Probabilistic Modeling of Platelet Aggregation: Effects of Activation Time and Receptor Occupancy

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Abstract

A mathematical model is constructed to predict the probability that a collision between two activated platelets results in doublet formation mediated by fibrinogen cross-bridges. The model is used to explore the effect of time from activation, looking at both simultaneous and non-simultaneous activation times. Also considered are the impact of blood fibrinogen concentration and various shear rates. The idea of hydrodynamic efficiency (P. Tandon and S. L. Diamond. 1997. *Biophys. J.*, 73:2819–2835.) is extended by varying the separation distance which is considered to be a collision. From fitting the model to data (Z. Xia and M. M. Frojmovic. 1994. *Biophys. J.*, 66:2190–2201.), it is found that the hydrodynamic efficiency corresponds to short interaction distances ($\approx$ 14 nm). The model predicts that the probability of forming a doublet increases quickly after activation, remains near its maximum for a significant time interval, and then declines. This may contribute to the regulation of the time and location of platelet aggregation, by ensuring that platelets are more likely to aggregate near an injury, rather than downstream in the vascular system. A newly activated platelet has a high probability of cross-bridging with an already activated platelet. Fibrinogen concentration strongly affects the time course and the equilibrium values of the aggregation probability. These results indicate the importance of considering the progression of the reaction between solution fibrinogen and surface receptors in determining a platelet's ability to aggregate.

Key words: fibrinogen, GPIIb/IIIa, capture probability, hydrodynamic efficiency, capture efficiency

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1 Symbols

a	Particle radius (μm)
α	Capture efficiency
b	Length of energy well of bound state (nm)
β	Product of parameters: $\phi_{\mathrm{r}} k^{+} t_{\mathrm{c}}$
c	Combination of parameters: $b/k_B T$ (pN^{-1})
C	Particle concentration
D	Diffusion coefficient of gpIIb-IIIa ($\mathrm{cm}^2\mathrm{s}^{-1}$)
ϵ_h	Hydrodynamic efficiency
f	Applied force to a bond (pN)
γ	Shear rate (s^{-1})
H	Platelet Hamaker coefficient (J)
J_d	Frequency of doublet formation (s^{-1})
J_s	Frequency of collisions with reference particle, as predicted by Smoluchowski (s^{-1})
k_B	Boltzmann constant
k_f	Forward reaction rate for surface receptors with ligand in solution ($\mathrm{M}^{-1}\mathrm{s}^{-1}$)
k_r	Reverse reaction rate for surface receptors with ligand in solution (s^{-1})
K_d	Fibrinogen-receptor dissociation constant (M)
k^{+}	Forward rate constant for fibrinogen binding with gpIIb-IIIa (s^{-1})
L_0	Concentration of Fg in solution (M)
N	Number of receptors in the contact area during a collision
P_a	Probability of aggregation
ϕ_i	Probability a receptor on cell i is occupied before the collision
ϕ_{fg_1}	Probability a pair of receptors on opposing cells have exactly one occupied and one free
ϕ_{r}	Probability a receptor lines up with a receptor on the opposing cell during a collision
p_b	Probability of a bond breaking before time t
p_f	Probability of a bond forming, given that the molecules are in apposition.
p_n	Probability of n cross-bridges at time t
s	Dimensionless separation distance
s_{coll}	Dimensionless separation distance which is considered a collision
T	Absolute temperature (K)
t_{c}	Contact time (s)
τ	Difference in activation times (s)
θ_p	Polar angle between colliding platelets
θ_a	Azimuthal angle between colliding platelets

2 Introduction

Hemostasis is the normal physiological response to blood vessel injury and is essential to maintaining the integrity of the vascular system. Hemostasis consists of two interacting processes, platelet aggregation and coagulation. The first involves cell-cell adhesion resulting in a platelet aggregate, and the second is an enzyme network that leads to the formation of a fibrin gel (clot) around the aggregating platelets. A malfunction of either process can lead to strokes, myocardial infarction, and other cardiovascular diseases. It is therefore important to understand these processes; this paper focuses on important aspects of platelet aggregation. Under normal conditions platelets passively circulate in the blood. In the event of damage to a vessel wall, chemicals are exposed which activate platelets causing them to stick to the injury site and to release chemicals, into the plasma, which can activate other platelets even if they do not directly contact the injury. Once activated platelets change shape from discoid objects to spiny spheres and express surface receptors which allow them to stick to one another to form a platelet aggregate.

Aggregating platelets are initially held together by the protein fibrinogen (Fg). Fibrinogen circulates in the blood at relatively high concentrations. Each platelet surface is covered with gpIIb-IIIa receptors which can bind fibrinogen, but only after the platelet is activated. Fibrinogen is an elongated, divalent molecule, possessing binding sites at each end. It acts to form a molecular bridge, holding two adjacent platelets together by binding each of its ends to gpIIb-IIIa receptors on different platelets. When two activated platelets collide, the probability that they will aggregate depends on the ability of fibrinogen bound on one cell to bind with a free receptor on the other cell. We call the probability that a collision between two isolated platelets results in the formation of fibrinogen cross-bridges the aggregation or capture probability.

The densities of unoccupied gpIIb-IIIa and bound fibrinogen on the surfaces of two colliding platelets play a role in determining the aggregation probability. After a platelet is activated, the receptors begin to bind fibrinogen at a rate which depends on the fibrinogen concentration. The densities of unoccupied and occupied receptors change with the time from activation, making the time since activation a factor in determining the probability that a collision between activated platelets results in the formation of a doublet. Previous mathematical models for the aggregation probability for platelets have not taken into account the effects of changing receptor occupancy (Potanin et al. 1993; Tandon and Diamond 1997).

Experimental studies of platelet aggregation show that the capture probability changes with the time from activation (Bell et al. 1989b; Goldsmith et al. 1994). Results from experiments with equal gpIIb-IIIa occupancy levels on colliding platelets (Bonnefoy et al. 2000; Xia and Frojmovic 1994) show that the ability of fibrinogen to form cross-bridges between colliding platelets is, in part, determined by the fraction of occupied receptors. These experiments measured the extent of aggregation at various times following the initiation of uniform shear flow and used these data to estimate the initial rate of aggregation. This rate therefore represents only the formation of aggregates and is not influenced by aggregate breakup. This motivated us to create a model of the aggregation probability which takes into account the densities of occupied and free receptors, which change as a function of time from activation, on the surfaces of the two colliding platelets.

Experimental measurements of the effect of receptor occupancy have only been done for the case when all platelets have the same fraction of occupied receptors. Platelets with different levels of occupied receptors result from their being activated at different times. As an aggregate forms on the arterial wall, new platelets continually arrive from upstream. In order for these new platelets to become part of the forming aggregate, they must interact with platelets which were activated some time earlier, and, as a result, have a different fraction of occupied receptors. Collisions between platelets at different stages of activation, and therefore with different levels of fibrinogen on their surfaces, could play an important role in the regulation of platelet aggregate formation.

We develop a mathematical model to predict the probability that a collision between two isolated, activated platelets results in cross-bridging by fibrinogen. The model accounts for the changing level of fibrinogen on the surfaces of the colliding platelets, as a function of the times from the activation of each of the platelets. The model predicts the probability of forming a doublet and considers the survival of the aggregate a separate event, consistent with the data from the experiments of Xia and Frojmovic (1994). We justify the assumption of separating formation and breaking by considering the time scales of these events, showing that doublet break-up occurs on a longer time scale than formation. We then use the model to explore what effect different activation times have on the aggregation process. Because the kinetics of the reaction between gpIIb-IIIa are determined, in part, by the concentration of fibrinogen, we make predictions based on the level of plasma fibrinogen. Finally we explore the effect that shear rate has on the aggregation probability.

Experiments testing the effects of receptor occupancy report the capture efficiency rather than

the capture probability (Bonnefoy et al. 2000; Xia and Frojmovic 1994). To understand the difference between these concepts, recall that Smoluchowski (1917) predicted the number of collisions for a dilute suspension of spherical particles under linear shear flow. He ignored the effects of hydrodynamic and colloidal forces on the motions of the particles. For a given particle, its collision cross-section is defined as an area upstream such that if another particle passes through that area, it will collide with the reference particle. Similarly the capture cross-section is defined as an area upstream such that if another particle passes through that area, it will collide and form a doublet with the reference particle. Smoluchowski assumed these areas are the same, meaning all collisions result in doublet formation. Using the Smoluchowski approximation, the collision cross-section is a circle of radius the sum of the radii of the colliding particles. The frequency of collisions is the flux through the collision cross-section. For equal-sized particles it is

$$J_s = \frac{32}{3} C \gamma a^3, \tag{1}$$

where C is the concentration of particles, γ is the shear rate, and a is the particle radius.

The capture efficiency, α, is defined as the ratio of the actual doublet formation rate to the collision rate predicted by Smoluchowski. Denoting the doublet formation rate by J_d,

$$\alpha = \frac{J_d}{J_s}. \tag{2}$$

Van de Ven and Mason (1977) compute the capture cross-section by integrating the velocity field forward in time from initial positions of large separation. These calculations take into account the effects of van der Waals and electrostatic forces on the particle motion. If the particles form a doublet, the initial position is part of the capture cross-section. Continuing this process for different initial positions, they determine the boundary of the capture-cross section and the value of J_d by computing the flux through this region. Zeichner and Schowalter (1977) compute the capture cross-section by integrating the velocity backwards in time from initial conditions corresponding to capture. This gives the capture cross-section and therefore the capture efficiency.

The capture efficiency is often misinterpreted as the capture probability, the fraction of collisions which result in the formation of a doublet. The capture efficiency is only equal to the capture probability provided that the collision frequency predicted by Smoluchowski is correct. Tandon and Diamond (1997) introduce the idea of hydrodynamic efficiency as a correction to the collision frequency. They define the hydrodynamic efficiency, ϵ_h, as the ratio of actual frequency of collisions

to the frequency predicted by Smoluchowski. The capture probability, P_a, is therefore related to the capture efficiency by

$$\alpha = \epsilon_h P_a. \tag{3}$$

The hydrodynamic efficiency can be interpreted as the fraction of collisions predicted by Smoluchowski which are actually collisions. One must be careful in defining the term collision. In this study we are concerned with the ability of fibrinogen to form cross-bridges. Fibrinogen bound to the surface of one platelet may be able to interact with receptors on another platelet at a distance larger than normally considered contact. Tandon and Diamond (1997) consider contact to occur at a distance 0.01 times the average radius of the colliding platelets. For equal sized platelets this value is roughly 14 nm. A fibrinogen molecule is approximately 50 nm long (Hantgan et al. 1994), which means that a collision could be defined as an encounter where two platelets come with 50 nm of each other. We explore this idea by computing the hydrodynamic efficiency as a function of the distance between platelets at which a collision is considered to occur.

Bell (1981) computes the capture probability for colliding cells cross-bridged by multivalent ligand. He assumes that the collision cross-section and collision frequency proposed by Smoluchowski are correct. From a colliding particle's initial position in the collision cross-section, he makes estimates of the number of bonds that will form and the number needed to overcome hydrodynamic forces acting to separate the cells after a collision. Based on these estimates, he assigns a probability of one to each initial position which will lead to stable cross-bridging. The capture probability is then given by the ratio of the flux through the region leading to successful cross-bridging to the total collision frequency predicted by Smoluchowski. These results do not take into account the correct hydrodynamics.

Tandon and Diamond (1997) modify Bell's method, but they include more detailed hydrodynamics and consider collisions between different sized particles. As mentioned previously, they draw the important connection between capture probability and capture efficiency by the introduction of the hydrodynamic efficiency. They do not consider the effects of differing levels of receptor occupancy in their model. The reaction between surface receptors and solution fibrinogen is taken to be in steady state. To model the changing number of receptors during neutrophil aggregation, Tandon and Diamond (1998) prescribed the number of available receptors as a piecewise linear function in time, which was determined from experimental data.

Long et al. (1999) compute the capture probability using a method similar to that of Bell, but with a different capture criterion. For each initial position in the collision cross-section, they compute the probability distribution for the number of cross bridges formed during the upcoming collision. This method assigns a probability between zero and one to each point in the collision cross-section. The capture probability is the flux, weighted by the probability measure, through the cross-section. They too ignore the detailed hydrodynamics.

Our model combines ideas from these previous studies. We use the idea of hydrodynamic efficiency from Tandon and Diamond, giving the relationship between capture efficiency and capture probability. We consider a variable collision distance to test possible long range effects due to the length of fibrinogen. We use a method similar to that of Long et al. (1999) for computing the probability distribution of the number of resulting cross-bridges, adapted to the case of two-way cross-bridging by a limited number of receptors on each cell. By considering the forces acting on the doublet and the time scales of formation and breaking, we show that bond breaking occurs on a longer time scale than bond formation. As a result, doublet formation and doublet survival can be considered two separate events, and we model only formation. The model takes into account the densities of occupied receptors on both cells at the time of collision. We use the model to test the effects of the time from activation, the fibrinogen concentration, and the shear rate on the probability of aggregation.

3 Hydrodynamic Efficiency

Consider two equal sized spherical particles. Let the undisturbed velocity field be $(\gamma y, 0, 0)$ in the absence of the spheres, where γ is the shear rate. Now let one particle center be at the origin and the other at (r, θ_p, θ_a), where θ_p is the polar angle and θ_a is the azimuthal angle. See Figure 1. The velocity of the second sphere relative to the first sphere was derived by Batchelor and Green (1972). In polar coordinates, it is

$$v_r = \frac{dr}{dt} = \gamma r(1 - \widehat{A}(r)) \sin^2 \theta_a \sin \theta_p \cos \theta_p + F_r \tag{4}$$

$$v_{\theta_p} = r \sin \theta_a \frac{d\theta_p}{dt} = \gamma r \left(\cos^2 \theta_p - \frac{\widehat{B}(r)}{2} \cos 2\theta_p \right) \sin \theta_a \tag{5}$$

$$v_{\theta_a} = r \frac{d\theta_a}{dt} = \gamma r(1 - \widehat{B}(r)) \sin \theta_a \cos \theta_a \sin \theta_p \cos \theta_p, \tag{6}$$

where $\widehat{A}$ and $\widehat{B}$ are dimensionless functions of the separation distance only, which we discuss later, and F_r represents forces acting in the radial direction, such as dispersion forces. We include only the van der Waals force in F_r. Other forces such as electrostatic forces and steric forces could also be included. Steric forces arise when two polymer coated spheres are in very close contact. Because the Debye length is on the order of 0.1 nm (Van de Ven 1989), electrostatic forces are short range. We therefore exclude these forces in our computation.

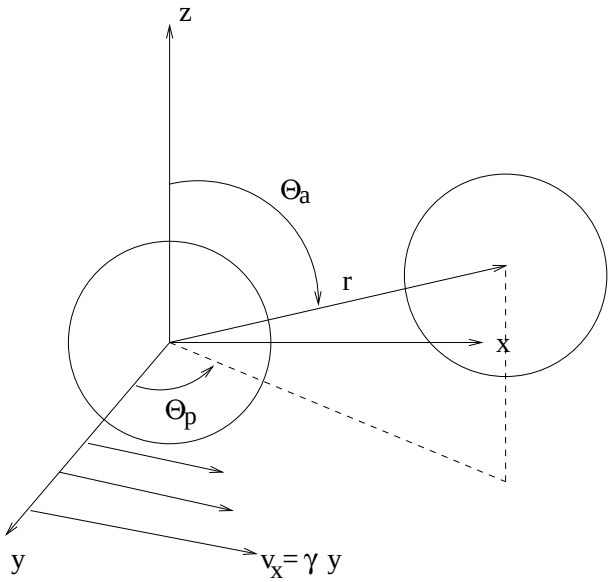


Figure 1: Two equal sized spherical particles in a linear shear flow, with one particle center at the origin. Note that the azimuthal angle is relative to the z-axis, which is the vorticity axis.

Using dimensionless variables defined by $s = r/a$ and $t^* = \gamma t$, where a is the radius of the particles, the equations (4)-(6) become

$$\frac{ds}{dt^*} = s(1 - A(s)) \sin^2 \theta_a \sin \theta_p \cos \theta_p + C(s) C_A F_A \tag{7}$$

$$\frac{d\theta_p}{dt^*} = \cos^2 \theta_p - \frac{B(s)}{2} \cos 2\theta_p \tag{8}$$

$$\frac{d\theta_a}{dt^*} = (1 - B(s)) \sin \theta_a \cos \theta_a \sin \theta_p \cos \theta_p. \tag{9}$$

Here, F_A is the dimensionless van der Waals force and $C(s)$ is a dimensionless function of the separation distance. The dimensionless parameter C_A is defined by

$$C_A = \frac{H}{36\pi\gamma\mu a^3}, \tag{10}$$

where H is the Hamaker coefficient, related to the magnitude of the van der Waals forces, and μ is the viscosity of the fluid. Various forms of the van der Waals force exist for two spheres. We use a form which is appropriate at both long and short distances:

$$F_A = \begin{cases} \dfrac{1+3.54p}{(s-2)^2(1+1.77p)^2} & \text{for } \; p<1 \\ \dfrac{1}{(s-2)^2}\left(\dfrac{0.98}{p}-\dfrac{0.434}{p^2}+\dfrac{0.0674}{p^3}\right) & \text{for } \; p\geq 1 \end{cases}, \tag{11}$$

where $p = 2\pi a(s-2)/\lambda$, and $\lambda = 100$ nm is the London wavelength (Van de Ven 1989).

Asymptotic forms of the functions $A(s), B(s)$, and $C(s)$ can be determined when the particles are extremely near each other or far apart (Batchelor and Green 1972). The midrange values can be approximated by matching with computational data. For our computations we take the forms given in (Tandon and Diamond 1997) for equal sized spheres. See Appendix A.

We designate the particle at the origin to be the reference particle. Define the collision cross-section as an area upstream such that if a second particle passes through this area, it will collide with the reference particle. The size of the collision cross-section depends on how close the particles must come for their interaction to be considered a collision. Call this distance $s_{\rm coll}$. We compute the collision cross-section as a function of $s_{\rm coll}$.

We choose to compute the collision cross-section by integrating forward in time rather than backward. We found that forward integration gives less numerical error. The computation is made by beginning the second particle at a location sufficiently far upstream so that its motion is negligibly affected by the presence of the other particle (we found the location $(-20, y^*, z^*)$ to suffice). For discrete points on the y^* axis, we begin with a test value of z^*. We integrate equations (7)-(9) forward in time until one of two events occurs: if $s < s_{\rm coll}$ for some time, the initial point is part of the collision cross-section, or if $\theta_p > 0$, the particles pass without colliding. The value z^* on the boundary of the collision cross-section is determined using a bisection algorithm and repeated integrations of the velocity equations. This procedure is repeated for different y^* values until the boundary of the collision cross-section in one quadrant is given as the curve $Z(y^*)$ in the y^*z^*-plane. The hydrodynamic efficiency is the ratio of the flux through this area to the flux through a quarter circle of twice the radius of the particles. The hydrodynamic efficiency is thus

$$\epsilon_h = \left(\int_0^2 y^*\sqrt{4-{y^*}^2}\; dy^*\right)^{-1}\left(\int_0^{Y^*} y^* Z(y^*)\; dy^*\right) = \frac{3}{8}\int_0^{Y^*} y^* z^*\; dy^*, \tag{12}$$

where Y^* is the point where the collision cross-section intersects the y^* axis. These flux integrals are of the form

$$\int_{\partial B} \mathbf{U} \cdot \mathbf{n} \, dS. \tag{13}$$

The dimensionless product $\mathbf{U} \cdot \mathbf{n}$ is simply y^* and the differential boundary element is $dS = Z(y^*) \, dy^*$.

4 Model Formulation

The aggregation probability is the probability that two colliding platelets form a doublet by forming fibrinogen cross-bridges. A successful collision is typically defined as one in which enough bonds form to overcome the hydrodynamic forces acting to separate the newly formed bonds (Bell 1981; Tandon and Diamond 1997). It is important to consider the time scale of bond rupture, relative to the time of the collision. If bonds break on a comparable time scale, then bond breaking must be accounted for in a model of capture probability. However if bond rupture occurs on a longer time scale, then it can be seen as an event separate from doublet formation. We present a model of this case, and therefore we define a successful collision as one in which at least one bond forms.

In order for fibrinogen to form a cross-bridge between two colliding platelets, a fibrinogen molecule bound to one cell must contact an available receptor on the other cell and bind. The time scales to consider in this process are the collision time, the reaction rate between solution ligand and surface receptors, the appropriate time scale for Brownian motion of surface receptors, the forward binding rate for the binding of singly bound fibrinogen to a nearby unoccupied receptor, and the rate of bond rupture. We compare the various time scales involved to determine what to include in our model.

Consider a collision between two isolated, spherical platelets. The local flow can be considered a linear shear flow disturbed by two particles. The mean contact time, $\langle t_c \rangle$, for two colliding, rigid spheres in linear shear flow of shear rate G is approximately (Bartok and Mason 1957):

$$\langle t_c \rangle = \frac{5\pi}{6} \gamma^{-1}. \tag{14}$$

Note that this average is computed assuming that particles follow straight line trajectories until the point of contact. We only use this value to estimate the order of magnitude of the contact time.

We compare our model with an experiment with a shear rate of 300 s^{-1} (Xia and Frojmovic 1994), and so the mean contact time is on the order of milliseconds.

4.1 Binding

The distance on the surface of the platelet that a gpIIb-IIIa complex travels by diffusion during contact is approximately $\sqrt{Dt_c}$, where D is the diffusion coefficient of gpIIb-IIIa in the platelet membrane and t_c is the contact time. The diffusion coefficient for gpIIb-IIIa, measured on the surface of megakaryocytes (platelet precursors), is on the order of 10^{-10} $cm^2 s^{-1}$ (Schootemeijer et al. 1997). The diffusivity on platelets may be lower due to interactions between the receptors and the platelet cytoskeleton. During contact the receptors do not diffuse more that a few nanometers, which is less than the size of the receptor head (Weisel et al. 1992). Therefore we ignore the diffusion of the receptors on the surface during the contact time. This is not to say that the receptor is perfectly stationary. Even if the base of the receptor is fixed in the platelet membrane, the receptor head may be constantly re-orienting due to thermal motion. We adsorb this reorientation into the forward rate constant.

The rate constant for the forward reaction between solution fibrinogen and surface gpIIb-IIIa is much faster than the reverse rate at physiological levels of fibrinogen. The time scale for the forward reaction is on the order of $(k_f L_0)^{-1}$, where k_f is the second order forward rate constant and L_0 is the fibrinogen concentration. This time is at least on the order of a tenth of a second, which is an order of magnitude greater than the collision time. When the molecules are in the proper orientation to bind, the reaction is extremely fast (Bell 1978). Therefore the reaction between surface receptors and solution fibrinogen occurs on a longer time scale than the collisions, and binding between properly aligned molecules is much faster. We therefore ignore the progression of the reaction between solution Fg and gpIIb-IIIa during contact, and focus on the probability that free receptors on one cell align with Fg bound to the opposing cell and bind.

4.2 Bond Rupture

Bond rupture is a stochastic event and can happen anytime after the formation of the bond. Ignoring any applied forces, the probability a bond breaks during a short interval is $k_r \Delta t$. Define $p_b(t)$ to be the probability that the bond has broken by time t. The change in probability over a

short interval of length Δt is the probability that the bond has not yet broken, $1 - p_b$, times the probability that it breaks $k_r \Delta t$. Taking the limit as $\Delta t \to 0$ gives,

$$\frac{dp_b}{dt} = k_r \left(1 - p_b\right); \quad p_b(0) = 0. \tag{15}$$

Note that the dissociation rate, k_r, is the inverse of the mean bond lifetime.

When an external force is applied to the bond, the dissociation rate is no longer constant. Bell (1978) introduced the exponential model

$$k_r(f) = k_r^0 \exp\left(bf/k_B T\right) = k_r^0 \exp\left(cf\right), \tag{16}$$

where f is the applied force, k_B is the Boltzmann constant, T is the absolute temperature, and b characterizes the length of the energy well of the bound state. Other forms of the force dependence on the breaking rate have been proposed (Dembo et al. 1988; Evans et al. 1991; Evans and Ritchie 1997; Piper et al. 1998). Our goal here is to determine only whether bond breaking is important on the time scale of the collision. We are not concerned with the exact form of the breaking rate's dependence on the applied force. We restrict our discussion to the exponential model because the sensitivity to force is contained in just one unknown parameter, c, which we need to estimate.

During the collision, the force acting on the bonds changes as the particles move relative to each other. Tha and Goldsmith (1986) derive expressions for the fluid force acting on two equal-sized spherical particles as a function of their size, their relative positions, the viscosity of the fluid, and the shear rate. For platelets of radius 1.4 μm, a viscosity of 1.3 cP, and a shear rate of 300 s^{-1} the maximum magnitude of the force on a bond is about 14.7 pN, and the mean, taken over all configurations, is 5.3 pN, ignoring compression. See Appendix B.

To estimate the importance of bond rupture, we consider a single bond under constant force for a time on the order of the contact time. Combining equations (15) and (16) under these assumptions, the probability of breaking is

$$p_b = 1 - \exp\left(-k_r^0 \exp\left(cf\right) t_c\right). \tag{17}$$

The unknown parameters are k_r^0 and c. To explore whether breaking is relevant to our model, we prescribe a force and contact time, and determine ranges of k_r^0 and c for which the probability of breaking is less than 0.01. Figure 2 shows k_r^0 vs. c for a contact time of 10 ms and an applied force of 15 pN. This force is the maximum force a bond would experience.

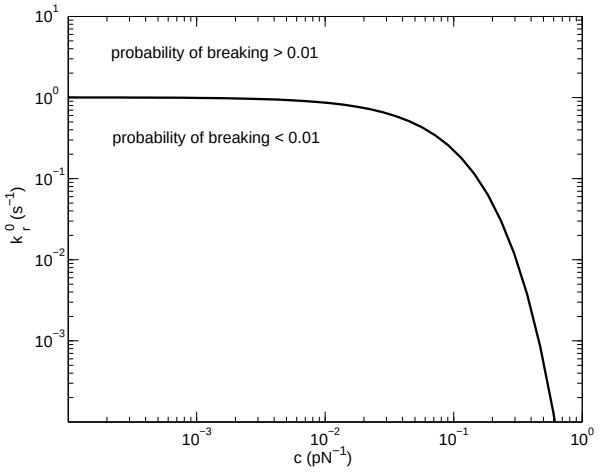


Figure 2: Ranges of k_r^0 and c for which the probability of a single bond breaking in less than 10 ms is less than 0.01 under a constant load of 15 pN.

The value of k_r^0 was determined by Huber et al. (1995) to be $4 \cdot 10^{-2}$ s^{-1}. Goldsmith et al. (2000), in a simulation of the breakup of doublets linked by gpIIb-IIIa-fibrinogen cross-bridges, estimates that the value of c should be less than 0.1 pN^{-1}. A second estimate of the value of c can be obtained from an experiment by Weisel (personal communication) to determine the breaking characteristics of a single bond between Fg and gpIIb-IIIa using optical tweezers. That value of c is close to the value estimated by Goldsmith et al. (2000). Comparing these estimates with Figure 2, suggests that the values of k_r^0 and c are well within the range that bond rupture can be ignored in our model of capture probability.

4.3 Aggregation Probability

For a given receptor on one cell to become part of a cross-bridge, three events must occur: (1) There must be a receptor located on the opposing cell surface in the same location, (2) One of the two receptors must be free and the other must be occupied by a Fg molecule, (3) The free receptor must bind with the free end of the Fg. Define ϕ_{r} and $\phi_{\mathrm{fg_1}}$ as the success probabilities of the first two events. Let $p_f(t)$ denote the probability a receptor and ligand have formed a bond by time t, where time zero is the time the molecules came into apposition. Assuming that receptors act independently of each other [1], the probability a given receptor forms a cross-bridge at time

[1] This assumption does not hold when bond rupture depends on the applied force.

t into the collision is the product $\phi_{\mathrm{r}}\phi_{\mathrm{fg_1}}p_f(t)$. Let p_n be the probability that n receptors form cross-bridges, and denote the total number of receptors on one cell in the contact area by N. The probability p_n satisfies the binomial distribution

$$p_n = \binom{N}{n} \left(\phi_{\mathrm{r}}\phi_{\mathrm{fg_1}}p_f\right)^n \left(1 - \phi_{\mathrm{r}}\phi_{\mathrm{fg_1}}p_f\right)^{N-n}. \tag{18}$$

Knowledge of the individual probabilities in the previous equation gives the distribution for the number of bonds, and therefore the probability of forming a doublet. We now discuss the expressions for these individual probabilities. Note that a common derivation for the probability distribution for the number of bonds involves the master equations (Chesla et al. 1998; Cozens-Roberts et al. 1990; Long et al. 1999; Piper et al. 1998). Our results could also be derived from master equations. See Appendix C.

Define $k^+\Delta t$ to be the probability a receptor and ligand form a bond in time Δt, given that they are in the vicinity of one another. Note that this rate constant includes local reorientation required for binding. Ignoring bond rupture, $p_f(t)$ satisfies

$$\frac{dp_f}{dt} = k^+(1 - p_f); \qquad p_f(0) = 0, \tag{19}$$

with solution

$$p_f(t) = 1 - \exp\left(k^+ t\right). \tag{20}$$

For short contact times $p_f(t) \approx k^+ t$.

The value of ϕ_r depends on the density of receptors in the contact area, which we assume is constant. The value of $\phi_{\mathrm{fg_1}}$ depends on the density of bound fibrinogen on the surface of each colliding platelet. As discussed previously, we assume that these densities are constant during the collision, but depend on the length of time from activation. Let ϕ_1 and ϕ_2 be the probabilities that a receptor is occupied just before a collision for each of the two cells. The probability that a pair of aligned receptors contains exactly one fibrinogen molecule is

$$\phi_{\mathrm{fg_1}} = \phi_1(1 - \phi_2) + \phi_2(1 - \phi_1). \tag{21}$$

The individual probabilities ϕ_1 and ϕ_2 are computed as follows.

Consider the cells in a solution in which the concentration of solution Fg, L_0, is sufficiently high so that reactions with individual cells do not change the concentration of the solution Fg.

The number of receptors on a cell is finite and fixed. Let k_f and k_r be constants such that the probability a free receptor becomes occupied in time Δt is $L_0 k_f \Delta t$ and the probability an occupied receptor becomes free is $k_r \Delta t$.

By the same reasoning used for equations (15) and (19), ϕ_i satisfies

$$\frac{d\phi_i}{dt} = k_f L_0 (1 - \phi_i) - k_r \phi_i; \qquad \phi_i(0) = 0, \tag{22}$$

where time zero is the time of activation. The solution is

$$\phi_i(t) = \left(\frac{k_f L_0}{k_r + k_f L_0} \right) \left[1 - \exp(-(k_r + k_f L_0)t) \right], \quad \text{for } i = 1, 2. \tag{23}$$

This equation can be rewritten as

$$\phi_i(t) = \left(\frac{L_0}{K_d + L_0} \right) \left[1 - \exp(-(K_d + L_0)k_f t) \right], \quad \text{for } i = 1, 2, \tag{24}$$

where $K_d = k_r / k_f$ is the dissociation constant. Note that for a fixed K_d value, the value of k_f only affects the time scale of the reaction. Using equations (21) and (24), the probability ϕ_{fg_1} can be obtained as a function of the activation times of the two colliding platelets. The bond dissociation is considered here because a platelet may have been activated for any length of time before the collision. Therefore this time scale may be much longer than the collision time.

Because we assumed that bond rupture occurs on a time scale longer than the scale of collisions, the probability a colliding pair of platelets forms a doublet, the aggregation probability, is given by the probability that they form at least one bond during the collision. The aggregation probability, P_a, is given by

$$P_a = 1 - p_0 = 1 - (1 - \phi_{\mathrm{r}} \phi_{\mathrm{fg}_1} p_f(t_c))^N \approx 1 - (1 - \phi_{\mathrm{r}} \phi_{\mathrm{fg}_1} k^+ t_c)^N \tag{25}$$

5 Comparison with experiment

Xia and Frojmovic (1994) report the capture efficiency as a function of the fraction of occupied receptors at a shear rate of $\gamma = 300\mathrm{s}^{-1}$. In order to obtain the capture efficiency, activated, fixed platelets were subjected to uniform shear using a micro-couette device. Samples of the suspension were removed at various points in time, immediately fixed, and the concentration of single platelets was measured. From fitting the data and differentiating, the initial rate of aggregation was obtained.

Because the suspension consisted of primarily singlets at the beginning of the experiment, the rate of doublet formation, J_d, at time zero equaled the rate of aggregation. The capture efficiency is then given by equation (2). Note that the reported capture efficiency is taken at time zero. As argued previously, doublet breakup occurs on a longer scale than doublet formation, and so bond breaking should have little effect on this measurement of capture efficiency.

The colliding platelets had the same fraction of occupied receptors. Let $\phi_1 = \phi_2 = x$ be the fraction of occupied receptors, and so $\phi_{\mathrm{fg}_1} = 2x - 2x^2$. Combining equations (3) and (25), the capture efficiency, α, is

$$\alpha = \epsilon_h \left(1 - (1 - 2\beta(x - x^2))^N\right), \tag{26}$$

where $\beta = k^+ t_c \phi_r$. The individual values of k^+ and ϕ_r cannot be determined by data fitting, which is why we use the parameter β.

Each platelet contains about 50,000 gpIIb-IIIa complexes (Charo et al. 1994). A fibrinogen molecule is approximately 50 nm long (Hantgan et al. 1994). Assuming that this is the maximum possible length of a cross-bridge between platelets and that each platelet has a radius on the order of 1μm, the surface area available for binding is a spherical cap which represents 1.25% of the total surface area. Therefore there are approximately 625 gpIIb-IIIa molecules on each platelet in the contact area.

Using the value of $N = 625$ and fitting equation (26) to the data in (Xia and Frojmovic 1994), gives $\epsilon_h = 0.2429$ and $\beta = 0.0187$.[2] The value we obtain for the hydrodynamic efficiency is consistent with the prediction of Tandon and Diamond, who compute $\epsilon_h = 0.226$ at a shear rate of 335 s^{-1} by a numerical simulation of particle paths. A plot of equation (26) with the data is shown in Figure 3. Note that when the receptors are between 20% and 80% occupied, the capture efficiency is almost identically equal to the hydrodynamic efficiency. Because the capture efficiency is the capture probability scaled by the hydrodynamic efficiency, the capture probability is very near one in this interval.

[2] We also tried both lower and higher values of N and found that this has the effect of changing only the value of β, leaving ϵ_h unchanged, while giving an identical fit to the data. Therefore our results are not sensitive to the choice of N.

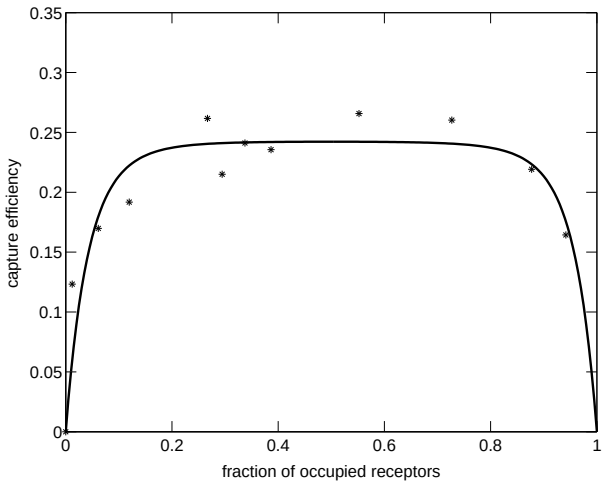


Figure 3: Fit of equation (26) to data from Xia and Frojmovic (1994).

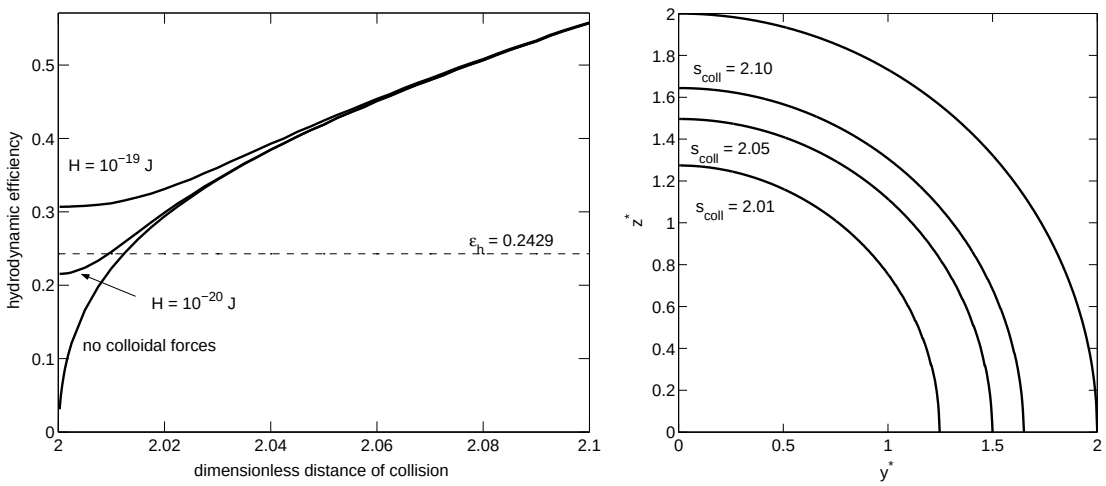
6 Results

6.1 Hydrodynamic Efficiency

We computed the hydrodynamic efficiency as a function of the separation distance at which a collision is defined to occur. The value of the dimensionless separation $s = 2$ corresponds to direct contact. We carried the computation out to $s = 2.1$, or one tenth of the radius. We made this computation for several values of the Hamaker coefficient, to explore how strongly the van der Waals forces influence the results. Comparing the hydrodynamic efficiency obtained from the data fit, $\epsilon_h = 0.2429$, to the graph in Figure 4 shows that this value corresponds to a collision distance around 2.01. It appears that platelets must come within one one-hundredth of a radius, about 14 nm, to be able to cross-bridge. The hydrodynamic efficiency is a function of the shear rate when colloidal forces are considered. Note that for small Hamaker coefficients or high shear rates (not shown), the values of hydrodynamic efficiency are similar to the values obtained by ignoring colloidal forces. We computed that $\epsilon_h = 1$ at collision distance of 2.31, meaning the capture efficiency equals the capture probability if collisions can occur even when the particles are about one third of a radius apart.

Also displayed in Figure 4 are one quadrant of the collision cross-sections for various values of the collision distance. Smoluchowski (1917) predicted a circular cross-section of twice the radius, by ignoring hydrodynamic and colloidal interactions. Note that the collision cross-sections we compute

are almost circular (the maximum z^* value is slightly larger than the maximum y^* value). The asymmetry is more pronounced for larger Hamaker coefficients and lower shear rates.



(a) (b)

Figure 4: (a) Hydrodynamic efficiency as a function of the distance which is considered a collision. $\gamma = 300$ s^{-1}, $\mu = 1.32$ cP, $a = 1.42$ μm. (b) The collision cross-section in one quadrant. The outermost curve is the capture cross-section predicted by Smoluchowski. $H = 10^{-20}$ J.

6.2 Time From Activation

The probability that a single receptor is occupied as a function of the time from activation is given in equation (24). The normal range of fibrinogen concentration is 2 - 4 g/L. We take $L_0 = 8.8$ μM, which corresponds to 3 g/L. The accepted value of the dissociation constant, $K_d = k_f/k_r$, is between 100 nM and 200 nM, and we use $K_d = 150$ nM. Huber et al. (1995) observed a fast forward rate of $3 \cdot 10^5$ (M s)$^{-1}$ upon first exposure of gpIIb-IIIa to fibrinogen, and a slower forward rate of $2 \cdot 10^4$ (M s)$^{-1}$ upon subsequent exposures. The slower value has been observed by others using a different technique (Muller et al. 1993; Erb et al. 1997). The forward rate *in vivo* has not been reported, but the forward rate is presumably in the range $2 \cdot 10^4$ - $3 \cdot 10^5$ (M s)$^{-1}$. We report our results for both the faster and slower rate. In Figures 5 – 8, times that appear in parentheses correspond to the slower rate.

6.2.1 Cells Activated at the Same Time

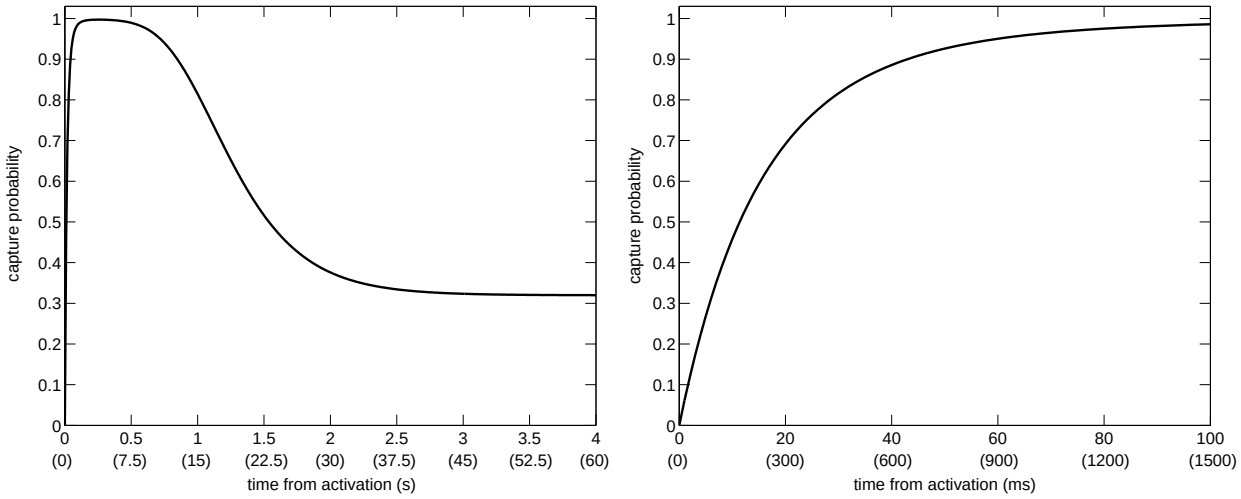
The capture probability as a function of time from activation for two cells activated simultaneously is shown in Figure 5. The capture probability quickly approaches its maximum value near one, and remains near one for some time before declining toward equilibrium. The rise and decline of the capture probability in Figure 5 correspond to receptor occupancy levels increasing to 20% and exceeding 80% respectively, as shown in Figure 3. The rise time is extremely fast because the reaction between solution fibrinogen and surface receptors proceeds quickly when the fraction of occupied receptors is low. The decline is much slower because the reaction is nearing equilibrium. Note that the equilibrium probability is nonzero because, as seen from equation (24), the fraction of occupied receptors approaches $L_0/(L_0 + K_d)$, which is less that one.

These results suggest that platelets activated at the same time have initially a very high probability to aggregate, but as time goes on and they move downstream, this probability becomes much lower. The quick rise to a maximum level of receptor occupancy may help platelets aggregate very soon after activation, presumably near an injury site. The much lower equilibrium probability ensures that platelets which are activated together, but which do not participate in aggregation, will nearly saturate their available receptors making them much less likely to aggregate downstream, away from the forming aggregate.

6.2.2 Cells Activated at Different Times

We are not aware of any previous experimental measurements or theoretical predictions concerning the probability of aggregation if the two colliding platelets have different levels of fibrinogen on their surfaces. Platelets with varying levels of occupied receptors result from their being activated at different times. As platelets from upstream arrive at the site of a forming aggregate, it is likely that recently activated platelets must collide with platelets which were activated some time ago and are already part of the wall-bound aggregate. Using our model, a difference in activation times can be simulated by letting the fraction of occupied receptors on one cell be $\phi_1(t)$ and on the other cell be $\phi_2(t + \tau)$, where ϕ_i is given in equation (24) and τ represents the difference in activation times. The capture probability as a function of the time from the activation of the cell activated most recently is displayed in Figure (6).

Differences in activation time have little impact on the capture probability on long time scales.



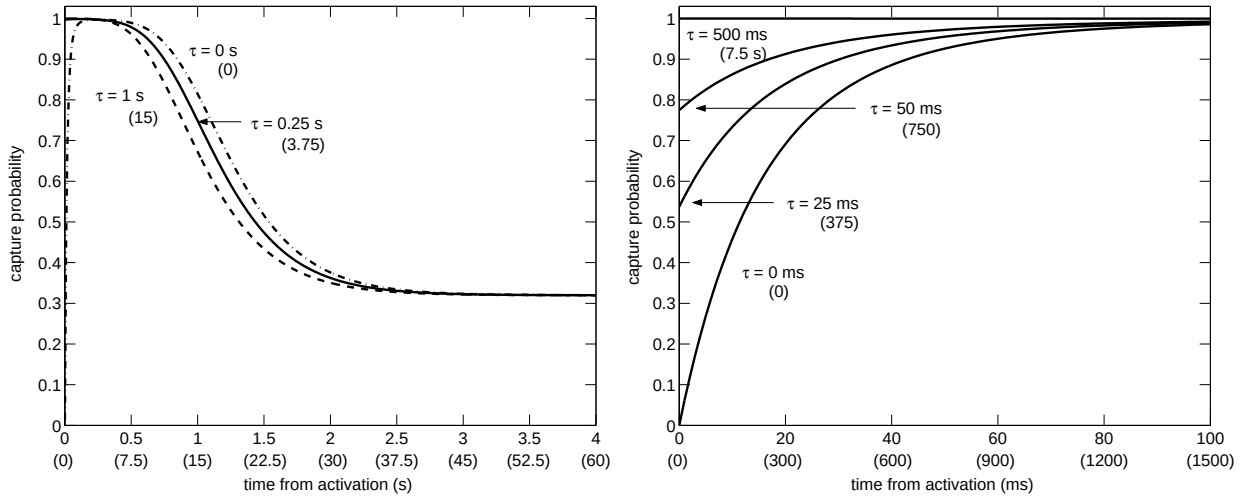
(a) (b)

Figure 5: Capture probability as a function of time for two cells activated at the same time. Times not in parentheses correspond to $k_f = 3 \cdot 10^5$ $(\mathrm{M\ s})^{-1}$, and times in parentheses correspond to $k_f = 2 \cdot 10^4$ $(\mathrm{M\ s})^{-1}$. (a) Long time scale shows initial rise, plateau, and decline toward equilibrium. (b) Short time scale shows the sharp rise upon activation.

The most significant difference is seen within a short time from activation of the second platelet. A platelet which has just been activated is much more likely to aggregate with a platelet activated some time ago than with another newly activated platelet. This result is not surprising because one colliding platelet is covered by fibrinogen available for cross-bridging and the other is covered by available receptors. As the difference in activation time increases, the initial probability approaches one, but there is little difference as the difference increases further, because the earlier activated cell is near equilibrium with solution Fg.

6.3 Varying Fg concentration

The fibrinogen concentration appears in both the time constant and equilibrium value for the probability that a surface receptor is occupied, given in equation (24). The capture probability as a function of the time from activation for two cells activated simultaneously for various fibrinogen concentrations is shown in Figure 7.



(a) (b)

Figure 6: Capture probability for two cells activated at different times. Time zero is the time from activation of the cell activated second. The difference in activation times is represented by τ. Times not in parentheses correspond to $k_f = 3 \cdot 10^5$ (M s)$^{-1}$, and times in parentheses correspond to $k_f = 2 \cdot 10^4$ (M s)$^{-1}$. (a) Different activation times have little affect on the long time scale. (b) For short times from activation, the difference in activation time has a significant effect.

Lower than normal fibrinogen concentrations delay cells reaching their optimum level of receptor occupancy for aggregation. Also surface receptors do not saturate with fibrinogen, and so the capture probability remains high for collisions between cells which have been activated for long periods of time. Higher than normal fibrinogen concentrations have the opposite effects. Activated platelets reach their optimum level of surface receptor occupancy quickly. Available surface receptors reach an effective equilibrium more quickly, in which almost all of the receptors are occupied. Collisions between platelets which have been activated for some time have a very low probability of successful binding.

6.4 Higher Shear Rate

An increase in shear rate has two effects on the probability of adhesion. The contact time decreases and the fluid force exerted on the potential doublet increases. Previous studies of capture

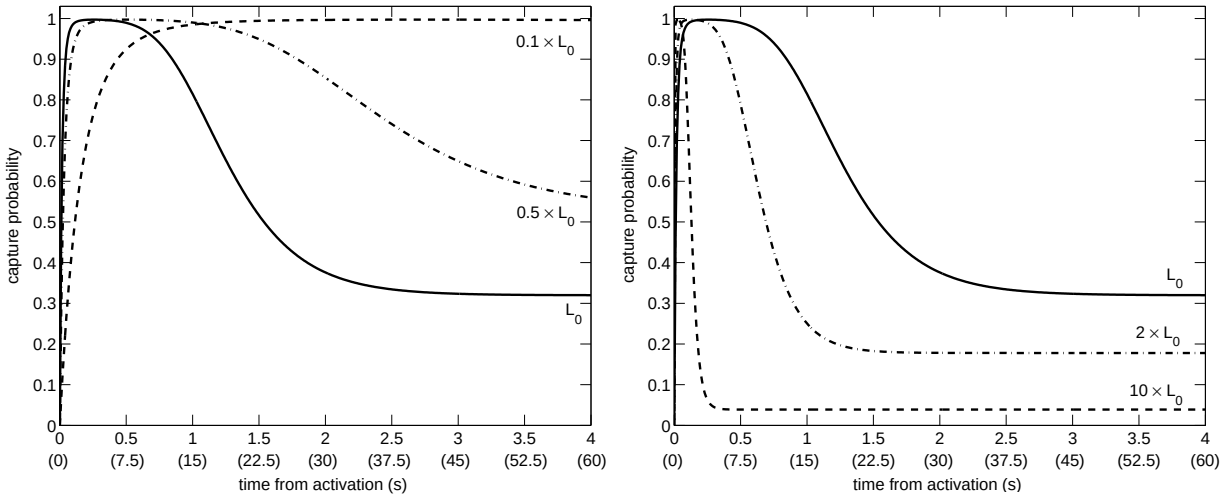


Figure 7: Capture probability for two cells activated at the same time for various concentrations of fibrinogen. $L_0 = 8.8\mu M$, γ =300 s^{-1}. Times not in parentheses correspond to $k_f = 3 \cdot 10^5$ (M s)$^{-1}$, and times in parentheses correspond to $k_f = 2 \cdot 10^4$ (M s)$^{-1}$. (a) Fibrinogen concentrations below normal. (b) Fibrinogen concentrations above normal.

probability required that the number of bonds needed for aggregation increase for higher shear (Bell 1981; Tandon and Diamond 1997). Because we are only modeling the probability of doublet formation and not doublet survival, the increased hydrodynamic force has little effect at the time of formation.

The mean encounter time is inversely proportional to the shear rate (Goldsmith and Mason 1967). Our parameter β is also inversely proportional to the shear rate, since it is proportional to the encounter time. The effects of different shear rates are explored by varying this parameter. The capture probability as a function of the fraction of occupied receptors (same on both cells) is displayed in Figure 8(a). As the shear rate increases, the maximum capture probability decreases, and the range of receptor occupancy levels near the maximum decreases. The capture probability as a function of time from activation is shown in Figure 8(b). At higher shear rates, the time to reach the maximum probability is slightly increased, while the time spent near the maximum and the equilibrium probability are decreased. The capture probability, for fixed receptor occupancy

levels, as a function of shear is shown in Figure 8(c). As the shear rate increases from low to high, the capture probability remains relatively constant for a range of shear rates before beginning to decline. The shear rate at which this decline begins depends on the fraction of occupied receptors.

7 Discussion

We have created a model to predict the probability that two colliding platelets form a doublet held together by fibrinogen cross-bridges. We refer to this probability as the aggregation or capture probability. The aggregation probability depends on the number of unoccupied gpIIb-IIIa complexes and bound fibrinogen on the surface of each platelet. After activation, the fraction of gpIIb-IIIa complexes occupied by fibrinogen changes as a function of time, and in turn the aggregation probability depends on the activation times of the colliding platelets. The forward reaction is relatively fast, but it is slow enough that collisions between platelets not yet in equilibrium with solution fibrinogen are of interest.

We estimated the importance of bond rupture on the time scale of doublet formation, taking into account the applied force on cross-bridges. At a shear rate of 300 s^{-1} in a fluid with the viscosity of plasma, the maximum force on a bond is on the order of 15 pN. The mean contact time is on the order of 10 ms. Using an exponential model for the force dependence of the breaking rate, we showed that a single bond under a constant force of 15 pN for 10 ms breaks with probability less than 0.01. Because a single bond, loaded by the maximal force a cross-bridge would experience, is unlikely to break, we conclude that it is reasonable to exclude bond rupture from our model of aggregation probability.

Experimental results exist for capture efficiency rather than capture probability. The two quantities are often equated, which would be correct only if the collision frequency predicted by Smoluchowski were correct. Tandon and Diamond (1997), using a numerical solution for the trajectories of colliding spheres, compute a correction to the capture efficiency, which they term hydrodynamic efficiency. An interpretation of the hydrodynamic efficiency is the fraction of collisions predicted by Smoluchowski which are actually collisions. This interpretation depends on the definition of a collision. In this study, we compute the hydrodynamic efficiency as a function of the inter-platelet distance which is considered a collision.

The capture efficiency as a function of occupied receptor fraction was observed to plateau at its maximum value for a large range of receptor occupancy levels (Bonnefoy et al. 2000; Xia and Frojmovic 1994). When the capture efficiency is equal to the hydrodynamic efficiency, the capture probability is equal to one. Therefore, the observed plateau of capture efficiency corresponds to a wide range of receptor occupancy levels which provide for capture probability near one. Our model for capture probability shows excellent agreement with these experimental results. In addition, the data fit yields a value for hydrodynamic efficiency which is consistent with the theoretical value for short range interaction.

For collisions between equal-sized spherical platelets at a shear rate of 300 s^{-1} and collision distances around the length of fibrinogen ($\approx$50 nm), the hydrodynamic efficiency is much higher than we obtained from fitting to data. The value we obtain corresponds to a collision distance around 14 nm, meaning the extension of fibrinogen from the surface is not a significant factor in increasing the capture probability. Moon et al. (1990) visualized platelet aggregates by electron microscopy. They observed fibrinogen molecules bound to the surface of single platelets, i.e., not participating in cross-bridging, extended 7 to 9 nm from the surface of the platelets. Also using electron microscopy, Weisel et al. (1992) visualized gpIIb-IIIa, removed from the platelet surface, binding to fibrinogen. They found that the angle between the heads of gpIIb-IIIa and fibrinogen to be approximately 98°, measured with respect to the long axis of fibrinogen. Assuming the same binding angle on the platelet surface, and a fibrinogen length of approximately 50 nm, fibrinogen bound at one end will extend just 7 nm above the surface of the platelet. Our value of hydrodynamic efficiency obtained from data fitting provides further evidence that the length of fibrinogen does not extend the collision radius of the platelet.

Letting the fraction of occupied receptors be a function of time, we make predictions for the aggregation probability as a function of time from the activation of the platelets. The time dependence of capture probability may play an important role in regulating the location of aggregation in the blood stream. According to our results, platelets are more likely to aggregate relatively soon after activation. At normal to high physiological levels of fibrinogen, platelets activated simultaneously significantly decrease their probability of cross-bridging with each other as most of their fibrinogen receptors become occupied. This appears to be a way of ensuring that platelets activated near a forming aggregate, but not participating in it, do not aggregate in the wrong place in the

circulatory system.

In the vicinity of a forming aggregate, collisions between platelets activated at different times are likely to occur. We use our model to assess the effect heterogeneous receptor occupancy levels have on the capture probability, by considering differences in activation times. The most striking effect is seen for newly activated platelets. Upon activation, the collisions most likely to result in a successful cohesion are those with another platelet which has been activated for some time. If platelets become activated near and collide with a forming aggregate, they have a high probability of joining it.

The rate of reaction between fibrinogen and gpIIb-IIIa complexes depends on the concentration of fibrinogen in the blood. Similarly, the steady state level of occupied receptors is a function of fibrinogen concentration. Our results predict that at low levels of fibrinogen, the available receptors do not saturate, which in turn implies that the capture probability does not decrease as time increases. In this case, the maximal probability is reached well after activation, so that platelets activated simultaneously have a higher probability of aggregating downstream rather than near the location of the aggregate. These results suggest that high concentrations of fibrinogen is a way to ensure that the peak probability is reached quickly following activation and that the receptors effectively saturate, preventing aggregation downstream.

Our model suggests that higher than normal fibrinogen concentrations do a better job of regulating the time and location of aggregation. On the other hand, high levels of fibrinogen in the blood are believed to be responsible for an increased risk of cardiovascular disease (Ernst and Resch 1993; Wilhelmsen et al. 1984). Cross-bridging activated platelets is not the only role of fibrinogen. For example, fibrinogen, cleaved by the enzyme thrombin into fibrin monomer, plays an essential role in coagulation. Together these results suggest that the body may regulate the level of fibrinogen to be sufficiently high to enusre proper aggregation, but not so high to cause medical problems associated with elevated levels of plasma fibrinogen. Platelets contain fibrinogen in their alpha granules, as well as other potential cross-bridging ligands, which is released into the plasma during activation (Walsh 1994). Thus, one of the purposes of secretion may be to increase the local concentration of fibrinogen at the time of activation. This would help a platelet reach its peak aggregation probability and later saturate its surface receptors quickly, without the danger of a high plasma fibrinogen concentration.

The capture efficiency as a function of shear rate was explored experimentally by Xia and Frojmovic (1994). They determine that as γ increases, the capture efficiency is inversely proportional to γ. Our model is consistent with this observation. Expanding equation (26),

$$\alpha = \epsilon_h \left(1 - (1 - C\gamma^{-1})^N\right) = \epsilon_h CN\gamma^{-1} + O(\gamma^{-2}), \tag{27}$$

where C is a constant which depends on the occupancy level and rate constants of fibrinogen binding. If colloidal forces are small, ϵ_h can be considered constant also. Therefore as $\gamma^{-1} \to 0$, our model also predicts that α is inversely proportional to the shear rate. We note that there is growing evidence (Frojmovic et al. 1997) that von Willebrand factor, rather than fibrinogen, is the dominant platelet cross-bridging molecule at high shear rates (above 1000 s^{-1}). Since our model and the experiments of Xia and Frojmovic (1994) involve only fibrinogen mediated cross-bridging, their results are not necessarily indicative of the capture probability at high shear rates.

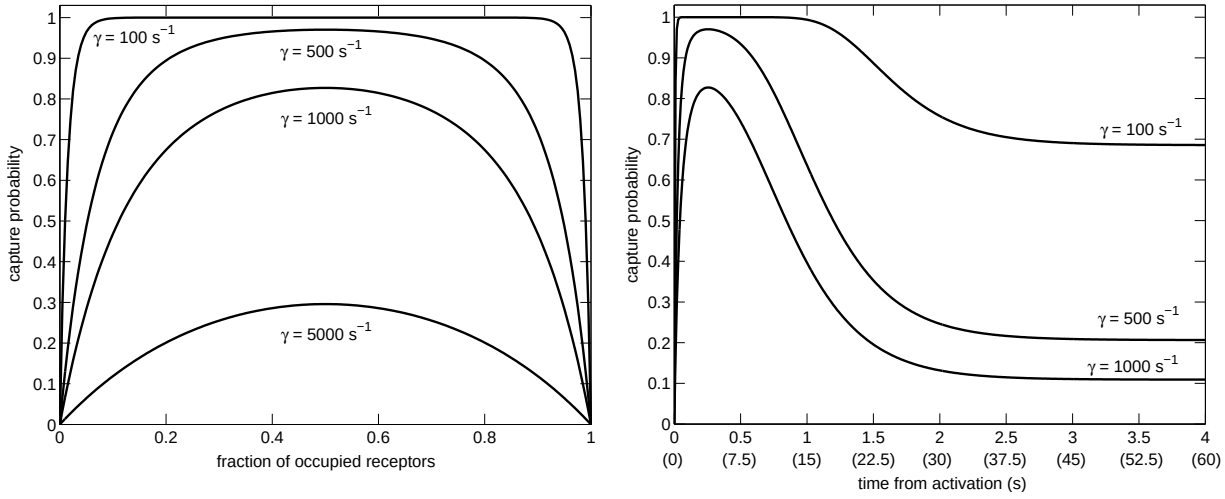
Several experiments report the capture efficiency as a function of time (Bell et al. 1989a; Bell et al. 1989b; Goldsmith et al. 1994). We attempted to compare our model results with the experimental results from these studies. The capture efficiencies reported in these papers were computed by measuring the number of single platelets remaining after some time interval during which the platelets were exposed to shear. During this time aggregates of various sizes may form and aggregates may break apart, and these events would affect the measured value of capture efficiency. Our model only considers collisions between individual platelets on a time scale much shorter that that of breakup. Therefore we were unable to compare our model with the data reported in these studies.

The capture efficiency is predicted theoretically by Potanin et al. (1993), using a force balance between fluid, colloidal, and biological forces. They predict a plateau region where the capture efficiency is relatively constant for shear rates between 10 and 1000 s^{-1}. According to our model, the peak capture probability is near one for a wide range of low to moderate shear rates, and so the capture efficiency should be nearly constant for those shear rates as well. Our model also predicts a plateau for low to moderate shear rates. The capture efficiency is plotted against shear rate in Figure 8(c) for two different fractions of occupied receptors, $\phi = 0.5$ and $\phi = 0.98$. The range of shear rates for which capture efficiency is essentially constant decreases as the fraction of occupied receptors deviates from 0.5. High shear rate has the effect of decreasing the range of receptor occupancy levels which provide for maximal capture probability, as shown in Figure 8(a).

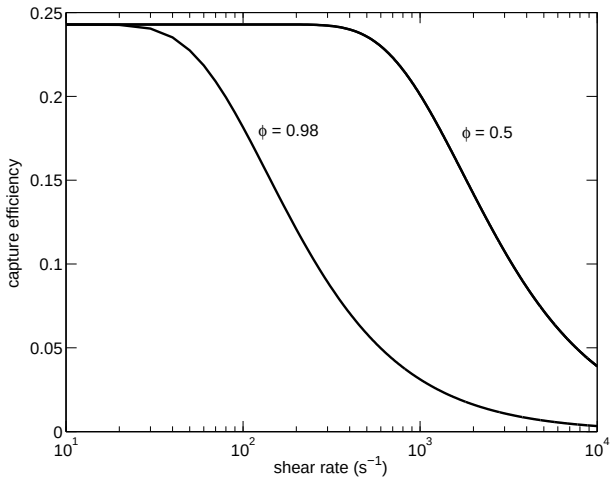
When the time of the reaction with solution fibrinogen is taken into account, the length of time near optimal probability decreases at higher shear rate, meaning the time since activation plays a more significant role in determining the capture probability.

Our model only predicts the initial probability of aggregation, not the probability that colliding platelets form a permanent doublet. When considering only the initial interaction, there is a symmetry to the results. A collision between two platelets with fractions of occupied receptors both equal to ϕ, has the same probability of forming a doublet as does a collision between platelets with fractions of occupied receptors $1 - \phi$. If ϕ is close to zero, the probability of initially forming a doublet is low due to a lack of available singly bound fibrinogen for cross-bridging. However the reaction with solution fibrinogen progresses rapidly, increasing the availability of bound fibrinogen. This is in contrast to the case where $\phi \approx 1$. The aggregation process is then limited by a lack of available receptors. As time goes on, more receptors will not become available. This means that while the two cases may have the same probability of forming a doublet initially, the subsequent events may be very different. A doublet for which $\phi \ll 1$ is more likely to form additional cross-bridges and therefore be less likely to come apart.

Our results suggest that the time from activation plays a significant role in determining the probability that two colliding platelets form a doublet. Even though we only consider events between isolated pairs of cells on short time scales, we believe the results of the model are suggestive of a mechanism for regulation of aggregation *in vivo*. We also show that the decrease in aggregation at high shear, seen in some experiments and often attributed to the increase in force, instead, may be the result of the reduction of the time that colliding platelets are together. In future studies of platelet aggregation, the progression of the reaction between solution fibrinogen and gpIIb-IIIa should be considered.



(a) (b)



(c)

Figure 8: (a) Capture probability for various shear rates as a function of occupied receptors. (b) Capture probability for various shear rates as a function of time from activation for two cells activated at the same time. Times not in parentheses correspond to $k_f = 3 \cdot 10^5$ (M s)$^{-1}$, and times in parentheses correspond to $k_f = 2 \cdot 10^4$ (M s)$^{-1}$. (c) Capture efficiency for a fixed receptor occupancy level.

A Expressions for A(s), B(s), C(s)

For the cases of very large separation distances and near contact, asymptotic forms of the functions $A(s)$, $B(s)$, and $C(s)$ are derived in (Batchelor and Green 1972). For intermediate scales, Tandon and Diamond (1997) give empirical forms which match the computational data in the literature within 2%. These expressions depend on the ratio of the radii of the two particles. We use these expressions with size ratio one.

$$A(s) = \begin{cases} 1 - 4.078(s-2) & s \leq 2.01 \\ \dfrac{1}{1 + 5.5676(s-2)^{1.0586}} & 2.01 < s < 3 \\ \dfrac{5}{s^3} - \dfrac{8}{s^5} & s \geq 3 \end{cases}$$

$$B(s) = \begin{cases} 0.404 + \dfrac{0.781}{\ln(s-2)} & s \leq 2.00075 \\ -0.3277(s-2)^{0.25} + 0.3497 & 2.00075 < s < 3 \\ \dfrac{16}{3s^5} & s \geq 3 \end{cases}$$

$$C(s) = \begin{cases} 2(s-2) & s \leq 2.01 \\ \dfrac{1}{1.0377(s-2)^{-0.8371} + 1} & 2.01 < s < 3 \\ 1 - 1.5s^{-1} + s^{-3} - 3.75s^{-4} & s \geq 3 \end{cases}$$

B Force on a Doublet

Expressions for the forces acting on a rigidly linked doublet in a shear flow were computed by Tha and Goldsmith (1986). The force normal to the doublet axis, F_{n}, and the tangential shear force F_{s}

are

$$F_{\rm n} = \alpha_3(h)\mu\gamma a^2 \sin^2\theta_a \sin 2\theta_p \tag{28}$$

$$F_{\rm s} = \alpha_{12}(h)\mu\gamma a^2 \sin\theta_a \left(\frac{\left(2\sin^2\theta_a\cos^2\theta_p - 1\right)^2 \sin^2\theta_p + \cos^2\theta_a\cos^2\theta_p}{1 - \sin^2\theta_a\cos^2\theta_p} \right)^{1/2}, \tag{29}$$

where $\alpha_3(h)$ and $\alpha_{12}(h)$ are functions of the separation distance h. For $h = 20$ nm, $\alpha_3 = 19.33$ and $\alpha_{12} = 7.02$. The maximum magnitude, $|F|_{\rm max}$, occurs when $\theta_a = \pi/2$ and $\theta_p = \pi/4$. In that case, all the force acts along the axis of the doublet, so that

$$|F|_{\rm max} = (F_{\rm n})_{\rm max} = \alpha_3(h)\mu\gamma a^2. \tag{30}$$

When the normal force is negative, the hydrodynamic forces are acting to move the particles closer together. Assuming that cross-bridges need not counter this compressive force, the force experienced by cross-bridges between the doublet is

$$F_{\rm cb} = \sqrt{(F_{\rm n}^+)^2 + (F_{\rm s})^2}, \tag{31}$$

where $F_{\rm n}^+$ is the normal force when it is positive and zero when it is negative. Averaging over all configurations of the doublet, the mean force acting on a cross-bridge is

$$\langle F_{\rm cb}\rangle = \frac{1}{4\pi}\int_0^{\pi}\int_0^{2\pi} F_{\rm cb}\; d\theta_p d\theta_a. \tag{32}$$

This integral can be computed numerically. Note that the average is not taken over orbits. This mean force does not represent the mean force experienced by a cross-bridge during rotation, but it is the mean over a population of doublets assuming that all configurations are equally likely.

C Master Equations

The traditional approach to computing the probability distribution for the number of bonds involves master equations. Our approach is equivalent under the assumptions of our model.

Suppose that M receptors have the opportunity to join into a cross-bridge. That is, each of these receptors is lined up with a receptor on the opposing cell, and exactly one of each pair of aligned receptors is bound to fibrinogen. Let Δt be a small time interval in which only a single binding event may occur. Let $p_{m|M}(t)$ be the probability that there are m cross-bridges at time

t given M receptors available for cross-bridging. Let $\Delta p_{m|M} = p_{m|M}(t+\Delta t) - p_{m|M}(t)$ be the change in this probability during a time interval of length Δt. Because only one binding event can take place, there are four ways a change in probability can occur: (1) A bond can form if there are $m-1$ existing bonds, (2) A bond can break if there are $m+1$ existing bonds, (3) A bond can form if there are m existing bonds, (4) A bond can break if there are m existing bonds. The first two cases increase the probability and the second two decrease it.

Define $k^+\Delta t$ to be the probability a receptor and ligand form a bond, given that they are in the proper spatial configuration to bind. Similarly, define $k_m^-\Delta t$ as the probability a cross-bridge breaks, which may depend on m. The total change in probability during time Δt is

$$\begin{aligned}\Delta p_{m|M} &= (k^+\Delta t)(M-(m-1))p_{m-1|M} - [(k^+\Delta t)(M-m) - (k_m^-\Delta t)m]p_{m|M} \\ &\quad + (k_m^-\Delta t)(m+1)p_{m+1|M} + O(\Delta t^2). \end{aligned} \tag{33}$$

Dividing both sides by Δt and taking $\Delta t \to 0$ gives the $M+1$ differential equations

$$\begin{aligned}\frac{dp_0|M}{dt} &= -(k^+M)p_{0|M} + k_m^- p_{1|M} \\ \frac{dp_{m|M}}{dt} &= k^+[M-(m-1)]p_{m-1|M} - [k^+(M-m) + k_m^- m]p_{m|M} \qquad \text{for } m = 1,\ldots,M-1 \\ &\quad + k_m^-(m+1)p_{m+1|M} \\ \frac{dp_{M|M}}{dt} &= k^+ p_{M-1|M} - (k_m^- M)p_{M|M}. \end{aligned} \tag{34}$$

Equations of this type are called master equations. Master equations were introduced by McQuarrie (1963) to predict the chemical kinetics of systems consisting of a small number of molecules. Various forms of the master equations have been used in modeling receptor ligand binding (Chesla et al. 1998; Cozens-Roberts et al. 1990; Long et al. 1999; Piper et al. 1998).

Because we have assumed that bonds break on a much longer time scale than we are modeling, k_m^- can be taken to be zero in (34). The resulting equations are easily solved for a given initial condition. Time zero is the time the platelets come together, and therefore the initial condition is $p_{0|M}(0) = 1$ and $p_{j|M}(0) = 0$ for $j \neq 0$. The solution is

$$p_{m|M}(t) = \binom{M}{m}(\pi(t))^m(1-\pi(t))^{M-m}, \tag{35}$$

where

$$\pi(t) = 1 - \exp\left(-k^+t\right). \tag{36}$$

For short times

$$\pi(t) \approx k^{+} t. \tag{37}$$

As before, ϕ_{r} is the probability a receptor lines up with a receptor on the opposing cell and ϕ_{fg_1} is the probability that exactly one of the two receptors is bound to fibrinogen. These probabilities are taken to be constant during the collision. The distribution for the number of receptors available for cross-bridging, M is the binomial distribution

$$\binom{N}{M} \left(\phi_{\mathrm{r}}\phi_{\mathrm{fg}_1}\right)^{M} \left(1 - \phi_{\mathrm{r}}\phi_{\mathrm{fg}_1}\right)^{N-M}, \tag{38}$$

where N represents the total number of receptors in the contact area.

Let p_n be the probability that n cross-bridges form during a collision. This is given by

$$p_n = \sum_{M=n}^{N} \binom{N}{M} \left(\phi_{\mathrm{r}}\phi_{\mathrm{fg}_1}\right)^{M} \left(1 - \phi_{\mathrm{r}}\phi_{\mathrm{fg}_1}\right)^{N-M} p_{n|M}, \tag{39}$$

which can be simplified to become

$$p_n = \binom{N}{n} \left(\phi_{\mathrm{r}}\phi_{\mathrm{fg}_1}\pi(t)\right)^{n} \left(1 - \phi_{\mathrm{r}}\phi_{\mathrm{fg}_1}\pi(t)\right)^{N-n}. \tag{40}$$

This is equivalent to equation (18).

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