Effect of Static Magnetic Field on Muscle Cells in Vitro

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ABSTRACT

An effect of a static magnetic field on proliferation and on orientation of cultured muscle cells has been studied *in vitro*. An experimental system was manufactured to apply a static magnetic field to muscle cell culture. The system consists of a neodymium magnet bar, a culture dish of 52 mm internal diameter contained in a transparent plastic chamber, and an inverted phase-contrast microscope. Air including carbon dioxide in five volume percent was introduced to the chamber to maintain atmosphere. C2C12 (Mouse myoblast) cells were suspended in Dulbecco's Modified Eagle's Medium with fetal bovine serum. The suspension was poured into the plastic dish placed on the stage of the microscope, where cells are exposed to the static magnetic field around the magnet bar at 29 degrees Celsius. For a comparative study, a part of the suspension was poured into the same kind of dish without exposure to the magnetic field. The number of cells, which adhered to the bottom of the culture dish, was traced according to the time (<120 min) during exposure to the magnetic field. The experimental results show that the cells tend to tilt to the direction of the static magnetic field of < 270 mT.

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

1. INTRODUCTION

Behavior of biological cells depends on various environmental factors, such as electric [1, 2], magnetic [3-9] and mechanical fields [10].

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* [1, 10]. The previous studies show the experimental design to evaluate the effect of alternating magnetic fields on cells [3-5]. Control methodology for adhesion and proliferation of cells would be applied to regenerative tissue technology.

In the present study, the effect of static magnetic stimulation on orientation of cultured muscle cells has been studied *in vitro*.

2. METHODS

Magnetic Field

An experimental system was manufactured to apply a magnetic field to muscle cell culture. The system consists of a neodymium magnet bar, a culture dish of 52 mm internal diameter contained in a transparent plastic chamber, and an inverted phase-contrast microscope. The neodymium magnet bar has a rectangular dimension of 4 mm × 8 mm × 52mm. A static magnetic field of < 270 mT was applied to cell culture with neodymium magnet bar, which is attached under the outer surface of the bottom plate of a culture dish. Air including carbon dioxide in five volume percent was introduced to the chamber to maintain atmosphere around the culture dish.

For comparative study, another similar experimental system was composed without magnet bar (Fig.1). The experiments of two comparative conditions were performed simultaneously with two systems.

The magnetic flux density was measured with a gauss meter (Model 5080, F. W. Bell Inc., USA).

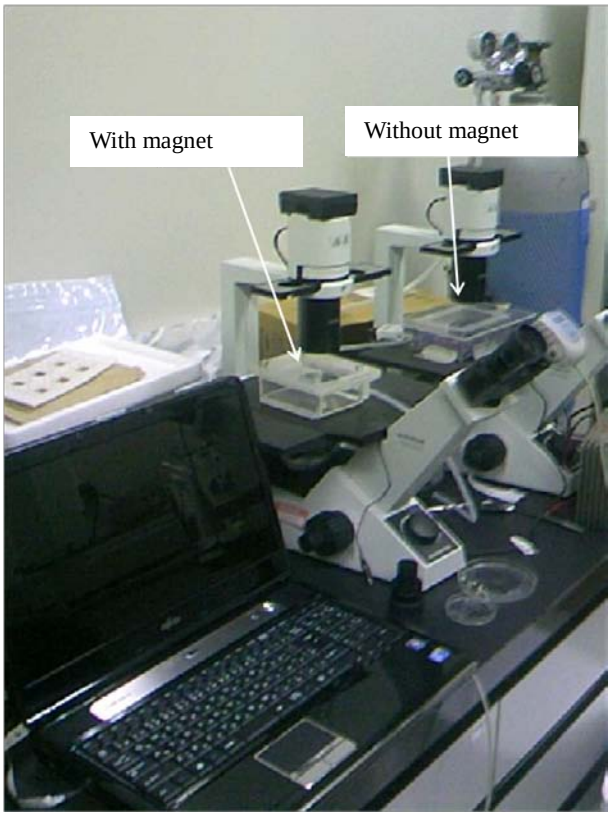

Fig. 1: Experimental system: with and without magnet.

Cell Culture

C2C12 (Mouse myoblast cell line originated with cross-striated muscle of C3H mouse) 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 density of cells in the suspension was adjusted to 38 thousands cells/$cm^{3.}$ The six milliliters of suspension was divided into two parts and each part of three milliliters was poured into each polystyrene dish of 52 mm internal diameter without collagen coating. The cells were cultured for 13 hours in a conventional incubator, where the partial pressure of carbon oxide and temperature were kept at 5 percent and 37 degrees Celsius, respectively.

For the successive 12 hours, one dish was set in the chamber with the magnet bar, and the other dish was set in the chamber without the magnet bar. A selected area near the magnetic bar was observed with the microscope every four hours (Fig. 2). In the test without magnetic field, the cells in a randomly selected area were observed. The room temperature was kept 29 degrees Celsius during the test.

Fig. 3 exemplified cells cultured in the static magnetic field. The arrow shows direction of the magnetic field (Fig.3). The angle between the longitudinal axis of each cell and the direction of the magnetic field was measured one by one.

In another test, cells were cultured for nine days in the medium with HS (horse serum) to observe differentiation, after cultured for three days in the medium with FBS. The cells were observed in three selected areas of different magnetic flux density: 15-45 mT, 60-90 mT, and 270 mT (Fig. 4).

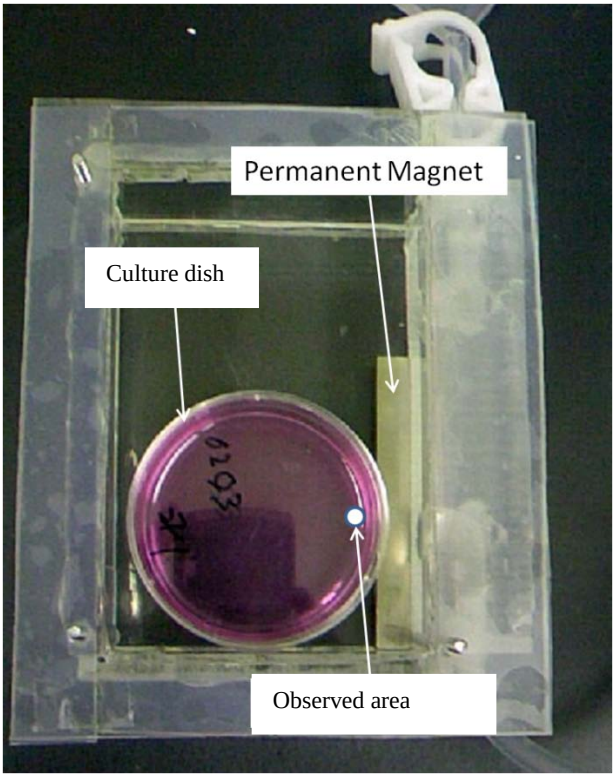

Fig. 2: Observed area is near the magnet bar in the culture dish contained in the transparent chamber.

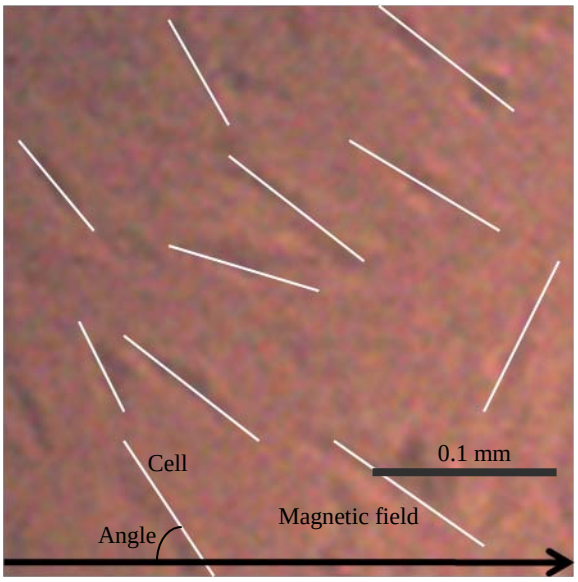

Fig. 3: Cells cultured in the static magnetic field.

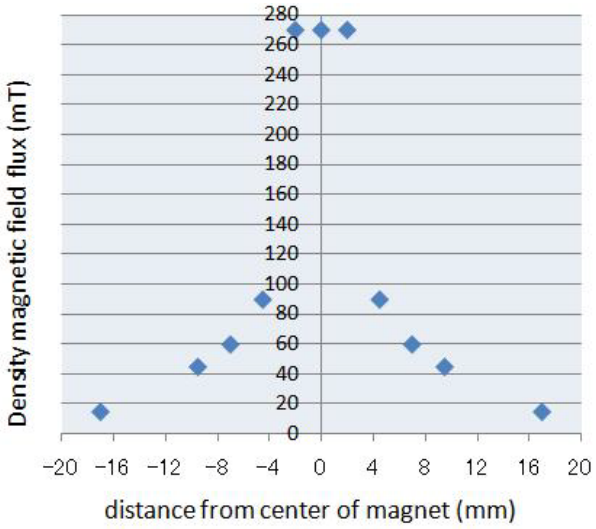

Fig. 4: Magnetic flux density in relation to distance from the centerline on the inner surface of the dish bottom, of which the magnet bar is attached on the outer surface.

3. RESULTS

Fig. 4 shows the magnetic flux density in relation to the distance from the line on the inner surface of the dish bottom, of which the magnet bar is attached on the outer surface. The results show that the maximum value of the magnetic flux density is 270 mT on the line, and that the magnetic flux density decreases rapidly as the distance increases.

Fig. 5 shows distribution of angles as a function of exposure time in the magnetic field. The figure shows that the angles tend to decrease with time.

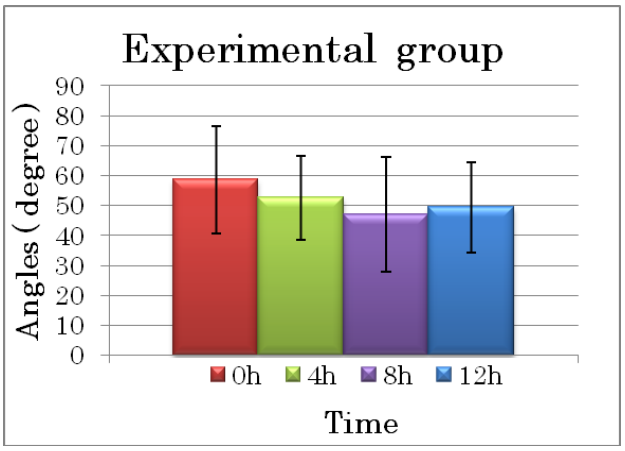

Fig. 5: Angles distribution as a function of exposure time in the magnetic field.

Figs. 6 exemplifies myotubes cultured for one day in the static magnetic field. The four pictures show C2C12 after one day of cultre in the medium with HS under each magnetic conditions: control, 15-45 mT, 60-90 mT, and 270mT (from top to bottom). Fig. 7 exemplifies myotubes cultured for eight days in the static magnetic field. The four pictures show C2C12 after eight days of cultre in the medium with HS under each magnetic conditions: control, 15-45 mT, 60-90 mT, and 270mT (from top to bottom). The cells were differentiated to myotubes regardless of the magnetic flux density.

4. DISCUSSION

The previous study shows that exposure to the static magnetic field of 80 mT for five days accelerates accumulation of actin and myosin in the differentiation process of L6 cells [7]. The study also shows that the static magnetic field of 80 mT for previous three days of differentiation affects on alignment of cells. Another previous study shows that the static magnetic field of 30 mT stimulates migration of cells.

The previous study shows that magnetic fields accelerate differentiation of cells.

The result in the previous study shows that the temperature increases form 25 degrees Celsius to 29 degrees Celsius for one hour exposure to the magnetic field and saturates at 29 degrees Celsius after one hour exposure [5]. When electric current applies to a coil, the coil generates not only magnetic field, but also thermal effect. The temperature of control study was adjusted to the same value as that of magnetic field study, so that the effect of magnetic field can be distinguished from the thermal effect.

The previous study shows that electric stimulation enhances differentiation of muscle cells [1, 2]. Another study shows mechanical stimulation improves tissue-engineered human skeletal muscle [10].

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 cellular collagen [6]. An

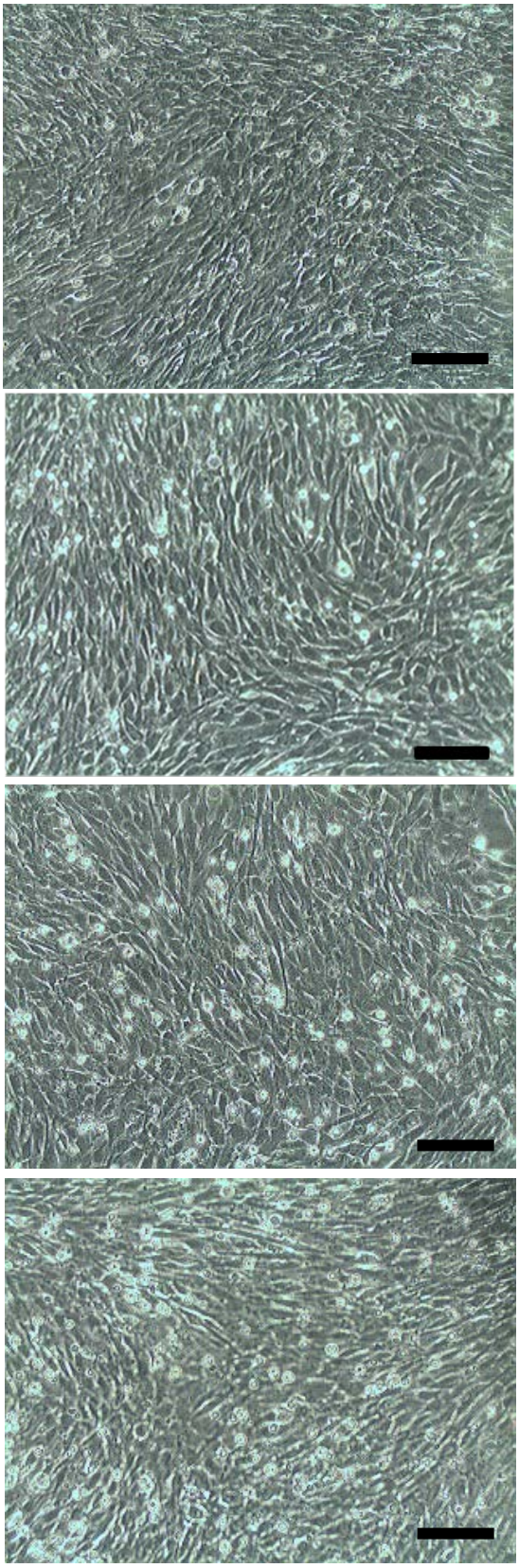

Fig. 6: C2C12 after one day of cultre in the medium with HS under each magnetic conditions: control, 15-45 mT, 60-90 mT, and 270mT. The bar shows 0.1 mm.

alternating magnetic field might affect on adhesive molecules on the cell membrane.

In the previous study, an alternating magnetic field of 1-4 mT at a period of 0.017 s was applied to the muscle cells, which tended to align to the direction of the magnetic field [3]. The slightly higher alternating magnetic field of 13 mT at a period of 0.01 s was applied to muscle cells in the present study, to compare results to that of the previous study. Distinguishing thermal effects, the effect of magnetic field is evaluated clearer in the present experimental system than in the previous one.

The previous study shows that adhesion of muscle cells to the bottom of the culture dish is accelerated with alternating magnetic fields.

5. CONCLUSION

The effect of a static magnetic field on orientation of cultured muscle cells to the culture plate has been studied *in vitro*. The experimental results show that proliferation of muscle cells is decelerated and the cells tend to tilt with a static magnetic field of < 270 mT.

6. ACKNOWLEDGMENT

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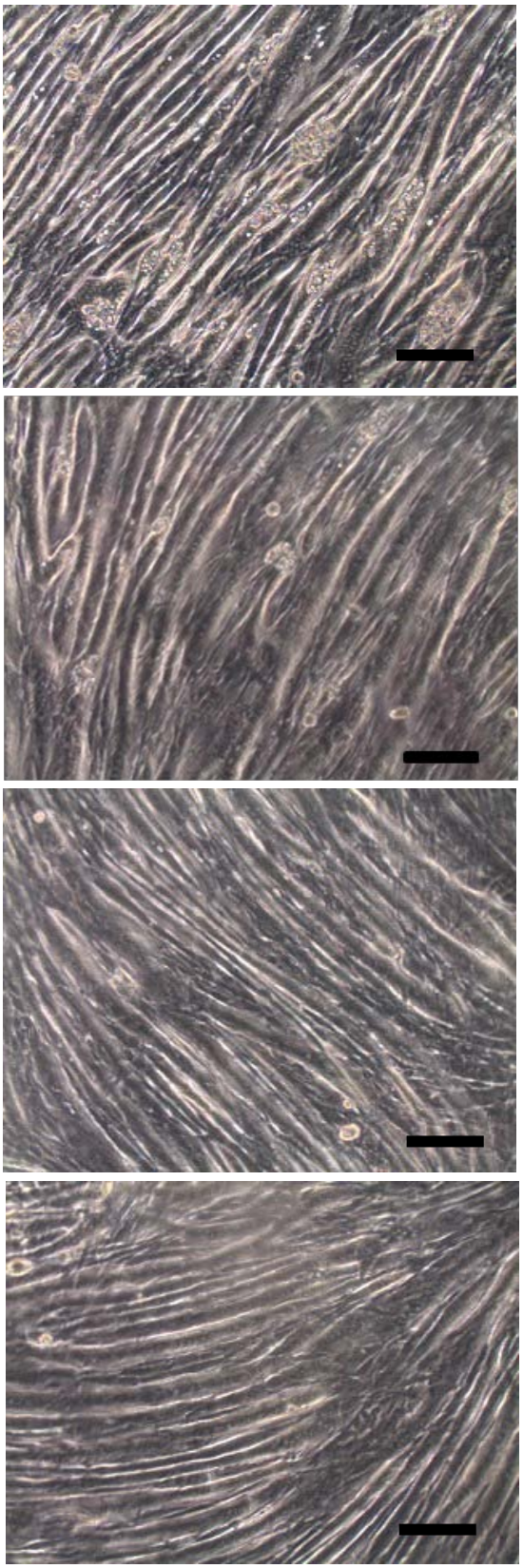

Fig. 7: C2C12 after eight days of cultre in the medium with HS under each magnetic conditions: control, 15-45 mT, 60-90 mT, and 270mT. The bar shows 0.1 mm.

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