Culture of Myoblast on Conductive Film with Electric Pulses

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ABSTRACT

The effect of stimulation of electric pulses through the scaffold on proliferation and on differentiation of myoblasts has been studied *in vitro*. A transparent thin film of indium tin oxide (ITO) coated on the glass was used for the conductive scaffold for the cell culture. C2C12 (mouse myoblast cell line originated with cross-striated muscle of C3H mouse) was cultured for 22 days on the film. Electric pulses (period 1 s, amplitude 1 V (0.0005 A), duration 0.001 s) were applied on the film for thirty minutes per day. During culture, the cells were observed with an inverted phase contrast microscope every day. The experimental results in 21 days of culture without electric pulses show that myoblast differentiate to myotube on ITO, although myoblasts do not differentiate to myotube on glass. On the scaffold with electric pulses in 21 days, myoblasts proliferate to confluent state, although they do not differentiate to myotubes.

Keywords: Biomedical Engineering, C2C12, Electric Pulse and ITO.

1. INTRODUCTION

Cell culture technique has been developed and several methodologies might clinically be applied to regenerative medicine [1]. C2C12 (mouse myoblast cell line) adheres to the scaffold, proliferates and differentiates to myotubes *in vitro* [2]. These behaviors of the cell depend on the micro property of the surface.

The effect of the surface of the scaffold on cell culture has been studied in the previous studies [3]. Several micro-fabrication processes have been designed to control adhesion of biological cells *in vitro* [4].

The muscle tissue is exposed to electric pulses in the biological body. The movement is also controlled with the electric pulses. The biological systems have ability to optimize themselves to their environment. The optimum electric stimulation has a potential to control growth of the muscle tissue, which might contribute to the regenerative medicine.

Indium tin oxide (ITO) is an inorganic compound of indium oxide (In_2O_3) and tin oxide (SnO_2). Because the transmittance in the visible light region is high, it is colorless and transparent in thin films. ITO is one of the useful materials, which is applied to the transparent electrode.

In the present study, the effect of stimulation of electric pulses through the scaffold of conductive film of ITO on proliferation and on differentiation of myoblasts has been studied *in vitro*.

2. METHODS

Conductive Scaffold

The indium tin oxide (ITO) film was used for the conductive scaffold for the cell culture. A transparent thin film of ITO is formed on the surface of a glass disk (Fig. 1). The thickness and the width of the film are 30 nm and 40 mm, respectively. The diameter and the thickness of the disk are 100 mm and 1 mm, respectively. The lead wire is soldered on the each end of the film to introduce the electric current.

Three small donut-rings of polydimethylsiloxane (PDMS, Dow Corning Corporation, MI, USA) are adhered with the affinity of surfaces to make the peripheral walls for the cell culture (Fig. 2). The outer diameter, the inner diameter and the thickness of the ring are 32 mm, 10 mm and 5 mm, respectively. The locations of the rings are on the ITO film, on the glass, and on the boundary area between the ITO film and the glass, respectively. The glass disk was placed in the dish of 100 mm diameter (Fig. 3).

Both on the ITO film and on the base of the glass, the purified water contact angles were measured by the contact angle analyzer (Phoenix-300, Meiwafosis Co., Ltd., Tokyo, Japan).

Electric Pulses

The electric pulses (period 1 s, amplitude 1 V, duration 0.001 s) were generated with an electric stimulator (SEN5201, Nihon Kohden Corporation, Japan). The stimulator was connected to the ITO film, and the pulses were introduced to the scaffold of cells for thirty minutes per day (Fig. 4). An electric resistance of 10 ohm is serially inserted between the ITO film and the stimulator (Fig. 5). The electric signals (V_1, V_2) were monitored by an oscilloscope during electric stimulation to the ITO film. V_1 and V_2 are the voltages between the terminals of electric stimulator, and between terminals of resistance, respectively. The electric stimulation was applied to the scaffold after adhesion of the cells by 24 hours of culture.

The temperature at the surface of the ITO film was measured by an infrared thermography (i5, FLIR Systems, Inc., Tokyo, Japan), before and after the introduction of electric pulses in the incubator.

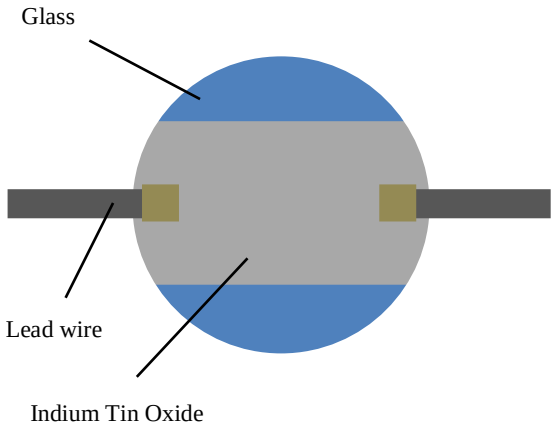

Fig. 1: ITO film on the disk of glass.

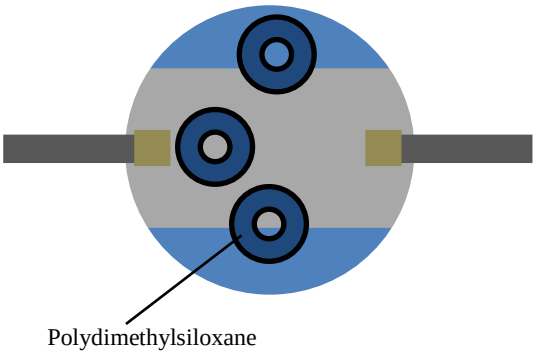

Fig. 2: PDMS rings are attached on the ITO and glass.

Fig. 3: Glass with ITO film in the dish of 100 mm diameter.

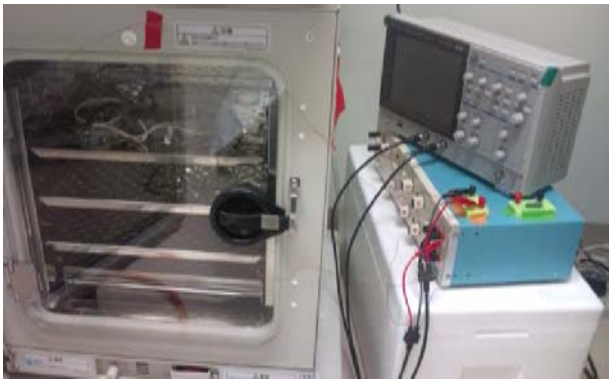

Fig. 4: Electric pulses introduced to scaffold of cells in incubator.

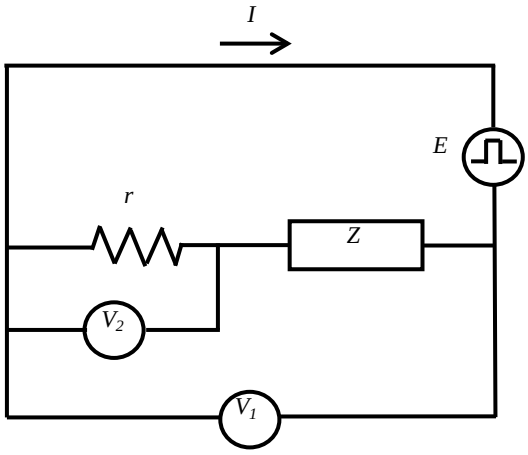

Fig. 5: Electric circuit during introduction of electric pulses (*E*) to scaffold (*Z*) of cells.

Cell Culture

C2C12 (Mouse myoblast cell line originated with cross-striated muscle of C3H mouse) of the ninth passage was used for the cell culture without electric stimulation, and C2C12 of the fourth passage was used for the cell culture with electric stimulation. D-MEM (Dulbecco's Modified Eagle Medium) containing 10% FBS (Fetal Bovine Serum) and 1% penicillin/ streptomycin was used for the medium. The cells were seeded on the scaffold with the density of 1000 cells/cm^2. Cells were cultured in the incubator at 310 K with 5% CO_2 for 21 days (with electric pulses for 30 minutes per day). The medium were refreshed every two days. Cells were observed with an inverted phase-contrast microscope (IX71, Olympus Co., Ltd., Tokyo) every day.

3. RESULTS

Fig. 6 shows the contact angle on the film of ITO (A) and on the base of the glass (B). The contact angle is smaller on film of ITO (61 degree) than on the glass (65 degree).

Fig. 7 shows the voltage monitored during introduction of electric pulses to the ITO film. The parameters of voltage between the terminals of electric stimulator are 1 s of period, 1 V of amplitude, and 0.001 s of duration, respectively. The

parameters of the voltage between terminals of resistance of 10 ohm are 1 s of period, 0.005 V of amplitude, and 0.001 s of duration. The 0.005 V of amplitude and 10 ohm of resistance makes 0.0005 A of current.

Fig. 8 shows the distribution of temperature around the ITO film on the glass, before (A) and after (B) introduction of electric pulses. The temperature at the center of ITO film is elevated from 33 degree centigrade to 36 degree centigrade in the incubator. The local temperature at the film is same as that at the glass during the introduction of electric pulses for one hour in the incubator. The three red rings in Fig. 8 correspond to the ring of PDMS, which has the rate of thermal emissivity different from the glass.

The cells are easily observed with the microscope through the transparent thin film of ITO on the glass surface (Figs. 9-11). The Cells adhere, extend pseudo, and proliferate both on polystyrene and on the ITO film.

Fig. 9 exemplifies cells on 11th day of culture without stimulation of electric pulses. Fig. 10 exemplifies cells on 21st day of culture without stimulation of electric pulses. C2C12 proliferated to the confluent state on the eighth day of culture on ITO, although C2C12 proliferated to the confluent state on the sixth day of culture on polystyrene dish. Myoblasts differentiated into myotubes on the 21st day of culture on ITO (Fig. 10B), although myoblasts differentiated into myotubes on the 11th day of culture on polystyrene dish (Fig. 9A).

Fig. 11 exemplifies cells on the 22nd day of culture with intermittent stimulation of electric pulses. C2C12 proliferated to the confluent state on the 15th day of culture on ITO with intermittent stimulation of electric pulses. Myoblasts do not differentiate into myotubes in 22 days of culture on ITO (Fig. 11B). Myoblasts do not proliferate to the confluent state in 22 days of culture on the glass (Fig. 11D).

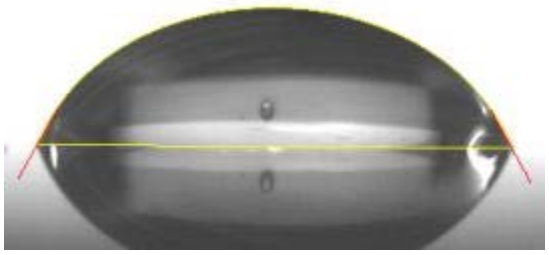

Fig. 6A: Contact angle on ITO film.

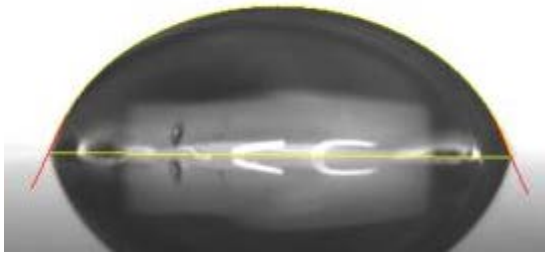

Fig. 6B: Contact angle on glass.

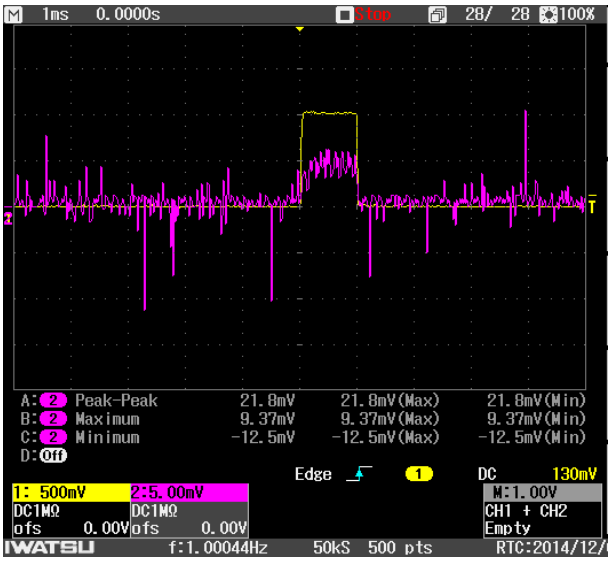

Fig. 7A: Electric pulse and voltage between terminals of resistance. Scale: Ordinate, 0.5 V (Channel 1) and 0.005 V (Channel 2); abscissa, 0.001 s.

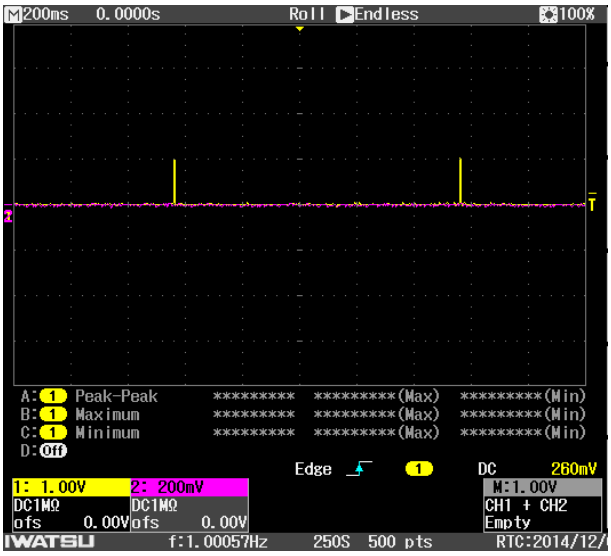

Fig. 7B: Electric pulse and voltage between terminals of resistance. Scale: Ordinate, 1 V (Channel 1) and 0.2 V (Channel 2); abscissa, 0.2 s.

4. DISCUSSION

The electric current was low so that the temperature at the surface of metal coated on the glass was kept around 310 K during the cell culture. From the current of 0.0005 A, and the voltage of 1 V, the resistance of the ITO film is estimated to be 2000 S^{-1}. The estimation makes of 0.0005 W of the electric power consumption at the ITO film during the pulse. Because the electric power consumption is very small and the electric current is pulsatile (short duration of pulse), the ITO film is not heated by the electric current.

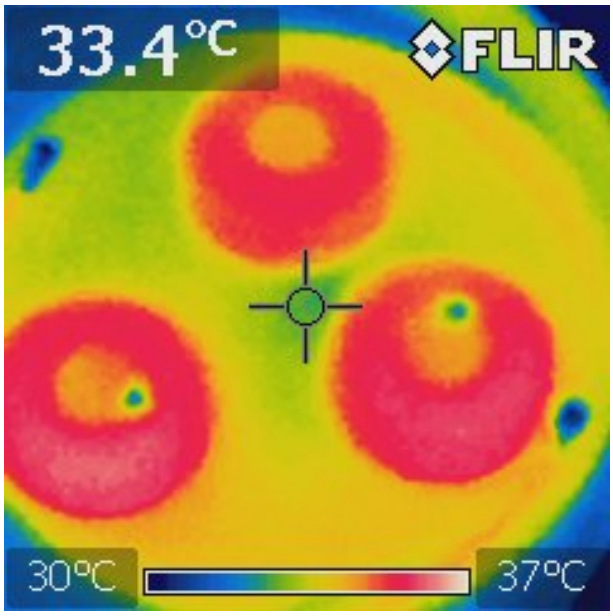

Fig. 8A: Temperature around ITO film before introduction of electric pulses. Temperature at the center of the film is 33.4 degree centigrade.

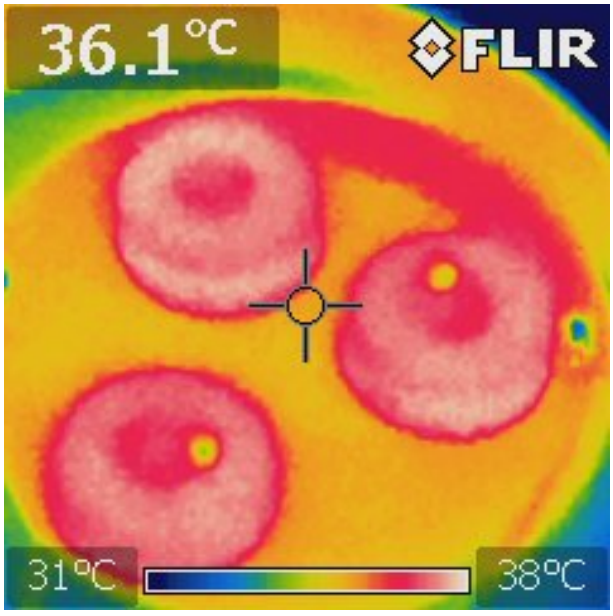

Fig. 8B: Temperature around ITO film after introduction of electric pulses for one hour. Temperature at the center of the film is 36.1 degree centigrade.

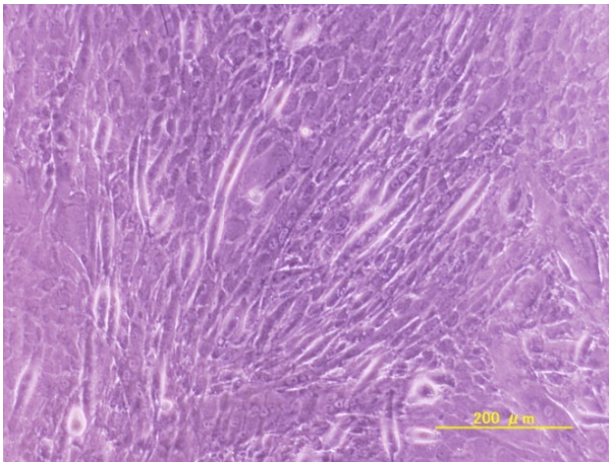

Fig. 9A: Myotubes on polystyrene dish on 11th day of culture without stimulation of electric pulses. Dimension from left to right is 1 mm.

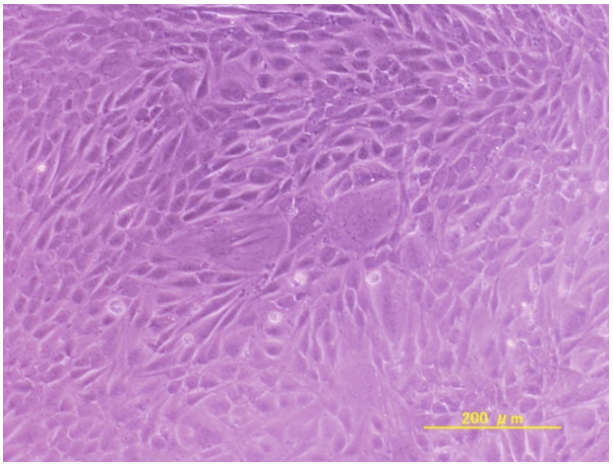

Fig. 9B: Myoblasts on ITO film on 11th day of culture without stimulation of electric pulses.

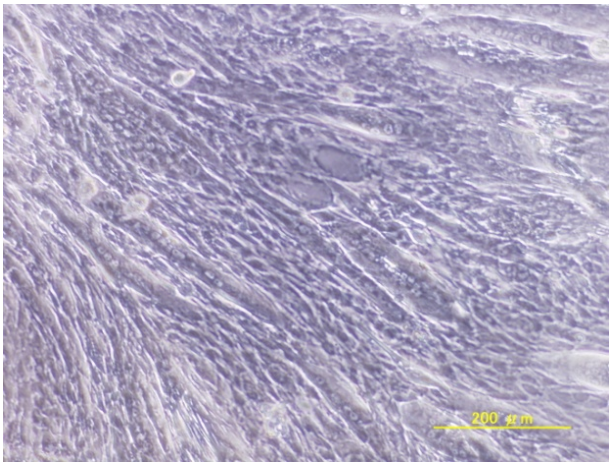

Fig. 10A: Myotubes on polystyrene dish on 21st day of culture without stimulation of electric pulses.

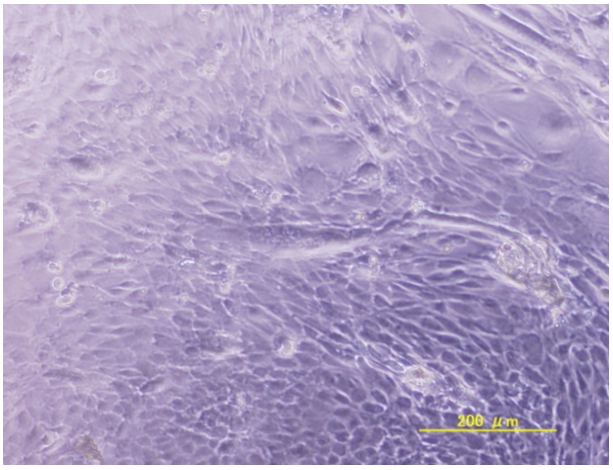

Fig. 10B: Myotubes on ITO film on 21st day of culture without stimulation of electric pulses.

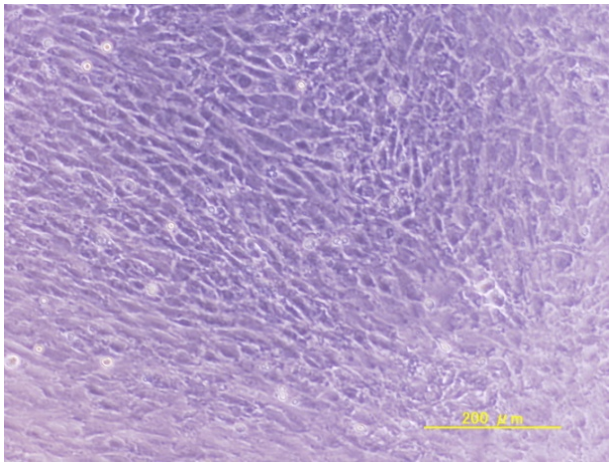

Fig. 10C: Cells on boundary area between ITO film and glass on 21st day of culture without stimulation of electric pulses.

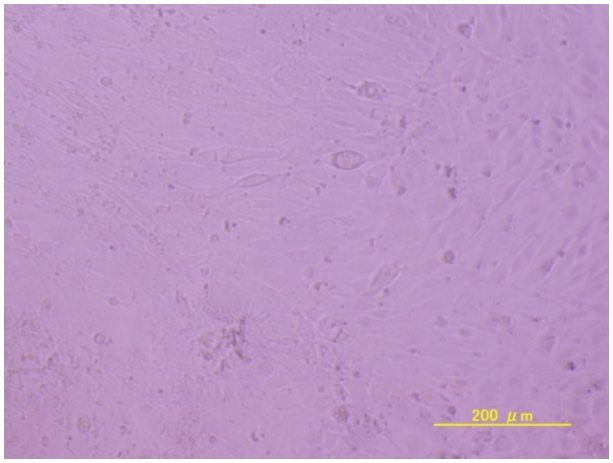

Fig. 10D: Cells on glass on 21st day of culture without stimulation of electric pulses.

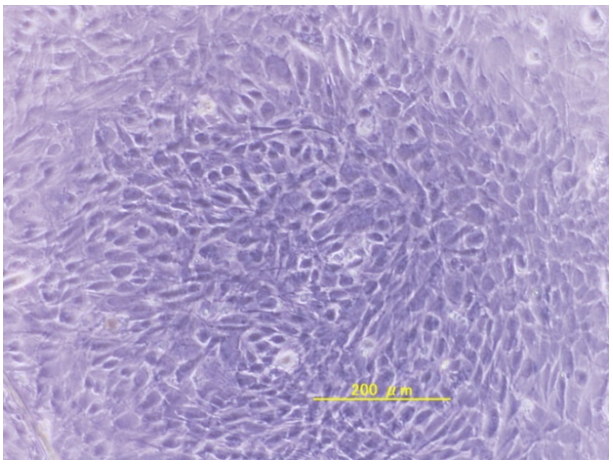

Fig. 11B: Cells on ITO film on 22nd day of culture with stimulation of electric pulses.

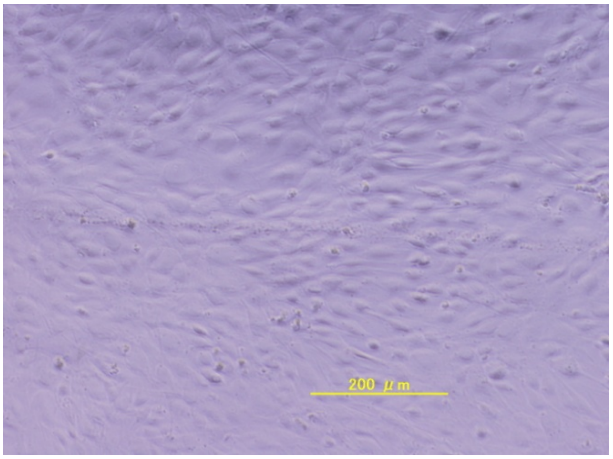

Fig. 11C: Cells on boundary area between ITO film and glass on 22nd day of culture with stimulation of electric pulses.

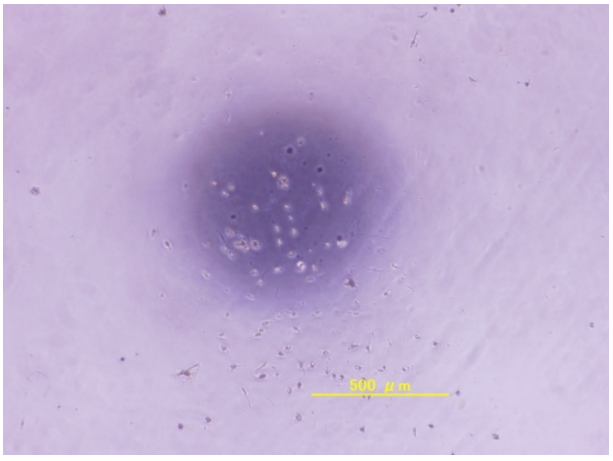

Fig. 11D: Cells on glass on 22nd day of culture with stimulation of electric pulses.

To inhibit the migration of cells on the glass of the culture disk, the area on the glass was divided by the ring of PDMS. The cells were cultured in each area within the ring.

The transparency of ITO film is good enough to observe the behavior of cells on the film. C2C12 is able to be cultured and differentiated on the ITO film.

The contact angle on the gold film deposited on PDMS depends on the process of deposition in the previous study [5]. The contact angle governs behavior of cells on the scaffold: adhesion, proliferation, and differentiation. The surface of ITO is more hydrophilic than the surface of glass in the present study. Proliferation of C2C12 tends to be faster on the ITO film than on the glass.

The behavior of cells depends on several kinds of environment: electric field [2], magnetic field [6], gravitational force [7], and shear flow [8, 9]. The microfluidic system has been applied to sort biological cells [10, 11], and to trap biological cells [12, 13]. The system also used to study local environment around the cultured cell. The micro pattern of the surface has been applied to study the surface effect of adhesion of cells [4, 14, 15].

The moderate electric stimulation might accelerate differentiation of myoblasts into myotubes [2]. In the present study, myoblasts do not differentiate into myotubes on ITO with electric pulses in 21 days, although they differentiate into myotubes on ITO in 21 days without electric pulses.

5. CONCLUSION

The effect of stimulation of electric pulses through the scaffold of conductive film on proliferation and on differentiation of myoblasts has been studied *in vitro*. A transparent thin film of indium tin oxide (ITO) coated on the glass was used for conductive scaffold for the cell culture. C2C12 (mouse myoblast cell line) was cultured for 22 days on the film. Electric pulses (period 1 s, amplitude 1 V (0.0005 A), duration 0.001 s) were applied on the film for thirty minutes per day. The experimental results show that myoblasts do not differentiate into myotubes on ITO with electric pulses in 22 days, although they differentiate into myotubes on ITO in 21 days without electric pulses.

6. ACKNOWLEDGMENT

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