Effect of Electric Field on Myocytes in Vitro

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ABSTRACT

An effect of an electric field on proliferation of cultured muscle cells has been studied *in vitro*. Two kinds of myocytes were exposed to electric stimulation: C2C12 (Mouse myoblast cell line originated with cross-striated muscle of C3H mouse) and L6 (rat skeletal muscle cell). The cells were suspended in Dulbecco's Modified Eagle's Medium. The suspension was exposed to the electric field between two electrodes made of platinum wire of 0.2 mm diameter at 25 degrees Celsius for five minutes a day. The electric pulses at a period of one second with a pulse width of one millisecond were generated with a function generator. Variation was made on amplitude of pulse between 10 mV and 100 mV. After electric stimulation for five minutes, cells were cultivated for one day in an incubator. For comparative study, a part of the suspension was poured into the same kind of dish without exposure to the electric field, and incubated. After incubation, the cells were observed with an inverted phase-contrast microscope, and the number of cells was counted. The experimental results show that proliferation decelerates with the amplitude of electric pulses lower than 0.1 V.

Keywords: Biomedical Engineering, Muscle Cells, Cell Culture, Electric Field and Proliferation

1. INTRODUCTION

Behavior of biological cells depends on various environmental factors, such as electric [1-3], magnetic [4] and mechanical fields [5, 6]. Electric pulses are generated with ion movement through membrane *in vivo*. The muscle tissue is controlled with electric stimulation under nerve system *in vivo* [7]. The electric stimulation has also been applied to a body in some medical treatments for rehabilitation.

Cell culture technique has been progressed and myoblasts have been clinically applied to ischaemic cardiomyopathy in the field of regenerative medicine. Acceleration technique for proliferation of cells has been studied to make muscle tissue *in vivo* and *in vitro* [8, 9]. Control methodology for proliferation and differentiation of cells would be applied to regenerative tissue technology.

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

2. METHODS

Electric Field

The cells were cultured in a culture plate, which has six wells of a flat bottom without coating of collagen. The internal diameter of each well is 35 mm.

The electrodes are made of platinum wire of 0.2 mm diameter, and attached on a polypropylene plate. When the plate covers the wells, a couple of electrodes in the rim position of each well generate an electric field in the medium. The wire has a square form in order to avoid concentration of the electric current, which might induce electrolysis. The cover plate has six square holes, through which the medium is observed during electric stimulation (Fig. 1).

Electric pulses of a period of one second with a pulse width of one millisecond were generated with a function generator (DF1906, NF Co. Ltd., Yokohama, Japan) (Fig. 2). Variation was made on amplitude of pulse: 10 mV, 50 mV and 100 mV.

Cell Culture

Two kind of muscle cells were used in the experiment: C2C12 (Mouse myoblast cell line originated with cross-striated muscle of C3H mouse) and L6 (rat skeletal muscle cell). Muscle cells were suspended in Dulbecco's Modified Eagle's Medium (D-MEM). Fetal bovine serum (FBS) was added to the medium with the volume rate in 10 percent of FBS and 90 percent of D-MEM. The 4 mL of suspension including forty thousands of cells was poured into each well.

To keep the temperature of 37 degrees Celsius and to keep the carbon dioxide content of five percent, an incubator was used for cultivation of cells. Fig. 3 shows the procedure of the experiment. After the cells were cultured in an incubator for three hours, electric pulses were applied for five minutes at room temperature. After additional 21 hours cultivation in an incubator, electric pulses were applied again for five minutes. The successive combined process of incubation for 24 hours and electric stimulation for five minutes was repeated two more times.

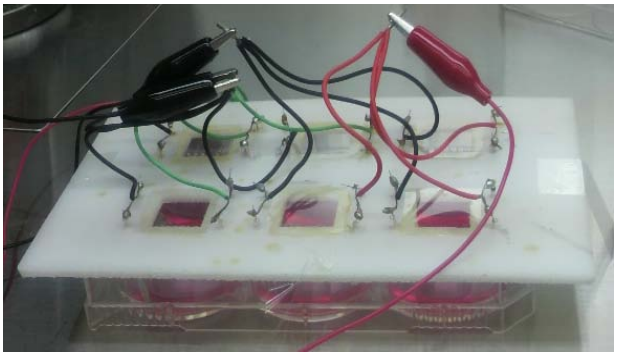

Fig. 1: Six pairs of electrodes and wells for cell culture.

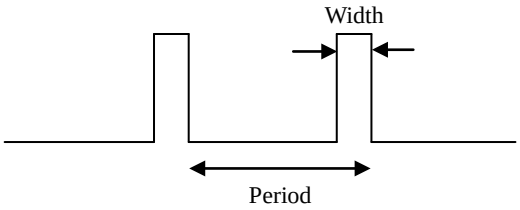

Fig. 2: Electric pulse.

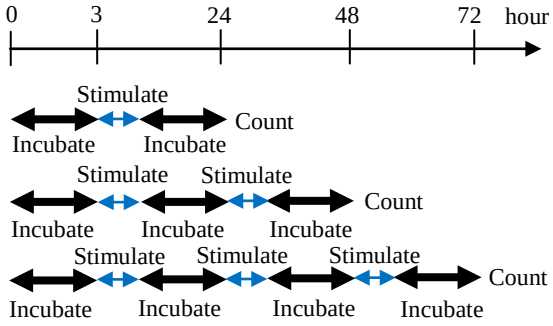

Fig. 3: Experimental Procedure.

At every time before electric stimulation for five minutes, the number of cells was counted in each well one by one. When the number of cells was counted, cells were exfoliated from the bottom of the well with trypsin treatment. The exfoliated cells were introduced into a counting chamber of 0.1 mm depth.

3. RESULTS

Figs. 4 and 5 show the number of C2C12 and of L6 in each well after cultivation for three hours, respectively. The number of cells scatters between thirty thousand and fifty thousand, which show the error range of the following data.

Figs. 6 and 7 show the number of C2C12 and of L6 in each well during cultivation for nine days. The column shows the mean value of four data, and the bar shows the range between the maximum value and the minimum value. Although the number of cells saturates in a week, the number of cells increases during five days in a sub-confluent state. The effect of electric field on proliferation of myocytes was investigated before sub-confluent state.

Figs. 8-10 exemplify C2C12 under electric stimulation of 0.1 V. Figs. 11-13 exemplify L6 under electric stimulation of 0.1 V. The figures show that cells proliferate to a sub-confluent state in three days of culture (Fig 10 and Fig. 13).

Fig. 14 shows the number of C2C12 tracings for three days with electric stimulation. Fig. 15 shows the number of L6 tracings for three days with electric stimulation. The column shows the mean value of four data, and the bar shows the range between the maximum value and the minimum value. The results show that proliferation decelerates with the amplitude (< 100 mV) of electric pulses both in C2C12 and in L6.

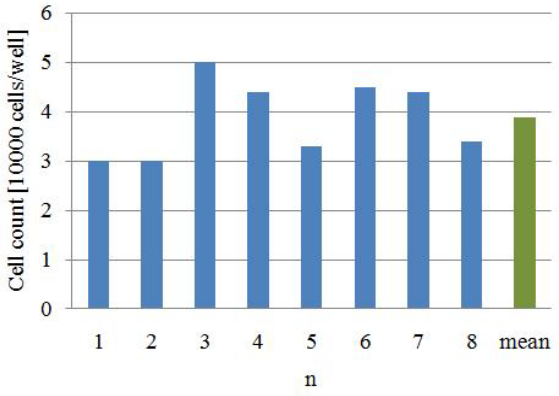

Fig. 4: Number of C2C12 after cultivation for three hours.

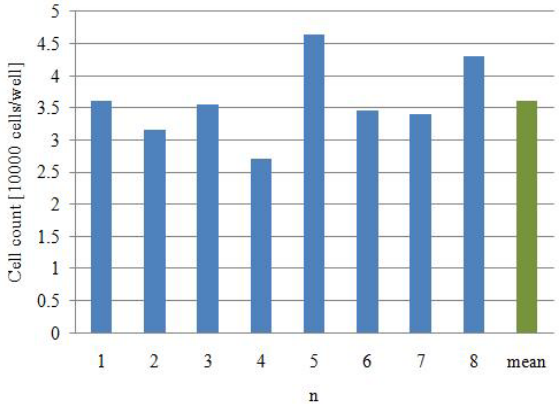

Fig. 5: Number of L6 after cultivation for three hours.

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 [5].

Several factors might govern adhesion of biological cells. The previous study shows that electric stimulation can restrict adhesion of muscle cells [2]. Another study shows an electromagnetic field affects on the cell [10]. An alternating magnetic field might affect on adhesive molecules on the cell membrane.

The muscle tissue is daily exposed to the field of electric pulses, which are signals for control of contraction. It serves a double purpose when the electric fields control regeneration of the tissue.

The present study shows that cells can proliferate after five minutes of electric stimulation. The experimental results show that proliferation decelerates with the amplitude of electric pulses. The results also show that deceleration of C2C12 proliferation is small with the pulse amplitude of 10 mV.

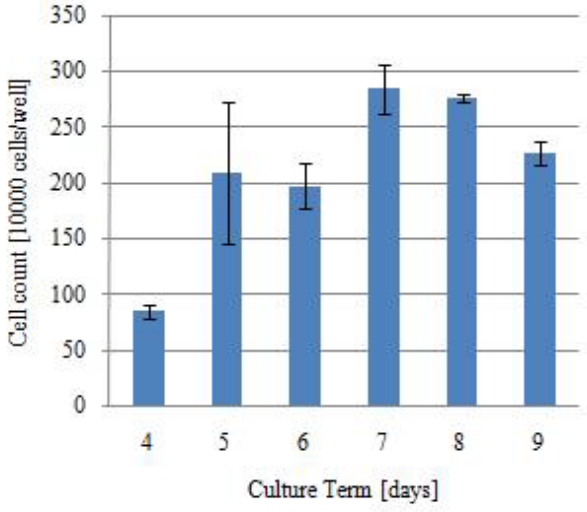

Fig. 6: Number of C2C12 tracings for nine days. n=4.

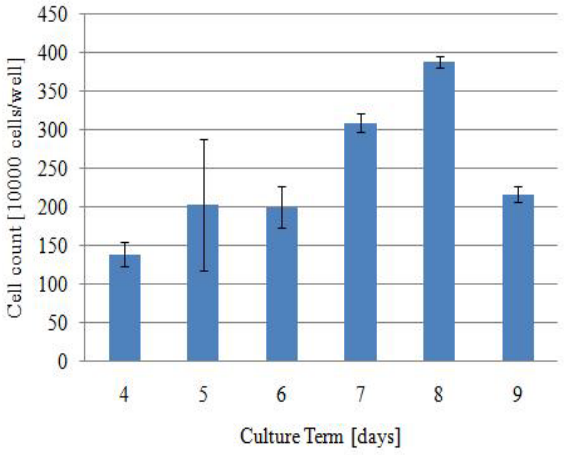

Fig. 7: Number of L6 tracings for nine days. n=4.

5. CONCLUSION

The effect of an electric field on proliferation of cultured myocytes has been studied *in vitro*. The experimental results show that proliferation decelerates with the amplitude (< 100 mV) of electric pulses.

6. ACKNOWLEDGMENT

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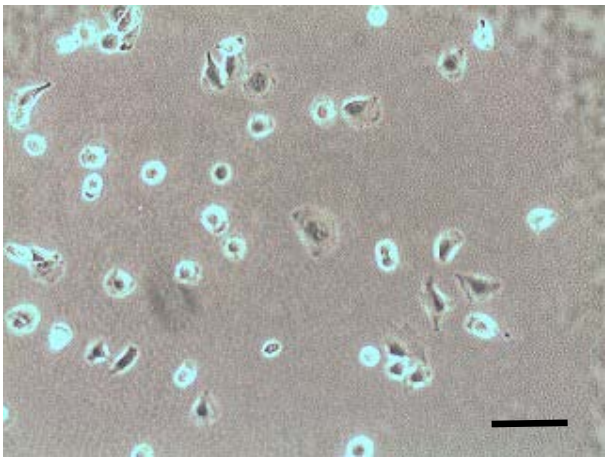

Fig. 8: C2C12 after three hours cultivation. The bar shows 0.1mm.

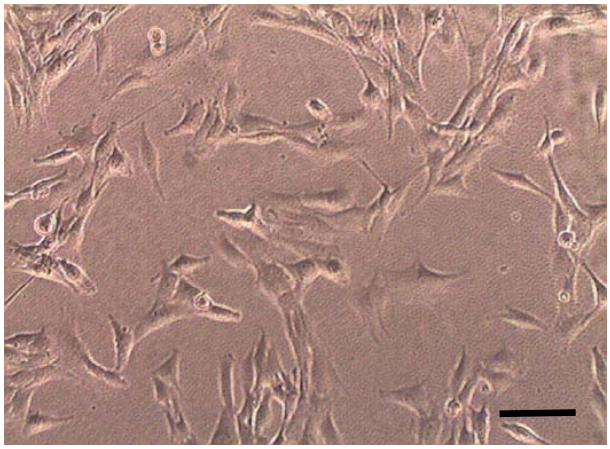

Fig. 9: C2C12 after 48 hours cultivation (electric stimulation of 0.1 V). The bar shows 0.1mm.

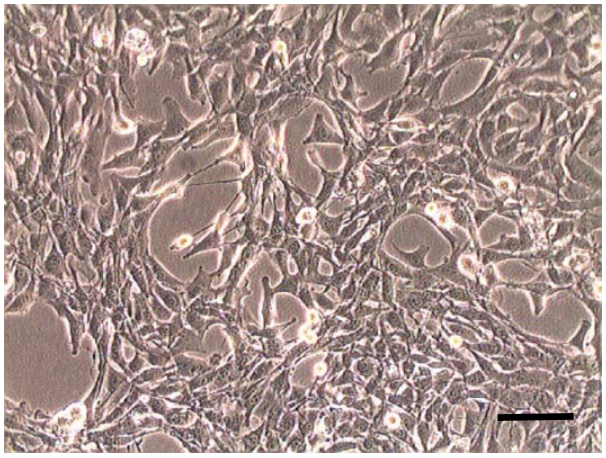

Fig. 10: C2C12 after 72 hours cultivation (electric stimulation of 0.1 V). The bar shows 0.1mm.

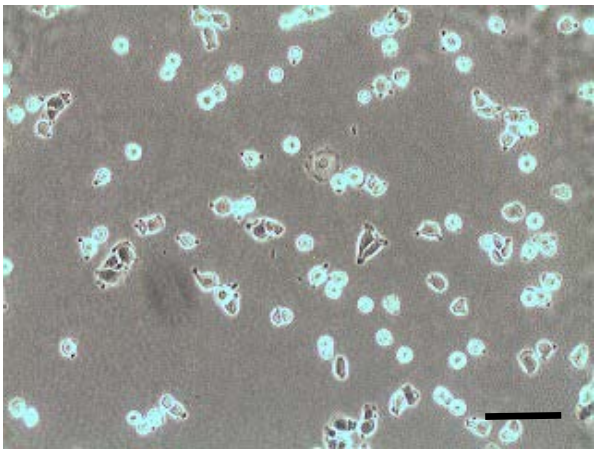

Fig. 11: L6 after three hours cultivation. The bar shows 0.1mm.

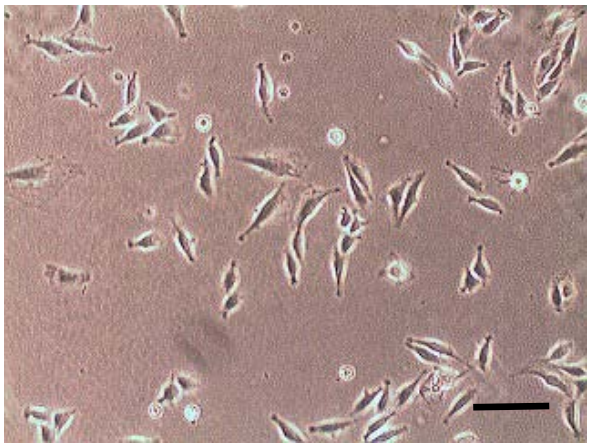

Fig. 12: L6 after 48 hours cultivation (electric stimulation of 0.1 V). The bar shows 0.1mm.

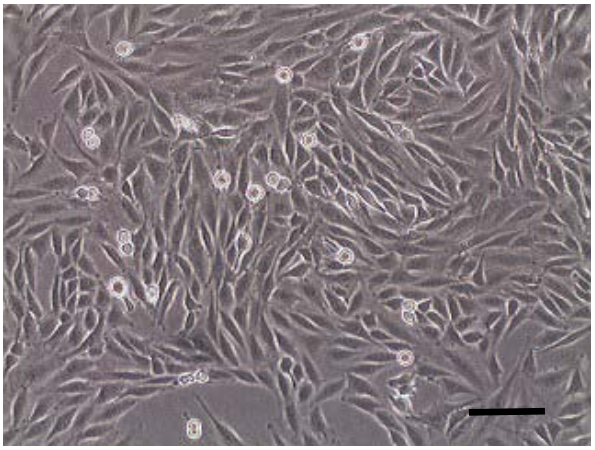

Fig. 13: L6 after 72 hours cultivation (electric stimulation of 0.1 V). The bar shows 0.1mm.

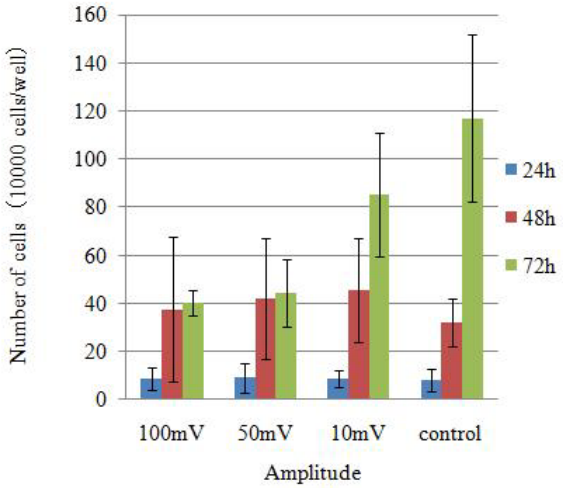

Fig. 14: Number of C2C12 tracings for three days. n=4.

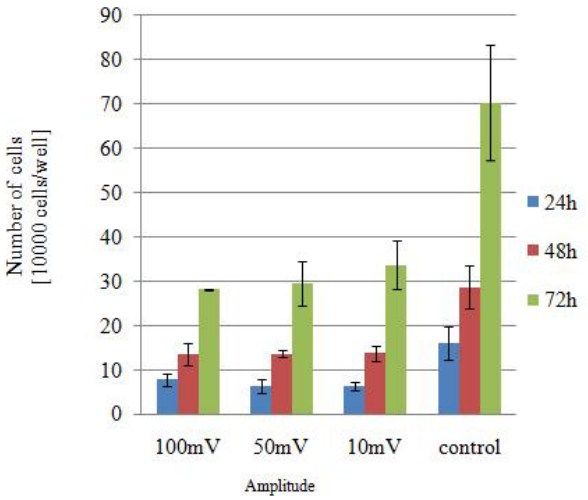

Fig. 15: Number of L6 tracings for three days. n=4.