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Rolling adhesion of leukocytes on soft substrates: Does substrate stiffness matter?

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ABSTRACT

Cell rolling on vascular endothelium under hydrodynamic blood flow is critical for realization of many physiological and pathological processes, such as inflammatory response and tumor metastasis. The blood-borne cells are in direct contact with the inner layer of endothelium, formed by a highly compliant layer of endothelial cells. The effect of endothelial stiffness on the adhesion and motion of rolling cells is poorly understood. Inspired by recent *in vitro* studies, here we implemented a computational method to model the specific adhesion of a rolling cell onto a soft substrate, subjected to a creeping shear flow. The substrate is modeled as an elastic half-space, coated with P- and E-selectin receptors with specific affinity for the complementary ligands located on the moving cell. Of particular importance is to predict the effect of substrate stiffness on cell adhesion and its kinematics and kinetics of motion. Simulation results show that the effect of substrate compliance is minimal when coated with P-selectin. Conversely, the trajectory of rolling cells on E-selectin coated substrates is sensitive to the substrate compliance. This is attributed to the moderation of binding forces applied by the soft substrate which leads to a higher average translational velocity of cells.

1. Introduction

The hallmark of acute inflammation and immune response is the adhesion of blood-borne leukocytes such as monocytes and neutrophils to endothelium and their infiltration into the affected tissues (Springer, 1994; Schall and Bacon, 1994; Langer and Chavakis, 2009). Leukocyte adhesion begins with rolling along activated endothelium, primarily mediated by specific binding between transmembrane glycoproteins on endothelial cells (ECs) and their complementary carbohydrate ligands expressed on the surface of leukocytes (Butcher, 1991; Nourshargh and Alon, 2014).[1] Selectins are a major family of EC receptors (Vestweber and Blanks, 1999; Ley, 2003). The known members of selectin family, namely P-, E-, and L-selectin, share many similarities in their primary structures (Rosen and Bertozzi, 1994; McEver, 2015). The extracellular domain of selectin is comprised from a lectin-like amino terminus domain and an epidermal growth factor domain, followed by two to nine consensus repeats with structural homology to the regulatory proteins.

Specific adhesion of selectins is afforded by the energetic affinity between their N-terminus domain and the oligosacharide moieties of cognate ligands (Zarbock et al., 2011). P-selectin glycoprotein ligand (PSGL-1), for example, is a highly conserved ligand for P-selectins and is constitutively expressed on all leukocytes (McEver, 2007; Carlow et al., 2009). Binding to selectin receptors regulates the capture and recruitment of leukocytes in the face of dislodging hemodynamic forces during their random encounters with postcapillary venules. Initial tethering and rolling are the first stages of multi-step paradigm of leukocyte adhesion (Springer, 1994; Ley and Tedder, 1995). Rolling of leukocyte has been observed and studied under *in vivo* and *in vitro* conditions (Sperandio et al., 2006). Intravital and trans-illumination microscopy techniques are often used for *in vivo* settings to directly monitor the leukocyte rolling on cerebral venules, under different physiological and pathological conditions (Mempel et al., 2004). Flow chamber assays are useful *in vitro* models to observe the interactions between flowing leukocytes and their substrates, under controlled flow conditions (Lawrence, 2001). Both *in vivo* and *in vitro* observations reveal the erratic nature of leukocyte rolling. Accordingly, the rolling of leukocytes is non-smooth and

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[1] The terminology for *ligands* and *receptors* is often used interchangeably in literature. Here, ligands explicitly refer to the adhesive molecules on circulating leukocytes and receptors are reserved for their binding partners on ECs.

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features a stop-and-go pattern, often referred to as *saltation* (Lawrence and Springer, 1991). This irregularity in cellular rolling is an attribute of the stochastic nature of ligand-receptor binding (Cheung et al., 2011).

The average velocity of rolling cells is an important indicator of adhesion dynamics between receptors and ligands. In leukocytes, the adhesion process is mediated by reversible bindings between ligands and receptors, with fast association and dissociation rates (Cheung et al., 2011). As a result, selectin-mediated bonds can be established and ruptured during the short encounters between ECs and marginated leukocytes. The forces exerted by the temporary bonds significantly reduce the velocity of rolling cells compared to that of non-adherent cells in the blood flow. The impact of physical chemistry of selectin bonds on rolling velocity of leukocyte is extensively studied in the past (Sundd et al., 2011). All three members of selectin family can take part in leukocyte rolling (Vestweber, 1993; Rosen and Bertozzi, 1994). P- and E- selectin are known for mediating the slow-rolling of leukocytes (Jung et al., 1996; Ley et al., 1998). *In vivo* studies of neutrophil rolling along the vascular endothelium, for example, shows that rolling velocity after cytokine induced inflammation is controlled by P-selectin with an average rolling velocity of 40 μm/s (Damiano et al., 1996). Conversely, L-selectin is known to be more efficient in mediating fast rolling of neutrophils (velocity $>$ 100 μm/s) (Jung et al., 1996; Stein et al., 1999). The rolling velocity of leukocytes is a function of the selectin site density too. Greatly increased expression of P-selectin under simulated inflammation condition leads to a reduction of leukocyte rolling velocity, presumably due to the increased number of established bonds and strong adhesion to the substrate (Lawrence and Springer, 1993).

It has been proposed that chemistry and elasticity of substrates are the orthogonal determinants of similar importance in regulation of cellular adhesion (Geiger, 2001; Engler et al., 2004). It is known that the function of focal adhesions in contractile cells is regulated by the stiffness of underlying substrate (Pelham and Wang, 1997). The focal adhesions of cells attached to a soft substrate are considerably smaller than those formed on a rigid surface. In general, cells adhered to stiffer substrates exhibit a more organized cytoskeleton and a larger spread area (Engler et al., 2004). The mechanical stiffness of substrate regulates other adhesion-dependent functions of cells too, including the lineage commitment, viability, and differentiation (Rowlands et al., 2008). The possible effect of substrate stiffness on rolling adhesion of leukocytes, however, is not particularly well documented in the literature. MacKay and Hammer, 2016 measured the rolling velocity and capturing efficiency of monocytes flowing on compliant hydrogels, functionalized with E- and P-selectins. Their observation indicates a noticeable variation in the rolling velocity of cells with changing the hydrogel stiffness, when coated with E-selectin. At a fixed E-selectin site density, decreasing hydrogel stiffness from 20 to 1 kPa led to a higher rolling velocity and lower capturing efficiency of the substrates. On the other hand, changing the stiffness of hydrogel coated with P-selectin did not significantly alter the rolling velocity of monocytes.

The experimental observations of MacKay and Hammer, 2016 are interesting given the fact that endothelial E- and P-selectin are often assumed to be functionally redundant and can replace each other in the process of recruiting leukocytes (Labow et al., 1994; Kunkel et al., 1996; Kunkel and Ley, 1996; Zarbock et al., 2011). These results have also a broader biological importance. ECs that line the inner surface of blood vessels are highly compliant. Measurements conducted with atomic force microscopy show that the average mechanical stiffness of an individual endothelial cell is on the order of 1 kPa (Ohashi and Sato, 2005; Stroka and Aranda-Espinoza, 2011). Therefore, unlike the rigid coverslips often used for *in vitro* experiments, endothelium presents a soft surface to the circulating leukocytes *in vivo*. In addition, understanding that how substrate stiffness mediates the kinematics of leukocytes rolling may further clarify the mechanism of leukocyte recruitment to atherosclerotic lesions. Atherosclerosis is a pathological disorder characterized by stiffening of vascular walls due the growth of lipid-rich lesions (Palombo and Kozakova, 2016). According to MacKay and Hammer, 2016, increased stiffness of arteries may directly contribute to recruitment of inflammatory-response immune cells in atherosclerosis.

To understand the mechanism underlying the sensitivity of rolling adhesion to substrate stiffness, one has to examine how substrate compliance mediates the binding mechanics between the cell and substrate. MacKay and Hammer (2016) proposed that high binding affinity between P-selectin and NH_2-terminus of PSGL-1 could be a possible reason for the lack of sensitivity of monocyte arrest to the substrate stiffness, when coated with P-selectin. PSGL-1 binds to P-selectin with at least 50-fold higher affinity than to E-selectin (Moore et al., 1994). The objective of this contribution is to examine the validity of this hypothesis using a mechanistic computational model for cell rolling, without integration of any signaling pathway. To this end, we used *Adhesive Dynamics* (AD), a computational algorithm developed by Hammer and coworkers (Hammer and Apte, 1992; Chang and Hammer, 1996; Kuo et al., 1997; Chang et al., 2000), to simulate the real time motion of a spherical particle at proximity of an adhesive wall. AD is frequently used to simulate the state of leukocytes adhesion under flow. It takes into account the hydrodynamic forces applied on the cell immersed in a creeping flow field, the non-specific interaction of cells with the substrate, and the stochastic nature of ligand-receptor binding between the cell and substrate.

2. Computational procedure

In what follows, we briefly describe the fundamentals of computational procedure used in the presented AD model. Further details about AD modeling are outlined in earlier contributions of Hammer and his coworkers. Leukocytes are idealized as rigid spherical objects with radius R_c, immersed in a viscous fluid (with viscosity μ) that undergoes a simple shear flow parallel to a smooth compliant substrate at a low Reynolds number (Fig. 1). The substrate is assumed to be a linear elastic material with Young's modulus E, coated with (E- or P-) selectin receptors. Since the soft substrate is formed by ECs (or hydrogels in experimental studies of MacKay and Hammer, 2016, it is further assumed to be incompressible).

2.1. Binding mechanics

Bond formation is a stochastic process that depends on k_f and k_r, the forward and reverse rates of reaction between binding partners. It has been found experimentally that the dissociation rate of a bond is modulated by the force developed in the bond. This force dependency is often represented by the model proposed by Zhurkov (1984) and Bell (1978)

$$k_r = k_{r0} \exp\left(\frac{\gamma f_b}{k_B T}\right) \tag{1}$$

where k_{r0} is the force-independent rate of dissociation, γ is the reactive compliance, f_b is the force developed in the bond, and $k_B T$ is the thermal energy. Under small pulling forces, bonds act like simple harmonic springs. Hence, their tensile force is assumed to depend linearly on the change in bond length

$$f_b = s_b(l_b - l_{b0}) \tag{2}$$

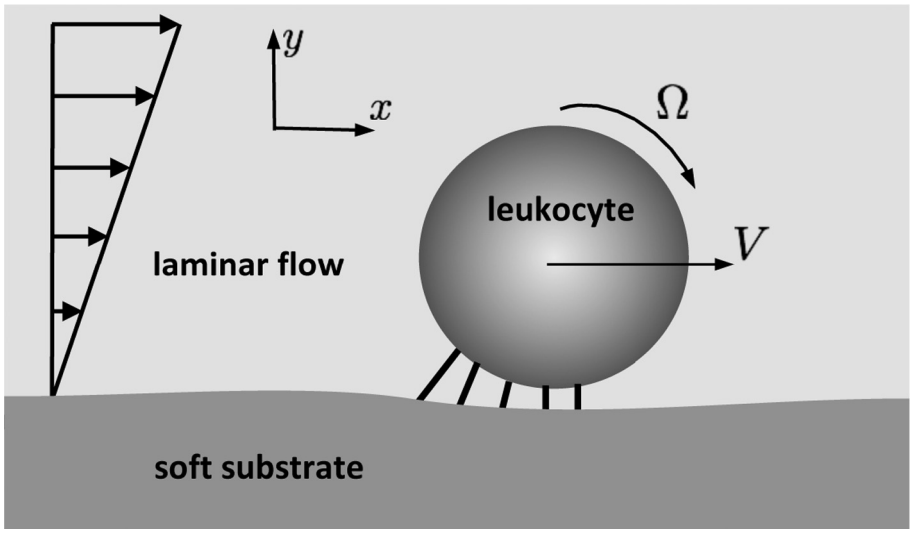

Fig. 1. Schematic illustration of leukocyte motion under a creeping viscous flow atop a soft adhesive substrate. The direction of laminar flow is along *x*-axis. Adhesion to the substrate is mediated by specific binding between the receptors of substrate and their cognate ligands, randomly placed on the leukocyte surface. The bonds are stretched as they resist the hydrodynamic drag experienced by the cell circulating with a translational and angular velocity of V and Ω, respectively.

where s_b represents the equivalent spring constant, l_b is the bond length after deformation, and l_{b0} is the bond length at rest.

The load transmitted through the strained bonds deforms the compliant substrate too. The local deformation at each binding site can be taken into account approximately by consideration of another linear spring placed in series with the spring representing the bond stiffness (Fig. 2). Considering this assembly, the overall mechanical rigidity of each binding site, $\bar{s}$, can be approximated as

$$\bar{s} = \frac{s_b s_s}{s_b + s_s} \tag{3}$$

where s_s shows the equivalent stiffness of the substrate. Therefore, the deformation is mostly localized in bonds unless s_s is comparable to s_b. The spring stiffness representing substrate rigidity is related to the Young's modulus following (Kendall, 1971)

$$s_s \approx \frac{E l_{b0}}{1 - \nu^2} \tag{4}$$

where ν shows the Poisson's ratio of the substrate. Since the substrate is taken to be incompressible, $\nu = 0.5$. The two-spring assembly shown by Fig. 2(b) represents as a simplified version of the model proposed by Paszek et al. (2009) for cell adhesion on compliant substrates.

To satisfy the principle of detailed balance, the forward rate of binding is expressed as (Bell, 1978)

$$k_f = k_{f0} \exp\left(\frac{s_b|\delta - l_{b0}|\left(\gamma - \frac{1}{2}|\delta - l_{b0}|\right)}{2k_B T}\right) \tag{5}$$

Here, k_{f0} is the intrinsic rate of reaction and δ is the separation distance between ligands and underlying receptors (Fig. 2).

2.2. Equilibrium condition

Neglecting the inertia of the small cell immersed in a creeping flow, the generalized equilibrium condition for the cell can be written as

$$\mathbf{F}^b + \mathbf{F}^n + \mathbf{F}^s = \mathbf{0} \tag{6}$$

where $\mathbf{F}^b$ is the resultant binding force exerted by all bonds, $\mathbf{F}^n$ shows the contributions of non-specific forces normal to the flow direction, and $\mathbf{F}^s$ is the hydrodynamic drag force applied by the

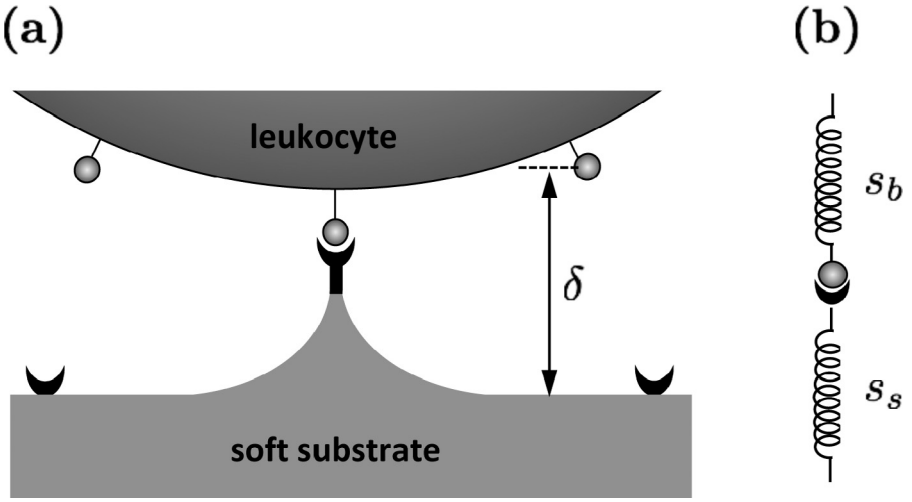

Fig. 2. (a) The compliant substrate is deformed at each binding site due to the tensile force developed at bonds during cell rolling. δ shows the separation distance between the free ligands and receptors. (b) The bond and deformable substrate are modeled as linear springs assembled in series with spring constants s_b and s_s, respectively.

shear flow. Note that the generalized equilibrium condition (6) includes the torque balance too. Different types of forces contributing in the non-specific repulsion between the cell and substrate (including gravity, van der Waals interaction, electrostatic, and steric stabilization forces) are described in Supplementary Material 1.

In theory, the hydrodynamic drag forces applied on the particle can be determined from (asymptotic) solutions of Stokes equations (Perkins and Jones, 1992). When the particle travels parallel to a rigid wall at steady state, it experiences no hydrodynamic lift force, due to the reversibility of Stokes flows (in time and space). When the substrate is deformable, the particle induces a pressure field that deforms the surface and breaks the symmetry of its profile (Sekimoto and Leibler, 1993; Skotheim and Mahadevan, 2004). In general, the surface is pushed downward in front of the moving particle and pulled upward at its posterior, due the asymmetry in pressure field. This non-symmetric profile yields a finite elasto-hydrodynamic lift force whose value depends on the gap distance between the particle and wall surface and the mechanical properties of the substrate (Urzay, 2010). If the substrate is incompressible, however, this elasto-hydrodynamic lift vanishes in the first order approximation of asymptotic solutions (Urzay, 2010), in which case the drag forces exerted on the particle are essentially similar to those induced by a rigid wall, as derived by Goldman et al., 1967a,b.

In view of linearity of Stokes equations, the equilibrium condition (6) can be written as

$$\mathbf{U} = \mathbf{M} \cdot \mathbf{F} \tag{7}$$

where **U** and **F** represent the generalized velocity and force vector, respectively. The velocity vector shows the translational and rotational velocity components of the cell in a Cartesian system (Hammer and Apte, 1992)

$$\mathbf{U} = (V_x, V_y, V_z, \Omega_x, \Omega_y, \Omega_z) \tag{8}$$

and the force vector shows the force and couple experienced by the cell (Hammer and Apte, 1992)

$$\mathbf{F} = \left(F_x^b + F^s, F_y^b + F^n, F_z^b, C_x^b, C_y^b, C_z^b + C^s\right) \tag{9}$$

Here, F^s and C^s are shear force and couple experienced by a stationary cell in a Couette flow field, receptively. That is (Goldman et al., 1967a)

$$F^s = 6\pi\mu R_c(R_c + d)\dot{\gamma}F^{s*} \tag{10}$$

$$C^s = 4\pi\mu R_c^3\dot{\gamma}C^{s*} \tag{11}$$

where $\dot{\gamma}$ is the shear rate and d is the gap distance between the cell and free surface. F^{s*} and C^{s*} are given by Goldman et al. as a function of d (Goldman et al., 1967a,b). **M** in Eq. (7) represents the 6×6 mobility matrix whose components are defined in Supplementary Material 2. Note that $\mathbf{M}^{-1}.\mathbf{U}$ shows the drag force experienced by the particle when it is moving with unknown velocity **U** in a quiescent fluid.

2.3. Monte Carlo procedure

The instantaneous cell velocity can be updated at different time steps using an Eulerian computational scheme. Since Eq. (1) holds for the constant pulling force f_b, each simulation has to be divided into small time steps during which f_b does not significantly change. Considering our computational abilities, we chose a time step of $\Delta t = 10^{-6}$ s. During each time step the following operations occur: (1) External forces on the cell are calculated. This includes the binding, non-specific, and drag forces experienced by the cell. (2) A Monte Carlo scheme updates the state of binding/unbinding between ligands and receptors. The probability of bond formation, P_f, and the probability of rupture, P_r, are given by (Hammer and Apte, 1992)

$$P_f = 1 - \exp(-k_f \Delta t) \tag{12}$$

$$P_r = 1 - \exp(-k_r \Delta t) \tag{13}$$

Random numbers, sampled between zero and one, are compared with the results of these probability functions. Bond formation or rupture will realize if the corresponding probabilities exceed the values of random numbers. (3) Components of the cell velocity vector are calculated using Eq. (7) and cell position within the flow field is updated at the end of each time step.

Table 1
Ranges and the chosen values of the parameters used for AD simulations (Hammer and Apte, 1992; Chang and Hammer, 1996; Chang et al., 2000; Caputo et al., 2007; Krasik et al., 2008).

Parameter	Definition	Range	Value
R_c	Cell radius	2–50 μm	5 μm
N_l	Ligand number	0.5×10^4–10^7	3500
μ	Viscosity	1–2 > gr/cm s	1 gr/cm s
s_b	Bond spring constant	0.5–5 dyn/cm	1 dyn/cm
l_{b0}	Equilibrium bond length	5–50 nm	25 nm
$\dot{\gamma}$	Shear rate	50–400 s^{-1}	100 s^{-1}

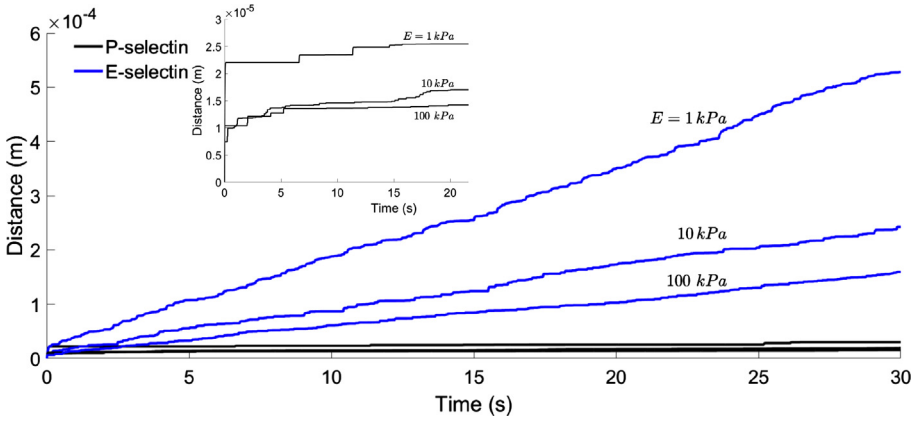

Fig. 3. Simulated track of a cell traveling on a soft substrate coated with E- and P-selectin. The traveled distance along x-axis is plotted as a function of time, considering three different Young's moduli for the substrate. The inset shows the magnified plots for P-selectin.

3. Results and discussion

Table 1 lists the values of model parameters used in simulations along with their biologically admissible ranges. Further model parameters required to characterize the non-specific forces are presented in Supplementary Material 2. The AD simulations are used to estimate the kinematics and kinetics attributes of an individual cell rolling on a soft substrate with a Young's modulus between 1 and 100 kPa, similar to the experiments by MacKay and Hammer (2016). Each simulation starts by positioning the cell above the substrate with a gap distance of 30 nm. After particle is set in motion, it is free to move in any direction but only its trajectory along the flow direction (x-axis in Fig. 1) will be reported here. A constant wall shear rate of 100 s^{-1} is used in all simulations. This matches the value often used for *in vitro* studies for which the leukocyte rolling was observed (Lawrence et al., 1987, 1990). No significant cell deformation is expected to occur at this shear rate (Jadhav et al., 2005). The particle size R_c is chosen to be comparable with the reported size of leukocytes (Downey et al., 1990). The intrinsic forward rate of reaction between selectin-ligand pairs, $k_{f0} = 84 s^{-1}$, is often used by other simulation studies (Chang et al., 2000). There are a few model parameters whose values are assumed to depend on the binding partners. Those include the intrinsic reverse rate of reactions, k_{r0}, and the reactive compliance

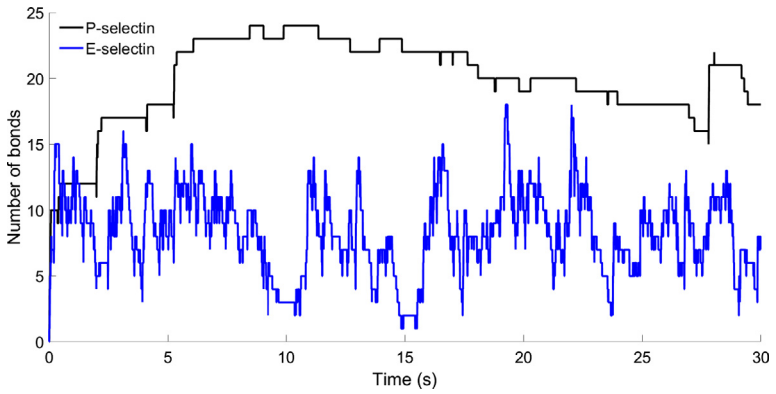

Fig. 4. Total number of established bonds between the cell and selectin coated substrates with a stiffness of $E = 10$ kPa.

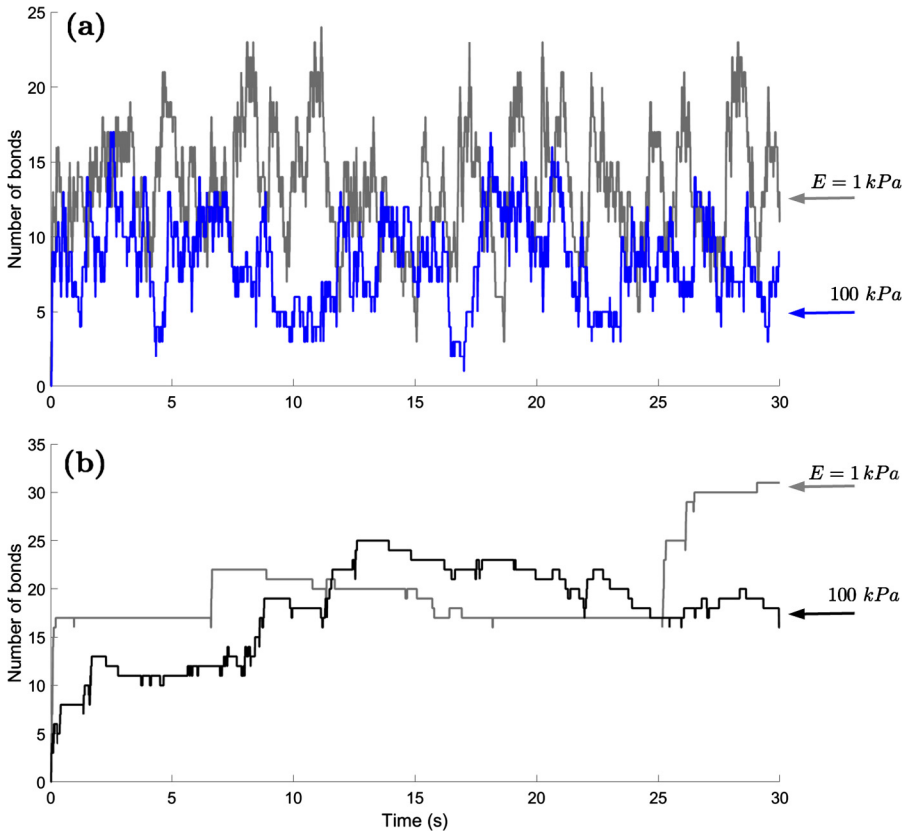

Fig. 5. The effect of substrate stiffness on the number of established bonds during rolling on (a) E- and (b) P-selectin coated substrates. The comparison is made between substrates with a stiffness of $E = 1$ and 100 kPa.

parameter γ. The chosen value of unstressed unbinding rate k_{r0} for P-selectin-ligand bonds is nearly two orders of magnitude less than E-selectin mediated bonds (0.022 s^{-1} vs 2.6 s^{-1} (Chang et al., 2000)). The reactive compliance length is taken to be 2.5 Å and 0.18 Å for the P- and E-selectin bonds, respectively (Chang et al., 2000). The simulations are programmed in Matlab® and carried out on parallel clusters in Ohio Supercomputer Center.

To begin, we calculated the cell trajectories over compliant substrates with different rigidities. AD simulations were performed for substrate rigidities of 1, 10, and 100 kPa, coated with E- and P-selectin. The cell displacements parallel to the flow direction are shown in Fig. 3. Similar to the experimental results of MacKay and Hammer (2016), predictions obtained from AD simulations exhibit a strong dependency between the traveled distance and substrate stiffness, when coated with E-selectin. On E-selectin coated substrates, cells show a stable rolling motion. That is, after formation of the first bond, the average velocity of cell decreases and reaches a steady value, indicating that the average rate of bond formation balances the rupture. Cell average velocity increases nearly three-folds upon reducing the substrate stiffness from 100 to 1 kPa. In contrast, the cell elicits a much weaker response to the alteration of substrate stiffness, when coated with P-selectin. After a short rolling period, cell is arrested by the substrate and exhibits negligible average translational velocity, irrespective of the substrate stiffness. Considering the stochastic nature of AD simulations, they were repeated multiple times in order to assess the reproducibility of predictions (see Supplementary Material 3).

The change in cell position with time is controlled by continuous formation and rupture of ligand-receptor bonds. Fig. 4 shows a comparison between the time history of bond formation, mediated by E- and P-selectin receptors, coating a substrate with $E = 10$ kPa. Substrates coated with P-selectin are capable of forming an appreciably larger number of bonds with the cell. Consistent with the lower rolling velocity on P-selectin coated substrates, this is due to the higher affinity of P-selectins towards the leukocyte ligands, implemented by much lower intrinsic off-rate k_{r0}. Further, a noticeable lag-time is observed between the formation and dissociation of P-selectin bonds, indicating the lasting survival of each bond. Fig. 5 shows the fluctuating number of bonds formed on substrates with the Young moduli of 1 and 100 kPa. On average, the number of bonds formed on E-selectin coated substrates is larger at substrate stiffness of 1 kPa (13 bonds versus 8). This seemingly stands at odds with the predictions made earlier for the average translational cell velocity, where it was shown that decreasing the substrate stiffness leads to a higher average velocity (Fig. 3). The growth in number of bonds, however, can be easily understood by evaluation of the binding force applied on the cell and its correlation with substrate stiffness. Following Eq. (3), the overall rigidity of a binding site, $\bar{s}$, decreases upon reduction of the equivalent substrate stiffness, down to a value comparable with that of a bond. The softer binding sites generate lower binding force at each bond. Fig. 6 depicts the evolution of the total binding force (F_b) of E-selectin bonds with time. The average value of F_b is clearly lower when the cell is adhered to a softer substrate. Thus, the probability

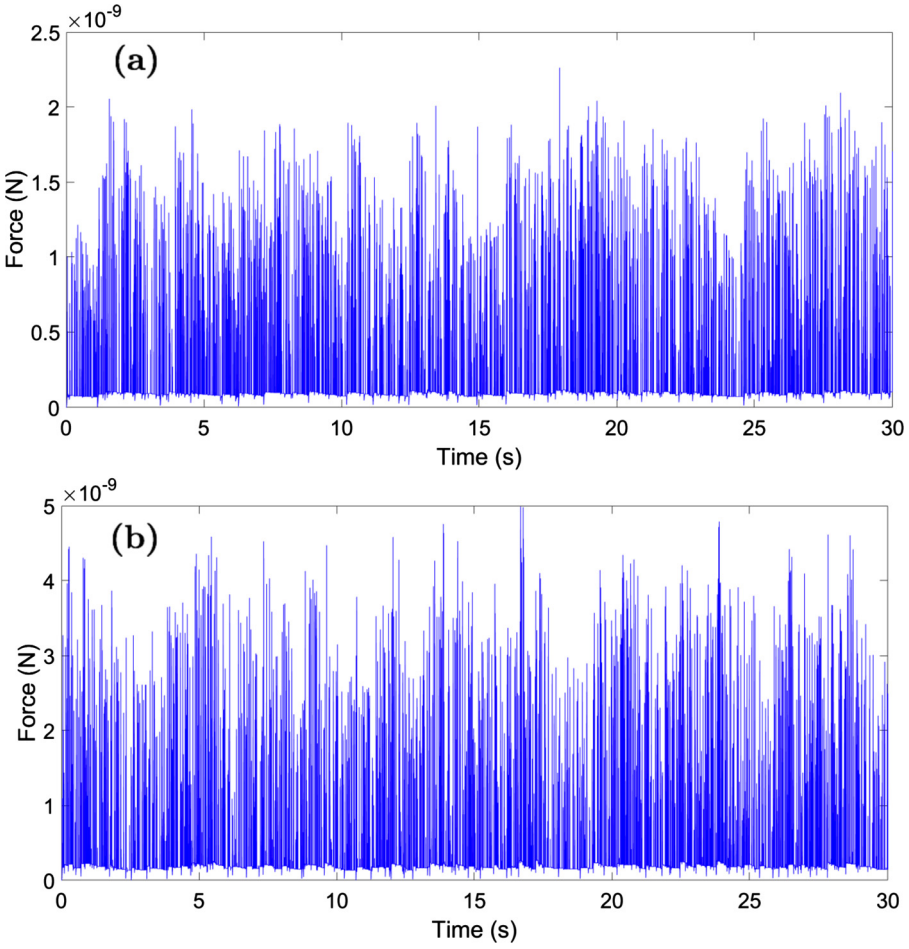

Fig. 6. The effect of substrate stiffness on the total binding force applied on a cell rolling on E-selectin coated substrates. The comparison is made between substrates with a stiffness of (a) $E = 1$ kPa and (b) $E = 100$ kPa.

of rupture, shown by Eq. (13), would be lower which results in a larger number of established bonds.

To better understand the cell kinematics and its dependency on substrate stiffness, the gap distance at the interface of cell and (undeformed) wall is calculated and shown in Fig. 7. Comparison between the simulation outputs shows a major difference between the rolling and arresting states of a cell. The steady fluctuation of gap distance between the cell and a P-selectin coated substrate implies that the hydrodynamic forces are not sufficiently strong to frequently break the firm bonds. Instead, the cell is only displaced slightly in the direction of dislodging forces and is pulled back by the resistive forces of bonds. On E-selectin coated substrates, bonds are frequently broken and reformed leading to a significant fluctuation of gap distance between the wall and moving cell. Close scrutiny of gap distances reveals a critical difference between their values on soft and stiff substrates, coated with E-selectin. On stiff substrates, cell shows alternating periods of rolling and arrest whereas such transient arrest periods appear to be very short-lived over a soft substrate. This can be more clearly illustrated by inspecting the instantaneous translational velocity of cell along the flow direction, as shown by Fig. 8. The fluctuation in traces is due to the resistive forces of bonds, being repeatedly

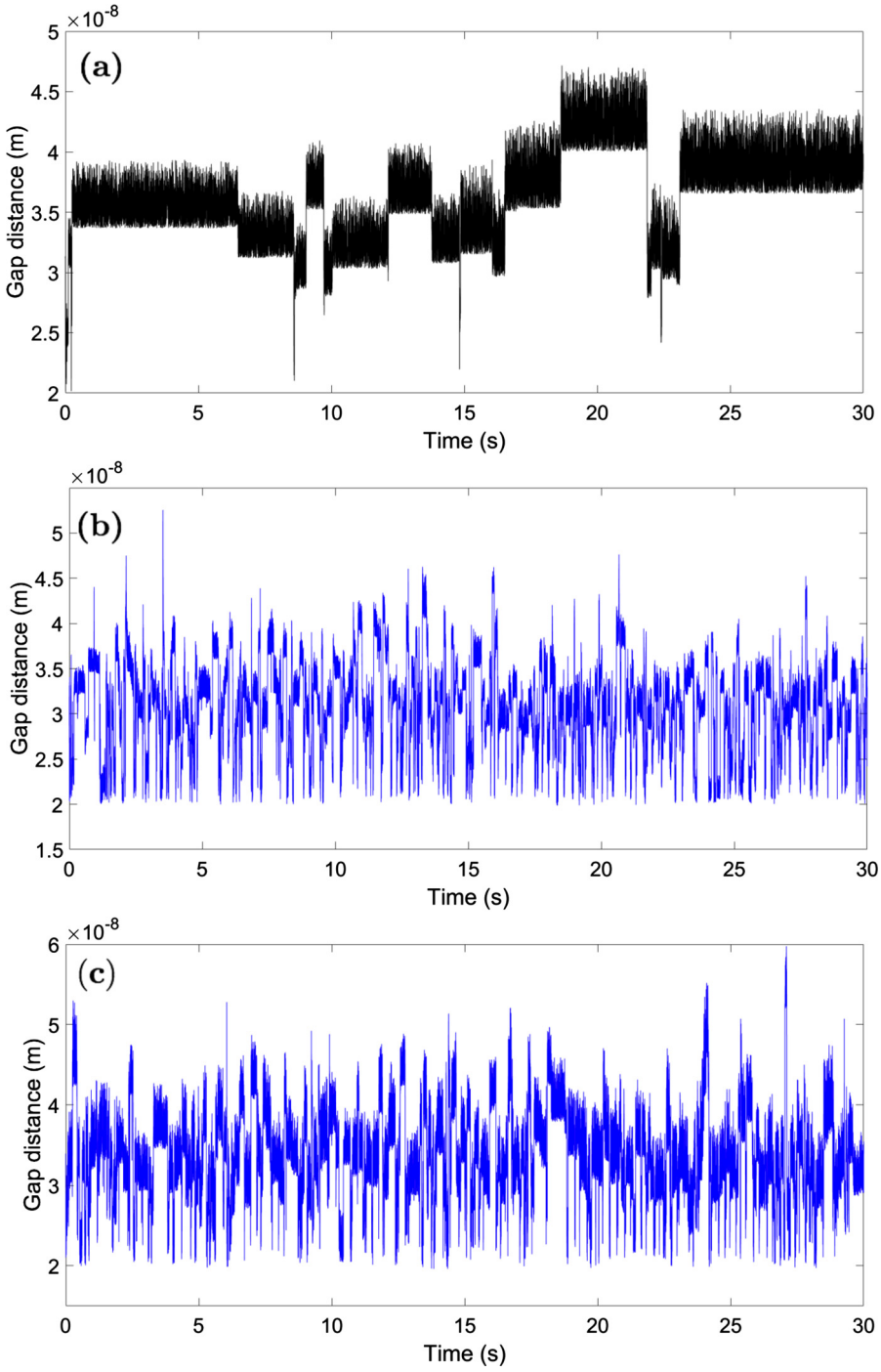

Fig. 7. The effect of substrate stiffness on the gap distance between the cell and selectin coated substrates. The comparison is made between substrates coated with (a) P-selectin with $E = 1$ kPa, (b) E-selectin with $E = 1$ kPa, and (c) E-selectin with $E = 100$ kPa.

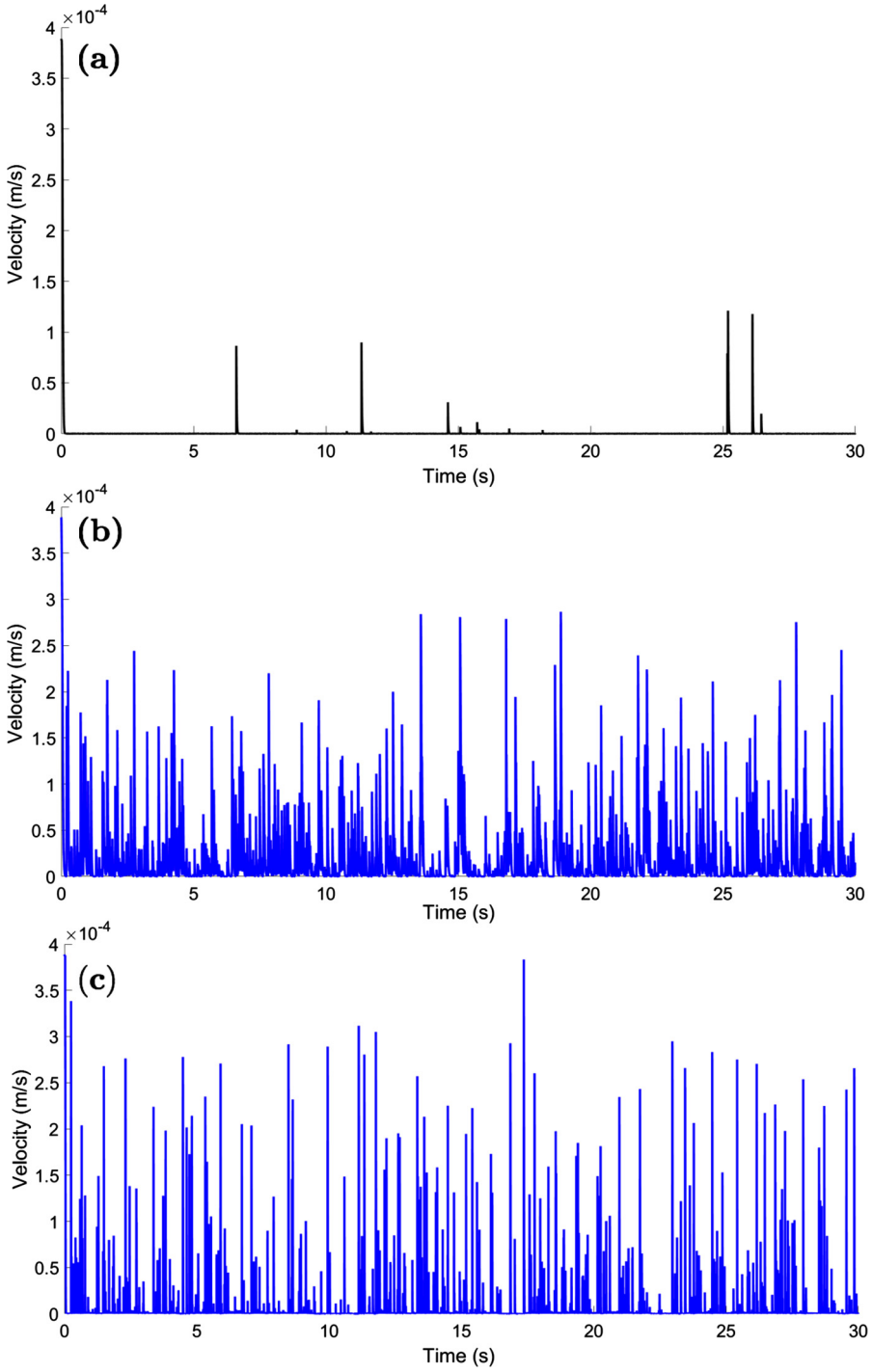

Fig. 8. Variation of instantaneous cell velocity with time during rolling on (a) P-selectin coated substrate with $E = 1$ kPa, (b) E-selectin coated substrate with $E = 1$ kPa, and (c) E-selectin coated substrate with $E = 100$ kPa.

broken and formed. Each momentary spike in cell instantaneous velocity is associated with the sudden rupture of adhesion. Prolonged pauses between the velocity jumps over the P-selectin coated substrate indicate the firm adhesion of arrested cells. On stiff substrates coated with E-selectin, these spikes are often separated by short periods of zero cell velocities. Such temporary pauses in cell motion are less frequently observed on soft substrates. This is the underlying reason for the longer trajectory of cells rolling on softer substrates, coated with E-selectin (Fig. 3). As the cell rolls, the bonds established at its trailing edge are further stretched. The resistive forces of the stretched bonds reduce the instantaneous (translational and angular) cell velocity. The rolling cell stops momentarily if the binding force is strong enough to balance the hydrodynamics drag. As shown in Fig. 6, the net binding force reduces upon decreasing the substrate stiffness from 100 to 1 kPa, concurrent with an increase in bond population (Fig. 5). Therefore, the tensile forces developed at each bond formed on the softer substrate ($E = 1$ kPa) is too weak to momentary halt the cell motion.

Taken together, the presented simulation outcomes imply that sufficiently low substrate rigidity could be a detrimental factor for the state of cell adhesion in shear flow. This can be illustrated

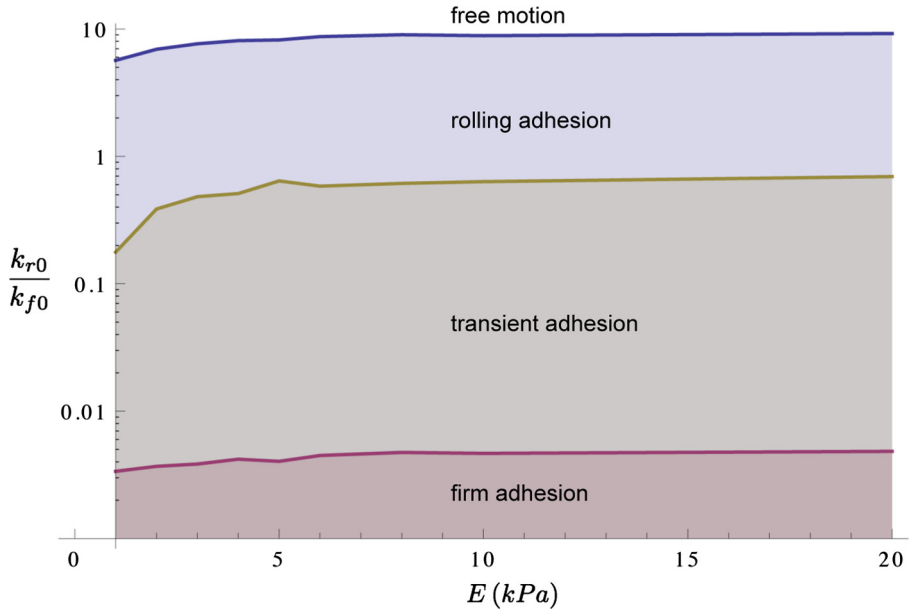

Fig. 9. The state diagram of adhesion constructed by consideration of interplay between substrate stiffness E and ratio k_{r0}/k_{f0}. Changing the substrate stiffness may influence the state of adhesion only on very compliant substrates.

differently using an adhesion state diagram that delineates the quantitative boundaries between the dynamic states of adhesion as a function of substrate stiffness. As shown by Fig. 9, four distinct regions can be specified in a state diagram of dynamic adhesion (Chang et al., 2000; Tees et al., 2002; Korn and Schwarz, 2008)[2]: (1) Free motion: when the mean translational speed of cell is greater than $0.95V_{hd}$ (V_{hd} is the hydrodynamic velocity). This condition does not imply that bonds cannot be established. Instead, it denotes a very high dissociation rate by which rupture happens before bonds are being stretched. (2) Arrest (firm adhesion): when the average translational velocity is smaller than $0.01V_{hd}$, in which case the unbinding is a rare event and cell remains motionless during a prolonged period of time. (3) Rolling adhesion: when $V > 0.01V_{hd}$ and $R_c\Omega/V > 0.8$. Under these conditions cell rolls on the substrate without a fractional stop time. (4) Transient adhesion: when $V > 0.01V_{hd}$ and $R_c\Omega/V < 0.8$. These conditions are associated with a rolling motion including alternating periods of short arrest. To construct the adhesion state diagram, the simulations were repeated with a grid of values for k_{r0}/k_{f0} and substrate rigidities ranging within several orders of magnitudes. The classification method mentioned above were used to parameterize the boundary curves. The boundary curve separating the firm adhesion region depends weakly on the change of substrate stiffness. This is consistent with our results indicating that firm adhesion to P-selectin coated substrates does not change appreciably with alteration of substrate rigidity. The curve separating the regions of rolling and transient adhesion is sensitive to the change of substrate stiffness, only when $E < 5$ kPa. A different state diagram for reactive compliance γ vs substrate stiffness is presented in Supplementary Material 4.

4. Concluding remarks

Inspired by recent experimental observations, we conducted a computational study to examine the effect of substrate rigidity on the kinematics and kinetics of cell translocation. We used AD as a powerful computational technique to simulate the motion of a spherical particle, representing a marginated leukocyte, on a soft substrate coated with P- and E- selectin. We used a mechanistic model for the effect of substrate compliance and ignored any signaling that may be activated within the cell upon adhesion to the soft substrate. Explicitly, we examined how stiffness controls cell trajectory, velocity, and the time history of bond formation. Simulation outputs displayed a trend similar to the experimental observation (MacKay and Hammer, 2016). Substrate stiffness had a minimal effect on the kinematics of rolling when coated with P-selectin. Cells were arrested by P-selectin receptors, almost immediately after being released, irrespective of the substrate stiffness. This was attributed to the high energetic affinity of P-selectin to the leukocyte ligands, providing a high survival probability for P-selectin bonds. In contrast, changing the stiffness of substrates coated with E-selectin led to a higher average velocity of cells. This transition was attributed to the weakening of binding forces when the substrates are sufficiently compliant.

The presented study is the first modeling approach that quantitatively elucidates the sensitivity of leukocyte motion to the rigidity of underlying substrate. While the results are in qualitative accord with the available experiments, further refinements have to be introduced to the model in future studies. The extent to which the wall stiffness mediates the kinematics of marginated cells may depend on other factors such as shear rate, ligand density, and cell size whose effects were not comprehensively studied in this work. In particular, the site density of binding partners and the shear rate are likely to influence the dynamic state of adhesion (see Supplementary Material 5 for discussion about the effect of ligand density). In addition, the catch-slip behavior of selectin bonds was not accounted for in this work. All members of selectin mediated bonds feature a catch-to-slip transition with increasing the force (Zhu et al., 2005; Snook and Guilford, 2010; Moshaei et al., 2019). This transition is particularly well documented for P-selectin/PSGL-1 bonds. The effect of catch behavior on leukocyte rolling is manifested by the *shear-threshold effect* (Yago et al., 2004); i.e., a threshold requirement for shear stress to facilitate stable rolling of leukocytes. This effect, however, is most prominently observed for leukocyte rolling supported by L-selectin bonds (Caputo et al., 2007) and is attributed to the allosteric regulation by an interdomain hinge of L-selectin (Lou et al., 2006; Beste and Hammer, 2008:). Other possible refinements include the con-

[2] Here we adopted the criteria introduced by Korn and Schwarz (2008).

sideration of viscoelastic response for microvillus tethers Caputo and Hammer (2005) (operating in series with the two-spring assembly shown by Fig. 2(a)) and the difference between the lengths of E- and P-selectin/PSGL-1 bonds. The latter is caused by extra subdomains in the extracellular portion of P-selectin. As noted by Patel et al. (1995), this may facilitate the interactions between P-selectin and PSGL-1 on leukocytes by widening the intermembrane space and debilitating the cell-cell repulsive interactions. Further, in formulating the model, there was a tendency to focus on the passive and mechanistic aspects of rigidity sensing and thereby no signaling pathway was incorporated in the model. Accumulated signals generated by ligated selectins are expected to progressively activate other cell ligands, such as β_2 integrins, and enhance their avidity (Kazuya et al., 1997; Hentzen et al., 2002; DiVietro et al., 2001). This signaling process can be integrated with the AD simulations (Krasik et al., 2008). In contractile cells, it is well known that substrate stiffness influences the activation of signaling molecules and their pathways (e.g., sityrosine phosphatase and kinase pathways (Giannone and Sheetz, 2006)). In case of leukocytes, it is yet to be determined whether changing substrate rigidity regulates any of the signaling pathways. When more experimental evidences are made available in the future, the model can be adapted to account for the possible signal transductions.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jbiomech.2019.05.004.

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