

# Effect of ELF Electrostimulation on Macrophage Scavenger Receptor

Muneyoshi Kagawa<sup>#1</sup>, Toshiyuki Shimooka<sup>\*2</sup>, Koichi Shimizu<sup>#3</sup>

<sup>#</sup>Graduate School of Information Science and Technology, Hokkaido University, Sapporo, Japan

<sup>1</sup>mkagawa@bme.ist.hokudai.ac.jp

<sup>3</sup>shimizu@bme.ist.hokudai.ac.jp

<sup>\*</sup>Faculty of Health and Medical Care, Saitama Medical University, Hidaka, Japan

<sup>2</sup>shimooka@saitama-med.ac.jp

**Abstract**—As an attempt to control immune functions using ELF electrostimulation, we studied the effects of electrostimulation on macrophage functions *in vitro*. We have reported that ELF electrostimulation affected the endocytic activity of macrophage and that humoral factors mediated this effect. In this study, as the first step to explore the mechanism, we examined the amount change of Macrophage Scavenger Receptors (MSR) caused by ELF electrostimulation (0.30 A/m<sup>2</sup>, 50Hz, 30 min). The increase of MSR was observed with statistical significance. The involvement of humoral factor in this phenomenon was also confirmed. This suggested that the MSR change mediated by the humoral factors is one of the mechanisms for the effect of electrostimulation on macrophage functions.

**Key words:** ELF electrostimulation, macrophage, macrophage scavenger receptor, humoral factor

## I. INTRODUCTION

The effects of ELF electromagnetic fields (ELF-EMFs) on physiological functions have been reported from various viewpoints. They include studies of effects on cell apoptosis and differentiation [1], cellular signal transduction [2], and levels of inducible HSP70 [3]. In these studies, different mechanisms were proposed for the effects. Walleczek proposed the effect on the Ca<sup>2+</sup> metabolism using thymocytes [4]. Lupke proposed the mediator of active oxygen in monocytes [5]. Most of them are on harmful effects. However, the electric field is used for clinical applications, such as therapeutic instruments using ELF electric field. We have pursued the possibility to control an immune function by ELF electrostimulation, and studied the effects on a macrophages fuction. We have investigated the effect of electrostimulation on macrophage *in vitro*. Macrophages play important roles in a human immune system. They show selective endocytic activity against an exogenous material and eliminate it. They produce free radicals such as reactive oxygen and NO, as well. There many diseased associated with the macrophages. For example, when they intake much ox-LDL, they become foam cells and induce arteriosclerosis. The excessive or insufficient productions of free radicals result in the body damages of the immunodeficiency, respectively. We have reported the effect of electrostimulation on the production of reactive oxygen and

NO [6,7]. We have also investigated the effect of endocytic activity. The endocytic activity was suppressed significantly by electrostimulation. To investigate the mechanism of this phenomenon, humoral factors were examined. After electrostimulation of macrophages, we collected the top clear layer that was separated through centrifugation. In this supernatant, the endocytic activity of macrophages was suppressed even though they had never experienced the electrostimulation [8].

In this study, we have examined the effect of electrostimulation on the amount of MSR on cell membrane to reveal the mechanism of the effect on endocytic activity. The MSR is the same endocytic receptor as an Fc receptor and mediates the endocytosis of nonspecific substance as latex beads and ox-LDL. Therefore, we examined the amount change of MSR on cell membrane. In addition, humoral factors were investigated to elucidate the mechanism of the effect.

## II. MATERIALS AND METHODS

### A. Macrophage Preparation

Peritoneal exudate macrophages were used in all the experiments. A normal mouse (Std:ddy, female, 7-11 weeks) was intra-peritoneally injected with thioglycollate medium. After 3-4 days the cells were harvested from the mouse abdomen. The cells were isolated and washed 3 times with Hanks' balanced salt solution by centrifugation. We prepared the cell culture solution (RPMI 1640 containing 10% FBS). The collected macrophages were suspended in the cell culture solution to make the cell density of 1.0-5.0 × 10<sup>6</sup> cells/ml.

### B. Electrostimulation System

The macrophages were stimulated with the electric current in the container insulated electrically from the electrodes to simulate the condition of electrostimulation from out side the human body. Figure 1 shows the appearance and the structure of the sample container. The container was made of acrylic resin with two copper electrodes. The electrodes were

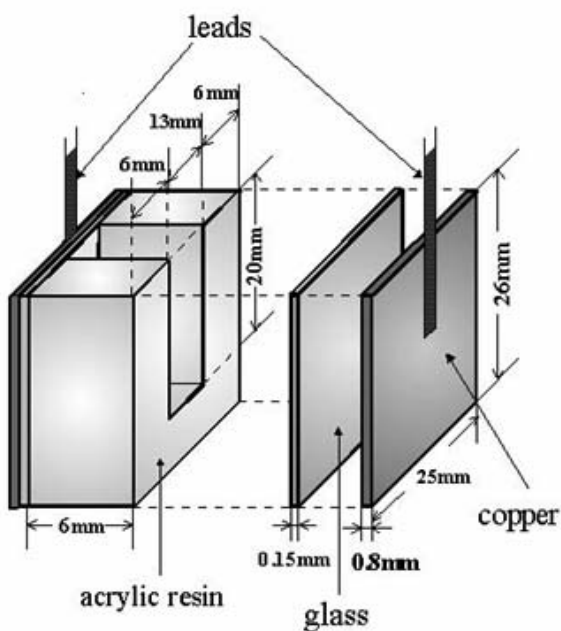
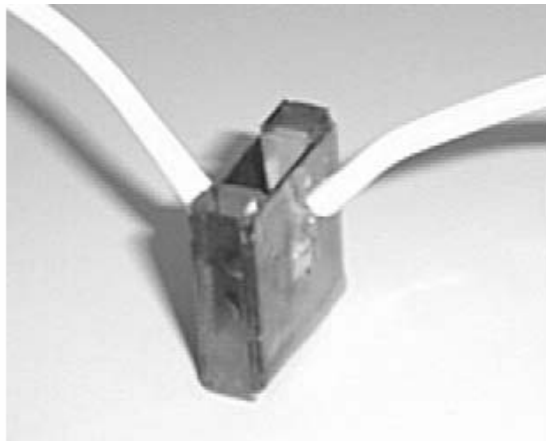


Fig.1 Appearance and structure of sample container to apply electrostimulation to cells suspension.

insulated with thin plates of glass. The outline of the electrostimulation system is shown in Fig.2. Two electrodes were connected to a high-voltage power source through a noise filter. As the power source, the power supply of a commercial apparatus for the electric field therapy was used (Healthtron, Hakuju Inst. Health Science, Maximum supply voltage 10kV). A sinusoidal AC signal of 50Hz was supplied to the apparatus with a waveform generator to make the high-voltage with the sinusoidal waveform of 50Hz. The sample container was placed in a shaking water bath at 37 °C during the experiment.

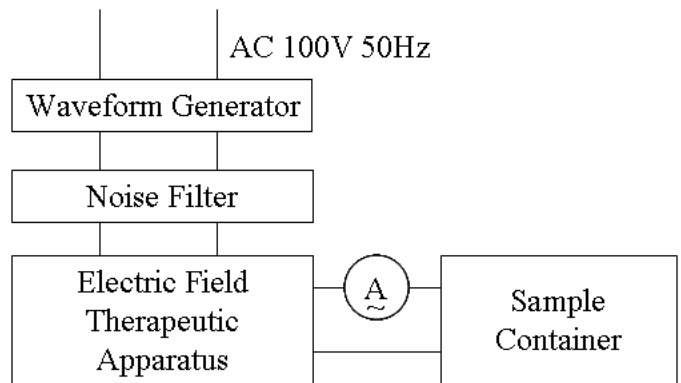


Fig.2 Schematic diagram of electrostimulation system.

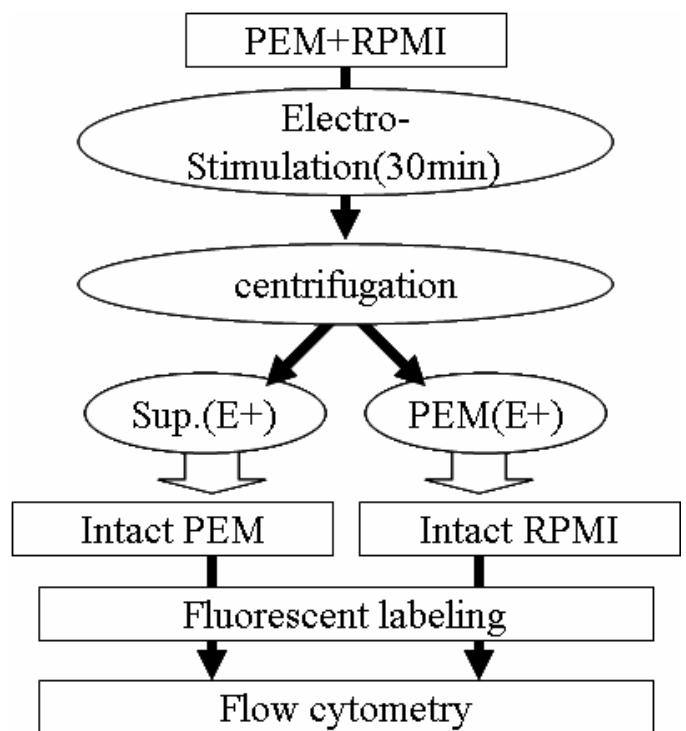


Fig.3 Flow of sample preparation and experiment to examine effect of humoral factors.

### C. Measurement of MSR Amount

Rat anti-mouse-CD204-antibody (MCA1322F, UK-Serotec Ltd.) was used to measure an MSR amount on cell membrane. Macrophages were separated into two groups, and electrostimulation ( $0.30 \text{ A/m}^2$ , 50Hz, 30min) was applied to the stimulation group. After the electrostimulation, the macrophages were labeled with the antibody at 0 °C with gentle shake. After the two hours culturing, the macrophages were isolated and washed 3 times with HBSS by centrifugation, and the macrophages were fixed with HBSS

containing 1% glutaraldehyde at 4 °C. The amount of the MSR was measured and analyzed by a flow cytometer (FACS Calibur Becton Dickinson and Company).

#### D. Association with Humoral Factors

We have reported that ELF electrostimulation affected endocytic activity, and that humoral factors were involved in the mechanism. To examine whether humoral factors affect the amount of MSR, we conducted the following experiment. Figure 3 shows the outline of the experiment.

The suspension of the macrophages was stimulated with electric current for 30 minutes. After the stimulation, the supernatant was separated from macrophages by centrifugation. The humoral factors in the supernatant might cause the change in endocytosis. The supernatant and the intact macrophages stored without electrostimulation, were mixed (tagged sup.(E+)). On the other hand, stimulated macrophage were mixed with the intact RPMI 1640 (tagged PEM(E+)). The suspension was cultured 30 minutes for the interaction of humoral factors with macrophages. After this, the macrophages were labeled in the same way as the one mention above.

### III. RESULTS AND DISCUSSION

Figure 4 shows the difference in fluorescent intensity between the stimulation and the sham groups. The ordinate is the fluorescent intensity. The same experiment was repeated 12 times, data of corresponding pair were connected with the solid lines. The  $p$ -values of the paired  $t$ -tests were  $p < 0.01$  showing statistical significance. This result suggested the increase of MSR amount by the ELF electrostimulation.

To elucidate the mechanism of this increase in the MSR amount, the effect of the humoral factors on the MSR amount was examined. Figure 5(a) shows the effect of supernatant from stimulated macrophage on the MSR amount. As shown in this figure, the slight increase in fluorescent intensity was observed. The  $p$ -values of the paired  $t$ -tests were  $p < 0.05$  showing statistical significance. It should be noted that the MSR of the non-stimulated macrophage did increase. Figure 5(b) shows the effect of the cell-body electrostimulation of macrophages on its MSR amount. In this case, fluorescent intensity of PEM(E+) was also larger than the sham groups ( $p < 0.01$ ).

As can be seen if Fig.5(a), a humoral factor is possibly involved in the MSR increase. The cytokine released from macrophage is a likely candidate of the humoral factor. The cytokine worked in our experiment is considered to be that stored in macrophage cells according to the period between the electrostimulation and the measurement. The macrophage cells were induced using thioglycollate medium. It was reported that the medium has the mRNA for TNF- $\alpha$ , IL-1 $\alpha$ , IL-1 $\beta$ , IL-10, IL-12 [9]. Lenten et al. conclude that the LPS-induced suppression of the scavenger receptor is mediated

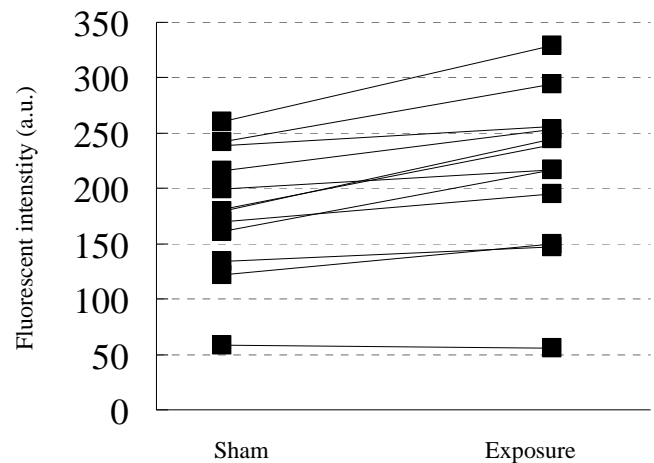
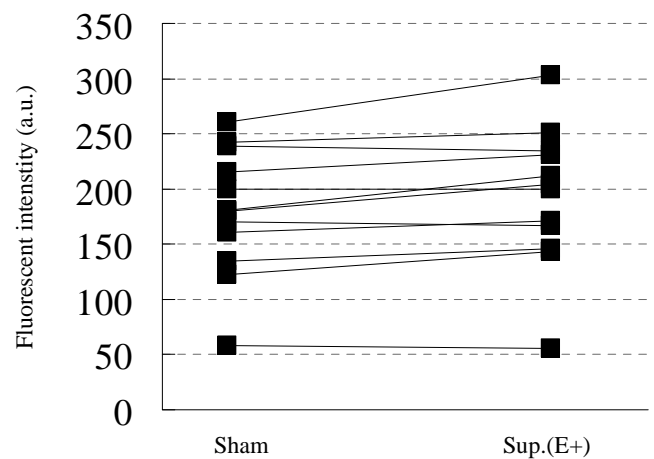
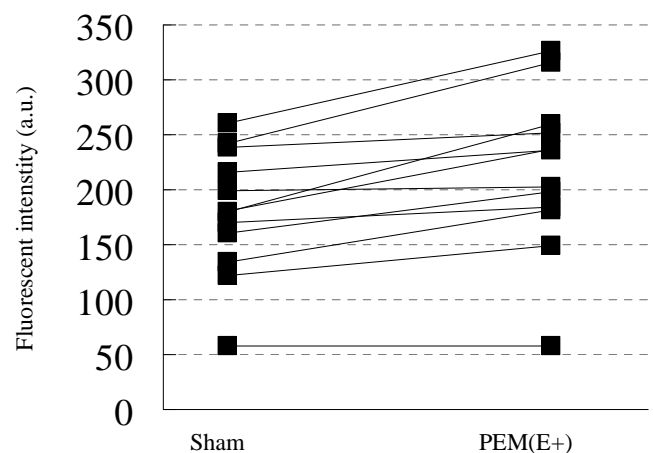


Fig.4 Effect of electrostimulation on MSR.



(a) Effect of supernatant from exposed cell on MSR amount.



(b) Effect of cell-body electrostimulation on its MSR amount.

Fig.5 Association of supernatant and PEM with MSR change.

primarily through TNF- $\alpha$  in human monocyte-macrophages [10]. In contrast, mouse macrophage lines increase SR-A expression in response to LPS [11]. Pessina et al. reported that pulsed EMF enhances induction of TNF- $\alpha$  by Peripheral Blood Mononuclear Cells [12]. We need to study the association of these cytokines to find the humoral factor related to the endocytic suppression and the MSR increase..

We have reported that the ELF electrostimulation suppressed the endocytic activity of macrophages. However, it is generally thought that the increase in MSR amount occurs when the amount of endocytosis increases. This implied that the amount of MSR on the cell surface is not related to the endocytic suppression. Further study is required on the effect of electrostimulation on the total amount of MSR and on endocytosis.

#### IV. CONCLUSION

With a view toward the control of immune function by ELF electrostimulation, the cellular functions were investigated *in vitro*. To investigate the mechanism of the change in macrophages endocytic activity, the effects of ELF electrostimulation on the MSR amount were investigated. The amount of MSR on cell membrane increased in ELF electrostimulation. The results suggested that humoral factors mediated this change.

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