Coated platelets introduce significant delay in onset of peak thrombin production: theoretical predictions

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Highlights

- A mechanistic model incorporating coated platelets sub-population is developed
- Coated platelet concentration follows dose-dependence on thrombin concentration
- Competitive binding of enzymes occurs only with (coated) platelet membrane
- Neglect of coated platelets significantly overestimates onset of peak thrombin

Coated platelets introduce significant delay in onset of peak thrombin production: theoretical predictions

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Abstract

Platelets play a crucial role in the initiation, progress, termination as well as regulation of blood coagulation. Recent studies have confirmed that not all but only a small percentage of thrombin-activated platelets ("coated" platelets) exhibit procoagulant properties (namely the expression of phosphatidylserine binding sites) required for the acceleration and progress of coagulation. A mechanistic model is developed for *in vitro* coagulation whose key features are distinct equations for coated platelets, thrombin dose-dependence for coated platelets, and competitive binding of coagulation factors to platelet membrane. Model predictions show significant delay in the onset of peak Va production, and peak thrombin production when dose-dependence is incorporated instead of a fixed theoretical maximum percentage of coated platelets. Further, peak thrombin concentration is significantly overestimated when either fractional presence of coated platelets is ignored (by 299.4%) or when dose-dependence on thrombin is ignored (by 24.7%).

Keywords: coagulation, coated platelets, dose-dependence, mechanistic model, thrombin

1. Introduction

The traditional view of blood coagulation is the "cascade" model which involves intricate interactions between coagulation factors and platelets, and

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proceeds in a sequential manner with regulatory feedback loops [28]. Coagulation cascade is initiated when, upon blood vessel injury, a transmembrane protein known as tissue factor (TF) is exposed on the subendothelium along with collagen [17]. Tissue factor triggers the series of enzymatic reactions leading to clot formation by first forming a procoagulant complex TF:VIIa or extrinsic tenase. The extrinsic tenase then activates factors IX and X which in turn bind their respective cofactors (factors VIIIa and Va, respectively) on procoagulant platelet surfaces to form the intrinsic tenase (IXa:VIIIa) and prothrombinase (Xa:Va) complexes, respectively. In parallel to the action of tissue factor, collagen promotes platelet adhesion and activation at the site of injury.

The cascade model well describes coagulation in *in vitro* settings where plasma proteases and platelets are uniformly mixed in a test tube. However, *in vivo*, the appropriate view of coagulation is of three stages - initiation, propagation, and termination- being spatially distributed, due to occurrence on different cell surfaces, and functional due to flow [36]. Whether *in vivo* or for whole blood samples *in vitro*, the enzymatic reactions contributing to propagation of coagulation in the cascade model are membrane-bound, the requisite membrane being provided by negatively-charged platelets [11]. By providing such a surface, the platelets not only aid in the formation of procoagulatory complexes (like intrinsic tenase and prothrombinase), but also localize thrombus formation [32]. The current understanding of the role of platelet membranes is quite different from that in the cascade model of coagulation. In the cascade model, most of the reactions are believed to take place in plasma while platelets only supply phospholipid surface for assembly of procoagulant complexes like intrinsic tenase & prothrombinase. However, according to [51], platelet membrane is central to procoagulant complex assembly starting with TF:VIIa:X, and including XI:IIa and later IX:VIIIa. It is also proved that only a sub-population of activated platelets (referred to as "coated" platelets [26], or CP in this paper) express phosphatidylserine (PS) which is essential for binding of coagulation enzymes. Further, the nature of the binding mechanism (whether competitive, reversible, or irreversible) and accessibility of binding sites (whether mapped one-to-one or competitive) are concepts that are being challenged [38].

An improvement of the mechanistic models describing blood coagulation, and especially the role of platelets, is essential for a more thorough understanding of the mechanisms behind coagulation. While the role of platelets in blood coagulation has been well studied for a long time now [29, 2, 18, 5], the con-

cept of only a sub-population of platelets (that is, "coated" platelets, or CP) being responsible for procoagulant activities is relatively new [26, 14, 34]. Formation of coated platelets has strong clinical implications [40, 39, 41], the study of which can be helpful in pharmacological intervention; it is therefore essential that such platelet heterogeneity be implemented in the mechanistic models of hemostasis.

In this paper, a mechanistic model of the *in vitro* coagulation cascade is developed whose distinctive feature is the inclusion of coated platelets (the activated sub-population of platelets). Further, the concentration of coated platelets follows dose-dependence on thrombin concentration [22]. Theoretically, this indicates a regulatory feedback relation between thrombin and coated platelet concentrations. In this mechanistic model, we assume platelet activation by thrombin alone to be significant for generation of procoagulant platelets: that is, the activation of platelets by other platelets does not contribute to generation of procoagulant platelets. A brief survey of the literature concerning coated platelets and their binding to coagulation factors is given in Section 2. This is followed by model development in Section 3. Model predictions, for initiation and peak of enzyme production, are obtained with and without dose-dependent coated platelets so as to ascertain the error due to non-inclusion. The results from this exercise are detailed and discussed in Section 4. Concluding remarks are made in Section 5.

2. Literature Survey

The extrinsic tenase complex (TF:VIIa) formed during the initiation phase of coagulation generates sub-picomolar amounts of fXa and fIXa. A small amount of prothrombin is then converted to thrombin by this platelet surface-bound fXa; this thrombin (once above a threshold value of 1nM [25]) binds to and activates platelets which provide the phosphatidylserine (PS) exposed surface for coagulation factor binding. In this way thrombin generates a positive feedback for its quantitative generation [30, 52]. Apart from the anionic binding sites, activated platelets also supply coagulation factors like factor V and factor XI [24, 50]. Panteleev et al [34] demonstrated that activation of platelets by thrombin alone generates a platelet sub-population capable of binding factors Va, IXa, Xa and VIIIa with great affinity.

Heterogeneity of activated platelets has provoked great interest among hematologists and clinicians alike [33]. That, upon activation, only a sub-population of platelets express PS is a reflection of the striking differences in the procoag-

ulant abilities of platelet subpopulations [20, 21, 55]. Kotova et al [22] studied the formation of coated platelets with respect to its dependency on thrombin concentration as well as on platelet concentration. They reported that the concentration of thrombin-activated coated platelets varies with thrombin concentration (for instance, 12% of the entire activated platelet concentration shows procoagulant properties when activated by 100nM thrombin, whereas 2% is procoagulant when activated by 1nM thrombin). There are now considerable experimental studies on understanding the formation [49, 48], regulation [22], and structural and functional properties of coated platelets [55, 1, 37]. Bouchard et al [8] were successful in measuring coated platelet sub-population using a flow cytometry-based assay. Topalov et al [49] studied the role of signal transduction pathways leading to the segregation of platelet sub-populations into PS-positive and PS-negative.

The view on binding of coagulation factors to (the procoagulant sub-population of) activated platelets has evolved from that incorporated by Kuharsky and Fogelson [23] -where specific numbers of binding sites are present for each enzyme/zymogen pair- to one in which all coagulation factors (except IIa) bind competitively to the (PS binding-) sites exposed on activated platelets [34][1]. This was confirmed by Podoplelova et al [37] who reported heterogeneity in distribution of coagulation factors on the surface of coated platelets. Moreover, coagulation factor binding to platelets is not always simply reversible. Podoplelova et al [38] have reported the two-step hysteresis-like binding of fXa with coated platelets, and formation of fXa-fX dimers. The same was shown for fVIII by [6].

There have been many models describing the role of procoagulant platelet surfaces in blood coagulation, the nature of their incorporation being different from one another: as an excess of activated platelets available for reactions to occur [19, 4], as a mathematical function representing the level of platelet activation [53, 13], as lipid surfaces [9], as explicitly distinct species [23, 47], and many more which incorporated transport to platelet surfaces by flow [35, 27, 54, 16]. Models like those developed by Susree & Anand [47] and Kuharsky & Fogelson [23] do incorporate separate terms for the reactions occurring on the platelet membranes as well as for those in plasma. However, these models assume that the entire population of activated platelets is

[1] Panteleev et al have employed such competitive binding, but only for factors X and II, in their model in [35] based on the study by Scandura et al [43]

procoagulant. In the model presented here, we incorporate the latest understanding of the role of platelets in coagulation namely a sub-population of activated platelets (known as coated platelets), thrombin dose-dependence of coated platelets, and competitive binding to platelet membrane during coagulation.

3. Mechanistic Model

We propose a pseudo-homogeneous model for *in vitro* coagulation cascade which incorporates a distinct procoagulant sub-population of activated platelets. In a pseudo-homogeneous model, the particle composition of blood is overlooked, and the heterogeneous system is treated as homogeneous by defining platelet concentration based on plasma volume : see [15] for classification of blood coagulation models.
The model consists of a set of 25 reaction equations that governs the generation and depletion of tissue factor (TF), resting & activated platelets (RP, AP), coagulation factors ($XIa/XI, IXa/IX, Xa/X, IIa/II, VIIIa/VIII, Va/V, VII/VIIa$), coated platelet surface-bound enzyme complexes ($TF:VII$, $TF:VIIa$, $VIIIa:IXa$, and $Va:Xa$), inhibitors ($ATIII$, $TFPI$, $Xa:TFPI$, and $\alpha 1INH$), and fibrin/fibrinogen (Ia/I). Initial concentrations of the reactants are those in human plasma. A 0.01% initial level of activation is assumed for enzymes : this assumption is a limitation which can be addressed using more advanced numerical procedures. Transport due to flow is neglected in this *in vitro* coagulation model.
The salient feature of the model is the manner and extent to which the role of platelets, particularly coated platelets, is incorporated:

- Platelets are modeled as a soluble phase unlike in the models developed in [23, 9, 27].
- Coated platelets are a sub-population of the entire activated platelet population. All surface-bound reactions occur on coated platelets **only** and not on all activated platelets.
- Concentration of coated platelets is explicitly thrombin dose-dependent.
- Competitive binding is assumed for all coagulation factors that bind to coated platelets (except IIa); the total number of binding sites per coated platelet is taken to be 25000 [34].

Each of the model equations is formulated in the following manner:

$$\frac{d[Y_i]}{dt} = G_i$$

where $[Y_i]$ denotes the concentration of the constituent Y_i, $\frac{d[Y_i]}{dt}$ denotes the time derivative of $[Y_i]$, and G_i is the net rate of production of Y_i as a result of enzymatic reactions (equal to the rate of production minus the rate of depletion).

We list here an equation showing competitive binding to coated platelets to clarify the distinctive features of this model.

$$[IXa^m] = \frac{25000[CP]\frac{[IXa]}{K_{D9}}}{1 + \frac{[IX]}{K_{D9}} + \frac{[IXa]}{K_{D9}} + \frac{[X]}{K_{D10}} + \frac{[Xa]}{K_{D10}} + \frac{[II]}{K_{D2}} + \frac{[VIII]}{K_{D8}} + \frac{[VIIIa]}{K_{D8}}}$$

The above equation represents competitive binding of factor IXa to the surface of coated platelets,'CP'.
Here $IX, X, II, VIII$ refer to the coagulation factors in their zymogen form, while $IXa, Xa, IIa, VIIIa$ refer to their corresponding enzymes. Superscript 'm' refers to 'membrane-bound', the membrane being found on the surface of coated platelets (CP). 25000 refers to number of binding sites shared competitively by the coagulation factors $IX/IXa, X/Xa, II$, and $VIII/VIIIa$ on the surface of CP ***only***. K_{Di} refers to dissociation constant of respective coagulation factor i (where $i : IX/IXa, X/Xa, II$, and $VIII/VIIIa$). We contrast this with the corresponding term for generation of IXa^m in the recently developed mechanistic model in [47]; the term is the following: $\frac{d[IXam]}{dt} = k_9^+ * 550 * [AP][IXa]$.
In the latter case IXa^m is generated by binding to a specified number of sites on any activated platelet, whereas in the model proposed here IXa^m is generated by competitive binding to only coated platelets.

The equations of the various reactions for coagulation, and equations governing coated platelets and bound factors as per the above assumptions, are outlined in Appendix A. Note that the reactions follow the form in [35].

Reaction rate constants are also listed in the tables in appendix. For an elaboration on the choice and sources of kinetic constants, we refer the readers to [46].

4. Results

4.1. Numerical Scheme

The system of ODEs and the initial conditions are non-dimensionalised as shown below:
t^*=t/T, T=840s, is the total time of simulation employed for the model
Y_i^*=Y_i/Y_i(t=0),
G_i^*=G_iT/Y_i(t=0).
The system of 25 ODEs is then solved using the 'ode15s' algorithm (meant for stiff ODEs) in MATLAB (version R2015b). The time-marching is performed using a time step of 10s until relative tolerance of each variable at each time step is 0.001 or absolute tolerance is 10^{-6} as built in the default MATLAB scheme. The simulations are performed and reported for 840s (i.e. 14 min).

4.2. Dose-dependence of coated platelets

Dose-dependence of coated platelet concentration on thrombin concentration is incorporated in the model. The dose-dependence curve is a linear fit to the data points (from [22]): (1nM, 2%), (10nM, 4%), and (100nM, 12%), and the equation is:

$$\delta = (0.00106 nM^{-1} \times [IIa]) + 0.00021$$

4.3. Model Validation

The model prediction for thrombin production in the presence of activated platelets is compared with the data from experiments obtained in Butenas et al [12] and shown in fig 1: a good match is seen. Note that the data pertains to platelets activated only by thrombin and no other exogeneous chemicals, and hence pertains to data for coated platelets. The average platelet count in humans is around 200,000/μL. For our model, we take a resting platelet concentration ([RP]) of 10nM to be the equivalent of this normal platelet count in human plasma (or normal plasma : NP), as explained in [3].

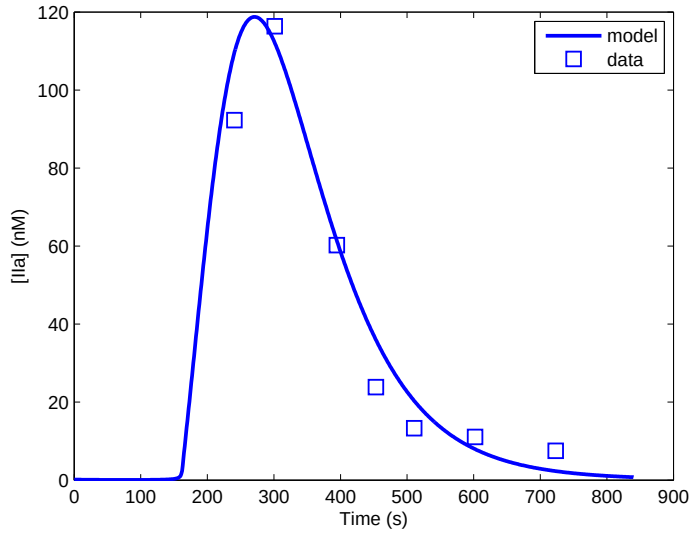

Figure 1: Thrombin production at [TF]=25pM and normal platelet count (i.e, 200,000 platelets/μL). Model compared with experimental data from Butenas et al., 2001, for reconstituted plasma.

4.4. Model Predictions

We document first the effect of increasing platelet count on concentration of coated platelets ([CP]): this has a direct consequence on availability of membrane binding sites during coagulation. As mentioned earlier, [RP] = 10nM for Normal Plasma (corresponding to 200,000 platelets/μL). Consequently, the [RP] of platelet-rich plasma (PRP) and platelet-poor plasma (PPP) are taken to be 50nM (corresponding to 1,000,000 platelets/μL) and 0.5nM (corresponding to 10,000 platelets/μL), respectively. The rise in [CP] is steeper (higher $\Delta[CP]/\Delta(Time)$) with increase in initial [RP] as seen in fig 2; though the rise in peak is consistent with what one expects, steep nature of the rise is a unique prediction. The shape of the profiles for [CP] also signify the dose-dependency on thrombin concentration (which follows a bell-shaped curve as well). The peak [CP] is much higher ($\approx$5 fold) in the case of PRP than that in normal plasma. Moreover, the rise in coated platelet production is observed much earlier in PRP (47s) when compared with that in normal plasma (162s). However, no peak is observed in the case of PPP.

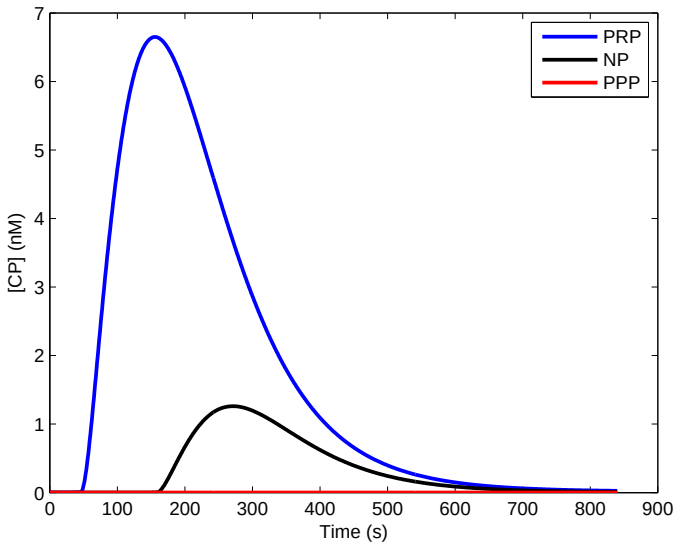

Figure 2: Effect of platelet concentration on coated platelet production ([TF]=25pM). Here PRP: Platelet-rich plasma, NP: Normal plasma, and PPP: Platelet-poor plasma.

Since the concentration of thrombin and the concentration of coated platelets produced are mutually dependent, we also document the peak production of thrombin at different fixed percentages of coated platelets. Figure 3 shows the profile of thrombin production with respect to time at different fixed percentages (δ) of coated platelets; δ corresponds to the fraction of activated platelets which are coated platelets, given by δ=[CP]/[AP]. We use the same symbol for this fraction as in [35] for ease of comparison. We observe that, while the time of peak thrombin production does not shift, the peak rises steadily keeping a nearly constant difference of about 17.0 nM for every increase of 2% in δ.

Figure 4 compares thrombin production profiles for three conditions: first, when the entire population of activated platelets is procoagulant ($\delta = 1.0$); second, when the concentration of coated platelets is fixed at the theoretical maximum ($\delta = 0.12$); and third, when the concentration of coated platelets is dose-dependent ($\delta = f([IIa])$). The second and third conditions correspond to the case of only coated platelets contributing to enzyme production and complex assembly. The results in figure 4 are the most salient model predic-

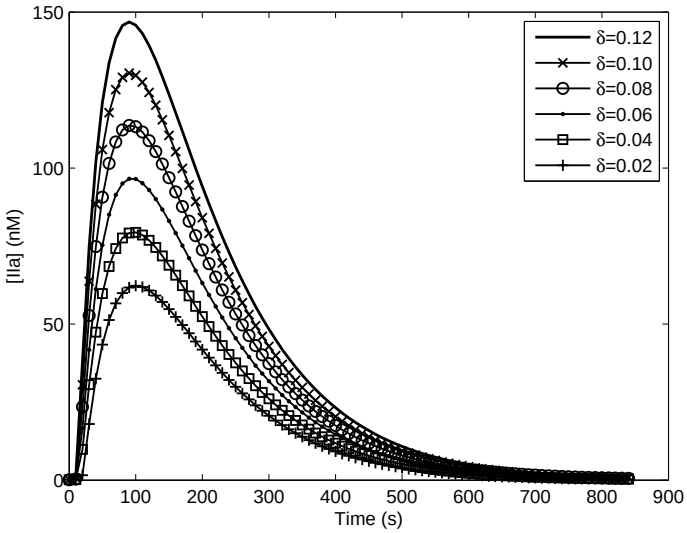

Figure 3: Thrombin production versus time at different fixed concentrations of coated platelets ([TF]=25pM). (Simulations are run only up to δ=0.12, the theoretical maximum for coated platelet percentage as given in Kotova, Ataullakhanov, & Panteleev, 2008)

tions because [IIa] increase is delayed for dose-dependent CP compared to 12% fixed concentration of CP (which is a theoretical maximum case). There is also significant overestimation of the peak thrombin concentration when either fractional presence of coated platelets (black versus red line: 299.4%) or their dose-dependence on thrombin (blue versus black line: 24.7%) is ignored.

We also report here, in figure 5, the delay as well as drop in peak thrombin concentration caused by decline in initial prothrombin concentration ($[\mathrm{II}]_0$). These predictions are obtained for the $\delta = f([IIa])$ case, and are representative of the delay introduced due to change in procoagulant concentrations in the model: this is relevant from the standpoint of physiology where initial concentrations vary from person to person.

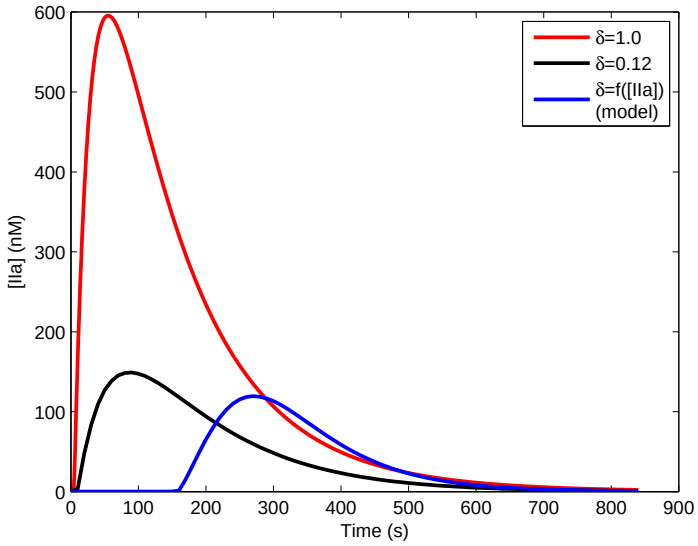

Figure 4: Thrombin production (at [TF]=25pM) at (i) $\delta = 1.0$ ([CP]=[AP]), (ii) $\delta = 0.12$ ([CP]=0.12×[AP]), and (iii) $\delta = f([IIa])$ ([CP]=f([IIa])×[CP]).

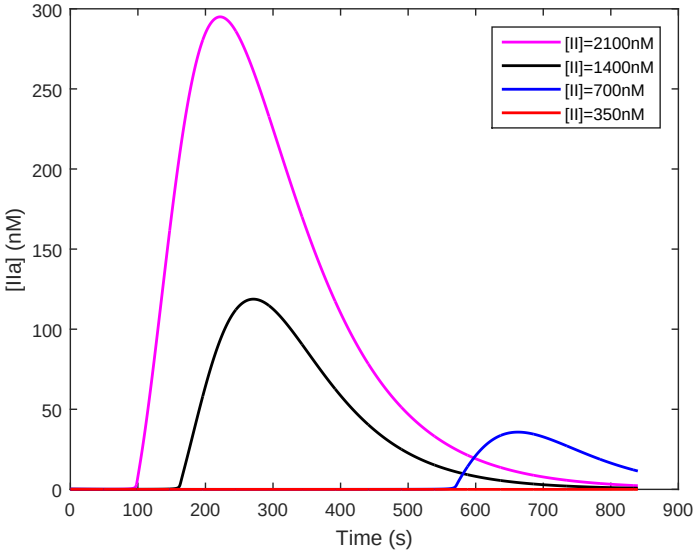

Figure 5: Thrombin production (at $\delta = f([IIa])$) with respect to different initial prothrombin concentrations: (i) $[II]_0$=2100nM (150%), (ii) $[II]_0$=1400nM (100%), (iii) $[II]_0$=700nM (50%), and (iv) $[II]_0$=350nM (25%).

Platelets play a crucial role in the coagulation cascade by providing the requisite surface for reactions to take place [32]. This forms the basis for the next set of model predictions reported in figures 6 & 7.
Figure 6 shows the dependence of thrombin production on prothrombinase concentration. As evident, for the given initial concentration of thrombin and prothrombinase, thrombin production is higher and is sustained for a longer period (in the time of simulation) in the $\delta = 1.0$ case (solid red line) than that in the cases with $\delta = 0.12$ and $\delta = f([IIa])$: we infer that not incorporating coated platelets and their dose-dependent formation in a model will lead to gross overestimation of thrombin generation curves as already seen in Figure 4.

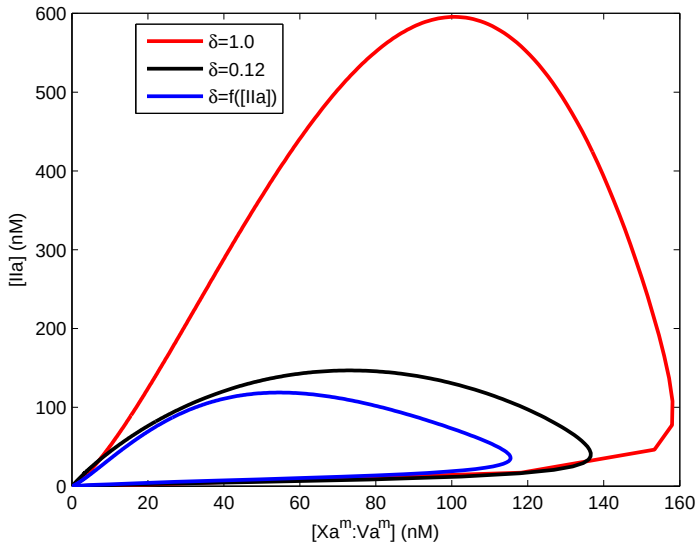

Figure 6: Thrombin production (at [TF]=25pM) with respect to prothrombinase concentration at (i) $\delta = 1.0$ ([CP]=[AP]), (ii) $\delta = 0.12$ ([CP]=0.12×[AP]), and (iii) $\delta = f([IIa])$ ([CP]=f([IIa])×[CP]).

Figure 7 documents the model predictions for production of factor Va which is a key enzyme required for propagation of coagulation. Factor Va production is slightly higher in the $\delta = 1.0$ case (solid red line) as compared with the $\delta = 0.12$ case (solid black line) and $\delta = $ f(IIa) case (solid blue line). Also, peak production of factor Va is much delayed in the $\delta = $ f(IIa) case (202s) than in the $\delta = 1.0$ and $\delta = 0.12$ cases (16s and 39s, respectively). These predictions may suggest a role for coated platelets in controlling an unwanted burst of thrombin production in the very beginning of the coagulation cascade.

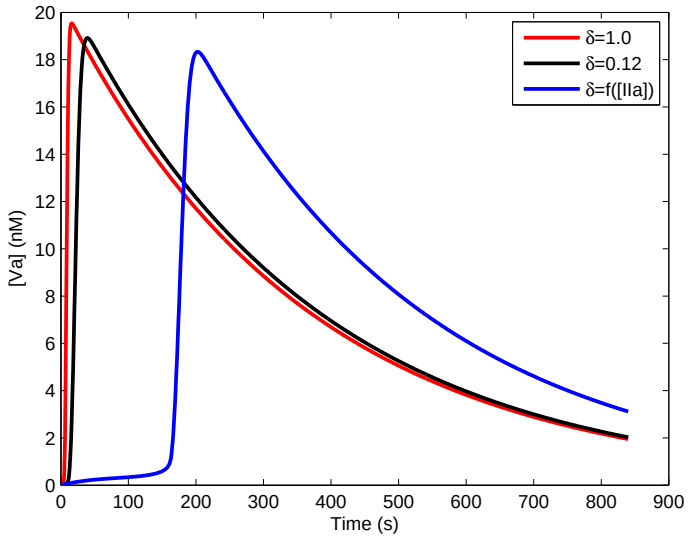

Figure 7: Factor Va production versus time (at [TF]=25pM) at (i) $\delta = 1.0$ ([CP]=[AP]), (ii) $\delta = 0.12$ ([CP]=0.12×[AP]), and (iii) $\delta = f([IIa])$ ([CP]=f([IIa])×[CP]).

5. Discussion

The mechanistic model developed in this work incorporates the role of coated platelets and also the competitive binding of enzymes to platelet membrane, during blood coagulation *in vitro*. The predictions of the proposed model reveal the significant differences in peak and duration of production of the key components - thrombin, prothrombinase and factor Va - when the entire population of platelets is assumed to be procoagulant instead of only the small sub-population as it actually happens: such overestimation is avoided by the proposed model. Predictions of the model developed by Xu et al., 2010, for thrombin generation (see fig 2(c) in supplementary material of [54]) also support this hypothesis of higher estimation of thrombin generation at 100% concentration of procoagulant surface binding sites.

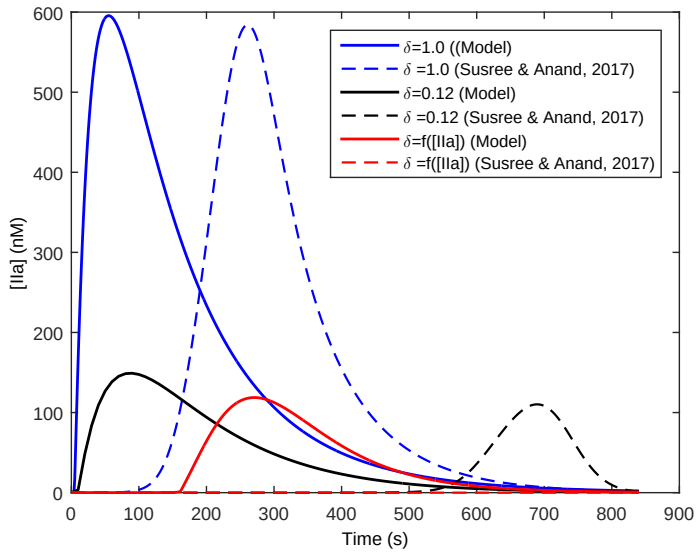

Figure 8: Thrombin production versus time (at [TF]=25pM) at (i) $\delta = 1.0$ ([CP]=[AP]), and (ii) $\delta = 0.12$ ([CP]=0.12×[AP]), for current model (solid lines) against model in Susree & Anand, 2017 (dotted lines).

Figures 8 presents a comparison of predictions of the current model with those from the model recently developed in [47]. As with the current model, there is a significant overestimation of thrombin concentration (both peak and duration) if coated platelets and thrombin dose-dependence are not incorporated. The differences in predictions between the model developed here

and the model in [47] is both quantitative (peak thrombin concentration is lesser and delayed for the Susree-Anand model) and qualitative (rise in thrombin concentration is less steep for the Susree-Anand model). In congruence with the experimental knowledge pertaining to coagulation, inclusion of thrombin-dose dependent procoagulant platelets as a fraction as well as inclusion of competitive binding of all relevant enzymes and zymogens is therefore important in accurately modeling clot formation and clotting times *in vitro*.

5.1. Limitations & Extensions

The model consists of 46 kinetic constants and initial conditions corresponding to 25 species for which ODEs are written; it is therefore practically difficult to match all conditions with biochemical reality when formulating the model. Parameter choice appropriate to the modeled conditions (in this case, *in vitro*) is of prime importance when developing a model [46]. Among the conditions discussed by Diamond [15], we have been able to satisfy (i) reaction network, (ii) reaction mechanism, and (iii) kinetic parameters, in the proposed model. However, the initial conditions selected for the model (0.01% activation of all enzymes) do not match the biochemical reality wherein only VIIa exists in activated form in the initial state and all the other enzymes are non-existent. In the present model, satisfying the initial conditions while using the numerical scheme in the inbuilt ode15s solver of MATLAB R2015b would need to be done by fitting more than one kinetic constant, which would in turn question the validity of the model: a more carefully selected numerical scheme need not have this limitation. Further, the variable binding mechanisms of enzymes with coated platelets, namely the formation of dimers, has been neglected, and these should be addressed while developing a more representative model. Since coated platelets are obtained by activation of platelets by only thrombin and collagen but not other activated platelets in plasma, experimental as well as mechanistic knowledge of platelet-activated platelets can be helpful. It can aid the diagnostic and therapeutic procedures by understanding better the role of heterogeneous platelet populations in blood coagulation.

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Appendix A. Model Reactions

$$G_{TF} = -k_{T7}^{+}[TF][VII] + k_{T7}^{-}[TF:VII] - k_{T7a}^{+}[TF][VIIa] + k_{T7a}^{-}[TF:VIIa] \quad \text{(A.1)}$$

$$G_{VII} = -k_{TF7}[TF:VIIa][VII] - \frac{k_{2,7}[IIa][VII]}{K_{M(2,7)} + [VII]} \quad \text{(A.2)}$$

$$G_{TF:VII} = k_{T7}^{+}[TF][VII] - k_{T7}^{-}[TF:VII] - k_{10,T7}[Xa][TF:VII] - \frac{k_{2,T7}[IIa][TF:VII]}{K_{M(2,7)} + [TF:VII]} \quad \text{(A.3)}$$

$$G_{VIIa} = k_{TF7}[TF:VIIa][VII] + \frac{k_{2,7}[IIa][VII]}{K_{M(2,7)} + [VII]} \quad \text{(A.4)}$$

$$G_{TF:VIIa} = k_{T7a}^{+}[TF][VIIa] - k_{T7a}^{-}[TF:VIIa] + k_{10,T7}[Xa][TF:VII] + \frac{k_{2,T7}[IIa][TF:VII]}{K_{M(2,7)} + [TF:VII]} - h_{7}^{AT}[ATIII][TF:VIIa] - h_{7}^{TP}[TFPI:Xa][TF:VIIa] \quad \text{(A.5)}$$

$$G_{XI} = -k_{eff}(1500 * [CP])[XI][IIa] \quad \text{(A.6)}$$

$$G_{XIa} = k_{eff}(250 * [CP])[XI][IIa] - h_{11(A3)}[XIa][ATIII] - h_{11(L1)}[XIa][\alpha_1 AT] \quad \text{(A.7)}$$

$$G_{IX} = -k_{eff(7,9)}[CP][VIIa][IX] - \frac{k_9[XIa][IX]}{K_{9M} + [IX]} \quad \text{(A.8)}$$

$$G_{IXa} = k_{eff(7,9)}[CP][VIIa][IX] + \frac{k_9[XIa][IX]}{K_{9M} + [IX]} - h_9[IXa][ATIII] \quad \text{(A.9)}$$

$$G_X = -k_{eff(7,10)}[CP][VIIa][X] - \frac{k_{10}[IXa^m][VIIIa^m][X^m]}{K_{D(TEN)}(K_{10M} + X^m)} \quad \text{(A.10)}$$

$$G_{Xa} = k_{eff(7,10)}[CP][VIIa][X] + \frac{k_{10}[IXa^m][VIIIa^m][X^m]}{K_{D(TEN)}(K_{10M} + X^m)} - h_{10}^{TP+}[TFPI][Xa] + h_{10}^{TP-}[Xa:TFPI] - h_{10}^{AT}[ATIII][Xa] \quad \text{(A.11)}$$

$$G_{II} = -k_{2t}[Xa][II] - \frac{(k_2/K_{2M} \times \delta)[Xa:Va^m][II^m]}{[CP]} \quad \text{(A.12)}$$

$$G_{IIa} = k_{2t}[Xa][II] - h_2[ATIII][IIa] + \frac{(k_2/K_{2M} \times \delta)[Xa:Va^m][II^m]}{[CP]} \quad \text{(A.13)}$$

$$G_{RP} = -\frac{k_p[RP][IIa]}{K_{pM} + [IIa]} \quad \text{(A.14)}$$

$$G_{AP} = \frac{k_p[RP][IIa]}{K_{pM} + [IIa]} \quad \text{(A.15)}$$

$$G_{VIII} = -\frac{k_8[IIa][VIII]}{K_{8M} + [VIII]} \quad \text{(A.16)}$$

$$G_{VIIIa} = \frac{k_8[IIa][VIII]}{K_{8M} + [VIII]} - h_8[VIIIa] \quad \text{(A.17)}$$

$$G_V = -\frac{k_5[IIa][V]}{K_{5M} + [V]} \quad \text{(A.18)}$$

$$G_{Va} = \frac{k_5[IIa][V]}{K_{5M} + [V]} - h_5[Va] \quad \text{(A.19)}$$

$$G_I = -\frac{k_f([IIa])[I]}{K_{fM}} \tag{A.20}$$

$$G_{Ia} = \frac{k_f([IIa])[I]}{K_{fM}} \tag{A.21}$$

$$G_{TFPI} = -h_{10}^{TP+}[Xa][TFPI] + h_{10}^{TP-}[Xa:TFPI] \tag{A.22}$$

$$\begin{aligned} G_{Xa:TFPI} = \; & h_{10}^{TP+}[Xa][TFPI] - h_{10}^{TP-}[Xa:TFPI] \\ & -h_7^{TP}[TF:VIIa][Xa:TFPI] \end{aligned} \tag{A.23}$$

$$\begin{aligned} G_{ATIII} = \; & -[ATIII](h_{10}^{AT}[Xa] + h_9[IXa] + h_2[IIa] \\ & +h_{T7}[TF:VIIa] + h_{11(A3)}[XIa]) \end{aligned} \tag{A.24}$$

$$G_{\alpha_1 AT} = -h_{11(L1)}[XIa][\alpha_1 AT] \tag{A.25}$$

Expansions of compact notations

$$\delta = (0.00106 \times [IIa]) + 0.00021$$

$$[CP] = \delta \times [AP]$$

$$[IXa^m] = \frac{25000[CP]\frac{[IXa]}{K_{D9}}}{1 + \frac{[IX]}{K_{D9}} + \frac{[IXa]}{K_{D9}} + \frac{[X]}{K_{D10}} + \frac{[Xa]}{K_{D10}} + \frac{[II]}{K_{D2}} + \frac{[VIII]}{K_{D8}} + \frac{[VIIIa]}{K_{D8}}}$$

$$[VIIIa^m] = \frac{25000[CP]\frac{[VIIIa]}{K_{D8}}}{1 + \frac{[IX]}{K_{D9}} + \frac{[IXa]}{K_{D9}} + \frac{[X]}{K_{D10}} + \frac{[Xa]}{K_{D10}} + \frac{[II]}{K_{D2}} + \frac{[VIII]}{K_{D8}} + \frac{[VIIIa]}{K_{D8}}}$$

$$[Xa : Va^m] = \frac{[Xa][Va^m]}{K_{D,PRO}\left(1 + \frac{[PS]}{K_i} + \frac{[Xa]}{K_{D,PRO}}\right) + [Va^m]}$$

$$[X^m] = \frac{25000[CP]\frac{[X]}{K_{D10}}}{1 + \frac{[IX]}{K_{D9}} + \frac{[IXa]}{K_{D9}} + \frac{[X]}{K_{D10}} + \frac{[Xa]}{K_{D10}} + \frac{[II]}{K_{D2}} + \frac{[VIII]}{K_{D8}} + \frac{[VIIIa]}{K_{D8}}}$$

$$[II^m] = \frac{25000[CP]\frac{[II]}{K_{D2}}}{1 + \frac{[IX]}{K_{D9}} + \frac{[IXa]}{K_{D9}} + \frac{[X]}{K_{D10}} + \frac{[Xa]}{K_{D10}} + \frac{[II]}{K_{D2}} + \frac{[VIII]}{K_{D8}} + \frac{[VIIIa]}{K_{D8}}}$$

$$[Va^m] = \frac{3000[CP][Va]}{K_{D5} + [Va]}$$

Table A.1: **Reactions & Kinetic Constants**

Reaction	Kinetic Constant	Description	References
$RP + IIa \xrightarrow{kp, KpM} AP$	$kp = 0.09s^{-1}$, $K_{pM} = 2.4nM$	platelet activation by IIa	[44]
$TF + VII \underset{k_{T7}^-}{\overset{k_{T7}^+}{\rightleftharpoons}} TF:VII$	$k_{T7}^+ = 3.2 \times 10^{-03} nM^{-1}s^{-1}$	binding of TF & VII	[47]
	$k_{T7}^- = 3.1 \times 10^{-03} s^{-1}$	dissociation of TF:VII	[47]
$TF + VIIa \underset{k_{T7a}^-}{\overset{k_{T7a}^+}{\rightleftharpoons}} TF:VIIa$	$k_{T7a}^+ = 0.023nM^{-1}s^{-1}$	binding of TF & VIIa	[47]
	$k_{T7a}^- = 3.1 \times 10^{-03} s^{-1}$	dissociation of TF:VIIa	[47]
$TF:VIIa + VII \xrightarrow{k_{TF7}} TF:VIIa + VIIa$	$k_{TF7} = 4.4 \times 10^{-04} nM^{-1}s^{-1}$	auto-activation of VII	[47]
$TF:VII + Xa \xrightarrow{k_{10,T7}} TF:VIIa + Xa$	$k_{10,T7} = 6.667 \times 10^{-03} nM^{-1}s^{-1}$	Xa-activation of TF:VII	[42]
$VII + IIa \xrightarrow{k_{2,7}, K_{M(2,7)}} VIIa + IIa$	$k_{2,7} = 0.061s^{-1}$ $K_{M(2,7)} = 2700nM$	IIa-activation of VII	[10]
$TF:VII + IIa \xrightarrow{k_{2,7}, K_{M(2,7)}} VIIa + IIa$	$k_{2,7} = 0.061s^{-1}$ $K_{M(2,7)} = 2700nM$	IIa-activation of TF:VII	[10]
$TF:VIIa + ATIII \xrightarrow{h_7^{AT}} TF:VIIa_i + ATIII$	$h_7^{AT} = 4.5 \times 10^{-07} nM^{-1}s^{-1}$	ATIII inactivation of TF:VIIa	[47]
$TF:VIIa + Xa:TFPI \xrightarrow{h_7^{TP}} TF:VIIa_i + Xa:TFPI$	$h_7^{TP} = 0.05nM^{-1}s^{-1}$	Xa:TFPI inactivation of TF:VIIa	[47]
$VIIa + IX \xrightarrow{k_{eff(7,9)}} VIIa + IXa$	$k_{eff(7,9)} = \frac{3.333 \times 10^{-06}}{\delta} nM^{-2}s^{-1}$	VIIa activation of IX	[35]
$IIa + XI \xrightarrow{k_{eff}} IIa + XIa$	$k_{eff} = 0.0005/\delta nM^{-2}s^{-1}$	IIa activation of XI	[35]
$XIa + ATIII \xrightarrow{h_{11(A3)}} XIi + ATIII$	$h_{11(A3)} = 2.667 \times 10^{-05} nM^{-1}s^{-1}$	ATIII inactivation of XIa	[4]
$XIa + \alpha_1 AT \xrightarrow{h_{11(L1)}} XIi + \alpha_1 AT$	$h_{11(L1)} = 2.167 \times 10^{-07} nM^{-1}s^{-1}$	α1AT inactivation of XIa	[4]
$IX + XIa \xrightarrow{k_9, K_{9M}} IXa + XIa$	$k_9 = 0.1833s^{-1}$ $K_{9M} = 160.0nM$	XIa activation of IX	[45]

Continued on next page

Table A.1 – continued from previous page

Reaction	Kinetic Constant	Description	References
$IXa + ATIII \xrightarrow{h_9} IXi + ATIII$	$h_9 = 2.223 \times 10^{-04} nM^{-1}s^{-1}$	ATIII inactivation of IXa	[47]
$VIIa + X \xrightarrow{k_{eff(7,10)}} VIIa + Xa$	$k_{eff(7,10)} = \frac{1.667 \times 10^{-04}}{\delta} nM^{-2}s^{-1}$	VIIa activation of X	[35]
$VIIIa + IXa \xrightleftharpoons{K_{D(TEN)}} VIIIa : IXa$	$K_{D(TEN)} = 0.56nM$	intrinsic tenase production	[35]
$VIIIa : IXa + X \xrightarrow{k_{10},K_{10M}} VIIIa : IXa + Xa$	$k_{10} = 8.334s^{-1}$ $K_{10M} = 63.0nM$	intrinsic tenase activation of X	[35]
$Xa + ATIII \xrightarrow{h_{10}} Xi + ATIII$	$h_{10}^{AT} = 3.05 \times 10^{-06} nM^{-1}s^{-1}$	ATIII inactivation of Xa	[47]
$Xa + TFPI \underset{h_{10}^{TP-}}{\overset{h_{10}^{TP+}}{\rightleftharpoons}} Xi + TFPI$	$h_{10}^{TP+} = 8.667 \times 10{-}04 nM^{-1}s^{-1}$ $h_{10}^{TP} = 3.33 \times 10{-}04 s^{-1}$	binding & dissociation of Xa, TFPI	[7]
$II + Xa \xrightarrow{k_{2t}} IIa + Xa$	$k_{2t} = 7.5 \times 10^{-06} nM^{-1}s^{-1}$	Xa-activation of II	[47]
$II + Xa : Va^m \xrightarrow{k_2,K_{2M}} IIa + Xa : Va^m$	$k_2/K_{2M} = 4.63 \times 10^{-05} s^{-1}$	prothrombinase activation of II	estimated to fit data in fig. 1
$IIa + ATIII \xrightarrow{h_2} IIi + ATIII$	$h_2 = 4.816 \times 10^{-06} nM^{-1}s^{-1}$	ATIII inactivation of IIa	[47]
$VIII + IIa \xrightarrow{k_8,K_{8M}} VIIIa + IIa$	$k_8 = 0.9s^{-1}$ $K_{8M} = 147.0nM$	IIa-activation of VIII	[47]
$VIIIa \xrightarrow{h_8} VIIIi$	$h_8 = 0.0037s^{-1}$	spontaneous decay of VIIIa	[47]
$V + IIa \xrightarrow{k_5,K_{5M}} Va + IIa$	$k_5 = 0.233s^{-1}$ $K_{5M} = 71.7nM$	IIa-activation of V	[47]
$Xa + Va^m \xrightarrow{K_{D,PRO}} Xa : Va^m$	$K_{D,PRO} = 0.118nM$	prothrombinase production	[35]
$Va \xrightarrow{h_5} Vi$	$h_5 = 0.0028s^{-1}$	spontaneous decay of Va	[47]
$I + IIa \xrightarrow{k_f,K_{fM}} Ia$	$k_f = 59.0s^{-1}$ $K_{fM} = 3160.0nM$	IIa-activation of fibrinogen	[47]

Other parameters [35]: $[PS]$[2]$= 346.0nM$, $K_i = 200.0nM$, ρ[3]$= 1000 molecules/platelet$.

Table A.2: **Dissociation constants for binding to membrane surface**

Coagulation Factor	**Dissociation Constant**	**References**
IX/IXa	K_{D9} =12.0 nM	[31]
X/Xa	K_{D10} =113.80 nM	[23]
II	K_{D2} =590.0 nM	[23]
VIII/VIIIa	K_{D8} =0.57 nM	[23]
V/Va	K_{D5} =2.98 nM	[23]

[2]phosphatidylserine
[3]molecules of factor V/platelet

The initial concentrations of the zymogens, inhibitors and platelets (Table A.3) have been obtained from literature. They have been used previously in [47].

Table A.3: **Initial Concentrations of the Proteins and Platelets**

Component	**Normal Conc.(nM)**
TF	variable
VII	10.0
TF:VII	0.0
VIIa	0.1
TF:VIIa	0.0
XIa	0.003
XI	30.0
IXa	0.009
IX	90.0
Xa	0.017
X	170.0
IIa	0.140
II	1400.0
RP	10.0
AP	0.001
VIIIa	0.00007
VIII	0.7
Va	0.002
V	20.0
I	7000.0
Ia	0.70
TFPI	2.5
Xa:TFPI	0.0
ATIII	3400.0
α1AT	45000.0

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