Effect of Extremely Low Frequency Magnetic Field on Cultured Skeletal Myoblasts

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ABSTRUCT

The effect of a magnetic field at an extremely low frequency on proliferation and orientation of cultured skeletal myoblasts has been studied *in vitro*. Skeletal muscle cells were isolated from a hind-limb quadriceps. The minced sample from a skeletal muscle was rinsed with a phosphate buffer solution, processed with an enzymatic digestion, centrifuged at 250 G, cultured in the medium for one hour, and filtered. The isolated cells were collected and seeded onto a dish coated with collagen. The culture dishes were placed parallel to the uniform alternating magnetic field, which was generated with the manufactured solenoid coil. The result shows that the cultured skeletal myoblasts tend to align to the direction of the magnetic field at the extremely low frequency.

Keywords: Bio-measurement, Magnetic field, Skeletal Myoblasts, Proliferation, Arrangement

1. INTRODUCTION

Cell culture technique has been progressed [1], and Myoblasts have been clinically applied to ischaemic cardiomyopathy [2]. Electrical and mechanical stimulation to cell culture have been investigated in the previous studies to control orientation of cells [3-5]. The previous study shows the experimental design to evaluate the effect magnetic field on cells [6-8]. The present experimental system has been designed to evaluate the effect of magnetic field on skeletal myoblasts *in vitro*. Reconstructed model in cultured muscle cells would be applied to investigation on muscle control in the biomedical engineering research field. The control methodology for orientation of cells might be applied to regenerated muscle tissue to achieve its effective electro mechanical function. In the present study, the effect of a magnetic field at an extremely low frequency on proliferation and orientation of cultured skeletal myoblasts has been studied *in vitro*.

2. METHODS

Experimental Equipment

The experimental equipment was manufactured to generate alternating magnetic fields at an extremely low frequency.

An alternating magnetic field (60 Hz, 1 mT – 4 mT) was generated through a solenoid with alternating voltage source of 6 V – 18 V. The solenoid consists of a cupper wire of 0.6 mm diameter coiled at 1800 turns around polymethyl-methacrylate cylinder of 85 mm diameter. The generated magnetic flux density is measured with a gauss-meter. Four dishes (radius, 35 mm) were placed in the center part of the cylinder so that the culture plane of the dish is parallel to the magnetic field (Fig. 1).

Another dish for control test was enclosed with the flexible sheet of micro-grain metal to shield the experimental space from the surrounding magnetic field.

In the experiment, both the magnetic flux density and frequency were controlled with the oscillator. The wave-forms of input voltage and output current are measured with an oscilloscope.

Fig. 1: Experimental equipment.

Cell Isolation and Culture

The culture medium consists of Eagle's MEM medium (including kanamycin), 10 percent of fetal bovine serum, fibrobrast growth factor, and was sterilized by an autoclave.

Figure 2 shows the experimental protocol. Adult rat skeletal muscle cells were isolated from a hind-limb quadriceps. The muscle in the femoral region was removed, and placed in a sterilized tube with a medium. The muscle was moved onto a dish with a saline solution and minced into pieces smaller than three cubic millimeter with the forceps scissors.

The sample was collected into a centrifugation tube, rinsed with a phosphate buffer solution (without calcium chloride and magnesium chloride) three times. An enzymatic digestion was processed for ten minutes shaking at 37 degrees centigrade with four milliliter of 0.25 percent trypsin and with an additional phosphate buffer of eight milliliter. After the enzymatic process was stopped with an additional medium solution of four milliliter, the top layer of sample was collected and cooled in ice. After centrifugation at 250 G at room temperature, the clear layer at the top was discarded and medium of two milliliter was added.

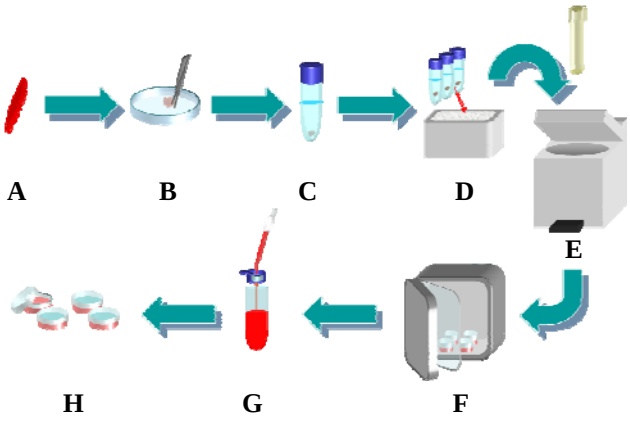

Fig. 2: Experimental protocol. A: muscle extraction, B: mince, C: trypsin process, D: cooling, E: centrifugation, F: incubation, G: filtration, H: cultivation.

The cells were dispersed with a pipette and expanded on a dish of 90 mm diameter, cultured in the medium of 15 milliliter at 37 degrees centigrade with carbon dioxide of five percent in an incubator. After cultivation for one hour, the suspension with cells was collected under gentle stirring with a pipette.

After the suspension passed through the filter of 40 micrometer holes, the isolated cells were collected and seeded onto a dish of 35 mm diameter coated with collagen at a density of 500 thousand cells per milliliter. The density was adjusted with a hemocyte-meter.

Cell Culture in Magnetic Field

Porcine skeletal myoblasts were collected from a femoral muscle. A piece of muscle tissue was extracted from the femoral muscle, minced, treated with trypsin, centrifuged, and filtered.

To isolate myoblasts, cells were cultured on a 100 mm dish coated with collagen in an incubator through four generations and identified with immunological stain technique of Desmin, which marks typical protein in muscle cells.

The cells from the fifth generation of cultured colony were used for the test. The myoblasts were cultured on dishes coated with collagen in each magnetic condition for three days. The results were evaluated about multiplication rate of cells, and the arrangement of cells.

The population of cells in the certain area in the dish was counted every twelve hours with a phase contrast microscope to observe proliferation. The acute angle between the magnetic field and the longitudinal axes of myoblast was also measured on each cell to evaluate the effect of magnetic field on orientation of cells. The plate, which has five holes of 1 mm diameter, was attached on the bottom of the dish to trace the same area.

3. RESULTS AND DISCUSSION

The intensity of surrounding magnetic fields sufficiently decreased with the flexible sheet of metal.

Figure 3 shows rat-cells observed with a microscope after cultivation for one hour. The centrifugation was processed at room temperature, because fat makes several colloidal massive structures at low temperature and interrupted collection of isolated cells (Fig. 3 (a)). The filter with holes of 40 micrometer was effective to isolate muscle cells (Fig. 3 (b)). The number of fibroblasts in the suspension decreases after cultivation for first one hour, because fibroblasts adhere faster than the muscle cells. Collagen coated on the dish promotes adhesion of muscle cells. Experimental results show that important factors, which govern isolation of muscle cells, are filtration and temperature during centrifugation.

Figure 4 shows isolated skeletal myoblasts.

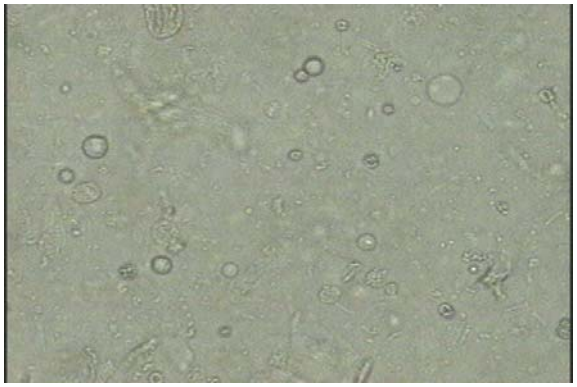

Fig. 3 (a): Cells after one hour cultivation.

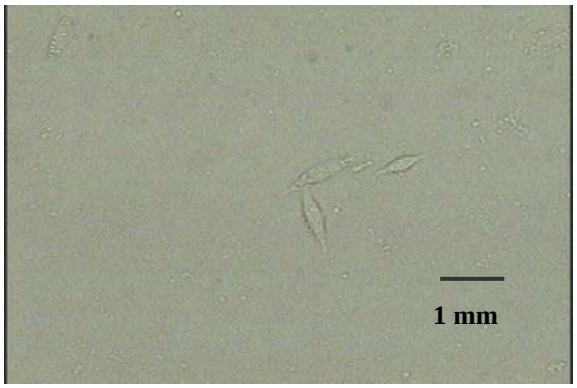

Fig. 3 (b): Cells after one hour cultivation (filter of 0.04 mm).

The results show that population of cells increases exponentially with time (Fig. 5) except in the magnetic field of 4 mT (Fig. 6).

The angles distribute randomly in the cells shielded from magnetic field, where they distribute at smaller angles in the cells exposed to the parallel magnetic field (Fig. 7). The result shows that the cultured skeletal myoblasts tend to align to the direction of the magnetic field at the extremely low frequency.

Experimental systems had been designed to improve magnetic conditions to study the effect of magnetic field on cell culture [6-8]. The uniform alternating magnetic field was controlled in the present experimental device with the solenoid coil and with the flexible sheet of micro-grain metal.

4. CONCLUSION

The experimental protocol about isolation of muscle cells and devices would be effective to study the effect of magnetic field on cultured muscle cells *in vitro*. The result shows that the cultured skeletal myoblasts tend to align to the direction of the magnetic field at the extremely low frequency.

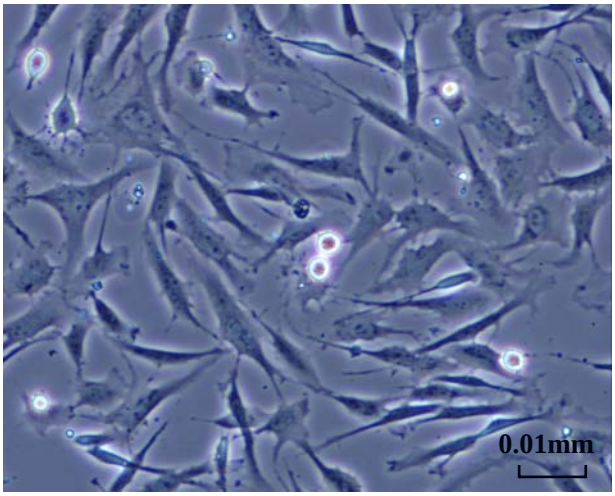

Fig. 4: Isolated myoblasts.

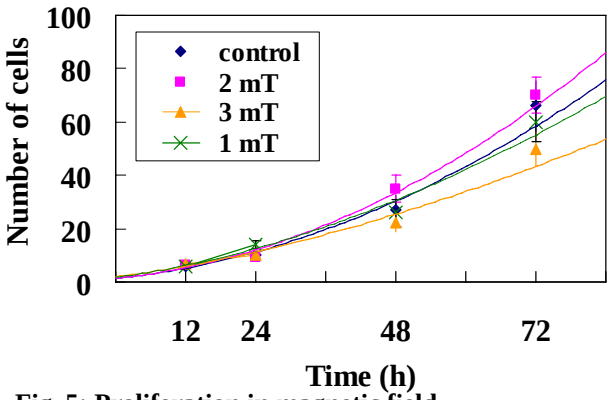

Fig. 5: Proliferation in magnetic field.

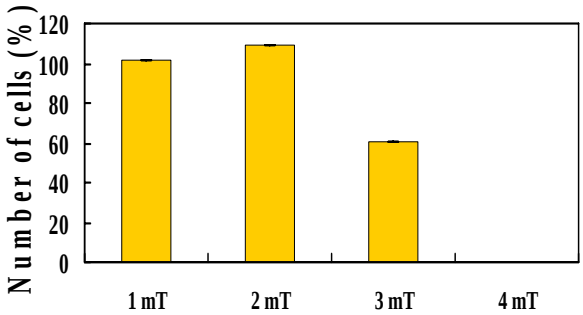

Fig. 6: Number of cells after 72 hours cultivation.

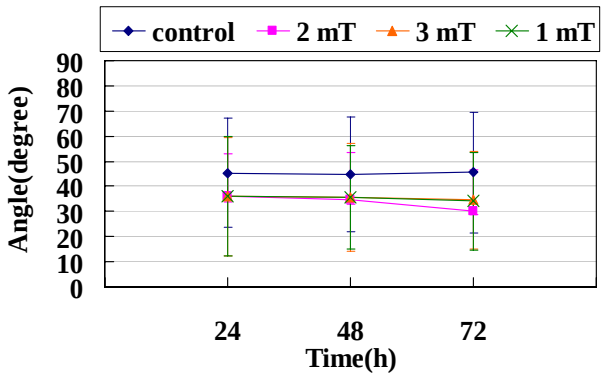

Fig. 7: Angles between cell and magnetic field.

5. ACKNOWLEDGMENT

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