Measurement of Periodical Contraction of Cultured Muscle Tube with Laser

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ABSTRACT

Periodical contraction of a cultured muscle tube has been measured with laser *in vitro*. C2C12 (mouse myoblast cell line) was cultured with High-glucose Dulbecco's Modified Eagle's Medium on a dish to make muscle tubes. Differentiation from myoblasts to myotubes was induced with an additional horse serum. Repetitive contraction of the tube was generated by electric pulses lower than sixty volts of amplitude with one milli-second of width through the electrodes of platinum, and observed with a phase-contrast microscope. A laser beam of 632.8 nm wavelength was restricted to 0.096 mm diameter, and applied to the muscle tubes on the bottom of the culture dish. Fluctuating intensity of the transmitted laser beam through the periodically contracting muscle tubes was measured, and its spectrum was analyzed. The analyzed data show that the repetitive contraction is synchronized with stimulation of the periodical electric pulses between 0.2 s and 2 s.

Keywords: Biomedical Engineering, Muscle Cell, Measurement, Laser, Periodical Contraction, Synchronization and Spectrum

1. INTRODUCTION

A biological myotube has potential to realize a light engineered actuator with high efficiency. Cell culture technique has enabled muscle cells cultured *in vitro* recently [1, 2]. Myoblasts differentiate to myotubes and reveal their contractile function *in vitro* [3, 4]. Myoblasts have been clinically applied to ischemic cardiomyopathy in the field of regenerative medicine. The technique also might be applied to engineered micro actuators *in vitro*. To realize an actuator of biological muscle cells, several methodologies have to be developed; such as arrangement of myotubes, control of movement and maintenance of performance.

Behavior of biological cells, on the other hand, depends on various environmental factors, such as electric, magnetic and mechanical fields. Acceleration techniques for orientation and proliferation of cells have been studied to make muscle tissue *in vivo* and *in vitro* [5]. Orientation of myotubes might affect on their controllability with electric stimuli [6]. The effect of electrical stimulation on adhesion and proliferation of muscle cells was studied in the previous study *in vitro* [7]. The effect of a magnetic field on adhesion of muscle cells to a culture plate was also studied in another study [8]. The mechanical stimulation by the flow of the medium might effect on cells behavior [9].

Several effects of electric stimulation have been reported on biological cells. The previous study shows that electric stimulation enhances differentiation of muscle cells [10]. Another study shows that mechanical stimulation improves tissue-engineered human skeletal muscle [11]. The previous study also shows that muscle cells can adhere and proliferate under electric stimulation with periodical pulses, and that adhesion of muscle cells can be controlled with the amplitude of pulse [7].

The previous study shows that the cultured myotubes contract when electric pulses are applied to the medium [12]. The repetitive contraction has been observed with an optical microscope. To study on their controllability, the movement has to be measured and analyzed in relation to the pulses applied. Laser technique is one of the effective methodologies to detect local movement of an object.

In the present study, an experimental system with a laser beam has been designed to measure micro repetitive movements of cultured muscle tubes *in vitro*.

2. METHODS

Muscle Tube

C2C12 (Mouse myoblast cell line, Fig. 1) was cultured with High-glucose Dulbecco's Modified Eagle's Medium (D-MEM) on a dish of 60 mm diameter to make muscle tubes. The bottom of the dish is coated with collagen type I. Cells were seeded with the ratio of two thousand cells per one square centimeter, and cultured in an incubator. The medium was replaced every two days. In the first term, fetal bovine serum (FBS) was added to the medium with the volume ratio in 10 percent of FBS and 90 percent of D-MEM to accelerate proliferation of cells. In the second term, FBS was switched to horse serum (HS) to induce differentiation of cells, before cells were proliferated to a sub-confluent state. The medium consists of seven percent of HS and 93 percent of D-MEM. The cells were observed with a phase-contrast microscope, when the medium was replaced.

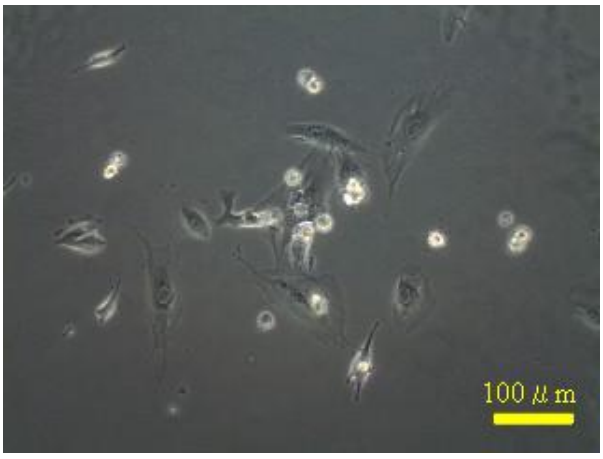

Fig. 1: Myoblasts, C2C12.

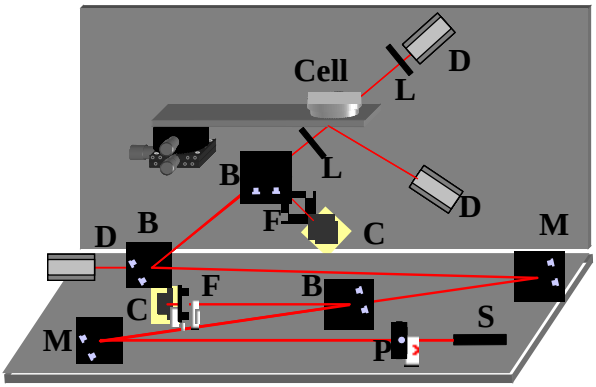

Fig. 2(a): Laser system: S, light source; B, beam splitter; C, CCD camera; M, total reflection mirror; P, deflection plate; F, absorptive neutral density filter; L, convex lens; D, detector.

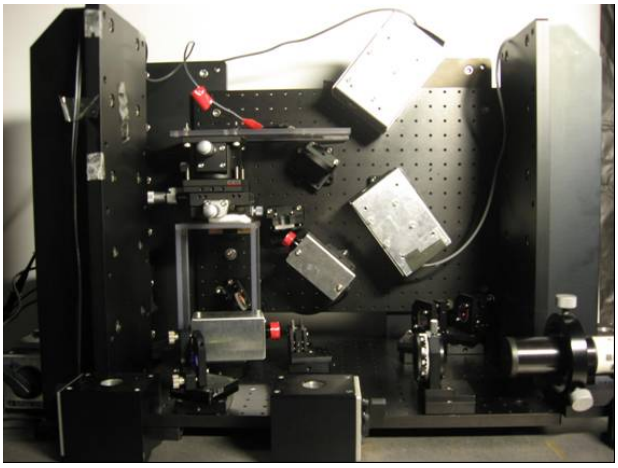

Fig. 2(b): External appearance of the laser system.

Measurement System

A measurement system (Fig. 2) with laser was manufactured. The system consists of a light source, three beam splitters, two charge-coupled device (CCD) cameras, two total reflection mirrors, a deflection plates, two absorptive neutral density filters, two convex lenses and two detectors. A helium neon laser head with a wavelength of 632.8 nm (Sigma Koki, LHP) is used for the light source. The beam is split with a plate beam-splitter, and the alignment of the beam is adjusted with the CCD camera. The diameter of the laser beam decreases to 0.096 mm through the convex lens, when the beam incidents the culture plate. A thin black film with a hole of 0.45 mm diameter is attached on the bottom of the culture dish to mark the observation area (Fig. 3). Intensity of the transmitted laser beam through the culture dish is measured with the photodiode detector. The both terminals of the diode are connected to an electric resistance of one mega-ohm. The voltage between two terminals is measured with a computer. The digitized voltage value is traced through an analog-to-digital converter with a sampling interval of one millisecond (Fig. 4). The spectrum of the traced serial data of 4096 (for four seconds approximately) was analyzed.

Electric Stimulation

Electrode is made of platinum wire of 0.2 mm diameter (Fig. 5).

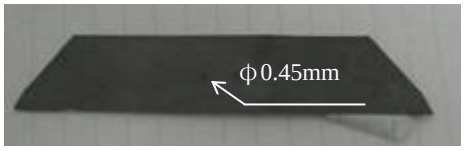

Fig. 3(a): Film attached on the bottom of the culture dish.

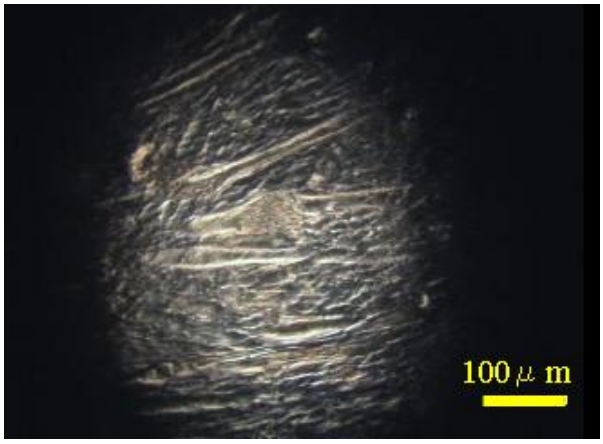

Fig. 3(b): Microscope view through a film attached on the bottom of the culture dish.

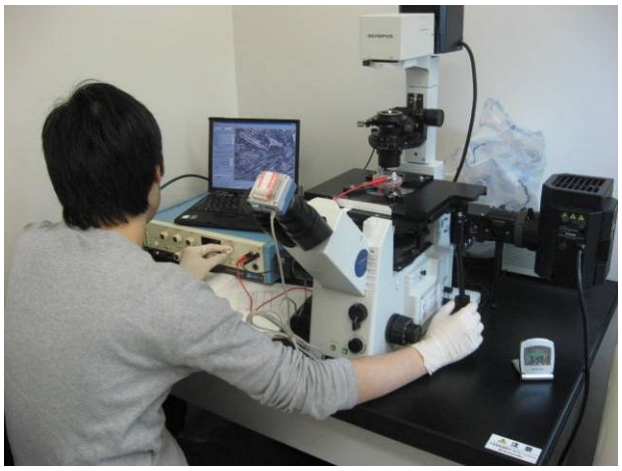

Fig. 4: Electric pulse generator (bottom), data storage computer, and phase-contrast microscope (right).

To fix the position of the tip of the electrode, the wire is inserted through a curved glass pipe of 0.6 mm inside diameter (1.0 mm outside diameter). A block of polymethyl methacrylate is used to fix each electrode at the fringe of the culture dish. To generate an electric field in the culture medium, two electrodes were dipped in the medium with the distance of 30 mm each other in a counter position (Fig. 6).

The electric stimulator (Nihon Kohden, SEM-4201) was used to generate periodical rectangular pulses (Fig. 4). Repetitive contraction of muscle tubes was induced with electric pulses of one milli-second width. Variation was made in the period (between 0.1 s and 2 s) and the amplitude (between 40 V and 60 V) of the repetitive pulses.

Fig. 5: Electrodes.

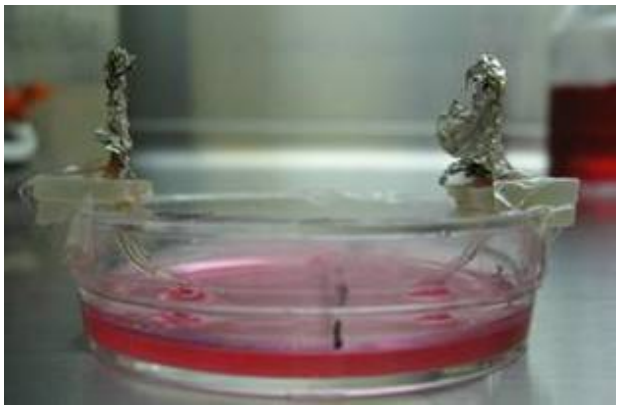

Fig. 6: Culture dish with electrodes.

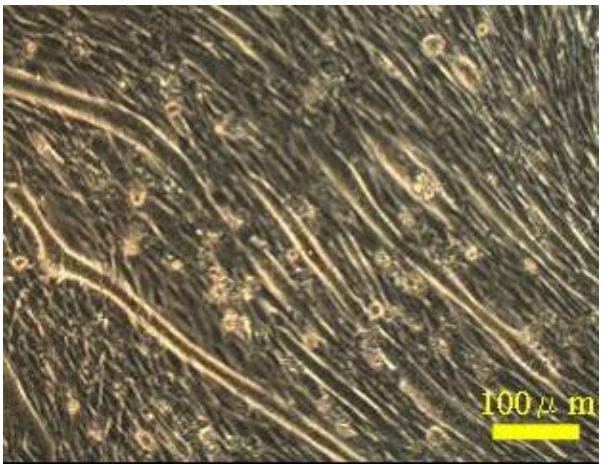

Fig. 7: Myotubes, C2C12.

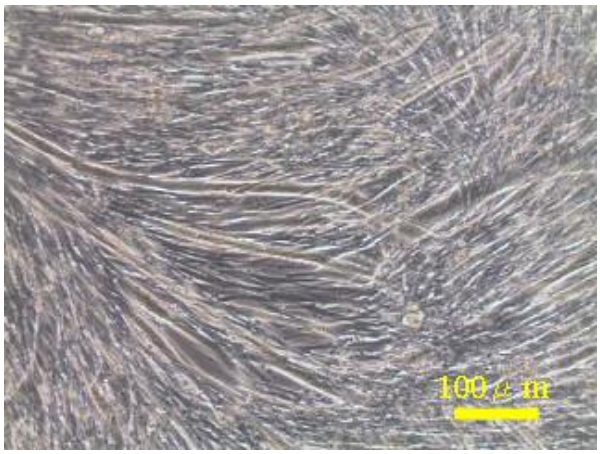

Fig. 8: Multiple layers of muscle tubes.

3. RESULTS

The sub-confluent state was observed in five days of cultivation. The myoblasts fused and differentiated to the myotubes, after the FBS in the medium was replaced with HS (Fig. 7).

After eleven days of cultivation, several contractive myotubes were observed, when the electric pulses were applied. The contractive muscle tube was formed on the second layer from the bottom of the culture dish (Fig. 8). The contractive muscle tubes were observed between the eleventh day and the thirty-eighth day of culture. Repetitive contraction of the muscle tube was observed with the phase-contrast microscope, when the periodical electric pulses between 0.5 s and 2 s were applied.

Fluctuating intensity of the transmitted laser beam was measured through the contracting muscle tubes, and the spectrum of intensity was analyzed. The digitized intensity tracings, the filtered tracings and their spectra are exemplified at period of one second in Figs. 9, Fig. 10 and Fig. 11, respectively. The peak spectrum was measured at the same frequency as that of electric pulses. The results show that the contraction is synchronized with stimulation of the periodical electric pulses between 0.2 s and 2 s. The peak spectrum was not clear at the pulse interval of 0.1 s. The results show that the fluctuating amplitude of the intensity decreases with frequency of the stimulating pulses and cannot be detected at the frequency of 10 Hz. Fig. 12 exemplifies the tracings of the intensity, when variation was made in the amplitude of electric pulses. The results show that fluctuating amplitudes of intensity does not significantly change with amplitude of the stimulating electric pulses. For the filtration of noise of high frequency, mean data during each term of 0.1 s are traced in Fig. 10. The steep onset at the contractive movement of myotubes (Fig. 10) generates spectra at the higher frequencies in Fig. 11.

4. DISCUSSION

The contractive muscle tube was observed in the second layer from the bottom of the dish. The muscle cells in the bottom layer might tightly be binding on the surface of the culture dish so that the cells cannot slide to generate contractive movement.

Fluctuating intensity of the transmitted laser beam includes noise, but can be successfully analyzed on the spectrum. The measurement system can detect periodical contraction of muscle tubes at the interval longer than 0.2 s. The muscle tube might be tetanized with periodical electric stimulation pulses at the interval shorter than 0.2 s. The repetitive movement cannot be detected during tetanization. The variation in amplitude of the contraction of muscle tubes could not be controlled with amplitude of the electric pulse between 40 V and 60 V. Relation between the number of contracting tubes and stimulation intensity is not clear, because the observation area is rather small.

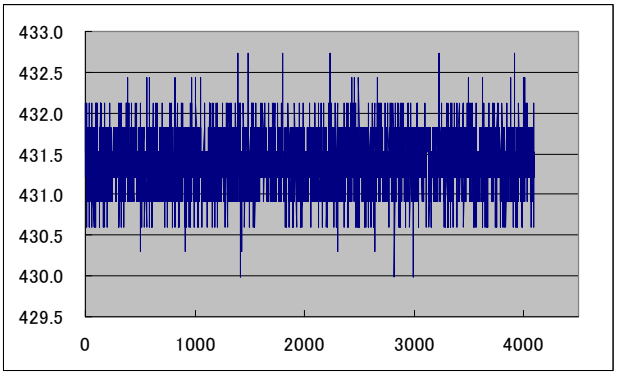

Fig. 9: Primary data digitized from intensity of transmitted laser: relation between intensity (mV, ordinate) and time (ms, abscissa) at period of 1 s.

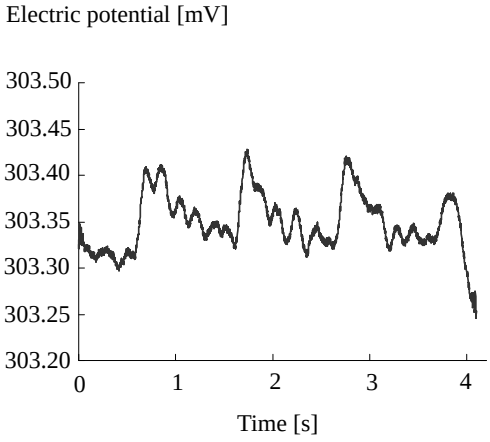

Fig. 10: Filtered voltage tracings from another trial at period of 1 s.

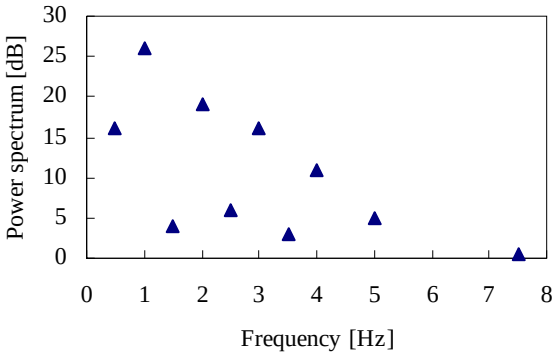

Fig. 11: Spectra of data shown in Fig. 10.

The sensitivity depends on the position between the laser beam and the muscle tube in the present measurement system, because the system can only detect variation of the transmitted laser intensity (Fig.13).

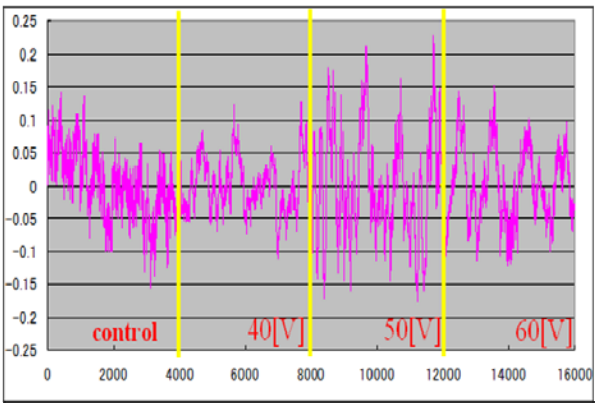

Fig. 12: Intensity tracings with variation of amplitude (V) of electric pulse.

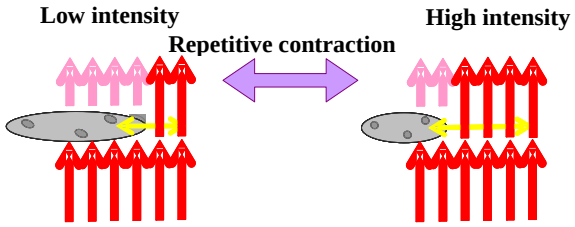

Fig. 13(a): Transmitted light intensity varies with contraction of a muscle tube (cross-sectional view).

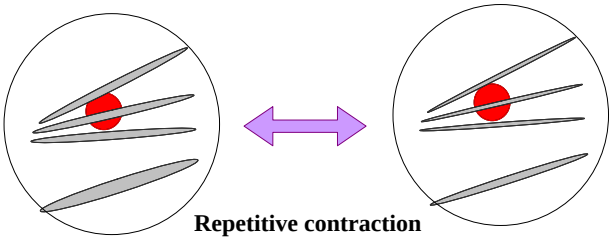

Fig. 13(b): Transmitted light intensity varies with contraction of muscle tubes (plain view).

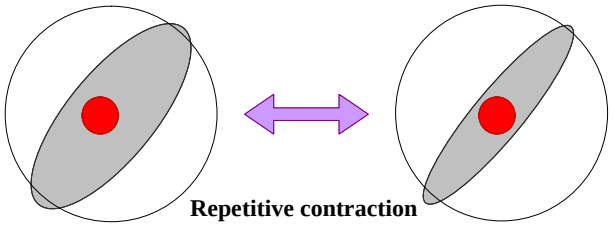

Fig. 13(c): Transmitted light intensity does not vary with contraction of muscle tubes (plain view).

The property of the muscle tubes has been examined *in vitro* in the previous study [1]. Electrical stimulation, on the other hand, has been applied in rehabilitation medicine.

To maintain the contractile movement of muscle tissue, continuous energy consumption is necessary, such as chemicals in the medium. To control the local environment around muscle

tissue [13, 14], development of non-invasive sensing methodology might be effective. The present system can be applied to detector for maintenance of controllability of muscle tubes.

5. CONCLUSION

Periodical Contraction of cultured muscle tube has been measured with laser *in vitro*. The results show that the repetitive contraction is synchronized with stimulation of the periodical electric pulses between 0.2 s and 2 s.

6. ACKNOWLEDGMENT

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