Culture of Myoblast on Micro Coil Spring with Electric Pulses

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ABSTRACT

Myoblasts have been cultured on a micro coil spring to control proliferation and differentiation on the scaffold with electric pulses *in vitro*. A micro coil spring made of the titanium wire of 0.085 mm was used for the scaffold for the cell culture. The coil spring has the dimension as follows: 0.65 mm diameter, 0.15 mm pitch, 5 mm length. C2C12 (mouse myoblast cell line) was seeded and cultured in Dulbecco's Modified Eagle Medium with 10 percent of fetal bovine serum. The electric pulses were applied to the coil spring for thirty minutes per day: the period of 1 s, the pulse width of 0.001 s, and the current amplitude of 0.02 A. The cells around the coil spring were observed with an inverted phase contrast microscope during the cell culture. The morphology of cells was observed with a scanning electric microscope at the end of the experiment. The experimental results show that the micro coil spring of titanium can be the scaffold of myoblasts and that electric stimulation might control proliferation and differentiation of myoblasts.

Keywords: Biomedical Engineering, C2C12, Micro Coil Spring, Electric Pulses, Cell Culture and Differentiation.

1. INTRODUCTION

The 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* [2-5]. 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 [6-9]. Several factors, which control adhesion of biological cells, have been studied *in vitro*.

Myoblast has been experimentally used to make a bio-actuator *in vitro* [10-17]. Several methods have been applied to control the bio-actuator: the electric stimulation, the electrode, and the scaffold.

In the present study, myoblasts have been cultured on a micro coil spring to control proliferation and differentiation on the scaffold with electric pulses.

2. METHODS

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

To control the scattering of the cell, the space around the coil spring is limited within a center hole (diameter of 10 mm) of the polydimethylsiloxane (PDMS) ring, which is placed on the bottom of the dish (Fig. 2A).

The lead wires of the both ends of the spring were fixed on the rim of the PDMS ring with the conductive tape to keep the position of the micro spring in the medium (Fig. 2B). Both ends of the titanium coil spring were connected to the platinum wire with the conductive paste (Dotite, D-550, Fujikura Kasei Co., Ltd., Tokyo, Japan) to introduce electric pulses.

Cell Culture
The tenth passage of C2C12 (Mouse myoblast cell line originated with cross-striated muscle of C3H mouse) was seeded in the area inside of the PDMS ring on the culture dish at the concentration of 10000 cells per cm^2.

The cells were cultured with the D-MEM (Dulbecco's Modified Eagle Medium) in the incubator for 30 days at 310 K with 5% of carbon dioxide gas (Fig. 3). The medium contains FBS (decomplemented fetal bovine serum) with the volume percent of 10. The medium also contains penicillin and streptomycin with the volume percent of one. The medium was changed every two days. The whole body of the micro coil spring was dipped in the culture medium during cell culture.

The cells were divided into three groups: A, B and C. In the group A, cells were cultured without electric pulses. In the group B, cells were cultured with electric pulses. The electric pulses are applied for thirty minutes per day after cell culture for 24 hours for adhesion to the scaffold. The cultured cells around the coil spring were observed in the groups A and B. In the group C, cells were cultured on the bottom of the culture dish without the coil spring to check the timing of differentiation from myoblasts to myotubes.

The electric stimulation was stopped on the seventh day. The coil spring was moved on the ninth day into the well without collagen coating and cells were cultured successively till the 31st day.

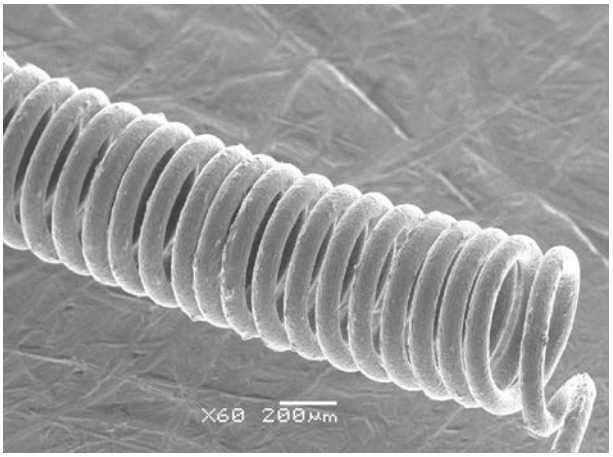


Fig. 1: Scanning electron-microscopic image of the micro coil spring. The bar shows 0.2 mm.

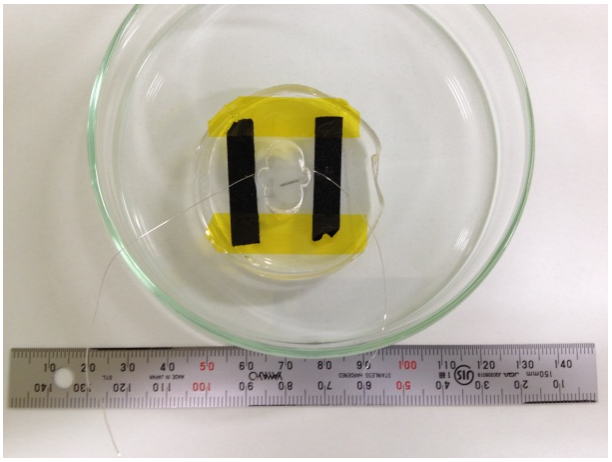


Fig. 2A: Micro coil spring placed in the space of PDMS ring contained in polystyrene dish, and connected to platinum wire.

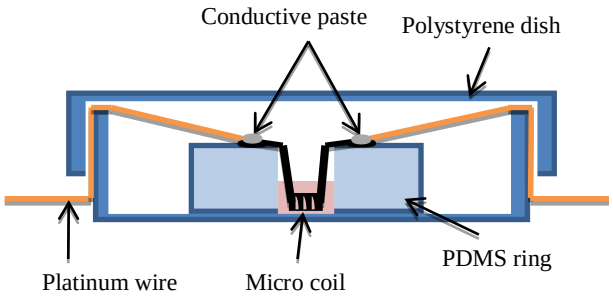


Fig. 2B: Micro coil spring placed in space of PDMS ring contained in polystyrene dish, and connected to platinum wire (cross section).

Electric Stimulation

The electric pulse *E* (period 1 s, amplitude 1 V, width 0.001 s) was generated with an electric stimulator (SEN5201, Nihon Kohden Corporation, Tokyo, Japan). The stimulator was connected to the coil *Z*, and the pulses were applied for thirty minutes per day for one week. An electric resistance *r* of 10 ohm is serially inserted between the coil spring and the stimulator (Fig. 4). The voltages (V_1 and V_2) were monitored by an oscilloscope during application of electric pulse to the coil spring. The electric current *I* (Fig. 4), which flows through *Z*, is calculated by Eq. 1.

$$I = V_2 / r \quad (1)$$

Temperature around the coil spring was measured by an infrared thermography (i5, FLIR Systems, Inc., Tokyo, Japan) during electrical stimulation.

Observation

The cells around the coil spring were observed with an inverted phase-contrast microscope (IX71, Olympus Co., Ltd., Tokyo) every day. In the group of electric stimulation, the cells were observed before and after electric stimulation for thirty minutes.

The morphology of the surface of the micro coil spring was observed by a scanning electron microscope (SEM, JSM6360LA, JEOL Ltd., Tokyo, Japan).

In the group C, Giemsa stain solution was applied to distinguish nuclei of myotubes on the twelfth day of culture.

Fig. 3: Cell culture on micro coil spring with electric pulses.

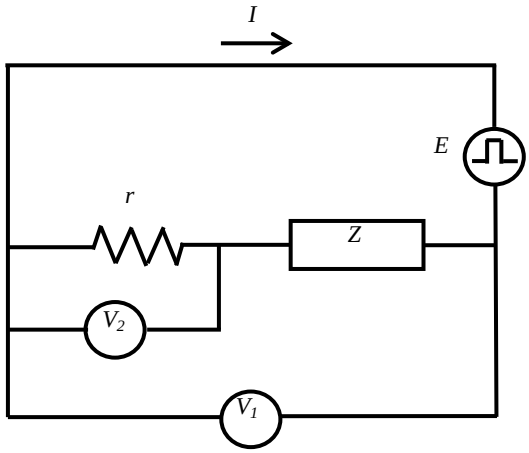


Fig. 4: Electric circuit for application of electric pulses (*E*) to coil spring (*Z*).

3. RESULTS

Fig. 5 shows electric signal tracings during application of electric pulses through the coil spring. The signal 1 in Fig. 5 shows the electric pulse *E* (period of 1 s, amplitude of 1 V, width of 0.001 s). The voltage between the ends of resistance of 10 ohm is 0.2 V, which makes current of 0.02 A (signal 2 in Fig. 5).

Fig. 6 shows temperature around the coil spring before and after application of electric pulses for thirty minutes. Fig. 6B shows that the temperature at the surface of the coil spring is 30.9 degree centigrade after application of electric pulses for thirty minutes. The heating effect of the electric pulses on the surface of the coil spring is small.

Fig. 7 exemplifies cells after cultivation for 24 hours around the coil spring. The figure shows that cells adhere both on the coil spring and on the bottom of the culture dish, even after application of the electric pulses for thirty minutes (Fig. 7B).

Fig. 8 shows that cells proliferate to make a confluent state in three days of culture without the coil spring.

Fig. 9 exemplifies cells after cultivation for seven days around the coil spring. The figure shows that the number of cells does not increase much around the coil spring both without (Fig. 9A) and with (Fig. 9B) the electric pulses.

In the group C, myoblasts differentiated to myotubes in eleven days of culture (Fig. 10). The myotubes with multiple nuclei are observed by Giemsa stain (Fig. 11).

Fig. 12 exemplifies cells after cultivation for thirty days around the coil spring. The cells on the bottom of the dish start to differentiate into myotubes.

Fig. 13 shows SEM image of the coil at the 31st day of culture. The figure shows that cells extend to make bridges between springs over the pitch of turns both without and with electric pulses. Myoblasts adhere around the coil spring of titanium, proliferate, extend to make bridges between wires over the pitch of turns, and make cylindrical layers around the coil.

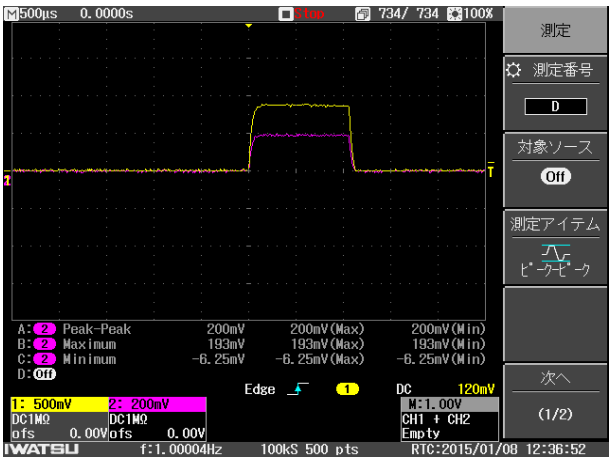
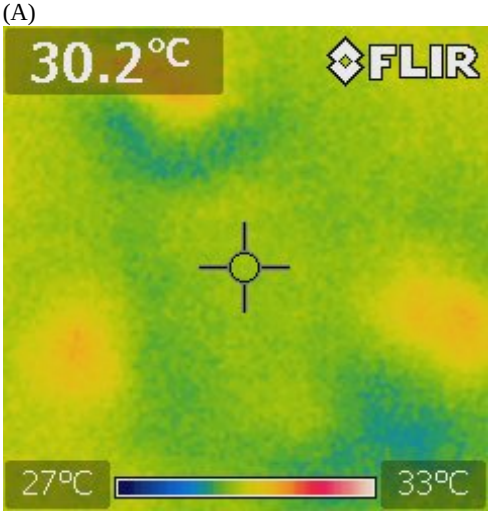


Fig. 5: Electric signals during application of electric pulses.

(A)



(B)

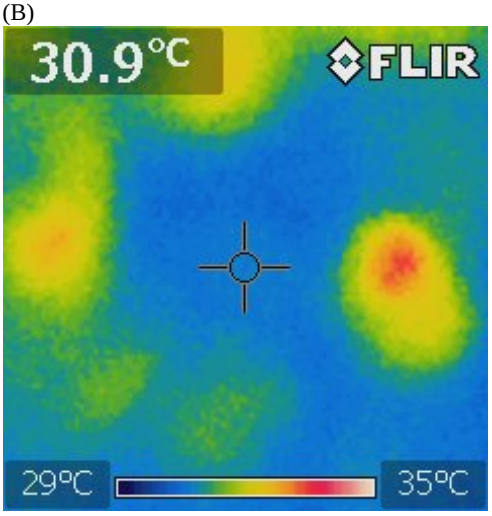


Fig. 6: Thermography around coil spring before (A) and after (B) electric pulse application for 30 min. The value shows temperature at the center. The coil spring is located at the center.

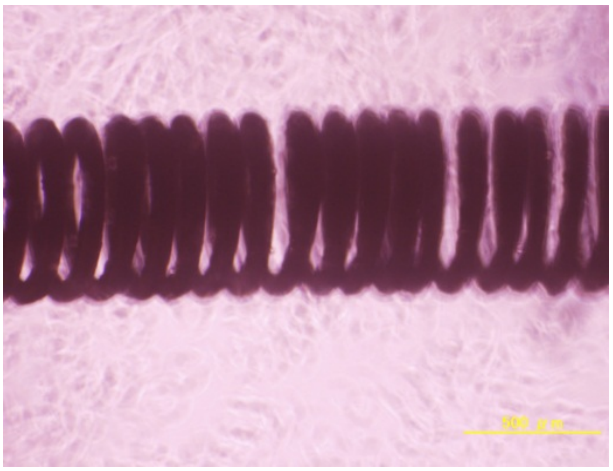


Fig. 7A: C2C12 cultured for 24 hours around coil spring without electric pulse application. Dimension from left to right is 2 mm.

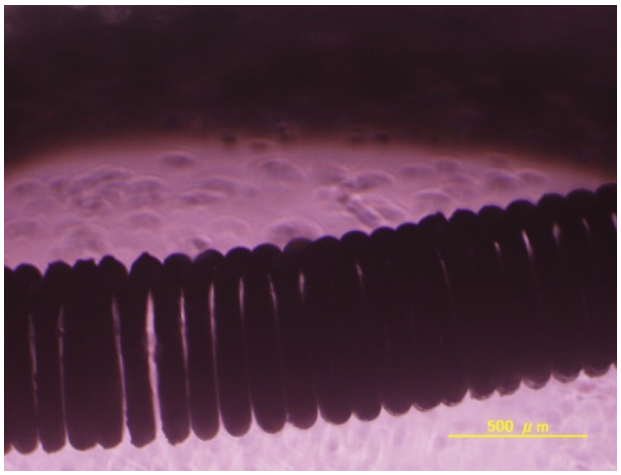


Fig. 7B: C2C12 after electric pulse application for 30 min on the second day of culture. Dimension from left to right is 2 mm.

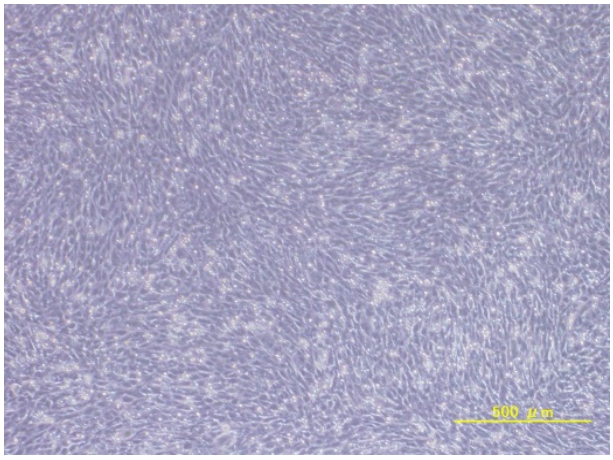


Fig. 8: C2C12 cultured for three days without coil spring. Dimension from left to right is 2 mm.

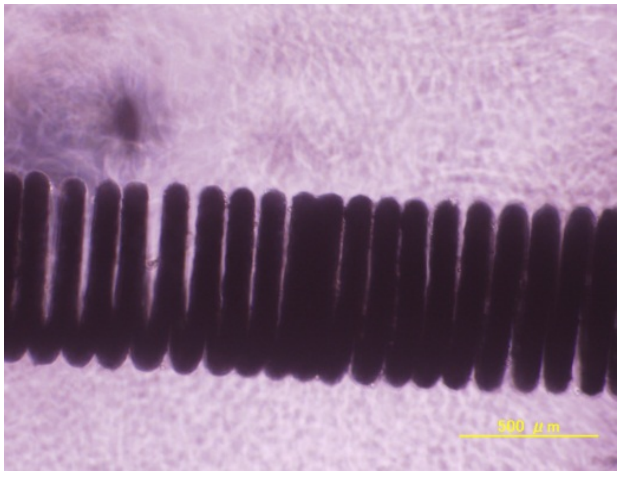


Fig. 9A: C2C12 cultured for seven days around coil spring without electric pulse application. Dimension from left to right is 2 mm.

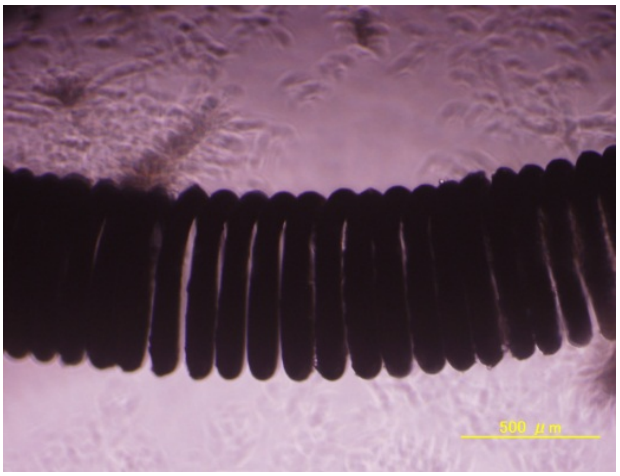


Fig. 9B: C2C12 cultured for seven days around coil spring with electric pulse application. Dimension from left to right is 2 mm.

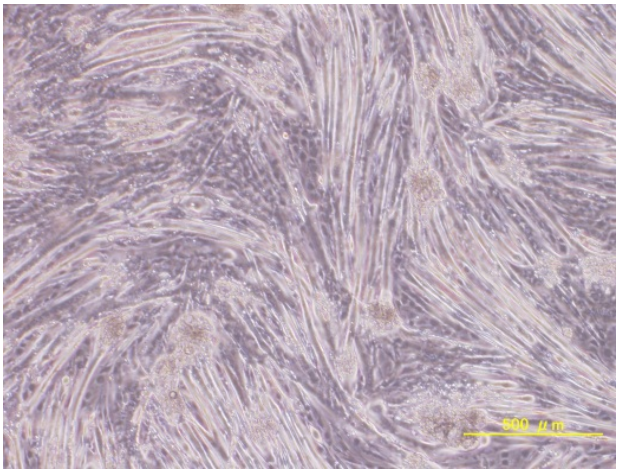


Fig. 10: C2C12 cultured for eleven days without coil spring. Dimension from left to right is 2 mm.

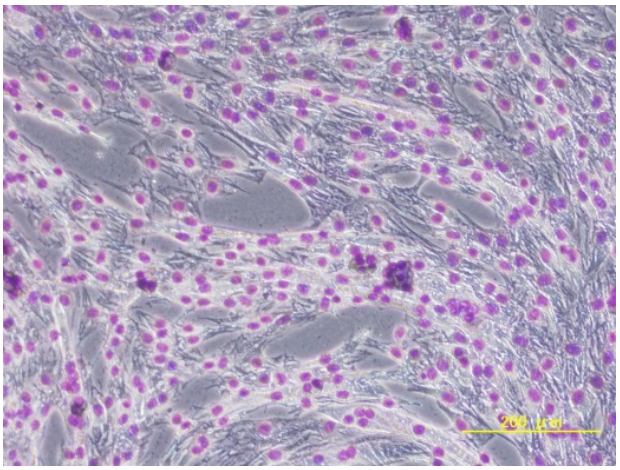


Fig. 11: C2C12 stained after culture for twelve days without coil spring. Dimension from left to right is 1 mm.

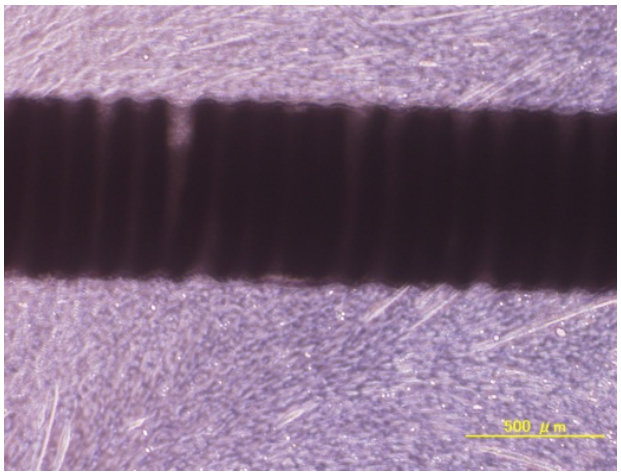


Fig. 12A: C2C12 cultured for thirty days around coil spring without electric pulse application. Dimension from left to right is 2 mm.

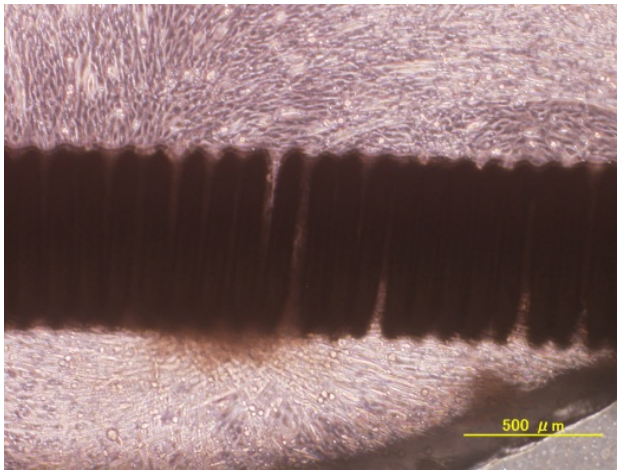


Fig. 12B: C2C12 cultured for thirty days around coil spring with electric pulse application. Dimension from left to right is 2 mm.

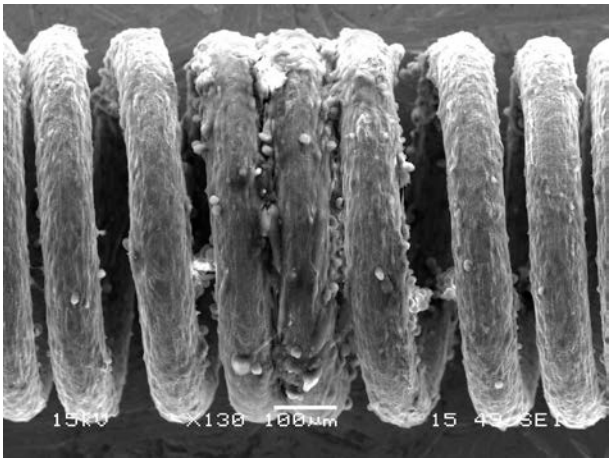


Fig. 13A: C2C12 cultured for thirty days on coil spring without electric pulse application. Dimension from left to right is 1 mm.

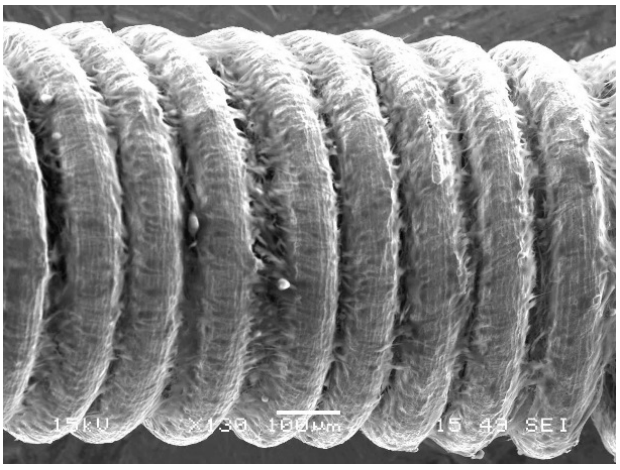


Fig. 13B: C2C12 cultured for thirty days on coil spring with electric pulse application. Dimension from left to right is 1 mm.

4. DISCUSSION

The cell culture technique was applied to make a bio-actuator combined with micro-machine technique in the previous studies [11, 12]. In the previous studies, the several kinds of acceleration technique to make orientation of cells were tried *in vitro*: with the shear flow [2, 3], with the gravitational force [4], with the nanofiber [7], or with the morphology of the surface [6, 8].

The micro coil spring gives a good scaffold for cell culture. The spiral morphology of the coil spring might make the spiral orientation of myotubes [15]. When cells make tissue, a space for supplying medium is necessary around cells. A coil spring has a spiral space along the wire: the interval of 0.085 mm between the wires. The space might give a path for the medium to approach to the cells.

The coil spring deforms in proportion to the force. The force generated in the muscle tissue cultured on the coil spring might be estimated by the displacement of the coil spring [15].

The movement of cultured myotubes is able to be controlled with electric pulses supplied to the medium. The laser system has been applied to measure the cyclic movement in the biological system [18].

Titanium is one of the materials, which has been used for biological application [19, 20]. 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.

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 [15].

The number of cells around the coil spring increases, after the ninth day of culture. Under the electric stimulation of pulses, the proliferation of cells might decrease [14].

Because the micro coil spring was slightly apart from the bottom of the culture dish, cells did not migrate from the

bottom of the dish to the coil spring. It is not easy to control the density of cells on the coil spring at the cell seeding. At the beginning of the culture, cells might easily migrate to the surface of the coil spring, when the coil spring is touching on the bottom of the dish. The coil spring is located closer to the bottom of the well, after the ninth day of culture in the present study.

The amount of the medium around the coil spring is more easily maintained in the wells than in the ring of PDMS, which might be one of the reasons why cells proliferate faster after the ninth day of culture in the present study.

The cells on the coil spring are hard to be observed by the optical microscope, because of the direction of the transmitted light through the three dimensional cell culture (Figs. 7, 9, 12). The extracellular matrix, which covers around the cells (Fig. 13), also makes the microscopic observation of cells difficult.

5. CONCLUSION

Myoblasts have been cultured on a micro coil spring to control proliferation and differentiation on the scaffold with electric pulses: the period of 1 s, the pulse width of 0.001 s, the current amplitude of 0.02 A, and thirty minutes per day. The experimental result shows that electric pulses tend to decrease the proliferation and differentiation of cells.

6. ACKNOWLEDGMENT

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