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ENHANCEMENT OF NEUTROPHIL MOTION BY SHOCK WAVES FOR DEVELOPING NEW CANCER IMMUNO-THERAPY –EFFECTS OF SURROUNDING LIQUID TEMPERATURE AND MAXIMUM PRESSURE ON NEUTROPHIL VELOCITIES–

Masaaki Tamagawa and Yoshikaze Tabata

Graduate School of Life Science and Systems Engineering
Kyushu Institute of Technology
Hibikino 2-4, Wakamatsu-ku, Kitakyushu 808-109, Japan
tama@life.kyutech.ac.jp

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Abstract. *This paper describes enhancement of neutrophil motion by shock waves for developing new cancer immuno-therapy with changing environmental conditions such as temperature and working pressure related to initial pressure ratio to activate immune-cells. To obtain the chemotaxis performance of neutrophil, the neutrophil velocity is measured by working gradient of cytokine concentration. In this paper, effects of temperature in the surrounding liquid and working pressure related to initial pressure ratio (maximum pressure of shock wave) on the velocities were investigated. After working 5 shock waves on the suspension liquid of neutrophils, the velocity was measured by image processing on the microscopy. It was concluded that the neutrophil velocities in case of low temperature (10°C) were higher than that in case of high temperature (26°C). The other factor such as duration time of these temperature was discussed. It was also concluded that there should be optimized strength of shock wave to obtain the highest velocity by changing the initial pressure with consideration of surrounding temperature, and the highest amplitude of neutrophil velocity was 1.5 times higher than that without shock wave.*

Keywords: Shock wave, Chemotaxis, Cancer immuno-therapy, Stimulus, Neutrophil

1. **Introduction.** Recently medical therapy and bio-industry by shock waves including the Extracorporeal Shock Wave Lithotripsy (ESWL) has been world-widely applied, and it is also used in the medical field of osteogenesis therapy and regenerative medicine [1]. On the other hand, more applications are required widely in other medical field or research field. They are such as safety research related to prevention of impact force acts on the head and bones due to falling of elderly people [2], and brain therapy including Alzheimer dementia [3] and cancer immuno-therapy without drugs.

As for the immuno-therapy, 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 a neutrophil (white blood cell) accesses at the place to cure. There are many previous researches related to this chemotaxis, but there are very few works related to driving force of chemotaxis by gradient of cytokine concentration. Most of them are related to motion on the wall and biochemical mechanism [4-14]. There are no topics to deal with the neutrophile's microswimming in water by physical mechanism. It means that there are no elucidations of the physical mechanism about this micro-swimming in these previous papers, and it is very difficult to apply the mechanism to develop new medical systems without physical understanding by using the method such as shock wave. Then

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it is necessary to elucidate the mechanism of enhancement of neutrophile motion by shock waves systemically.

In our previous investigations [15], we focus on the fundamental mechanism by assuming Marangoni effect for micro particulate, and it was found that the sign of concentration gradient on the membrane is an important factor for locomotion of neutrophil.

By the way, cancer immuno-therapy naturally cures by promoting a patient's own immunity. In general, an immune function is increased by cultivating in vitro after taking out a patient's immunological cells, and immuno-cells is returned to the patient's body. In this cultivating process, the process should be accelerated by stimulus to shorten these cultivation time.

In this investigation, the possibility of shock wave's enhancing the immune function that immune-cells originally possess is examined (Figure 1). Although it has been confirmed that the number of cells increases when underwater shock wave works on endothelial cells in our previous investigations [16], but the influence of shock waves on immune cells has not been elucidated yet. In this paper, the degree of immune function of neutrophils by shock waves is investigated by measuring the underwater neutrophil velocity. Especially, the effects of the maximum pressure of shock waves on degree of neutrophil velocity related to chemotaxis factors are examined. The final purpose of this investigation is to find out the optimizing the stimulus method to promote neutrophil velocity and develop new cancer immuno-therapy.

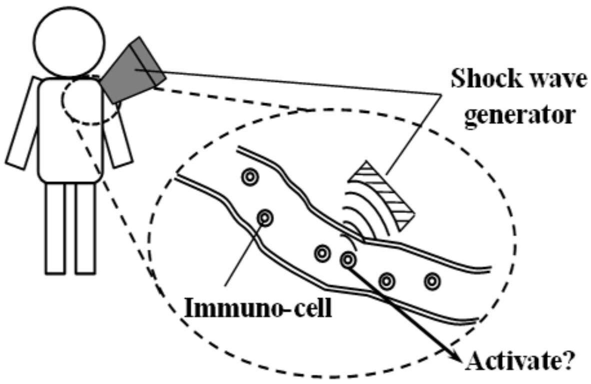
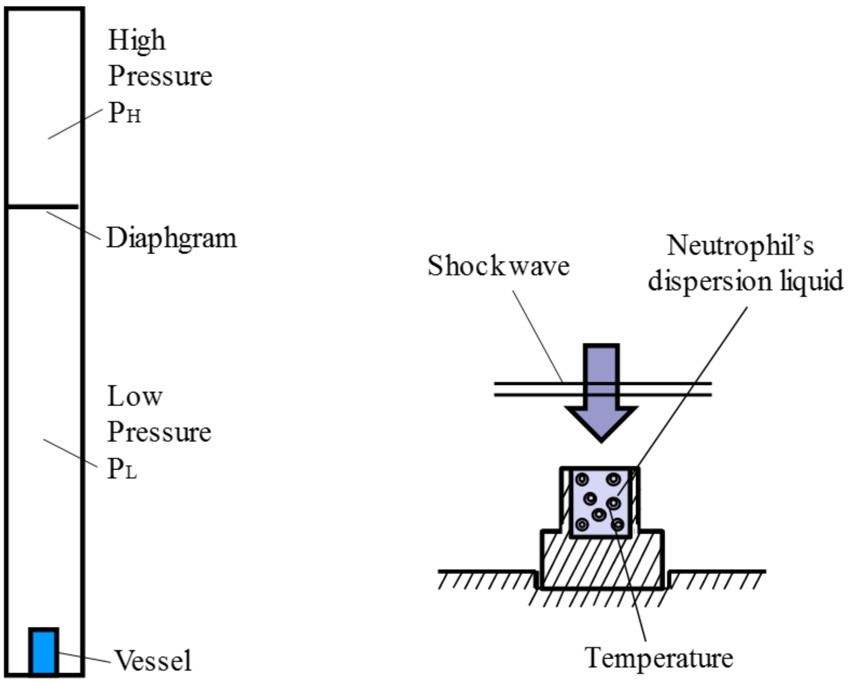


Figure 1. Concept of cancer immuno-therapy by applying shock waves

In this paper, experimental apparatus and methods to find out the effects of initial pressure ratio (maximum pressure) of shock waves on neutrophil velocity were shown in Section 2, and effect of surrounding temperature on neutrophil velocity and effects of initial pressure ratio on neutrophil velocity were shown and discussed in Section 3, and the concluding remarks were described in Section 4.

2. **Experimental Apparatus and Methods.**

2.1. **Shock wave's working on neutrophils.** The test vessel is filled with neutrophil dispersion derived from pig's fresh blood, and plane shock waves are applied to the neutrophils in it by using a shock tube (Figure 2(a)). In the experimental conditions, the initial pressure ratio of shock waves (the ratio of the initial low pressure to the initial high pressure of the shock tube) is from 2 to 3.5 to add the proper stimulus. The reason why the pressure range is proper is derived previous experimental conditions [16] for working shock wave to avoid the cell damage. And the shot number of shock waves is 5 to show the results of the proper stimulus clearly. Figure 3 shows typical pressure history in case of initial pressure ratio 2.5. As shown in this figure, maximum pressure is determined according to the initial pressure ratio.



(a) Shock tube (b) Neutrophil suspension in the vessel

FIGURE 2. Neutrophil velocity with initial pressure and duration time

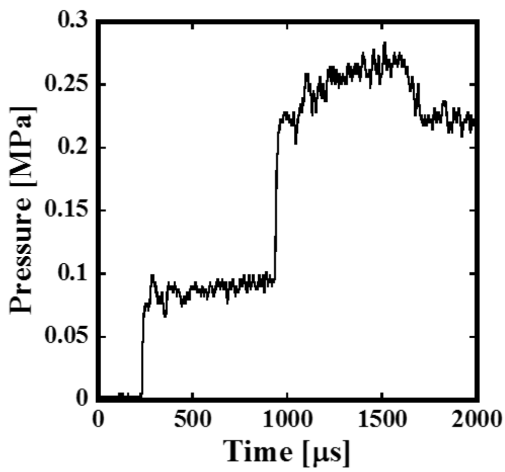


FIGURE 3. Typical pressure history at the vessel including neutrophil suspension in case of initial pressure ratio 2.5

During the neutrophil's suspending in the vessels located at the end of shock tube (Figure 2(b)), effects of two kinds of the surrounding temperature in the vessel on the neutrophil velocity are examined in case of the high temperature (26°C) and the low temperature (10°C). Here two kinds of duration time of each temperature such as 10 mins and 6 hours are used, and the effects of each time on the neutrophil velocity are also examined.

After shock wave's applying, the neutrophil dispersion is heated for observation of motion in a thermostatic chamber maintained at 38°C.

2.2. **Observation of neutrophil motion and PTV analysis.** To diffuse the chemotactic cytokine IL-8 from the pipette, the volume (100 mL) of the worked neutrophil dispersion is located on a glass slide (Figure 4). In this experiment, the cytokine concentration is 10 ng/mL, and the volume was 5 μL. Then the motion of neutrophils toward the

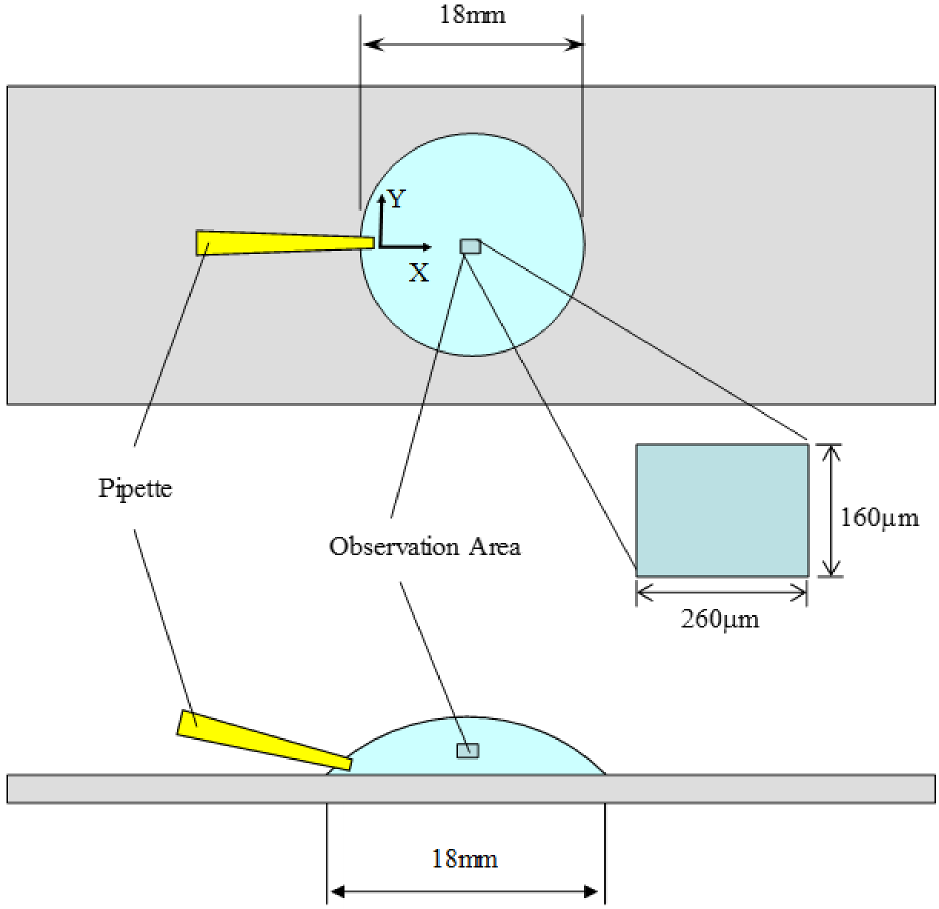
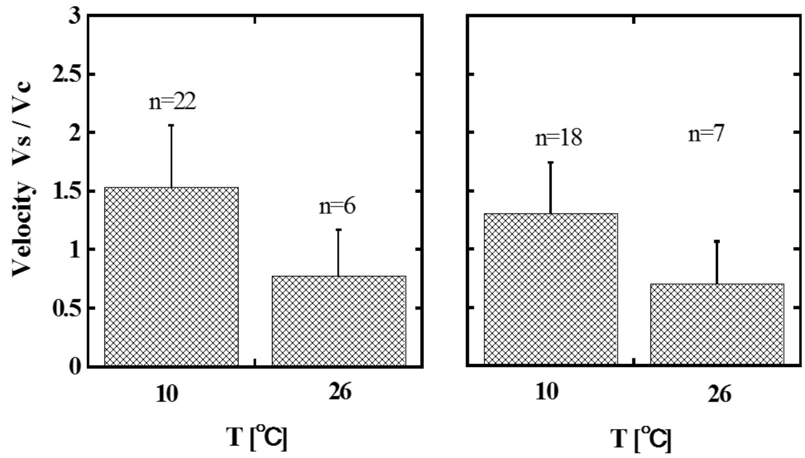


FIGURE 4. Observation area on the microscope and the pipette to diffuse cytokine concentration

direction of high cytokine concentration is observed by a microscope with a CCD camera. After getting the movies of these motions, PTV (Particle Tracking Velocimetry) analysis is performed by using the neutrophils as a tracer (PTV analysis software named DIPP MOTION (Detect Corporation)). The coordinates of tracer neutrophils are calculated by this movie and the velocities of each neutrophil are determined in this software.

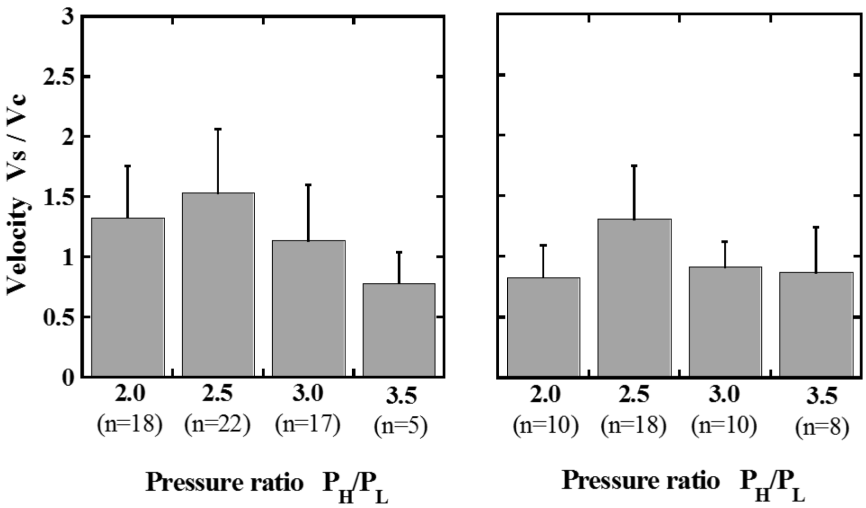
3. Results and Discussion.

3.1. **Effect of surrounding temperature on neutrophil velocity.** Figure 5 shows the neutrophil velocity with and without shock wave when the surrounding temperature is changed in case of initial pressure ratio 2.5. Figure 5(a) shows the case that the heating duration time of the neutrophil dispersion before the shock wave's applying is 10 mins, and Figure 5(b) shows the case that the duration time is 6 hours. The vertical axis represents the dimensionless velocity Vs/Vc, where Vs is the neutrophil velocity by shock wave and Vc is the neutrophil velocity of the control group (without shock wave), and the horizontal axis represents the surrounding temperature before shock wave's working. n represents the number of neutrophils measured during the experiment. From Figures 5(a) and 5(b), it is found that the motion velocity increases 1.5 times when the surrounding temperature is kept to be low (10°C) and the shock wave is applied. On the other hand, when the shock wave is applied while keeping the surrounding temperature high (26°C), the motion velocity is less than that of the control group. This is considered to be due to the fact that the elasticity of cell membranes becomes to be larger by keeping the surrounding temperature low. If the elasticity of cell membrane is high, deformation of cell is small, and the pressure and stress wave are propagating quickly, and it is considered that the low



(a) 10 mins (b) 6 hours

FIGURE 5. Neutrophil velocity with surrounding temperature and duration time



(a) 10 mins (b) 6 hours

FIGURE 6. Neutrophil velocity with initial pressure and duration time

temperature is more effective than the high temperature to enhance the motion velocity. As a result, temperature is more sensitive to the motion velocity. From these results, the surrounding temperature is kept at 10°C in the next section.

3.2. **Effects of initial pressure ratio on neutrophil velocity.** Figure 6 shows the effect of the initial pressure ratio on the neutrophil velocity. In this figure, the vertical axis represents the dimensionless velocity Vs/Vc and the horizontal axis represents the initial pressure ratio, as the same definition in Figure 5. In Figures 6(a) and 6(b), it is found that the dimensionless velocity Vs/Vc increases until the initial pressure ratio is 2.5, and decreases when the pressure ratio is less than 2.5 or 3.0. It is considered that the stimulation to neutrophils by the shock waves is not sufficient when the initial pressure ratio is 2.0. On the contrary, the stimulation is excessive due to lower velocity when the initial pressure ratio is more than 3.0. It is also found that the dimensionless velocity tends to be smaller in Figure 6(b) when the heating duration time is longer in each initial pressure ratio. It is considered that neutrophil activation is decreasing due to the increase in heating duration time.

From these results, it is found that the dimensionless velocity of neutrophils by the shock wave working is maximum at the initial pressure ratio of 2.5, which is the moderate stimulus to neutrophils.

4. **Conclusion.** In this paper, shock waves were used to enhance or activate the chemotactic function of neutrophils, and the degree of enhancement was examined by measuring the neutrophil velocity. The following things are concluded.

1) The effect of the shock wave on the neutrophil velocity can be obtained more clearly in case of surrounding low temperature (10°C) than that in case of high temperature (24°C).

2) There should be optimized strength of shock wave to obtain the highest velocity by changing the initial pressure with consideration of surrounding temperature. From this investigation, the highest amplitude of neutrophil velocity is 1.5 times higher than that without shock wave.

3) It was suggested that the activation of neutrophils itself decreases with the increase of heating duration time.

In future, the experiments using ultrasonic waves should be done to examine the effects of frequency and continuous wave on the neutrophil velocity to develop new cancer immune-therapy.

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REFERENCES

[1] K. Hanawa, K. Ito, K. Aizawa, T. Shindo, K. Nishimiya, Y. Hasebe, R. Tuburaya, H. Hasegawa, S. Yasuda, H. Kanai and H. Shimokawa, Low-intensity pulsed ultrasound induces angiogenesis and ameliorates left ventral dysfunction in a porcine model of chronic myocardial ischemia, *PloS One*, vol.9, no.8, 2014.

[2] M. Tamagawa, H. Matsuura, M. Nakano, M. Yamanaka and M. Kubota, A basic study on safety evaluation in the event of human collision or fall, *Journal of BMFSA*, vol.13, no.2, pp.97-104, 2011.

[3] K. Eguchi, T. Shindo, K. Ito, T. Ogata, R. Kurosawa, Y. Kagaya, Y. Monma, S. Ichijo, S. Kasukabe, S. Miyata, T. Yoshikawa, K. Yanai, H. Taki, H. Kanai, N. Osumi and H. Shimokawa, Whole-brain low-intensity pulsed ultrasound therapy markedly improves cognitive dysfunctions in mouse models of dementia – Crucial roles of endothelial nitric oxide synthase, *Brain Stimulation*, vol.11, pp.959-973, 2018.

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

[5] H. L. Goldsmith, T. A. Quinn, G. Drury, C. Spanos, F. A. McIntosh 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.

[6] B. Albert, D. Bray, J. Lewis, M. Raff, K. Roberts and J. D. Watson, *Molecular Biology of the Cell*, 3th Edition, Garland Publishing, New York and London, 1994.

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

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

[9] 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.

[10] 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.

[11] C. T. Mierke, The matrix environmental and cell mechanical properties regulate cell migration and contribute to the invasive phenotype of cancer cells, *Reports Prog. Phys.*, vol.82, no.6, 2019.

[12] Q. Wang and H. G. Othmer, Analysis of a model microswimmer with applications to blebbing cells and mini-robots, *J. Math. Biol.*, vol.76, pp.1699-1763, 2018.
[13] G. Wang and T. Galli, Reciprocal link between cell biomechanics and exocytosis, *Traffic*, vol.19, no.10, pp.741-749, 2018.
[14] W. Strychalski and R. D. Guy, Intracellular pressure dynamics in blebbing cells, *Biophys. J.*, vol.110, no.5, pp.1168-1179, 2016.
[15] M. Tamagawa and T. Hirose, Numerical simulation of transport process of cytokine concentration on a neutrophil membrane to elucidate mechanism of chemotaxis, *ICIC Express Letters, Part B: Applications*, vol.7, no.1, pp.31-36, 2016.
[16] M. Tamagawa and N. Ishimatsu, Effects of shock waves on acceleration of cell growth rate by shock tube, *Proc. of IMECE 2008*, pp.655-656, 2008.