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NUMERICAL SIMULATION OF TRANSPORT PROCESS OF CYTOKINE CONCENTRATION ON A NEUTROPHIL MEMBRANE TO ELUCIDATE MECHANISM OF CHEMOTAXIS

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Abstract. *To develop propulsion mechanism for micro-machine in liquid is an important topic such as medical inspection machine in a blood pipe. It is very difficult to put small engine or motor in a micro-scale machine. So it is necessary to develop new mechanism for this propulsion. Chemotaxis, the motion of neutrophil (white blood cell) in liquid, is famous phenomena in immunology. In general, the motion of the neutrophil is considered to be driven by concentration gradient of cytokine, which is attractant substance. However, it has not been elucidated yet. This paper describes the mechanism of neutrophil's propulsion by concentration gradient of cytokine. Especially, propagation of cytokine on neutrophil's membrane, which has been obtained in our experiment, was investigated by numerical simulation for elucidating the mechanism of motion. Using transport equations of concentration on the membrane and outer fluid region, the numerical simulation was done. As a result, the concentration gradient on the membrane changes from negative to positive by changing "diffusion coefficient" and "adsorption-desorption coefficient" of neutrophil's membrane. This change corresponds to our previous experimental result.*

Keywords: DDS, Numerical analysis, Chemotaxis, Concentration gradient, Cytokine

1. **Introduction.** Inflammation reaction is a very important role as immune response in human body. In this reaction, once the inflammation occurred, the cytokine (chemokine; concentration matter) is delivered from the inflammation place. Then neutrophil (white blood cell) accesses at the place to cure. In this case, the neutrophile moves to the place that has large gradient of cytokine concentration. This is so called chemotaxis of neutrophile. There are many previous researches related to this chemotaxis, but there is very few work related to driving force of chemotaxis by gradient of cytokine concentration. Most of them are related to motion on the wall or biochemical mechanism [1-7]. Then the driving force of this motion in liquid has not been elucidated yet.

Generally the particulate in liquid on the gradient of concentration has the force driven by so called "concentration Marangoni effect" (Figure 1). However, it is not elucidated whether the mechanism of an inorganic particulate is the same as the driving force of neutrophil or not.

By the way, this mechanism can be applied to the drug delivery systems (DDS). Once the chemokine or concentration is generated at the affected part, the capsule with chemotactic function moves to this place automatically. So it is preferable for DDS to carry the particles with drug efficiently to the affected part in human body. Then it is necessary to understand and clarify the mechanism of neutrophile motion by gradient of chemokine concentration.

The final purpose of this investigation is to elucidate the driving force of particulate by concentration Marangoni effects by experimental and computational work.

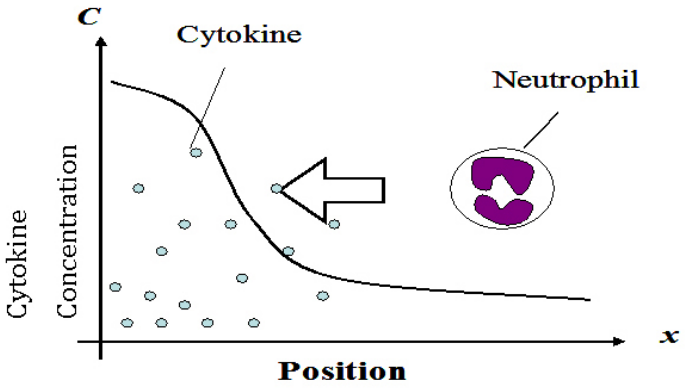


FIGURE 1. Concept of chemotaxis using concentration Marangoni effect

In this paper, after reviewing the physical model of neutrophil and previous experiments (Section 2 and Section 3), the computational model of membrane for sensing cytokine concentration was constructed. Especially, the model of transportation process of cytokine concentration on neutrophil's membrane was constructed (Section 4). Using transport equations of concentration on the membrane and outer fluid region, the numerical simulation was done. And the effects of concentration gradient on the diffusion process of the membrane and surrounding fluid were obtained and discussed (Section 5). Finally, conclusions and further investigation are made (Section 6).

2. **Driving Force by Surface Tension Gradient.** Supposing the particulate sphere in liquid under the surface tension gradient, the driving force F of particulate in liquid by the surface tension is obtained from circular integration of surface tension gradient.

$$F = \int_C \left(\frac{d\sigma}{dx}\right) dx \tag{1}$$

where σ shows surface tension. And surface tension is a function of concentration and temperature. If the temperature is constant, the gradient of surface tension is expressed as follows,

$$\left(\frac{d\sigma}{dx}\right) = \left(\frac{\partial\sigma}{\partial C}\right)_T \left(\frac{dC}{dx}\right) \tag{2}$$

where C shows concentration. From this relation, the driving force in liquid is caused by the concentration (the surface tension) gradient. This is so called concentration Marangoni effect.

3. **Observation of Concentration Diffusion and Neutrophil Motion by Fluorescence.** In our previous experiments [8], to prove the neutrophil motion by concentration gradient, the experimental observation was done using the simple system as shown in Figure 2. In this system, after dropping the cytokine at the liquid area of neutrophil suspension, the concentration of cytokine is diffusing in the suspension liquid. The motion of neutrophil was taken by the video movie. The cytokine with fluorescent material (FITC) was observed as intensity level in CCD image. Bottom picture in Figure 2 shows diffusion process of cytokine and motion of neutrophil. It was found that the high intensity region can be observed around the neutrophil. It shows that the cytokine concentration attaches to the membrane of neutrophil. Figure 3 shows intensity gradient (concentration gradient) history on the membrane of neutrophil. It was found that gradient is changing from negative to positive value, and oscillating concentration was increasing suddenly. From this time, the neutrophil begins to move from left to right.

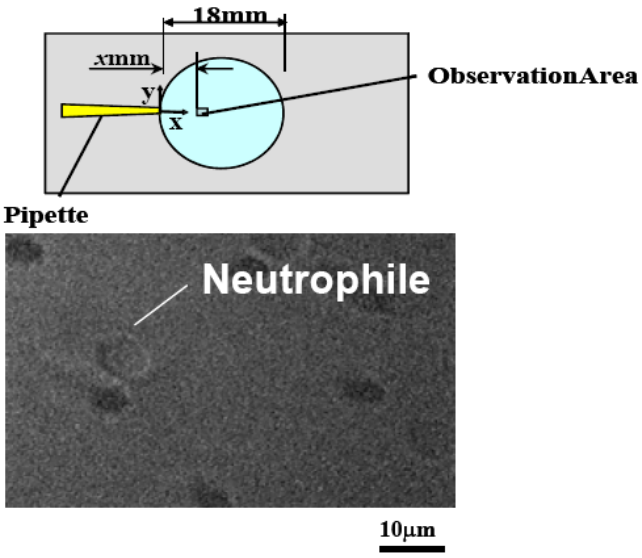


FIGURE 2. Observation system of neutrophil motion by concentration (cytokine) gradient on the prepared slide

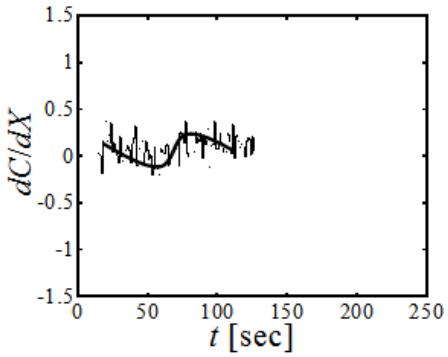


FIGURE 3. Concentration gradient history on the membrane after passing incident concentration gradient

As a most important result, it was found that the diffusion process of concentration on the membrane is faster than that around the membrane. Then it was considered that the concentration gradient of cytokine on the membrane is "positive" by this faster transportation of concentration on the membrane. It is necessary to check the sign of the concentration gradient as it is important role in this mechanism.

4. **Computational Model for Transport Process of Concentration on Membrane.** To construct the model to simulate the concentration transport on the membrane and in the surrounding fluid, the model of resemble phenomena such as surfactant around a bubble is adopted. Cuenot et al. [9] proposed the model of slightly soluble surfactants on the flow around a spherical bubble. This model includes the effects of surface tension on the surface of the bubble, and concentration of surfactants. In this model, the flow field is divided into two regions, i.e., as neutrophil membrane and surrounding fluid. Especially, the concentration is also transported on the membrane like a fluid motion. For both regions, they are continuum equation, Navier-Stokes equations and transport equation for concentration. The following concentration transport equations on the membrane and in the surrounding fluid are applied in this model,

$$\frac{\partial \Gamma}{\partial t} + \nabla_s \cdot (V_s \Gamma) = D_s \nabla_s^2 \Gamma - D(\nabla C)_s \cdot N \tag{3}$$

$$\frac{\partial C}{\partial t} + V \cdot \nabla C = D\nabla^2 C \tag{4}$$

where Γ [ng/ml] is cytokine concentration on the membrane, C [ng/ml] is cytokine concentration in surrounding fluid, D_s [m^2/s] is diffusion coefficient of the membrane, D [m^2/s] is diffusion coefficient of surrounding fluid, and V_s and V indicate advection velocity. In this paper, as these advection velocities are small, they are assumed to be 0. Adsorption coefficient h [m^2/s] is also taken into account in the boundary condition on the membrane surface.

As for the computational regions, the whole region is 75 [μm] $\times$ 40 [μm], the diameter of neutrophil d is 12 [μm] and the membrane thickness t_n is 0.5 [μm] (Figure 4). For initial conditions, the concentration distribution is set up as shown in Figure 4. The concentration around the inlet C_0 is 10 [ng/ml], and the other concentration C_0 is 0 [ng/ml]. As for the boundary conditions, flux j_n at outer region ∂C_1 and inner ∂C_3 is 0, and continuous condition at the interface ∂C_2.

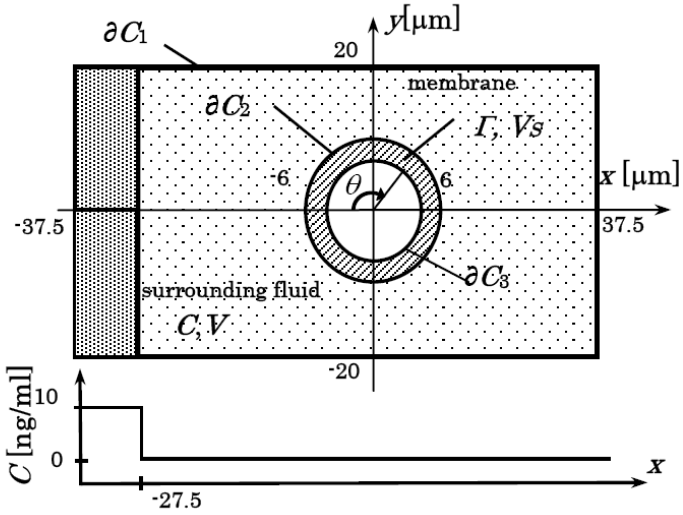
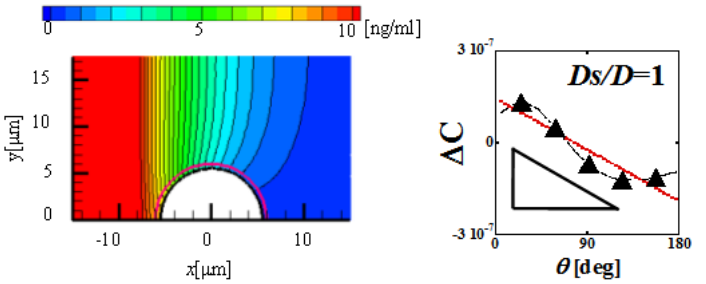


FIGURE 4. Computational model for analyzing the transport process of concentration around the neutrophil with membrane and initial concentration profile along x axis

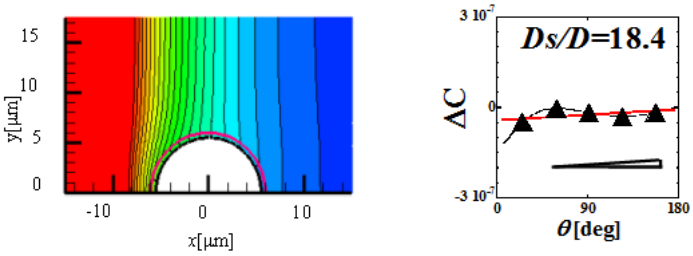
As the effects of D and D_s on Marangoni effect are large, the diffusion coefficient of membrane D_s/D and adsorption coefficient h are used as parameters.

5. **Results and Discussions.** In this case, by changing the diffusion coefficient of membrane D_s/D and adsorption coefficient h, the gradient of concentration can be checked.

Figure 5 shows the effects of concentration coefficient ratio D_s/D on concentration contour in the surrounding fluid and the membrane after 30 seconds, and concentration gradient along the circumference on the membrane. Figure 5(a) and Figure 5(b) show the case of $D_s/D = 1$, 18.4, respectively. In this case, adsorption coefficient h is set to be 1.9×10^{-7}. In this figure, the lateral axis shows angle on the membrane, and the longitudinal axis shows cytokine concentration or concentration difference. From this figure, it is found that the sign of gradient changes from negative to positive due to the diffusion ratio. Especially, in case of $D_s/D = 18.4$, the gradient is positive on the membrane although the gradient of the surrounding fluid is positive. This result means that the driving force is obtained by concentration gradient alternatively if the diffusion of the membrane is larger than that of surrounding fluid. In fact, the positive gradient is also observed in the previous experiment.

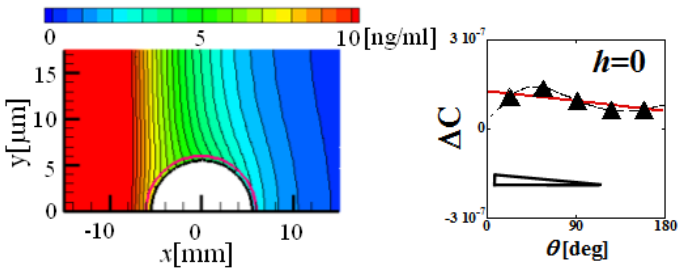


(a) $D_s/D = 1.0$

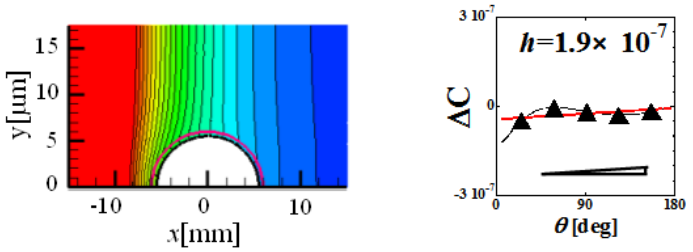


(b) $D_s/D = 18.4$

FIGURE 5. Computational result for (1) concentration contour after 30 seconds and (2) concentration profile by changing the diffusion coefficient on membrane and surrounding fluid: (a) $D_s/D = 1$ and (b) $D_s/D = 18.4$



(a) $h = 0$



(b) $h = 1.9 \times 10^{-7}$

FIGURE 6. Computational result for (1) concentration contour after 30 seconds and (2) concentration profile by changing the adsorption coefficient of the membrane: (a) $h = 0$ and (b) $h = 1.9 \times 10^{-7}$

Figure 6 shows the effects of adsorption coefficient h on concentration contour in the surrounding fluid and the membrane after 30 seconds, and concentration gradient along the circumference on the membrane. In this case, diffusion coefficient ratio D_s/D is set to be 18.4. From this figure, it is also found that there is the same trend for sign of

concentration gradient in case of higher adsorption coefficient. It means positive gradient occurs at high adsorption on the membrane.

From these results, there should be regions that the gradient of cytokine concentration on the membrane changes from negative to positive when the diffusion coefficient ratio D_s/D is increasing or adsorption coefficient h is increasing.

6. **Conclusions.** Assuming that the difference of diffusion process corresponds to the surface tension difference, which leads to driving force by Marangoni effect, the numerical analyses of transport of cytokine concentration on the membrane and in the surrounding fluid are done. And it is concluded that the concentration gradient on the membrane changes from negative to positive by increasing "diffusion coefficient" and "adsorption-desorption coefficient" of neutrophil's membrane. This change corresponds to our previous experimental result. It suggests that the fluidity of the membrane is important.

Further investigations for effects of neutrophil's rotating and advection of the surrounding fluid are needed.

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REFERENCES

[1] P. R. Ebrahimzadeh, Neutrophil chemotaxis in moving gradients of fMLP, *Journal of Leukocyte Biology*, pp.651-661, 2000.

[2] H. L. Goldsmith, T. A. Quinn, G. Drury, C. Spanos, F. A. Mclntosh and S. I. Simon, Dynamics of neutrophil aggregation in Couette flow revealed by videomicroscopy: Effect of shear rate on two-body collision efficiency and doublet lifetime, *Biophysical Journal*, vol.81, pp.2020-2034, 2001.

[3] B. Albert, D. Bray, J. Lewis, M. Raff, K. Roberts and J. D. Watson, *Molecular Biology of the Cell 3E*, 1999.

[4] S. K. W. Dertinger, D. T. Chiu, N. L. Jeon and G. M. Whitesides, Generation of gradients having complex shapes using microfluidic networks, *Analytical Chemistry*, vol.73, pp.1240-1246, 2001.

[5] D. Kim and C. L. Haynes, Neutrophil chemotaxis within a competing gradient of chemoattractants, *Analytical Chemistry*, vol.84, pp.6070-6078, 2012.

[6] R. A. Jannat, M. Dembo and D. A. Hammer, Traction forces of neutrophils migrating on compliant substrates, *Biophysical Journal*, vol.101, pp.575-584, 2011.

[7] M. B. Byrne, Y. Kimura, A. Kapoor, Y. He, K. S. Mattam, K. M. Hasan, L. N. Olson, F. Wang, P. J. A. Kenis and C. V. Rao, Oscillatory behavior of neutrophils under opposing chemoattractant gradients supports a winner-take-all mechanism, *PLoS ONE*, vol.9, no.1, 2014.

[8] M. Tamagawa and K. Matsumura, Fundamental investigations of driving force of a neutrophile in liquid using concentration Marangoni effect for developing microcapsules in drug delivery dystems, *2008 ASME Fluids Engineering Conference*, pp.553191-553194, 2008.

[9] B. Cuenot et al., The effects of slightly soluble surfactants on the flow around a spherical bubble, *Journal of Fluid Mechanics*, vol.339, pp.25-53, 1997.