Analysis of microvascular thrombus mechanobiology with a novel particle-based model

Original Article

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29 **Abstract**

30 Platelet accumulation at the site of vascular injury is regulated by soluble platelet agonists,
 31 which induce various types of platelet responses, including integrin activation and granule
 32 secretion. The interplay between local biochemical cues, mechanical interactions between
 33 platelets and macroscopic thrombus dynamics is poorly understood.

34 Here we describe a novel computational model of microvascular thrombus formation for detailed
 35 analysis of thrombus mechanics. Adopting a previously developed two-dimensional particle-
 36 based model focused on the thrombus shell formation, we revise it to introduce platelet agonists.
 37 Blood flow is simulated via computational fluid dynamics approach. In order to model soluble
 38 platelet activators, we apply Langevin dynamics to a large number of non-dimensional virtual
 39 particles. Taking advantage of the available data on platelet dense granule secretion kinetics, we
 40 model platelet degranulation as a stochastic agonist-dependent process.

41 The new model qualitatively reproduces enhanced thrombus formation due to granule secretion
 42 in line with *in vivo* findings and provides a mechanism for thrombin confinement at the early
 43 stages of aggregate formation. Our calculations also predict that release of dense granules results
 44 in additional mechanical stabilization of the inner layers of the thrombus. Distribution of the
 45 inter-platelet forces throughout the aggregate reveals multiple weak spots in the outer regions of
 46 thrombus, which are expected to result in mechanical disruptions at the later stages of thrombus
 47 formation.

48 INTRODUCTION

49 Thrombus formation is an elaborate mechanism aimed at haemorrhage arrest that might
50 otherwise cause excessive intratissular bleeding. However, unrestrained hemostatic response
51 might lead to blood vessel occlusion and life-threatening embolism (Jackson, 2011). Platelets,
52 being the principal structural elements of the arterial thrombus, rapidly respond to biochemical
53 and biomechanical cues at the site of the injury and form the aggregate that is primarily
54 stabilized by platelet-platelet interactions. Platelets largely define early mechanics of the
55 thrombus outer shell (Babushkina et al., 2015, Kaneva et al., 2021), are vital for determining the
56 occlusive scenarios (Ahmed et al., 2020, Belyaev et al., 2015) and contraction-driven re-
57 arrangement of thrombus architecture (Nechipurenko et al., 2019), and provide a protected
58 environment for blood coagulation to occur (Podoplelova et al., 2016, Tosenberger et al., 2013).
59 Platelet activation is driven by receptor-mediated signaling, and soluble platelet agonists are now
60 largely recognized as key regulators involved in arterial thrombus formation and stabilization
61 (Sachs and Nieswandt, 2007).

62 For instance, thrombin and adenosine 5'-diphosphate (ADP) are potent inducers of platelet
63 activation. Under the hemostatic response thrombin is produced in coagulation reaction cascade,
64 whereas ADP is contained within platelet dense granules and is released through exocytosis
65 along with multiple other compounds, including ATP, serotonin and polyphosphates.

66 Experimental insights into heterogeneous organization of arterial thrombi gave rise to further
67 understanding of the impact the platelet activators have on hemostasis (Stalker et al., 2013).
68 Thrombin is believed to be almost entirely responsible for the formation of the thrombus core,
69 the innermost layers of highly-activated platelets, whereas ADP and thromboxane A₂ are
70 principal regulators of the unstable thrombus shell assembly. How thrombin activity, platelet
71 degranulation and agonist-induced platelet activation altogether orchestrate dynamics of
72 thrombus formation is still unclear.

73 Theoretical modeling is widely used to study hemostasis due to the complexity of the system in
 74 question (Babushkina et al., 2015, Belyaev et al., 2015, Kaneva et al., 2021, Nechipurenko et al.,
 75 2019, Nechipurenko et al., 2020, Tosenberger et al., 2013, Xu et al., 2017, Yazdani et al., 2017).
 76 We have previously developed a particle-based model of arterial thrombus shell formation to
 77 address the state of adhesive receptors in different layers of the growing thrombi (Kaneva et al.,
 78 2021). However, since the model only considered the formation of thrombus shell, platelet
 79 activation was assumed to be an explicitly time-dependent process. To elaborate on the subject
 80 of thrombus heterogeneity, we modified the model and introduced explicit description of key
 81 platelet agonists. For the aim of this study, we adopted the platelet-platelet interaction
 82 mechanism and integrated it with the modeling approach we developed previously to describe
 83 platelet-flow interactions using quasi-steady flow approximation (Trifanov et al., 2018). The
 84 next step was supplementing this framework with a virtual particle model of agonist kinetics and
 85 a model of agonist-induced platelet activation.

86 Using the developed modeling framework, we demonstrate enhanced thrombus formation due to
 87 release of platelet dense granules. We also show that secretion provides additional mechanical
 88 stabilization of the inner layers of the thrombus. Using the model, we provide a possible
 89 explanation for the confinement of thrombin activity in space as being result of intrathrombus
 90 flows at the early stages of thrombus formation.

91

92 **MATERIALS AND METHODS**

93 **Model framework**

94 In the model, we consider the following processes that impact or drive thrombus formation:
 95 hydrodynamics of the blood flow, agonist-induced platelet activation, platelet-flow and platelet-
 96 platelet interactions, thrombin generation, secretion of ADP (through degranulation), and
 97 transport of thrombin and ADP (Fig. 1A).

Hydrodynamics of the blood flow is described using quasi-steady flow approximation (see *Supplemental text A*) and blood (except platelets) is described is continuous media with Navier-Stokes equations. Platelets are described as particles traveling within the media, and platelets that constitute the aggregate are considered as stationary 2-dimensional discs with effective hydrodynamic radius. Agonists-induced platelet activation is described in a following manner: virtual particles that represent chemical species, change parameters of the mechanical interaction between platelets (see *Supplemental texts B* and *D* for details). Thrombin generation is described as a constant flux of thrombin-associated particles from the thrombus formation initiation region (the injury site). Secretion of ADP is described as generation of ADP-associated particles at the site of particular platelet. Transport of APD and thrombin is described using the Langevin dynamics of corresponding particles (see *Supplemental text C*).

The model comprises three distinct modules that interchange information about the current state of the system (Fig. 1B). Computational fluid dynamics (CFD) module recalculates pressure and velocity fields based on positions of platelets provided by particle dynamics (PD) module. Current hydraulic resistance in computational domain is used to update inlet parabolic flow boundary condition. Pressure and velocity fields obtained from CFD module by solving stationary Navier-Stokes equations are considered stationary in the PD module and used to calculate forces exerted on the platelets by the flow for the PD module. Information from CFD module is also used by agonist kinetics (AK) module to update concentration maps for agonists (see *Supplemental text E*). Platelet activation states are then updated in the PD module using concentration maps provided by AK module, and are in turn used to calculate inter-platelet forces. PD module is in the core of the model framework: here the positions of platelets are updated by numerical integration of the equations of motion, which comprise platelet-flow, platelet-wall and platelet-platelet forces and torques. Thrombin concentration also determines probability of degranulation for individual platelets, and signals for degranulation events are passed down to AK module to generate ADP.

124 The details on each module and simulation procedures are described in Supplemental texts A-E.

125

126

127 **RESULTS**

128 **1. Thrombin-driven hemostatic response leads to the formation of a stable platelet** 129 **aggregate**

130 To investigate the dynamics of thrombus formation driven solely by thrombin – a potent platelet
131 agonist, we first analyzed the simplified case with a constant thrombin flux emanating from
132 injury site as described in *Supplemental text C*. We also implemented explicit thrombin-
133 dependent activation mechanism as described in *Supplemental text D* and simulated first 90
134 seconds of thrombus formation. Due to the stochastic nature of the model, this and following
135 calculations were performed several times. Thrombus height was increased gradually, slowing
136 down and reaching 12.25 ± 0.15 micrometers by the end of the simulation (Fig. 2A, black curve,
137 Supplemental Video 1, 2). No thrombus disruptions were observed over the course of any
138 simulation. The observed increase in stability of thrombus in comparison to disruptive thrombus
139 shell (Kaneva et al., 2021) arises from the reinforcement of platelet-platelet bonds embedded in
140 the model to reflect high potency of thrombin as platelet activator.
141 Thus, the model recapitulates formation of a stable thrombus “core” as observed in experiments,
142 where only thrombin activity is retained.

143

144 **2. Thrombin concentration gradient rapidly reaches steady state and limits thrombus** 145 **propagation in the absence of granule secretion**

146 We next examined whether spatial distribution of thrombin concentration is the limiting factor
147 that determines the height of the thrombus in case when no other agonists are present. For this
148 purpose, we analyzed the distribution of thrombin-particles in a large pre-formed platelet
149 aggregate. Thrombin concentration decreases exponentially with distance from the injury site,

150 and the gradient reaches steady state during first 3 seconds of simulation (Fig 3B), not much
151 faster than does concentration gradient obtained in simulations with growing thrombus,
152 described in a previous section (Fig. 3C). Comparing thrombin gradients from the two
153 computational experiment settings, we conclude that it is thrombin gradient that limits the extent
154 of thrombus growth in case when other agonists are absent. The mechanisms responsible for
155 exponential decrease of thrombin concentration in space were further investigated and described
156 below.

157

158 **3. Platelet degranulation induced by thrombin enhances thrombus formation**

159 Platelet dense granule secretion plays essential role in hemostasis as has been shown both *ex vivo*
160 and *in vivo* (Israels et al., 1990, Meng et al., 2015). Disorders associated with abnormal
161 degranulation result in impaired thrombus shell formation, such as in mice with Hermansky-
162 Pudlak syndrome (Hermasky and Pudlak, 1969, Meng et al., 2015). To gain general
163 understanding of the mechanism by which secreted agents affect formation of the thrombus, we
164 focused on particular component of the dense granules – ADP and added platelet secretion to the
165 model of thrombin-induced thrombus formation as described in *Supplemental text D*. Similar to
166 the simulations described in the first section, we modeled 90 seconds of thrombus formation.
167 Due to the burst-like character of granule secretion process (as separate granules are secreted),
168 we observed burst-like kinetics of ADP generation and washout (Fig. 3B-D, Supplemental Video
169 4). Thrombus growth intensified compared to thrombin-only induced thrombus formation as
170 seen by steeper increase in thrombus height and resulting thrombus height of 18.1 ± 0.4
171 micrometers compared to 12.25 ± 0.15 microns (Fig. 2A, grey and black curves). Considering
172 that ADP gradient exceeds out of the thrombus region in the direction opposite the blood flow,
173 we infer that this enhancement results from ADP-mediated platelet recruitment due to ADP-
174 induced platelet activation, described in the *Supplemental text D*.

175 Thus, the described model demonstrates that release of ADP from platelet dense granules
176 accelerates thrombus formation, in line with *in vivo* data.

177

178 **4. High permeability of thrombus results in thrombin confinement effect due to** 179 **convection-mediated dilution**

180 To investigate the mechanisms responsible for thrombin confinement effect, we next analyzed
181 the distribution and magnitude of plasma flows throughout the aggregate. In order to avoid non-
182 realistic barriers for the flow due to the two-dimensional nature of the model, we decreased the
183 hydrodynamic radius of each platelet during velocity field calculations (Fig. 4 A, C). The
184 obtained geometry results in two-dimensional porosity of approximately 0.8, representing the
185 upper bound of the 3d porosity measured for the outer layers of thrombus *in vivo* (Stalker et al.,
186 2013). The heatmap of velocity magnitude values inside the aggregate at the 90 s of simulation
187 demonstrates complex velocity field with an average value of 0.05 mm/s (Fig. 4 C, D). The
188 estimate of the Peclet number for thrombin transport inside thrombus in current model exceeds
189 unity, suggesting the significant effect of intrathrombus flow on thrombin concentration
190 gradient, as evidenced in Figure 3A, B and thus explaining the exponential decrease of thrombin
191 concentration as a function of distance from the injury site (Fig. 3 B, C). Importantly, the Peclet
192 number for ADP transport is less than unity due to much higher diffusion coefficient and results
193 in rapid diffusion-driven redistribution of ADP through the aggregate and hence – increased
194 platelet recruitment at the outer regions of the aggregate.

195

196

197 **5. Particle-based approach uncovers complex distribution of the tensile forces in** 198 **heterogeneous thrombus**

199 The complex dynamics of arterial thrombus observed *in vivo* is driven by the interplay between
200 platelet-platelet and platelet-flow interactions. The values of critical tensile forces between

201 platelets largely depend on the degrees of their integrins activation, which, in turn, depend on the
 202 local biochemical and biomechanical stimulus, experienced by these platelets.

203 To estimate the magnitudes and distribution of platelet-flow interactions we calculated the
 204 hydrodynamic drag forces within the aggregate. Analysis of the forces acting on the platelets in
 205 thrombus is depicted on Fig. 4E. Expectedly, these forces are much higher for the outer platelets
 206 of the aggregate, where the values of the shear rate are maximal.

207 To get deeper insight into the distribution of the mechanical forces, which stabilize the
 208 aggregate against the flow, we took advantage of the particle nature of the model and analyzed
 209 individual inter-platelet forces in thrombus. Interestingly, both repulsion and attraction forces
 210 between platelets show complex distribution with many “hot pairs”, wherein these forces are
 211 significantly increased (Fig. 5 B, C). The values of critical forces for given pair of platelets in
 212 our model is proportional to the product of platelet activation degrees (see *Supplemental text D*).

213 The distribution of the critical tensile forces throughout the thrombus (Fig. 5A) qualitatively
 214 corresponds to the distribution of platelet activation levels (Supplemental Video 3). These forces
 215 are maximal at the base of the thrombus, where activation levels are higher, and decrease in the
 216 outer layers of the aggregate. To evaluate the safety margin S for each pair of platelets we used a
 217 simple formula:

218 $S=1-F/F_{\max}$,

219 where F is the tensile force for given pair of platelets (derived from Morse potential), and $F_{\max}$ is
 220 the critical force for these platelets given their activation states.

221 Thus, the higher values of S (close to unity) correspond to the “safe” pairs – where current
 222 tensile forces are much lower than critical, while values closer to zero reflect “weak spots” –
 223 where platelet-platelet contact is likely to rupture in case if external forces are increased.

224 The distribution of the safety margin values throughout the aggregate (Fig. 5D), also
 225 demonstrates numerous “weak spots”. Interestingly, these weak spots are mainly located in the

226 outer domain of the aggregate formed in the presence of both thrombin and ADP, but are still
227 present in the innermost domain in case of thrombin-driven thrombus formation.

228

229

230

231 **DISCUSSION**

232 Understanding the coupling between local biochemical/biomechanical stimuli and platelet-
233 platelet interactions is crucial for elucidation of the basic mechanisms that regulate complex
234 dynamics of arterial thrombus formation. Here we describe a novel *in silico* approach for
235 investigation of the interplay between local biochemical stimuli, platelet-platelet interactions and
236 the overall dynamics of thrombus under arterial blood flow.

237 In order to get deeper understanding of the processes that drive the formation of heterogeneous
238 thrombus structure, we have significantly advanced the particle-based model, which was recently
239 used to describe the plasticity of the arterial thrombus shell (Kaneva et al., 2021). The developed
240 modeling framework considers the change of the blood flow due to formation of the thrombus,
241 platelet integrin activation in response to thrombin and ADP, transport of thrombin and ADP, the
242 secretion of ADP in response to thrombin, as well as two types of platelet-platelet interactions.

243 The model was built in a bottom-up manner and its particle nature allows thorough and
244 mechanistic analysis of the ongoing processes on the level of single platelets. However, such
245 level of description results in high computational complexity of the model and thus makes it
246 perfectly suitable for description of microvascular thrombosis on physiologically relevant
247 timescales of several minutes.

248 Here we demonstrate that ADP, being secreted by platelets in response to thrombin, is rapidly
249 distributed throughout the thrombus through diffusion process and leads to activation of the
250 platelets that adhere to thrombus surface through primary stochastic interactions. Thus, dense

251 granule secretion, which delivers ADP, results in significant increase of thrombus size, in line
 252 with *in vivo* findings (Meng et al., 2015).

253 Our results suggest a possible mechanism of thrombin confinement on the early stages of
 254 aggregate formation – when clot contraction is negligible. Intrathrombus plasma velocities result
 255 in advection-mediated dilution of thrombin, limiting the size of the thrombin-induced thrombus.

256 Rapid stabilization of thrombin gradient inside thrombus is in line with experimental data
 257 obtained in mice: linear increase in thrombin sensor fluorescence observed over initial 120 s of
 258 thrombus growth *in vivo* suggests that total amount of thrombin in the thrombus remains
 259 constant over the indicated time period (Welsh et al., 2014).

260 Particle-based nature of the model allowed us to analyze the distribution of inter-platelet forces
 261 throughout the aggregate and conclude that dense granule secretion leads to additional
 262 stabilization of the innermost thrombus region. However, the ADP-dependent inter-platelet
 263 contacts in the outer layers of thrombus demonstrate multiple weak spots that might lead to
 264 thrombus disruptions at the later stages of thrombus formation.

265 The described model represents a unique computational instrument that couples local
 266 biochemical cues to the inter-platelet interactions and granule secretion. It exploits physically
 267 clear variables and parameters and is generally built in the bottom-up manner based on
 268 experimental data, including data on ADP-dependent activation of platelet integrins.

269

270 However, the current version of the model has several important limitations that will be
 271 addressed in future: it does not consider contraction of the thrombus, which is known to
 272 significantly reduce local aggregate permeability and hence alter the transport of species inside
 273 the aggregate (Tomaiuolo et al., 2014). The model does not explicitly account for coagulation
 274 reactions and fibrin formation, which might significantly impact local mechanical stability and
 275 thrombin transport. It does not consider such processes, as thromboxane A2 synthesis and
 276 release, and the activation of platelets by this substance (including granule release). We also did

not account for additional platelet activation due to outside-in signaling (mechanically induced activation) or GPVI-induced signaling.

Despite these limitations, the developed instrument represents a valuable tool for the thorough analysis of the platelet mechanics that drives the dynamics of hemostatic response in arterioles and venules.

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REFERENCES

1. Ahmed, M. U., Kaneva, V., Loyau, S., Nechipurenko, D., Receveur, N., Le Bris, M., Janus-Bell, E., Didelot, M., Rauch, A., Susen, S., Chakf^v©, N., Lanza, F., Gardiner, E. E., Andrews, R. K., Panteleev, M., Gachet, C., Jandrot-Perrus, M., & Mangin, P. H., 2020. Pharmacological Blockade of Glycoprotein VI Promotes Thrombus Disaggregation in the Absence of Thrombin. *Arteriosclerosis, thrombosis, and vascular biology*, 40(9), 2127-2142.

- 303 2. Babushkina, E. S., Bessonov, N. M., Ataullakhanov, F. I., & Panteleev, M. A., 2015.
304 Continuous Modeling of Arterial Platelet Thrombus Formation Using a Spatial
305 Adsorption Equation. PloS one, 10(10), e0141068.
- 306 3. Belyaev, A. V., Panteleev, M. A., & Ataullakhanov, F. I., 2015. Threshold of
307 microvascular occlusion: injury size defines the thrombosis scenario. Biophysical
308 journal, 109(2), 450-456.
- 309 4. Hermansky F, Pudlak P., 1959. Albinism associated with hemorrhagic diathesis and
310 unusual pigmented reticular cells in the bone marrow: report of two cases with
311 histochemical studies. Blood. 1959; 14:162-169.
- 312 5. Israels S.J., McNicol A., Robertson C., Gerrard J.M., 1990. Platelet storage pool
313 deficiency: diagnosis in patients with prolonged bleeding times and normal platelet
314 aggregation. British journal of haematology, 75(1), 118-121.
- 315 6. Jackson, S, 2011. Arterial thrombosis-insidious, unpredictable and deadly. Nat Med 17,
316 1423-1436.
- 317 7. Kaneva, V. N., Dunster, J. L., Volpert, V., Ataullahanov, F., Panteleev, M. A., &
318 Nechipurenko, D. Y., 2021. Modeling Thrombus Shell: Linking Adhesion Receptor
319 Properties and Macroscopic Dynamics. Biophysical journal, 120(2), 334-351.
- 320 8. Meng, R., Wu, J., Harper, D. C., Wang, Y., Kowalska, M. A., Abrams, C. S., Brass, L.
321 F., Poncz, M., Stalker, T. J., & Marks, M. S., 2015. Defective release of α granule and
322 lysosome contents from platelets in mouse Hermansky-Pudlak syndrome models. Blood,
323 125(10), 1623-1632.
- 324 9. Nechipurenko, D. Y., Receveur, N., Yakimenko, A. O., Shepelyuk, T. O., Yakusheva, A.
325 A., Kerimov, R. R., Obydenyy, S. I., Eckly, A., Lvon, C., Gachet, C., Grishchuk, E.
326 L., Ataullakhanov, F. I., Mangin, P. H., & Panteleev, M. A., 2019. Clot Contraction
327 Drives the Translocation of Procoagulant Platelets to Thrombus Surface. Arteriosclerosis,
328 thrombosis, and vascular biology, 39(1), 37-47.

- 329 10. Nechipurenko, D. Y., Shibeko, A. M., Sveshnikova, A. N., & Panteleev, M. A., 2020. In
330 Silico Hemostasis Modeling and Prediction. *Hamostaseologie*, 40(4), 524-535.
- 331 11. Podoplelova, N. A., Sveshnikova, A. N., Kotova, Y. N., Eckly, A., Receveur, N.,
332 Nechipurenko, D. Y., Obydennyi, S. I., Kireev, I. I., Gachet, C., Ataullakhanov, F. I.,
333 Mangin, P. H., & Panteleev, M. A., 2016. Coagulation factors bound to procoagulant
334 platelets concentrate in cap structures to promote clotting. *Blood*, 128(13), 1745-1755.
- 335 12. Sachs, U. J., & Nieswandt, B., 2007. In vivo thrombus formation in murine models.
336 *Circulation research*, 100(7), 979-991.
- 337 13. Stalker, T. J., Traxler, E. A., Wu, J., Wannemacher, K. M., Cermignano, S. L., Voronov,
338 R., Diamond, S. L., & Brass, L. F., 2013. Hierarchical organization in the hemostatic
339 response and its relationship to the platelet-signaling network. *Blood*, 121(10), 1875-
340 1885.
- 341 14. Tomaiuolo, M., Stalker, T. J., Welsh, J. D., Diamond, S. L., Sinno, T., & Brass, L. F.,
342 2014. A systems approach to hemostasis: 2. Computational analysis of molecular
343 transport in the thrombus microenvironment. *Blood*, 124(11), 1816-1823.
- 344 15. Tosenberger, A., Ataullakhanov, F., Bessonov, N., Panteleev, M., Tokarev, A., &
345 Volpert, V., 2013. Modelling of thrombus growth in flow with a DPD-PDE method.
346 *Journal of theoretical biology*, 337, 30-41.
- 347 16. Trifanov, P. V., Kaneva, V. N., Strijhak S. V., Panteleev, M. A., Ataullakhanov, F. I.,
348 Dunster, J., Voevodin, V. V., Nechipurenko, D. Y., 2018. Developing Quasi-Steady
349 Model for Studying Hemostatic Response Using Supercomputer Technologies.
350 *Supercomputing Frontiers and Innovations*, Vol.5, ,Ññ4, p.67-72.
- 351 17. Welsh, J.D., T.J. Stalker, R. Voronov, R.W. Muthard, M. Tomaiuolo, S.L. Diamond, and
352 L.F. Brass., 2014. A systems approach to hemostasis: 1. The interdependence of
353 thrombus architecture and agonist movements in the gaps between platelets. *Blood*. 124:
354 1808 LP - 1815.

18. Xu, S., Xu, Z., Kim, O. V., Litvinov, R. I., Weisel, J. W., & Alber, M., 2017. Model predictions of deformation, embolization and permeability of partially obstructive blood clots under variable shear flow. *Journal of the Royal Society, Interface*, 14(136), 20170441.
19. Yazdani, A., Li, H., Humphrey, J. D., & Karniadakis, G. E., 2017. A General Shear-Dependent Model for Thrombus Formation. *PLoS computational biology*, 13(1), e1005291.

FIGURE LEGENDS

Figure 1. Model framework. (A) Diagram depicting interdependent modules within the model. The insert shows schematics of ADP generation through degranulation. (B) Data transfer between modules and separation of calculations into CPU- and GPU-hosted. Information about current positions of virtual particles together with velocity fields obtained from hydrodynamical module and degranulation signals is passed to GPU where positions of virtual particles are updated. Data is then transferred back to the CPU to calculate concentration map and update state of activation of platelets (see the text).

Figure 2. Dynamics of thrombus formation in the model. (A) Dynamics of thrombus growth as represented by thrombus height (in micrometers) versus model time (in seconds). Black curve corresponds to thrombin-only induced thrombus formation and grey curve is related to thrombus formation in the presence of both thrombin influx and platelet secretion. N=3 for each curve. (B-C) Snapshots of states of activation of platelets after 60 seconds of simulation for thrombin-induced thrombus formation (B) and thrombin and ADP-induced thrombus formation (C). First and second row show only the fraction of numerical value of the extent of activation induced by

380 thrombin and ADP, correspondingly. Third row depicts overlapping of thrombin- and ADP-
381 stimulated regions where platelets activated by both agonists are colored as a shade of green.
382

383 **Figure 3. Thrombin concentration gradient.** (A) Concentration map of thrombin in grown
384 thrombus. Thrombin concentration gradient is shown for the center of the injury site denoted by
385 white vertical axis y and its exponential fit as obtained for a preformed thrombus (B) and for a
386 growing thrombus during its formation (C). Zero value at horizontal axis corresponds to the
387 injury site. Different curves correspond to different stages of the simulation. In both
388 representative simulations (B-C) concentration gradient rapidly reaches the steady state.
389

390 **Figure 4. ADP concentration maps.** (A-C) Spatial distribution of ADP at successive stages of
391 platelet formation, at 30, 60 and 90 seconds of the simulation. Color corresponds to ADP
392 concentration in μM . Degranulation events as one seen in (B) follow burst-like kinetics (see
393 Supplemental Video 4).
394

395 **Figure 5. Intrathrombus plasma flows.** (A) The velocity field and mesh in computational
396 region. As thrombus propagates into the vessel, the flow becomes obstructed and inlet velocity
397 decreases to maintain constant pressure drop in computational domain, though local flow
398 velocity increases up to 14 mm/s compared to initial inlet velocity of 8.85 mm/s. This creates
399 high shear rate at the boundary of the thrombus. Note that platelet radii are decreased (see the
400 text). (C) Flow velocity profile taken in the center of aggregate denoted by black axis in (A). (B)
401 Intrathrombus velocity field normalized to 0.5 mm/s and corresponding velocity profile.
402 Velocity drops to zero inside platelets. (E) Hydrodynamic forces imposed on individual platelets.
403 Platelets on the surface of the thrombus are exposed to the higher drag forces.
404

Figure 6. Distribution of inter-platelet forces through the aggregate. Left column corresponds to thrombus formed under thrombin-only stimulation, right column corresponds to thrombus formed under both thrombin and ADP stimulation. (A) Critical tensile forces between pairs of platelets as allowed for by their current state of activation. Platelets are centers of Morse potential field, and the force is proportional to the product of the extents of activation of interacting platelets. (B) Attraction forces between pairs of platelets which can be interpreted as tensile forces. (C) Repulsion between pairs of platelets. (D) Safety margin of a platelet-platelet bonds defined as $S = 1 - F/F_{\max}$, where F is attraction force between platelets and $F_{\max}$ is the critical force for this pair of platelets. Dark red bonds correspond to weak spots of thrombus where attraction forces are close to critical ones. (E) Torque imposed on individual platelets by their neighbors through stochastic springs representing vWF-mediated interaction. On the surface of the thrombus this torque counteracts the flow-induced torque and hence prevents lateral rolling of newly attached platelets under the shear stress.

Conflict of interest statement

The authors state that they have no conflict of interest.

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B

Only thrombin

Thrombin and ADP

A

B

C

D

E

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