

Effect of Centrifugal Force on Cell Culture

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ABSTRACT

An effect of a mechanical force field on cell culture has been studied *in vitro*. C2C12 (mouse myoblast cell line originated with cross-striated muscle of C3H mouse) was cultured on a polystyrene plate coated with collagen for 13 days in the mechanical force field. To apply the mechanical force field to the cells on the plate, the plate was inserted into a centrifugal tube. The tube was placed in a conventional centrifugal machine, to set the surface of the culture plate in the parallel position to the centrifugal field. The shape and the orientation of the cells were observed with a phase contrast microscope during the cell culture. The experiments show that C2C12 extends pseudo, proliferates to the confluent manner and differentiates to myotube even under continuous stimulation of 270 times of the gravitational force.

Keywords: Biomedical Engineering, C2C12, Cell Culture and Centrifugal Force Field.

1. INTRODUCTION

Cell culture technique has been developed and several methodologies have been clinically applied to regenerative medicine. The acceleration technique for orientation, proliferation and differentiation of cells has been studied to make tissue *in vivo* or *in vitro* [1-9, 12]. Control methodology for orientation, proliferation and differentiation of cells would be applied to the regenerative tissue technology.

The mechanical stress is one of the interested factors in the environment of cells, because they receive mechanical force *in vivo*. The mechanical stress on cells might induce various responses: deformation, migration, proliferation, and differentiation. Several methods have been designed to apply the mechanical stress to cells [2-10, 16].

A transmission point of the stress to a specimen is important. In many studies, the stress is applied to a scaffold [2-5]. When fixation between the cell and the scaffold is not enough, the stress is not transmitted to the cell. A mechanical field, on the

other hand, can be used to apply a continuous stress to a specimen [6-10, 13]. The specimen fixed at a position receives the shear stress in the mechanical field.

The biological cells receive gravitational force on the earth. An astronaut needs exercise before standing, when he comes back from the space station. The characteristic of muscle tissue might change in the space station, where the tissue does not receive gravitational force [11].

In the present study, the effect of mechanical stimulation on cultured cells has been studied in centrifugal force field *in vitro*.

2. METHODS

Excess Gravitational Force Field

The excess gravitational force was applied to cultured cells with the centrifugal force (Fig. 1). Cells were cultured on a polystyrene plate (30 mm × 8.5 mm × 2 mm) for two weeks in the centrifugal force field. The plate has a line on the back side of the culture surface to mark the observation point. To apply the mechanical force field to the cells on the plate, the plate was inserted into a centrifugal tube. The tube (Fig. 3) was set on an angled rotor in the centrifugal machine (CN-1040, Matsuuraseisakusyo. Ltd, Tokyo, Japan) (Fig. 4). The rotor (turning radius of 8 cm) was rotated with the constant speed slower than 1750 revolutions per minute, which makes the excess gravitational force lower than 270 G at the surface of the culture plate. The angle between the directions of the centrifugal force and the surface of the cell culture plate is 1 rad. The force is divided into two ingredients, which are the tangential force and the normal force at the culture plate. The centrifugal force of 270 G makes 140 G of the tangential force and 230 G of the normal force. To keep the balance of the rotator in the centrifugal machine, two tubes were placed in the counter position each other. The centrifugal machine was placed in an incubator to keep the partial pressure of bicarbonate of 5 % at 310 K. The tube is shield with Parafilm (Pechiney Plastic Packaging Company, IL), through which gas passes. For comparison, some tubes were shield with the plastic screw cap.

Cell Culture

C2C12 (mouse myoblast cell line originated with cross-striated muscle of C3H mouse) of the passage between the third and the sixth was used in the test. C2C12 was cultured with the D-MEM (Dulbecco's Modified Eagle Medium) containing 10% FBS. The medium containing penicillin and streptomycin was refreshed every two days.

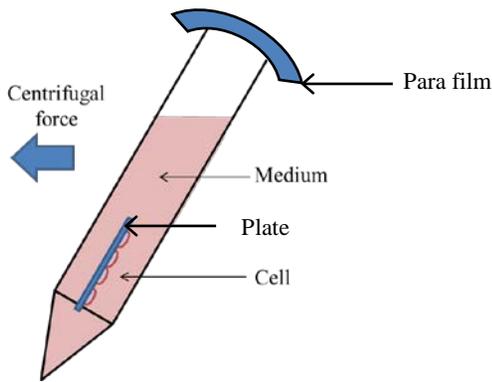


Fig. 1: Centrifugal force (arrow) applied on cell culture in tube.

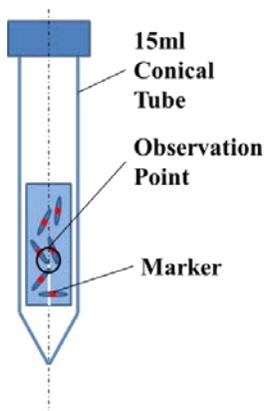


Fig. 2: Cell culture in tube. Mark to trace the same area of culture.



Fig. 3: Centrifugal machine in incubator.

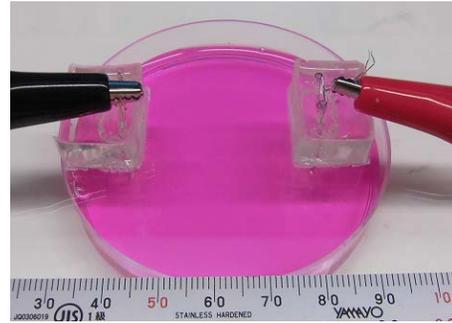


Fig. 4: Electrodes for stimulation.

The polystyrene plate cut from the bottom of a culture dish is dipped for 24 hours in ethanol for disinfection. After rinsed with a phosphate buffer solution, the plate was placed in the culture dish of six wells, and the cells were seeded at the density of 3000 cells/cm². After the cells were cultured for 24 hours without centrifugal stimulation, the plate was moved from the dish to the tube and exposed to mechanical stimulation of the centrifugal force for two weeks. The medium was changed every two days.

Morphological Study

Density and morphology of cells was observed with an inverted phase-contrast microscope (IX71, Olympus, Tokyo) every 24 hours during the test. During the observation, the rotation of the centrifugal machine was stopped, and the plate with cells was moved from the tube to the dish filled with the medium.

Electric Stimulation

At the end of tests, myotubes were stimulated with electric pulses to confirm differentiation of C2C12. The culture plate was moved from the tube to the dish. The electric pulse (interval shorter than 0.5 s, amplitude lower than 25 V, duration 0.002 s) was generated with an electric stimulator (SEN5201, Nihon Kohden Corporation, Japan). The pulses were applied with a couple of electrodes of platinum wire of 0.1 mm diameter, which was dipped into the medium of the culture dish (Fig. 4). The movement of the myotubes was observed with the inverted phase-contrast microscope.

3. RESULTS

The experiments show the following results.

Cells deform from the round shape to the extended shape, which shows adhesion on the culture plate in 24 hours (Fig. 5(A)). The cells are sparse on the plate, because most of cells adhere to the bottom of the dish.

C2C12 begins to fuse to make myotube after centrifugation sealed with the cap for one day at 5.6 G (Fig. 5(B)). Centrifugal force is downward direction in the following figures. Myotubes are oriented not only to the parallel direction to the centrifugal force field, but also perpendicular direction to the centrifugal force field. Most of cells are exfoliated after centrifugation sealed with the cap for four days (Figs. 5(C)&(D)). The result at 89 G (Fig. 6) is similar to that at 270 G (Figs. 7(C), 8(C), 9(C)).

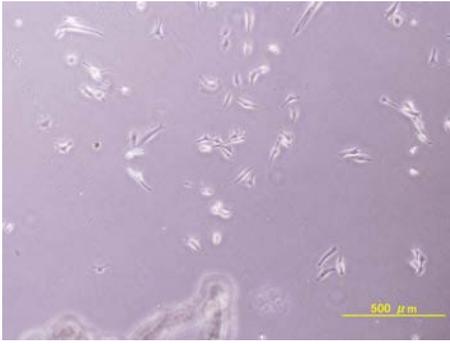


Fig. 5(A): C2C12 before centrifugation of 5.6 G (250 rpm) with cap for 24 hours. Dimension from left to right is 2 mm.

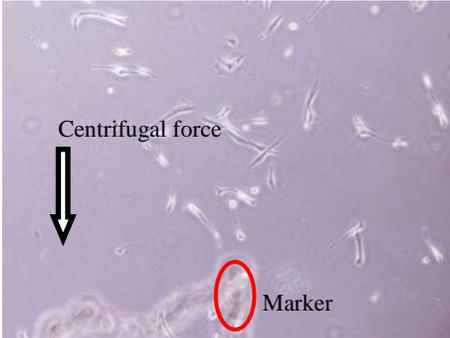


Fig. 5(B): C2C12 after centrifugation at 5.6 G for one day. Centrifugal force is downward direction in the following figures.

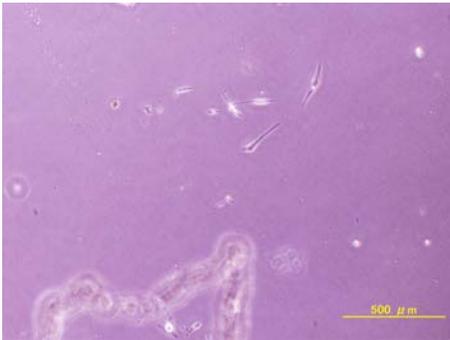


Fig. 5(C): C2C12 after centrifugation of 5.6 G with cap for two days. Dimension from left to right is 2 mm.

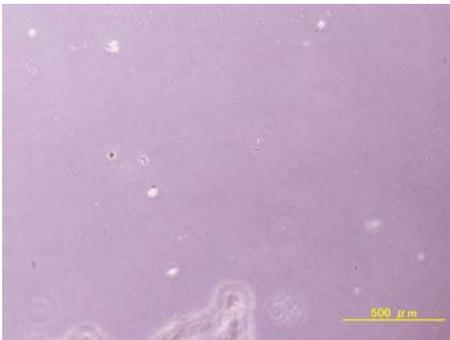


Fig. 5(D): C2C12 after centrifugation of 5.6 G with cap for four days. Dimension from left to right is 2 mm.

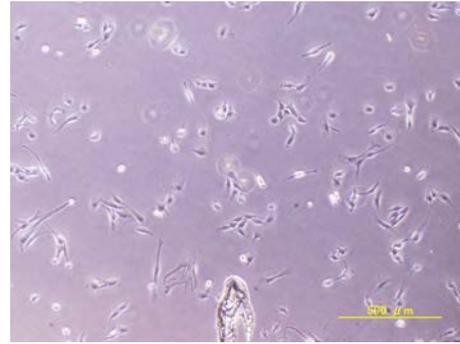


Fig. 6(A): C2C12 before centrifugation of 89 G with cap for 24 hours. Dimension from left to right is 2 mm.

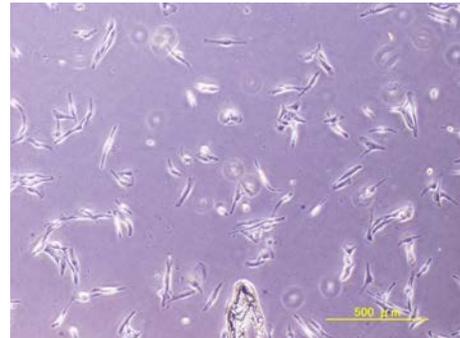


Fig. 6(B): C2C12 after centrifugation of 89 G with cap for one day. Dimension from left to right is 2 mm.



Fig. 6(C): C2C12 after centrifugation of 89 G with cap for two days. Dimension from left to right is 2 mm.

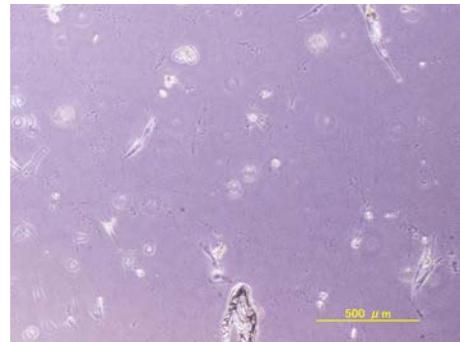


Fig. 6(D): C2C12 after centrifugation of 89 G with cap for four days. Dimension from left to right is 2 mm.

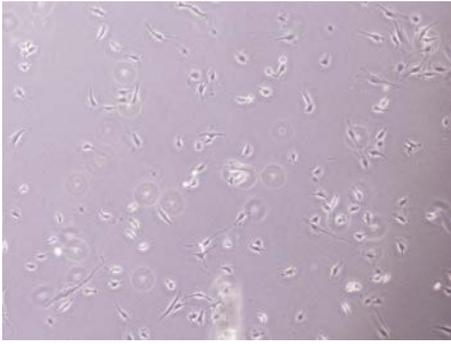


Fig. 7(A): C2C12 before centrifugation of 270 G with film for 24 hours. Dimension from left to right is 2 mm.



Fig. 7(B): C2C12 with film for 24 hours. Dimension from left to right is 2 mm.

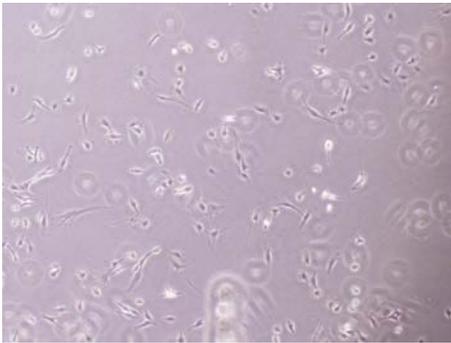


Fig. 7(C): C2C12 before centrifugation of 270 G with cap for 24 hours. Dimension from left to right is 2 mm.

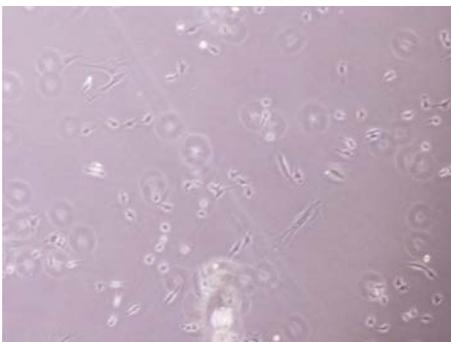


Fig. 7(D): C2C12 with cap for 24 hours. Dimension from left to right is 2 mm.

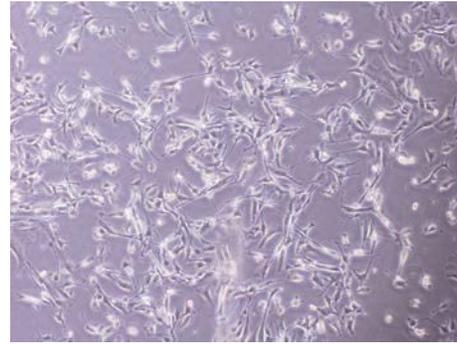


Fig. 8(A): C2C12 after centrifugation of 270 G with film for 4 days. Dimension from left to right is 2 mm.

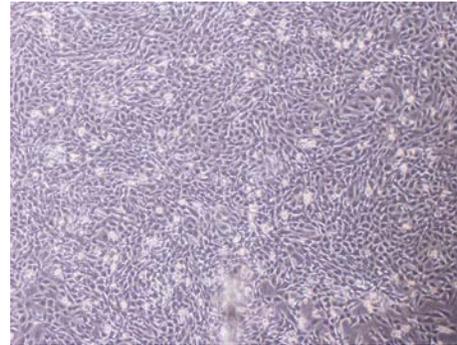


Fig. 8(B): C2C12 with film for 4 days. Dimension from left to right is 2 mm.

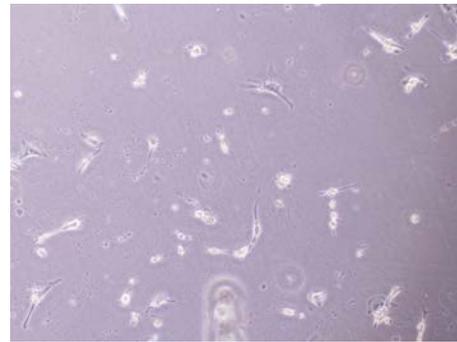


Fig. 8(C): C2C12 after centrifugation of 270 G with cap for 4 days. Dimension from left to right is 2 mm.

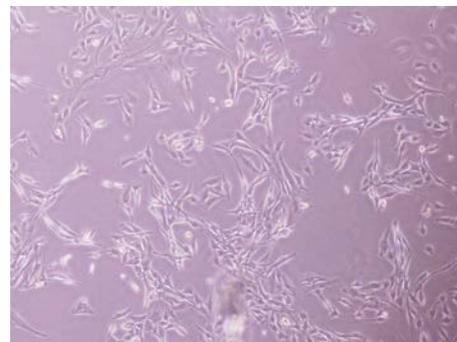


Fig. 8(D): C2C12 with cap for 4 days. Dimension from left to right is 2 mm.

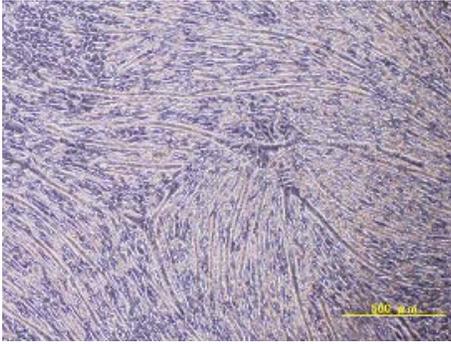


Fig. 9(A): C2C12 after centrifugation of 270 G with film for 12 days. Dimension from left to right is 1 mm.

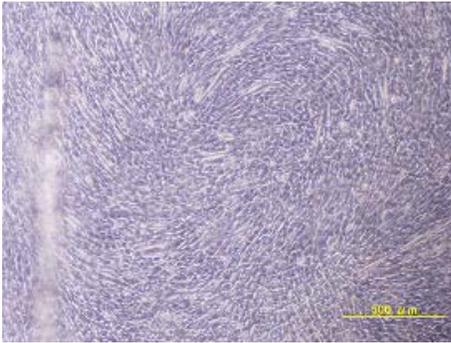


Fig. 9(B): C2C12 with film for 12 days. Dimension from left to right is 2 mm.

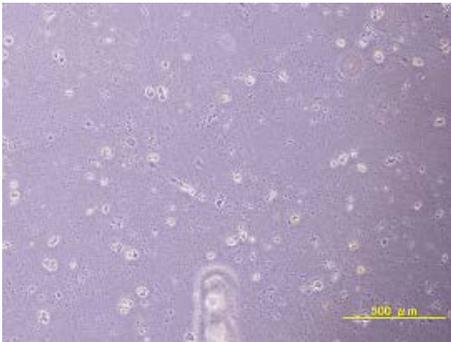


Fig. 9(C): C2C12 centrifugation of 270 G with cap for 12 days. Dimension from left to right is 2 mm.

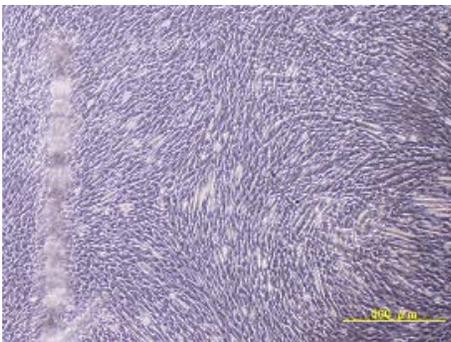


Fig. 9(D): C2C12 with cap for 12 days. Dimension from left to right is 2 mm.

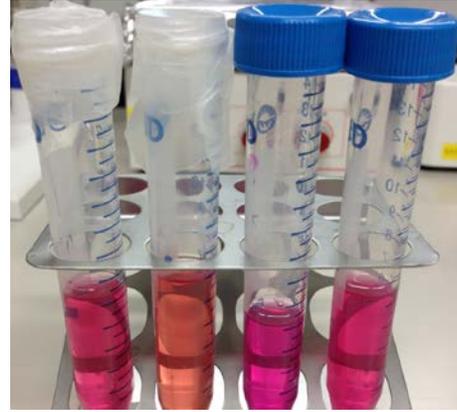


Fig. 10: Medium after two days of cell culture: centrifugation with film (A), rest with film (B), centrifugation with cap (C), rest with cap (D).

When the tube sealed with the film, C2C12 proliferate to the confluent manner and differentiates to myotube even under continuous stimulation of 270 times of the gravitational force for 12 days (Figs. 7(A), 8(A), 9(A)).

Without excess gravitational force, C2C12 proliferate to the confluent manner and differentiates to myotube even sealed with cap (Figs. 7(D), 8(D), 9(D)).

C2C12 differentiates to myotube. The repetitive cyclic constriction of the myotube is observed in 13 days of culture, when the electric pulses are applied to the medium (Fig. 9(A)).

In two days of cell culture, the color of the medium in the tube sealed with the cap does not change much (Fig. 10(C&D)), although that in the tube sealed with the film changes (Fig. 10(B)).

4. DISCUSSION

The culture plate of polystyrene is cut from the bottom of the culture dish, because the material is standard in the cell culture. Even after dipped in ethanol, C2C12 is proliferated and differentiated on the plate.

The response of biological system to the microgravity field has been studied using a space satellite. The cell cycle might extend in the space. C2C12 differentiation might be accelerated and the myotube might align to the direction perpendicular to the centrifugal field in the atmospheric partial pressure [7, 8].

Compared to the controlled partial pressure in the incubator, the atmospheric partial pressure might decelerate proliferation of C2C12 (Fig. 8(B) & (D)), although that might accelerate differentiation of C2C12 (Fig. 8(A)).

When mechanical stimulation is applied to the scaffold, the whole stimulation cannot always be transmitted to the cells. When the tension applied to a scaffold, the deformation of scaffold generates compression and shear in the different direction simultaneously [14]. The parallelepiped chamber is convenient to observe the response of cells under controlled

shear stress [8]. To apply continuous uniform mechanical stimulation to the cells, centrifugal force is used in the present study [6].

Both acceleration of proliferation and orientation of cells are important targets in the research field of regenerative medicine on the cultured biological tissue. The previous study shows that the behavior of cells depends on the electric [1] and magnetic stimulation [12]. Another study shows that mechanical stimulation improves a tissue-engineered human skeletal muscle [2]. The results of the study will contribute to acceleration technique in regenerative medicine

The previous studies show that a mechanical field governs behavior of cells [13-16]. The shear flow governs the orientation of endothelial cells [7, 9]. The shear stress affects the orientation of the smooth muscle cells in the biological tissue [3]. The direction of the mechanical field affects fibroblasts [5]. The effect of shear flow on orientation of cells depends on the kinds of cells [7]. Although HUBEC orients along the stream lines, C2C12 tilts from the stream lines to make myotubes. The previous study shows orientation of cells perpendicular to the stretch direction [4].

Too strong mechanical stimulation damages cells. The moderate mechanical stimulation, on the other hand, might accelerate differentiation of cells [6]. The mechanical stimulation can decrease proliferation of cells [6]. The mechanical stress also exfoliates several cells, which makes vacancy around the adhesive cell. The differentiation might be optimization of cells to the changing environment. The mechanical stress can accelerate differentiation of C2C12 to make myotubes [6].

5. CONCLUSION

The effect of gravitational force on orientation of cell has been studied *in vitro*. To apply excess gravitational force on cells, the cell culture tube was set in a conventional centrifugal machine. The experimental results show that C2C12 extends pseudo, proliferate to the confluent manner and differentiates to myotube even under continuous stimulation of 270 times of the gravitational force.

6. ACKNOWLEDGMENT

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