Effect of Electric Field Direction on Contractile Movement of Longitudinally Oriented Myotubes Cultured in Vitro

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ABSTRACT

The effect of electric field direction on contraction of longitudinally oriented myotubes has been studied *in vitro*. The oriented myotubes were made by the cultivation in the vortex flow around a silicone rubber disk in the culture dish swung by a rotating tilted plate at 30 revolutions per minute. C2C12 (Mouse myoblast cell line) cells are cultured in the Dulbecco's Modified Eagle's Medium for seven days. The horse serum was added to differentiate the muscle cells to myotuybes. After the cultivation, periodical electric pulses (1 ms pulse width with a period of 0.1 s) were applied to the medium with a pair of electrodes of platinum wire of 0.2 mm diameter. The myotubes were tetanized, when the electric pulses were applied. The distance between traceable points on a myotube in longitudinal direction was measured during the microscopic observation. Variation was made on the angle between the longitudinal direction of myotube and the direction of the electric field. The experimental results show that the amplitude of contraction is maximized when the direction of the electric field is parallel to the longitudinal direction of myotubes, and that the rate increases when the myotubes are oriented parallel each other.

Keywords: Biomedical Engineering, Muscle Cells, Cell Culture, Electric Field, Tetanic Contraction, Vortex Flow and Orientation

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* [2-5]. Reconstructed myotubes have a potential to be applied to a micro actuator. Orientation of myotubes might affect on their controllability with an electric stimulation.

Several effects of electric stimulation have been reported on biological cells. 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 previous study also 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 [5].

In the present study, the effect of electric field direction on contraction of longitudinally oriented myotubes has been studied *in vitro*.

2. METHODS

Cell Culture in Vortex Flow

The oriented myotubes were made by the cultivation in the vortex flow [6] (Fig. 1). A silicone rubber disk (22 mm diameter, 3 mm thick) was attached on the bottom at the center of a plastic culture dish of 35 mm internal diameter. The

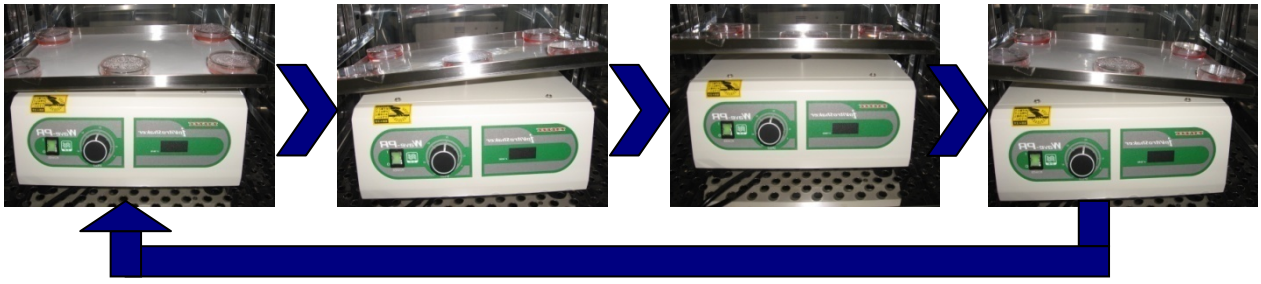

Fig. 1: Cultivation in the vortex flow. Rotation of tilted disk generates swing motion in incubator.

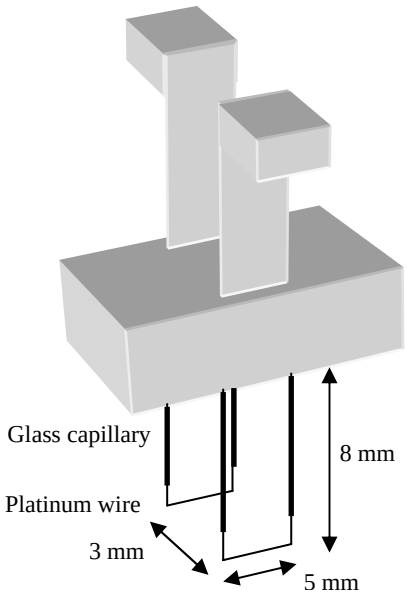

Fig. 2: Electrodes.

silicone rubber disk is stuck on the bottom of the dish with affinity between their surfaces without bond.

A polystyrene culture dish was used in the first group, while a temperature-responsive polymer (Poly-N-isopropylacrylamide) [7] coated dish was used in the second group.

The culture dish was placed on the plate, which tilted by 0.1 rad to the horizontal plane. The plate rotated to generate a swing motion of 30 revolutions per minute (WAVE-SI, Taitec, Co., Ltd., Koshigaya). The motion produces a vortex flow in the medium in the dish. C2C12 (Mouse myoblast cell line originated with cross-striated muscle of C3H mouse) cells were suspended in the Dulbecco's Modified Eagle's Medium. The suspension of 2 mL was poured into the dish and cultured in an incubator for seven days, while the plate was continuously rotating to make a steady vortex flow around the silicone rubber disk in the medium (Fig. 1).

Fetal bovine serum (FBS) was added to the medium with the volume ratio in 10 percent of FBS and 90 percent of D-MEM. The medium was exchanged every two days. Since the second day of culture, FBS was changed to the horse serum (HS) to differentiate the muscle cells to myotuybes. HS was added with the volume ratio in 7 percent of HS and 93 pecent of D-MEM.

Control cells were cultured and differentiated in the dish, which was placed in the incubator without exposure to the flow.

Electric Stimulation

After the cultivation, periodical electric pulses were applied to the medium to generate contraction of myotubes at 27 degrees Celsius.

A block of electrodes was manufactured. The block consists of a pair of electrodes of platinum wire of 0.2 mm diameter and a plastic block of supporter (Fig. 2). The wire was inserted through a glass capillary, which passes through the block to be fixed at the position. The distance is 3 mm between two electrodes of rectangle of 5 mm width in opposed position each other. The electrodes of a couple of parallel platinum wires generate a uniform electric field between them.

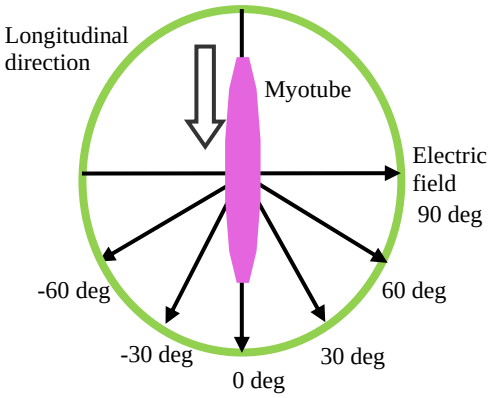

Fig. 3: Electrodes rotate on the cap of culture dish to select angle to myotube.

The block was placed on the cap with a rectangular hole (10 mm width × 12 mm) of the culture dish, to be able to be rotated for adjusting its direction. The electrode of wire was dipped with 2 mm depth, and positioned above 2 mm from the bottom of the culture dish. Several radial lines are marked on the cap to keep the direction of electrode (Fig. 3). The electrodes dipped in the medium, and the periodical electric pulses were introduced. The rectangular repetitive pulses, which have a pulse width of 1 ms with a period of 0.1 s and amplitude of 40 V, were generated by the electric stimulator (SEM-4201, Nihon-Koden Co., Ltd., Tokyo) (Fig. 4).

Contraction Measurement

The myotubes were tetanized, when the electric pulses were applied. The culture dish was took down from the rotating plate in the incubator at 37 degrees Celsius, and placed on the inverted microscope. The dish was placed on the heater to maintain temperature at 27 degrees Celsius in the room temperature of 26 degrees Celsius (Fig. 5). The distribution of the temperature around the culture dish was measured by an infrared thermography. The movement of myotubes was observed at 27 degrees Celsius, while the electric pulses were applied.

The distance between traceable points on the myotube in

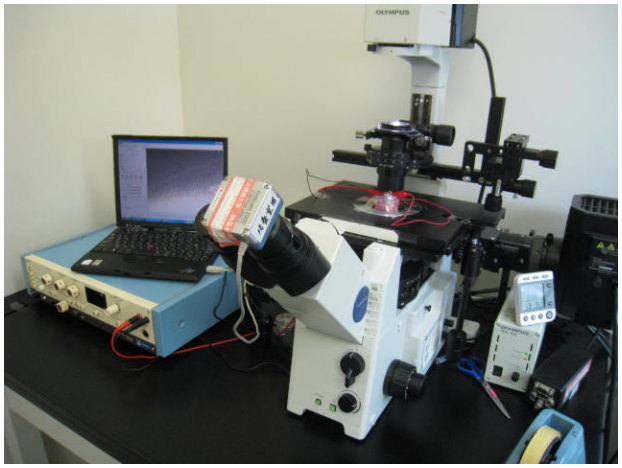

Fig. 4: Myotube contraction measurement system.

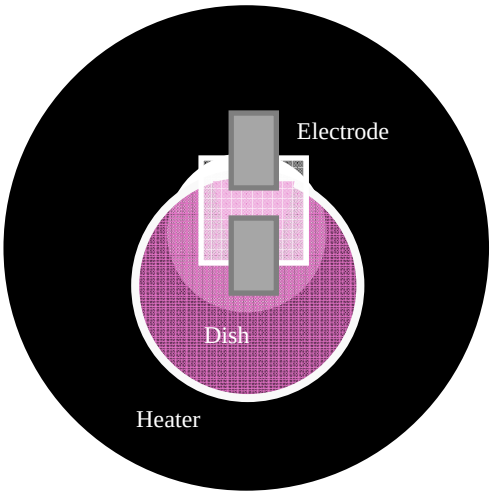

Fig. 5: Dish on the heater plate for temperature control.

longitudinal direction was measured during the microscopic observation (Fig. 8). The distance after tetanic contraction is compared to that before tetanic contraction. The traceable points were selected in the myotube, where bigger contraction occurs. Variation was made on the angle between the longitudinal direction of myotube and the electric field direction (Fig. 3).

Measurement was applied to three conditions of myotubes: the culture on the polystyrene dish in the vortex flow, the culture on the temperature-responsive polymer coating dish in vortex flow, and the culture on the polystyrene dish without flow.

The contraction ratio (R) is calculated by Eq. 1.

$$R = (L0 - L1) / L0 \quad (1)$$

In Eq. 1, L0 is the distance before tetanic, and L1 is the distance after tetanic.

3. RESULTS

The muscle cells proliferated to the sub-confluent state in one day of incubation. The oriented myotubes beyond the area of 1 mm square were observed around the silicone disk in seven days

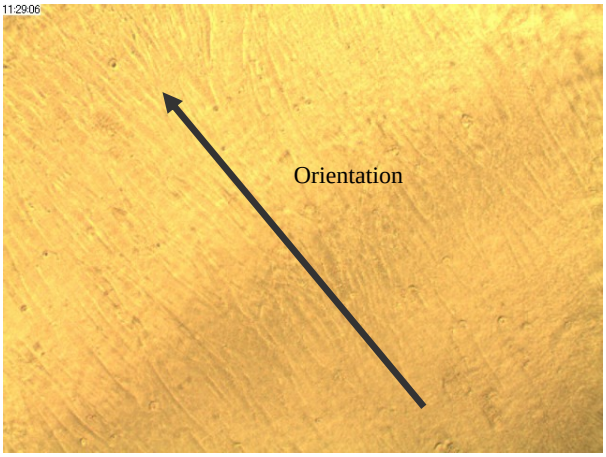

Fig. 6: Oriented myotubes by vortex flow. Distance between left to right is 0.8 mm in the figure.

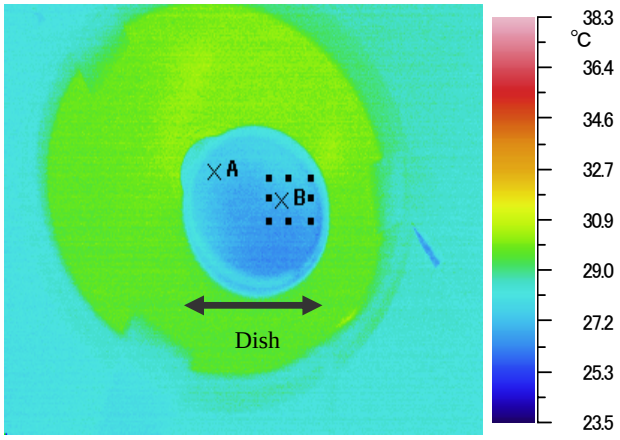

Fig. 7: Distribution of temperature around the culture dish.

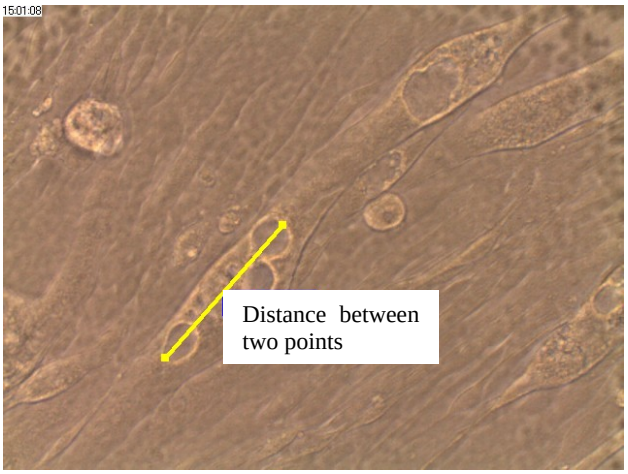

Fig. 8: Distance between traceable points on the myotube. Distance between left to right is 0.8 mm in the figure.

with flow (Fig. 6), where directions of myotubes were random in the culture without flow.

Fig. 7 exemplifies the distribution of the temperature around the culture dish on the heater plate measured by an infrared thermography, which shows the temperature around the culture dish was 27 degrees Celsius.

Fig.8 exemplifies traceable points on the myotube to measure the contraction ratio.

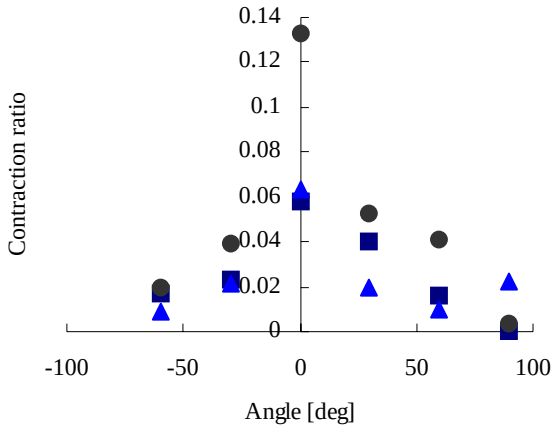

Fig. 9: Relation between contraction ratio and angle: square, control; triangle, flow on temperature-responsive polymer; circle, flow on polystylene.

Fig. 9 shows the relation between contraction ratio and the angle. The angle of "zero" corresponds to the direction of the electric field parallel to the longitudinal direction of myotube, and that of "90" corresponds to the direction of the electric field perpendicular to the longitudinal direction of myotube, respectively. The data of square show the contraction ratio of myotubes cultured without flow. The data of triangle show the contraction ratio of myotubes cultured with flow on the temperature-responsive polymer coating dish, and the data of circle show that on the polystyrene dish, respectively.

The experimental results show that the amplitude of tetanic contraction is maximized when the direction of electric field is parallel to the longitudinal direction of myotubes, and that the ratio increases when the myotubes are oriented parallel each other on the polystyrene dish.

The cuture layer including myotubes on the temperature -sensitive-polymer-coating slightly lifted up from the bottom of the dish at 28 degrees Celsius. The experimental result shows that contraction ratio of myotubes on the temperature sensitive polymer decreases compared with those on the polystylene. The contraction occurs even at 90 degree, when myotubes lift on the temperature-responsive polymer coating dish (Fig. 9).

4. DISCUSSION

The present study shows that the amplitude of contraction is maximized when the direction of electric field is parallel to the longitudinal direction of myotubes. The electric potential difference across the cell membrane might be maximized when the direction of electric field is parallel to the longitudinal direction of myotubes.

The periodical electric pulses were applied to the culture medium in the present study. The previous study shows that myotube repeats contraction and relaxation synchronized with the periodical electric pulses, and that myotube is tetanized with the electric pulses at period of 0.1 s [9].

Contraction is observed in the second layer from the bottom layer. Contraction in the bottom layer cannot be observed, because the myotubes might be strictly attached to the polystyrene surface of the dish in the bottom layer.

The experimental result shows that contraction occurs even at 90 degree, when myotubes are lifted up on the temperature sensitive polymer coating form the bottom of the dish. The contraction might occur combined with that of adjacent myotubes, which are not perpendicular to the electric field.

The temperature sensitive polymer Poly-N-isopropylacrylamide is hydrophilic below 32 degrees Celsius [7]. The polymer reversibly changes conformation in the water. When the medium is cooled down from 37 degrees Celsius to 20 degrees Celsius, the cultured cells are exfoliated from the polymer-coated bottom of the dish. Temperature of medium was kept at 27 degrees Celsius to keep lifted condition of myotubes in the culture dish in the present experiment.

The myotubes might shrink a little and the tension in myotubes might be released, when they are lifted with the temperature sensitive polymer. The relaxation might decrease the contraction ratio in the test. The lift, on the other hand, might help contraction of myotubes even in the electric field perpendicular to the longitudinal direction of the myotubes.

5. CONCLUSION

The effect of electric field direction on contraction of longitudinally oriented myotubes has been studied *in vitro*. The experimental results show that the amplitude of repetitive contraction is maximized when the direction of electric field is parallel to the longitudinal direction of myotubes, and that the ratio increases when the myotubes are highly oriented.

6. ACKNOWLEDGMENT

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