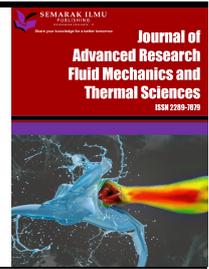




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Effects of Neutrophil Pseudopods Generation on the Propulsion Mechanism

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ABSTRACT

In vivo, neutrophil motion is known to be propelled by concentration gradient of cytokine. However, the mechanism of propulsion has not been elucidated yet in the previous research. Based on the assumption that neutrophil is propelled by the concentration Marangoni effect, concentration gradient of cytokine on the neutrophil membrane and the neutrophil motion is investigated experimentally. In our previous investigations, it was found that neutrophil generate pseudopods for infiltration of organs near the wall. In this study, to investigate the phenomenon that neutrophil pseudopods generation near the wall, a microscopy-based observation experiment is conducted. The distance between the neutrophils and the wall and geometrical characteristics of the neutrophils are measured. From the results, the following three findings were obtained. First, arithmetic mean roughness and root mean square roughness are higher at distances of 2 and 4 μm from the wall surface. Second, skewness and kurtosis are highest value at distances of 2 μm from the wall surface. Third, neutrophil generates pseudopods at 4 μm from the wall surface and completely change shape at 2 μm . These findings suggest that near-wall interactions promote pseudopod-induced roughening that is likely to influence transport on the neutrophil membrane and, consequently, propulsion in cytokine gradients.

1. Introduction

Development of medical micro robotic devices have been focused over the past decades for targeted delivery, precision surgery, sensing and nanosurgery [1-4]. Designing a miniaturized, versatile robot less than a few micrometers in size would allow access to the entire human body, enabling new procedures down to the cellular level and more precise and efficient local diagnosis and treatment. For example, robotic surgical systems such as the da Vinci system can translate the surgeon's hand movements into precise movements of small instruments inside the patient's body. The technology to safely deliver compounds across biological barriers is being studied as drug delivery systems (DDS), and its effects on various diseases, especially tumour, cardiovascular, and respiratory diseases, are attracting attention. Despite great expectations, most of the past demonstrations are primarily in vitro conditions. Because there are several major issues including

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propulsion through complex media (such as blood, mucus, and vitreous) as well as deep tissue imaging and control in vivo [5-7]. Microscale swimmer's propulsion including spermatozoa and kind of bacteria are often considered as the power source of micro robotic devices. Particularly, neutrophil is highly biocompatible and unharmed in vivo. It has been reported that the efficiency of drug delivery to inflammatory regions has been significantly improved by utilizing neutrophils as drug carriers [8].

Neutrophil is induced in the direction of high cytokine concentrations. The chemotaxis of neutrophils in the blood is an important component of the immune system in vivo, but the detailed driving mechanism is still unclear. In our previous studies, assuming that neutrophil moves by the concentration Marangoni effect, concentration gradient of cytokine on the surrounding fluid and neutrophil membrane is experimentally and theoretically examined [9,10]. The results showed that the concentration gradient on the neutrophil membrane repeats positive and negative values while the concentration gradient in the surrounding fluid is constant.

On the other hand, neutrophils take a spherical shape when far from the vessel wall and generate pseudopods when close to vessel wall. However, the effect of pseudopods on the underwater propulsion mechanism has not been clarified. In this study, the relationship of the distance from the wall and some geometrical features of neutrophils is examined with roughness indexes used in engineering. To calculate roughness indexes, neutrophil motion and distribution of cytokine concentrations are observed with a microscopy. Measured roughness indexes are arithmetic mean roughness R_a , root mean square roughness R_{ms} , skewness R_{sk} , kurtosis R_{ku} , which are often used in the engineering field.

2. Neutrophil Driving Mechanism

Since the size of neutrophil is from 10 to 15 μm , neutrophil is regarded as a microparticle [11]. Some microscale particles like a neutrophil in a liquid move by the Concentration Marangoni effect. It is a phenomenon that interfacial tension on the microparticles changes when solute concentration in liquid surrounding microparticle is non-uniform [12,13]. The force F acting on the microparticles is expressed as the sum of the interfacial tensions σ working on their surfaces with proportionality constant α as follows:

$$F = \alpha \left(\frac{dC}{dx} \right) \quad (1)$$

The force F is expressed by the microparticles velocity V from Stokes' resistance with proportionality constant β law as follows:

$$F = \beta V \quad (2)$$

Since Eq. (1) and Eq. (2) are equal, V is proportion to F with constant k as follows:

$$V = k \left(\frac{dC}{dx} \right) \quad (3)$$

In this study, the effect of the pseudopods generation on this proportional relationship is examined. Neutrophil has the property of generating pseudopods near the inner wall of blood vessels as shown in Figure 1(a). To investigate the effect of shape difference as shown in Figure 1(b), (c), the distance from the wall and roughness indexes are measured.

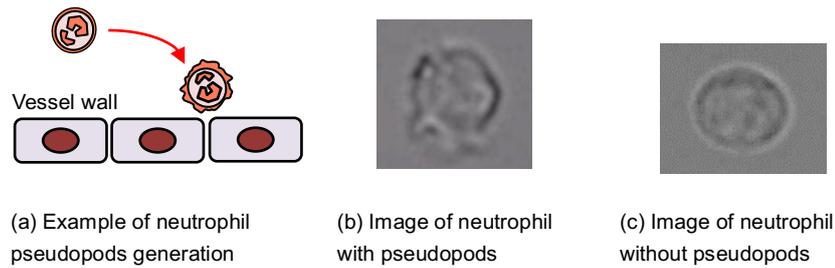


Figure 1: Neutrophil pseudopods generation

3. Methodology

Observation method

Observational experiments are done with reference to our previous study [14]. As shown in Figure 2, 10 ng/ml of cytokine solution is inserted by pipette into a dispersion containing neutrophils. A concentration gradient is formed around neutrophils, and neutrophils in liquid are induced to move toward higher concentrations of cytokine. The diffusion of cytokine and the neutrophil motion is photographed by CCD camera installed under the cover glass. Cytokines in solution labelled with fluorescent substances emit with exciting light, and the brightness distribution is measured. Analyzing the brightness distribution around neutrophil, the roughness indexes are calculated. To observe neutrophils with and without pseudopods, the distance H between the neutrophil and the wall (cover glass) is also measured from the amount of revolution of the microscope handle during focus adjustment as shown in Figure 3. Neutrophils are observed at $H = 2 \sim 10 \mu\text{m}$.

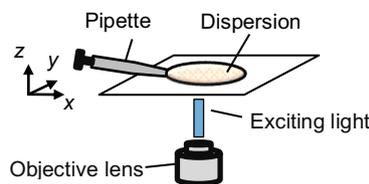


Figure 2: Schematic diagram of the experiment

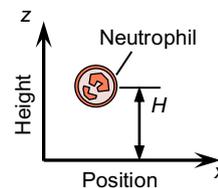


Figure 3: Distance from the wall

Shape index of neutrophil surface

To calculate the roughness indexes from captured neutrophil images, the outline of neutrophil is obtained. The outline is created by the following method (i)~(iv) using the Canny method [15,16].

(i) Smoothing using a Gaussian filter

The neutrophil image shown in Figure 4(a) is smoothed to Figure 4(b) by applying Gaussian filter $g(x,y)$ with scale σ . Gaussian distribution function is defined as follows:

$$g(x,y) = \frac{1}{2\pi\sigma^2} \exp\left(-\frac{x^2+y^2}{2\sigma^2}\right) \quad (4)$$

(ii) Intensity of brightness value gradient

The intensity of the luminance gradient in a two-dimensional image $|G|$ is defined from the luminance derivative in the horizontal (G_x) and vertical direction (G_y) as follows:

$$|G| = \sqrt{G_x^2 + G_y^2} \quad (5)$$

(iii) Non-maximum value suppression

Non-maximum suppression is a method to extract only the ridge of a mountain with gradient intensity. The non-maximum portions of the contour are deleted from the contiguous pixels in the normal direction.

(iv) Hysteresis thresholding

The binarization process is done using two luminance thresholds. The luminance values along the contour are judged to be greater or less than the threshold values. Points below the small threshold are deleted, and points above the large threshold are defined as contours. Points with luminance values between the two thresholds are referred to the neighboring points, and if they are connected to a contour that exceeds the large threshold, they are considered as contours.

The contour obtained by these processes is shown in Figure 4(c). Using the obtained contour coordinates and the center of gravity position, the outline profile curve (Figure 4(d)) is obtained by plotting the radius r in the circumferential direction. The mean radius of the neutrophil r_m is expressed with the circumference of the neutrophil l as follows:

$$r_m = \frac{1}{l} \int_0^l r dx \quad (6)$$

Neutrophil shape is evaluated with roughness indexes: arithmetic mean roughness R_a , root mean square roughness R_{ms} , skewness R_{sk} , and kurtosis R_{ku} , which are used as indices of the roughness of the uneven surface. Arithmetic mean roughness R_a is the average of the absolute value along the distribution. R_a is defined by Eq. (7).

$$R_a = \frac{1}{2\pi} \int_0^{2\pi} |r(\theta) - r_m| d\theta \quad (7)$$

The root mean square R_{ms} is a measure of distribution of the same dimension as energy. R_{ms} is defined by Eq. (8).

$$R_{ms} = \sqrt{\frac{1}{2\pi} \int_0^{2\pi} |r(\theta) - r_m|^2 d\theta} \quad (8)$$

Skewness R_{sk} is a measure of the degree to which the distribution of a random variable deviates from a normal distribution that is symmetrical. R_{sk} is defined by Eq. (9).

$$R_{sk} = \frac{1}{R_{ms}^3} \sqrt{\frac{1}{2\pi} \int_0^{2\pi} |r(\theta) - r_m|^3 d\theta} \quad (9)$$

Kurtosis R_{ku} is a measure of the combined weight of the distribution tail's relative to the center of the distribution. Kurtosis R_{ku} is defined by Eq. (10).

$$R_{ku} = \frac{1}{R_{ms}^4} \sqrt{\frac{1}{2\pi} \int_0^{2\pi} |r(\theta) - r_m|^4 d\theta} - 3 \quad (10)$$

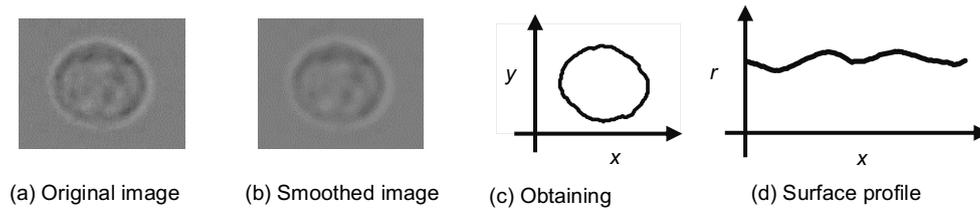


Figure 4: Procedure for obtaining surface profile curves

4. Results and Discussion

Figure 5(a) shows the relationship between the distance from the wall and roughness indexes. In engineering, R_a represents the level of friction on the surface [17]. R_a is higher at $H = 2, 4 \mu\text{m}$. There is a statistically significant difference between the results for $H \leq 4 \mu\text{m}$ and those for $H > 4 \mu\text{m}$. This result means that pseudopods are generated, and neutrophils change to a more frictional shape when the distance between the neutrophil and the wall is less than $4 \mu\text{m}$. There is a possibility that cytokines are less efficient at flowing to the surface of the membrane and don't generate concentration gradient when neutrophil is near the wall. Figure 5(b) shows the relationship between the distance from the wall and root mean square roughness. R_{rms} represents the energy caused by friction on a surface [18]. R_{rms} is statistically higher at $H = 2, 4 \mu\text{m}$ and the value is almost 0 above $H = 6 \mu\text{m}$. This trend is similar to Figure 5(a). This result means that neutrophil has a smooth shape with

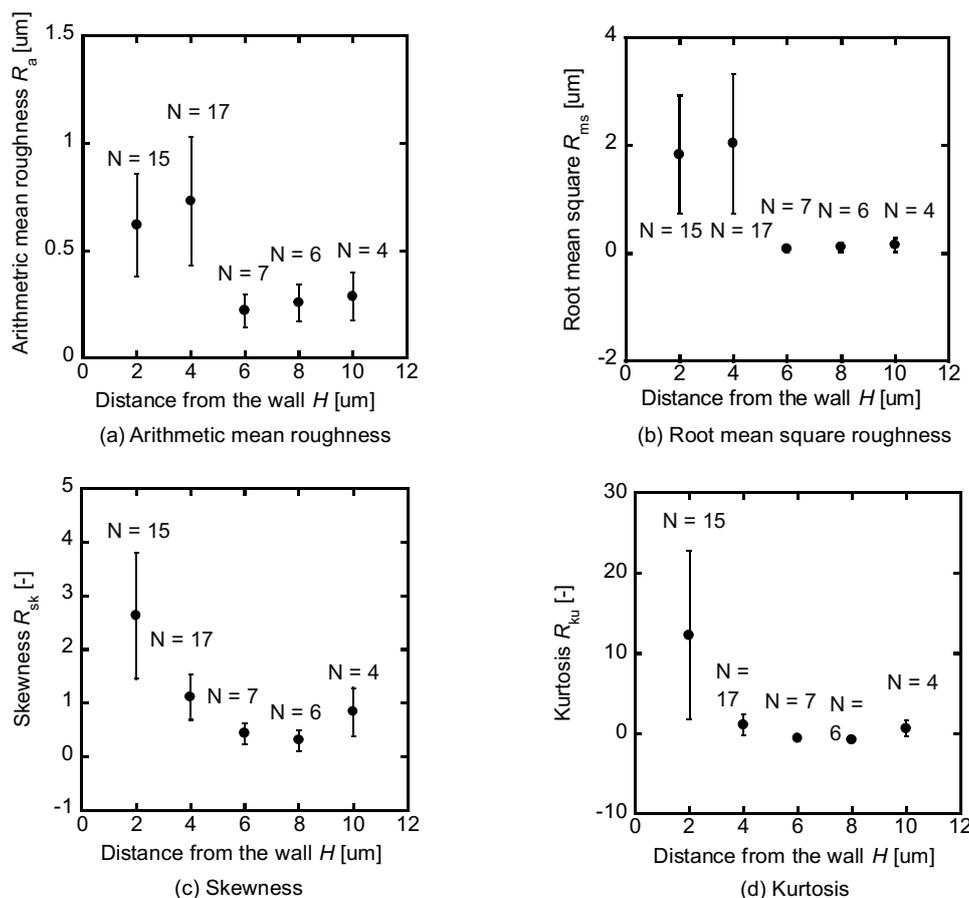


Figure 5: Relationship between distance from wall and roughness indexes

very little frictional energy above $H = 6 \mu\text{m}$. Neutrophils are known to behave more like proliferation and differentiation near blood vessels than migration. There is a possibility that neutrophil energy is used for locomotion in water and for cellular processes such as proliferation and differentiation at the wall surface. Figure 5(c) shows the relationship between the distance from the wall and skewness. R_{sk} is an index used, for example, to indicate the stiffness of a metallic material [19]. R_{sk} shows higher value when $H = 2 \mu\text{m}$, different from Figure 5(a) and Figure 5(b). The result suggests that when $H = 2 \mu\text{m}$, neutrophil is less rigid and more sensitive to influence from surrounding fluid. However, because of the large variability (large error bars), any apparent difference at $H = 2 \mu\text{m}$ cannot be regarded as statistically significant. Figure 5(d) shows the relationship between the distance from the wall and kurtosis. R_{ku} represents the randomness of the distribution in the field of statistics [19,20] R_{ku} shows the highest value when $H = 2 \mu\text{m}$ and the value is almost 0 for the others. This result indicates that neutrophils are spiky at $H = 2 \mu\text{m}$. It suggests that at $H = 4 \mu\text{m}$, there is friction on the surface due to the generation of short pseudopods, but the change in shape is small. This trend is similar to that in Figure 5(c), and no statistically significant difference is observed.

In summary the significant differences in R_a and R_{ms} across the $H = 4 \mu\text{m}$ boundary imply that neutrophils may initiate pseudopod formation at approximately $H = 4 \mu\text{m}$. Although R_{sk} and R_{ku} indicate a potentially more pronounced morphological change at $H = 2 \mu\text{m}$; however, no statistically significant differences were observed. In vivo, cytokine-driven neutrophil velocity is known to decrease near vessel walls; consistent with this, our results suggest that pseudopod generation under near-wall confinement may contribute to reduced motility.

However, the present experimental setup uses a cover glass as the wall surface rather than a vessel wall, and the imaging geometry primarily captures the cell surface facing the cover glass. Therefore, pseudopods extending perpendicular to the cover glass (toward the liquid phase) may be under-detected, which can limit the accuracy of roughness-based shape evaluation. To improve morphological quantification, future experiments should include imaging from the liquid-side (top view) and additional shape descriptors.

In addition, calcium imaging will be performed using Fura-2 AM as a direct and experimentally justified follow-up to probe intracellular dynamics associated with pseudopod formation. Specifically, we will examine whether near-wall conditions (e.g., $H = 2$ vs. $4 \mu\text{m}$) are accompanied by distinct intracellular Ca^{2+} responses, which may mechanistically link geometric remodeling to changes in motility.

5. Conclusion

In this study, the relationship between distance from wall and geometric features of neutrophil was investigated by experimental approaches. As a result, the following issues are concluded.

- I. Arithmetic mean roughness and root mean square roughness are higher at distances of 2 and $4 \mu\text{m}$ from the wall surface.
- II. Skewness and kurtosis showed the highest value at distance of $2 \mu\text{m}$ from the wall surface however, no statistically significant differences were observed.
- III. Neutrophil generates pseudopods at distance of $4 \mu\text{m}$.

As the future work, to elucidate the relationship between the internal and external flow of neutrophils, observation experiment of calcium ion flow inside neutrophils using Fura-2 AM solution will be done. However, the current experiment has a methodological limitation: the wall distance H was estimated from the microscope focus-handle rotation, which may introduce systematic error due to mechanical backlash and subjective focal-plane selection. This uncertainty may increase the variability of higher-order indices (e.g., skewness and kurtosis). Future work should include

independent calibration of H (e.g., using a z-axis calibration standard) and complementary imaging from the liquid side or 3D imaging to better capture cell morphology.

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References

- [1] Schroeder, D. A. Heller, M. M. Winslow, J. E. Dahlman, G. W. Pratt, R. Langer, T. Jacks and D. G. Anderson, "Treating metastatic cancer with nanotechnology" *Nature Reviews Cancer*, Vol. 12, 2012, pp. 39–50.
- [2] J. Li, B. E.-F. de Ávila, W. Gao, L. Zhang and J. Wang, "Micro/nanorobots for biomedicine: Delivery, surgery, sensing, and detoxification," *Science Robotics*, Vol. 2, No. 4, 2017, pp. 180–184.
- [3] H. Alemzadeh, J. Ramen, N. Leveson, Z. Kalbarczyk and R. K. Iyer, "Adverse Events in Robotic Surgery: A Retrospective Study of 14 Years of FDA Data," *PLOS ONE*, Vol. 11, No. 4, 2016, e0151470.
- [4] J. Zhang, Z. Chen, R. K. Kankala, S. B. Wang and A. Z. Chen, "Self-propelling micro-/nano-motors: Mechanisms, applications, and challenges in drug delivery," *International Journal of Pharmaceutics*, Vol. 596, No. 1, 2021, pp. 1–14.
- [5] X. Lin, Z. Wu, Y. Wu, M. Xuan and Q. He, "Self-Propelled Micro-/Nanomotors Based on Controlled Assembled Architectures," *Advanced Materials*, Vol. 28, Issue 6, 2015, pp. 1060–1072.
- [6] X. Kawamura, M. Nomura, J. Hayase, Y. Iwakiri, A. Nishikimi, R. Takayanagi, Y. Fukui and H. Sumitomo, "The Cell Polarity Protein mNsc Regulates Neutrophil Chemotaxis via a Noncanonical G Protein Signaling Pathway," *Developmental Cell*, Vol. 26, Issue 3, 2013, pp. 292–302.
- [7] H. Li, F. Peng, X. Yan, C. Mao, X. Ma, D. A. Wilson, Q. He and Y. Tu, "Medical micro- and nanomotors in the body," *Acta Pharmaceutica Sinica B*, Vol. 13, Issue 2, 2023, pp. 517–541.
- [8] Zhang, C. Ling, L. Pang, Q. Wang, J. Liu, B. Wang, J. Liang, Y. Guo, J. Qin and J. Wang, "Direct Macromolecular Drug Delivery to Cerebral Ischemia Area using Neutrophil-Mediated Nanoparticles," *Theranostics*, Vol. 7, Issue 13, 2017, pp. 3260–3275.
- [9] R. Obana and M. Tamagawa, "Experimental and Theoretical Study of Propulsion Mechanism with Neutrophil Rotation by using Concentration Marangoni Effect," *Journal of Advanced Research in Fluid Mechanics and Thermal Sciences*, Vol. 108, No. 1, 2023, pp. 114–125.
- [10] R. Obana and M. Tamagawa, "Evaluation of Neutrophil's Rotation on Transport Process of Membrane Concentration and its Underwater Propulsion by CFD Analysis," *Journal of Advanced Research in Fluid Mechanics and Thermal Sciences*, submitted.
- [11] Amulic, C. Cazalet, G. L. Hayes, K. D. Metzler and A. Zychlinsky, "Neutrophil Function: From Mechanisms to Disease," *Annual Review of Immunology*, Vol. 30, 2012, pp. 459–489.
- [12] K. Fujisaki, "Marangoni Effects in Surfactant Solution," *Biophysical Journal*, Vol. 34, Issue 4, 1995, pp. 389–394.
- [13] V. Sternling and L. E. Scriven, "Interfacial turbulence: Hydrodynamic instability and the Marangoni effect," *AIChE Journal*, Vol. 5, Issue 4, 1959, pp. 514–523.
- [14] R. Obana and M. Tamagawa, "Effects of underwater plane shock waves on neutrophil propulsion," *Journal of Biomechanical Science and Engineering*, Vol. 17, No. 3, 2022, pp. 22-00083.
- [15] K. N. Hansson and S. Hansson, "Skewness and Kurtosis: Important Parameters in the Characterization of Dental Implant Surface Roughness—A Computer Simulation," *International Scholarly Research Notices*, Issue 1, 2011, pp. 1–6.
- [16] J. Canny, "A Computational Approach to Edge Detection," *IEEE Transactions on Pattern Analysis and Machine Intelligence*, Issue 6, 1986, pp. 679–698.
- [17] A. B. Faia-Torres, S. Guimond-Lischer, M. Rottmar, M. Charnley, T. Goren, K. Maniura-Weber, N. D. Spencer, R. L. Reis, M. Textor and N. M. Neves, "Differential regulation of osteogenic differentiation of stem cells on surface roughness gradients," *Biomaterials*, Vol. 35, Issue 33, 2014, pp. 9023–9032.
- [18] Duparré, J. Ferre-Borrull, S. Glied, G. Notni, J. Steinert and J. M. Bennett, "Surface characterization techniques for determining the root-mean-square roughness and power spectral densities of optical components," *Applied Optics*, Vol. 41, Issue 1, 2002, pp. 154–171.
- [19] N. Tayebi and A. A. Polycarpou, "Modeling the effect of skewness and kurtosis on the static friction coefficient of rough surfaces," *Tribology International*, Vol. 37, Issue 6, 1986, pp. 679–698.
- [20] R. Dwyer, "Use of the kurtosis statistic in the frequency domain as an aid in detecting random signals," *IEEE Journal of Oceanic Engineering*, Vol. 9, Issue 2, 1984, pp. 85–92.