesicles, the on-rate is about $10^7$ M$^{-1}$sec$^{-1}$ and the off-rate is about $1.0 \text{ sec}^{-1}$ [33] giving a dissociation constant of about $10^{-7}$ M. To estimate on- and off-rates for the higher-affinity binding of FX to platelets, we keep the on-rate the same as for vesicles and adjust the off-rate to give the correct dissociation constant. The rates for FIX binding with platelets are taken to be the same as for FX binding. (b) We assume binding constants for FIXa binding to the specific FIXa binding sites are the same as for shared sites. (c) FV binds with high-affinity to phospholipids (PCPS) [33] and we use the same rate constants reported there to describe FV binding to platelets. (d) The $K_d$ for FVIII binding with platelets is taken from [17]. We set the off-rate $k_{off}^8$ for FVIII binding to platelets equal to that for FX binding to platelets, and calculate the on-rate $k_{on}^8$. (e) For prothrombin interactions with platelets, $K_d$ is reported to be $5.9 \cdot 10^{-7}$ M [34]. We choose $k_{2}^{off}$ and set $k_{2}^{on} = k_{2}^{off}/K_d$. (f) Estimated as described in [5] (g) $K_d = 10 \text{ nM}$ [35]. (h) $K_d = 1.7 \text{ nM}$ [21].

<table>
<thead>
<tr>
<th>Reaction</th>
<th>M$^{-1}$sec$^{-1}$</th>
<th>sec$^{-1}$</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>Binding</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(of -, to -)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Factor IX,Plt)</td>
<td>$k_{9}^{on} = 1.0 \cdot 10^7$</td>
<td>$k_{9}^{off} = 2.5 \cdot 10^{-2}$</td>
<td>a</td>
</tr>
<tr>
<td>(Factor IXa,Plt)</td>
<td>$k_{9}^{on} = 1.0 \cdot 10^7$</td>
<td>$k_{9}^{off} = 2.5 \cdot 10^{-2}$</td>
<td>a</td>
</tr>
<tr>
<td>(Factor X,Plt)</td>
<td>$k_{10}^{on} = 1.0 \cdot 10^7$</td>
<td>$k_{10}^{off} = 2.5 \cdot 10^{-2}$</td>
<td>b</td>
</tr>
<tr>
<td>(Factor Xa,Plt)</td>
<td>$k_{10}^{on} = 1.0 \cdot 10^7$</td>
<td>$k_{10}^{off} = 2.5 \cdot 10^{-2}$</td>
<td>a</td>
</tr>
<tr>
<td>(Factor Va,Plt)</td>
<td>$k_{5}^{on} = 5.7 \cdot 10^7$</td>
<td>$k_{5}^{off} = 1.0$</td>
<td>c</td>
</tr>
<tr>
<td>(Factor Va,Plt)</td>
<td>$k_{5}^{on} = 5.7 \cdot 10^7$</td>
<td>$k_{5}^{off} = 1.0$</td>
<td>c</td>
</tr>
<tr>
<td>(Factor VII,Plt)</td>
<td>$k_{8}^{on} = 5.0 \cdot 10^7$</td>
<td>$k_{8}^{off} = 0.17$</td>
<td>d</td>
</tr>
<tr>
<td>(Factor VIIIa,Plt)</td>
<td>$k_{8}^{on} = 5.0 \cdot 10^7$</td>
<td>$k_{8}^{off} = 0.17$</td>
<td>d</td>
</tr>
<tr>
<td>(Factor II,Plt)</td>
<td>$k_{2}^{on} = 1.0 \cdot 10^7$</td>
<td>$k_{2}^{on} = 5.9$</td>
<td>e</td>
</tr>
<tr>
<td>(Factor IIa,Plt)</td>
<td>$k_{2}^{on} = 1.0 \cdot 10^7$</td>
<td>$k_{2}^{on} = 5.9$</td>
<td>e</td>
</tr>
<tr>
<td>(Factor XI,Plt)</td>
<td>$k_{11}^{on} = 1.0 \cdot 10^7$</td>
<td>$k_{11}^{off} = 0.017$</td>
<td>h</td>
</tr>
<tr>
<td>(Factor XIa,Plt)</td>
<td>$k_{11}^{on} = 1.0 \cdot 10^7$</td>
<td>$k_{11}^{off} = 0.017$</td>
<td>h</td>
</tr>
<tr>
<td>Reaction</td>
<td>M$^{-1}$sec$^{-1}$</td>
<td>sec$^{-1}$</td>
<td>sec$^{-1}$</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>--------------------</td>
<td>-----------</td>
<td>-----------</td>
</tr>
<tr>
<td>Activation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(of -, by -)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(FV, FXa)</td>
<td>$k^+<em>m\cdot e</em>{10}^m = 1.0 \cdot 10^8$</td>
<td>$k^- m \cdot e_{10}^m = 1.0$</td>
<td>$k_{cat}^{m}m \cdot e_{10}^m = 4.6 \cdot 10^{-2}$</td>
</tr>
<tr>
<td>(V, Ia)</td>
<td>$k^+<em>m \cdot e</em>{2}^m = 1.73 \cdot 10^7$</td>
<td>$k^- m \cdot e_{1}^m = 1.0$</td>
<td>$k_{cat}^{m}e_{2}^m = 0.23$</td>
</tr>
<tr>
<td>(FVIII, FXa)</td>
<td>$k^+<em>m \cdot e</em>{10}^m = 5.1 \cdot 10^7$</td>
<td>$k^- m \cdot e_{10}^m = 1.0$</td>
<td>$k_{cat}^{m}m \cdot e_{10}^m = 2.3 \cdot 10^{-2}$</td>
</tr>
<tr>
<td>(FVIII, FIIa)</td>
<td>$k^+<em>m \cdot e</em>{10}^m = 2.64 \cdot 10^7$</td>
<td>$k^- m \cdot e_{10}^m = 1.0$</td>
<td>$k_{cat}^{m}e_{1}^m = 0.9$</td>
</tr>
<tr>
<td>(FX, FVIIa:FIXa)</td>
<td>$k^+<em>m \cdot e</em>{10}^m = 1.31 \cdot 10^8$</td>
<td>$k^- m \cdot e_{10}^m = 1.0$</td>
<td>$k_{cat}^{m}e_{10}^m = 20.0$</td>
</tr>
<tr>
<td>(FX, FVIIa:FIXa$^+$)</td>
<td>$k^+<em>m \cdot e</em>{10}^m = 1.31 \cdot 10^8$</td>
<td>$k^- m \cdot e_{10}^m = 1.0$</td>
<td>$k_{cat}^{m}e_{10}^m = 20.0$</td>
</tr>
<tr>
<td>(FII, FVa:FXa)</td>
<td>$k^+<em>m \cdot e</em>{10}^m = 1.03 \cdot 10^8$</td>
<td>$k^- m \cdot e_{10}^m = 1.0$</td>
<td>$k_{cat}^{m}e_{10}^m = 30.0$</td>
</tr>
<tr>
<td>(FXI-FXI, FIIa)</td>
<td>$k^+<em>m \cdot e</em>{10}^m = 2.0 \cdot 10^7$</td>
<td>$k^- m \cdot e_{10}^m = 1.0$</td>
<td>$k_{cat}^{m}e_{10}^m = 1.3 \cdot 10^{-4}$</td>
</tr>
<tr>
<td>(FX, FXIa)</td>
<td>$k^+<em>m \cdot e</em>{11}^m = 0.6 \cdot 10^7$</td>
<td>$k^- m \cdot e_{11}^m = 1.0$</td>
<td>$k_{cat}^{m}e_{11}^m = 0.21$</td>
</tr>
<tr>
<td>Binding</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(of -, with -)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(FVIIa, FIXa)</td>
<td>$k_{ten}^{m} = 1.0 \cdot 10^8$</td>
<td>$k_{ten} = 0.01$</td>
<td>$k_{ten} = 0.01$</td>
</tr>
<tr>
<td>(FVIIa, FIXa$^+$)</td>
<td>$k_{ten}^{m} = 1.0 \cdot 10^8$</td>
<td>$k_{ten} = 0.01$</td>
<td>$k_{ten} = 0.01$</td>
</tr>
<tr>
<td>(FVa, FXa)</td>
<td>$k_{pro}^{m} = 1.0 \cdot 10^8$</td>
<td>$k_{pro} = 0.01$</td>
<td>$k_{pro} = 0.01$</td>
</tr>
</tbody>
</table>

Table M-VI: REACTIONS ON PLATELET SURFACES (a) $k_{cat}^{m} e_{10}^m = 0.046$ sec$^{-1}$ and $K_M = 10.4 \cdot 10^{-9}$ M [36]. (b) The rate constants for thrombin activation of FV on platelets are assumed to be the same as in plasma. (c) $k_{cat}^{m} e_{10}^m = 0.023$ sec$^{-1}$ and $K_M = 2.0 \cdot 10^{-8}$ M [29]. (d) The rate constants for thrombin activation of FVIII on platelets are assumed to be the same as in plasma. (e) The formation of the tenase and prothrombin complexes is assumed to be very fast with $K_d = 1.0 \cdot 10^{-10}$ M [37]. (f) $k_{cat}^{m} e_{10}^m = 20$ sec$^{-1}$ and $K_M = 1.6 \cdot 10^{-7}$ M [38]. (g) $k_{cat}^{m} pro = 30$ sec$^{-1}$ and $K_M = 3.0 \cdot 10^{-7}$ M [39]. (h) $k_{cat}^{m} e_{10}^m = 1.3 \cdot 10^{-4}$, $K_M = 50$ nM [30]. Rate constants apply also for thrombin-activation of Plt-FXIa-FXI. (i) $k_{cat}^{m} e_{10}^m = 0.21$, $K_M = 0.2 \mu$M [31, 32]. Rate constants apply also for activation of platelet-bound FIX by Plt-FXIa-FXIIa.
Table M-VII: INHIBITION REACTIONS (a) We estimate these parameters based on the half-lives of Factors FIXa, FXa, FIIa in plasma [40] and assume that the rate of FXIa inactivation is the same as that of FXa and thrombin. (b) For inhibition of FVa by APC, $k_{\text{cat}}^{\text{e}5} \cdot \text{APC} = 0.5$ sec$^{-1}$ and $K_M = 12.5 \cdot 10^{-9}$ nM and $[\text{PC}] = 65$ nM [43]. (c) From [42]. (d) $K_d = 0.5$ nM and $[\text{PC}] = 65$ nM [43]. (e) $k_{\text{PC} \cdot \text{TM} \cdot e_{5}^{c}} = 0.167$ sec$^{-1}$, $K_M = 0.7 \cdot 10^{-6}$ M [44].

<table>
<thead>
<tr>
<th>Reaction</th>
<th>$M^{-1}$sec$^{-1}$</th>
<th>sec$^{-1}$</th>
<th>sec$^{-1}$</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inactivation (of -, by -)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(FIXa, AT)</td>
<td>$k_{\text{AT} \cdot e_{9}}^{\text{in}} = 0.1$</td>
<td></td>
<td></td>
<td>a</td>
</tr>
<tr>
<td>(FXa, AT)</td>
<td>$k_{\text{AT} \cdot e_{10}}^{\text{in}} = 0.1$</td>
<td></td>
<td></td>
<td>a</td>
</tr>
<tr>
<td>(FIIa, AT)</td>
<td>$k_{\text{AT} \cdot e_{2}}^{\text{in}} = 0.2$</td>
<td></td>
<td></td>
<td>a</td>
</tr>
<tr>
<td>(FXIa, AT)</td>
<td>$k_{\text{AT} \cdot e_{11}}^{\text{in}} = 0.2$</td>
<td></td>
<td></td>
<td>a</td>
</tr>
<tr>
<td>(APC, FVa)</td>
<td>$k_{\text{e}5^{c} \cdot \text{APC}}^{+} = 1.2 \cdot 10^{8}$</td>
<td>$k_{\text{e}5^{c} \cdot \text{APC}}^{-} = 1.0$</td>
<td>$k_{\text{e}5^{c} \cdot \text{APC}}^{\text{cat}} = 0.5$</td>
<td>b</td>
</tr>
<tr>
<td>(APC, FVIIIa)</td>
<td>$k_{\text{e}8^{c} \cdot \text{APC}}^{+} = 1.2 \cdot 10^{8}$</td>
<td>$k_{\text{e}8^{c} \cdot \text{APC}}^{-} = 1.0$</td>
<td>$k_{\text{e}8^{c} \cdot \text{APC}}^{\text{cat}} = 0.5$</td>
<td>b</td>
</tr>
<tr>
<td>Binding (of -, with -)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(TFPI, FXa)</td>
<td>$k_{\text{TFPIA} \cdot e_{10}}^{+} = 1.6 \cdot 10^{7}$</td>
<td>$k_{\text{TFPIA} \cdot e_{10}}^{-} = 3.3 \cdot 10^{-4}$</td>
<td></td>
<td>c</td>
</tr>
<tr>
<td>(TFPla, TF:FVIIa)</td>
<td>$k_{\text{TFPIA} \cdot e_{7}}^{+} = 1.0 \cdot 10^{7}$</td>
<td>$k_{\text{TFPIA} \cdot e_{7}}^{-} = 1.1 \cdot 10^{-3}$</td>
<td></td>
<td>c</td>
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<tr>
<td>(TM, Thrombin)</td>
<td>$k_{\text{TM} \cdot e}^{+} = 1.0 \cdot 10^{8}$</td>
<td>$k_{\text{TM} \cdot e}^{-} = 5.0 \cdot 10^{-2}$</td>
<td></td>
<td>d</td>
</tr>
<tr>
<td>Activation (of -, by -)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(PC, TM:Thrombin)</td>
<td>$k_{\text{PC} \cdot TM \cdot e_{5}^{c}}^{+} = 1.7 \cdot 10^{6}$</td>
<td>$k_{\text{PC} \cdot TM \cdot e_{5}^{c}}^{-} = 1.0$</td>
<td>$k_{\text{PC} \cdot TM \cdot e_{5}^{c}}^{\text{cat}} = 0.16$</td>
<td>e</td>
</tr>
<tr>
<td>Reactants</td>
<td>$M^{-1}\text{sec}^{-1}$</td>
<td>$\text{sec}^{-1}$</td>
<td>Note</td>
<td></td>
</tr>
<tr>
<td>-----------------------------------------------</td>
<td>--------------------------</td>
<td>-------------------</td>
<td>------</td>
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</tr>
<tr>
<td>Unactivated platelet adhering to SE</td>
<td>$k_{\text{adh}}^+=2 \cdot 10^{10}$</td>
<td>$k_{\text{adh}}^-=0$</td>
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<tr>
<td>Activated platelet adhering to SE</td>
<td>$k_{\text{adh}}^+=2 \cdot 10^{10}$</td>
<td>$k_{\text{adh}}^-=0$</td>
<td>a</td>
<td></td>
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<tr>
<td>Platelet activation by platelet in solution</td>
<td>$k_{\text{act}}^\text{plt} = 3 \cdot 10^8$</td>
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<td>b</td>
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<tr>
<td>Platelet activation on SE</td>
<td>$k_{\text{act}}^\text{plt} = 3 \cdot 10^8$</td>
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<td>b</td>
<td></td>
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<tr>
<td>Platelet activation by thrombin</td>
<td>$k_{\text{act}}^{\text{th}}=0.50$</td>
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Table M-VIII: PLATELET TRANSITIONS (a) Estimated from data in [45, 46] as described in [6]. (b) Estimated from data in [47] as described in [6]. SE=subendothelium.
References


1 SUPPLEMENTARY MATERIAL

Title: Synergy Between Tissue Factor and Exogenous Factor XIa in Initiating Coagulation
Authors: Leiderman, Chang, Ovanesov, Fogelson

The simulations performed for this Supplement were conducted using the mathematical model described in [1]. A complete listing of the reactions included in the model and of the parameter values used in the simulations is in the Methods and Materials Supplement (Tables M-I – M-VII). See [1] for a listing of the model’s differential equations, and [1, 2, 3] for further discussions of the model’s derivation and behavior. The mathematical model simulates the clotting response due to a small injury to a vessel wall, as depicted in Fig. M-I in the Methods and Materials Supplement.

1.1 Effect of Shear Rate on Synergistic Thrombin Burst

Here we show the results of simulations at shear rates 50/sec and 10/sec. These should be compared with Fig. 1 in the main paper which shows results for shear rate 100/sec. The figures demonstrate that the synergistic response occurs at shear rates much lower than that looked at in the main paper.
Figure S-I: Effects of TF and FXIa on intravascular thrombin generation in *in silico* experiments for shear rates 50/sec and 10/sec. Plasma thrombin concentration at 10 min (Top Left) and 20 min (Top Right) for $[\text{TF}]_d$ between 0 and 1 fmol/cm$^2$ and $[\text{E-FXIa}]$ between 0 and 20 pM and shear rate 50/sec. Plasma thrombin concentration at 10 min (Bottom Left) and 20 min (Bottom Right) for $[\text{TF}]_d$ between 0 and 1 fmol/cm$^2$ and $[\text{E-FXIa}]$ between 0 and 20 pM and shear rate 10/sec. Each point on the heat-maps was obtained from a single simulation with specified values of $[\text{TF}]_d$ and $[\text{E-FXIa}]$. Platelet count 250,000/µL.
1.2 Rate of thrombin increase during burst

To better understand the sensitivity of the maximum relative rate of thrombin increase during a burst shown in Fig. 1H, in Fig. S-II we plot the plasma thrombin, and platelet prothrombinase and tenase concentrations from these simulations in a different way. For each simulation, we determined the time $t_1$ at which the thrombin concentration reached 1 nM, which we regard as the start of the thrombin burst, and we plotted the concentrations starting at $t_1$ as a function of the elapsed time $t_{\text{elapsed}} = t - t_1$. Thus the plots for the different simulations were aligned with $t_{\text{elapsed}} = 0$ indicating when for each simulation, the thrombin concentration reached 1 nM. Fig. S-II shows that there was little difference among the prothrombinase concentrations at the start of the burst, but that the tenase concentrations at that time were clustered into distinct groups determined by [E-FXIa]. Within a cluster, the variation with $[\text{TF}]_d$ was small. The different tenase concentrations at the start of the bursts were the cause of the subsequent differences in rates of increase of prothrombinase and thrombin during the bursts. Higher concentrations of E-FXIa led to greater availability of Plt-FIXa early in the simulations and to the higher concentrations of tenase at the starts of the corresponding bursts. For these simulations, the availability of Plt-FVIIIa, due to activation by Plt-FXa, was sufficient at all $[\text{TF}]_d$ considered.

Figure S-II: Concentrations as functions of the elapsed time since the thrombin concentration reached 1 nM for $[\text{TF}]_d = 10.0, 3.0, 1.0, 0.5, 0.1, 0.02$ fmol/cm$^2$ and $[\text{E-FXIa}] = 2, 5, 10, 20$ pM. Each cluster of curves corresponds to one of the $[\text{E-FXIa}]$ values. (A) Plasma thrombin concentrations vs elapsed time. (B) Prothrombinase concentrations vs elapsed time. (C) Tenase complex concentrations vs elapsed time. The tenase and prothrombinase concentrations include those of the complexes bound to their substrates, FX and prothrombin, respectively. Platelet count 250,000/µL. Shear rate 100/sec.

Here, we show the results of a series of simulations conducted to examine the relative rate of increase of thrombin during a thrombin burst in situations without E-FXIa. Fig. S-III shows that this rate increases with increasing $[\text{TF}]_d$. Here, $[\text{TF}]_d$ is the dominant determinant, early in each simulation, of the availability
of both Plt-FVIIIa and Plt-FIXa, and thus the rate of formation of the platelet-bound tenase complexes.

Figure S-III: For simulations with no exogenous E-FXIa, time course of plasma thrombin concentration for $[\text{TF}]_d = 25$ (blue), 20 (cyan), 15 (red), 10 (black), 7.5 (magenta), 5 (green) fMol/cm$^2$. Dashed lines show 1 nM and 15 nM concentrations. The steepness of the curves between these levels increases with $[\text{TF}]_d$. Platelet count 250,000/µL. Shear rate 100/sec.
1.3 Effect of FIXa binding sites on platelets

Simulations were performed in which i) FIX had 250 binding sites and FIXa had 250 binding sites per activated platelet, and ii) in which each activated platelet had 500 binding sites for which FIX and FIXa compete. Results are shown in Fig. S-IV. They should be compared with Fig. 1 in the main paper which shows results from simulations in which each activated platelet had 250 binding sites for which FIX and FIXa compete and 250 additional sites to which only FIXa could bind. The synergistic response to low \([\text{TF}]_d\) and \([E-\text{FXIa}]\) is seen for all of these cases.

![Images of heatmaps showing thrombin generation](image)

**Figure S-IV:** Effects of TF and FXIa on intravascular thrombin generation in *in silico* experiments for different assumptions about FIXa binding sites. Plasma thrombin concentration at 10 min (Top Left) and 20 min (Top Right) for \([\text{TF}]_d\) between 0 and 1 fmol/cm² and \([E-\text{FXIa}]\) between 0 and 20 pM when each platelet has 250 binding sites only for FIX and 250 binding sites only for FIXa. Plasma thrombin concentration at 10 min (Bottom Left) and 20 min (Bottom Right) for \([\text{TF}]_d\) between 0 and 2.5 fmol/cm² and \([E-\text{FXIa}]\) between 0 and 20 pM when each platelet has 500 binding sites for which FIX and FIXa compete. Each point on the heat-maps was obtained from a single simulation with specified values of \([\text{TF}]_d\) and \([E-\text{FXIa}]\). Platelet count 250,000/µL.
References


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<tr>
<th>Initial Coagulation Stimulus</th>
<th>Thrombin Generation Assay</th>
<th>in silico Thrombin Generation Under Flow</th>
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<td>Tissue Factor AND Exogenous FXIa</td>
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