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1 Title page

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

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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

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

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

change of blood properties due to blood clotting. Kuharsky and Fogelson [25] proposed an integrated model for thrombin generation under a simplified flow field, using a system of 59 equations that also included the binding of substances and the localization of reactions on surfaces. . In following publications by the same group additional processes were incorporated in the model: the alteration of the rheological properties of blood due to clotting, by modelling platelet-platelet and platelet-wall interaction as reversible elastic links. [26]. ; the APC mechanism and the transport of substances between plasma and endothelium cells [27]. Additionally, a small scale discrete model using an immersed boundary method [28] for platelets was utilized to develop a continuous model for platelet aggregation [29]; the later was also applied in simulations with pulsating flow in an idealized two dimensional vessel bifurcation [30] and with the inclusion of transport within the thrombus, was used to demonstrate the effects of flow conditions and the quantity of TF exposed on thrombus growth [31]. A similar model was presented by Xu et al [32], which later included a cellular pot model [33] for discrete cells and an energy-based stochastic process for cell motion. The simulation involved differentiation of cell movements depending on fibrin levels and cell-cell or cell-surface interaction and bonds. The model was used to evaluate the role of fVII in venous thrombus formation [34] and to examine the impact of pulsating flow and the non-Newtonian characteristics of blood on thrombus growth [35]. Anand et al [36] presented another multi-process model that used a viscoelastic model to simulate flow for both free vessel lumen and clot. This model also incorporated the activation of platelets due to excessive shear stress and fibrin production and lysis. In a similar work, a model for the viscosity of blood depending on fibrin concentration was proposed and used in a three-dimensional simulation of blood coagulation in a tube [37].

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

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

Materials and methods

Thrombus modelling

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

equations of Table 1. These equations express the concentration of 4 species in a zero-dimensional system. The outcome is the temporal evolution of thrombin concentration. The reaction rate constants of the model are derived directly from thrombin generation assays and can be adjusted within a reasonable range in order to reproduce the results of thrombin generation assays for a wide range of cases including haemophilia. The adjustment can be 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	$[AP^{(f)}]$	$+(k_{AP}^{IIa} + k_{AP}^{AP} \cdot [AP^{(f)}]) \cdot [RP^{(f)}]$

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

The described physical system corresponds to a well-mixed solution, where the spatial distributions of the concentration of all species are uniform. At the same time each of the reactions and the respective reaction rates correspond to processes occurring either in plasma, on the membrane of activated platelets or at the sites where TF is expressed according to the cell based approach [7]. This is achieved with the use of different reaction rate constants for the areas with activated platelets and the additional reaction terms for 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	$+ \left((k_{bi,surf}^{AP} \cdot A_f + k_{bi,RP}^{AP} \cdot [RP^{(b)}] + k_{bi,AP}^{AP} \cdot [AP^{(b)}]) \cdot [RP^{(f)}] \right) - k_{diss}^{AP} [AP^{(b)}]^2$ $+ k_{AP}^{IIa} [RP^{(b)}] - k_{AP}^{AP} ([AP^{(b)}] + [AP^{(f)}]) \cdot [RP^{(f)}] + k_{AP}^{surf} [RP^{(b)}]$
	act	

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

Coupling with flow

For the simulations including flow, the concentrations ($C_i^{(f)}$) of the blood circulating species were calculated using the convection-diffusion equation with source terms (S_i) (equation 1). For immobilized species ($C_j^{(b)}$) the source term expresses the temporal evolution (equation 2).

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

Equation 1

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

Equation 2

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

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

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

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

Geometry reconstruction

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

Flow field

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

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

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

Results

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

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

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

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

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

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

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

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

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

In MI1 and STA geometries, the transition from initiation to amplification phase was fast and levels of thrombin concentration that are considered sufficiently high to cause platelet activation ($>1.2\text{nM}$) were present downstream the reacting area only after the amplification. In these models the downstream propagation was observed after the initiation phase. In MI2 geometry the process had much slower progress and while in specific points downstream the reacting surface thrombin exceeds the threshold value during the initiation phase. The simulations were stopped 2 minutes after the downstream propagation and after maximum thrombin concentration had obtained a constant value, approximately 1.5 minutes after the downstream propagation. In all cases the process was limited in a small zone near the vessel wall, even in the presence of vortices.

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

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

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

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

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

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

Conclusions

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

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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.

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

Figure captions

Figure 1: **Phenomenological thrombin generation model**. The simplified cell based concept used for the development and application of the thrombin sub-model under flow. The different colours of arrows indicate the processes that are lumped in each reaction rate 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

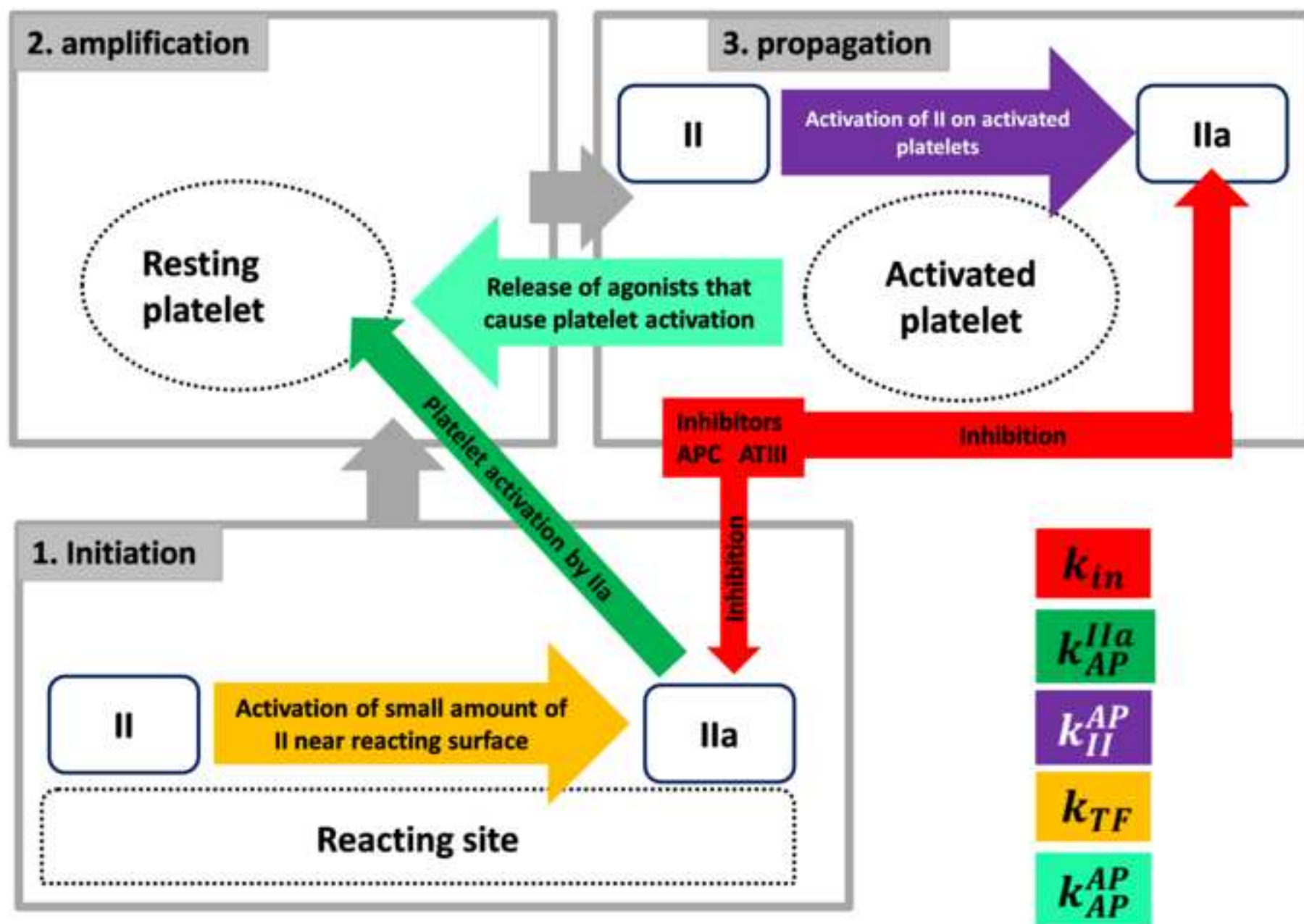
541 conditions of simulations in LAD geometries ($t < 6\text{mins}$ and $\gamma_w \approx 240s^{-1}$). The dashed line
542 corresponds to the full surface coverage.

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.

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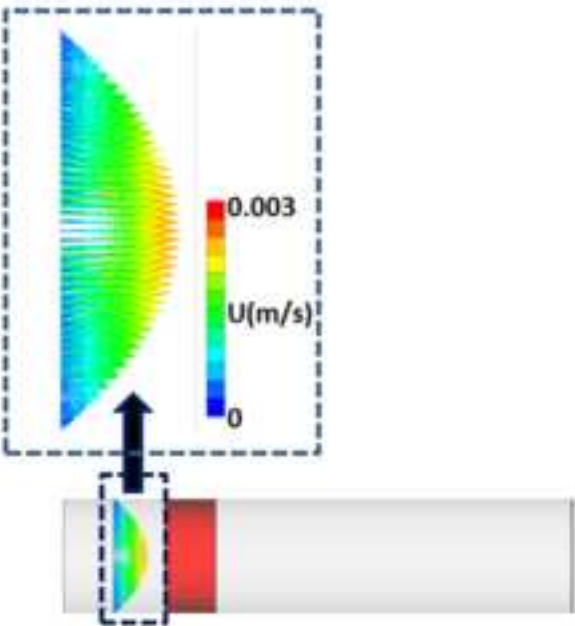
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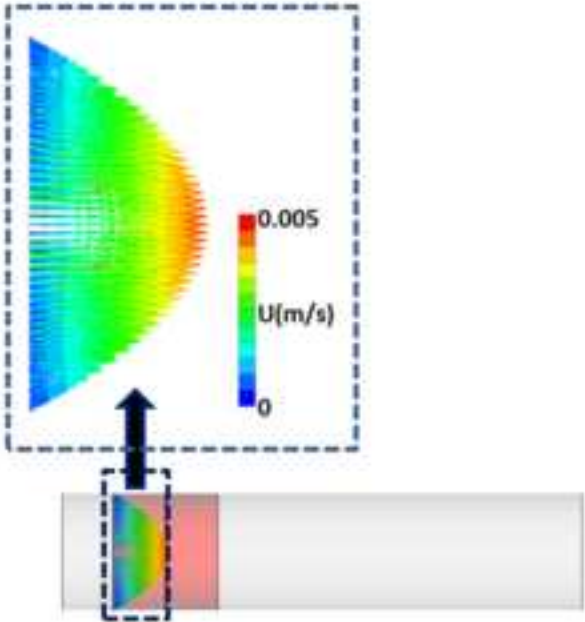


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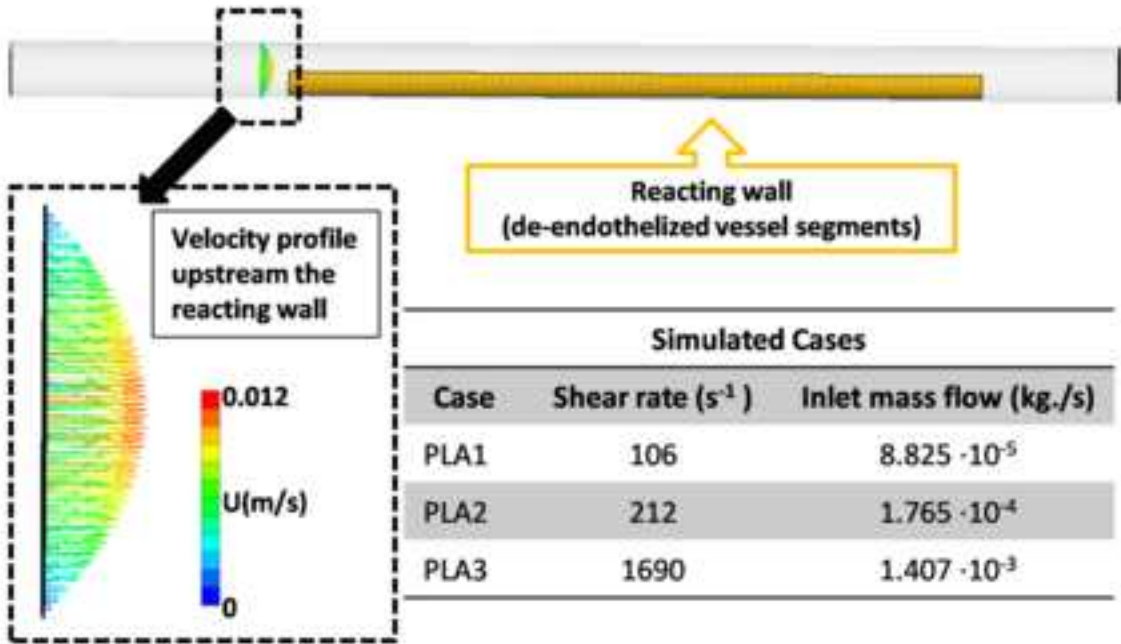
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Diameter	0.45mm
RW length	0.4mm
Shear	40s^{-1}
Mass flow	$3.70 \cdot 10^{-7}\text{kg/s}$

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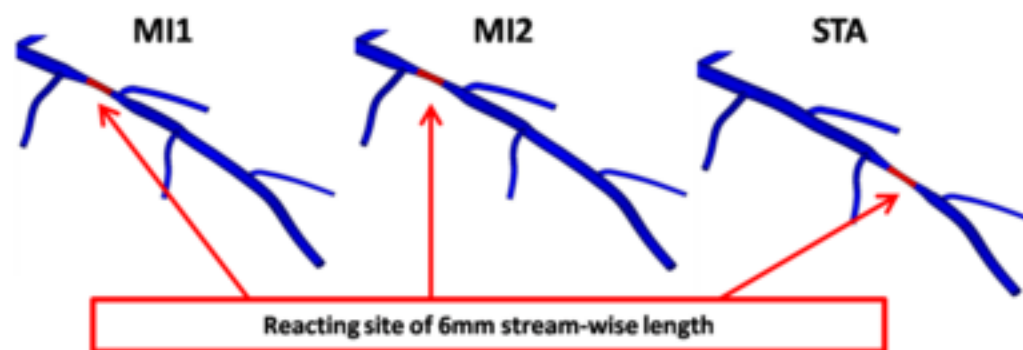
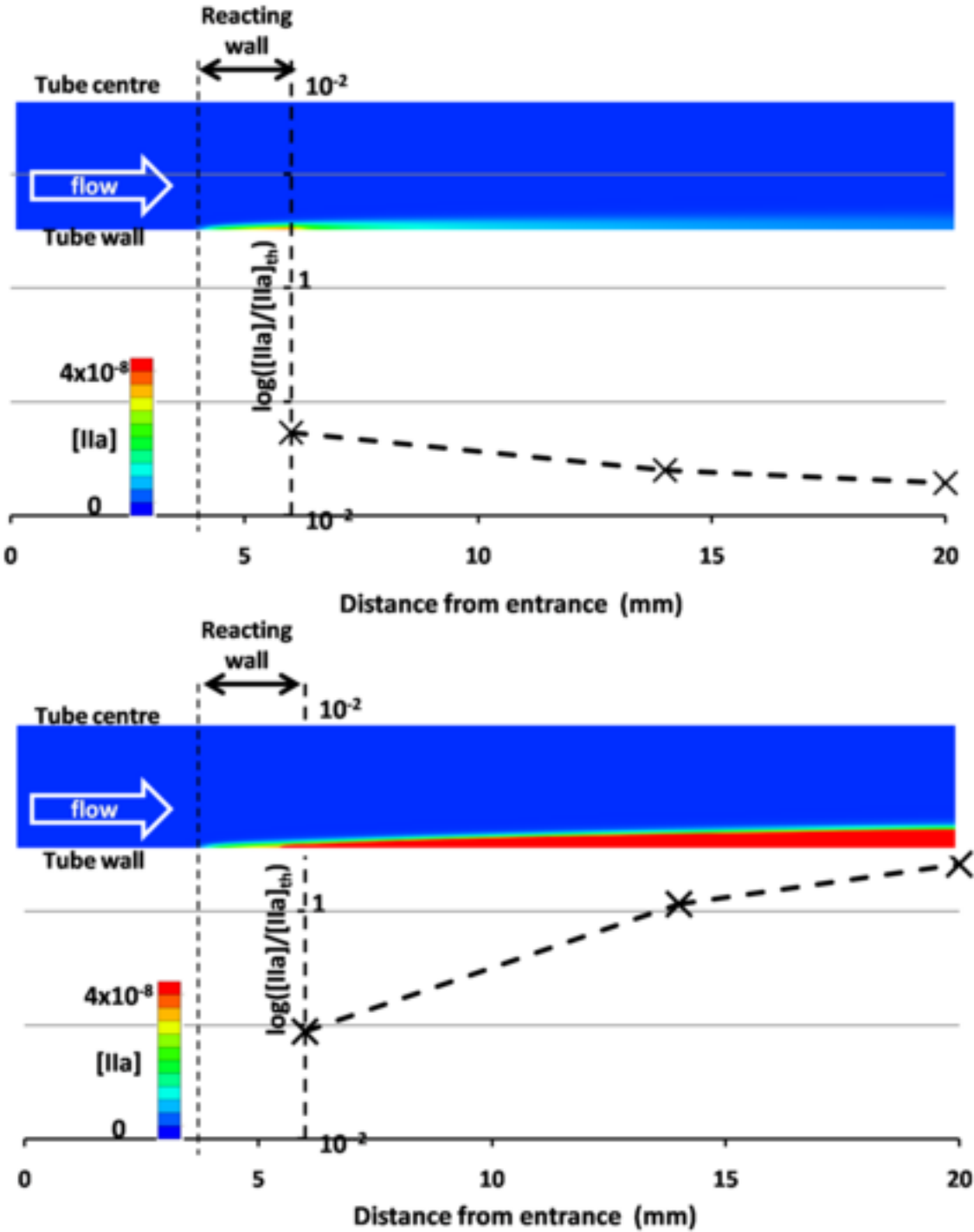
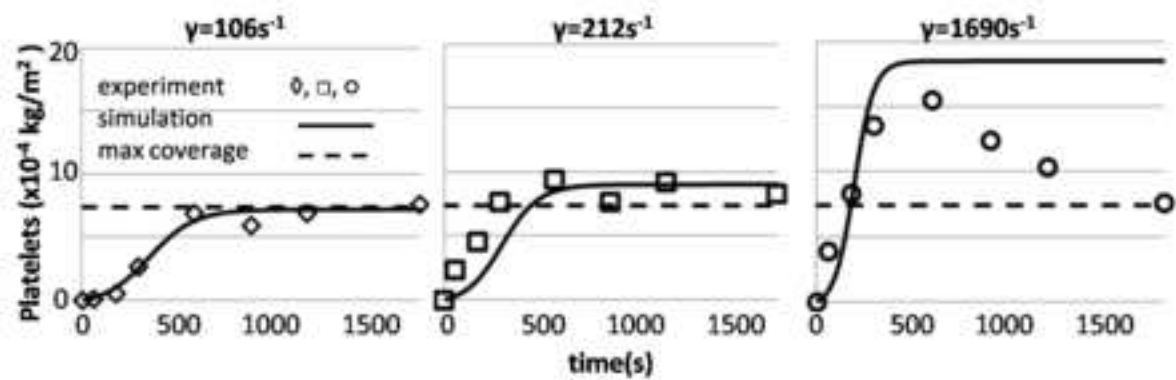


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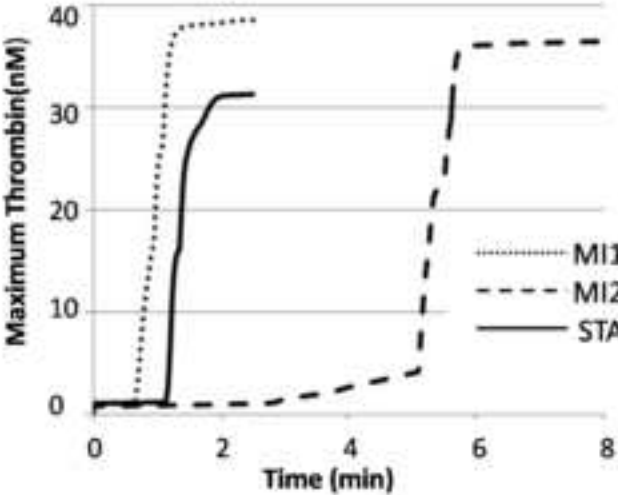
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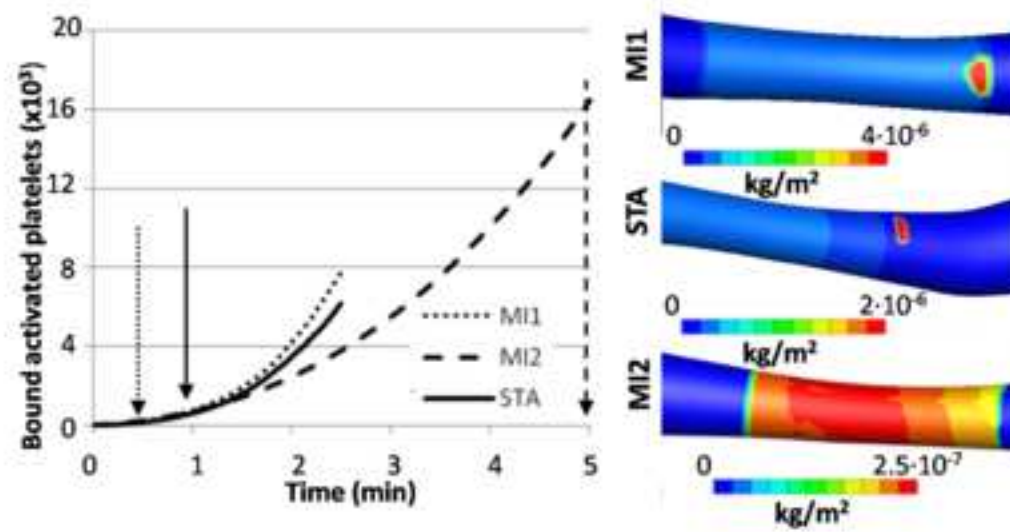
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Supplementary data

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