Environmental Design of Muscle Cell Culture for Micro-actuator

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ABSTRUCT

An interdisciplinary research project on "Application of cultured muscle cells on medical engineering" has been proposed between engineering and medicine to be applied to actuators. In the present study, environment of muscle cell culture has been designed to accelerate cells proliferation, differentiation, and arrangement to be applied to micro-fabricated scaffolds with magnetic and electric fields *in vitro*. In the magnetic fields test, isolated muscle cells from a hind-limb quadriceps of an adult miniature pig were cultured in the controlled magnetic field for 72 hours. The culture dishes were placed parallel to the uniform alternating magnetic field, which was generated by a manufactured solenoid coil with a cooling water circulation system. In the electric stimulation test, L6 rat's myoblasts were cultured and electrically stimulated between two electrodes of platinum wire with 4 V rectangular pulse waves for ten minutes every two days for one week. On the other hand, cell culture scaffolds have been designed and fabricated with a stereo-lithography system. The designed environment has possibility to accelerate cells

proliferation, differentiation, and arrangement to be applied to micro-fabricated scaffolds.

Keywords: Biomeasurement, Cell-Culture, Muscle Cell, Environment, Micro-Actuator, Magnetic Field, Electric Stimulation

1. INTRODUCTION

An interdisciplinary research project on "Application of cultured muscle cells on medical engineering" has been proposed between engineering and medicine to be applied to actuators.

Cell culture technique has been progressed [1], and myoblasts have been clinically applied to ischaemic caridiomyopathy in the field of regenerative medicine [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 [6-10].

The engineering on robotics has progressed, on the other hand, with biomimetic studies, which has been applied to control methodology in multidisciplinary fields. A lot of simulation models have been proposed in the biomedical engineering field [11].

Micromachining technique has also been progressed with optic technology and been tried to be applied to the biomedical engineering field.

Biological muscles control smooth movements, and have high efficiency to generate power with its light structure, which might bridge to an ideal actuator.

In the present study, environment of muscle cell culture for micro-actuator has been designed to accelerate cells proliferation, differentiation, and arrangement to be applied to micro-fabricated scaffolds with magnetic and electric fields *in vitro*.

2. METHODS

Magnetic field test

An alternating magnetic field between 2 mT and 3 mT for 72 hours is generated around a coil with alternating electric current. To generate a magnetic field, a solenoid coil of 1800 turns was manufactured. The coil was enclosed with the flexible sheet of micro-grain metal to shield the experimental space from the surrounding magnetic field (Fig. 1). Cooling water of 33 degrees Celsius was circulated around the coil in the incubator to control temperature in the culture dish during exposure to the magnetic field (Fig. 2). In the experiment, both the magnetic flux density and frequency were controlled with the oscillator in sinusoidal wave of 60 Hz. The wave-forms of input voltage and output current are measured with an oscilloscope.

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. Adult miniature pig skeletal muscle cells were isolated from a hind-limb quadriceps [8, 9]. The muscle in the femoral region was removed,

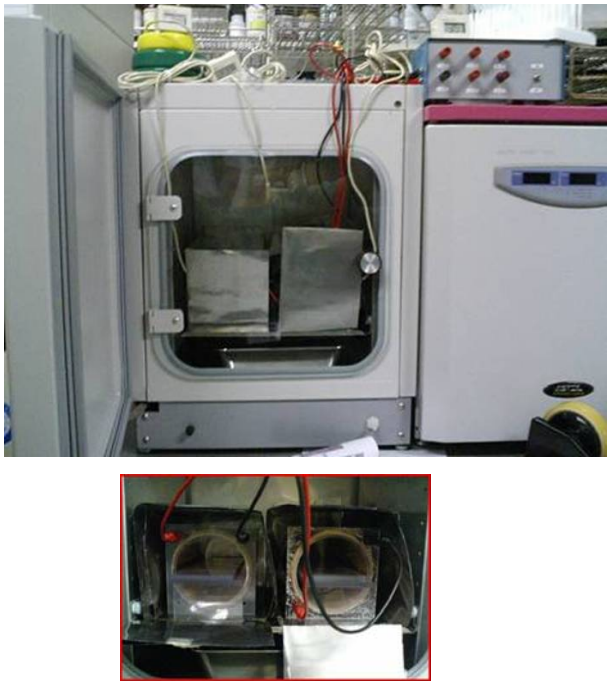


Fig. 1: Experimental system for magnetic field.

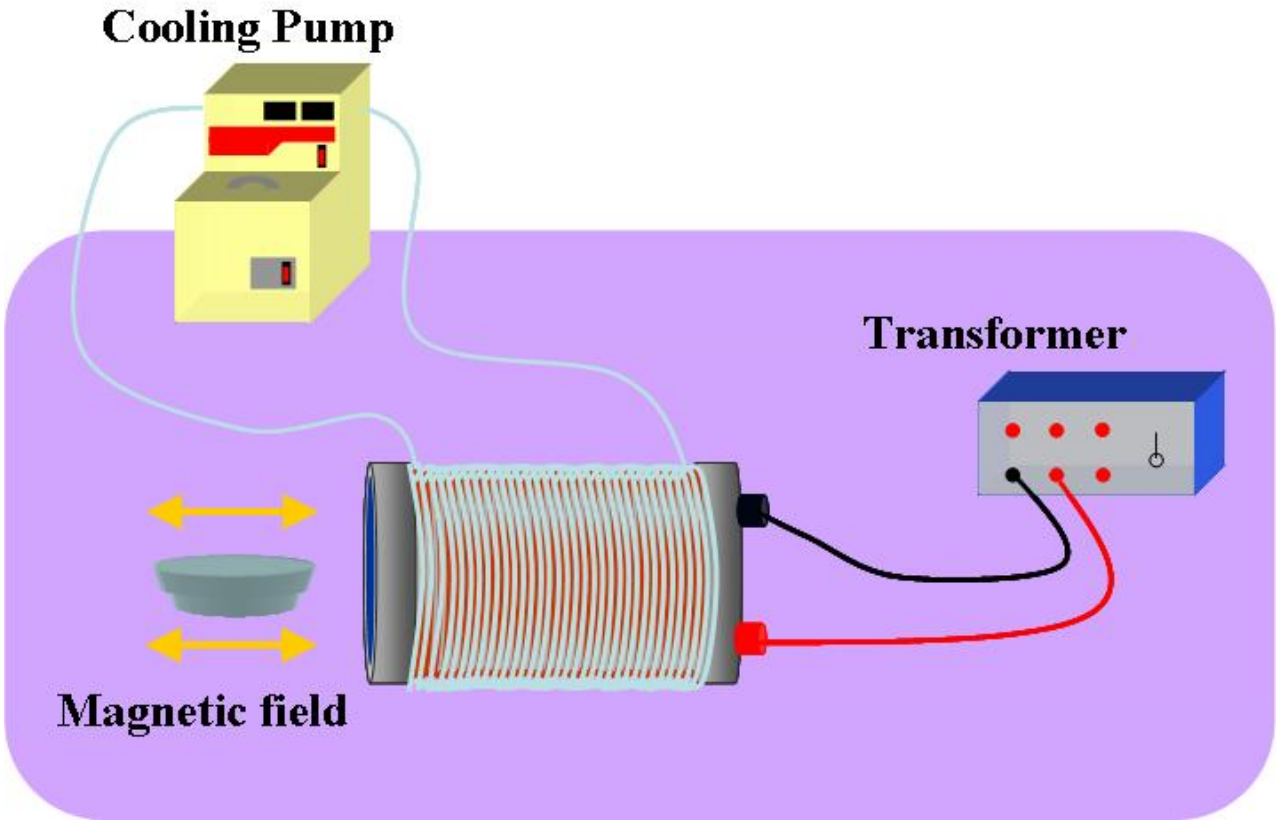


Fig. 2: Cooling system of cell culture during exposure to magnetic field.

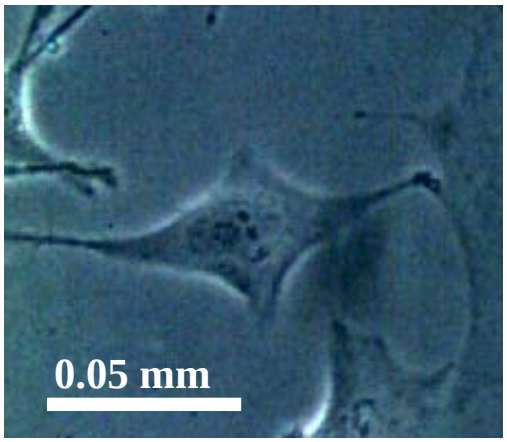


Fig. 3. L6 rat's myoblast.

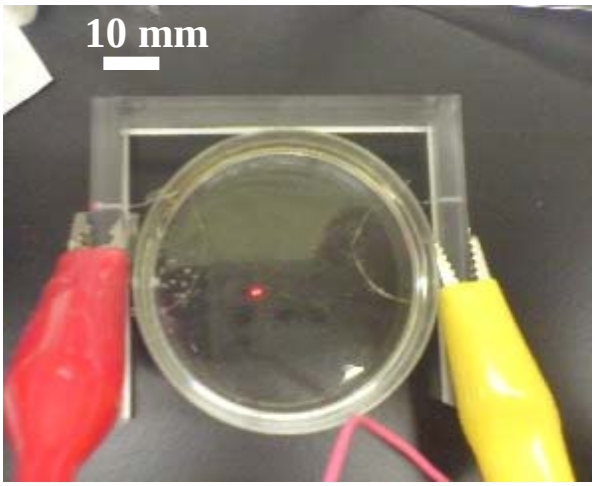
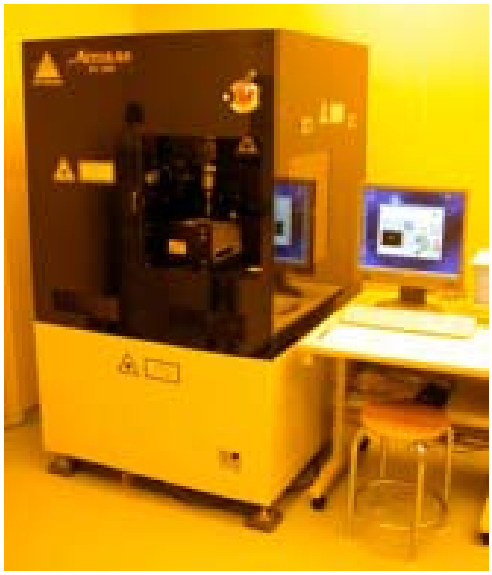


Fig. 4: Electric stimulation apparatus.

and placed in a sterilized tube with a medium. The muscle was moved onto a dish with a saline solution and minced into pieces with the forceps scissors. After an enzymatic digestion was processed with trypsin, the suspension was filtered and collected cells were seeded onto a dish of 35 mm diameter coated with collagen at a density of 50 thousand cells per milliliter. The density was adjusted with a hemocyte-meter. Dulbecco's Modified Eagle's Medium with 10 % of fetal bovine serum and with 0.02 mg/mL of fibroblast growth factor-basic was used for the culture medium.

The experiment with magnetic field was performed with cells between sixth and tenth



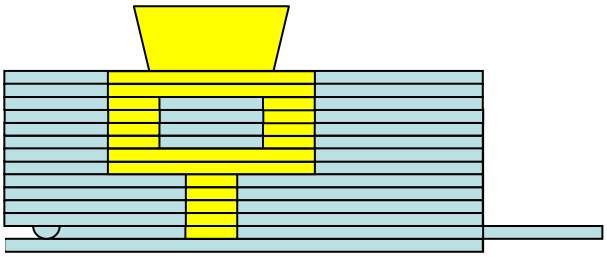


Fig. 5: Stereo-lithography system. Acrytic plastic layers are accumulated one by one.

generation during the successive subculture. The pig's skeletal myoblasts were cultured on dishes coated with collagen in each magnetic condition. The results were evaluated about multiplication rate of cells, and the arrangement of cells.

Electric stimulation test

L6 rat's myoblasts (Fig. 3) cultured on the dish without collagen coating were used in the experiment for electric stimulation. Dulbecco's Modified Eagle's Medium with 10 % of fetal bovine serum was used for the culture medium. Platinum wires of 0.1 mm diameter were used for electrodes (Fig. 4). After cells proliferated into confluent on the culture dish, cells were electrically stimulated with 4 V rectangular pulse waves of 1.0 ms at the frequency of 0.5 pulse per second for ten minutes every two days for one week.

Micromachining

Cell culture scaffolds have been designed and

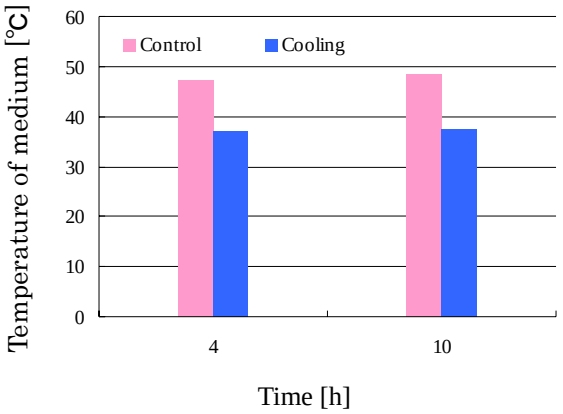


Fig. 6: Cooling effect.

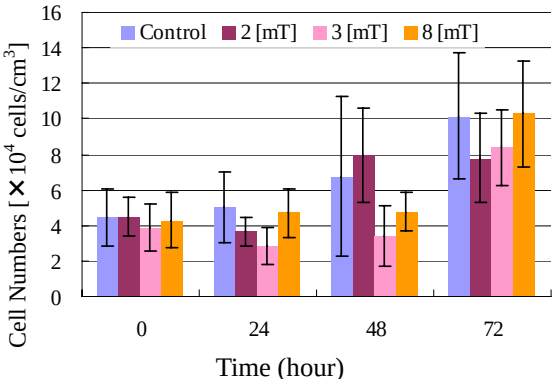


Fig. 7: Proliferation in magnetic field.

fabricated with a stereo-lithography system (Fig. 5). The scaffolds consist of collagen bridges and acrylic plastic anchors.

3. RESULTS AND DISCUSSION

Temperature in the culture dish was successfully controlled around 37 degrees Celsius during exposure to the magnetic fields of 3 mT with the aid of circulation of the cooling water (Fig. 6).

The results show that magnetic field does not affect on cells proliferation (Fig 7), and that cells tend to tilt to the direction of the magnetic field [10]. Cultured cells formed a mass in several days after confluent state in monolayer (Fig. 8).

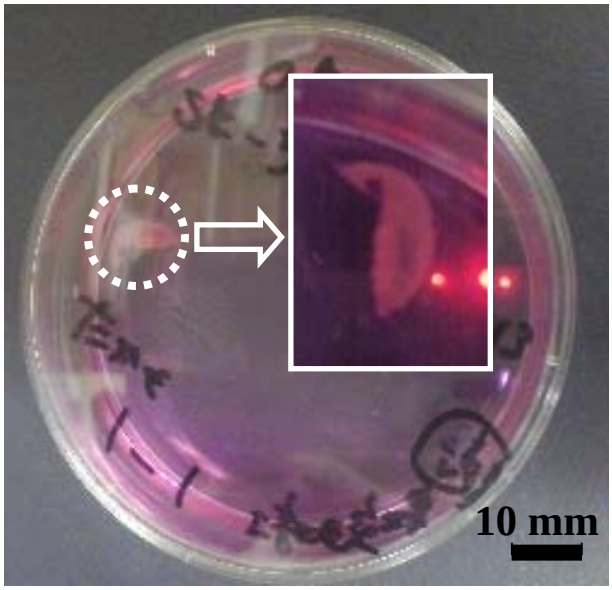
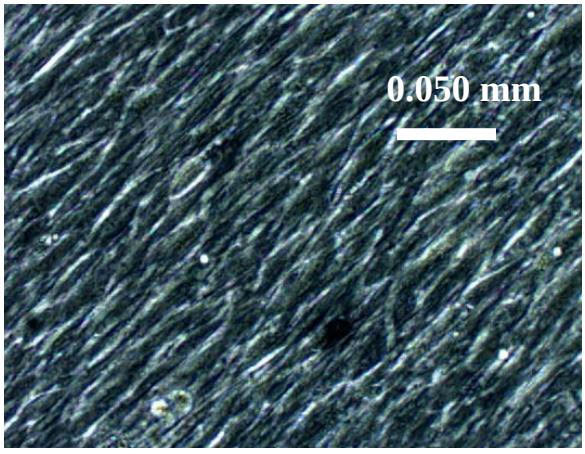


Fig. 8: Mass formed after confluent state.



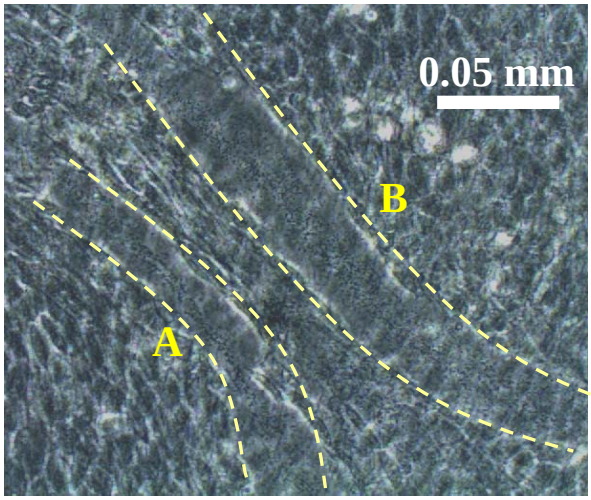


Fig. 9: L6 rat's myoblasts proliferated, arranged (upper), fused and formed myotubes (lower A & B).

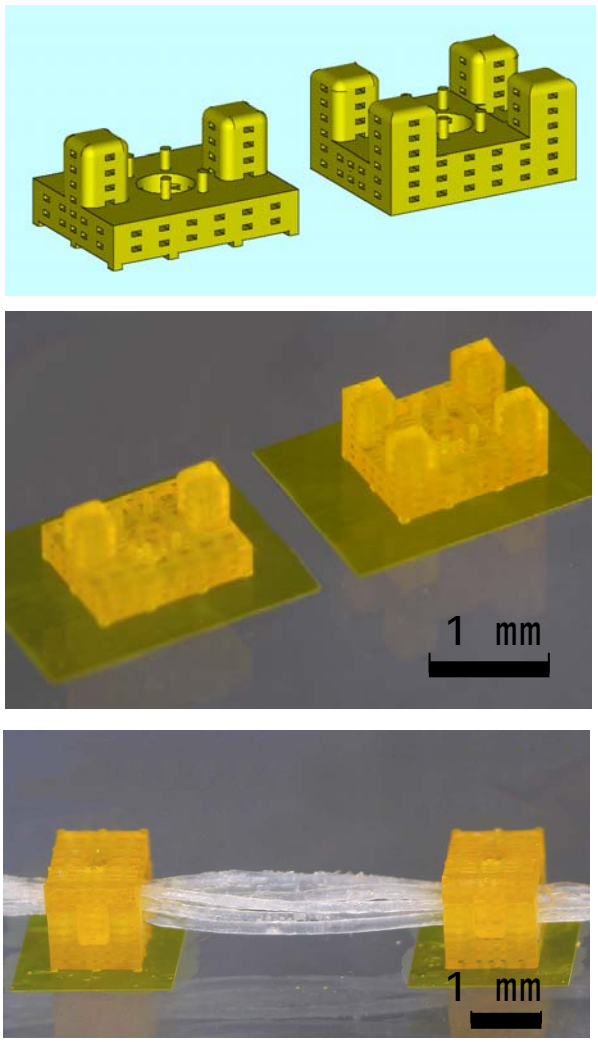


Fig. 10: Scaffold fabricated with stereo-lithography system; design (upper), anchor (center), with collagen bridge (lower).

Myotubes were formed in 19 days from confluent layer formation of myoblasts under the electric stimulation (Fig. 9). Myoblasts fusion and myotubes formation might be accelerated with the electric stimulation.

The scaffold was successfully fabricated with a stereo-lithography system (Fig. 10).

Reconstructed model in cultured muscle cells would be applied to investigation on muscle control in the biomedical engineering research field.

4. CONCLUSION

Environment of muscle cell culture for micro-actuators has been designed with magnetic and electric fields *in vitro*. The designed environment has possibility to accelerate cells proliferation, differentiation, and arrangement to be applied to micro-fabricated scaffolds.

5. ACKNOWLEDGMENT

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