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Rupture of single receptor-ligand bonds: A new insight into probability distribution functions

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Abstract

Single molecule force spectroscopy is widely used to determine kinetic parameters of dissociation by analyzing bond rupture data obtained via applying mechanical force to cells, capsules, and beads that are attached to an intermolecular bond. The current analysis assumes that the intermolecular bond force is equal to the externally applied mechanical force. We confirm that viscous drag alone or in combination with cellular deformation resulting in viscoelasticity modulates bond force so that the instantaneous intermolecular bond force is not equivalent to the applied force. The bond force modulation leads to bond rupture time and force histograms that differ from those predicted by probability distribution functions (PDFs) using the current approach. A new methodology that accounts for bond force modulation in obtaining PDFs is presented. The predicted histograms from the new methodology are in excellent agreement with the respective histograms obtained from Monte Carlo simulation.

INTRODUCTION

Cell adhesion mediated by surface-bound receptor-ligand interactions [or so-called 2D binding] plays a pivotal role in diverse biological processes, including thrombosis [1], tumor metastasis [2], arteriosclerosis [3], and inflammation [4, 5]. Changes in cell adhesion can be the defining event in a wide range of diseases, including cancer [6], arthritis [7], osteoporosis, and atherosclerosis [8]. Receptor-ligand interactions that mediate cellular adhesion are often subjected to forces that regulate their dissociation via modulating off-rates. The bond lifetime of receptor-ligand bonds and how much force they withstand are controlled by off-rates. Therefore, one should be able to determine off-rates from either bond lifetime or dissociation force measurements. Specifically, in the first type of measurement bond lifetimes are measured in a range of constant forces and this method was used to obtain the first experimental estimate of $k_{\text{off}}(f)$ for a unimolecular interaction between P-selectin and PSGL-1 [9]. In the second type of method, dissociation forces are measured in a range of constant loading rates, which has been popularized by the theory of dynamic force spectroscopy (DFS) analysis [10].

Bond dissociation kinetics and forced bond rupture have been investigated theoretically and experimentally using various approaches or assays, e.g., flow chamber [9,11–14], biomembrane force probe (BFP) [15, 16], atomic force microscopy (AFM) [17–21], microneedles [22], micropipette aspiration [23, 24], optical tweezers [25], magnetic tweezers [26, 27], fluorescence recovery after photobleaching (FRAP) [28], and

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microcantilever [29]. The bond rupture data is analyzed by constructing rupture force histogram to determine the most probable rupture force at a given loading rate [30]. This in turn is utilized to extract kinetic parameters of dissociation from linear or nonlinear least-squares fits to a probability distribution function (PDF) [29, 30]. In the current approach the PDFs are obtained by assuming that the intermolecular bond force is equal to the applied force [25, 29–31] which could be true in absence of bond force modulation due to viscous drag alone or due to viscoelasticity arising from the complex interplay of cellular deformation and viscous drag acting on the cell. The bond rupture force histograms could differ when bond force modulation is taken into account to obtain PDF. In this work we show that the viscous drag alone or in combination with cellular deformation resulting in viscoelasticity affects bond dynamics and therefore the derived bond rupture time and force histograms.

In the current approach to obtain PDF it is assumed that the entire work done by the applied force solely affects the free energy of the bond, a direct consequence of quasi-static force equilibrium assumption [25, 29–31]. In presence of viscous drag acting on the cell we might expect this quasi-static force equilibrium at very slow loading rates but not at high loading rates. At very slow loading rates the cell would move with a very small velocity and hence drag force acting on it would be negligible. In presence of considerable viscous forces, part of the entire work done by the applied force is lost in the form of viscous dissipation, the work done against viscous forces [32]. We should note that for a single receptor-ligand bond between two cells or between a cell and the extracellular matrix (ECM) viscous dissipation depends on the size of the cell, the viscosity of the media, and the velocity of the cell which in turn depends on the applied loading rate [32]. If the cell is modeled as an elastic (deformable) material then its radius will vary under an applied load. Hence, it is expected that in presence of considerable viscous forces there will be a non-linear decrease of the free energy for dissociation under an applied load in contrast to a linear decrease an assumption embedded in the current approach [25, 29–31]. Consequently, this would lead to a modified bond kinetics dependent on the viscous dissipation resulting in modified rupture time and force histograms. In this work we demonstrate that due to bond force modulation the bond rupture time and force histograms differ from those predicted by PDFs using the current approach [25, 29–31]. The accuracy of extracted off-rate relevant to real physiological conditions depends not only on the accurate construction of bond rupture force histogram but also on fitting bond rupture data to an accurate PDF. We present a new methodology that accounts for bond force modulation to obtain PDFs. The bond rupture time and force histograms so obtained from the new methodology are in excellent agreement with the respective histograms obtained from Monte Carlo simulation for a given set of conditions.

MODEL AND METHOD

A schematic model of the DFS process is presented in Fig. 1. Figure 1 shows our model of a spherical (radius = R) polymorphonuclear (PMN) leukocyte containing a Newtonian fluid of density ρ and viscosity μ , suspended in a Newtonian fluid with the same density and viscosity. The bond is formed between a ligand (L-selectin) molecule on the substrate and a receptor (P-selectinglycoprotein-ligand-1 (PSGL-1)) molecule concentrated on the tip of PMN microvilli which are microscopic cellular membrane protrusions that increase the surface area of cells, and are involved in a wide variety of functions, including absorption, secretion, cellular adhesion, and mechanotransduction [33, 34]. Thus PMN cell is adhered to the substrate by a single L-selectin–PSGL-1 bond [35]. The bond is modeled as a linear spring having spring constant σ . We consider four cell-microvillus models of increasing deformability (elasticity): **(a)** solid cell with solid microvillus **(b)** solid cell with deformable microvillus **(c)** deformable cell with solid microvillus **(d)** deformable cell with deformable microvillus. As soon as a bond between the cell and the substrate is formed we apply a force

$F(t) = R_f t$, where R_f is the loading rate, on the surface of the cell along the line perpendicular to the substrate causing bond stretching and eventually bond rupture (see Fig. 1). We consider irreversible forced bond rupture. Once the bond is ruptured the cell separates from the substrate and the simulation is terminated.

Micro-mechanical Model of Detachment of Solid (Inelastic) PMN Cell

In Fig. 1 if the PMN cell is modeled as solid (undeformable) then we can write an analytical expression for time varying bond stretch by utilizing Newton's second law of motion:

$$\rho \frac{4}{3} \pi R^3 \frac{d^2 x}{dt^2} = F(t) - \sigma x(t) - 6\pi\mu R \frac{dx}{dt}, \quad (1)$$

where x is the displacement of the cell center as shown in Fig. 1. The first term on the left, the second and the third terms on the right side represent inertial force, bond force and drag force arising from the relative motion between the cell and the surrounding fluid, respectively. In this work we do not consider viscous force acting on a force measurement probe of the DFS process such as a cantilever in AFM etc. [36]. At any instant of time the magnitude of the drag force acting on the cell depends on the viscosity μ of the media, the

radius R of PMN cell, and the relative velocity $\frac{dx}{dt}$ of the cell which in turn depends on R_f .

Letting $a = \rho \frac{4}{3} \pi R^3$, $b = 6\pi\mu R$, and $c = \sigma$ the above equation becomes

$$a \frac{d^2 x}{dt^2} + b \frac{dx}{dt} + c x(t) = F(t). \quad (2)$$

For $F = R_f t$ the solution for $x(t)$ is

$$x(t) = c_1 \exp(At) + c_2 \exp(Bt) + \frac{R_f}{c} \left(t - \frac{b}{c} \right), \quad (3)$$

where

$$A = \frac{-b + \sqrt{b^2 - 4ac}}{2a}, B = \frac{-b - \sqrt{b^2 - 4ac}}{2a}, \quad (4)$$

and c_1, c_2 are constants of integration that can be determined using the initial conditions $x(0) = x'(0) = 0$ giving

$$c_1 = -c_2 + \frac{R_f}{c} \left(\frac{b}{c} \right), c_2 = \frac{1}{A - B} \frac{R_f}{c} \left(A \frac{b}{c} + 1 \right). \quad (5)$$

Note that $x(t)$ is the bond stretching when the microvillus is solid. When the microvillus is viscoelastic it can be extended (deform) and may form a tether when the force is greater than 45 pN [34]. In the extension regime the solution for $x(t)$ is still given by Eq. 3 but with

modified $a = \left(1 + \frac{\sigma}{\sigma_m} \right) \rho \frac{4}{3} \pi R^3$, $b = \left(1 + \frac{\sigma}{\sigma_m} \right) 6\pi\mu R$, and $c = \sigma$. Similarly, in the tether regime the solution for $x(t)$ is still given by Eq. 3 but with modified

$a = \rho \frac{4}{3} \pi R^3$, $b = \frac{\sigma}{\mu_m} \rho \frac{4}{3} \pi R^3 + 6\pi\mu R$, and $c = \sigma + \frac{\sigma}{\mu_m} 6\pi\mu R$. Thus knowing $x(t)$ we can simulate the detachment of receptor-ligand bond as a function of loading rate using the Monte Carlo method [37]. Note that by neglecting both viscous drag and inertia Eq. (1) reduces to $\sigma x(t) =$

$F(t)$, i.e., the instantaneous bond force is equal to the applied force, an inherent assumption in the current approach [25, 29–31].

3-D simulation of Adhesion and Detachment of Deformable PMN Cell

In addition the PMN cell in Fig. 1 is modeled as 3-D deformable capsule containing a Newtonian fluid of density ρ and viscosity μ suspended in a Newtonian fluid with the same density and viscosity. The adhesion and detachment of deformable PMN cell suspended in a Newtonian fluid is simulated by simultaneously: 1) solving the Navier-Stokes (N-S) equation by immersed boundary method (IBM) for motion of deformable capsule suspended in a Newtonian fluid; 2) solving the constitutive equation using the finite element method for the neo-Hookean membrane of the spherical capsule; and 3) simulating the formation and rupture of receptor (PSGL-1)-ligand (L-selectin) bond using Monte Carlo method with kinetic rate constant based on the Bell model. For details of these three steps and numerical implementation the reader is advised to refer to our previous article [38] dealing with the binding and rupture of multiple bonds in response to an externally applied force.

Monte Carlo simulation of receptor-ligand interactions

We model the bond between adhesion molecules (ligand-receptor) as a Hookean spring, so the chemical force on the cell due to the bond, f , is given by

$$f = \sigma(x_m - \lambda) = \sigma x(t) \quad (6)$$

where σ is the bond spring constant, x_m is the stressed bond length, $x(t)$ is the bond stretch, and λ is the equilibrium bond length. There are three kinetic models, namely, the Dembo model [39], the Bell model [40], and the two-state model [41] that are commonly used to simulate bond formation and rupture. We simulate bond formation and rupture utilizing the Bell model. The work presented in this manuscript is relevant to determination of the Bell model parameters utilizing a methodology described by Evans and Ritchie [15]. In the Bell model the force dependent on-rate, k_f , and the off-rate, k_r , are given by [40]

$$k_f = k_f^0 \exp \left[\frac{f(x_\beta - 0.5x)}{k_b T} \right], \quad (7)$$

$$k_r = k_r^0 \exp \left(\frac{x_\beta f}{k_b T} \right), \quad (8)$$

where x_β is the reactive compliance, $f = \sigma x(t)$ is the force acting on the bond, k_f^0 and k_r^0 are the on-rate and off-rate at an equilibrium distance λ , T is the absolute temperature, and k_b is the Boltzmann constant. We use Monte Carlo simulation to incorporate the stochastic nature of ligand-receptor interactions. In the simulation during a time interval Δt , the probability (P_b) that a ligand will bind with its complementary receptor is given by [37]

$$P_b = 1 - \exp(-k_f \Delta t). \quad (9)$$

Similarly the probability for bond rupture is given by [37]

$$P_r = 1 - \exp(-k_r \Delta t). \quad (10)$$

At each time step, the probabilities of bond formation and rupture are compared to random numbers (P_{ran1} and P_{ran2}) lying between 0 and 1. $P_b > P_{\text{ran1}}$ indicates bond formation whereas $P_r > P_{\text{ran2}}$ indicates bond rupture.

Determination of Probability Distribution Function

The probability $P_b(t)$ for the bond to remain intact at time t under an applied force f follows the following time evolution equation [31]

$$\frac{dP_b}{dt} = -k_r(f)P_b, \quad (11)$$

where $k_r(f)$ is the off-rate. The distribution of rupture forces, or the probability distribution function, $P_d(f)$ is related to $P_b(t)$ by the following relation [31]

$$P_d(f) df = -\frac{dP_b}{dt} dt. \quad (12)$$

Integration of Eqn. 11 in conjunction with the off-rate given by Eqn. 8 and by assuming the instantaneous bond force is equal to the applied force, i.e., $f = R_f t$, gives the following explicit expressions for $P_b(f)$

$$P_b(f) = \exp \left[-\frac{k_r^0}{R_f \beta} \{ \exp(f\beta) - 1 \} \right], \quad (13)$$

which in turn along with Eqn. 12 provides the following explicit expressions for $P_d(f)$

$$P_d(f) = \frac{k_r^0}{R_f} \exp(f\beta) \exp \left[-\frac{k_r^0}{R_f \beta} \{ \exp(f\beta) - 1 \} \right], \quad (14)$$

where $\beta = \frac{x\beta}{k_b T}$. In this case the average bond rupture time, $\langle T \rangle$, and the average bond rupture force, $\langle F \rangle$, are given by

$$\langle T \rangle = \frac{1}{R_f \beta} \exp \left[\frac{k_r^0}{R_f \beta} \right] E_1 \left(\frac{k_r^0}{R_f \beta} \right), \quad \langle F \rangle = R_f \langle T \rangle, \quad (15)$$

where $E_1(x)$ is the exponential integral, $E_1(x) = \int_1^\infty \frac{e^{-xt}}{t} dt$ [42]. In our new method the instantaneous bond force is given by the bond stretch times the bond spring constant, i.e., $f = \sigma x(t)$. The off-rate (Eqn. 8) written in terms of the bond stretch $x(t)$ then becomes

$$k_r(t) = k_r^0 \exp[\sigma \beta x(t)]. \quad (16)$$

Thus in this case the expression for $P_b(t)$ obtained from Eqn. 11 is

$$\ln(P_b) = -k_r^0 \int_0^t \exp[\sigma \beta x(t)] dt = -k_r^0 \Psi(t), \quad (17)$$

where $x(t)$ is given by Eqn. 3 when the cell is modeled as undeformable. When the cell is modeled as deformable then in that case it is impossible to write an explicit expression for $x(t)$ given the complex interplay of cellular elasticity and hydrodynamics. In this case for a given set of conditions we generate a unique $x(t)$ from our 3-D IBM simulation code for deformable cell [38]. Thus in our new method the expression for probability distribution function, $P_d(t)$, written in terms of bond stretch is given by

$$P_d(t) \sigma dx(t) = k_r^0 \exp[\sigma \beta x(t)] \exp \left[-k_r^0 \Psi(t) \right] dt. \quad (18)$$

Hence at any time t knowing $x(t)$ one can plot $P_d(t)$ versus t and $P_d(t)$ vs $\sigma x(t)$ resulting in rupture time distribution and rupture force distribution, respectively. The average rupture time and the average rupture force are given by

$$\langle T \rangle = \int_0^\infty \sigma dx(t) P_d(t) t \text{ and } \langle F \rangle = \int_0^\infty \sigma dx(t) P_d(t) \sigma x(t), \text{ respectively.}$$

Model and Computational Parameters

We simulated bond formation and rupture between the PMN cell and the substrate using the parameter values listed in Table 1. For L-selectin–PSGL-1 bonds the Bell model parameters are taken from Ref. [43]. A time step of 10^{-7} s is used in all these simulations. We repeated Monte-Carlo simulation for 10^6 times at a given loading rate to get statistically accurate rupture time and force histograms. A detailed ultrastructural study of Bruehl et al. [44] has shown that the actual length of microvilli varies from 0.15 to 0.7 μm . In our work, we use the average length of microvilli as 0.35 μm . The electrostatic and steric stabilization forces have been neglected because these forces exist in biological systems at distances from 50 to 250 Å [37].

RESULTS

Intermolecular Bond Force for Various Cell and Microvillus Models

Results similar to those shown in Fig. 2 were originally presented by Gupta and Eggleton [45] at $R_f = 10^5$ pN/s. In this work we present similar results at $R_f = 5 \times 10^6$ pN/s for the sake of completeness of this article. Fig. 2 shows time variation of applied force and intermolecular bond force obtained from various cell and microvillus models (a) – (d) at $R_f = 5 \times 10^6$ pN/s. The applied load shown as the solid line increases linearly with time. When both the cell and microvillus are modeled as undeformable, there is a short lag time after which the intermolecular bond force increases nearly linearly with time as shown by dashed lines in Fig. 2. In this case viscous drag alone modulates intermolecular bond force. The intermolecular bond force increases non-linearly for all other cell and microvillus models considered in this work. In these viscoelasticity arising from the complex interplay of cellular deformation and viscous drag modulates intermolecular bond force. It is seen that irrespective of the cell and microvillus model used, at any time the intermolecular bond force is lower than the applied load [45]. The difference in applied load and the intermolecular bond force is the lowest when both cell and microvillus are modeled as undeformable, while it is the largest when both cell and microvillus are modeled as deformable [45]. Similar sets of results are obtained for $R_f = 10^3 - 10^8$ pN/s (see Supplementary Figure S1). However, it is found that for a given cell and microvillus type modeled the difference in the applied load and the intermolecular bond force decreases as R_f decreases, implying that in the limit of infinitely slow loading rate the difference might vanish (see Supplementary Figure S2). It is apparent from Fig. 2 that the rupture force histograms constructed based on the applied force will differ from those constructed based on the intermolecular bond force as shown in Fig. 3. It is seen from Fig. 3(a) that as we increase cellular deformability the peak (the most probable rupture force) in the rupture force histograms constructed based on the applied force shifts rightward towards larger forces. On the other hand the peak in the rupture force histograms constructed based on the intermolecular bond force shifts leftward towards smaller forces as seen from Fig. 3(b). It is apparent from Fig. 3 that in DFS experiments involving live cells the measurement of the most probable rupture force based on the applied force would result in inaccurate estimation of kinetic off-rate given the most probable rupture force based on the bond force could be different.

Bond Rupture Force and Rupture Time Histograms for Various Cell and Microvillus Models

Figures 4(a)–4(f) show the bond rupture force histograms obtained from the current approach ($f = R_f t$) and those obtained from the new methodology ($f = \sigma x(t)$) for various cell and microvillus models at loading rates $R_f = 10^3, 10^4, 10^5, 10^6, 10^7$, and 10^8 pN/s, respectively. It should be noted that the current approach does not account for the cellular deformation to obtain PDF hence results in a unique bond rupture force histogram at a given R_f . On the other hand the bond rupture force histograms obtained from the proposed new methodology vary with cell-microvillus models (a) to (d) with increasing cellular deformation. It is seen that at a given R_f the bond rupture histogram obtained from the current approach and that obtained from the new methodology for cell-microvillus model (a) are nearly identical. This is attributed to the fact that for cell-microvillus model (a) bond force modulation is the lowest which is caused by viscous drag alone as discussed above. However, once we increase the level of cellular deformation from cell-microvillus models (b) to (d) the bond rupture force histograms obtained from the new methodology start deviating from the bond rupture force histogram obtained from the current approach at a given loading rate. The higher the cellular deformation, the higher the deviation. In this case viscoelasticity arising from the complex interplay of cellular deformation and viscous drag modulates intermolecular bond force as discussed above resulting in such deviation.

For the Bell model the most probable rupture force, f^* , as a function of the applied loading rate can be obtained by differentiating Eq. (14) with respect to f to give [29]:

$$f^* = \frac{1}{\beta} \ln \left(\frac{\beta R_f}{k_r^0} \right). \quad (19)$$

For the L-selectin–PSGL-1 bonds $1/\beta = 267.51$ pN for the parameter values listed in the

Table 1. It is apparent that for meaningful f^* one must meet $\frac{\beta R_f}{k_r^0} \geq 1$. In other words for $R_f < \frac{k_r^0}{\beta} \approx 2.3 \times 10^3$ pN/s the most probable rupture force becomes unrealistic, i.e., the peak (the most probable rupture force) in the rupture force histogram disappears. Therefore, for the L-selectin–PSGL-1 bonds the bond rupture force histograms obtained from the Monte Carlo simulation utilizing the Bell model do not show a peak at $R_f = 10^3$ pN/s as seen from Fig. 4(a). It is apparent from Figs. 4(b)–4(f) that the most probable rupture force at a given loading rate differs from that given by the current approach due to the intermolecular bond force modulation and it varies with the nature of cell-microvillus model. In Figs 4(a)–4(f) we also show the corresponding bond rupture force histograms obtained from the Monte Carlo simulation. The predicted bond rupture force histograms from the new methodology are in excellent agreement with the respective histograms obtained from Monte Carlo simulation for a given cell-microvillus model at a given loading rate. Note that there is a slight kink in the bond rupture force histograms when bond force ≈ 45 pN. This is attributed to transition of microvillus from extension regime to tether regime (see Fig. 2).

The corresponding bond rupture time histograms are shown in Figs. 5(a)–5(f). Once again it should be noted that the current approach does not account for the cellular deformation hence results in a unique bond rupture time histogram at a given R_f . On the other hand the bond rupture time histograms obtained from the proposed new methodology vary with cell-microvillus models (a) to (d) with increasing cellular deformation. It is seen from Figs. 5(a)–5(f) that as we increase the level of cellular deformation from cell-microvillus models (b) to (d) the bond rupture time histograms obtained from the new methodology start deviating from the bond rupture time histogram obtained from the current approach at a given loading rate. The higher the cellular deformation, the higher the deviation. Further it is

seen that for a given cell-microvillus model the deviation in the bond rupture time histograms increases as we increase the loading rate. In Figs 5(a)–5(f) we also show the corresponding bond rupture time histograms obtained from the Monte Carlo simulation. The predicted bond rupture time histograms from the new methodology are in excellent agreement with the respective bond rupture time histograms obtained from the Monte Carlo simulation at a given loading rate for a given cell-microvillus model.

Average Bond Rupture Force and Rupture Time for Various Cell and Microvillus Models

Tables 2, 3, 4, 5, 6, and 7 list the average bond rupture force and the average bond rupture time obtained from the current approach ($f = R_f t$) and those obtained from the new methodology ($f = \sigma x(t)$) for various cell and microvillus models at loading rates $R_f = 10^3$, 10^4 , 10^5 , 10^6 , 10^7 , and 10^8 pN/s, respectively. Once again it should be noted that the current approach does not account for the cellular deformation hence results in a unique average bond rupture force and a unique bond rupture time at a given R_f . On the other hand the average bond rupture force and the average bond rupture time obtained from the proposed new methodology vary with cell-microvillus models (a) to (d) with increasing cellular deformation. In particular, the average bond rupture force decreases with increasing cellular deformation at a given loading rate. While the average bond rupture time increases with increasing cellular deformation at a given loading rate. Such viscoelastic effects arising from the interplay of cellular deformation and viscous drag on the average bond rupture force and the average bond rupture time can not be characterized based on the current approach. In Tables 2–7 we also list the average bond rupture force and the average bond rupture time obtained from the Monte Carlo simulation for a given cell-microvillus model. The average bond rupture force and the average bond rupture time obtained from the new methodology are in excellent agreement with the respective average bond rupture force and the average bond rupture time obtained from the Monte Carlo simulation at a given loading rate for a given cell-microvillus model. Note that the high values of average bond rupture forces are due to a low value of x_β for L-selectin–PSGL-1 bonds [43]. At a given loading rate the average bond rupture force for Streptavidin-biotin bonds ($x_\beta = 5.1 \text{ \AA}$, $k_r^0 = 0.013 \text{ s}^{-1}$ for $R_f < 10^4$ pN/s and $x_\beta = 1.0 \text{ \AA}$, $k_r^0 = 26.9 \text{ s}^{-1}$ for $R_f > 10^4$ pN/s [43]) is found to be nearly five times lower than the average bond rupture force for L-selectin–PSGL-1 bonds (see Supplementary Figure S3). Hence, at a given loading rate the magnitude of average bond rupture force depends on the type of molecular interaction.

CONCLUSIONS

The detachment of PMN cell adhered to the substrate by a single L-selectin-PSGL-1 bond was simulated using a Monte Carlo approach. Using these simulations, we successfully confirmed that viscous drag alone or in combination with cellular deformation resulting in viscoelasticity modulates the externally applied load such that the instantaneous intermolecular bond force is not equivalent to the externally applied load [45]. Specifically, we confirmed that irrespective of the cell and microvillus model used the intermolecular bond force is always lower than the applied load, i.e., the bond does not experience the exact applied load at any instant of time [45]. The difference in applied load and the intermolecular bond force based on the bond stretch is the lowest when both the cell and microvillus are modeled as solid (undeformable), while it is the largest when both cell and microvillus are modeled as elastic (deformable) [45]. Bond force modulation results in bond rupture time and force histograms that differ from those given by the current approach. We modified the current approach to account for bond force modulation in obtaining bond rupture time and force histograms. The histograms obtained from our modified approach are in excellent agreement with those corresponding histograms obtained from the Monte Carlo simulation at the same condition.

We have successfully demonstrated that the most probable rupture force at a given loading rate differs from that given by the current approach due to the intermolecular bond force modulation and varies with the nature of cell-microvillus model. In light of the findings presented in this work it is reasonable to say that the kinetic off-rate determined from the current (DFS) approach would not represent the true kinetic off-rate relevant to physiological conditions because of the bond force modulation due to viscous drag alone or in combination with cellular deformation resulting in viscoelasticity. Measuring the actual bond force and then constructing bond rupture force histograms would lead to the true kinetic off-rate relevant to physiological conditions. Irrespective of the nature of the cell and its surrounding, measuring bond length as a function of time once and then constructing PDF using the new methodology presented in this work would lead to accurate extraction of kinetic parameters of dissociation relevant to real physiological conditions.

In this work we considered bond force modulation solely due to viscous drag acting only on the PMN cell. Specifically, we did not consider bond force modulation due to viscous drag acting on a force measurement probe of the DFS process. It is apparent that inclusion of viscous drag acting on a force measurement probe in our model would contribute to an additional bond force modulation. However, what is important is the “insight” learned from our work, namely, viscous drag acting on the cell (or any other component of the DFS process for that matter) can lead to bond force modulation resulting in bond rupture force histograms that differ from those predicted by PDF using the current approach where it is assumed that at any instant the bond force is given by the instantaneous applied force. We presented a new methodology that accounts for bond force modulation to obtain PDF.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

The bond force modulation leads to different force and time histograms

We present a new theory that accounts for force modulation to obtain histograms

Predicted histograms agree with those obtained from the Monte Carlo simulation

Figure 1.

A schematic model of the DFS process. PMN cell is adhered to the substrate through binding between PSGL-1 (receptor) and L-selectin (ligand) molecules.

Figure 2.

Time variation of the applied force (solid line), intermolecular bond force for cell-microvillus models (a) to (d) obtained from simulation at $R_f = 5 \times 10^6$ pN/s for the model parameters listed in Table 1.

Figure 3.

Rupture force histograms based on (a) applied force (b) intermolecular bond force at $R_f = 5 \times 10^6$ pN/s for the legend corresponding to Fig. 2.

Figure 4.

Bond rupture force histograms at (a) $R_f = 10^3$ pN/s, (b) $R_f = 10^4$ pN/s, (c) $R_f = 10^5$ pN/s, (d) $R_f = 10^6$ pN/s, (e) $R_f = 10^7$ pN/s, (f) $R_f = 10^8$ pN/s.

Figure 5.

Bond rupture time histograms at (a) $R_f = 10^3$ pN/s, (b) $R_f = 10^4$ pN/s, (c) $R_f = 10^5$ pN/s, (d) $R_f = 10^6$ pN/s, (e) $R_f = 10^7$ pN/s, (f) $R_f = 10^8$ pN/s.

TABLE I

Parameter values used in the model

Parameter	Definition	Value
R	PMN radius	$3.75 \mu\text{m}$
L_{mv}	Length of microvillus	$0.35 \mu\text{m}$
λ	Receptor-ligand bond length	$0.07 \mu\text{m}$
k_f^0	Unstressed on-rate	3 s^{-1}
k_r^0	Unstressed off-rate	8.6 s^{-1}
x_β	Reactive compliance	$0.16 \text{ \AA}$
σ	Spring constant	4 dyn/cm
Eh	Membrane stiffness	3.0 dyn/cm
σ_m	Microvillus spring constant	$43 \text{ pN}/\mu\text{m}$
μ_m	Effective viscosity of tether	$11 \text{ pN.s}/\mu\text{m}$
μ	Fluid viscosity	0.8 cP
ρ	Fluid density	1 g/cc
T	Temperature	310 K

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TABLE II

Average rupture force (pN) and average rupture time (s) at $R_f=10^3$ pN/s.

Cell-microvillus model	$f = R_f t$		$f = \alpha x(t)$			
	$\langle F \rangle$	$\langle t \rangle$	$\langle F \rangle$ (simulation)	$\langle F \rangle$ (theory)	$\langle t \rangle$ (simulation)	$\langle t \rangle$ (theory)
(a)	86.70	8.67×10^{-2}	86.83	86.69	8.68×10^{-2}	8.67×10^{-2}
(b)	86.70	8.67×10^{-2}	85.92	85.90	8.69×10^{-2}	8.70×10^{-2}
(c)	86.70	8.67×10^{-2}	84.53	84.67	8.88×10^{-2}	8.89×10^{-2}
(d)	86.70	8.67×10^{-2}	82.37	82.74	9.10×10^{-2}	9.13×10^{-2}

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TABLE III

Average rupture force (pN) and average rupture time (s) at $R_f=10^4$ pN/s.

Cell-microvillus model	$f = R_f t$		$f = \sigma x(t)$			
	$\langle F \rangle$	$\langle t \rangle$	$\langle F \rangle$ (simulation)	$\langle F \rangle$ (theory)	$\langle t \rangle$ (simulation)	$\langle t \rangle$ (theory)
(a)	373.64	3.74×10^{-2}	373.95	373.59	3.74×10^{-2}	3.74×10^{-2}
(b)	373.64	3.74×10^{-2}	372.73	372.67	3.76×10^{-2}	3.76×10^{-2}
(c)	373.64	3.74×10^{-2}	356.02	356.27	3.88×10^{-2}	3.89×10^{-2}
(d)	373.64	3.74×10^{-2}	338.92	338.47	4.25×10^{-2}	4.27×10^{-2}

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TABLE IV

Average rupture force (pN) and average rupture time (s) at $R_f=10^5$ pN/s.

Cell-microvillus model	$f = R_f t$		$f = \sigma x(t)$			
	$\langle F \rangle$	$\langle t \rangle$	$\langle F \rangle$ (simulation)	$\langle F \rangle$ (theory)	$\langle t \rangle$ (simulation)	$\langle t \rangle$ (theory)
(a)	880.79	8.81×10^{-3}	880.93	880.73	8.83×10^{-3}	8.82×10^{-3}
(b)	880.79	8.81×10^{-3}	880.77	880.79	8.89×10^{-3}	8.89×10^{-3}
(c)	880.79	8.81×10^{-3}	811.98	811.89	9.94×10^{-3}	9.94×10^{-3}
(d)	880.79	8.81×10^{-3}	753.79	754.16	1.11×10^{-2}	1.11×10^{-2}

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TABLE V

Average rupture force (pN) and average rupture time (s) at $R_f=10^6$ pN/s.

Cell-microvillus model	$f = R_f t$		$f = \sigma x(t)$			
	$\langle f \rangle$	$\langle t \rangle$	$\langle F \rangle$ (simulation)	$\langle f \rangle$ (theory)	$\langle t \rangle$ (simulation)	$\langle t \rangle$ (theory)
(a)	1474.61	1.47×10^{-3}	1474.73	1474.39	1.49×10^{-3}	1.49×10^{-3}
(b)	1474.61	1.47×10^{-3}	1477.35	1477.35	1.50×10^{-3}	1.51×10^{-3}
(c)	1474.61	1.47×10^{-3}	1391.72	1391.75	1.74×10^{-3}	1.74×10^{-3}
(d)	1474.61	1.47×10^{-3}	1344.86	1345.91	1.96×10^{-3}	1.95×10^{-3}

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TABLE VI

Average rupture force (pN) and average rupture time (s) at $R_f=10^7$ pN/s.

Cell-microvillus model	$f = R_f t$		$f = \sigma x(t)$			
	$\langle f \rangle$	$\langle t \rangle$	$\langle F \rangle$ (simulation)	$\langle f \rangle$ (theory)	$\langle t \rangle$ (simulation)	$\langle t \rangle$ (theory)
(a)	2087.11	2.09×10^{-4}	2087.07	2086.70	2.26×10^{-4}	2.26×10^{-4}
(b)	2087.11	2.09×10^{-4}	2121.53	2121.70	2.31×10^{-4}	2.32×10^{-4}
(c)	2087.11	2.09×10^{-4}	2013.97	2014.69	2.90×10^{-4}	2.90×10^{-4}
(c)	2087.11	2.09×10^{-4}	1963.80	1967.00	3.36×10^{-4}	3.37×10^{-4}

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TABLE VII

Average rupture force (pN) and average rupture time (s) at $R_f=10^8$ pN/s.

Cell-microvillus model	$f = R_f t$		$f = \sigma x(t)$			
	$\langle f \rangle$	$\langle t \rangle$	$\langle F \rangle$ (simulation)	$\langle f \rangle$ (theory)	$\langle t \rangle$ (simulation)	$\langle t \rangle$ (theory)
(a)	2702.61	2.70×10^{-5}	2680.49	2680.68	4.37×10^{-5}	4.37×10^{-5}
(b)	2702.61	2.70×10^{-5}	2866.25	2866.88	5.81×10^{-5}	5.81×10^{-5}
(c)	2702.61	2.70×10^{-5}	2583.91	2586.65	6.95×10^{-5}	6.94×10^{-5}
(d)	2702.61	2.70×10^{-5}	2567.68	2570.20	7.61×10^{-5}	7.61×10^{-5}