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A simplified mathematical model for thrombin generation

Papadopoulos Kostis, Gavaises Manolis and Chris Atkin

School of Engineering and Mathematical Sciences

City University London

Northampton square, London

EC1V 0HB

Room: C171
Phone: +44 (0) 20 7040 8115
Fax: +44 (0) 20 7040 8566
Email: Papadopoulos.Konstantinos.1@city.ac.uk :
M.Gavaises@city.ac.uk

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3 **Abstract**

4 A new phenomenological mathematical model based directly on laboratory data for thrombin

5 generation and having a patient-specific character is described. A set of the solved equations

6 for cell-based models of blood coagulation that can reproduce the temporal evolution of

7 thrombin generation is proposed; such equations are appropriate for use in Computational

8 Fluid Dynamics (CFD) simulations. The initial values for the reaction rates are either taken

9 from already existing model or experimental data, or they can be obtained from simple

10 reasoning under certain assumptions; it is shown that coefficients can be adjusted in order to

11 fit a range of different thrombin generation curves as derived from thrombin generation

12 assays. The behaviour of the model for different platelet concentration seems to be in good

13 agreement with reported experimental data. It is shown that the reduced set of equations used

14 represents to a good approximation a low-order model of the detailed mechanism and thus it

15 can represent a cost-effective and-case specific mathematical model of coagulation reactions

16 up to thrombin generation

17 **Keywords:**

18 CFD, coagulation, simulation, thrombus

19

20 **Introduction**

21 The formation of thrombus in blood is involved in a number of life threatening situations like

22 Coronary Artery Disease and mechanical heart valve complications; it is a multi-scale

23 phenomenon both in respect of time and space, involving a number of biochemical

24 substances, blood circulating minerals and cellular responses. While the formation of blood

25 clot is a physiological response of human body to vessel injury, it can be initiated when blood

26 contacts certain substances like those exposed after the rupture of atheromatous plaques in

27 stenosed vessels [Rauch, Osende et al. 2001] and when pathological flow conditions prevail

28 in a region [Nesbitt, Westein et al. 2009]. Between the initiation and the formation of a

29 thrombus, a series of enzymatic reactions takes place also known as coagulation cascade

30 [Furie and Furie 2008], classically divided in three parts: (1) the extrinsic or Tissue Factor

31 (TF) pathway, (2) the intrinsic or contact pathway and the (3) common pathway. In every

32 step of the process a circulating zymogen is activated, with the activation reaction being

33 catalysed by the products of previous steps. However, as most of these enzymatic reactions

34 take place on cell membranes, the current approaches for coagulation are cell-based models

35 and the process is divided in three discrete phases, initiation, amplification and propagation.

36 Thrombin (factor IIa) and platelets play critical roles in the coagulation process. Thrombin in

37 the final step catalyses the conversion of fibrinogen (factor I) to fibrin (factor Ia), a protein

38 that through polymerization creates a mesh clot that also traps circulating blood cells. In

39 addition, thrombin activates factor XIII (that forms bonds that crosslink the fibrin strands

40 [Mosesson 2005]), causes the activation of platelets [Brass 2003], the activation of factors V

41 and VIII and their inhibitor protein C (APC). Platelets on the other hand, after activation by

42 chemical or mechanical stimulation [Jesty, Yin et al. 2003] become adhesive and form

43 aggregates on the materials exposed after arterial damage [Badimon, Badimon et al. 1986] or

44 plaque rapture [Fernández-Ortiz, Badimon et al. 1994; Reininger, Bernlochner et al. 2010] or

45 in flowing blood. In addition they play a major role in thrombin formation [Rosing, van Rijn

46 et al. 1985; Monroe, Hoffman et al. 2002] and they enhance the coagulation process by

47 supporting on their membrane some of the coagulation reactions [Smith 2009], releasing

48 chemical substances and micro-particles [Rendu and Brohard-Bohn 2001] that influence the

49 progress of coagulation and activate other platelets.

50 The advance of computational techniques and increase of computational power have made

51   possible the emergence of in silico studies that reproduce a part of or the whole process in

52   greater or lesser detail, simulating either the whole process of thrombus formation or only the

53   coagulation reaction system up to thrombin or fibrin production. The first mathematical

54   simulation of thrombin and fibrin generation in plasma used exponential time functions as

55   fixed inputs for concentration of some enzymes [Willems, Lindhout et al. 1991]. In vitro

56   measurements of the reaction rate constants [Lawson, Kalafatis et al. 1994] were used for the

57   development of a system of 20 reactions, including formation and breakage of complexes, in

58   a study that mainly focused on the effect of variation of the concentration of different factors

59   [Jones and Mann 1994]. A similar model was proposed for the intrinsic pathway including

60   fibrin production and APC inhibition mechanism, and was used to investigate threshold

61   values for some enzymes and the spatial propagation of coagulation from the reacting site due

62   to diffusion [Zarnitsina, Pokhilko et al. 1996; Zarnitsina, Pokhilko et al. 1996]. Subsequent

63   work included more chemical substances and biochemical processes [Hockin, Jones et al.

64   2002] up to thrombin production, resulting in a system consisting of 27 reactions and 42

65   reaction rate constants that later was combined with a Monte Carlo simulation method, in

66   order to detect changes to the cascade initiation behaviour, due to small variation of the

67   concentration of enzymes induced by the stochastic approach [Lo, Denney et al. 2005]. At the

68   same time some studies used simulations to investigate a specific part of the coagulation

69   cascade, as the function of positive feedback loops and threshold concentrations for cascade

70   initiation[Beltrami and Jesty 1995], the triggering threshold with respect to Tissue Factor

71   Pathway Inhibitor (TFPI) [Xu, Hu Xu et al. 2005] or the inhibition mechanism of APC [Qiao,

72   Xu et al. 2004].

73   The studies that simulate thrombus formation and growth, simultaneously with blood flow

74   and concentration of related substances, necessitate a less detailed sub-model for the

75   coagulation cascade. While for the first studies of this kind the production rates of substances

76   were mainly modelled as fluxes or with the use of few reactions [Hubbell and McIntire 1986;

77   Folie and McIntire 1989], the increase of computational power allowed more complicated

78   multi-scale and multi-phase models to emerge, that include an integrated coagulation sub-

79   model. The authors of [Kuharsky and Fogelson 2001] proposed an integrated model of

80   thrombus formation under flow conditions, taking into account the localization of reactions

81   on surfaces, with the inclusion of the available binding sites on cell membranes for enzymes

82   and using a system of 59 equations to simulate the coagulation system up to thrombin

83   generation. A study that modelled platelet-platelet and platelet-wall interaction as reversible

84   elastic links demonstrated the influence of these interactions on the flow field and predicted

85   thrombus evolution and emboli formation [Fogelson and Guy 2004]. The initial model was

86   later improved with the addition of the APC mechanism and the transport of substances

87   between plasma and endothelium cells [Fogelson and Tania 2005]. The same concepts for

88   cells and reactions were combined with an immersed boundary method [Lai and Peskin 2000;

89   Peskin 2002] for modelling platelet movement and the interaction between platelet membrane

90   sites and chemicals or endothelium. The results of this micro-scale model were also used to

91   develop a continuous model for platelet aggregation (with platelets as continuous phase with

92   movement limitations) describing the alterations in blood flow due to the presence of

93   aggregated platelets [Fogelson and Guy 2008]. The macro-scale model was tested in

94   simulations with pulsating flow in an idealized two dimensional vessel bifurcation [Yang,

95   Lewis et al. 2004]. The continuous model, with coupling of flow with thrombus growth and

96   including flow and transport within the thrombus, was used to demonstrate the effects of flow

97   conditions and the quantity of TF exposed in thrombus growth [Leiderman and Fogelson

98   2011]. Anand et al [Anand, Rajagopal et al. 2003; Anand, Rajagopal et al. 2005] presented

99   another multi-process model that used a viscoelastic model to simulate flow for both free

100   vessel lumen and clot. This model also incorporated the activation of platelets due to

101 excessive shear stress and fibrin production and lysis. In a similar work, a model for the

102 viscosity of blood depending on fibrin concentration was proposed and used in a three-

103 dimensional simulation of blood coagulation in a tube with a reacting site; in this study the

104 area where fibrin concentration exceeded a specific value interpreted as the area occupied by

105 the clot[Bodnár and Sequeira 2008]. In [Xu, Chen et al. 2008] another multi-scale model was

106 proposed that included a cellular pot model [Marée, Grieneisen et al. 2007] for discrete cells

107 and cell movement was simulated through an energy-based stochastic process. The

108 simulation involved differentiation of cell movements depending on fibrin levels and cell-cell

109 or cell-surface interaction and bonds. The model was used to evaluate the role of fVII in

110 venous thrombus formation due to vessel injury [Xu, Lioi et al. 2010] and to examine the

111 impact of pulsating flow and the non-Newtonian characteristics of blood on thrombus growth

112 [Xu, Chen et al. 2009].

113 While the detailed description of coagulation included in these works makes them appropriate

114 for studying the influence of different factors, unfortunately it increases dramatically the

115 computational cost; thus published applications mainly refer to small two dimensional

116 regions (~100μm) while the dimensions of computational regions for studying thrombus

117 formation in a coronary artery or in mechanical heart valves are much larger (typically, the

118 diameter of the coronary artery is about 4mm while the diameter of the aortic root is of some

119 cm) with the flow distribution being three-dimensional and strongly time dependent

120 preventing use of simplified flow models. In addition, these models do not have patient-

121 specific characteristics, as the use of reaction rate constants derived from experiments do not

122 allow the significant variability of thrombin generation observed for different individuals

123 [Oliver, Monroe et al. 1999]. At the same time, it has been shown that the resulting thrombin

124 generation curve predicted by such models under steady state conditions can be simulated in

125 different ways by a much simpler system of 6 equations [Wagenvoord, Hemker et al. 2006].

126   As the process between the initial stimulation and the formation of thrombin consist the main

127   part of the coagulation reactions, our motivation is to develop a phenomenological model for

128   thrombin formation that would be efficient enough to be used with three dimensional

129   Computational Fluid Dynamic (CFD) simulations while also being adjustable in order to

130   reflect measured differences existing in data of different individuals. A case-specific

131   simplified model like this, though not including the full biochemical details of the process,

132   could be used for comparison of different cases of clinical interest.

133   **Materials and methods**

134   The aim of the study is to propose a set of equations that describe the thrombin generation in

135   blood using the minimum possible number of parameters but which are able to describe with

136   acceptable accuracy the whole process. The model is validated against experimental results

137   for thrombin generation in vitro from Thrombin Generation Assays (TGA). The proposed

138   model for thrombin production is based on the cell-based models of coagulation[Smith 2009];

139   the full description of the biochemical processes are mainly based on [Hoffman and Monroe

140   2001]. For a detailed modelling of coagulation the localization of the different reactions

141   makes the task more complicated, as it requires taking into account additional parameters

142   such as the binding rate of the substances on cell membranes and the expression,

143   concentration and availability of appropriate binding sites on the cell surfaces. For the

144   development of a simplified model however, it can function as an advantage, as the processes

145   can be grouped in respect to the location they occur (on platelet surface, on the vessel wall or

146   in plasma). This approach is rigorous in cases where the transport of reactants is mainly due

147   to convection such as arterial flow conditions, or in cases where the different species are well

148   mixed. The generation of thrombin and generally the coagulation process is mainly attributed

149   to activated platelets, while the initiation phase is localized on the reacting site of the vessel

150   surface. The burst of thrombin generation is considered to occur when the small amounts of

151   thrombin produced during the initiation phase cause thrombin concentration to exceed a

152   threshold value. The threshold values used are within the range of thrombin concentration

153   values that have been reported in [Rosing, van Rijn et al. 1985] as being capable of causing

154   platelet activation, 0.5 to 1.2nM of thrombin (0.05 to 0.12U/ml) . The equations of the model

155   are shown in Table 1. The initial values for the reaction rate constants, shown in Table 2, are

156   taken either from already existing models or from experimental studies.

157   As the proposed model used a reduced number of 4 reactions, each reaction rate represents

158   the integral effect of more than one actual process. Also, 'activated platelets' actually

159   represent platelet activity, in the sense that 100% activated platelets implies maximum

160   platelet activity rather than the fact that all platelets are activated. Figure 1a illustrates the

161   actual biochemical reactions that influence each constant while Figure 1b the reduced model.

162   The model is structured as follows: During the initiation phase thrombin generation is

163   described by a slow first order reaction. This reaction, which is localized on the TF-bearing

164   cells, is used to describe the whole pro-coagulant activity that occurs on the TF-bearing cells

165   − formation of the TF-VIIa complex, activation of factors IX, X and V. In this reaction is also

166   included the inhibition of the amounts of IXa, Xa that leave the cell surfaces by TFPI in

167   plasma. The initial value for the reaction rate constant was estimated using the lag times

168   reported in [Lawson, Kalafatis et al. 1994; van't Veer and Mann 1997]. Inhibition of thrombin

169   during this phase is also modelled as a first-order reaction, with the value of the reaction rate

170   constant for thrombin inhibition calculated from the IIa ·ATIII inhibition reaction, using the

171   initial bulk concentration of ATIII ($k_{in} = k_{in,ATIII} \cdot \varphi_{ATIII,0} = 1.71 \cdot 10^{-2} s^{-1}$), as this

172   reaction is the major process of thrombin inhibition .(Thrombin inactivation is 77% by anti-

173   thrombin, 14% a_2 macroglobulin and 9% by minor inhibitors [Hemker and Béguin 1995]).

174   With this setup for the initiation phase, the model describes the threshold behaviour of the

175   initiation of blood coagulation in respect to TF concentration[Okorie, Denney et al. 2008;

176   Shen, Kastrup et al. 2008], as there is a minimum value of activation constant capable of

177   causing thrombin burst and, as this constant is related to the TF concentration (see

178   discussion), a threshold value for TF concentration.

179   Beyond the point that thrombin concentration has reached the threshold value, the conversion

180   of prothrombin to thrombin is mainly attributed to platelets after their activation. This process

181   is modelled as a second-order reaction, with the initial values for the reaction rate constant

182   used from [Rosing, van Rijn et al. 1985; Sorensen, Burgreen et al. 1999]. Platelets can be

183   activated either by thrombin, if its concentration is greater than the threshold value, or

184   directly by other activated platelets. Thrombin in concentration about 1nM can initiate the

185   activation of platelets [Liu, Freedman et al. 1994], from 0.5nM for minimum activity to

186   1.2nM for maximal [Rosing, van Rijn et al. 1985]. Platelet activation by thrombin is

187   modelled as a first-order reaction that is initiated when thrombin concentration reaches the

188   threshold value. The activation of platelets by activated platelets represents the activation by

189   platelet-released substances and is modelled as a second order reaction. the reaction rate

190   constants for the activation of platelets used were found in [Kuharsky and Fogelson 2001]. In

191   the case of TF induced coagulation the contribution of the later reaction is negligible, but it

192   can make the model capable of being used to describe shear induced coagulation.

193

194   Inhibition of thrombin during the propagation phase is again modelled as a first-order

195   reaction. The reaction rate constant for the inhibition of thrombin in plasma is not the same as

196   for the initiation phase; here we must note that this constant is significantly smaller than the

197   one used in [Leiderman and Fogelson 2011] for modelling this reaction in the same manner,

198   which was based on thrombin half-life in plasma. However, as the activation of protein C that

199   acts as an inhibitor to the coagulation process mainly occurs on the endothelium cells, for the

200 areas near the endothelium cells the value used for thrombin inhibition is larger, with the

201 exact value derived from the adjustment of the model with the use of TGA results. As

202 demonstrated in Figure 2, the model using the initial parameter values gives reasonable

203 results. By adjusting the values of the constants and parameters of the model within

204 physiological limits, the model can reproduce, to a good level of accuracy, actual thrombin

205 production, by considering the four main parameters of a thrombin generation assay: lag-time

206 (Tlag), maximum concentration (Cmax), time until thrombin concentration reaches the

207 maximum (Tmax) and the estimated thrombin potential (ETP); the last one is represented by

208 the surface under the curve in a thrombin concentration vs time graph. The initial attempts of

209 adjustments were performed manually, but in general the adjustment can be done using the

210 following procedure and assumptions.

211 Platelet response is very fast compared to the other processes included in the model

212 [Frojmovic, Mooney et al. 1994; Frojmovic, Mooney et al. 1994]. With the approximation

213 that all platelets are activated as soon as thrombin concentration reaches the threshold value,

214 the system of the equations can be analytically solved, giving the equation that describes

215 thrombin concentration through time. This equation has the general form (see appendix 1a):

$$[IIa](t) = \frac{B_i}{A_i - C_i}(e^{A_i t} - e^{C_i t}) = F(t)$$

216 The equation is similar to the equation proposed in [Wagenvoord, Hemker et al. 2006] but in

217 a non-uniform platelet distribution the results will vary in space. Here the constants A, B and

218 C depend both on the model parameters and TAG results, and they have different values for

219 the initial phase and the propagation phase.

220 Using the parameters of the TGA and this function we obtain the equations

$$F(Tlag) = [IIa]_{thr}$$

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$$F(Tmax) = Cmax$$

$$\frac{dF}{dt}(Tmax) = 0$$

$$\int_0^\infty F(t)dt \cong \int_{Tlag}^{t\infty} F(t)dt = ETP$$

221 From this set of equations the constants of the model can be approximated numerically,

222 making the resulting model equations able to reproduce with the curve of a TGA with given

223 parameters. In most of the following results only the constants related to thrombin production

224 $k_{II}^{AP}, k_{surf}, k_{in}$ (see Table 1) were adjusted. In case data from the whole curve is available,

225 the constants can be approximated as described in appendix 1b.

Results

227 We first applied the model using the initial values for the constants; while the resulting curve

228 has the shape of a typical thrombin generation curve, the actual values were significantly

229 higher than the typical results of TGA for fresh platelet rich plasma as reported in

230 [Gerotziafas, Depasse et al. 2005]. Performing simulations for different time steps, we found

231 that the results of the model are identical for time steps below 0.5s (Figure 2). As a whole

232 heart cycle at rest conditions is about 0.8s (for 75 bpm), the maximum magnitude of the time

233 steps for simulating pulsating flow is much smaller, thus in terms of temporal discretization,

234 the coupling of the model with CFD simulations can be straightforward.

235 Subsequently we applied the model in 8 different cases, denoted as Case1-8. In the plots,

236 when both model and experimental results are represented, model results are named 'C#

237 model' and the experimental results 'C# exp'. The equations for the adjustment of the

238 constants were solved in MATLAB, while the adjustment of the platelet related constants was

239 done manually using Excel and Systems Biology Toolbox 2 [2006]. Case 1 represents the

240   tuning of the model constants in order to reproduce typical TGA results as reported in

241   [Gerotziafas, Depasse et al. 2005] and the resulting curve (C1 model) is shown in Figure 3

242   and Figure 4, compared with curves representing slower thrombin generation. In the absence

243   of inhibition (case 2 and case 3), the time interval between the end of the initiation phase and

244   the moment that thrombin concentration reaches its maximum value is 2-4 min, depending on

245   the constant for thrombin activation by activated platelets, and the results match

246   approximately the experimental thrombin production curves found in [Lawson, Kalafatis et

247   al. 1994] (Figure 5).

248   The next test (Case 4) involved adjusting the model constants in order to fit an arbitrary

249   thrombin generation curve. The experimental curve was found in [Hemker, Giesen et al.

250   2003]. Figure 6, shows that the model predicts the experimental curve with good accuracy.

251   For future application of the model under flow conditions characterised by non-uniform

252   concentrations of platelets, it has been considered important to test its behaviour with given

253   constants for varying concentration of platelets near to the physiological values. We adjusted

254   the constants so that the model approximated the TGA results for platelet concentration 150 x

255   10^9 pl/L (physiological values are 200-400 x 10^9 pl/L) and then applied the model with the

256   same setup for two other values of platelet concentrations; Cases 5-7 use the same values for

257   reaction rate constants with platelet concentration 400, 150 and 100 x 10^9 pl/L respectively.

258   The results shown on Table 3 are within the range of values reported in [Gerotziafas, Depasse

259   et al. 2005] although the dependence of maximum concentration of thrombin on platelet

260   concentration seems a little stronger than for the in vitro experiments. The resulting curves

261   are shown in Figure 3. Further increase of platelet concentration resulted in higher values for

262   maximum thrombin concentration and small decrease of Tmax, without significant effect on

263   ETP. If the constants describing platelet activation are also suitably modified, the equations

264   can match curves that depict slower thrombin formation like the ones reported in [Allen,

265   Wolberg et al. 2004] for lower platelet concentrations (75 x 10^9 pl/L) as shown in Figure 4.

266   The values of the model constants after modification, in order to fit different cases, are shown

267   in Table 4 and for all cases the values are within a reasonable range.

268   **Discussion**

269   This work presents a phenomenological model for thrombin generation that is flexible

270   enough to reproduce a wide range of cases and simple enough to be used for modelling

271   thrombin generation in large scale CFD simulations − in contrast with the most recently

272   suggested models. The four equations of the model are based on the principle assumption that

273   all reactions occur either on the platelet surface or on the reacting site of the vessel wall.

274   The reactions related to platelet activation actually represent the transition from the initiation

275   to the propagation phase. As the curves used to reproduce a wide range of TGA results

276   correspond to different experiments, these constants vary significantly between two groups of

277   Cases. In the experiments for Cases 1-4, where phospholipids have been used as a substrate

278   for enzymatic reactions, this transition is much faster than for Cases 5-8 where human

279   platelets have been used. For Cases 5-8 the values of these constants have been adjusted

280   manually. It is interesting that the calculated time until platelets reach half of the maximum

281   activity for Case 8 is 5-10 min, in agreement with [Allen, Wolberg et al. 2004]. However, as

282   reported recently [Ninivaggi, Apitz-Castro et al. 2012], TGA results in whole blood resemble

283   the curves of Case 1 and 4, (possibly because red blood cells that are not included in most

284   TGA also contribute to thrombin generation [Ninivaggi, Apitz-Castro et al. 2012; Whelihan

285   and Mann 2013]) so this modification of the parameters related to platelet activation is not

286   necessary for physiological cases and the model can be calibrated as described above. On the

287   other hand with the use of this modification the model could also approximate pathological

288   cases as haemophilia or thrombin generation after anticoagulation treatment. In contrast to

289   the methods described and reviewed in [Brummel-Ziedins 2013] where the effect of the

290   variation of each factor concentration and activity is investigated, this model uses only the

291   information included in the TGA curve, thus it has some limitations. For a given value of

292   inhibition reaction rate, has a maximum lag time that it can reproduce if the reported lag time

293   is greater than this maximum value, modification of the inhibition constant is required.

294   Curves corresponding to pathological situations as severe haemophilia A [Wagenvoord,

295   Hemker et al. 2006]) can be approximated. As factor VIII is mainly involved in the

296   propagation phase, it is expected that in order to reproduce curves for different fVIII

297   concentrations the related constants (k_{in}, k_{AP}^{IIa} and k_{IIa}^{AP}) should be modified. As shown in

298   Figure 8, this can by done ((the concentrations of VIII have been calculated using 12.8 hours

299   as half-life of fVIII [van Dijk, van der Bom et al. 2005]). `At first sight, the most significant

300   inaccuracy of the model (when the initial values of the parameters are used) is that the time

301   interval between the initiation of thrombin burst and maximum concentration of thrombin is

302   smaller than the one reported in TGAs (2.8min compared to 2min predicted by the model)

303   when adjusting only the three previously mentioned parameters. The increase of thrombin

304   concentration predicted by the model is sharper compared to the experimental data and the

305   shapes of the two curves differ for this time interval. That can be fixed by adjusting manually

306   the constants related to platelet activation as we did for Cases 4-8. However the experimental

307   data, while in all studies presented in the form of curves, in some cases actually correspond to

308   measurements in discrete time intervals and the resulting curves are obtained through data

309   fitting. In Figure 7 a plot of three curves is shown, for the time interval of thrombin

310   concentration increase, obtained from the same model results. In one case (dense) the time

311   interval between two data points is 2s, while for the other two cases (sparse1 and sparse2) is

312   30s and 20s respectively –reasonable time intervals between the withdrawal of two samples.

313   It is obvious that curve fitting on experimental values, used in studies prior to the introduction

314   of continuous monitoring of thrombin generation, while making the results more presentable

315   can also give incorrect information on the actual evolution of the process between two

316   measurements, so differences between modelled and experimental curves do not necessary

317   indicate inadequacy of the model.

318   The constant that represents the initial thrombin production rate k_{surf} (for a given value of the

319   inhibition rate) is mainly determined by the lag time. While there is dependence of different

320   experiments' results on the exact composition of the samples and the triggering substance

321   used, for the case described in [van't Veer and Mann 1997; Gerotziafas, Depasse et al. 2005],

322   there seems to be a clear relationship (R^2=0.989) between TF concentration and k_{surf} for the

323   same experimental conditions and TF concentrations between 1 and 30pM:

$$k_{surf} = k_{surf,max}\left(A + \text{B}\ln\left(\frac{[TF]}{[TF_{max}]}\right)\right)$$

324   The constant values are $A = 0.996 \approx 1, B = 0.0372$ while for the aforementioned study the

325   maximum calculated value for the rate of thrombin generation during the initiation phase is

326     $k_{surf,max} = 7.91 \cdot 10^{-6} s^{-1}$ and the relationship can approximately be written as:

$$k_{surf} = k_{surf,max}\left(1 + 0.0372 \cdot \ln\left(\frac{[TF]}{[TF_{max}]}\right)\right)$$

327   These relationship gives good result when estimating k_{surf} in other studies with similar

328   methodology, but for studies using recombinant TF:VIIa as trigger for the coagulation

329   process, while the a good correlation between k_{surf} and trigger concentration can be

330   established, (R^2>0.98) the resulting formulas are different.

331   For the case of thrombus formation on a reacting site of a blood vessel wall, the term

332   representing the slow phase of thrombin generation corresponds to a surface reaction term.

333   The reaction rate for the surface reaction can be calculated with the use of the reported

334   surface TF concentration in atheromatous plaques (33pg/cm^2) [Bonderman, Teml et al. 2002]

335   and the molecular weight of (46,000Da approximately [Arabinda Guha 1986]). For a

336   computational cubic cell of e.g. 100μm this numbers would lead to a TF concentration of

337   about 70pM resulting to $k_{surf} = 9.75 \cdot 10^{-6} s^{-1}$. Sub-threshold concentration of thrombin

338   actually represents also the products of the previous steps of coagulation. At the same time

339   this approach allows a slow rate of fibrin production before the burst of thrombin and a

340   realistic prediction of clotting time. As TGA results demonstrate inter-laboratory variation

341   [Van Veen, Gatt et al. 2008], this model, based on TGA results and with its simplified

342   character, does not claim to reproduce with precise accuracy thrombin generation but offers a

343   way to model thrombin generation that (1) has comparative value, in the sense that it can be

344   adjusted to describe different rates of thrombin generation, and (2) can be easily coupled with

345   CFD simulations. We believe that these equations, if combined with two more, one

346   describing fibrin formation and one describing platelet deposition on the reacting site of the

347   vessel, can be used as a thrombus formation model for three dimensional CFD simulations in

348   blood vessels. The results of such a model can be used to compare the evolution of thrombus

349   formation in different cases and for different thrombogenic potential of human blood. Finally,

350   as the model describes the production of thrombin in blood in a phenomenological way, it

351   does not require additional information regarding the concentration of different factors and

352   the details for every reaction in the coagulation system and it can be calibrated and applied

353   directly for different cases based only on the parameters or the curve of the TGA (or the

354   curve itself).

355   **Declarations**

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357   Funding: None

358   Ethical approval: Not required

359

360   (4,585 words)

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522   temporal dynamics of intrinsic pathway of blood coagulation. II. Results."
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524

525   **Appendix: Equations' solution:**

526   a. Under the assumption that all platelets are instantly activated when thrombin reaches

527   its threshold value, the concentration of platelets is zero when thrombin concentration

528   is below the threshold value and equal to the resting platelet concentration when

529   thrombin exceeds the threshold values. The equations describing thrombin and

530   prothrombin concentration become:

$$\frac{\partial[IIa]}{\partial t} = -k_{in}[IIa] + k_{tot} \cdot [II]$$

$$\frac{\partial[II]}{\partial t} = -k_{tot} \cdot [II]$$

531 Here k_{tot} represent the total rate of prothrombin conversion to thrombin and includes both the

532 production on the reacting site and on the platelet surfaces:

$$k_{tot} = k_{surf} + k_{II}^{AP} \cdot [PL] \,, if \ [IIa] > [IIa]_{th}$$

$$k_{tot} = k_{surf}, \qquad\qquad if \ [IIa] \leq [IIa]_{th}$$

533   This leads to a direct solution for prothrombin concentration:

$$[II](t) = [II](t = 0) \cdot e^{-k_{tot} \cdot t}$$

534   The D.E. describing thrombin concentration becomes:

$$\frac{\partial [IIa]}{\partial t} = -k_{in}[IIa] + k_{tot} \cdot [II]_o \cdot e^{-k_{tot} \cdot t}, or \ \frac{dx}{dt} = Ax - CBe^{Ct}$$

$$where \ A = -k_{in}, B = \ [II]_o \ and \ C = -k_{tot}$$

535   The equation can be rewritten as follows,

$$\frac{dy}{dt} = (A - C) \cdot y - CB, where \ y = x \cdot e^{-Ct}$$

536   The last expression, after manipulation leads to an (approximate because of the aforementioned

537   assumption) analytic solution for the temporal evolution of thrombin concentration (X):

$$X(t) = \frac{e^{Ct}}{A - C} \left[((A - C)X_0 e^{-Ct_0} - CB)e^{(A-C)t} + CB \right] \ or$$

$$[IIa](t) = \frac{e^{-k_{tot} \cdot t}}{k_{tot} - k_{in}} \left[\left((k_{tot} - k_{in})[IIa]_0 e^{k_{tot} \cdot t_0} + k_{tot} \cdot [II]_o \right) e^{(k_{tot} - k_{in}) \cdot t} - k_{tot} \cdot [II]_o \right]$$

538

539   For $[IIa](t = 0) = 0$ the equation gets the simplified form:

$$[IIa](t) = \frac{k_{tot} \cdot [II]_o}{k_{in} - k_{tot}} (e^{-k_{tot} t} - e^{-k_{in} \cdot t})$$

540   b. In the case the data from the whole curve is available, there is another way for

541   obtaining the model constants. The reaction rate is obtained by solving (numerically)

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542   the last equation:

$$[IIa]_{th} - \frac{k_{surf} \cdot [II]_o}{k_{in} - k_{surf}} \left(e^{-k_{surf}Tlag} - e^{-k_{in} \cdot Tlag} \right) = 0$$

543   As the activated platelet concentration is approximately,

$$[AP] = [RP](0) \left(1 - e^{-(t-Tlag)k_{AP}^{IIa}} \right), t > Tlag$$

544   Analytical expression can be obtained for prothrombin concentration versus time,

545        $[II](t) = [II](t = 0) \cdot e^{-k_{tot}(t) \cdot t}$ and therefore for the differential equation

546   describing thrombin evolution for t>Tlag, $\frac{d}{dt}[IIa] = -k'_{in} \cdot [IIa] + k_{tot}(t) \cdot [II](t)$

547   and the constants can be obtained numerically using an iterative process.

List of Figures

549   **Figure 1**: Schematic representation of cell based model for blood coagulation (reproduced

550   from Smith et al 2009). (a) and the reduced model (b). The different coloured arrows

551   represent the actual processes that are lumped in each reaction rate constant   . Red:

552   Inhibition of thrombin in plasma by ATIII and near the vessel wall by APC. Green: Platelet

553   activation by thrombin. Purple: thrombin production by activated platelets. Yellow:

554   Thrombin production in plasma near the reacting site on vessel wall, includes all the reactions

555   from binding of VIIa on TF up to the generation of small amounts of IIa and also (mostly) the

556   inhibition of all other species except thrombin. Turquase: Activation of platelets by

557   substances released by activated platelets such as ADP.

558   **Figure 2**: Results of the model for different time steps. For time steps below 0.5s the results

559   coincide.

560   **Figure 3**: Behaviour of the model for different initial platelet concentrations.

561   **Figure 4:** Adjustment of the model in order to reproduce slower thrombin generation,

562   experimental data.[1]

563 **Figure 5:** Model results for thrombin production without inhibition, adjusted to fit the results

564 reported in[27]

565 **Figure 6:** Capability of the model to reproduce an arbitrary thrombin generation curve[20]

566 **Figure 7**: Dependence of the shape of the curve on the time interval between two data

567 points. While the initial results are the same curve fitting on data produces significantly

568 different curves

569 **Figure 8**: Reproduction of thrombin generation curves corresponding to different fVIII

570 concentrations and the normalized modification of the related constants.

571

572   **Tables, figures**

573   Table 1: Equations of the model

Thrombin (IIa)	$\dfrac{\partial[IIa]}{\partial t} = -k_{in}[IIa] + (k_{surf} + k_{II}^{AP} \cdot [AP]) \cdot [II]$
Prothrombin (II)	$\dfrac{\partial[II]}{\partial t} = -(k_{surf} + k_{II}^{AP} \cdot [AP]) \cdot [II]$
Activated Platelets (AP)	$\dfrac{\partial[AP]}{\partial t} = k_{AP}^{AP} \cdot [AP] \cdot [RP] + k_{AP}^{IIa} \cdot [RP]$
Resting Platelets (RP)	$\dfrac{\partial[RP]}{\partial t} = -k_{AP}^{AP} \cdot [AP] \cdot [RP] - k_{AP}^{IIa} \cdot [RP]$

574

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575   Table 2: Constants and parameters

Process	Constant symbol	Initial value (S.I.))	Reference
Thrombin generation by activated platelets	k_{II}^{AP}	$0.0856 - 1.81 s^{-1}$	[Rosing, van Rijn et al. 1985; Sorensen, Burgreen et al. 1999]
Thrombin generation on reacting surface	k_{surf}	$10^{-5} s^{-1}$	n/a
Platelet activation by thrombin	k_{AP}^{IIa}	$0, if\ [IIa] < [IIa]_{thr}$ $0.5, if\ [IIa] \geq [IIa]_{thr}$	[Kuharsky and Fogelson 2001]
Platelet activation by activated platelets	k_{AP}^{AP}	$5.24 \cdot 10^{-2} s^{-1}$	[Kuharsky and Fogelson 2001]
Thrombin inhibition*	k_{in}	$1.71 \cdot 10^{-2} - 0.2 s^{-1}$	[Hockin, Jones et al. 2002; Leiderman and Fogelson 2011]
Thrombin concentration threshold for platelet activation	$[IIa]_{thr}$	$1.75 - 4.18 \cdot 10^{-8} kg/kg$ (0.5-1.2nM or 0.05-0.12U/ml)	[Rosing, van Rijn et al. 1985]

576

577   Table 3: Results of the model for different cases

		Tlag (min)	Tmax (min)	Cmax (nM)	ETP (nM·min)
Case 1	experiment	3.6 ± 0.8	7.4±1.8	164±50	1321±330
	model	3.7	6.8	165	1364
Case 2	experiment	1.5	3.5	$(1.5 \cdot 10^3)$	n/a
	model	1.5	4	$(1.5 \cdot 10^3)$	n/a
Case 3	experiment	0.67	2.25	$(1.5 \cdot 10^3)$	n/a
	model	0.65	2.38	$(1.5 \cdot 10^3)$	n/a
Case 4	experiment	3.5	4.8	199	735
	model	3.7	4.6	196	710
Case 5	experiment	5±0.5	11±2.7	161±38	1633±81
	model	5.8	9	190	1718
Case 6	experiment	5.5±0.5	11±0.2	98±40	1316±255
	model	6	9.4	91	1370
Case 7	experiment	5.8±0.7	13±0.9	72±38	1135±300
	model	6	9.6	62	1118
Case 8	experiment	15	27	101	2012
	model	13.3	25.3	102	1934

578

579

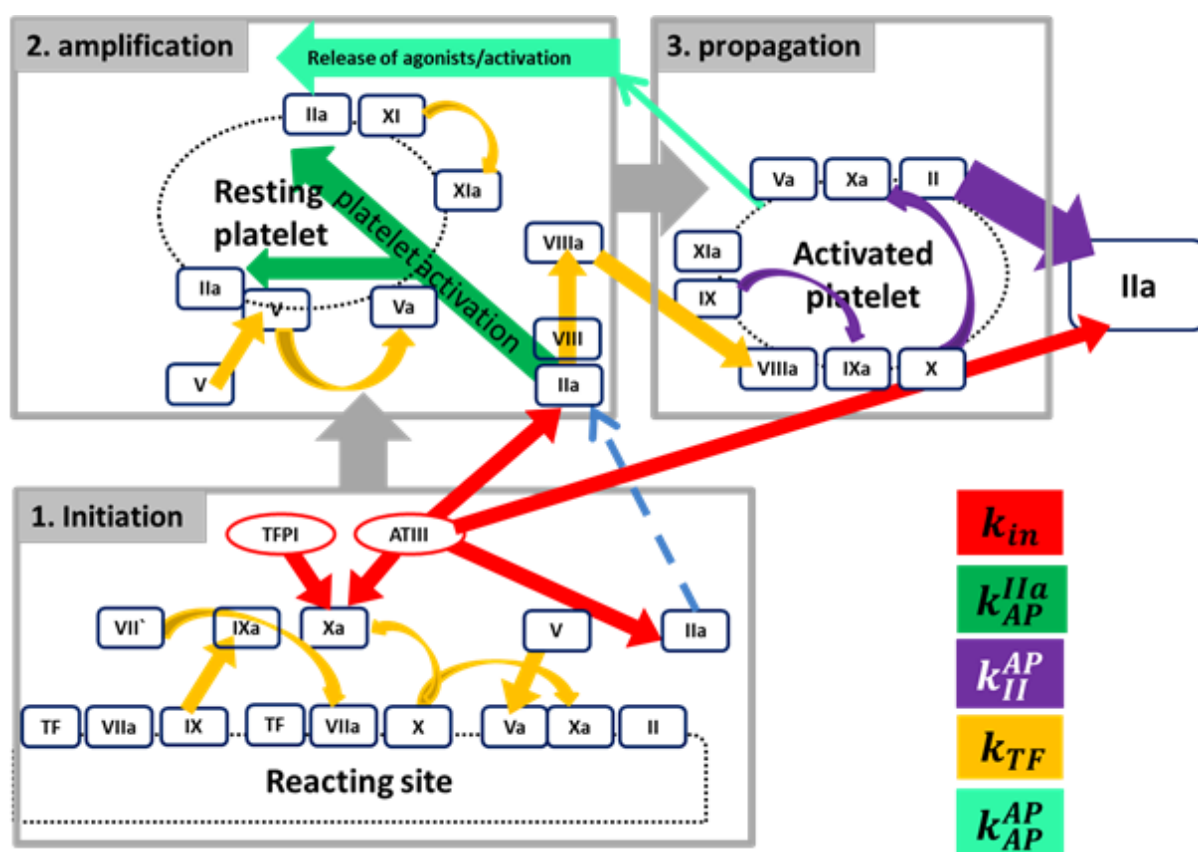Papadopoulos et al-*Model for thrombin generation*
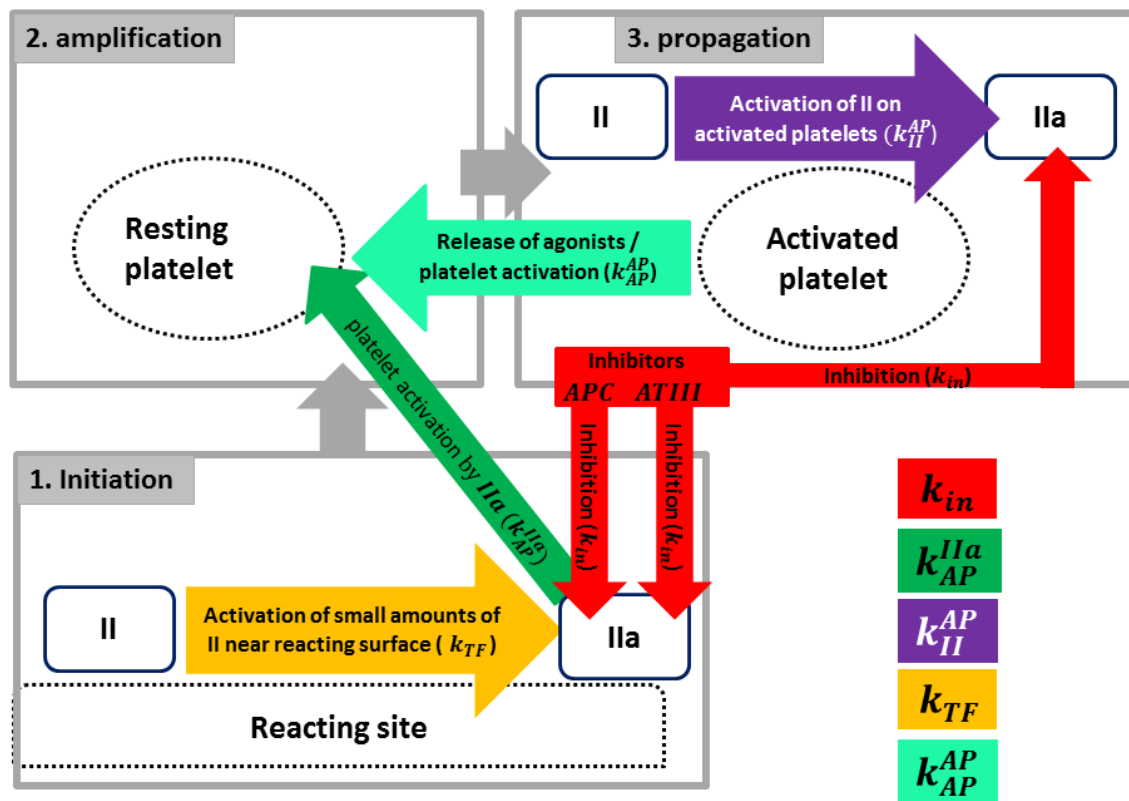
580

581   Table 4: The variation of the model constants after adjustment in order to reproduce different

582   cases

Constant	Initial estimations	Case 1	Case 2	Case 3	Case 4	Cases 5-7	Case 8
$k_{surf}(s^{-1})$	10^{-5}	$7.367 \cdot 10^{-6}$	$9.162 \cdot 10^{-6}$	$1.511 \cdot 10^{-5}$	$7.367 \cdot 10^{-6}$	$7.223 \cdot 10^{-6}$	$4.06 \cdot 10^{-7}$
$k_{II}^{AP}(s^{-1})$	$0.0856 - 1.81$	0.73	2.8	4	1.55	0.525	3.6
$k_{in}(s^{-1})$	$1.71 \cdot 10^{-2} - 0.2$	0.032	0	0	0.052	0.0262	0.024
$k_{AP}^{IIa}(s^{-1})$	$0.2 - 0.5$	-	-	0.5	0.5	0.002	0.0018

583

584   **Figure 1a**

585

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586 **Figure 1b**

587

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588 **Figure 2**

589

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590 **Figure 3**

591

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592 **Figure 4**

593

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594   **Figure 5**

Thrombin generation without inhibition

595

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596 **Figure 6**

597

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598 **Figure 7**

curves for different sample density

599

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600   **Figure 8**

Thrombin generation for different fVIII concentrations

Normalized variation of constants				
fVIII (IU/ml)	47.2	12.5	1.6	0.1
kact	0.263	0.956	0.978	1
kpla	1	0.0369	0.0131	0.00789
kin	0.147	0.197	0.279	1

601