A Microvillus Based Approach to Model Cell Rolling

By

Suman Bose
B.Tech, Department of Mechanical Engineering
Indian Institute of Technology, Kharagpur, India, 2007

SUBMITTED TO THE DEPARTMENT OF MECHANICAL ENGINEERING IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE IN MECHANICAL ENGINEERING
AT THE
MASSACHUSETTS INSTITUTE OF TECHNOLOGY

SEPTEMBER 2009

Signature of Author_____
Department of Mechanical Engineering
August 21, 2009

Certified by_____
Professor Rohit N. Karnik
d'Arbeloff Assistant Professor of Mechanical Engineering
Thesis Supervisor

Accepted by_____
Professor David E. Hardt
Graduate Officer, Department of Mechanical Engineering

(This page is left blank intentionally)

A Microvillus Based Approach to Model Cell Rolling

By
Suman Bose

Submitted to the Department of Mechanical Engineering on August 21, 2009 in Partial Fulfillment of the Requirements for the degree of Master of Science in Mechanical Engineering

Abstract

Cell rolling is a physiological phenomenon, which allows leukocytes to attach to activated vascular endothelium and reach sites of inflammation. A novel approach to model cell rolling is presented in this thesis. The model incorporates all the aspects known to be important to rolling in a semi-analytical framework making it computationally efficient. Bond kinetics have been used to define microvillus attachment probability which is in turn used to find out the net force on the cell. Deformability is also taken into account by an empirical relation which allows shear modulation of cell-surface contact area. The model showed excellent agreement with experimental results over a wide range of shear stresses. Using the model, the effects of cell deformability and microvillus structure have been studied and its implications discussed. The model was also used to predict rolling of microspheres, which showed reasonable agreement with experiments. Finally, the contribution of different features towards stabilization of rolling was elucidated by simulating different hypothetical cases with contributions from different cellular features.

Thesis supervisor: Prof. Rohit N. Karnik
Title: d'Arbeloff Assistant Professor of Mechanical Engineering

(This page is left blank intentionally)

Acknowledgements

First, I would like to express my gratitude to my advisor Prof. Rohit Karnik for the support he has provided me for this work. Rohit, has been like a mentor to me in every sense and provided guidance with a calm mind even at the most rough times. His passion for knowledge and values in life has truly inspired me and shaped my personality largely. I have learnt a lot from him from the discussions that we had, and the time we spent. I would also like to acknowledge my friends Sankha, Jongho, Sumeet, Chia, Jason and others who made my MIT experience enjoyable and fulfilling. Lastly, I would like to thank my mom, dad and god for without them nothing would seem to have any purpose.

Suman Bose
Cambridge, Massachusetts
2009

(This page is left blank intentionally)

Table of Contents

List of figures

(This page is left blank intentionally)

Chapter 1. Introduction

Leukocytes or white blood cells are a part of the immune system responsible for fighting against pathogens. Recruitment of leukocytes from blood and homing to the site of infection is a challenging task for which nature has evolved a sophisticated mechanism. Inflammation sites release cytokines that activate the endothelial cells and lead to expression of glycoproteins known as selectins and other signaling molecules on their surface (3, 4). Leukocytes in resting state posses ligands for selectins on their surface enabling them to attach to activated endothelium (5-7). Selectin mediated adhesive bonds have high formation rate and breakage rate which enables transient bonds to form between the leukocytes and the endothelium (8). Bonds form at the leading edge of the cell and break at the trailing edge resulting in the cell to 'roll' forward. Selectin mediated rolling is followed by cytokine activation of leukocytes, firm adhesion through integrins and finally extravasation. Figure 1 illustrates the different stages of the inflammatory cascade.

Cell rolling has also been implicated in the trafficking of other cell types such as lymphocytes, platelets, hematopoietic and mesenchymal stem and progenitor cells, and metastatic cancer cells (6, 9, 10). Cell rolling is thus important for understanding physiological processes including inflammatory response, homing of stem cells, and metastasis of cancer. Recently, cell rolling has been used as a method for separation of cells, and holds promise for therapeutic and diagnostic applications. (11, 12). A systematic study of cell rolling is thus important for understanding many physiological process and development of new separation technologies.

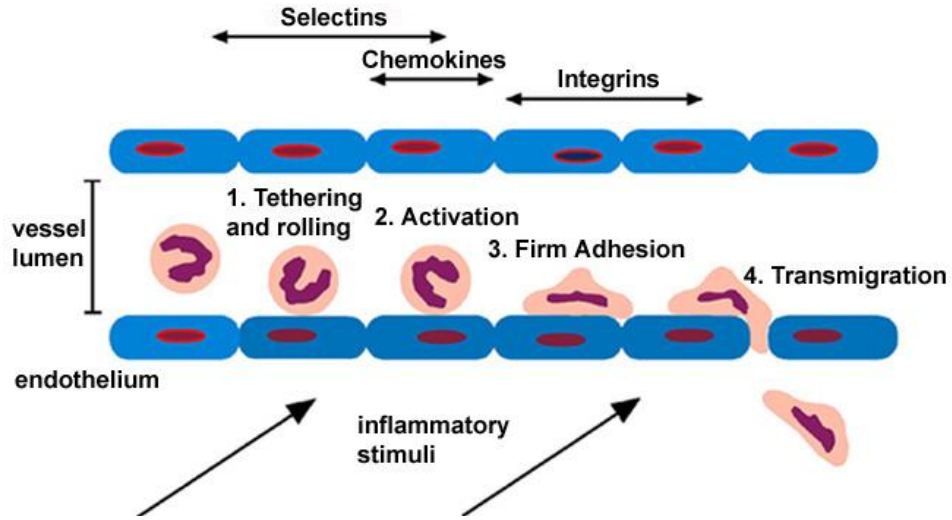

Figure 1. The inflammatory cascade illustrating capture, rolling, activation and extravasation of leukocytes from blood. Adapted from *http://www.mpi-muenster.mpg.de/nvz/wilde.shtml*

The complexity of cell rolling has been revealed through in vivo and in vitro experiments and a number of remarkable features have become known. Binding kinetics of receptors and sensitivity to force, mechanics of microvilli and the cell, and receptor clustering have evolved specialized properties to ensure very robust cell rolling over a range of shear stresses (see figure 2). However, several aspects of cell rolling are not completely understood: For example, What role do cellular features like deformability play in cell rolling? what are the effects of microvillus ? and importantly, what is the mechanism for stabilization of rolling at high shear rates? Rolling behavior has been mimicked in vitro using cell-free systems (ligand-coated microspheres) to resolve some of these issues by separating the cellular contributions from molecular contributions to rolling (13). However, the strong coupling between various parameters such as ligand density and microvillus rheology that affect rolling makes it extremely difficult, if not impossible, to

study the effect of individual parameters experimentally without affecting the others.

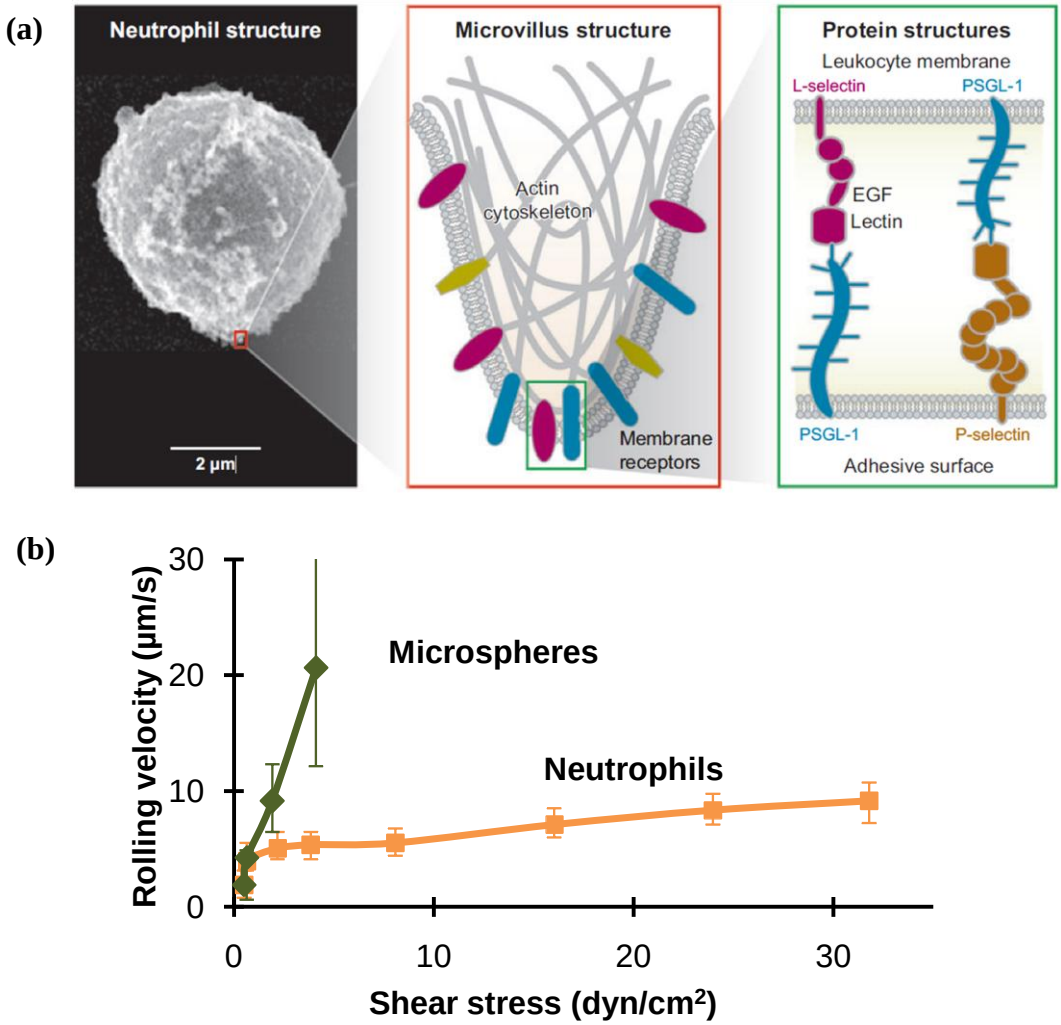

Figure 2. Rolling of neutrophils. (a) Neutrophils are covered with small protrusions called microvilli (shown in the electron micrograph). Microvilli tips are decorated with transmembrane receptors (like PSGL-1, L-Selectin) which can bind to complementary ligand molecules on the endothelium or an artificial substrate. Adapted from (1). (b) Neutrophils show extremely stable rolling on P-selectin surface over a wide range of shear stress while ligand coated microspheres roll with high velocities only until moderate shear stress. Data reproduced from Yago.et.al (2)

Thus, models of cell rolling serve an important role in exploring the effects of different parameters on cell rolling. Coupled with experiments, models can give new insights into the biophysics of cell rolling.

In this study, we present a semi-analytical model of cell rolling that includes all the biophysical features – deformability, force-dependent molecular interactions, microvillus dynamics and receptor clustering – that are implicated in cell rolling. Using a novel approach, the deterministic kinetics of bond formation have been integrated into a probabilistic framework governing the transport of surface-tethered microvilli. The model agrees very well with earlier experimental results both quantitatively and qualitatively. The model has been used to study some of the intriguing aspects of rolling like the effect of different cellular features on rolling and interesting trends have been discussed. Lastly, the model was applied to predict rolling behavior of ligand coupled microspheres where it showed agreement with experiments.

Chapter 2. Background

Cell rolling was studied *in vitro* by Lawrance et.al (3) by rolling neutrophils on P-selectin incorporated lipid bilayers. They observed that cells could roll on selectin surfaces in a steady manner with stable velocities similar to that observed *in vivo*. Puri.et.al (14) compared the kinetics of rolling for different selectin molecules (P, E and L-selectin) and concluded that the interaction of P-selectin was strongest. P-selectin glycoprotein ligand-1 (PSGL-1) was first identified by Moore (7) as a ligand for P-selectin present on neutrophils. It was also observed that 70% of PSGL-1 was located on the tips of microvilli on neutrophils. Similar observation showing clustering of L-selectin was also later made by Bruehl (15). In order to mimic cell rolling, Hammer and co-workers used ligand coated microspheres that rolled on selectin coated surfaces (16). Although the microspheres could attach and roll on the selectin surfaces, they could not show stable rolling as seen with cells and often rolled with higher velocities. Several aspects of neutrophils like ligand dimerization (17) and microvilli tethering (transition of microvillus rheology to viscous dominated regime) (18) were also been implicated in cell rolling. Although some of the aspects of cell rolling have been studied experimentally, proper investigations of different features have been largely left to modeling and simulation studies.

A series of theoretical and computational models have been developed over the past two decades to explore cell rolling. Among the first models of cell rolling was the peeling dynamics model by Dembo et al (19). They considered only the trailing edge of the cell and modeled the cell membrane as inextensible and elastic and the bonds as

Hookean springs. Coupling the reaction kinetics with the bending mechanics of the membrane, constitutive relations governing membrane peeling were obtained. The model was used to predict transient and steady rolling characteristics of cells. The stochastic nature of cell rolling was first studied by Cozen-Roberts (20, 21) and later by Zhao (22) who modeled bond formation and breakage as probabilistic events, which was used to predict the instantaneous rolling velocity of the cell. These early models could simulate the stochastic motion of the cell seen through experiments (23), but they excluded many relevant biophysical interactions such as microvillus mechanics, receptor clustering, and cell deformability, thus limiting the usefulness of these models. Adhesive dynamics developed by Hammer and co-workers (24) was the first detailed model of cell rolling which modeled the reaction kinetics as probabilistic events and also accounted for van der Walls attraction, electrostatic interactions, gravitational forces, steric stabilization, microvillus structure and glycocalyx interaction. Adhesive Dynamics could recreate the transient 'stop and go' motion of the cells and calculate the mean and the variance in velocity. It was also used to study the effects of different parameters like bond properties, number of receptors on microvillus tips etc. Later versions of the model incorporated Bell's equation for calculating force-dependent bond dissociation (8, 25), which revealed the sensitive dependence of the rolling velocity on bond kinetics. Using the same model, Caputo et al. (26) studied the effects of receptor clustering and microvillus rheology, and found that rolling was slowest at a microvillus viscosity close to that measured experimentally.

Although AD is the most advanced model of cell rolling, it is computationally intensive, making it difficult to rapidly examine the effects of different parameters on cell

rolling. Moreover, Adhesive Dynamics models the cell as a rigid sphere neglecting its deformability which is known to play an important role in the rolling response (27). Semi-analytical models are more suited for parametric studies because of their simplicity and flexibility. Although semi-analytical models may not enable detailed simulations like Adhesive Dynamics, they can be used to gain deeper understanding into the physics of cell rolling.

A simple semi-analytical model was developed by Tozeren and Ley (28), where the cell was modeled as a rigid sphere with the assumption that bonds between the cell and the surface were stressed everywhere except at the leading edge of the cell. Bond formation and disassociation rates were assumed to be independent of applied force and obtained by fitting the model to experimental data. The authors showed that bond length and flexibility were critical parameters for rolling to occur. Although an useful model, it suffered from unphysical assumptions like compression of bonds, high on and off rates and mechanical properties of the cell. Later, Krasik and Hammer (29) presented an improved semi-analytical model of cell rolling based on insights obtained from Adhesive Dynamics simulations. Their model assumed cells to be rigid spheres, with the load being carried only by bonds at the trailing edge of the cell. The rate of the transport of bonds to the trailing edge was equated with the force-dependent dissociation rate, leading to an estimation of the net force acting on the cell due to the strained bonds. Parametric study of cell rolling was made using different non-dimensional numbers and a relationship between various parameters necessary for rolling was discussed. Although the rolling velocity at different shear stresses predicted by the model was numerically close to experimental results, they were qualitatively different. The model predicted a monotonic

increase in rolling velocity with shear stress that was almost exponential in nature. On the contrary, experimental data showed an initial rapid increase in rolling velocity with shear stress (upto 2 dyn/cm^2) followed by a steady rolling velocity with little or no increase with shear stress (3). This phenomenon of stabilization of rolling at high shear stress has also been observed in other studies for upto shear stress of 35 dyn/cm^2 (2, 14). Although features like microvillus extension (18), PSGL-1 dimerization (17) and other cellular and molecular properties (2) have been implicated in stabilization of rolling, a clear understanding is yet to be obtained. Thus the qualitative disagreement between the Karsik model (29) and experimental observations was probably because microvillus and cell deformation were not accounted in the model.

Chapter 3. Model Description

We consider a cell rolling at a steady velocity V under a fluid flow with shear rate $\dot{\gamma}$ (Figure. 3). The cell has flexible microvilli on its surface which posses adhesion molecules on their tips (7, 15). Deformability of the cell allows for a considerable surface area of the cell to come in close proximity to the ligand coated substrate and the microvilli can bind to the substrate. As the cell rolls forward, the attached microvilli extend, exerting a tensile force both on the receptor-ligand bonds and on the cell. The force carried by the microvillus is distributed equally amongst the existing number of bonds on its tip which in turn governs the lifetime of the bonds and ultimately the time span for which the microvillus remain bound to the substrate. At any given instant of time, the sum of forces exerted by all the microvilli on the cell balances the fluid drag force, enabling the cell to roll with a steady velocity. The details of each aspect of the model are described below.

3.1 Model of the cell

The cell is modeled as a sphere of radius R and the rolling substrate (or endothelium) as a flat plane. The cell being deformable forms a circular contact area of radius r with the surface. Extensible structures known as microvilli exist on the cell surface at a density of N_m μm^{-2} each with a tip area of A_m. Adhesion molecules (e.g. PSGL-1) are expressed on the surface of the cell at an average density of $\bar{N}_R$. However, consistent with previous electron microscope studies (7, 15), we assume that the adhesion

molecules are localized on the tips of the microvilli. Thus, the actual receptor density on the microvillus tip will be much higher than the average density and is given by $N_R = \bar{N}_R / (N_m A_m)$, $N_m A_m$ representing the fraction of the cell surface actually contacting the substrate and is an indicator of receptor distribution. This number can vary between 0 and 1 , a lower value representing a tighter packing of adhesion molecules either due to fewer or thinner microvilli.

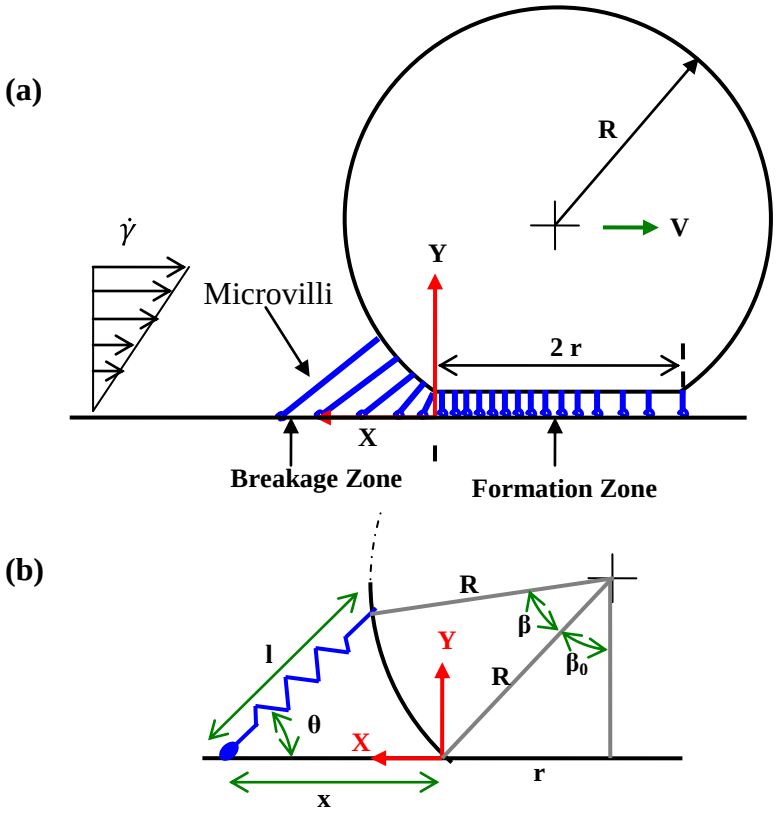

Figure 3. (a) Schematic diagram of the model. (b) Microvillus extension.

3.2 Cell Deformability

Experiments and numerical studies have shown that fluid shear modulates the contact area of the cell during rolling. The contact area increases with fluid shear initially but saturates to a stable value at large shear rates (30, 31). Since an accurate prediction of the contact area is not possible through a semi-analytical treatment, we used an empirical relation to incorporate the effect of shear modulation of contact area. We assume that the cell maintains a finite contact area with the substrate even at zero shear stress because of the microvilli and flexible adhesion molecules present on its surface. As the shear stress increases, the contact area increases remaining circular in shape until it saturates at a maximum value. Then the contact radius at any given shear stress is approximated by the relation

$$r = r_{\max} - \left(r_{\max} - r_{\min}\right)\exp\left(-\tau/\tau_o\right) \tag{1}$$

r_{min} and r_{max} are the minimum and maximum contact radius of the cell , τ is the fluid shear stress while τ_o is a measure of rigidity of the cell indicating how the contact radius changes from r_{min} to r_{max}. A conservative estimate of r_{min} can be made by calculating the area of contact due to microvilli. We consider the cell as a hard sphere with stiff microvilli present on its surface. At zero shear stress, the cell is expected to rest on three microvilli. Since the average distance between the microvilli are approximately 1 μm, r_{min} is taken as 0.5 μm. We can also reach a similar value by considering the flexibility of the ligand only. Assuming the cell as a hard sphere coated with highly flexible ligands, the contact area would be defined as the region where the ligands are not extended and hence are force free. This happens upto a point where the separation between the sphere

surface and the substrate is less than the natural length of the ligand. For neutrophil with radius of 4 μm and PSGL-1, a homodimeric ligand of around 50 nm length (28, 31) present on its surface, we calculated r_{min} to be approximately 0.5 μm. Other properties like r_{max} and τ_o are found from comparison with experiments.

3.3 Microvillus Mechanics

Microvilli play an important part in the rolling process by acting as a mechanical linkage between the adhesive bond and the cell body. Micropipette experiments by Shao et.al (32, 33) showed that neutrophil microvillus undergoes elastic dominated extension at low pulling force but behaves like a viscous material under a large force. Both microvillus extension and tethering have been implicated in stabilization in rolling of neutrophils under high shear stress (18, 34), and its effects shown though numerical studies (26). However, the extension model for microvillus used in the previous cell rolling studies (26, 29) is valid only for static forces and is inaccurate under dynamic loading. In this paper the microvillus is modeled by a three-element model consisting of a series of a spring (stiffness K_m) and damper (viscosity η_m), both in parallel to a second spring (stiffness K_c) (see figure 4). Transmembrane proteins like PSGL-1, L-selectin are anchored to the cytoskeleton (35) and hence any force applied to the molecule is transmitted both to the cytoskeleton and the membrane. The cytoskeletal extension is elastic in nature and is represented by the spring K_c while the viscoelastic membrane is represented by the spring K_m and damper η_m in series. The membrane and the cytoskeleton contribute individually to carry the load on the molecule - hence a parallel

arrangement of their representative elements in our model. Microvillus undergoing extension suffers a transition to a viscous dominated regime after the pulling force exceeds a critical value (33, 36), and is believed to be caused by the uprooting of the PSGL-1 molecule from the cytoskeleton (36). This phenomenon is know as tethering. In order to incorporate tethering in our model, we assumed that once force in the cytoskeletal spring (K_c) reaches a critical value of F_0 the cytoskeletal link is severed and the force in that component is clipped at F_0. Hence, when the pulling force is large or the microvilli are pulled to a long length a viscous dominated behavior is observed, which gives similar results to experimental observations (32, 33, 36, 37). Using this model, the constitutive relation at a steady rolling velocity is given by

$$\begin{cases} V\dfrac{dF_m}{dx}+\dfrac{K_m}{\eta_m}F_m=\dfrac{K_mK_c}{\eta_m}l+\left(K_m+K_c\right)V\dfrac{dl}{dx} & \text{for } K_cl\le F_0 \\ V\dfrac{dF_m}{dx}+\dfrac{K_m}{\eta_m}\left(F_m-F_0\right)=K_mV\dfrac{dl}{dx} & \text{for } K_cl>F_0 \end{cases} \tag{2}$$

Where l is the microvillus extension, F_m is the force exerted on the microvillus and F_0 is the transition force. Comparing the behavior of the model with previously published results of Shao (33) we assign the values of the cytoskeletal spring K_c to 43 pN/μm and the membrane damper η_m as 11 pN-s/μm. The resulting model, which has K_m as the only unknown parameter, was fitted with the experimental data for viscoelastic relaxation of a micovillus by Xu (37) which yielded a value of 200 pN/μm for K_m. Although another three element model with a different arrangement of the elements has been used by Xu (37), we chose to use the current model because of its physical significance which

correlates directly with the biology and its ability to predict microvillus behavior both before and after transition to tethering.

The microvillus is attached to the surface through the receptor-ligand bonds which also carries its load. Assuming that the load is shared equally between the existing bonds on the microvillus tips, the force per bond can be obtained from $f_b = F_m / (N.A_m)$ where f_b is the force per bond on the microvillus tip and N is the density of bonds on the tip.

3.4 Kinetics of bond formation

We consider two distinct regions in this model – the 'formation zone' where the microvilli are stress free allowing bond formation and breakage to occur at intrinsic rates, and the 'breakage zone' where the microvilli are stretched and only bond dissociation occurs (see figure 1). The bond disassociation rate k_b is dependent on the force on the bond as given by Bell's model (25) $k_b = k_b^0(r_c f_b / k_B T)$ where k_b^0 is the intrinsic breakage rate, r_c is the reactive compliance (that corresponds to the range of the interaction), f_b is the force on a single bond and $k_B T$ is the thermal energy. Pinning the coordinate system at the trailing end of the cell as shown in figure 3, the kinetic rates in formation zone ($x<0$) are $k_f = k_f^0$ and $k_b = k_b^0$ while those in the breakage region ($x \geq 0$) are $k_f = 0$ and k_b obtained from the Bell's equation. At steady state, the kinetic equation governing bond formation can be expressed as

$$-V\frac{dN}{dx} = k_f\left(N_R - N\right)\left(N_L - N\right) - k_b N \tag{3}$$

where N is the bond density on the tip of a microvillus positioned at x, N_R is the actual receptor density on the microvillus tip, N_L is the ligand density on the surface, k_f is the formation rate, and k_b is the breakage rate.

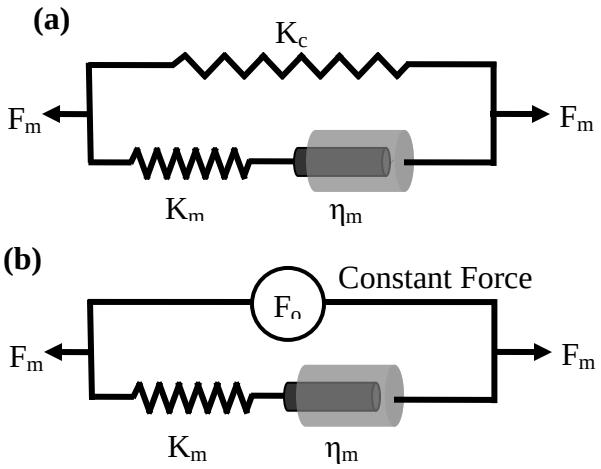

Figure 4. Viscoelastic model of microvillus for a force less than (a) and greater than (b) the threshold force F_o.

3.5 Kinematic relations

The extension of a microvillus is expressed in terms of the cell geometry and the distance between the point of attachment of the microvillus to the surface and the cell. We assume that the cell itself does not undergo any deformation except for the contact area, and that the microvilli are unstretched at the trailing edge ($x=0$) (see Figure. 3b). At a given location x, defining $\beta_0 = \sin^{-1}(r/R)$ and $\beta = x/R$, the microvillus extension l and the angle θ between the microvillus and the surface (see figure. 3b) can be expressed as

$$l = \sqrt{\left(x + r - R\sin(\beta + \beta_0)\right)^2 + \left(R\cos\beta_0 - R\cos(\beta + \beta_0)\right)^2}$$
$$\theta = \tan^{-1}\left(\frac{R\cos\beta_0 - R\cos(\beta + \beta_0)}{x + r - R\sin(\beta + \beta_0)}\right) \quad (4)$$

3.6 Force balance

The net force exerted on the cell due to the receptor-ligand bonds is obtained by integrating the forces due to individual microvilli. The probability of bound state of a microvillus, defined as $\phi(x)$, is equivalent to the probability of existence of *atleast* one bond on the microvillus tip. Since bond formation only occurs inside the contact region, a microvillus starts with a certain number of bonds from the trailing edge and gradually loses more bonds as it moves further into the breakage zone. Thus the probability of existence of a bond can be defined as $p_i = N/N_0$ where N_0 is the bond density that the microvillus started with, in current case at x=0. Defining the total number of bonds a microvillus starts with before entering the breakage zone as $z = N_0 A_m$, the probability that the microvillus is attached to the substrate or in bound state is given by

$$\phi = 1 - (1 - p_i)^z \quad (5)$$

The net bond force in x-direction is then obtained from

$$\frac{dF_x}{dx} = \phi . F_m . N_m . (2r) . \cos\theta \quad (6)$$

where F_x is the x-component of the net force exerted by the bonds on the cell. The definition of the bound state enables us to compute many interesting parameters like local microvillus density, average microvillus length, simply by using the bound state

probability as a weighing function. The definition of probabilistic weights eliminates the need for arbitrarily defining the extent of the breaking region as with earlier models (27, 38).

Under steady state, the external fluid flow imposes a drag force on the cell, which balances the net force exerted on the cell due to receptor-ligand bonds. The drag force is calculated from Goldman's equation (39).

$$F_x = 1.7\left(6\pi\tau R^2\right) \tag{7}$$

where τ is the fluid shear stress, and R is the radius of the cell. Eqn. (7) assumes that the cell is stationary, which enables a simple expression of the drag force. This assumption is not in serious error as velocities of rolling cells are typically an order of magnitude smaller than the fluid flow velocity at a distance R away from the surface. It is noteworthy that Eqn. (7) was derived for a hard sphere and employs lubrication theory – a situation not physically valid for cell rolling since cells are deformable. Computational models have shown that although the drag force scales linearly with shear stress, the pre-factor (which is 1.7 in Eqn. (10)) is smaller for a deformed cell than an undeformed sphere. Although computational models are required to obtain the exact drag force, Eqn. (10) provides a reasonable approximation.

Chapter 4. Numerical scheme

The formulation presented above involves simultaneous solution of coupled ordinary differential equations along with nonlinear equations. Hence, we have adopted a novel iterative scheme which is accurate and computationally inexpensive. Our approach involves iterating the rolling velocity in order to find the desired fluid shear stress. From the target shear stress, the contact radius is calculated from Eqn. (1) and a rolling velocity V is assumed. Based on rolling velocity, the microvillus force history is obtained by solving Eqn. (2) along with the kinematic relations in Eqn. (4). The force history is used to get the bond kinetics from Eqn. (3) with the initial condition of $N = 0$ at $x = -2r$, which is then used to get the value of ϕ at each location. Then, Eqn. (6) is solved to obtain F_x, which gives the value of shear stress τ using Eqn. (7). Depending on the error of the calculated value of shear stress, the rolling velocity is updated and the process is repeated till the iterations converge. A binary search algorithm was employed to search for the rolling velocity in the above-mentioned manner with a maximum error in shear stress as 0.1-1%. All the ordinary differential equations were solved using a variable order solver in MATLAB with error tolerance of 10^{-4} and maximum step size of 10^{-2}. The convergence of the scheme was fast and required about 10-30 s to converge for a single value of shear stress on a PC. Thus, this scheme easily allowed for a parametric study with little computational resources.

Chapter 5. Results

In the present study, we use our model to study the rolling of neutrophils on P-selectin coated surfaces mediated through the PSGL-1 ligands present on the neutrophil surface. The numerical values for the parameters used in the simulations along with their source references are listed in Table 1. Although the numerical values for most of the parameters were taken directly from experimental studies available in literature, deformability and the microvillus properties have been estimated indirectly as described in previous sections.

Table 1. Numerical values of parameters used in simulation (unless otherwise mentioned).

Symbol	Definition	Value	Source
R	Cell Radius	4 μm	(26)
r_{max}	Maximum contact radius	2 μm	Best case match with (14)
r_{min}	Minimum contact radius	0.5 μm	Estimated
τ_0	Deformability factor	15 dynes/cm^2	Best case match with (14)
K_c	Microvillus spring constant representing cytoskeletal stiffness	43 pN/μm	Comparison with (33)

K_m	Microvillus spring constant representing membrane stiffness	200 pN/μm	Fitting with data of (37)
F_0	Transition force	45 pN	(33)
η_m	Membrane viscosity	11 pN-s/μm	Comparison with (32, 33)
k_f^0	Intrinsic bond formation rate	0.1 $\mu m^2/s$	(29)
k_b^0	Intrinsic bond breakage rate	1 s^{-1}	(40)
r_c	Reactive compliance of bond	0.5 Å	(40)
T	Temperature	300 K	
$\bar{N}_R$	Average receptor density on cell	24 μm^{-2}	(26)
N_L	Ligand density on substrate	100 μm^{-2}	
N_m	Microvillus density on cell	4 μm^{-2}	(7, 26)
A_m	Area of microvillus tip	0.02 μm^2	(7, 26)

5.1 Comparison with experiment

In order to validate the model we compared our numerical results with the experimental data of Puri et.al (14). The ligand density was matched with that used in the experiment (N_L =90 μm^{-2}) and the rolling velocity was obtained for different fluid shear

stresses. The same process was repeated for different values of r_{max} and τ_0 and we found that the best case match was for r_{max} = 2 μm and τ_0= 15 dynes/cm^2 which are quantitatively reasonable. The comparison plotted in Figure 5, shows an excellent agreement between the experimental and numerical data both at low and high shear stress. In the following sections, we used the model to study the contributions of specific cellular features to cell rolling to answer some interesting questions.

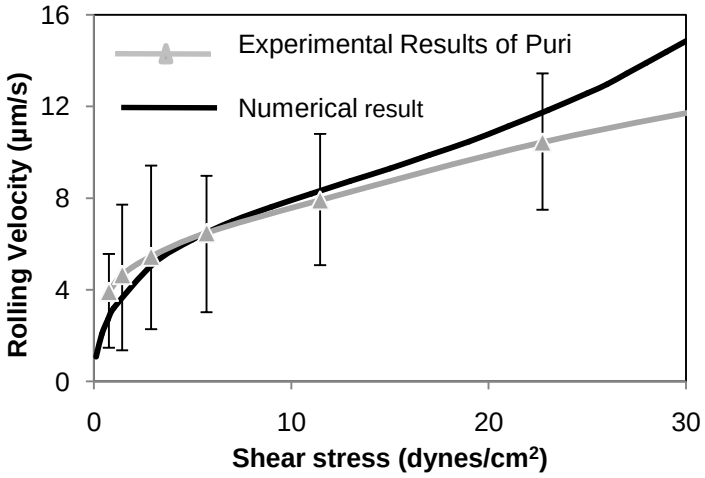

Figure 5. Comparison of model with experimental data of neutrophil rolling on P-Selectin by Puri et.al (14). The ligand density is 90 sites/μm^2.

5.2 Effect of cell deformability on rolling

Deformability has long been identified as an important feature in cell rolling. Experiments with live cells and fixed cells showed marked difference in rolling characteristics between the two cases (2) indicating the importance of cell deformability. However, cellular deformation and the contact area are known to be modulated by the fluid shear (31). We used our model to explore how this modulation of deformation

affects the rolling characteristics of a cell. In our model τ_0 represents the rigidity of the cell and determines how strongly fluid shear changes the contact radius (see figure 3). We compared the variation of rolling velocity with fluid shear for different values of τ_0 (see figure 6) and found that the change in contact area can profoundly affect the rolling behavior of the cell. While extremely rigid cells ($\tau_0=\infty$) showed unstable rolling with high rolling velocity that increased almost exponentially with increase in shear stress, highly deformable cells ($\tau_0=0$) exhibited stable rolling with only moderate increase in rolling velocity with increase in shear stress. Interestingly the effect of shear modulation was prominent in moderately deformable cells (intermediate values of τ_0) which exhibited a rapid increase in rolling velocity at low shear stresses (< 5 dyn/cm^2) but attained steady rolling velocity, similar to the highly deformable case, at higher shear stresses. In fact, Yago. et al (2) observed that K562 cells coupled with PSGL-1 could roll on P-selectin surface at high shear stress only when they were alive, and rolling was impaired when the cells were fixed. Although, we could not recreate their experiment because of the uncertainly of cellular and structural properties of K562 cells, the experimental observation qualitatively agrees with our prediction. An important fact that this result shows is that shear modulation of contact area helps the neutrophils in maintaining a stable rolling velocity over a wide range of shear stress. This phenomenon might have a biological relevance as it provides a mechanism to prevent aggregation of cells at places of low shear stresses like junctions of arteries and veins.

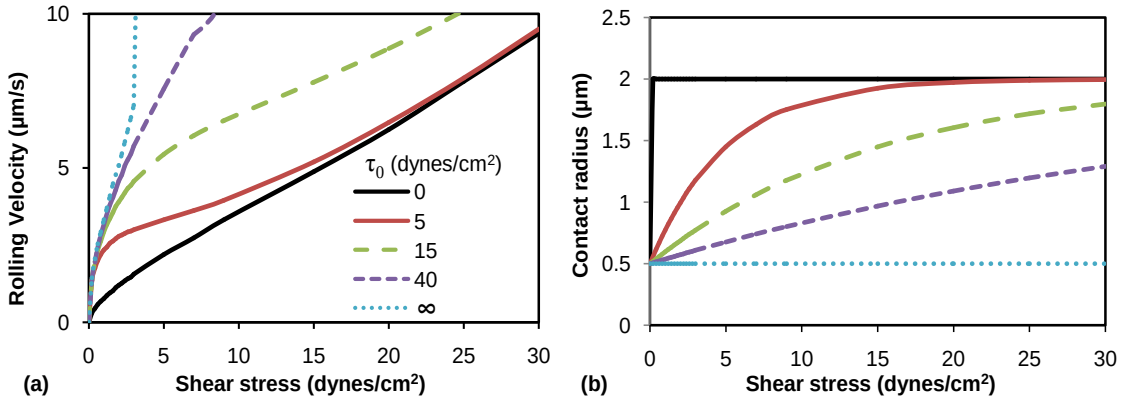

Figure 6. Effect of deformability on cell rolling. Rolling velocity as a function of shear stress (a) is plotted for several values of τ_0. The modulations of the radius of contact for these values of τ_0 are also shown (b).

5.3 Role of microvillus and localization of receptors

The structure and function of microvilli have been shown to be important for cell rolling and have been extensively studied before (26). Tethering of microvilli, where their rheology shifts from elastic to viscous dominated regime, has been attributed to stabilization of rolling at high shear stresses (18). In this study, we investigate some important mechanical aspects of the microvillus and study its implications on cell rolling. Microvilli localize the adhesion molecules on their tips resulting in a co-operative effect during bond breakage. As a microvillus moves from the leading edge through the formation and breakage zone, the number of bonds formed on its tips changes. The history of the number of bonds on present on a microvillus tip at different locations (x-distance) is plotted in figure 7a. The shear stress was 2 dyn/cm^2 and the corresponding

contact radius was approximately 0.69 μm. Bonds form in the contact region, reach equilibrium quickly and start to break as soon as they enter the breakage zone (x>0). Interestingly, the number of bonds in the breakage region falls steadily upto a point after which there is a sharp drop until no bonds are left. This behavior results from the co-operative load sharing by the bonds whereby the force on a bond is reciprocal to the number of surviving bonds. As the number of bonds on the tip starts reducing, the load on the surviving bonds increases leading to faster breakage. Previous models which do not account for microvilli, did not observe this effect (28). Instead of a sharp breakage of bonds, an exponential decay of bonds typical of first order reaction was seen. Our results are also agreement with experimental results of Ramchandran (18) where the tethers of around 2μm were observed at a shear stress of 2 dynes/cm^2.

Microvilli attached at each location contribute towards the net force experienced by the cell depending on its extension. In order to find the relative contribution of microvillus at each location towards the net force, we plotted the x-direction force distribution given by the right side of Eqn. (6) as a function of x (see figure 7b). We found that the force distribution peaks at an intermediate value x showing that the microvilli located at that position have a maximum contribution to the net force on the cell. It should be remembered that the force distribution depends on the force carried by each microvillus and the microvillus density. As we move away from the trailing edge, the extension of the microvillus increases along with the force carried by it. However, the number of attached microvilli (given by the product of $N_m\varphi$) also decreases with increasing extension (see figure 7b). Hence, an optimum exists because of the two opposing effects.

Tethering of microvilli is believed to be important in stabilizing rolling at high shear stress (18). Tethered microvilli carry a nearly constant load and prevent any increase of force on the bonds, thus increasing the lifetime of the bonds. We plotted the fraction of the total attached microvillus that tethered along with rolling velocity against shear stress as shown in figure 7c. We found that tethering started only for shear stress in excess of 0.7 dynes/cm^2 and continued to increase with increasing shear. However, at higher shear stresses (> 15 dyn/cm^2) the tethering fraction was stable at about 40% and increased only modestly with increase in shear. It is known that tethers grow into more complex structures over time (18) which might explain the stabilization at high shear stress. However including such effects in the model is difficult and outside the scope of the present study.

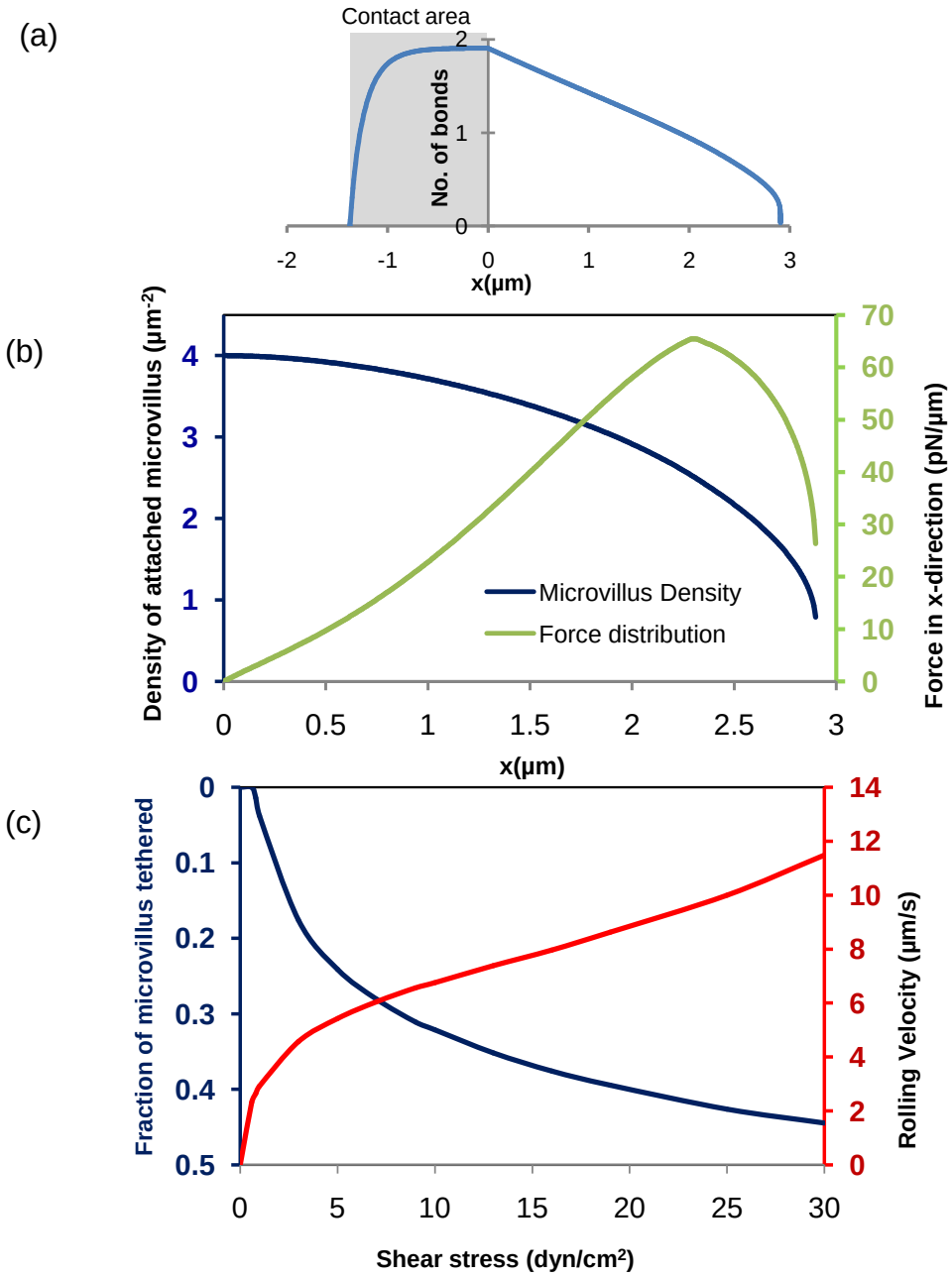

Figure 7. (a) Number of bonds on microvillus tip at different positions (b) Number of attached microvillus per μm^2 at different distance from the trailing edge (blue) and the x-direction force distribution (green). (c) Fraction of the total microvilli that are tethered at a given instant of time at different shear stress (blue) and corresponding rolling velocity vs shear stress (red). The shear stress was 2 dyn/cm^2 in all the cases.

An important aspect of the microvillus structure is localization of the adhesive receptors on its tip. Redistribution of receptors has two direct implications – it increases the receptor concentration to very high values on the tip and it allows the bonds to share load leading to a co-operative effect. To study the first effect we varied the microvillus tip area keeping their density (N_m) constant. This represents the case when the microvilli on the cell become thinner but their numbers remain the same. The result is plotted in Figure 8a which shows that on decreasing the tip area, the rolling velocity remains constant upto a certain value after which it increases sharply and the cell can no longer support rolling. The observation can be explained based on the bond density. The only parameter that variation of A_m affects is the local concentration of the receptor at the tip N_R. As long as N_R is lower than the ligand density N_L, all the available receptors can form bonds and contributes toward the load. However, at lower values of A_m, N_R is much larger than N_L and bond formation is compromised due to unavailability of ligands. Hence rolling becomes unstable as the tip area decreases. The critical tip area below which rolling would be unstable can be found as $A_m^* \sim N_R/(N_L N_m)$ which for the present case gives 0.05 μm^2. The predicted trend can clearly seen in figure 8a.

We studied another interesting scenario where each microvillus splits into two or more smaller microvilli, their total tip area ($N_m A_m$) remaining constant. This arrangement keeps the local receptor concentration at the tip N_R constant, but changes the number of receptors on each microvillus thus changing their co-operative effect. Rolling velocity was plotted against microvillus density, keeping the product $N_m A_m$ constant at 0.08 (see figure 8b). Surprisingly we found that optimum rolling existed only when the number of receptors on microvillus tip was between 8 to 60. Either increase or decrease in receptor

number resulted in an increase in rolling velocity upto a point where rolling became unstable. We believe two independent mechanisms results in this behavior. As the number of receptors per microvillus tip decreases, the co-operative effect is lost and lifetime of bonds decreases, resulting in faster breakage of bonds and higher rolling velocity. On the other extreme, if too many bonds are present on a single microvillus, the number of available microvilli reduces (since $N_m A_m$ is constant). As a result, even if the lifetime of individual microvilli is longer, their collective effort or the net force reduces and rolling velocity is higher.

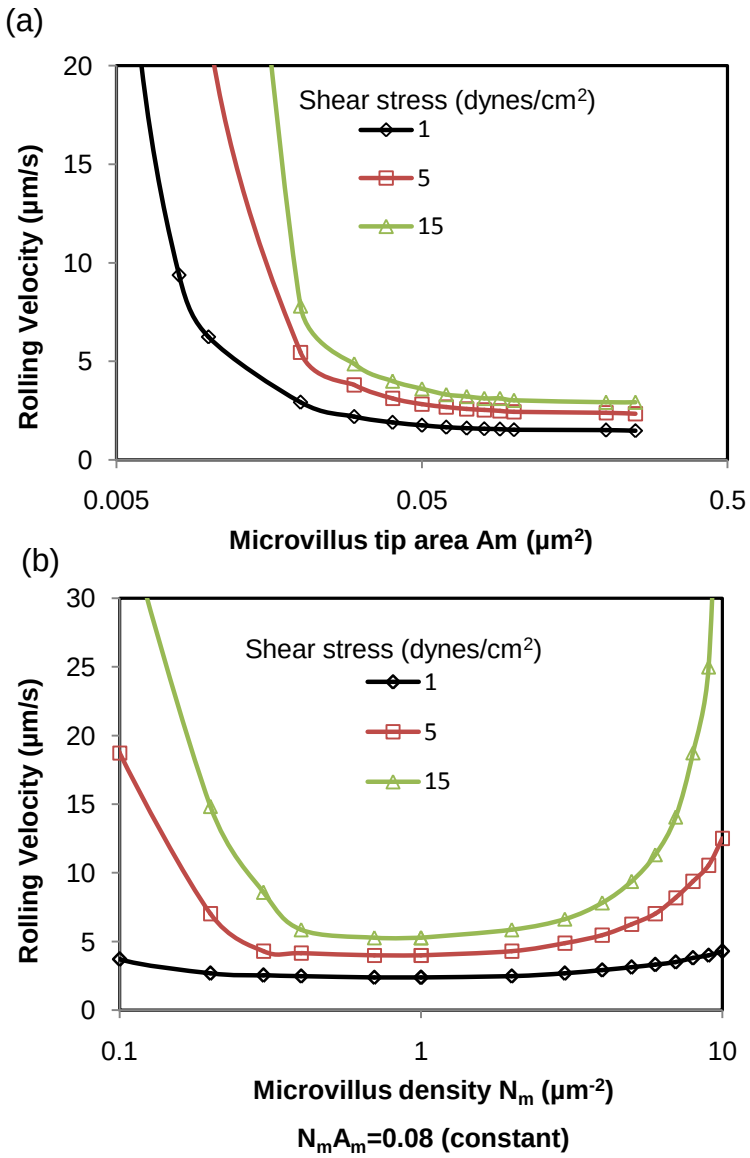

Figure 8. (a) Effect of the microvilli tip area on rolling velocity. Different markers and colors represent different shear stresses. All other properties are kept constant. (b) Effect of number of microvilli on rolling velocity for constant receptor density on microvillus tip and constant total tip area (N_mA_m) .Total number of receptors per unit area was kept constant.

5.4 Case study: Rolling of cells and microspheres

In this last section, we apply our model to different situations and answer some interesting questions. We used our model to simulate rolling of neutrophils and microspheres and compared the results with experimental data of Yago et.al (2). The ligand density (P-selectin) of the substrate was adjusted to 145 μm^{-2} and the neutrophil radius was set to 4.25 μm as reported. Other parameters were used as reported in Table 1. In order to simulate rolling of microspheres we changed the cell radius to 3 μm, and set τ_0 to a large number (i.e. the contact radius was fixed at 0.5 μm). The receptor density (N_R) was taken as 203 μm^{-2} corresponding to 23,000 sPSGL-1 molecules per particle measured during the experiment (2). Microspheres have random distribution of bonds and no microvilli. Instead the bonds acts as molecular springs to carry load (41). We considered this by setting $N_m=N_R$ and assumed the bonds to be pure elastic with spring constant 1000 pN/μm (K_c=1000 and K_m=0) which is in the range of molecular springs (41). Figure 9(a) shows a good agreement between the experimental and numerical results for both the neutrophil and microsphere asserting the flexibility of the model to describe different systems.

One of the intriguing aspects of cell rolling is the ability of the cell to roll steadily with almost constant velocity over a wide range of shear stress. In order to understand this behavior, Yago et.al (2) performed a series of experiments with sPSGL-1 coated microspheres and neutrophils, and studied the contribution of molecular and cellular features separately. Their results showed that the molecular properties, although necessary, are not sufficient to provide stable rolling and cellular properties are important

in this context. It also showed that deformability is very important for the cell to roll stably. Although this study could elucidate the difference between cellular and molecular features, the relative importance between the different cellular features was not clear. Studies had independently confirmed the importance of microvilli in stabilizing of rolling (18). Hence, we wanted to investigate the relative contribution of deformability of the cell and microvillus dynamics on stabilization of rolling.

In order to compare the contribution of microvillus and deformability towards stable rolling, we simulated three hypothetical cases – first, where the neutrophils can extend microvilli but are not deformable, second, where the neutrophils are deformable but cannot extend microvilli (like a deformable microsphere uniformly coated with receptors) and third, with no deformation or microvilli (microspheres with same radius and receptor density as neutrophils). While the first case was simulated by setting τ_0 to a large value, for the second case we switched the microvillus properties to those of elastic molecular springs similar to those used for microspheres. The third case was simulated by using the same procedure as used before with microspheres, but with the parameter values similar to neutrophils ($N_m = N_R = 24\ \mu m^{-2}$, $A_m = 1/N_m$). We found that in all the three hypothetical cases the cell could not roll over the full range of shear stresses (0-10 dyn/cm^2) (See figure 9(b)) and the plotted lines ended abruptly referring to the region where rolling cannot be supported according to our model. Rolling was worse when none of the cellular features were present and became progressively better as deformability and microvilli extension was incorporated. It seemed to be the case that microvilli extension were more potent in stabilizing rolling than deformability alone. Deformability acts to increase the contact length and hence the total number of bonds formed. However,

because of high rate of formation, equilibrium is attained very quickly and any advantage gained by added deformability is not useful unless rolling velocity is high. On the other hand, microvillus extension actually increases the bond lifetime in the breakage region directly helping in carrying more load. This might explain the observed effect of the two cellular properties.

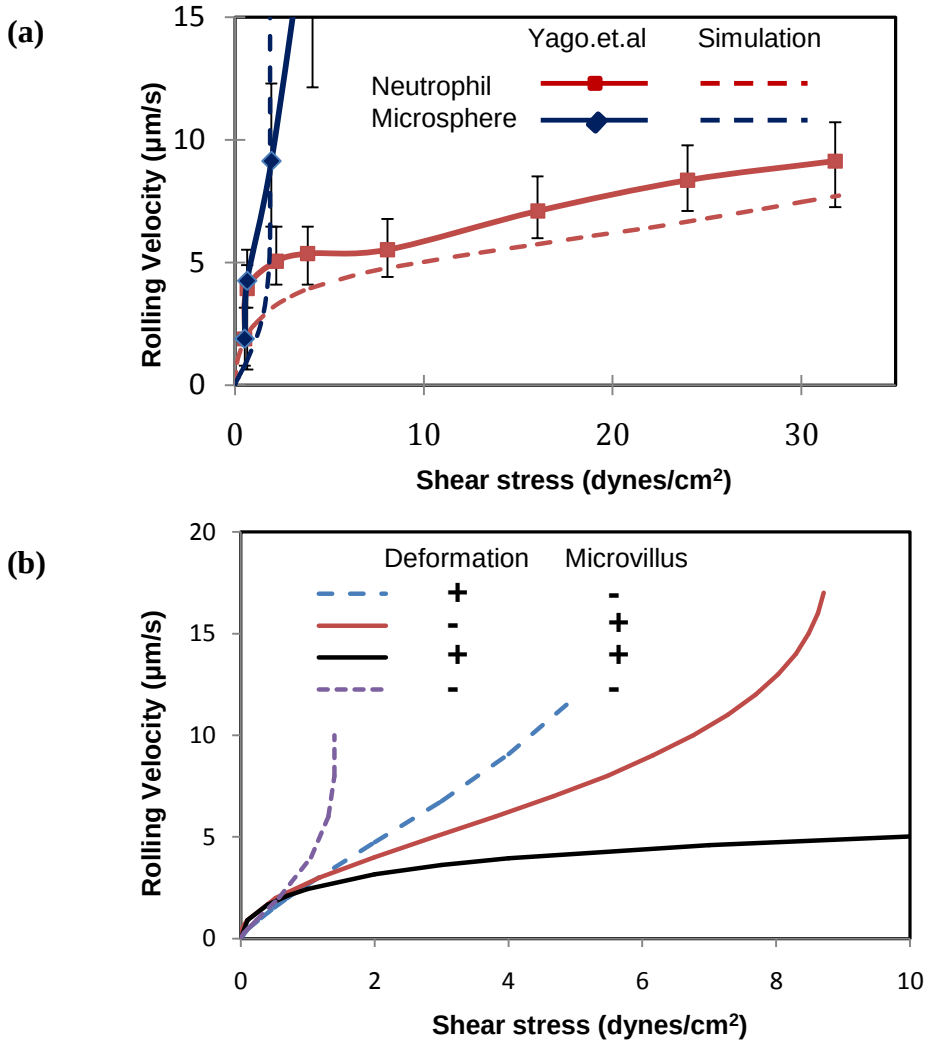

Figure 9. Contribution of different cellular features to stability of cell rolling. (a) Comparison of rolling of neutrophils (radius 4.25 µm) and sPSGL-1 microspheres (3 µm radius) rolling on P-selectin coated surface (145 sites/µm^2) with experimental results of Yago (7). (b) Hypothetical case of rolling of neutrophils without deformation, without microvillus or both, presented along with normal neutrophils to evaluate the relative contribution of the two features in rolling.

Chapter 6. Conclusion

Cell rolling is an extremely complex phenomenon brought into effect by orchestration of different molecular and cellular features of the cell and its surrounding. Although our knowledge of cell rolling has progressed tremendously in the last decade with insight into the biophysics of molecular features, some very basic questions remain unanswered. In the current study, we have presented a model of cell rolling that considers all the major relevant phenomenon which are known to affect cell rolling. The semi-analytical nature of the model provides it with simplicity and makes it easily implementable. The model could recreate rolling of neutrophils over a wide range of shear stresses with excellent agreement with experimental data, not achieved previously by any other model. Our study showed that the stability of rolling over a wide shear stresses is a result of multiple cellular features. Shear modulation of contact area ensures that rolling velocities are moderate even at low shear stresses while microvillus extension along with increased deformation maintains stability in high shear stress regime. The model could also recreate the rolling of microspheres which points towards its flexibility. However, a few deviations from experimental results for rolling of microspheres was observed. Since microspheres are devoid of the cellular features, their rolling behavior is critically dependent on properties like bond kinetics, and an accurate prediction would require advanced stochastical simulations. Nevertheless, the results were in qualitative agreement.

References

1. Thomas, W. 2008. Catch bonds in adhesion. Annual Review of Biomedical Engineering 10:39-57.
2. Yago, T., A. Leppanen, H. Y. Qiu, W. D. Marcus, M. U. Nollert, C. Zhu, R. D. Cummings, and R. P. McEver. 2002. Distinct molecular and cellular contributions to stabilizing selectin-mediated rolling under flow. Journal of Cell Biology 158:787-799.
3. Lawrence, M. B., and T. A. Springer. 1991. Leukocytes roll on a selectin at a physiological flow-rates - distinction from and prerequisite for adhesion through integrins. Cell 65:859-873.
4. Lasky, L. A. 1995. Selectin-Carbohydrate Interactions and the Initiation of the Inflammatory Response. Annual Review of Biochemistry 64:113-139.
5. Kansas, G. S. 1996. Selectins and their ligands: Current concepts and controversies. Blood 88:3259-3287.
6. Ley, K. 2003. The role of selectins in inflammation and disease. Trends in Molecular Medicine 9:263-268.
7. Moore, K. L., K. D. Patel, R. E. Bruehl, F. G. Li, D. A. Johnson, H. S. Lichenstein, R. D. Cummings, D. F. Bainton, and R. P. McEver. 1995. P-Selectin Glycoprotein Ligand-1 Mediates Rolling of Human Neutrophils on P-Selectin. Journal of Cell Biology 128:661-671.
8. Chang, K. C., D. F. J. Tees, and D. A. Hammer. 2000. The state diagram for cell adhesion under flow: Leukocyte rolling and firm adhesion. Proceedings of the National Academy of Sciences of the United States of America 97:11262-11267.

9. Orr, F. W., H. H. Wang, R. M. Lafrenie, S. Scherbarth, and D. M. Nance. 2000. Interactions between cancer cells and the endothelium in metastasis. Journal of Pathology 190:310-329.

10. Springer, T. A. 1994. Traffic signals for lymphocyte recirculation and leukocyte emigration - the multistep paradigm Cell 76:301-314.

11. Greenberg, A. W., and D. A. Hammer. 2001. Cell separation mediated by differential rolling adhesion. Biotechnology and Bioengineering 73:111-124.

12. Karnik, R., S. Hong, H. Zhang, Y. Mei, D. G. Anderson, J. M. Karp, and R. Langer. 2008. Nanomechanical control of cell rolling in two dimensions through surface Patterning of receptors. Nano Letters 8:1153-1158.

13. Eniola, A. O., P. J. Willcox, and D. A. Hammer. 2003. Interplay between rolling and firm adhesion elucidated with a cell-free system engineered with two distinct receptor-ligand pairs. Biophysical Journal 85:2720-2731.

14. Puri, K. D., E. B. Finger, and T. A. Springer. 1997. The faster kinetics of L-selectin than of E-selectin and P-selectin rolling at comparable binding strength. Journal of Immunology 158:405-413.

15. Bruehl, R. E., T. A. Springer, and D. F. Bainton. 1996. Quantitation of L-selectin distribution on human leukocyte microvilli by immunogold labeling and electron microscopy. Journal of Histochemistry & Cytochemistry 44:835-844.

16. Brunk, D. K., D. J. Goetz, and D. A. Hammer. 1996. Sialyl Lewis(x)/E-selectin-mediate rolling in a cell-free system. Biophysical Journal 71:2902-2907.

17. Ramachandran, V., T. K. Epperson, T. Yago, M. U. Nollert, C. Zhu, R. D. Cummings, and R. P. McEver. 2001. PSGL-1 dimerization stabilizes cell rolling

on P-selectin in shear flow. Arteriosclerosis Thrombosis and Vascular Biology 21:709-709.

18. Ramachandran, V., M. Williams, T. Yago, D. W. Schmidtke, and R. P. McEver. 2004. Dynamic alterations of membrane tethers stabilize leukocyte rolling on P-selectin. Proceedings of the National Academy of Sciences of the United States of America 101:13519-13524.

19. Dembo, M., D. C. Torney, K. Saxman, and D. Hammer. 1988. THE REACTION-LIMITED KINETICS OF MEMBRANE-TO-SURFACE ADHESION AND DETACHMENT. Proceedings of the Royal Society of London Series B-Biological Sciences 234:55-83.

20. Cozensroberts, C., D. A. Lauffenburger, and J. A. Quinn. 1990. Receptor-Mediated Cell Attachment and Detachment Kinetics .1. Probabilistic Model and Analysis. Biophysical Journal 58:841-856.

21. Cozensroberts, C., J. A. Quinn, and D. A. Lauffenburger. 1990. Receptor-Mediated Cell Attachment and Detachment Kinetics .2. Experimental-Model Studies with the Radial-Flow Detachment Assay. Biophysical Journal 58:857-872.

22. Zhao, Y. H., S. Chien, and R. Skalak. 1995. A Stochastic-Model of Leukocyte Rolling. Biophysical Journal 69:1309-1320.

23. Alon, R., S. Q. Chen, K. D. Puri, E. B. Finger, and T. A. Springer. 1997. The kinetics of L-selectin tethers and the mechanics of selectin-mediated rolling. Journal of Cell Biology 138:1169-1180.

24. Hammer, D. A., and S. M. Apte. 1992. Simulation of Cell Rolling and Adhesion on Surfaces in Shear-Flow - General Results and Analysis of Selectin-Mediated Neutrophil Adhesion. Biophysical Journal 63:35-57.

25. Bell, G. I. 1978. Models for Specific Adhesion of Cells to Cells. Science 200:618-627.

26. Caputo, K. E., and D. A. Hammer. 2005. Effect of microvillus deformability on leukocyte adhesion explored using adhesive dynamics simulations. Biophysical Journal 89:187-200.

27. Lei, X., M. R. Lawrence, and C. Dong. 1999. Influence of cell deformation on leukocyte rolling adhesion in shear flow. J Biomech Eng-T Asme 121:636-643.

28. Tozeren, A., and K. Ley. 1992. How do selectins mediate leukocyte rolling in venules? Biophys. J. 63:700-709.

29. Krasik, E. F., and D. A. Hammer. 2004. A semianalytic model of leukocyte rolling. Biophysical Journal 87:2919-2930.

30. Dong, C., and X. X. Lei. 2000. Biomechanics of cell rolling: shear flow, cell-surface adhesion, and cell deformability. Journal of Biomechanics 33:35-43.

31. Dong, C., J. Cao, E. J. Struble, and H. W. Lipowsky. 1999. Mechanics of leukocyte deformation and adhesion to endothelium in shear flow. Annals of Biomedical Engineering 27:298-312.

32. Shao, J. Y., and R. M. Hochmuth. 1996. Micropipette suction for measuring piconewton forces of adhesion and tether formation from neutrophil membranes. Biophysical Journal 71:2892-2901.

33. Shao, J. Y., H. P. Ting-Beall, and R. M. Hochmuth. 1998. Static and dynamic lengths of neutrophil microvilli. Proceedings of the National Academy of Sciences of the United States of America 95:6797-6802.

34. Schmidtke, D. W., and S. L. Diamond. 2000. Direct observation of membrane tethers formed during neutrophil attachment to platelets or P-selectin under physiological flow. Journal of Cell Biology 149:719-729.

35. Snapp, K. R., C. E. Heitzig, and G. S. Kansas. 2002. Attachment of the PSGL-1 cytoplasmic domain to the actin cytoskeleton is essential for leukocyte rolling on P-selectin. Blood 99:4494-4502.

36. Evans, E., V. Heinrich, A. Leung, and K. Kinoshita. 2005. Nano- to microscale dynamics of P-selectin detachment from leukocyte interfaces. I. Membrane separation from the cytoskeleton. Biophysical Journal 88:2288-2298.

37. Xu, G., and J. Y. Shao. 2008. Human neutrophil surface protrusion under a point load: location independence and viscoelasticity. American Journal of Physiology-Cell Physiology 295:C1434-C1444.

38. Krasik, E. F., and D. A. Hammer. 2004. A semianalytic model of leukocyte rolling. Biophysical Journal 87:2919-2930.

39. Goldman, A. J., R. G. Cox, and H. Brenner. 1967. Slow viscous motion of a sphere parallel to a plane wall--II Couette flow. Chemical Engineering Science 22:653-660.

40. Alon, R., D. A. Hammer, and T. A. Springer. 1995. LIFETIME OF THE P-SELECTIN-CARBOHYDRATE BOND AND ITS RESPONSE TO TENSILE FORCE IN HYDRODYNAMIC FLOW. Nature 374:539-542.

41. Chang, K. C., and D. A. Hammer. 2000. Adhesive dynamics simulations of sialyl-Lewis(x)/E-selectin-mediated rolling in a cell-free system. Biophysical Journal 79:1891-1902.