Myotube Cultured on Micro Coil Spring

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ABSTRACT

Myoblasts have been cultured on a micro coil spring to estimate forces generated among the tissue of cultured myotubes. A micro coil spring made of titanium wire of 0.085 mm was used for the scaffold for the cell culture. The coil has the dimension as follows: 0.65 mm diameter, 0.05 mm pitch, 5 mm length. C2C12 (mouse myoblast cell line originated with cross-striated muscle of C3H mouse) was seeded at the concentration of 10000 cells per cm^2. The cells around the coil were observed with an inverted phase contrast microscope. The experiment shows the following results. Cells are able to adhere around the coil, proliferate, differentiate into myotube, make cylindrical layer around the coil, and bridge between the pitches of coils. The force, which is generated in myotubes pulling the wires between pitches, is estimated to 0.01 N.

Keywords: Biomedical Engineering, C2C12, Micro Coil and Differentiation.

1. INTRODUCTION

Cell culture technique has been developed and several methodologies have been clinically applied to regenerative medicine [1]. The acceleration technique for orientation and proliferation of cells has been studied to make a biological tissue *in vivo* or *in vitro* [2-4]. Control methodology for behavior of cells would be applied to regenerative tissue technology: orientation, proliferation and differentiation.

The effect of the surface of the scaffold on cell culture has been studied in the previous studies [5, 6]. Several factors, which control adhesion of biological cells, have been studied *in vitro* [7].

When cells make a tissue, they might be pulling each other to keep the morphology. The force generated in the tissue might be estimated with the restoring force of the scaffold.

In the cell culture, myoblasts differentiate to myotubes, which have potential to generate repetitive contraction with stimulation of electric pulses. When the scaffold has resistance to contraction, the myotubes make movement of repetitive contraction with the scaffold.

In the present study, myoblasts have been cultured on a micro coil spring to estimate forces generated among the tissue of cultured myotubes.

2. METHODS

Micro Coil
A micro coil spring (Hi-Lex Corp., Takarazuka, Japan) made of titanium wire of 0.085 mm diameter was used for the scaffold for the cell culture (Fig. 1). The coil has the dimension as follows: 0.65 mm diameter, 0.15 mm pitch, 5 mm length.

Cell Culture
The micro coil spring was placed in the micro-plate of 24 wells of flat bottom of polystyrene (Fig. 2). The internal diameter of each well is 15 mm.

The sixteenth passage of C2C12 (Mouse myoblast cell line originated with cross-striated muscle of C3H mouse) was seeded at the concentration of 10000 cells per cm^2.

C2C12 was cultured with the D-MEM (Dulbecco's Modified Eagle Medium) in the incubator for 37 days at 310 K with 5% of carbon dioxide gas. The medium contains FBS (fetal bovine serum) with volume percent of 10. The medium also contains penicillin streptomycin with volume percent of one.

The whole body of the micro coil was dipped in the culture medium during cell culture.

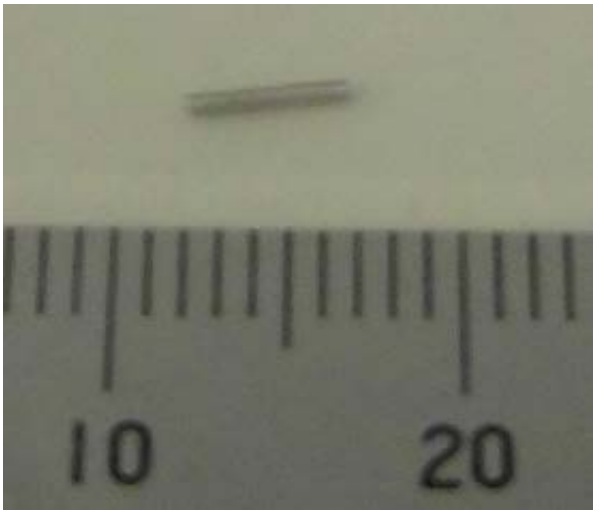

Fig. 1: Micro coil spring.

Fig. 2: Micro-plate of 24 wells.

After four days of culture, the coil was moved to another micro-plate to be separated from the cells on the bottom of the micro-plate. The cells on the coil were successively cultured for another four weeks. The medium was changed every two days. The content of the medium was changed to D-MEM (Dulbecco's Modified Eagle Medium) containing 5% HS (horse serum) and 1% penicillin streptomycin on the thirteenth day of culture.

The cells around the coil spring were observed with an inverted phase-contrast microscope (IX71, Olympus Co., Ltd., Tokyo) every day.

Spring Constant

The spring constant of the coil (k [N/m]) is calculated by Eq. 1.

$$k = F / x = G d^4 / (8 N D^3) \quad (1)$$

In Eq. 1, F is force [N], x is displacement [m], G is modulus of transverse elasticity of the wire [Pa], d is diameter of wire [m] N is number of turns, and D is diameter of the coil [m].

$$E = 2 G (1+n) \quad (2)$$

In Eq. 2, E is Young's modulus, and n is Poisson's ratio. In titanium, $E = 10^{11}$ Pa and $n = 0.3$ make $G = 4 \times 10^{10}$ Pa.

In the micro coil spring of titanium used in the present study, $G = 4 \times 10^{10}$ Pa, $d = 0.085$ mm, $N = 30$ turns, and $D = 0.65$ mm make $k = 40$ N/m.

When the dimension of a pitch of the coil varies 0.01 mm, the local force at one turn is 0.01 N.

3. RESULTS

Fig. 3 exemplifies C2C12 cultured for two days. In the figure, most of cells are on the bottom of the dish. After the coil was moved to another micro plate, many cells are attached on the coil.

Cells start to adhere to the coil in four days, and proliferate to cover the surface of the coil in five days of culture (Fig. 4). In two weeks of culture, some of cells exfoliate, when the coil is slightly vibrated during handling of culture. Most of cells, however, keep adhesion to the coil in three weeks of culture. Several myotubes are observed around the coil in two weeks of culture (Fig. 5). Most of myotubes tend to be oriented in the parallel direction to the wire of the coil.

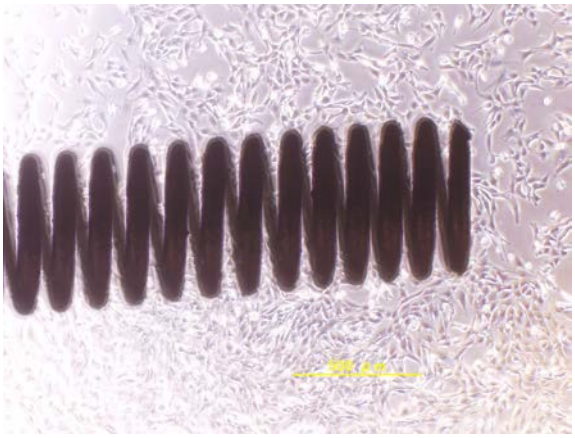

Fig. 3: C2C12 cultured 2 days around micro coil. Dimension from left to right is 2 mm.

Fig. 4: C2C12 cultured 5 days on micro coil. Dimension from left to right is 2 mm.

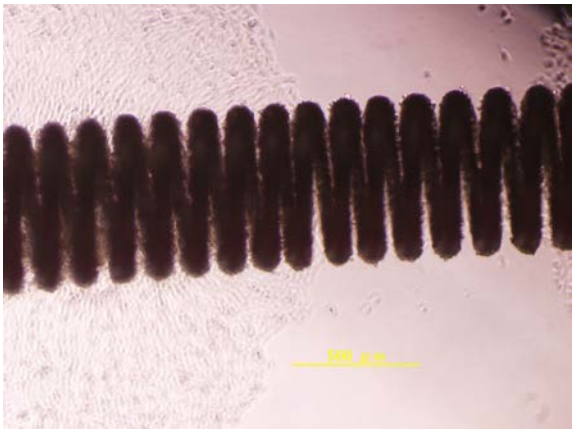

Fig. 5: C2C12 cultured 9 days on micro coil. Dimension from left to right is 2 mm.

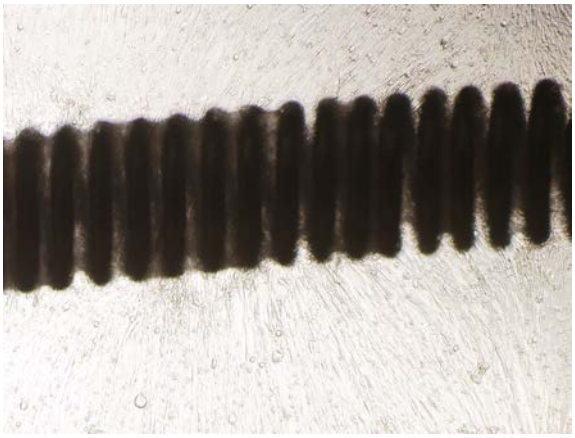

Fig. 6: C2C12 cultured 17 days on micro coil. Dimension from left to right is 2 mm.

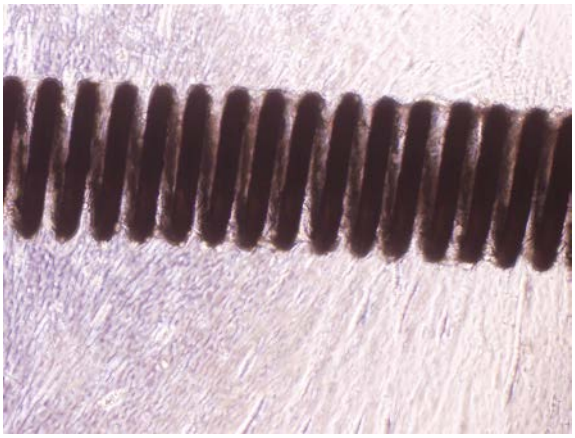

Fig. 7: C2C12 cultured 29 days on micro coil. Dimension from left to right is 2 mm.

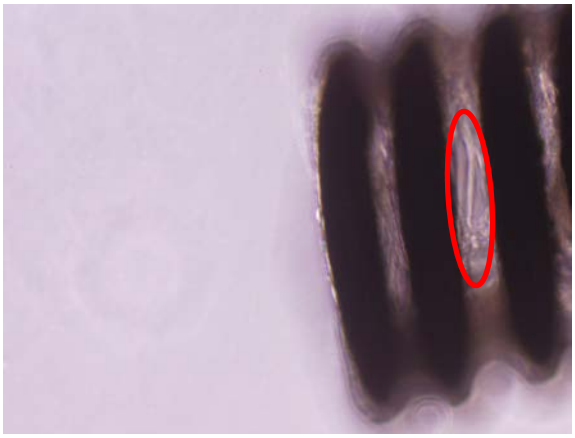

Fig. 8: Myotube on micro coil. Dimension from left to right is 1 mm.

Some of the pitches of the coil decrease at the level of 0.01 mm, when several myotubes are formed around the coil (Figs. 6 & 7).

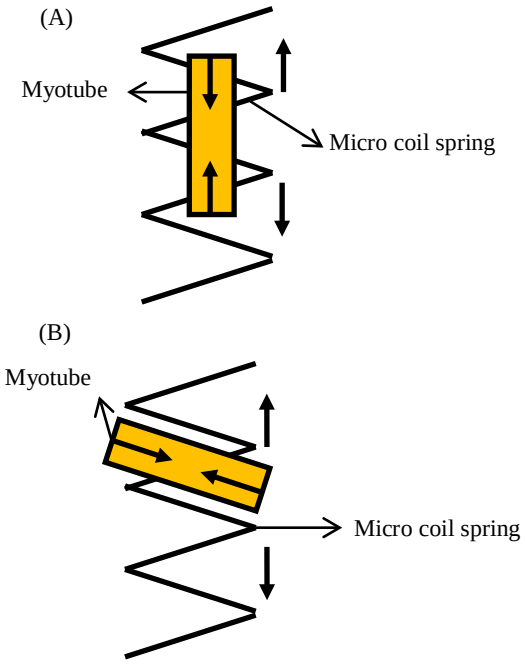

Fig. 9: Hybrid actuator of micro coil spring with myotube: axial (A) and spiral (B) direction.

4. DISCUSSION

After separation from the cells on the bottom of the micro-plate, most of cells are kept on the coil in the present experiment. The cells proliferate, and differentiate keeping contact with the coil.

When cells make tissue, a space for supplying medium is necessary around cells. A coil has a spiral space along the wire. The space might give a path for the medium approach to the cells.

In the previous studies, the several kinds of acceleration technique to make orientation of cells were tried *in vitro*: with the shear flow [2], with the gravitational force [3], with the nanofiber [8], or with the morphology of the surface [5, 6]. The micro coil spring gives a good scaffold for cell culture. The spiral morphology of the coil might make the spiral orientation of myotubes (circle in Fig. 8).

C2C12 is able to adhere and proliferate on the surface of the micro coil spring of titanium. The cells are also able to differentiate into myotubes around the coil spring.

The coil spring deforms in proportion to the force. The force generated in the muscle tissue cultured on the coil might be estimated by the displacement of the coil. The force generated in the tissue in the present experiment is estimated to 0.01 N by the displacement of 0.01 mm of the pitch of the coil. The force might be generated between the myotubes or in the myotube. The estimated force by the deformation of the coil is axial direction of the coil. The direction of the force generated in myotube might be longitudinal direction of the myotube, which is oriented to the spiral direction of the coil (Fig. 8).

The movement of cultured myotubes is able to be controlled with electric pulses supplied to the medium [9]. If the coil generates resistant force against the contractive force generated by myotubes, the coil covered with myotubes has a potential to make a repetitive contractile actuator [10-12]. The laser system has been applied to measure the cyclic movement in the biological system [13].

Titanium is one of the materials, which has been used for biological application [14]. Titanium has been implanted to human body as a strut of valves, a root of teeth, pins in orthopedic treatment, and a part of joint.

The morphology of micro channel has simulated the lymph system in the circulatory system *in vivo*. In several studies, permeability has been tried to control in designing artificial vessels. The micro spring has a potential to be applied to the scaffold for the micro channel.

The experimental results will contribute to estimate forces generated in the tissue of myotubes.

5. CONCLUSION

Myoblasts (C2C12) have been cultured on a micro coil spring to measure forces generated among the tissue of cultured myotubes. A micro coil spring (0.65 mm diameter, 0.05 mm pitch, 5 mm length) made of titanium wire of 0.085 mm was used for the scaffold for the cell culture. The experiment shows that cells are able to adhere around the coil, proliferate, differentiate into myotube, make cylindrical layer around the coil, and bridge between the pitches of coils. The force, which is generated in myotubes pulling the wires between pitches, can be estimated with the spring constant.

6. ACKNOWLEDGMENT

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