



City Research Online

City St George's, University of London

Citation: Papadopoulos, K., Gerotziapas, G. T. & Gavaises, M. (2017). Modelling of thrombin generation under flow in realistic left anterior descending geometries. *Medical Engineering & Physics*, 50(1), pp. 50-58. doi: 10.1016/j.medengphy.2017.10.001

This is the accepted version of the paper.

This version of the publication may differ from the final published version. To cite this item please consult the publisher's version.

Permanent repository link: <https://openaccess.city.ac.uk/id/eprint/19885/>

Link to published version: <https://doi.org/10.1016/j.medengphy.2017.10.001>

Copyright and Reuse: Copyright and Moral Rights remain with the author(s) and/or copyright holders. Copies of full items can be used for personal research or study, educational, or not-for-profit purposes without prior permission or charge, unless otherwise indicated, provided that the authors, title and full bibliographic details are credited, a hyperlink and/or URL is given for the original metadata page and the content is not changed in any way. For full details of reuse please refer to [City Research Online policy](#).

1 Title page

2

3 Modelling of thrombin generation under flow in realistic left anterior
4 descending geometries

5

6

7

8

9

10

11 Dr Konstantinos P. Papadopoulos, City University London

12 Prof. Manolis Gavaises, City University London

13 Dr Grigoris T. Gerotziafas, Pierre and Marie Curie University- Paris 6

14

15

16

17 Corresponding author: Dr Konstantinos P. Papadopoulos

18 Email: Konstantinos.Papadopoulos.1@city.ac.uk, costis.papa@gmail.com

19 Telephone: +44 (0) 7580564404/ +30 6973498714

20 Fax: +44 207 040 8101

21

22 Address: City University London
23 SEMS
24 EC1V 0HB
25 Northampton square
26 London
27 United Kingdom

28

29

30 **Abstract**

31 Currently there are no available methods for prediction of thrombotic complications in
32 Coronary Artery disease. Additionally, blood coagulation tests are mainly performed in a
33 steady system while coagulation in vivo occurs under flow conditions. In this work, a
34 phenomenological model for coagulation up-to thrombin generation is proposed; the model
35 is mainly based on the results of thrombin generation assays and therefore it can account
36 for the variation of the coagulability that is observed in different individuals. The model is
37 applied on 3 cases of left anterior descending arteries (LAD) with 50% maximum stenosis
38 placed at a different location and have been statistically assessed as of different
39 complication risk. The simulations showed that parameters of thrombin generation assays
40 obtain different values when they refer to thrombin generation under realistic coronary flow
41 conditions. The flow conditions prevailing locally because of the geometric differences
42 among the arterial trees can lead to different initiation times and thrombin production rates
43 and it also alters the spatial distribution of the coagulation products. Similarly, small changes
44 of the coagulation characteristics of blood under identical flow conditions can allow or
45 prevent the initiation of coagulation. The results indicate that combined consideration of
46 geometry and coagulation characteristics of blood can lead to entirely different conclusions
47 compared to independent assessment of each factor.

48 **Introduction**

49 Coronary artery disease (CAD) is the formation of plaques in the interior of the coronary
50 vessel walls. This condition often leads to thrombus related complications that make CAD
51 the leading cause of mortality worldwide [1]. Thrombus formation in coronary artery . is
52 believed to be triggered by the rupture of an atheromatous plaque and subsequent
53 exposure of tissue factor (TF) and collagen. The triggering is followed by a series of

54 biochemical reactions that result in the activation of fibrin by thrombin and the formation of
55 the clot that narrows or blocks the flow in the coronary artery. As some of these reactions
56 occur much faster on the membrane of platelets and endothelium cells the contemporary
57 description of the process is cell-based[7]. Intracoronary ultrasound findings have suggested
58 that a ruptured plaque does not necessary lead to thrombosis [3-5]. This can be attributed to
59 any of the three factors, traditionally known as Virchow's triad, that influence the process:
60 (1) specific conditions on vessel's wall (2) coagulability of blood and (3) local flow conditions
61 [2].

62 . During the last three decades, there were several attempts to investigate aspects of the
63 process of thrombus formation using computational simulations. Based on in-vitro
64 experiments on the enzymatic reactions (indicatively[12]) zero-dimensional models that
65 reproduce the temporal evolution of the coagulation system have been developed., The first
66 model consisted of 14 reaction rate constants, describing the activation and inhibition of
67 four coagulation factors [13], while following works included up to 50 constants and focused
68 on the extrinsic [14, 15] or the intrinsic [16] pathway. Such models were used to investigate
69 specific parts of the coagulation process, as the function of positive feedback loops and
70 threshold concentrations for the initiation of the process [17], the triggering threshold with
71 respect to Tissue Factor Pathway Inhibitor (TFPI) concentration [18], the inhibition
72 mechanism of APC [19] or the effect of stochastically induced small variation of enzymes
73 concentration [20]. For the simulation of thrombus formation the temporal evolution of the
74 process is coupled with the diffusion and transport of the substances, initially as fluxes or
75 with the use of few reactions [21, 22]. The model of Sorensen et al, although focused on
76 platelet aggregation, could also fit into this category [23]. While there are recent works using
77 simplified reaction models, [24]. the trend is towards more complicated multi-scale and
78 multi-phase models. These models, in addition to the set of equations that represents the
79 reactions, may incorporate the movement of cells, the localization of equations and the

80 change of blood properties due to blood clotting. Kuharsky and Fogelson [25] proposed an
81 integrated model for thrombin generation under a simplified flow field, using a system of 59
82 equations that also included the binding of substances and the localization of reactions on
83 surfaces. . In following publications by the same group additional processes were
84 incorporated in the model:the alteration of the rheological properties of blood due to
85 clotting, by modelling platelet-platelet and platelet-wall interaction as reversible elastic
86 links. [26]. ;the APC mechanism and the transport of substances between plasma and
87 endothelium cells [27]. Additionally, a small scale discrete model using an immersed
88 boundary method [28] for platelets was utilized to develop a continuous model for platelet
89 aggregation

90 [29]; the later was also applied in simulations with pulsating flow in an idealized two
91 dimensional vessel bifurcation [30] and with the inclusion of transport within the thrombus,
92 was used to demonstrate the effects of flow conditions and the quantity of TF exposed on
93 thrombus growth [31]. A similar model was presented by Xu et al [32], which later included a
94 cellular pot model [33] for discrete cells and an energy-based stochastic process for cell
95 motion. The simulation involved differentiation of cell movements depending on fibrin levels
96 and cell-cell or cell-surface interaction and bonds. The model was used to evaluate the role
97 of fVII in venous thrombus formation [34] and to examine the impact of pulsating flow and
98 the non-Newtonian characteristics of blood on thrombus growth [35]. Anand et al [36]
99 presented another multi-process model that used a viscoelastic model to simulate flow for
100 both free vessel lumen and clot. This model also incorporated the activation of platelets due
101 to 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 [37].

104 For the case of coronary thrombosis, a typical value for the diameter of the artery is around
105 4mm and the flow is strongly three-dimensional and time dependent, preventing utilization
106 of simplified flow fields. Additionally there is significant variability of the response of the
107 coagulation system observed for different individuals [38]. From the reviewed works, only
108 the latest was applied on 3D geometries, while the typical used regions are 2D simplified
109 geometries with dimensions of $100 \times 100 \mu\text{m}^2$. In most studies the flow field is simplified and
110 predefined. Finally, the inclusion of a large number of processes makes the application of
111 the models computationally expensive while at the same time they require a large amount
112 of experimental data in order to be adapted for different patients. Due to these limitations
113 of the existing methods there is no connection between modelling of thrombus formation
114 and clinical practice.

115 In this work, we propose a model for coagulation under realistic flow conditions up to the
116 stage of thrombin generation that compensates for some of these difficulties. The proposed
117 phenomenological model has the following characteristics: (1) computational effectiveness,
118 so that it can be coupled with transient flow simulations; (2) ability to obtain patient specific
119 character in a manner that can be directly related to clinical practice, drawing data directly
120 from clinical tests.

121 **Materials and methods**

122 **Thrombus modelling**

123 For the description of the coagulation process the cell based approach (Figure 1) was
124 followed. The computational model was realized in three steps: (i) a zero dimensional sub-
125 model for thrombin generation was developed; (ii) the thrombin-sub model was modified
126 for application under flow and (iii) a sub-model for platelet aggregation under flow was
127 added. For the simulation of the coagulation reactions up to thrombin generation our
128 previously published phenomenological model was used [39], consisting of the 4-lumped

129 equations of Table 1. These equations express the concentration of 4 species in a zero-
 130 dimensional system. The outcome is the temporal evolution of thrombin concentration. The
 131 reaction rate constants of the model are derived directly from thrombin generation assays
 132 and can be adjusted within a reasonable range in order to reproduce the results of thrombin
 133 generation assays for a wide range of cases including haemophilia. The adjustment can be
 134 performed either manually or via a repetitive algorithm.

Thrombin	$[IIa]$	$-k_{in,0}[IIa] + (k_{II}^{AP} \cdot [AP^{(f)}]) \cdot [II]$
Prothrombin	$[II]$	$-(k_{II}^{AP} \cdot [AP^{(f)}]) \cdot [II]$
Resting platelets in flow	$[RP^{(f)}]$	$-(k_{AP}^{IIa} + k_{AP}^{AP} \cdot [AP^{(f)}]) \cdot [RP^{(f)}]$
Activated platelets in flow	$[AP^{(f)}]$	$+(k_{AP}^{IIa} + k_{AP}^{AP} \cdot [AP^{(f)}]) \cdot [RP^{(f)}]$

135 Table 1: Lumped reactions of the thrombin generation submodel for the blood
 136 circulating species.

137 The described physical system corresponds to a well-mixed solution, where the spatial
 138 distributions of the concentration of all species are uniform. At the same time each of the
 139 reactions and the respective reaction rates correspond to processes occurring either in
 140 plasma, on the membrane of activated platelets or at the sites where TF is expressed
 141 according to the cell based approach [7]. This is achieved with the use of different reaction
 142 rate constants for the areas with activated platelets and the additional reaction terms for
 143 the areas near the reacting vessel surface.

$[IIa]$		$-k_{in,add}[IIa] + (k_{TF,surf} + k_{II}^{AP}[AP^{(b)}])[II]$
$[II]$		$-(k_{TF,surf} + k_{II}^{AP}[AP^{(b)}])[II]$
$[RP^{(f)}]$	bind	$-\left((k_{bi,surf}^{RP} \cdot A_f + k_{bi,RP}^{RP} \cdot [RP^{(b)}] + k_{bi,AP}^{RP} \cdot [AP^{(b)}]) \cdot [RP^{(f)}]\right)$ $- k_{AP}^{AP} \cdot [AP^{(b)}] \cdot [RP^{(f)}]$
	act	
$[AP^{(f)}]$	bind	$-\left((k_{bi,surf}^{AP} \cdot A_f + k_{bi,RP}^{AP} \cdot [RP^{(b)}] + k_{bi,AP}^{AP} \cdot [AP^{(b)}]) \cdot [AP^{(f)}]\right)$ $+ k_{AP}^{AP} \cdot [AP^{(b)}] \cdot [RP^{(f)}]$
	act	

$[RP^{(b)}]$	bind	$+ \left((k_{bi,surf}^{RP} \cdot A_f + k_{bi,RP}^{RP} \cdot [RP^{(b)}] + k_{bi,AP}^{RP} \cdot [AP^{(b)}]) \cdot [RP^{(f)}] \right) - k_{diss}^{RP} [RP^{(b)}]^2$ $- k_{AP}^{IIa} [RP^{(b)}] - k_{AP}^{AP} ([AP^{(b)}] + [AP^{(f)}]) \cdot [RP^{(f)}] - k_{AP}^{surf} [RP^{(b)}]$
	act	
$[AP^{(b)}]$	bind	$+ \left((k_{bi,surf}^{AP} \cdot A_f + k_{bi,RP}^{AP} \cdot [RP^{(b)}] + k_{bi,AP}^{AP} \cdot [AP^{(b)}]) \cdot [RP^{(f)}] \right) - k_{diss}^{AP} [AP^{(b)}]^2$ $+ k_{AP}^{IIa} [RP^{(b)}] - k_{AP}^{AP} \cdot ([AP^{(b)}] + [AP^{(f)}]) \cdot [RP^{(f)}] + k_{AP}^{surf} [RP^{(b)}]$
	act	

144 Table 2: The additional reaction terms in the computational cells attached to the
145 reacting part of the vessel wall. They describe the activation (act) and binding (bind)
146 of platelets and the additional thrombin generation due to surface TF exposure and
147 bound activated platelets. Each reaction rate constant of the form k_B^A simulates the
148 effect of substance B on the concentration of A.

149 .

150 Coupling with flow

151 For the simulations including flow, the concentrations ($C_i^{(f)}$) of the blood circulating species
152 were calculated using the convection-diffusion equation with source terms (S_i) (equation 1).

153 For immobilized species ($C_j^{(b)}$) the source term expresses the temporal evolution (equation
154 2).

$$\frac{\partial C_i^{(f)}}{\partial t} + \nabla(C_i^{(f)} \cdot \vec{u}) = D \nabla^2 C_i^{(f)} + S_i$$

155 Equation 1

$$\frac{\partial C_j^{(b)}}{\partial t} = S_j$$

156 Equation 2

157 The quantity of bound species is expressed in surface concentration (kg/m^2), while the
158 circulating species in volume concentration (kg/kg). In the reactions involving transition of a
159 species from circulating to bound state and vice versa the actual mass of the involved
160 species in the computational cell is calculated, using the volume of the computational cell or
161 the reacting surface. Then the fraction of the species that is binding or unbinding is
162 computed using the appropriate reaction rate constant.

163 During in vivo coagulation, TF is located on the vessel wall during initiation and therefore the
164 bulk initiation reaction used in the thrombin sub-model needs to be replaced by a surface
165 reaction. In order to obtain a value for this surface reaction we exploited the experimental
166 results of Shen et al [40] regarding the threshold behaviour of coagulation initiation under
167 flow. This study revealed that the initiation of coagulation under flow occurs only when the
168 stream-wise length of the reacting surface is sufficiently large and the wall shear rate is
169 sufficiently low. We simulated the described experimental cases (Figure 2) and we adjusted
170 the surface reaction rate constant to achieve initiation of coagulation for the above-
171 threshold experimental setups and at the same time not to have initiation for the below-
172 threshold setups.

173 The platelet aggregation sub-model was developed based on the results of Badimon et al
174 [41] regarding the binding of platelets on de-endothelized vessel stripes under flow. The
175 experimental setup was reconstructed and three cases with different wall shear rate values
176 were simulated (Figure 3) as described in the experiment. The aggregation rate constant
177 used for the platelets was shear-dependent and for the specific part there was no distinction
178 between resting and activated platelets.

179 As the proposed coagulation model is phenomenological, each of the used reactions stands
180 for a number of actual processes. The outcome of the model is the temporal evolution of
181 thrombin generation. 'Prothrombin' in the sense used in the model represents the coagulant
182 potential of blood. In the same manner, the surface initiation reaction incorporates all the
183 processes that intervene between the expression of TF and the activation of thrombin.
184 Similarly, the activated platelets account for all the additional activity involved in the
185 amplification phase.

186 **Geometry reconstruction**

187 Finally, the developed model was applied in realistic LAD geometries under flow conditions.
188 Three different geometrical models, MI1, MI2 and STA with maximum radius reduction 50%,
189 were constructed [42] using the centrelines and the diameters of the vessels as obtained
190 from coronary angiographies. The geometries have been previously assessed as of different
191 complication risk [43] based on statistical correlation of the occurrence of acute coronary
192 syndromes to the location of the stenosis and the existence of bifurcations within the
193 affected lesion. In the STA geometry there are no bifurcations involved in the stenotic lesion
194 and it is considered of low complication risk while in MI1 and MI2 geometries there are
195 bifurcations within the stenotic lesion and they are considered of higher risk regarding acute
196 coronary complications [44]. A reacting area of 6mm stream-wise length was defined on
197 each of the three geometries with the centre of the reacting area being at the point of
198 maximum radius reduction (Figure 4).The transient flow field for one cardiac cycle was
199 obtained using CFD techniques [42].

200 **Flow field**

201 For the velocity \vec{u} at each point a predefined time dependent value was used in all
202 simulations. The flow field was calculated by solving the incompressible Navier-Stokes
203 equations with the use of the commercial software ANSYS FLUENT and the details have been
204 previously published [42, 45]. In brief, blood was modelled as a single-phase Newtonian fluid
205 with density 1060kg/m^3 and viscosity $3.5 \cdot 10^{-3}\text{Pa} \cdot \text{s}$. Aortic pressure pulse was applied at
206 the inlet of the geometry and outflow resistance boundary conditions at the outlets. The
207 time scale of the cardiac cycle is of the order of 1s, approximately 5000 times smaller
208 compared to the timescale of the coagulation model. As the model is mainly sensitive to the
209 wall shear rate and it has been shown that wall shear mainly depends on the average flow
210 rate rather than the exact shape of the inlet pulse[46, 47], a simplified inflow pulse

211 consisting of 9 time instances of the original cardiac pulse was used. The total inflow of the
212 used pulse was 0.85% elevated, compared to the mass flow inlet of the initial pulse.

213 All the simulations were performed using an i7 intel processor in serial computing (single
214 core computations). Simulating the flow for three cardiac pulses lasted approximately one
215 day, while the application of the biochemical model under transient flow conditions required
216 approximately five days for simulating real time of some minutes.

217 Results

218 We started the simulations of the experiments of Shen et al [40] with an estimated value for
219 the reaction rate constant for the surface induced initiation, based on geometrical
220 assumptions. Using trial and error we approximated the surface initiation of the coagulation
221 with the value $k_{TF,surf} = 1.785 \cdot 10^{-6} \frac{kg}{m^2} \cdot s^{-1}$. This constant represents the thrombin
222 production rate from a surface where TF is expressed, per surface unit. Using this value for
223 the surface initiation reaction, the simulations for the above threshold experimental cases
224 resulted in initiation of the coagulation process, while in the simulations of the sub-
225 threshold setups the concentration of thrombin was found below the minimum value that
226 leads to platelet activation after 200s of perfusion (Figure 5), in accordance to the simulated
227 experiments [40].

228 A number of sub-threshold setups were also simulated to test the behaviour of the model
229 (Table 1). As the initiation was considered a surface reaction, the threshold condition for the
230 transition from the initiation to the amplification phase was also modified and was
231 expressed as amount of thrombin per surface unit $[IIa]_{th}^{(s)} = 3.44 \cdot 10^{-9} \cdot nmol/mm^2$
232 instead of being expressed as volume concentration (1.2nM). This modification implies that
233 the initiation occurs in a reaction zone of approximately $3\mu m$ above the reacting surface.
234 This approach also enforces the mesh independence of the model. As the surface thrombin
235 generation is proportional to the reacting surface, using a transition criterion based on the

236 volume concentration of thrombin would lead to strong dependence of the transition on the
 237 volume of the computational cell. The simulations also revealed that from the wall shear
 238 rate value (γ_w) and the stream-wise length of the reacting area (L_{SWR}), we can derive a
 239 quantity, previously defined as coagulation activation index [42], $CAI = L_{SWR}/\gamma_w$ that is
 240 related to the initiation of coagulation. CAI is proportional to the residence time of the blood
 241 components over the reacting surface. As shown in Table 1, for a given value of $k_{TF,surf}$ a
 242 small modification of the parameters can allow or prevent the initiation of coagulation,
 243 depending on the change of CAI value.

Case	WSR (s^{-1})	$L_{SWR}(m)$	$k_{TF,surf}$	CAI ($10^{-5}m \times s$)	initiation
SA Basic case 1	25	$2 \cdot 10^{-4}$	$1.785 \cdot 10^{-6}$	0.8	YES
SB Basic case 2	40	$4 \cdot 10^{-4}$	$1.785 \cdot 10^{-6}$	1	YES
SA with lower reaction rate constant	25	$2 \cdot 10^{-4}$	$1.775 \cdot 10^{-6}$	0.8	NO
SA with elevated shear rate	30	$2 \cdot 10^{-4}$	$1.785 \cdot 10^{-6}$	0.625	NO
SB with elevated shear rate	60	$4 \cdot 10^{-4}$	$1.785 \cdot 10^{-6}$	0.667	NO

244 Table 3: The basic cases used for the calculation of the surface initiation reaction
 245 rate were SA and SB. SA was the case with minimum CAI and required the
 246 maximum value of $k_{TF,surf}$ for initiation. The following three cases as many others
 247 not presented were simulated in order to confirm that SA and SB were actually
 248 threshold setups for the initiation and that a small modification of the parameters to
 249 the direction of reduced CAI does not allow initiation.

250 The platelet aggregation model was tested for the three different flow conditions described
 251 in the simulated experiment. The experimental results of platelet aggregation regarding the
 252 maximum amount of bound platelets and the initial aggregation rate were accurately
 253 approximated by the simulations as shown in Figure 6. The model failed to predict the

254 disaggregation of platelets that was observed in high shear rate conditions after 10 min of
 255 perfusion. However this happens more likely due to stress accumulation as the aggregate is
 256 continuously subject to high shear stress values ($1690s^{-1}$) while for the transient coronary
 257 flow used in this study the average wall shear rate is much lower ($235s^{-1}$).

258 For the two sets of simulations described above, the flow field was obtained by solving the
 259 incompressible Navier-Stokes equations in FLUENT ANSYS, while fixed mass flowrates were
 260 used for both the inlet and the outlet of computational domain. As the interaction between
 261 flow and biochemical reactions is not considered, the biochemical models were applied on
 262 steady predefined flow field.

263 For the simulations in the LAD geometries the setup of the coagulation model was identical,
 264 with the processes regarding thrombin generation tuned in order to correspond to typical
 265 values of TGA. For the simulations under flow we calculated the parameters of TGA: the lag
 266 time (Tlag), the time until thrombin reaches its maximum concentration (ttP), the maximum
 267 concentration of thrombin C_{max} and the production rate of thrombin and as an additional
 268 characteristic parameter of the simulations was considered the moment when thrombin
 269 concentration exceeded the threshold value for the transition of coagulation from the
 270 initiation to the amplification phase ($1.2nM$) at a point downstream the reacting site
 271 (downstream propagation time or DP time). The values of these parameters for the TGA and
 272 the three LAD models are summarized in Table 2. While all three models had similar
 273 maximum values of thrombin concentration, the temporal evolution was different for each
 274 model (Figure 7).

	Lag time (min)	Time to peak (min)	$C_{max}(nM)$	Production rate (nM/s)	DP time (min)
TGA	3.6 ± 0.8	7.4 ± 1.8	164 ± 50	1	n/a

STA	1	2	31	0.49	1.2
MI1	0.5	1.3	38	0.77	0.8
MI2	5	5.9	35	0.68	2.9

275 **Table 4: Influence of flow on thrombin generation parameters.** The values of the
276 main parameters characterizing thrombin generation as calculated for the three LAD
277 models compared to the standard TGA values.

278 In MI1 and STA geometries, the transition from initiation to amplification phase was fast and
279 levels of thrombin concentration that are considered sufficiently high to cause platelet
280 activation ($>1.2\text{nM}$) were present downstream the reacting area only after the amplification.

281 In these models the downstream propagation was observed after the initiation phase. In
282 MI2 geometry the process had much slower progress and while in specific points
283 downstream the reacting surface thrombin exceeds the threshold value during the initiation
284 phase. The simulations were stopped 2 minutes after the downstream propagation and after
285 maximum thrombin concentration had obtained a constant value, approximately 1.5
286 minutes after the downstream propagation. In all cases the process was limited in a small
287 zone near the vessel wall, even in the presence of vortices.

288 The difference in the temporal evolution is caused by the different distribution of bound
289 activated platelets on the reacting surface of the vessel wall (Figure 8). While the total
290 amount increases in a similar manner for all three geometries, in MI1 and STA geometry
291 there is a small area at the end of the reacting boundary where the amount of bound
292 activated platelets is more than one order of magnitude higher than the average. On the
293 contrary, in MI2 geometry activated platelets are uniformly distributed on the reacting
294 surface and this has as a result a prolonged initiation phase and a large amount of activated
295 platelets at the instance of the transition to the amplification phase. This large amount of
296 bound activated platelets in MI2 geometry explains the observation of above threshold
297 ($>1.2\text{nM}$) thrombin concentration before the main amplification phase.

298 Discussion

299 Application of the coagulation model under flow resulted in lower maximum concentration
300 of thrombin compared to the TGA. The initiation time was also different than the TGA
301 values, in MI1 and STA significantly smaller while in MI2 significantly larger. Thrombin
302 generation rate though was similar for all cases and close to the TGA value (Table 2). As TGA
303 parameters have absolute and not only comparative value, these findings indicate that the
304 results of steady state coagulation tests should be somehow translated under flow
305 conditions. Perfusion tests require a relatively large amount of blood and are difficult to
306 standardize, therefore the easier way to correlate the results of steady state clinical tests to
307 coagulation under flow is via computer simulations.

308 The results of the coagulation model on the different geometries highlight the importance of
309 local flow conditions on the evolution of the process. The simulations were performed with
310 identical setup of the coagulation model therefore all three cases assume identical
311 behaviour of blood regarding coagulability. However, in MI2 case the temporal evolution
312 was much slower and the local conditions at the moment of the transition from initiation to
313 amplification phase were different, as thrombin concentration and amount of bound
314 activated platelets were elevated compared to the other two cases. This shows that the
315 coagulation tests provide only a part of the important information regarding the condition of
316 a patient as similar results might lead to entirely different situations. Again, this can be
317 assessed only in-silico, as any experiments or clinical tests are not feasible.

318 During the calibration of the model under flow conditions it became obvious that the
319 initiation phase is very sensitive to the surface thrombin production rate and the local value
320 of the wall shear. At first site this indicates that the exact concentration of TF in the
321 atheromatous plaque may play an important role in the initiation and the transition to the
322 amplification phase. However, the dependence of the initiation rate on TF concentration

323 according to experimental results [48] is logarithmic [42] so the actual reaction rate is not
324 that sensitive to small variations of TF concentration. As there is available information for
325 the surface concentration of TF on atheromatous plaques [49] an experiment similar to the
326 one performed by Shen et al [40] with known surface concentration of TF on the reacting
327 surface can lead to a value for this constant that will be appropriate for general use in the
328 cases when plaque rupture or vessel injury is assumed.

329 In MI2 case the prolonged initiation phase was combined with high concentration of bound
330 activated platelets in the reacting area. A prolonged lag phase of thrombin generation in a
331 thrombin generation assay is mainly related to hypocoagulable states. Increased lag time is
332 observed in cases with reduced stimulation or increased inhibition of thrombin [50]. On the
333 contrary, the application of the coagulation model under flow shows that a prolonged
334 initiation phase leads to increased accumulation of activated platelets at the reacting site.
335 These bound platelets will become activated during the propagation phase and contribute
336 locally to thrombin generation. The observed mechanism indicates that it is very possible
337 that the prolonged lag phase indicates increased risk for thrombotic complications in vivo,
338 provided that an initiation stimulus is present a fact that also supported by recent clinical
339 findings [51].

340 It is interesting to note that while flow seems to have an important role, the whole process
341 of coagulation in LAD models was limited into a small boundary layer near the vessel wall.
342 The concentration of thrombin and activated platelets becomes approximately zero in a
343 small distance from the vessel wall. As Peclet number is too high ($>10^4$) even in a small
344 distance ($\sim 3\mu\text{m}$) from the vessel wall, diffusion was not expected to have significant role
345 under arterial flow conditions but the restriction of coagulation products near the wall
346 occurs also in the areas where vortices are present. Therefore, according to our findings the

347 more important aspect of flow for coagulation is wall shear rate, while the possible
348 contribution of recirculation zones to the process was not confirmed by this work.

349 As the main concept in this work is the correlation of coagulation models with clinical tests,
350 in the future we intent to modify the proposed mathematical model for coagulation in a
351 manner that the platelet aggregation sub-model will be also calibrated based on existing
352 clinical platelet assays. We also intend to add the fibrin activation and polymerization part of
353 the process and correlate the model with thromboelastography tests (TEG). Finally, we also
354 hope, through partnership with a biochemical lab, to achieve in vitro quantitative validation
355 of the method by performing perfusion tests in coronary models. However, the findings of
356 this study indicate that it would be useful for clinical practice to co-estimate the prevailing
357 flow conditions and blood coagulability for each patient, and that this co-estimation can be
358 performed via numerical simulations.

359 **Conclusions**

360 In this study we proposed a method for modelling coagulation under flow up to thrombin
361 generation, based partly on clinical tests. We demonstrated that it is feasible to build a
362 coagulation model based on clinical tests instead of laboratory experiments and therefore
363 achieve a patient specific and more importantly clinically relevant simulation of blood
364 coagulation. The application of the model revealed that certain parameters that characterize
365 thrombin generation in TGA tests obtain different values for thrombin generation under
366 realistic flow conditions. Additionally, we showed that identical behaviour of blood can lead
367 to different temporal evolution of coagulation depending on the geometry of the coronary
368 and the flow conditions. As experiments in-vivo on coronary thrombosis are not feasible and
369 relative measurements are also extremely limited, the findings of this study demonstrate
370 that mathematical simulation of coagulation in a case specific manner is a promising and

371 inexpensive pathway towards the assessment of coronary disease and can contribute to the
372 prognosis of thrombotic complications.

373 **Conflicts of interest**

374 None.

375 **Funding:**

376 Part of this work was funded by EPSRC, as a PhD scholarship

377 **Ethical approval:** not required

378

379 **References**

- 380 [1] Roger VL, Go AS, Lloyd-Jones DM, Adams RJ, Berry JD, Brown TM, et al. Heart disease and
381 stroke statistics--2011 update: a report from the American Heart Association. *Circulation*.
382 2011;123:e18-e209.
- 383 [2] Lee KW, Lip GY. Acute coronary syndromes: Virchow's triad revisited. *Blood coagulation*
384 & fibrinolysis. 2003;14:605-25.
- 385 [3] Hong MK, Mintz GS, Lee CW, Kim YH, Lee SW, Song JM, et al. Comparison of coronary
386 plaque rupture between stable angina and acute myocardial infarction: a three-vessel
387 intravascular ultrasound study in 235 patients. *Circulation*. 2004;110:928-33.
- 388 [4] Maehara A, Mintz GS, Bui AB, Walter OR, Castagna MT, Canos D, et al. Morphologic and
389 angiographic features of coronary plaque rupture detected by intravascular ultrasound.
390 *Journal of the American College of Cardiology*. 2002;40:904-10.
- 391 [5] Rioufol G, Finet G, Ginon I, Andre-Fouet X, Rossi R, Vialle E, et al. Multiple atherosclerotic
392 plaque rupture in acute coronary syndrome: a three-vessel intravascular ultrasound study.
393 *Circulation*. 2002;106:804-8.
- 394 [6] Heemskerk JW, Bevers EM, Lindhout T. Platelet activation and blood coagulation.
395 *THROMBOSIS AND HAEMOSTASIS-STUTTGART*-. 2002;88:186-94.
- 396 [7] Smith SA. The cell-based model of coagulation. *Journal of Veterinary Emergency and*
397 *Critical Care*. 2009;19:3-10.
- 398 [8] Hathcock JJ. Flow Effects on Coagulation and Thrombosis. *Arteriosclerosis, Thrombosis,*
399 *and Vascular Biology*. 2006;26:1729-37.
- 400 [9] Nesbitt WS, Westein E, Tovar-Lopez FJ, Tolouei E, Mitchell A, Fu J, et al. A shear gradient-
401 dependent platelet aggregation mechanism drives thrombus formation. *Natural Medicines*.
402 2009;15:665-73.
- 403 [10] Yin W, Shanmugavelayudam SK, Rubenstein DA. The effect of physiologically relevant
404 dynamic shear stress on platelet and endothelial cell activation. *Thrombosis Research*.
405 2011;127:235-41.
- 406 [11] Goto S, Ikeda Y, Saldívar E, Ruggeri ZM. Distinct mechanisms of platelet aggregation as a
407 consequence of different shearing flow conditions. *Journal of Clinical Investigation*.
408 1998;101:479-86.
- 409 [12] Lawson JH, Kalafatis M, Stram S, Mann KG. A model for the tissue factor pathway to
410 thrombin. I. An empirical study. *Journal of Biological Chemistry*. 1994;269:23357-66.
- 411 [13] Willems G, Lindhout T, Hermens WT, Hemker H. Simulation Model for Thrombin
412 Generation in Plasma. *Pathophysiology of Haemostasis and Thrombosis*. 1991;21:197-207.
- 413 [14] Jones KC, Mann KG. A model for the tissue factor pathway to thrombin. II. A
414 mathematical simulation. *Journal of Biological Chemistry*. 1994;269:23367-73.
- 415 [15] Hockin MF, Jones KC, Everse SJ, Mann KG. A Model for the Stoichiometric Regulation of
416 Blood Coagulation. *Journal of Biological Chemistry*. 2002;277:18322-33.
- 417 [16] Zarnitsina VI, Pokhilko AV, Ataullakhanov FI. A Mathematical model for the spatio-
418 temporal dynamics of intrinsic pathway of blood coagulation. I. The model description.
419 *Thrombosis Research*. 1996;84:225-36.
- 420 [17] Beltrami E, Jesty J. Mathematical analysis of activation thresholds in enzyme-catalyzed
421 positive feedbacks: application to the feedbacks of blood coagulation. *Proceedings of the*
422 *National Academy of Sciences*. 1995;92:8744-8.
- 423 [18] Xu C, Hu Xu X, Zeng Y, Wen Chen Y. Simulation of a mathematical model of the role of
424 the TFPI in the extrinsic pathway of coagulation. *Computers in Biology and Medicine*.
425 2005;35:435-45.

426 [19] Qiao YH, Xu CQ, Zeng YJ, Xu XH, Zhao H, Xu H. The kinetic model and simulation of blood
427 coagulation--the kinetic influence of activated protein C. *Medical Engineering & Physics*
428 2004;26:341-7.

429 [20] Lo K, Denney WS, Diamond SL. Stochastic Modeling of Blood Coagulation Initiation.
430 *Pathophysiology of Haemostasis and Thrombosis*. 2005;34:80-90.

431 [21] Hubbell JA, McIntire LV. Platelet active concentration profiles near growing thrombi. A
432 mathematical consideration. *Biophysical Journal*. 1986;50:937-45.

433 [22] Folie BJ, McIntire LV. Mathematical analysis of mural thrombogenesis. Concentration
434 profiles of platelet-activating agents and effects of viscous shear flow. *Biophysical Journal*.
435 1989;56:1121-41.

436 [23] Sorensen EN, Burgreen GW, Wagner WR, Antaki JF. Computational Simulation of
437 Platelet Deposition and Activation: I. Model Development and Properties. *Annals of*
438 *Biomedical Engineering*. 1999;27:436-48.

439 [24] Lobanov AI, Starozhilova TK. The Effect of Convective Flows on Blood Coagulation
440 Processes. *Pathophysiology of Haemostasis and Thrombosis*. 2005;34:121-34.

441 [25] Kuharsky AL, Fogelson AL. Surface-Mediated Control of Blood Coagulation: The Role of
442 Binding Site Densities and Platelet Deposition. *Biophysical Journal*. 2001;80:1050-74.

443 [26] Fogelson AL, Guy RD. Platelet-wall interactions in continuum models of platelet
444 thrombosis: formulation and numerical solution. *Mathematical Medicine and Biology*.
445 2004;21:293-334.

446 [27] Fogelson AL, Tania N. Coagulation under Flow: The Influence of Flow-Mediated
447 Transport on the Initiation and Inhibition of Coagulation. *Pathophysiology of Haemostasis*
448 *and Thrombosis*. 2005;34:91-108.

449 [28] Peskin CS. The immersed boundary method. *Acta Numerica*. 2002;11:479-517.

450 [29] Fogelson AL, Guy RD. Immersed-boundary-type models of intravascular platelet
451 aggregation. *Computer Methods in Applied Mechanics and Engineering*. 2008;197:2087-104.

452 [30] Yang XS, Lewis RW, Zhang HY. Finite Element Analysis of Fogelson's Model for Platelet
453 Aggregation. 2004.

454 [31] Leiderman K, Fogelson AL. Grow with the flow: a spatial-temporal model of platelet
455 deposition and blood coagulation under flow. *Mathematical Medicine and Biology*.
456 2011;28:47-84.

457 [32] Xu Z, Chen N, Kamocka MM, Rosen ED, Alber M. A multiscale model of thrombus
458 development. *Journal of The Royal Society Interface*. 2008;5:705-22.

459 [33] Marée AM, Grieneisen V, Hogeweg P. The Cellular Potts Model and Biophysical
460 Properties of Cells, Tissues and Morphogenesis. In: Anderson AA, Chaplain MJ, Rejniak K,
461 editors. *Single-Cell-Based Models in Biology and Medicine: Birkhäuser Basel*; 2007. p. 107-
462 36.

463 [34] Xu Z, Lioi J, Mu J, Kamocka MM, Liu X, Chen DZ, et al. A Multiscale Model of Venous
464 Thrombus Formation with Surface-Mediated Control of Blood Coagulation Cascade.
465 *Biophysical Journal*. 2010;98:1723-32.

466 [35] Xu Z, Chen N, Shadden SC, Marsden JE, Kamocka MM, Rosen ED, et al. Study of blood
467 flow impact on growth of thrombi using a multiscale model. *Soft Matter*. 2009;5:769-79.

468 [36] Anand M, Rajagopal K, Rajagopal KR. A Model for the Formation and Lysis of Blood
469 Clots. *Pathophysiology of Haemostasis and Thrombosis*. 2005;34:109-20.

470 [37] Bodnár T, Sequeira A. Numerical simulation of the coagulation dynamics of blood.
471 *Computational and Mathematical Methods in Medicine*. 2008;9:83-104.

472 [38] Oliver JA, Monroe DM, Roberts HR, Hoffman M. Thrombin Activates Factor XI on
473 Activated Platelets in the Absence of Factor XII. *Arteriosclerosis, Thrombosis, and Vascular*
474 *Biology*. 1999;19:170-7.

- 475 [39] Papadopoulos KP, Gavaises M, Atkin C. A simplified mathematical model for thrombin
476 generation. *Medical Engineering & Physics*. 2014;36:196-204.
- 477 [40] Shen F, Kastrup CJ, Liu Y, Ismagilov RF. Threshold Response of Initiation of Blood
478 Coagulation by Tissue Factor in Patterned Microfluidic Capillaries Is Controlled by Shear
479 Rate. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2008;28:2035-41.
- 480 [41] Badimon L, Badimon JJ, Galvez A, Chesebro JH, Fuster V. Influence of arterial damage
481 and wall shear rate on platelet deposition. Ex vivo study in a swine model. *Arteriosclerosis,
482 Thrombosis, and Vascular Biology*. 1986;6:312-20.
- 483 [42] Papadopoulos KP. Flow effect on thrombus formation in stenosed coronary arteries: a
484 computational study: City University London; 2015.
- 485 [43] Katritsis DG, Pantos J, Efstathopoulos E. Hemodynamic factors and atheromatic plaque
486 rupture in the coronary arteries: from vulnerable plaque to vulnerable coronary segment.
487 *Coronary Artery Disease*. 2007;18:229-37.
- 488 [44] Katritsis DG, Efstathopoulos EP, Pantos J, Korovesis S, Kourlaba G, Kazantzidis S, et al.
489 Anatomic Characteristics of Culprit Sites in Acute Coronary Syndromes. *Journal of
490 Interventional Cardiology*. 2008;21:140-50.
- 491 [45] Papadopoulos KP, Gavaises M, Pantos I, Katritsis DG, Mitroglou N. Derivation of flow
492 related risk indices for stenosed left anterior descending coronary arteries with the use of
493 computer simulations. *Medical Engineering & Physics*.
- 494 [46] Johnston BMJaPR. Simulations of pulsatile blood flow in tapered S-shaped in-plane and
495 out-of-plane coronary arteries. 18th World IMACS / MODSIM Congress, Cairns, Australia.
496 2009.
- 497 [47] Myers JG, Moore JA, Ojha M, Johnston KW, Ethier CR. Factors Influencing Blood Flow
498 Patterns in the Human Right Coronary Artery. *Annals of Biomedical Engineering*.
499 2001;29:109-20.
- 500 [48] Gerotziafas GT, Depasse F, Busson J, Leflem L, Elalamy I, Samama MM. Towards a
501 standardization of thrombin generation assessment: The influence of tissue factor, platelets
502 and phospholipids concentration on the normal values of Thrombogram-Trombinscope
503 assay. *Thrombosis Journal*. 2005;3.
- 504 [49] Bonderman D, Teml A, Jakowitsch J, Adlbrecht C, Gyöngyösi M, Sperker W, et al.
505 Coronary no-reflow is caused by shedding of active tissue factor from dissected
506 atherosclerotic plaque. *Blood*. 2002;99:2794-800.
- 507 [50] Castoldi E, Rosing J. Thrombin generation tests. *Thrombosis Research*. 2011;127,
508 Supplement 3:S21-S5.
- 509 [51] Smid M, Dielis A, Winkens M, Spronk H, Van Oerle R, Hamulyak K, et al. Thrombin
510 generation in patients with a first acute myocardial infarction. *Journal of Thrombosis and
511 Haemostasis*. 2011;9:450-6.

512

513 Figure captions

514 Figure 1: **Phenomenological thrombin generation model**. The simplified cell based concept

515 used for the development and application of the thrombin sub-model under flow. The

516 different colours of arrows indicate the processes that are lumped in each reaction rate

517 constant used. K_{in} : Thrombin inhibitor; k_{AP}^{IIa} : Activation of platelets by thrombin; k_{II}^{AP} :

518 Thrombin activation due to activated platelets; k_{TF} ; Initiation by exposed TF k_{AP}^{AP} : Activation
519 of platelets due to the presence of activated platelets

520 Figure 2: **Surface initiation simulations.** The experimental setup of the two basic cases used
521 for the calculation of the rate constant for the surface initiation reaction. The length
522 upstream the TF bearing surface was sufficiently long in order for a parabolic velocity profile
523 to be developed.

524 Figure 3: **Platelet aggregation simulations.** The geometry and the flow parameters used for
525 the calibration and validation of platelet aggregation sub model

526 Figure 4: **Figure 4: Location of reacting lesion in coronary models.** The three LAD models
527 used for the application of the thrombus formation, MI1 MI2 and STA. The maximum
528 stenosis was 50% radius reduction compared to the health LAD model. The stream-wise
529 length of the reacting surface is 6mm in all cases and the centre of the reacting area is at the
530 peak of the stenosis.

531 Figure 5: **Above threshold vs sub-threshold setup.** Comparison of an above threshold (up)
532 and a sub-threshold (down) setup regarding the initiation of coagulation. In the above
533 threshold setup, the concentration of thrombin is increasing downstream the reacting
534 surface and is sufficiently high ($>1\text{nM}$) to cause platelet activation and the results
535 correspond to 120s perfusion time.

536 Figure 6: **Platelet aggregation for different shear rates.** The performance of the platelet
537 aggregation sub-model. The simulations follow the experimental results regarding the initial
538 deposition rate and the maximum deposition. For the case with high shear rate the model
539 fails to reproduce the disaggregation of the platelets after 10min of perfusion. However, in
540 this case both perfusion time and wall shear rate are extremely high compared to the

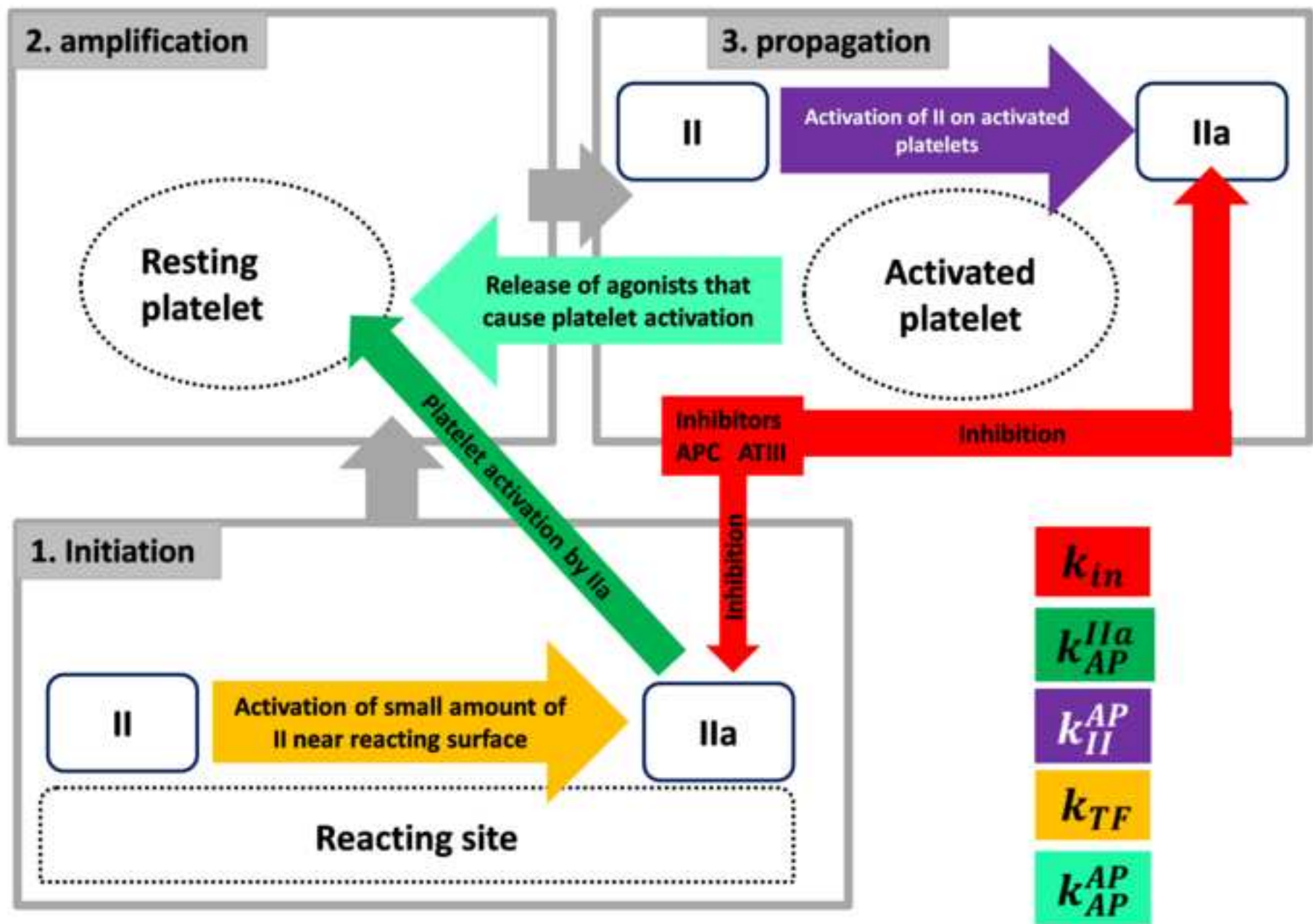
541 conditions of simulations in LAD geometries ($t < 6\text{mins}$ and $\gamma_w \approx 240\text{s}^{-1}$). The dashed line
542 corresponds to the full surface coverage.

543 Figure 7: **Maximum thrombin concentration.** Temporal evolution of maximum thrombin
544 concentration for the three LAD models. For all models maximum thrombin concentration is
545 approximately 25% of the TGA value. For MI1 and STA the initiation phase is significantly
546 short while for MI2 case is long compared to TGA.

547

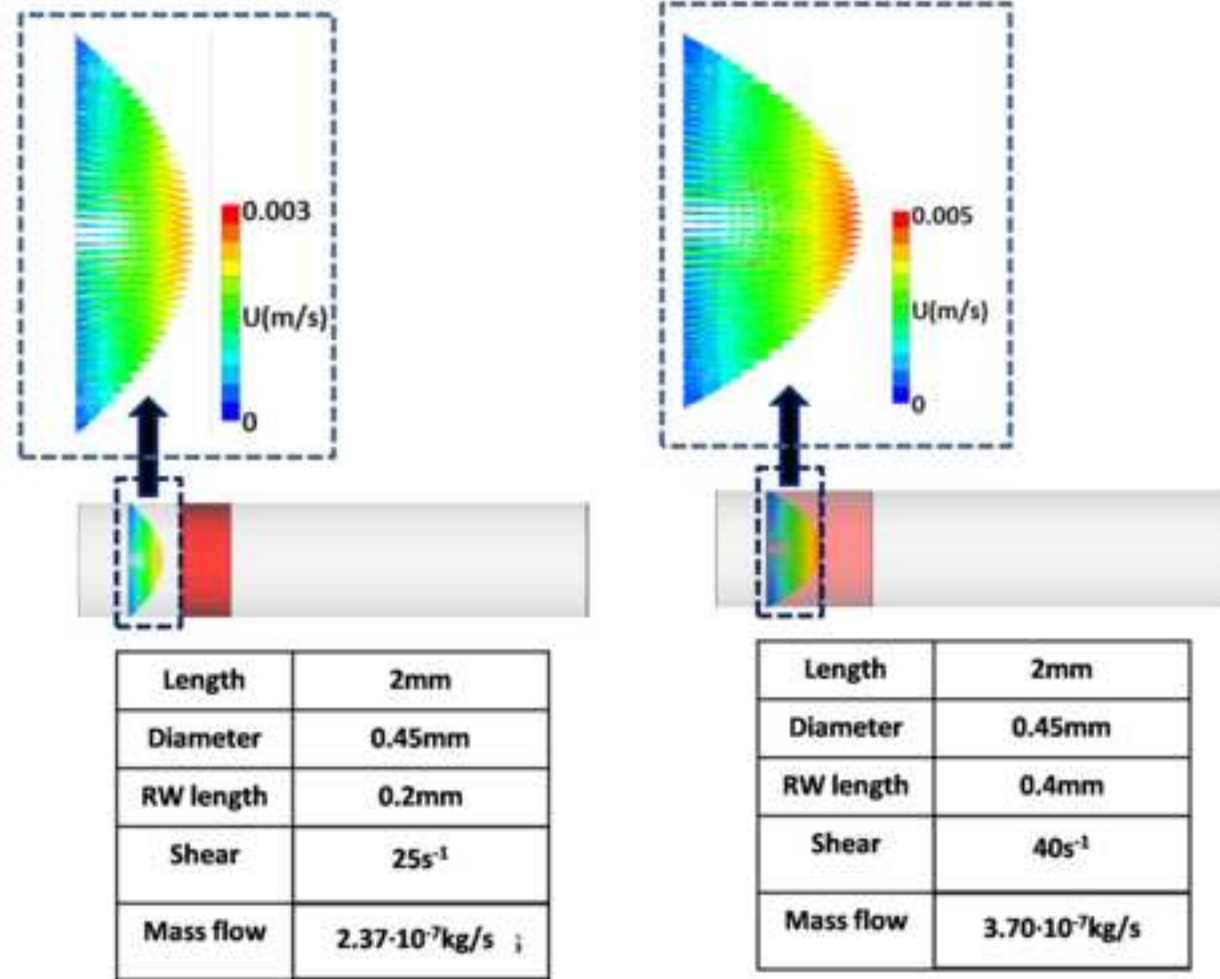
548

Figure
[Click here to download high resolution image](#)



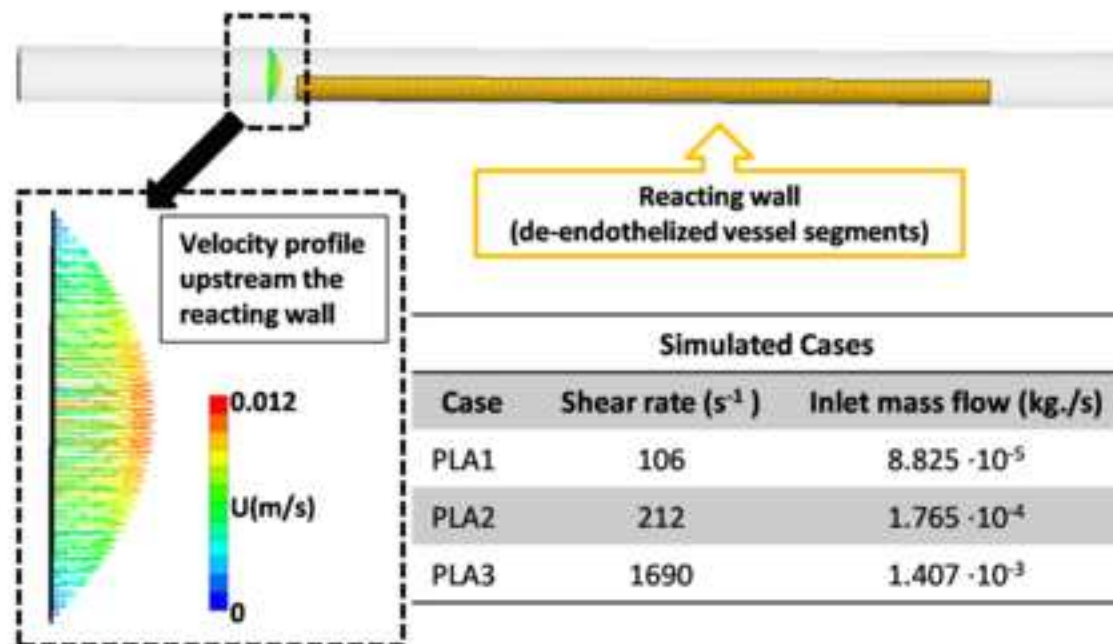
Figure

[Click here to download high resolution image](#)



Figure

[Click here to download high resolution image](#)



Figure

[Click here to download high resolution image](#)

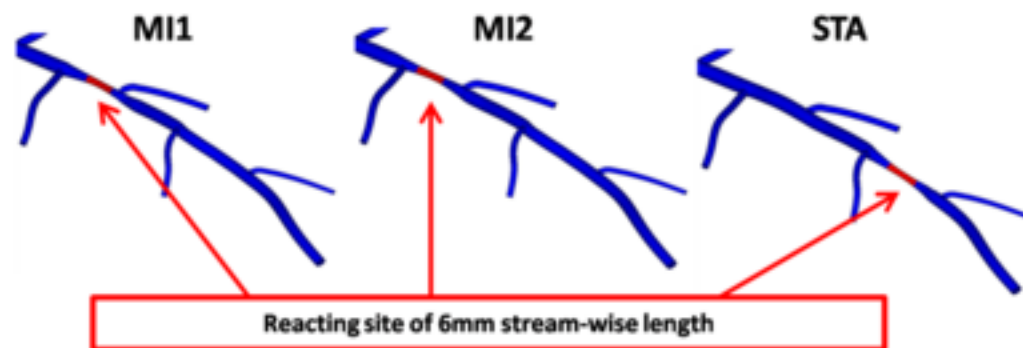
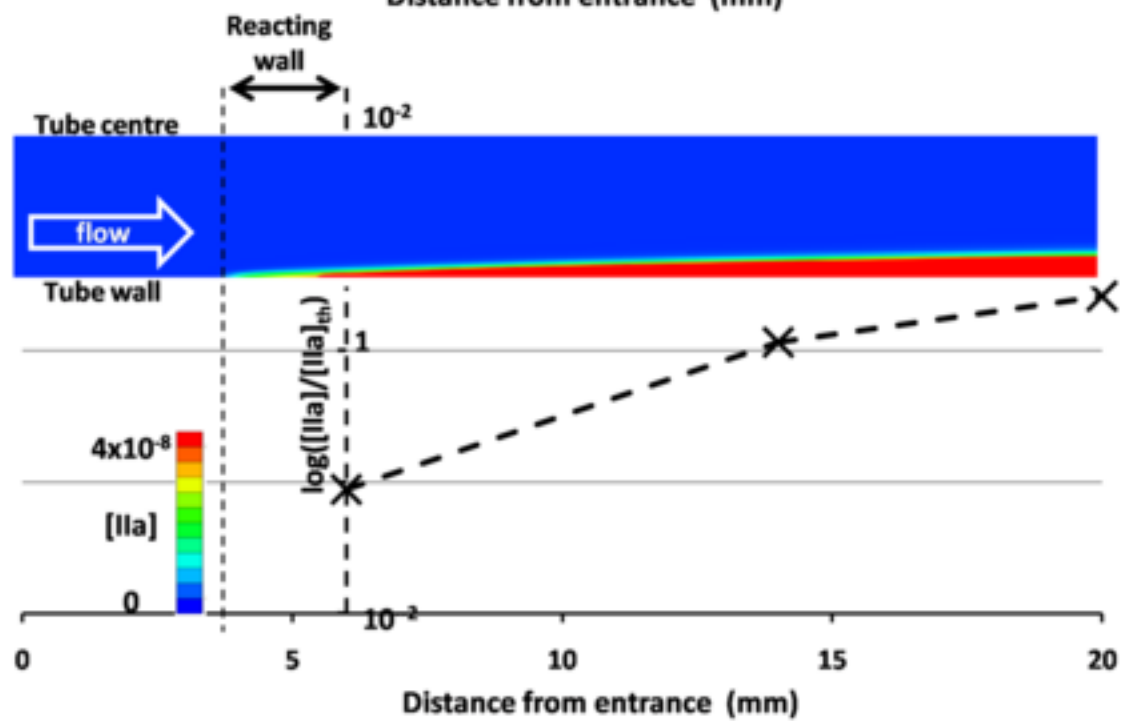
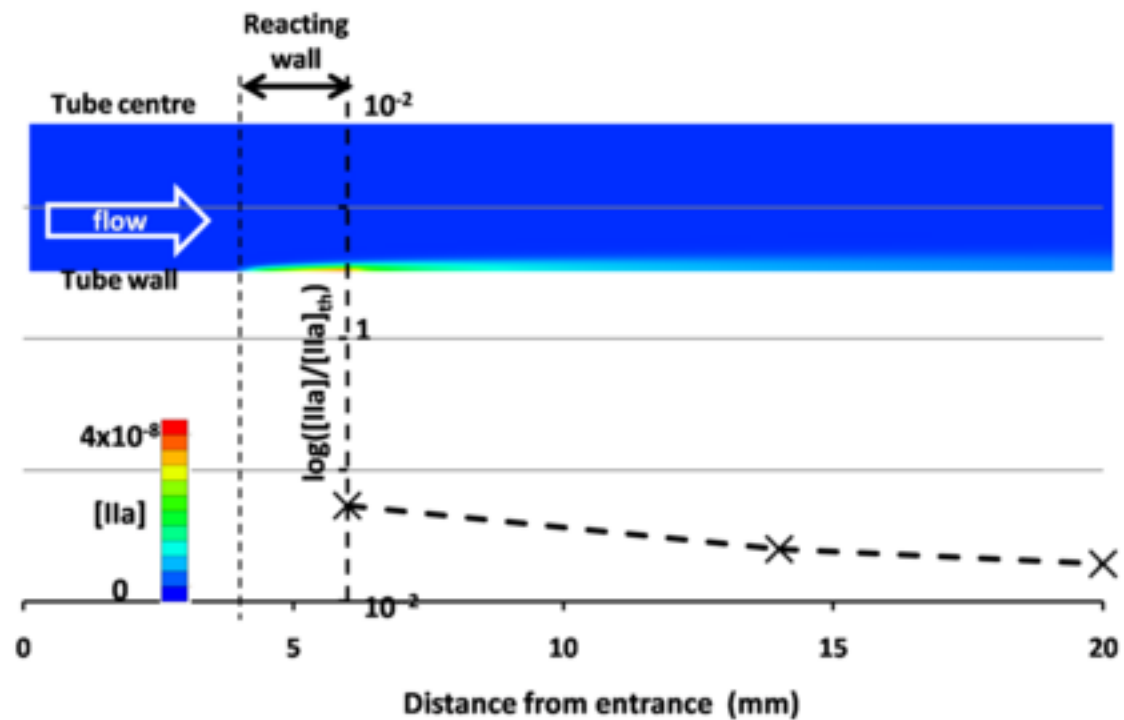
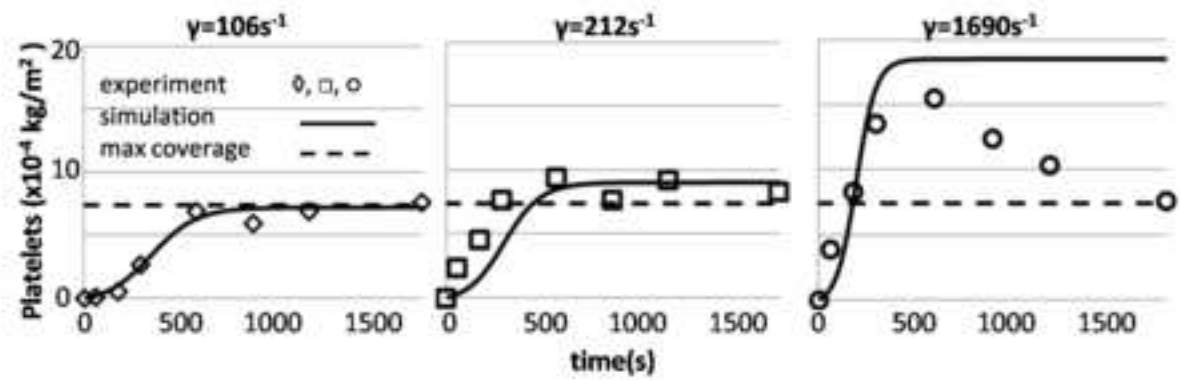


Figure
[Click here to download high resolution image](#)



Figure

[Click here to download high resolution image](#)



Figure

[Click here to download high resolution image](#)

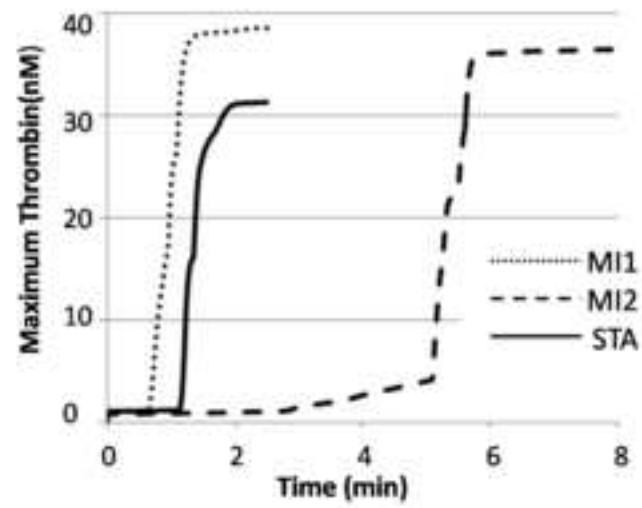
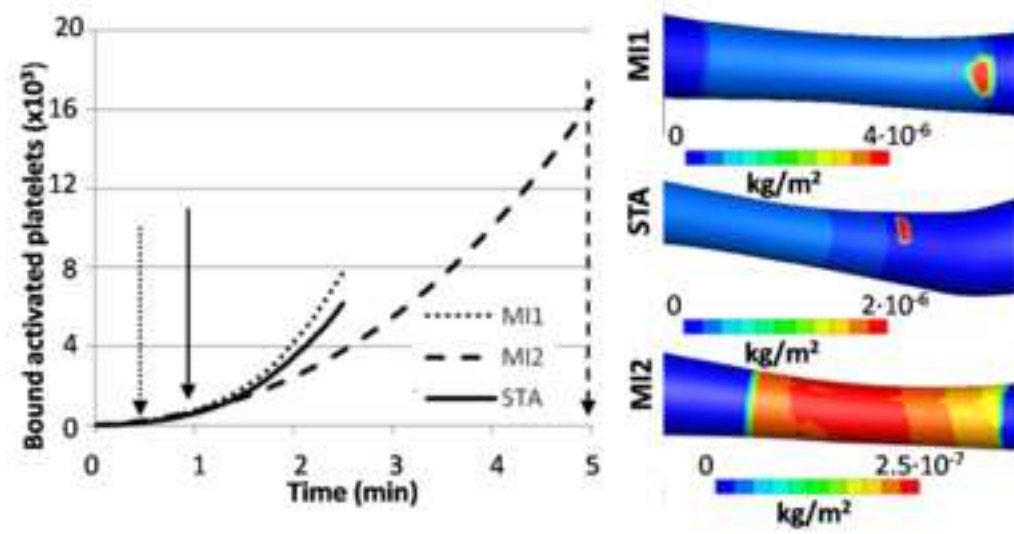


Figure
[Click here to download high resolution image](#)



Supplementary data

[Click here to download Supplementary data: Appendix.docx](#)