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Blood flow velocity effects and role of activation delay time on growth and form of platelet thrombi

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Mural thrombi are composed dominantly of platelets and develop under a blood flow. Portions can break off and are carried in the blood flow as emboli. Thrombus growth rates are affected by the velocity of the blood flow, but they do not simply increase with it, they exhibit a maximum, with subsequent decrease. Whereas this variation indicates an interaction of biochemical and physical processes, studies have concentrated widely on understanding only the biochemical processes. Here we show results of simulation of thrombus formation in 3D flows by accounting for the movements of individual platelets. Each platelet follows prescribed rules for interactions while the local flow around the thrombus continuously adjusts to the growing structure of the thrombus, also when embolization occurs. With an activation delay time assigned to each platelet we demonstrate the dependence of thrombus growth rate on blood velocity as found experimentally by Begent and Born [Begent N, Born GV (1970) *Nature* 227:926–930]. With activated platelets having mutual tensile action sustainable up to a prescribed distance we achieve thrombus growth faster than with shorter maximum distances that make a thrombus less porous; when the prescribed maximum distance is large enough the thrombus shape is not like a “hill” but like a “carpet.” We find that thrombus growth rate is enhanced by modest pulsatility but less so when pulsations are amplified in part because of more embolization.

direct numerical simulations | platelet aggregation | *in vivo* comparison | stochastic modeling

Acute thrombogenesis in a flowing bloodstream can occur on damaged tissues in normal circulation (1). It has also been observed in blood flow over vascular prostheses (2) and in artificial internal organs, such as prosthetic heart valves (3). The thrombi are composed predominantly of platelets, and they can develop even in the presence of systemic anticoagulants such as heparin (4).

Blood flow velocity effects were investigated systematically *in vivo* by Begent and Born (1), who obtained quantitative data on thrombus growth rates for a range of blood flow rates. Their study remains the clearest time-resolved *in vivo* study of the effect of blood flow rates on thrombus formation. Richardson (5) subsequently proposed that Begent and Born's observations were consistent with a shear-flow aggregation process (6, 7) in which an activation delay time of the platelets is allowed for, a delay time between each platelet's close encounter with the thrombus and its development of ability to adhere to the thrombus, and which was estimated then to be the order of 0.1–0.2 s. A predicted consequence of this finding was that the height-to-length ratio for thrombi would be lower in blood flows, where a significant fraction of the activated platelets escaped the primary thrombus before their activation delay time had elapsed; this was demonstrated later by Born and Richardson (8). More recent experiments by Petrishchev and Mikhailova (9) *in vivo* and van Gestel *et al.* (10) *in vitro* produced qualitatively similar results as the ones of Begent and Born but they lack the quantitative details required for validating simulation results.

At the time of Begent and Born's studies there were handicaps to carrying the implications further. The biological handicap was the lack of specific knowledge of cell membrane channels and

receptors, and therefore of cell mechanics by which an activation delay time could be mediated (and varied). The computational handicap was that computing capability then available was inadequate to consider the movements of, say, 50,000 individual platelets in a blood flow where thrombus growth is initiated at one location on a wall. This latter handicap now has diminished, and this article describes what is found in running simulations of the Begent and Born flow situations and what can be predicted about thrombus formation in pulsatile flows. The latter is a circumstance important to clinical conditions such as thrombo-embolic stroke and myocardial infarction involving thrombus formation on fissured atherosclerotic plaques in carotid and coronary arteries.

Numerical Simulations

Simulations representing 3D blood flow were performed for a 50- μm -diameter straight tube, with 500- μm length at several different steady blood flow rates. Platelets are considered uniformly distributed in the inflow, with the latter having a parabolic velocity profile at entry. The mean velocity distribution alters downstream, over time, as a thrombus forms on the wall and acts as an obstacle to the flow; there is an interaction between the flow and the structure formed by the thrombus. The time-dependent computations continuously update the geometry of the thrombus with regard both to size and shape. This procedure was followed for blood flow rates both below and above the rate, which is expected to provide the maximum relative rate of growth. This approach provided simulation results, which can be compared with the experimental data of Begent and Born (1).

Simulations were extended to investigate the effect of pulsatility of blood flow. The inflow was prescribed to have a steady component and a simple harmonic component, the amplitude of the fluctuating component being ε times the steady component, with values of ε ranging from 0.1 to 0.7. The frequency ω of the fluctuating component was 1 Hz. The product of the activation delay time τ and the frequency ω is a dimensionless parameter. Both might be modified pharmacologically and largely independently, so behavior of thrombus growth at other values of the product are prospectively interesting to determine whether there are zones of behavior worth targeting and reasons for possible differences in thrombus growth between small animal (higher ω) and large animal (lower ω) observations, while τ differs less between the relevant species.

Biological Model

With the mode of supply of ADP used by Begent and Born (1), one can expect that there is a period in which the concentration of ADP builds up parallel to the axis of the blood vessel, and therefore the axial location at which the ADP concentration caused by the iontophoresis is large enough to initiate activation of platelets advances upstream for a while. The footprint of ADP concentration around the outside of the vessels Begent and Born used was likely

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decades, in many experimental studies the flow conditions are not recorded or controlled for, nor are electron micrographs of thrombi routinely reported. Such incompleteness of experimental information makes it difficult to improve representational details applied in computer modeling (see *Simulation Methodology*). It has been noted that in the use of light-dye methods for thrombus initiation the vessel-wall injury varies with the intensity of the light applied, but reports of light intensity used, and of the extent of primary vascular injury, are often missing. There are, of course, some representational details used in the model that may be robust, in the sense that modifications of values of some parameters may make modifications of outcome that are too small to distinguish by presently available methodology in experiments [the standard deviations in the thrombus growth rates as a function of blood velocity measured by Petrishchev and Mikhailova (9) are comparable with variations that arise in our computational model, but also imply that such experiments may be limited for comparing with small refinements of the model]. For example, in modeling the interplatelet attractive forces it seems sufficient for the present to use one fixed force-distance relation for a fixed finite time, although in practice it must vary over time [likely connected in part to the variations of platelet cytosolic free calcium with time (10)] and be associated with a mix of engaged cell-surface receptors and composition of linking molecules. In modeling platelet-platelet repulsive forces, modifications to the platelet membrane stiffness (as by α -tocopherol) may require a corresponding adjustment, but potentially also to the attractive force because α -tocopherol also down-regulates GPIIb promoter activity (18). If a negative feedback regulation associated with platelet-borne MMP-9 (19) is confirmed to play a role, it could be necessary to include its effect in the activation aspects of the model used here. If there are subpopulations with different reactivities, as implied by Van Gestel *et al.* (10), they, too, can be incorporated by adaptation of the model. In a study to assess thromboembolism associated with pulsatile flow, Sukavaneshvar *et al.* (20) observed in an *ex vivo* model that flow pulsatility induced increases in both thrombosis and embolism.

The model may lead to improvements in assessing the impact of various platelet-active drugs, observed and measured in specific flow conditions, in altering thrombus formation in a variety of other flow conditions. Hopefully, our model will spur new experiments to determine some of the parameters of thrombi previously overlooked and lead to models more sophisticated in representation of biochemical and physical processes that matter most.

Simulation Methodology

For our simulation, we assumed platelets in concentration of 300,000 per mm^3 , 3 μm equivalent diameter, a vessel diameter of 50 μm , parabolic velocity profile at the inlet and mean blood flow velocities of 100–800 $\mu\text{m/s}$, overlapping the major range of Begent and Born's experimental conditions (1). Applying the force coupling method (21), the equation of motion is

$$\rho \frac{\partial \mathbf{u}}{\partial t} = -\nabla p + \mu \nabla^2 \mathbf{u} + \mathbf{F}, \quad [2]$$

$$\nabla \cdot \mathbf{u} = 0, \quad [3]$$

$$\mathbf{F} = \sum_{n=1}^N \mathbf{F}^n \Delta(\mathbf{x} - \mathbf{Y}^n), \quad [4]$$

where the coupled force associated with the n th platelet is distributed around its center-point \mathbf{Y}^n as

$$\Delta(\mathbf{x} - \mathbf{Y}^n) = (2\pi\sigma^2)^{-3/2} e^{-\frac{(\mathbf{x}-\mathbf{Y}^n)^2}{2\sigma^2}}, \quad \sigma = \frac{a}{\sqrt{\pi}}, \quad [5]$$

with a being the platelet radius, with the force vector

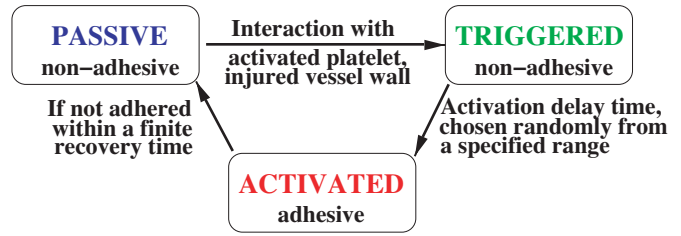


Fig. 5. In our model, platelets can be in three different biological states: passive, triggered, and activated. In the passive state, platelets are not adhesive, which is a normal state of the platelets in blood. If a passive platelet interacts with an injured wall or an activated platelet it becomes triggered and after an activation delay time it becomes activated and adhesive. The activation delay time is chosen uniformly at random from a specified range. If after a finite recovery time an activated platelet does not adhere to anything it returns back to a passive state.

$$\mathbf{F}^n = -\frac{4}{3} \pi a^3 (\rho_p - \rho) \frac{d\mathbf{Y}^n}{dt} + \mathbf{F}_{\text{interaction}}, \quad [6]$$

and the postsuperscript is annotation designating the n th platelet, not a power. The first term allows for the difference between the density of platelets in comparison to an equivalent volume of the suspending fluid, and the second term representing the force exerted between the n th platelet and other platelets or the vessel wall surface. The individual platelet velocity is obtained from a local, volume-averaged fluid velocity as

$$\mathbf{V}^n = \frac{d\mathbf{Y}^n}{dt} = \int \mathbf{u} \Delta(\mathbf{x} - \mathbf{Y}^n) d\mathbf{x}. \quad [7]$$

The interaction force term includes, for any platelet, a repulsive portion when the platelet is approaching and has come within a specific distance of another platelet or wall surface. If both the n th platelet and another (or the location on the wall) it encounters are in the active state the link is created between them. No more than one link can be created between each pair of activated platelets (or the activated platelet and the wall). For activated platelets with links in addition to the repulsive force, there is also an attractive force, shown at the larger distances of separation; this is zero at smaller, finite distances to permit loose packing of mutually adhering platelets. The looseness of packing is significant for a thrombus because it provides a space through which the suspending fluid can seep. It also recognizes the linking role of fibrinogen without attempting to model it in detail. We note that Guy and Fogelson (22) have made computational estimations regarding the role of fibrinogen, based on the collision of two spherical particles with fibrinogen available in the suspending fluid, one particle being already activated, and consider bond completion and bond rupture; because of the many platelets considered in our model, a simpler form of relation that does not assess aspects of fibrinogen concentration is used here.

In our model, each platelet carries with it an activation-distance corona. Before a platelet is triggered it is tested at each step in time to determine whether an activated platelet has its center within twice the activation-distance corona of its own center; if so, the platelet is coded as having its activation triggered, and within a time span (freshly) randomly chosen between two time interval limits it becomes activated (see Fig. 5). (These are larger than the time step used in the flow computations.) The finite extent (1.5 platelet radius) of the activating corona beyond the perimeter of the platelet allows in this model for diffusion of activators such as ADP without attempting a more exact determination of the convective field involved. If an activated platelet has not adhered within a finite recovery time it returns to its initial passive state. Throughout these

conditional aspects the model is intended to make practical allowances for physico-physiological aspects in ways that avoid requirement of concurrent solutions for e.g., time-dependent ADP transport to be generated as these would be computationally prohibitive at the present time. Much detailed biochemistry is condensed, and only the associated major changes in physical forces are modeled here. The model is chosen to allow computations to march forward in time, without iteration. Each time a computation is performed with constant values of parameters and the same flow rate, there is some variation from the results of otherwise similar runs when the random-number generator for determining individual platelet activation delay times is freshly seeded. Thus, replication runs are made to explore the corresponding variability of thrombus growth.

The governing flow equations are solved by using the spectral/hp element solver NEKTAR (23) marching forward in time steps, the position vectors for all of the platelets being updated (and all associated near-neighbor conditions being checked) for each time step. The force coupling method seems more reliable when the average particle density is modest, and so the suspension of red cells is treated as a continuum. The impact of this assumption is believed to be small because the Smoluchowski number, ratio of shear gradient encounter rate to random-walk collision rate, for platelets in the flow considered here is quite high, and an augmentation of platelet diffusivity would not have a significant effect. We focus on the onset of the aggregation and consider small aggregates of size $\approx 100 \mu\text{m}$. For aggregates of this size the increase of platelet near-wall concentration caused by the presence of the red blood cells should have minor effects on the results obtained here (24). The few other cells typical of whole blood such as the leukocytes are omitted from the model as being too low in number density to have a significant dynamic effect. Even so, using this model has consumed significant amounts of supercomputer time (hundreds of thousands of hours) over a period of 2 years, including sensitivity studies.

At the beginning of the computation, the fluid in the vessel is empty of platelets but the entering flow has them approximately uniformly distributed in it. However, by the time there are platelets sufficiently close to the seed platelets on the vessel wall to be activated, there is full priming of the flow at smaller radii with platelet-laden blood. To initiate thrombus growth at a known location, typically three activated platelets were placed close together on the vessel wall at the initiation of flow. (In pulsatile flows of large amplitude, these were observed to oscillate somewhat back and forth in the flow direction, a natural consequence of the model and sometimes seen in actual flows.) Approaching platelets, which come close enough to these, become triggered; while a few triggered platelets escape downstream, many become activated while still close enough to adhere, and through continuous repetition of this process the platelets aggregate as a mural thrombus.

Interaction potentials applied with soft matter are typically quadratic. Hence, our interaction force term ($F_{\text{interaction}} = F_1 + F_2 + F_3 + F_4$) is piecewise linear, the part for closer approach having

the repulsion force rising linearly with decreasing distance. Specifically, the force acting on the platelet approaching the wall or another platelet is

$$F_1(r) = 2\alpha \left(1 - \frac{r/a}{R}\right)$$

for $r/a \leq R$, where a is the platelet radius and r is a distance from the center of the platelet to the wall or the surface of the other platelet. The force is directed along the normal to the wall or the vector connecting the centers of the platelets. From the distance at which that has become zero, a region of zero force is applied, $F_2(r) = 0$ for $R \leq r/a \leq L$ (representing where connecting proteins, especially fibrin, may be slack), beyond which an attractive force rises linearly with increasing distance,

$$F_3(r) = \alpha \left(\frac{r/a}{L} - 1\right)$$

for $L \leq r/a \leq M$, representing a combination of connecting protein/filopodia and cell membrane elasticity. The force between activated platelets rises up to a point where typically the connections are not long enough to reach, or have broken from, the neighbor platelet, with the force dropping to zero at an out-of-range position,

$$F_4(r) = \alpha \left(\frac{M}{L} - 1\right) \frac{B - r/a}{B - M}$$

for $M \leq r/a \leq B$. The attraction force is directed along the vector between the center of the platelet and the attachment point on the wall or the center of another platelet. The values of parameter R , L , and B are set to 1, 1.5, and 3.5, respectively, while

$$M = \frac{L + B}{2};$$

the constant α is set to $4 \times 10^{-9} N$. These parameters were chosen based on physical considerations and systematic simulations to evaluate the sensitivities of the model. For example, we have performed 36 simulations for three different values of α corresponding to $\times 1/10$, $\times 1/3$, and $\times 3$ the above value (at flow rate $400 \mu\text{m/s}$), and all results were within the range established by the value of $\alpha = 4 \times 10^{-9} N$ at the same flow rate. Finally, the activation delay time is assumed randomly uniformly distributed over the range 0.1 to 0.2 s, selected for each platelet once, but differently in otherwise replicate runs. The platelet recovery time is set to 5 s (5).

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- Begent N, Born GV (1970) *Nature* 227:926–930.
- Harker LA, Marzec UM, Kelly AB, Chronos NRF, Sundell IB, Hanson SR, Herbert JM (1998) *Circulation* 98:2461–2469.
- Acar J, Jung B, Boissel JP, Samama MM, Michel PL, Teppe JP, Pony JC, LeBreton H, Thomas D, Isnard R, et al. (1996) *Circulation* 94:2107–2112.
- Hirsh J, Raschke R, Warkentin TE, Dalen JE, Deykin D, Poller L (1995) *Chest* 108:S258–S275.
- Richardson PD (1973) *Nature* 245:103–104.
- Smoluchowski MZ (1917) *Phys Chem* 92:129–168.
- Thill A, Veerapaneni S, Simon B, Wiesner M, Bottero JY, Snidaro D (1998) *J Colloid Interf Sci* 204:357–362.
- Born GVR, Richardson PD (1980) *J Membr Biol* 57:87–90.
- Petrishchev NN, Mikhailova IA (1995) *Microvasc Res* 49:12–16.
- Van Gestel MA, Heemskerk JW, Slaaf DW, Heijnen VV, Sage SO, Reneman RS, Oude Egbrink MG (2002) *J Vasc Res* 39:534–543.
- Zucker MB (1989) *Methods Enzymol* 169:117–133.
- Falati S, Gross P, Merrill-Skoloff G, Furie BC, Furie B (2002) *Nat Med* 8:1175–1181.
- Polanowska-Grabowska R, Gear AR (1992) *Proc Natl Acad Sci USA* 89:5754–5758.
- Falati S, Liu Q, Gross P, Merrill-Skoloff G, Chou J, Vandendries E, Celi A, Croce K, Furie BC, Furie B (2003) *J Exp Med* 197:1585–1598.
- Ni H, Yuen PS, Papalia JM, Trevithick JE, Sakai T, Fassler R, Hynes RO, Wagner DD (2003) *Proc Natl Acad Sci USA* 100:2415–2419.
- Evans E (2001) *Annu Rev Biophys Biomol* 30:105–128.
- Poole JC, Sanders AG, Florey HW (1958) *J Pathol Bacteriol* 75:133–143.
- Chang SJ, Lin JS, Chen HH (2000) *Free Radical Biol Med* 28:202–207.
- Sheu JR, Fong TH, Liu CM, Shen MY, Chen TL, Chang Y, Lu MS, Hsiao G (2004) *Br J Pharmacol* 143:193–201.
- Sukavaneshvar S, Zheng Y, Rosa GM, Mohammad SF, Solen KA (2000) *ASAIO J* 46:301–304.
- Maxey MR, Patel BK (2001) *Int J Multiphas Flow* 27:1603–1626.
- Guy RD, Fogelson AL (2002) *J Theor Biol* 219:33–53.
- Karniadakis GE, Sherwin SJ (2005) *Spectral/hp Element Methods for CFD* (Oxford Univ Press, Oxford).
- Eckstein EC, Belgacem F (1991) *Biophys J* 60:53–69.