FLEXURAL STIFFNESS, MOBILITY, AND EXTENSIONAL STIFFNESS OF SINGLE HUMAN NEUTROPHIL MICROVILLI

Jin-Yu Shao
Jinbin Xu

Department of Biomedical Engineering
Washington University in Saint Louis
Saint Louis, MO

INTRODUCTION

To fight infection or injury, human neutrophils first roll on the endothelium before their firm adhesion and transendothelial migration. Neutrophil microvilli, which are membrane folds or projections on the neutrophil surface, facilitate the rolling by concentrating adhesion mediators like L-selectin (CD62L) and P-selectin glycoprotein ligand-1 (PSGL-1 or CD162) on their tips [1, 2]. There are about five microvilli per micrometer squared on a neutrophil surface. Due to the shear stress of the blood flow, microvilli are extended or even transformed into long membrane tubes (tethers) during the rolling of neutrophils to help stabilize the rolling. Without microvilli or an optimum shear stress, the neutrophil arrest on the endothelium is not as efficient. It has been shown that L-selectin is anchored to the cytoskeleton through interactions with α-actinin, a major actin-binding protein, while the cytoplasmic tail of PSGL-1 binds to moesin, a linker protein that possesses both membrane- and actin-binding domains [3, 4]. The loss of the cytoplasmic tail of L-selectin or PSGL-1 abolishes the effective rolling of leukocytes on ligand-coated substrates [5, 6].

Neutrophil microvilli are typically 0.35 μm in length and 0.1-0.2 μm in diameter with a cylindrical shape, while the diameter of a human neutrophil is about 8.5 μm. L-selectin and PSGL-1 are estimated to be about 15 nm and 50 nm long respectively outside the cell membrane. A normal neutrophil can roll for ~90 s and 270 μm until adhering firmly to the endothelium [7]. Before rolling on the endothelium, a neutrophil translates and rotates simultaneously, but moves smoothly and freely in the bloodstream. Because of the richness of microvilli, when a neutrophil touches an endothelial cell surface, the initial contact point is very likely the tip of a microvillus. If a bond forms between the microvillus and endothelium, a horizontal force and a clockwise torque will develop on the cell because of the flowing blood. It is known that a microvillus can be extended as much as 250% with a spring constant about 43 pN/μm if the pulling force is between 17 pN and 45 pN [8]. However, not much is known about how it will respond to being bent and how it will respond to being pulled at smaller forces.

METHODS AND MATERIALS

Anti-CD62L or anti-CD162-coated latex beads were incubated with human neutrophils isolated from venous or finger prick blood. Two types of bead are used: polystyrene beads coated with goat anti-mouse IgG (Fc specific) purchased from Bangs Laboratories (Fishers, IN; ~3.22 μm in diameter), and carboxylate-modified fluorescent beads purchased from Molecular Probes (Eugene, Oregon; ~40 nm in diameter). The thermal fluctuation of the beads was tracked with the single particle tracking technique at a resolution of about 5 nm.

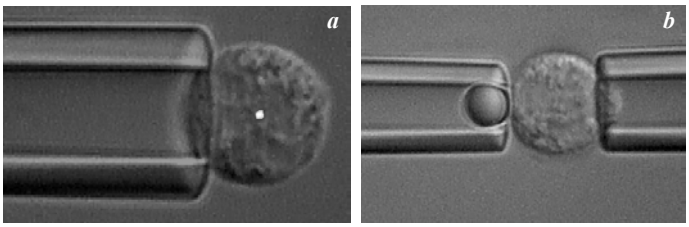

Fig. 1. Micrographs showing how to measure the flexural (*a*) and extensional (*b*) stiffness of neutrophil microvilli.

The flexural or extensional stiffness (k_F or k_E) was calculated with

$$k_F \text{ or } k_E = \frac{k_B T}{\langle \delta x^2 \rangle}, \quad (1)$$

where $\sqrt{\langle \delta x^2 \rangle}$ is the root mean square of the bead displacement (x), k_B is the Boltzmann constant, and T is the temperature. For the cases where the bead traveled on the spherical cell surface, we calculated the spherical arc length (λ) as the distance that the bead traveled.

RESULTS

Flexural Stiffness (k_F)

As shown in Fig. 1*a*, some anti-CD62L-coated beads adhered to the neutrophil surface. If beads were not bound to the neutrophil surface,

they would float around and would only stay in the focal plane shortly. In our control experiment, the beads coated with purified general type IgG_1 (Sigma, Saint Louis, MO) were not found on neutrophils. Figure 2 shows the tracked positions of a bead that adhered to a neutrophil surface. It is obvious that the bead was fluctuating around an equilibrium position. In this case, $\sqrt{\langle \delta x_\theta^2 \rangle} = 49$ nm and $\sqrt{\langle \delta x_\varphi^2 \rangle} = 51$ nm (θ and φ are two orthogonal directions of a spherical coordinate system), which correspond to 1.7 pN/μm and 1.6 pN/μm for the flexural stiffness of this microvillus. We analyzed 43 cases for k_F. After three outliers were removed, an average value of 9.5 ± 7.8 pN/μm (mean ± standard deviation; $n = 40$) was obtained for k_F.

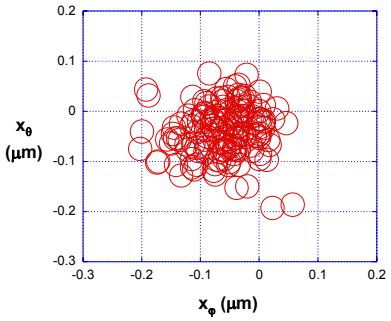

Fig. 2. The tracked positions of a 40-nm bead on a neutrophil surface. The tracking resolution was determined by tracking some beads fixed on a piece of cover glass. The diameter of the 40-nm bead is drawn to scale.

Mobility

When the bead that adhered to the neutrophil was tracked, the bead often appeared to move in a certain direction. Figure 3 shows the tracked positions of a bead that apparently was moving on the neutrophil surface. At least three pervious studies have shown that more than 80% of L-selectin are concentrated on the tips of microvilli, so the bead motion very likely indicates that neutrophil microvilli are not immobile structures on the neutrophil surface. In 15 seconds, the bead traveled about 0.3 μm. The correlation between λ^2 and t shows a motion patter similar to protein diffusion. Therefore, an equivalent "diffusion coefficient" (D_{eq}) can be calculated for the motion of neutrophil microvilli from the following equation:

$$\lambda^2 = 4D_{eq}t\,, \qquad (2)$$

where t is time. For the case shown in Figure 3, a linear fit resulted in a D_{eq} of 0.0015 μm²/s. After removing four outliers out of 23 measurements, we obtained an average of 0.0030 ± 0.0018 μm²/s (mean ± standard deviation; $n = 19$) for D_{eq}.

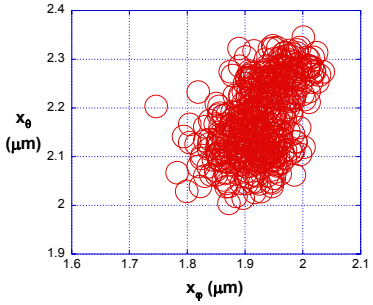

Figure 3. The tracked trajectory of a bead adherent to a neutrophil surface.

Extensional Stiffness (k_E)

As shown in Fig. 1*a*, a neutrophil was repetitively contacted by an anti-CD162-coated bead at an adhesion frequency less than 25%. When the adhesion occurred, the bead very likely adhered to a single microvillus because of this low adhesion frequency. Figure 4 shows first a contact that did not result in adhesion, then a contact that did result in an adhesion event where the microvillus in contact was pressed down and recovered to its natural equilibrium position. The bead fluctuation was tracked for about 15 seconds, then the adhesion was broken and the bead moved away at a small aspiration pressure. The equilibrium position of the bead seemed to change slowly possibly due to the mobility of the microvillus. Therefore, we chose a time interval (from 5.34 s to 9.63 s as shown in Figure 4 where the bead equilibrium position did not seem to change to calculate $\sqrt{\langle \delta x^2 \rangle}$ and k_E. In this case, $\sqrt{\langle \delta x^2 \rangle} = 16.7$ nm and $k_E = 14.5$ pN/μm. After four outliers were removed from 49 measurements, a value of 23.7 ± 9.2 pN/μm (mean ± standard deviation; $n = 45$) was obtained for k_E.

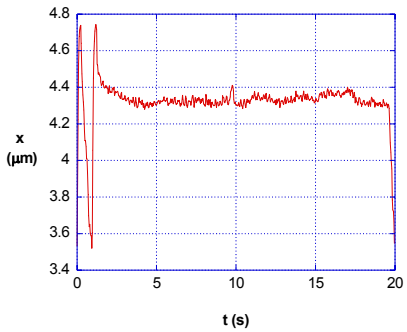

Figure 4. The tracked positions of a 3.22-μm bead adherent to a neutrophil as shown in Figure 1*b*.

CONCLUSIONS

The flexural stiffness of a neutrophil microvillus is on the same order of magnitude as its extensional stiffness. Neutrophil microvilli, which are mobile and strain-hardening structures, can be easily pressed down when neutrophils roll on the endothelium or contact other surfaces.

ACKNOWLEDGEMENTS

This work was supported by the Whitaker Foundation (RG-99-0289) and the National Institutes of Health (R01-HL69947).

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