

Effect of Electric Stimulation on Adhesion and Proliferation of Cultured Muscle Cells

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ABSTRACT

The effect of electric stimulation on adhesion and proliferation of cultured muscle cells has been studied *in vitro*. C2C12 (Mouse myoblast cell line) cells are cultured with Dulbecco's Modified Eagle's Medium and stimulated for 5min with electric pulse. Variations were made on the amplitude of the pulse. The number of cells, which adhered to the bottom of the culture dish, was counted according to the time (<180 min) after finishing electric stimulation. To observe cells proliferation, the number of cells was also counted at the time (<72 hours) after electric stimulation for five minutes. The experimental results show that adhesion is restricted with electric pulse of the higher amplitude of ten volts.

Keywords: Biomedical Engineering, Muscle Cells, Cell Culture, Electric Stimulation, Adhesion, Proliferation and Pulse

1. INTRODUCTION

Electrical stimulation has been applied in rehabilitation medicine. The effect of electrical stimulation on muscle cells was studied in previous study *in vitro* [1]. Behavior of biological cells depends on various environmental factors, such as electric, magnetic and mechanical fields.

Cell culture technique has been progressed and myoblasts have been clinically applied to ischaemic cardiomyopathy in the field of regenerative medicine. Acceleration technique for orientation and proliferation of cells has been studied to make muscle tissue *in vivo* and *in vitro* [2-4]. The previous studies show the experimental design to evaluate the effect of magnetic fields on cells [5, 6]. Control methodology for adhesion and proliferation of cells would be applied to regenerative tissue technology.

In the present study, the effect of electric stimulation on adhesion and proliferation of cultured muscle cells has been studied *in vitro*.

2. METHODS

Cell Culture

C2C12 (Mouse myoblast cell line originated with cross-striated muscle of C3H mouse) was cultured with Dulbecco's Modified Eagle's Medium (D-MEM). The cells were seeded in the polystyrene micro-plate, which has six wells with the flat bottom of 35 mm diameter without collagen coating (Fig. 1). Fetal bovine serum (FBS) was added to the medium with the volume rate in 10 percent of FBS and 90 percent of D-MEM. Three milliliter of medium was contained in each well.

Electric Stimulation

The electrode was made of platinum wire of 0.2 mm diameter, which was nestled along the fringe of each well with 18 mm long above 1 mm from the bottom. The wire was inserted through a glass capillary, which passes through the cap of the well to be fixed at the position. The terminal of the capillary was melted, which seals the terminal to stop capillary phenomenon so that the medium is protected from

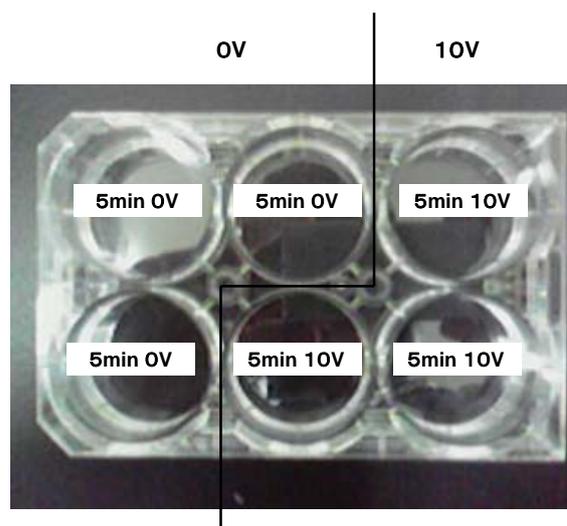


Fig. 1: Six wells with flat bottom of 35 mm diameter.

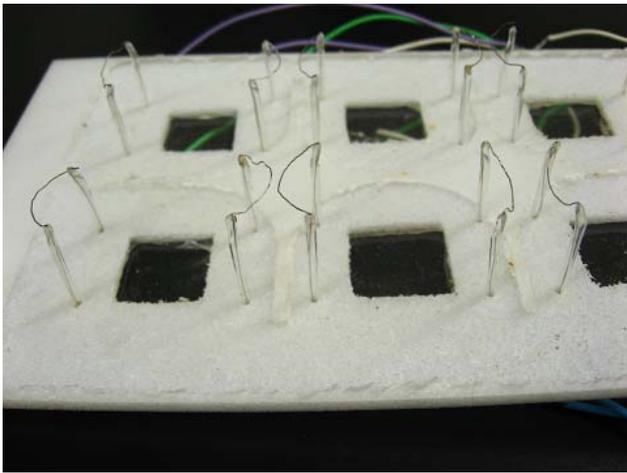


Fig. 2: Electrodes fixed on the cap of culture dish.

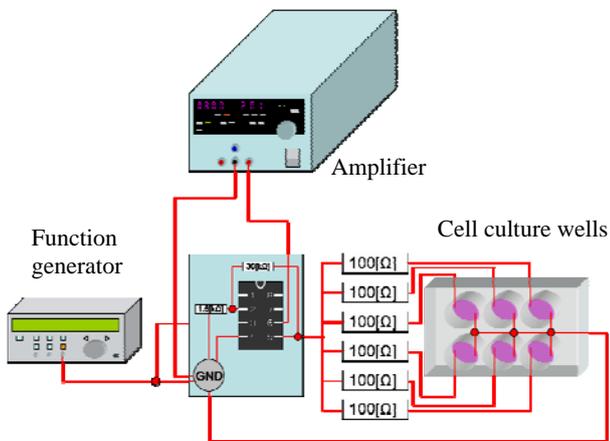


Fig. 3: Experimental system.

contamination. Two electrodes in opposed position each other were dipped in the medium of each well (Fig. 2).

Electric rectangular pulses, which have 0.1 s width and 1 s period (10 Hz), were generated with a function generator to be applied to culture medium (Fig. 3 & 4). Variation was made in amplitude of the electric pulse between 1 and 10 V with an amplifier. Control cells were not exposed to the electric stimulation.

Adhesion

Cells were exfoliated from the plate of the culture dish with trypsin and seeded in the well with 10000 cells per square centimeter. Immediately after cells seeding, electrodes were dipped, and electric pulses were applied for five minutes. To trace the numbers of cells, which adhered on the bottom after finishing electric stimulation for five minutes, medium was suck away of each well one-by-one at 5, 15, 30, 60, 180 minuets, respectively (Fig. 5). To wash away the cells, which had not adhered to the bottom of the well, medium of 3 mL was added, gently stirred in the well, and discarded. At 180 minutes, cells in every well were exfoliated with trypsin, and counted the number for each well. In the control study, no electric

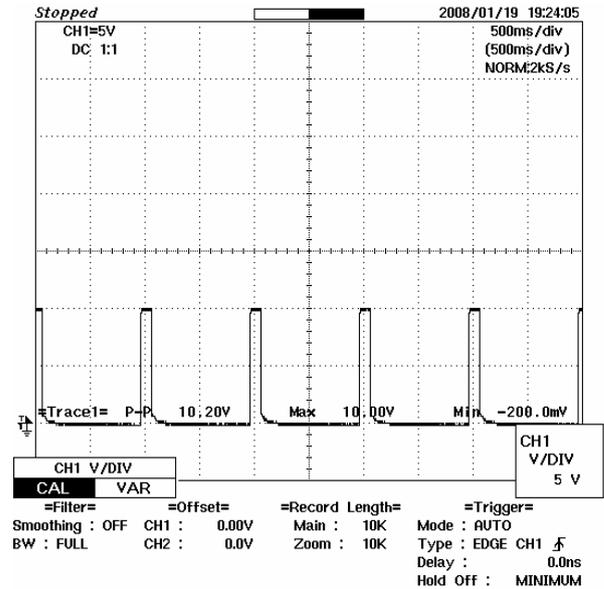


Fig. 4: Electric pulse tracings: width 0.1 s, period 1 s, and amplitude (difference between peak and base values) 10 V.

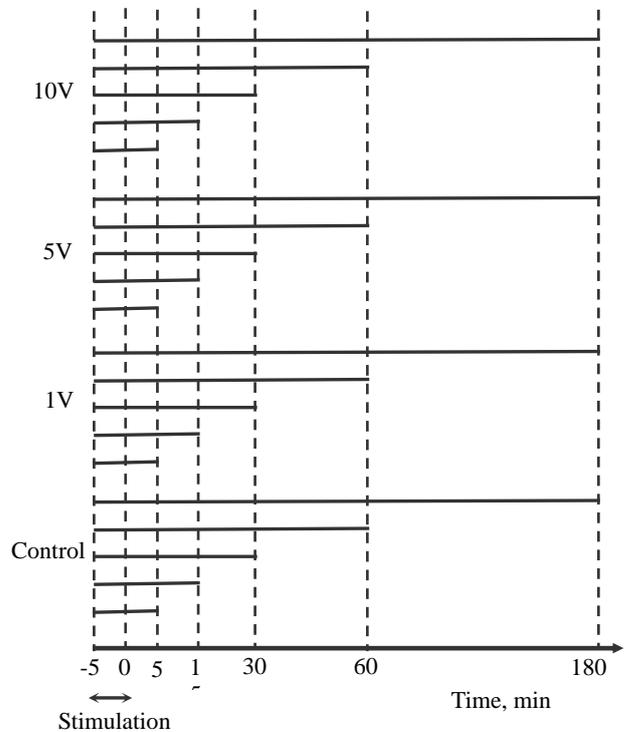


Fig. 5: Experimental protocol for cell adhesion.

stimulation was applied to the medium after cell seeding.

Proliferation

Electric stimulation was applied at three hours after seeding. Numbers of the cells were counted at 24, 48, 72 hours, after the electric stimulation for five minutes. In the control study, no electric stimulation was applied to the medium after cell seeding.

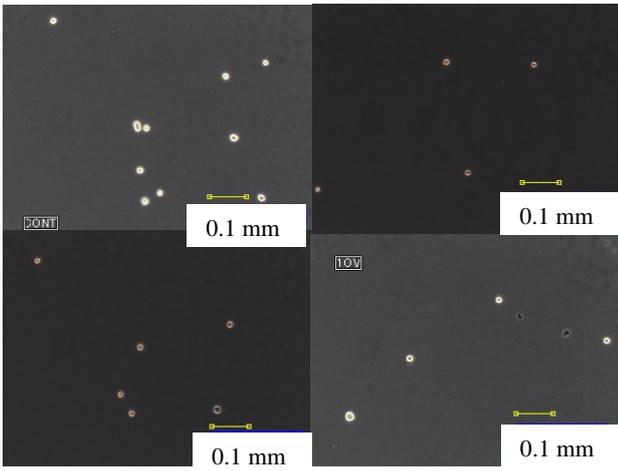


Fig. 6: Cells at 5 min after electric stimulation: control (upper left), 1 V (upper right), 5 V (lower left) and 10 V (lower right).

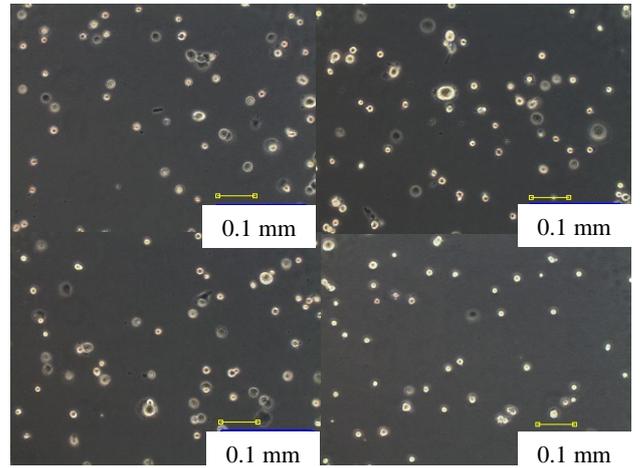


Fig. 9: Cells at 60 min after electric stimulation: control (upper left), 1 V (upper right), 5 V (lower left) and 10 V (lower right).

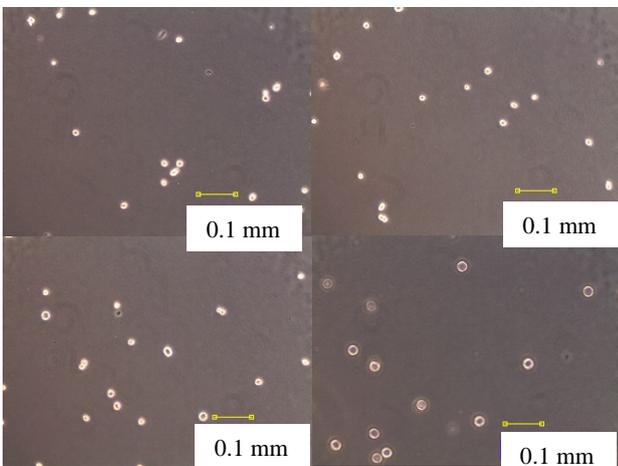


Fig. 7: Cells at 15 min after electric stimulation: control (upper left), 1 V (upper right), 5 V (lower left) and 10 V (lower right).

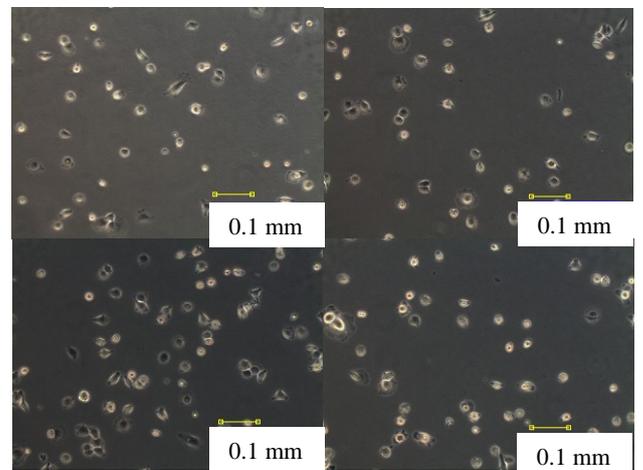


Fig. 10: Cells at 180 min after electric stimulation: control (upper left), 1 V (upper right), 5 V (lower left) and 10 V (lower right).

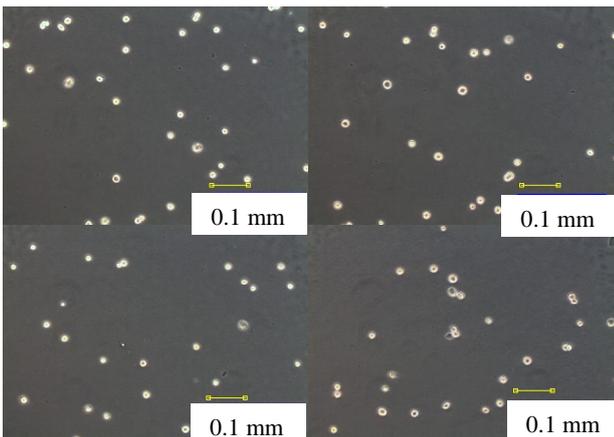


Fig. 8: Cells at 30 min after electric stimulation: control (upper left), 1 V (upper right), 5 V (lower left) and 10 V (lower right).

3. RESULTS

In the experiment on adhesion, the number of adhered cells was small at 5 minutes (Fig. 6). After 15 minutes, the number of adhered cells decreased with electric stimulation (Figs. 7-10). The number of cells was small at every hour with electric stimulation of ten volts. The number of cells increased with time, and almost saturated at 60 minutes (Fig. 11).

In the experiment of proliferation, the number of cells decreased with amplitude of electric pulses, although the increase rate of the number of cells was approximately same regardless of the voltage of electric pulses (Figs. 12-16).

The experimental results show that adhesion is restricted and delayed with electric pulse of the higher amplitude to ten volts.

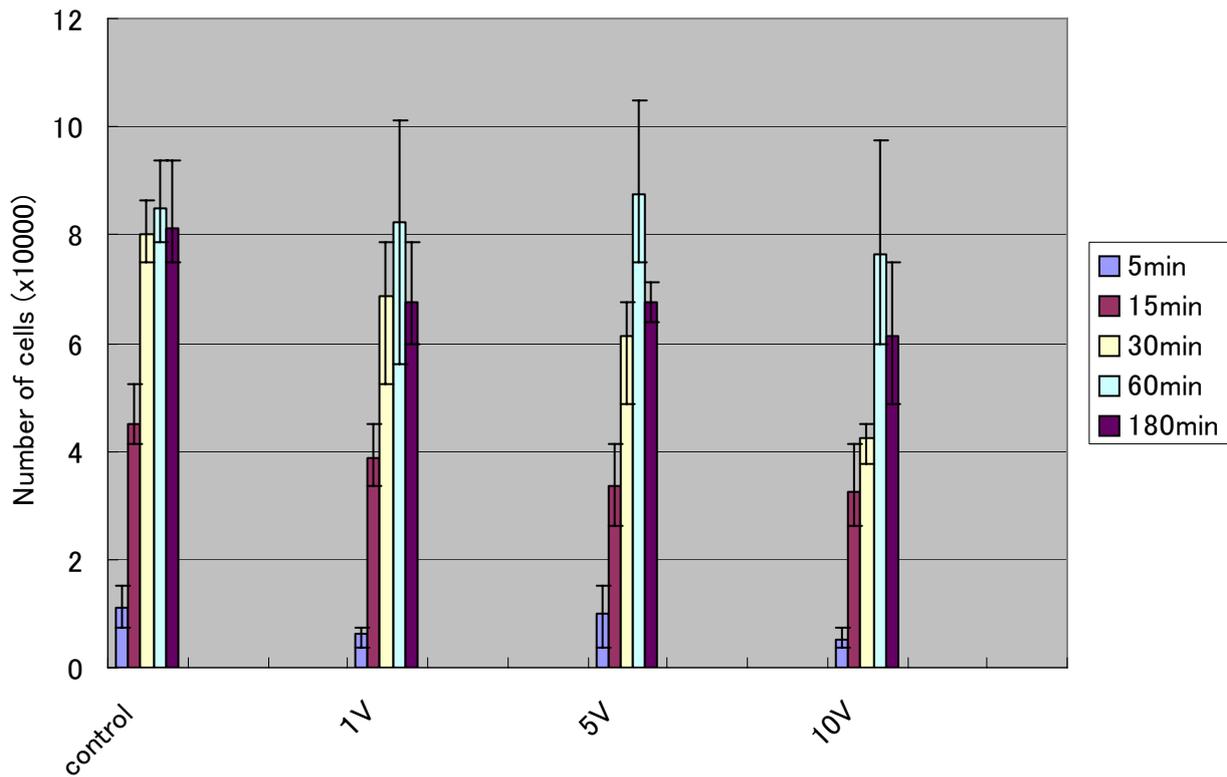


Fig. 11: Number of adhered cells after electric stimulation. Each bar shows minimum and maximum value.

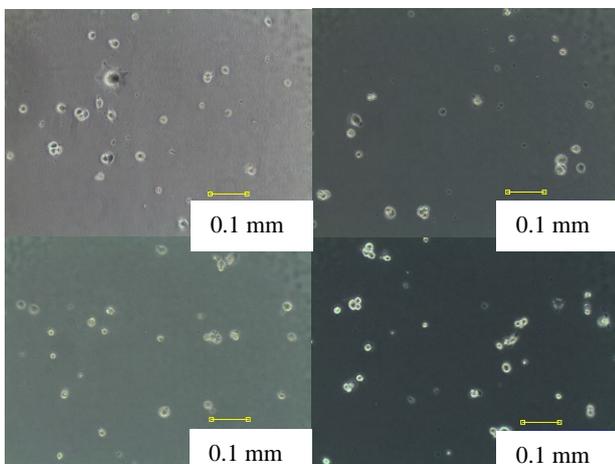


Fig. 12: Cells immediately after electric stimulation of 5 min: control (upper left), 1V (upper right), 5V (lower left) and 10 V (lower right).

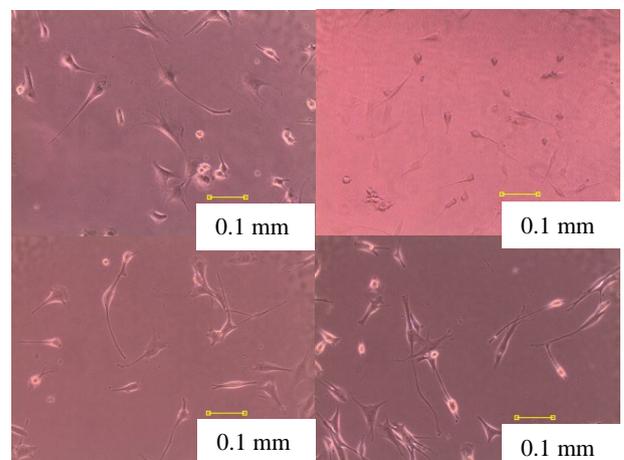


Fig. 13: Cells at 24 hours after electric stimulation: control (upper left), 1V (upper right), 5V (lower left) and 10 V (lower right).

4. DISCUSSION

The previous study shows that electric stimulation enhances differentiation of muscle cells [1]. Another study shows mechanical stimulation improves tissue-engineered human skeletal muscle [4].

The present study shows that muscle cells can adhere and proliferate under electric stimulation with periodical pulses, and that adhesion of muscle cells can be controlled with the amplitude of pulse.

The terminal of the capillary was melted and sealed to stop

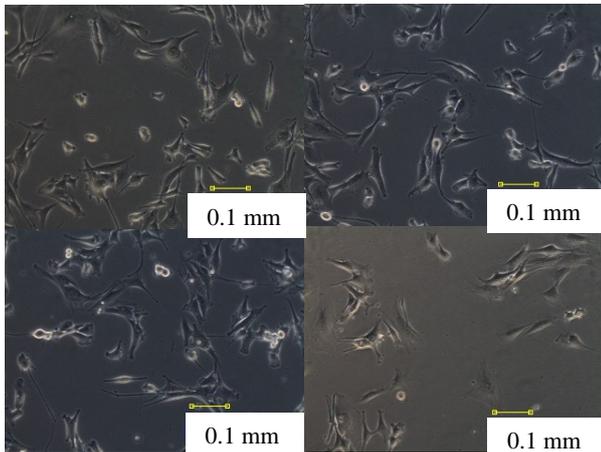


Fig. 14: Cells at 48 hours after electric stimulation: control (upper left), 1V (upper right), 5V (lower left) and 10 V (lower right).

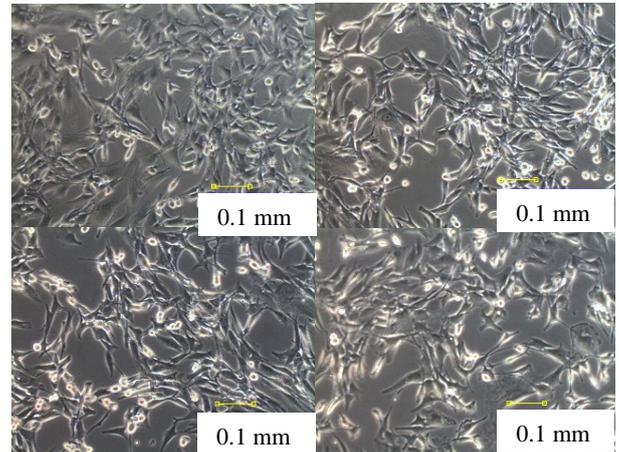


Fig. 15: Cells at 72 hours after electric stimulation: control (upper left), 1V (upper right), 5V (lower left) and 10 V (lower right).

capillary phenomenon. The treatment protects the medium from contamination.

5. CONCLUSION

The effect of electric stimulation on adhesion and proliferation of cultured muscle cells has been studied *in vitro*. The experimental results show that adhesion is restricted with electric pulse of 10 Hz at the higher amplitude to ten volts.

6. ACKNOWLEDGMENT

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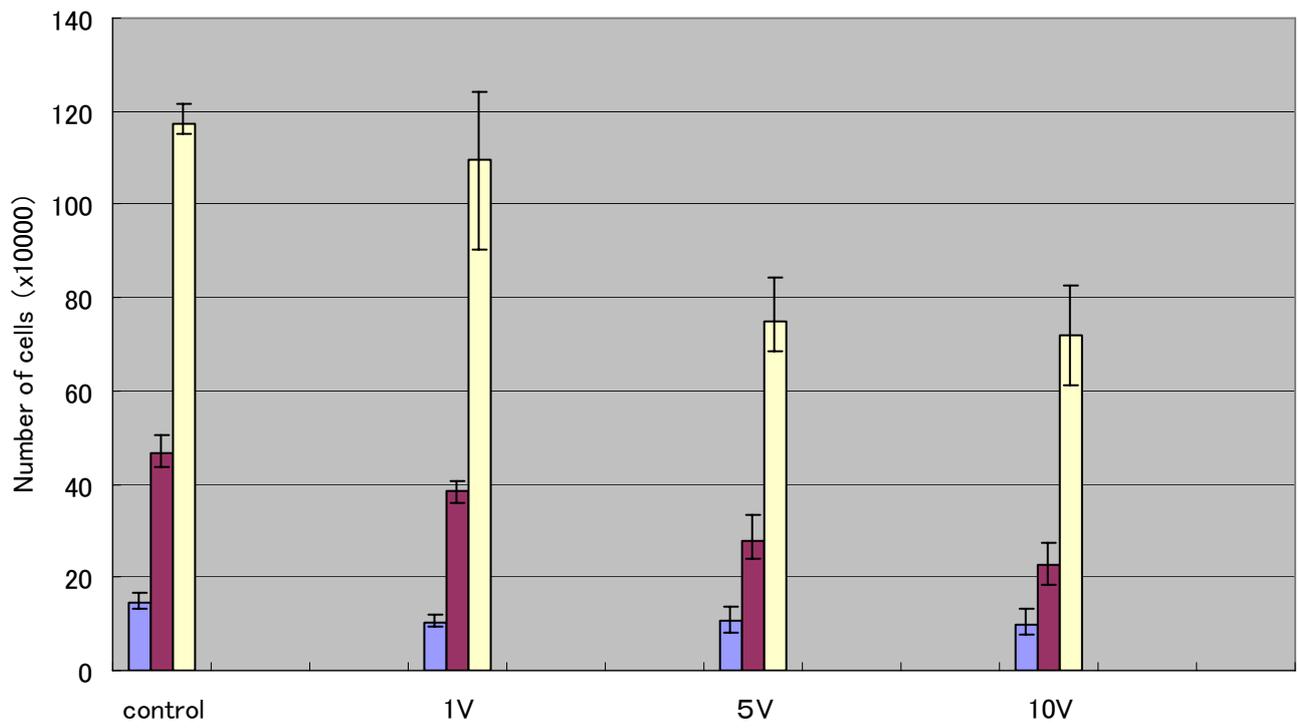


Fig. 16: Number of cells after electric stimulation. Three columns show 24, 48, 72 hours from left to right, respectively. Each bar shows minimum and maximum value.