Effect of Magnetic Field on Adhesion of Muscle Cells to Culture Plate

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ABSTRACT

The effect of a magnetic field on adhesion of cultured muscle cells to the culture plate has been studied *in vitro*. An experimental system was manufactured to apply a magnetic field to muscle cell culture. The system consists of a couple of solenoid coils, a culture dish of 52 mm internal diameter, and an inverted phase-contrast microscope. The solenoid coil generates the alternating magnetic field of 13 mT of the effective value at a period of 0.01 s with the electric current of the rectangular pulses. C2C12 (Mouse myoblast cell line originated with cross-striated muscle of C3H mouse) cells were suspended in Dulbecco's Modified Eagle's Medium. The suspension was poured into the plastic dish placed on the stage of the microscope. The culture dish was exposed to the magnetic field between the solenoid coils at 29 degrees Celsius. For comparative study, a part of the suspension was poured into the same kind of dish without exposure to the magnetic field at 29 degrees Celsius. The number of cells, which adhered to the bottom of the culture dish, was traced according to the time (<130 min) during exposure to the alternating magnetic field. The experimental results show that adhesion is accelerated with alternating magnetic field of 13 mT.

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

1. INTRODUCTION

Behavior of biological cells depends on various environmental factors, such as electric [1], magnetic [2] and mechanical [3] 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* [4, 5]. The previous studies show the experimental design to evaluate the effect of magnetic fields on cells [6, 7]. Control methodology for adhesion and proliferation of cells would be applied to regenerative tissue technology.

In the present study, the effect of magnetic field on adhesion of cultured muscle cells has been studied *in vitro*.

2. METHODS

Magnetic Field

The experimental system consists of a couple of solenoid coils, a culture dish of 52 mm internal diameter, and an inverted phase-contrast microscope (Fig. 1). The magnetic field is applied to the culture dish with the solenoid coil. The solenoid coil was manufactured with copper wire of 0.5 mm diameter (Fig. 1). The wire is coiled 1000 turns around a plastic cylinder solid, which has a diameter of 10 mm and a length of 500 mm.

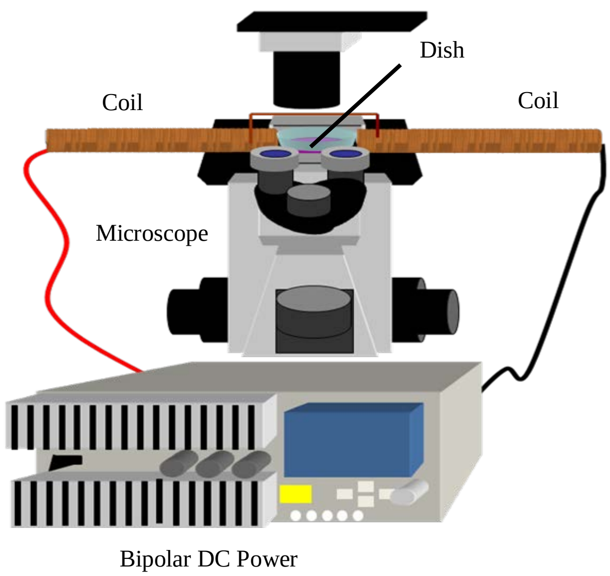


Fig. 1: Experimental system.

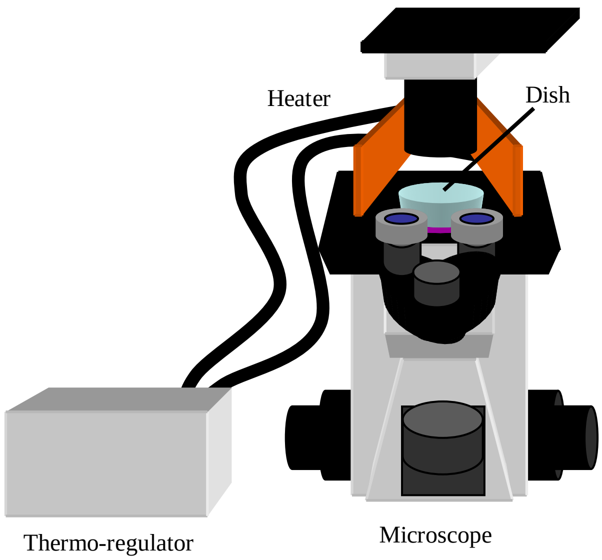


Fig. 2: Control study with thermo-regulator.

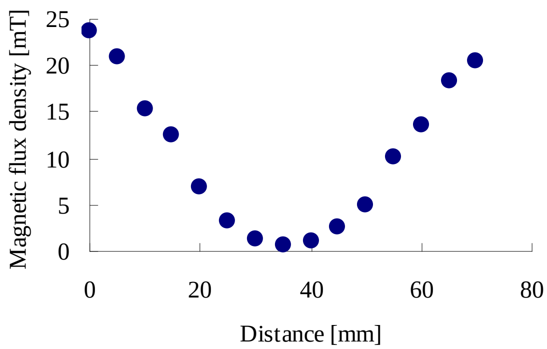


Fig. 3: Magnetic flux density in relation to distance from the right end of the left coil to the left end of the right coil.

The magnetic flux density was measured with a gauss meter (Model 5080, F. W. Bell Inc., USA). The alternating magnetic field of 13 mT of effective value at a period of 0.01 second is generated with alternating electric current, which is measured at the observation point in culture dish. Alternating electric power, which have 0.01 second period, are generated with Bipolar DC Power (BP4610, NF Co. Ltd., Yokohama, Japan).

The culture dish is placed between two coils so that their longitudinal symmetry lines are duplicated each other. The distance between two coils is 70 mm to leave space for the culture dish.

Temperature
The temperature of the liquid in the culture dish during experiment was adjusted to the same level (29 degrees Celsius) between two conditions: with and without magnetic field. Distilled water of three milliliter was contained in the culture dish of 52 mm internal diameter to measure distribution of temperature. The distribution of temperature at the surface of the water in the culture dish between two coils during exposure to the magnetic field was measured with an infra-red thermo camera (Neo Thermo, TVS-700, NEC Avio Infrared Technologies Co. Ltd., Tokyo, Japan). The temperature was traced for 160 minutes from the start of the magnetic field exposure, while the room temperature was maintained at 25 degrees Celsius.

In the control study, no magnetic stimulation was applied to the medium after cell seeding. To adjust the temperature without exposure to the magnetic field same as that with exposure to the magnetic field, control dish was placed between the plate type electric heaters with a thermo-regulator (TF4-10, Keyence Co. Ltd., Osaka, Japan) (Fig. 2). The distribution of temperature at the surface of the water in the culture dish placed on the heater without the magnetic field was measured with an infra-red thermo camera, while the heater was controlled to 29 degrees Celsius.

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 three milliliters of suspension, which was kept at 29 degrees Celsius in the water bath before test, was poured into the polystyrene dish of 52 mm internal diameter without collagen coating in 2 million cells/cm^2.

Ten sectors of 4 mm square in the culture dish were randomly selected to trace the behavior of the muscle cells in the fixed area under the microscopic observation. The time until the first adhesion of cell was measured in each sector. The numbers of cells, which adhered on the bottom in each sector of the dish during continuous exposure to the magnetic field, were counted at 15, 30, 45, 60, 75, 90, 105, 120 minutes, respectively.

At the end of exposure to the magnetic field for 130 minutes, five sectors of 4 mm square were selected independent of observed sectors for 120 minutes. The numbers of cells, which adhered on the bottom in each sector of the dish, were counted after exposure to the magnetic field for 130 minutes, respectively.

The ratio (R) of number of adhered cells to number of total cells is calculated by Eq. 1.

$$R = A / T \quad (1)$$

In Eq.1, A is the number of the adhered cells and T is the number of total cells in the sector.

3. RESULTS

Fig. 3 shows the magnetic flux density in relation to the distance from the right end of the left coil. The distance of 35 mm corresponds to the middle point between two coils in Fig. 3. The observation point is the distance of 15 mm, where the magnetic flux density is 13 mT.

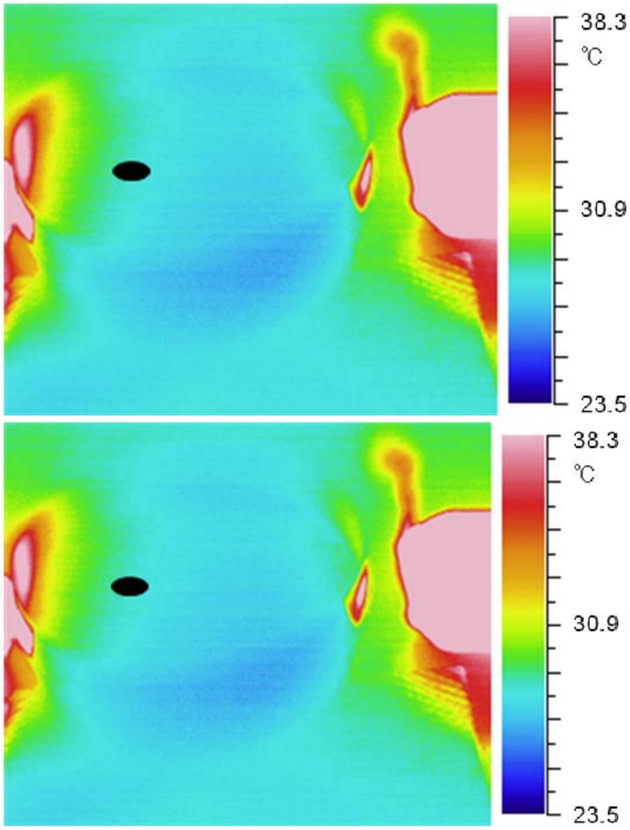


Fig. 4: Distribution of temperature between coils. Upper, 30 min; Lower, 60 min. Distance between left and right is 87 mm in the figure.

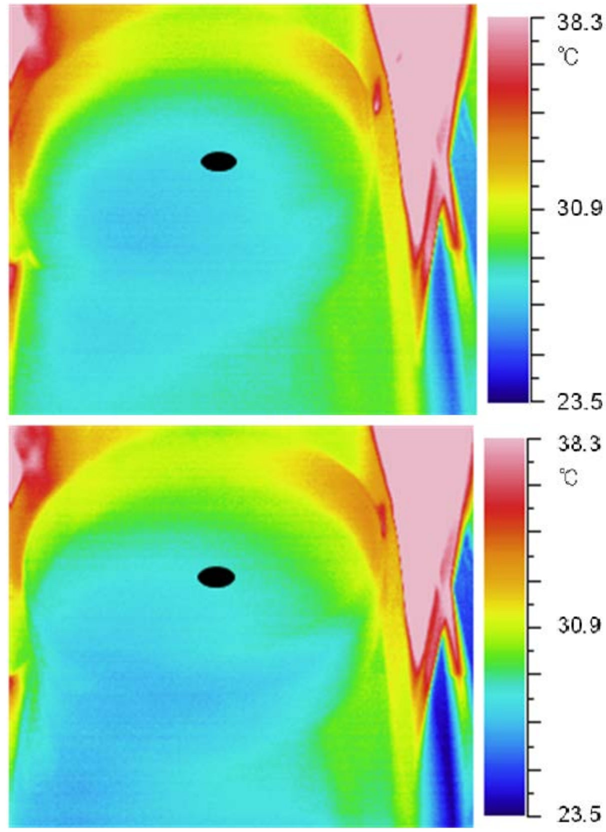


Fig. 5: Distribution of temperature between heaters. Upper, 30 min; Lower, 60 min. Distance between left and right is 87 mm in the figure.

Fig. 4 exemplifies the distribution of temperature at the surface of the water in the culture dish between two coils during exposure to the magnetic field, where the upper figure shows the distribution of temperature after exposure for thirty minutes and the lower figure shows that for sixty minutes. The uniform temperature area in the middle of the figure corresponds to the surface of the water in the dish of 52 mm diameter (Figs. 3, 4). The horizontal distance is 86 mm in Figs. 3 and 4. The figure shows area of higher temperature at the coil (left and right in Fig. 3). The black filled circle marks the point of measure for Fig. 5. The point was selected as a typical point for observation of cells in the dish.

Fig. 5 exemplifies the distribution of temperature at the surface of the water in the culture dish placed between the heaters. The black filled circle marks the point of measure for Fig. 6. The high temperature area around the culture dish corresponds to the surface of the heater.

Fig. 6 exemplifies tracings of temperature at the surface of the water in the culture dish (Fig. 3) for 160 minutes from the start of the magnetic field exposure, while the room temperature was maintained at 25 degrees Celsius. The result 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.

Fig. 7 exemplifies tracings of temperature at the surface of the water in the culture dish (Fig. 4) between the heaters for 160 minutes from the start of the heating, while the room temperature was maintained at 25 degrees Celsius. The result shows that the temperature increases form 25 degrees Celsius to 29 degrees Celsius for one hour heating and saturates at 29 degrees Celsius after one hour heating. The results show that the arranged heater works well to adjust the condition of the temperature in the liquid in the culture dish to the same level as that exposure to the magnetic field.

Fig. 8(a) exemplifies muscle cells in the medium during exposure to the magnetic fields with comparison to control study (Fig. 8(b)). The floating cells are rounded, while adhered cells are deformed. With the focus of their fringe, the adhered cells are able to be distinguished from floating cells.

Fig. 9 shows the time for the first cell adhesion. The test was repeated 13 times, and each result is displayed at each case in Fig. 9. In the most of cases, the time is shorter in magnetic field than in control. The experimental results show that the cells tend to adhere faster with the magnetic field than without magnetic field.

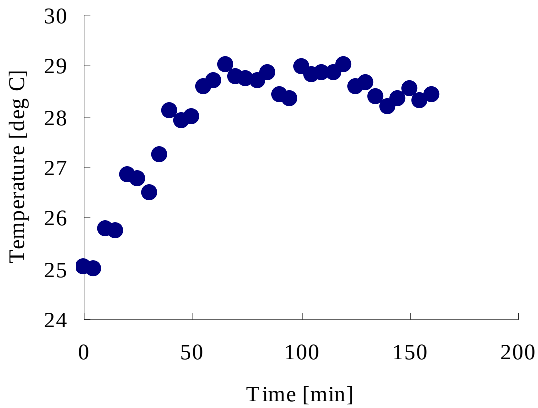


Fig. 6: Tracings of temperature at marked point of Fig. 4.

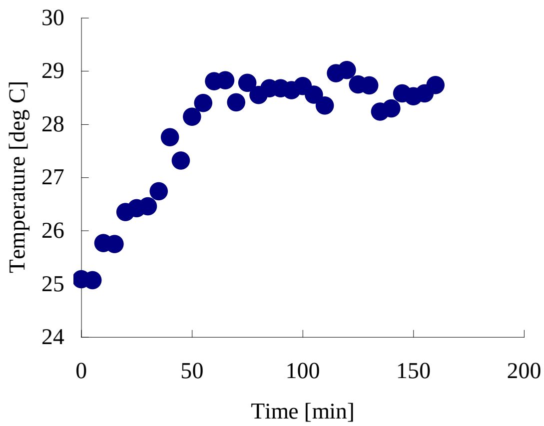


Fig. 7: Tracings of temperature at marked point of Fig. 5.

Fig. 10 shows the ratio of cells adhesion in relation to exposure time to the magnetic field. The results show that the ratio of adhesion increases with culture time and that the ratio increases earlier with the magnetic field than without magnetic field. The ratio of cells adhesion saturates for 2 hours exposure to the magnetic field (Fig. 10). Each bar shows the range between the maximum and minimum values in Fig. 10.

Fig. 11 shows the ratio of cells adhesion after exposure to the magnetic field for 130 minutes. The test was repeated 13 times, and each result is displayed at each case in Fig. 11. The ratio of cells adhesion was counted at five sectors in the area marked in Figs. 4&5 in each case. In the most of cases, the ratio is higher in magnetic field than in control. The results show that the ratio of adhesion for 130 minutes is higher with the magnetic field than without magnetic field.

The experimental results show that adhesion is accelerated with alternating magnetic fields.

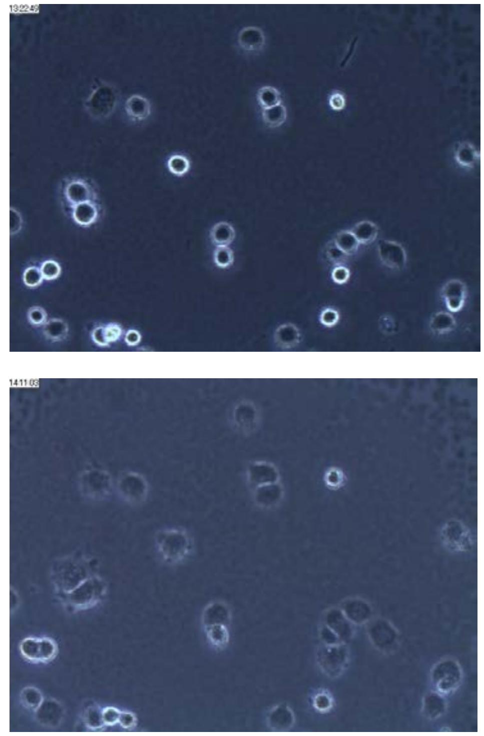


Fig. 8(a): Muscle cells exposed to magnetic field for 30 min (upper) and for 60 min (lower). Distance between left to right is 0.8 mm in the figure.

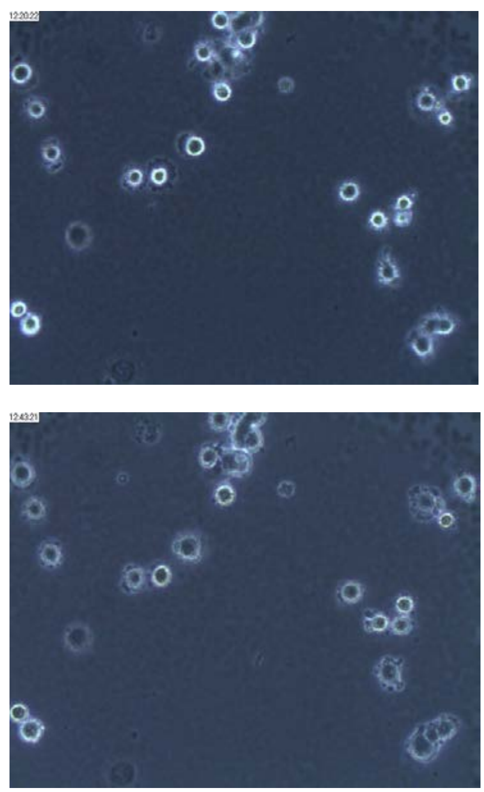


Fig. 8(b): Muscle cells without exposure to magnetic field for 30 min (upper) and for 60 min (lower). Distance between left to right is 0.8 mm in the figure.

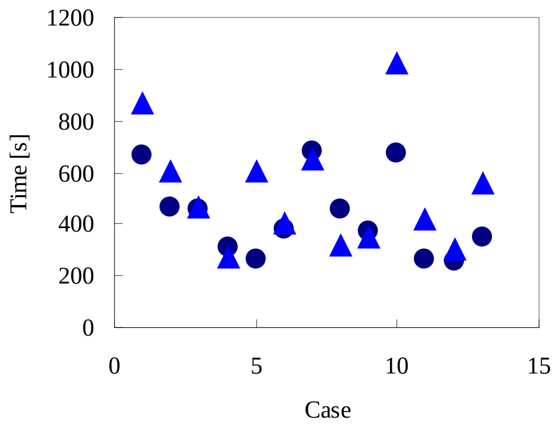


Fig. 9: Time for the first cell adhesion. Circle, with magnetic field; triangle, control.

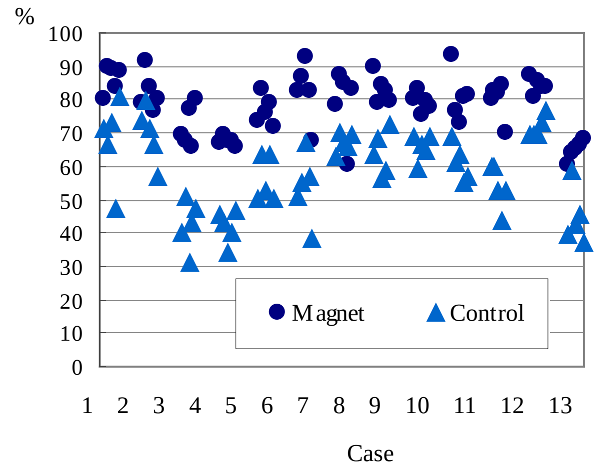
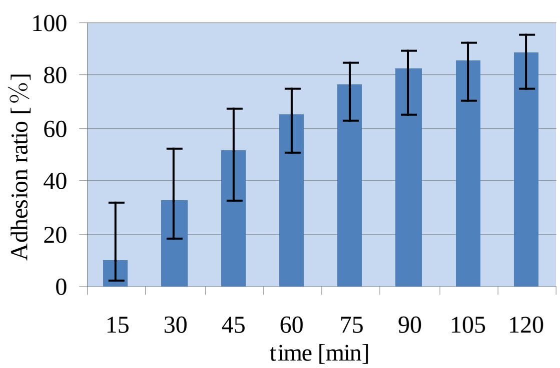


Fig. 11: Ratio of cells (%) adhesion after exposure to the magnetic field for 130 minutes.



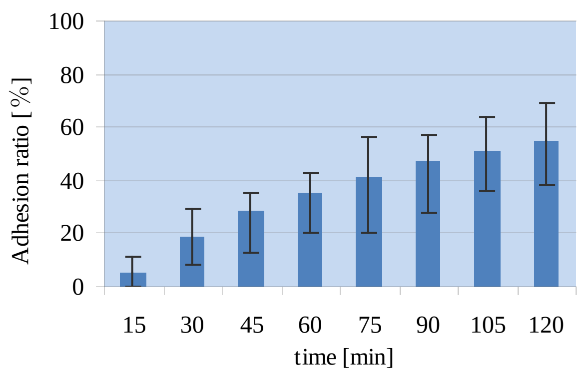


Fig. 10: Ratio of cells adhesion in relation to exposure time to the magnetic field (upper), and that of control study.

4. DISCUSSION

The length of the cylinder is enhanced to 500 mm for the diameter of 10 mm, because the larger ratio between length and diameter approximates uniform magnetic field between two solenoid coils.

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 [4, 8]. 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 [8]. Another study shows an electromagnetic field affects on the cellular collagen [9]. An alternating magnetic field might affect on adhesive molecules on the cell membrane.

An experimental system of a uniform alternating magnetic field was designed with the Helmholtz coil in another study [10].

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 [9]. 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 present 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 magnetic field on adhesion of cultured muscle cells to the culture plate has been studied *in vitro*. The experimental results show that adhesion is accelerated with alternating magnetic field of 13 mT.

6. ACKNOWLEDGMENT

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