Simultaneous Measurement System for Local Medium pH and Movement of Contracting Myotubes in Vitro

Kousuke KIDA, Shigehiro HASHIMOTO, Eiji YAMADA, Masahide OKADA, Shuichi MOCHIZUKI, Toshia FUJISATO, Tomohiro SAHARA, Mieko OHSUGA
Department of Biomedical Engineering, Osaka Institute of Technology, Osaka, 535-8585, Japan
hashimoto@bme.oit.ac.jp http://www.oit.ac.jp/bme/~hashimoto

and

Hajime OTANI
Cardiovascular Center, Kansai Medical University, Moriguchi, Japan

and

Kiyoshi YOSHINAKA
Department of Bioengineering, The University of Tokyo, Tokyo, Japan

ABSTRACT

An optical measurement system has been designed to measure Local pH of the medium and a movement of a contracting cultured myotube simultaneously *in vitro*. A light source of a light-emitting diode and a helium neon laser was applied to the culture medium to measure pH in the medium and the movement of the contracting myotube, respectively. The intensity of 558 nm wavelength of the transmitted light from the diode was measured to detect pH in the medium, which includes phenol red. The intensity of 632.8 nm wavelength of the transmitted light from the laser was measured to detect the cyclic contractile movement of the cultured myotube. C2C12 (mouse myoblast) cells were cultured and differentiated to myotubes. The cyclic contraction of the myotube was induced with electric pulses applied through the medium. The movement was measured with the laser beam under observation with an inverted phase contrast microscope, while pH was measured with the diode. The results show that the designed system was effective to measure local medium pH and movement of contracting myotubes simultaneously *in vitro*.

Keywords: Biomedical Engineering, Muscle Cells, Cell Culture, pH and Laser

1. INTRODUCTION

Biological muscle has a potential to realize a light small actuator with high efficiency. Recent progress in tissue engineering realizes the muscle cell culture technique. The cultured muscle cell has a potential to be applied to a micro actuator. The previous studies show that mouse muscle cells differentiate to myotubes and their periodical contraction is controlled with electric pulses applied through the medium. The movement can be measured with a laser beam [1].

The value of pH is one of the useful indicators to control the content of the medium for cell culture [2]. When the contents of the medium vary with energy consumption during muscle contraction, pH decreases to acidosis. The conventional pH probe is invasive and too large in scale to measure local pH value in cell culture. To design a control method for environment to maintain a contractive movement of the biological muscle tissue, the value of pH could be an indicator [3].

Most of medium, on the other hand, contains phenol red for pH indicator, which enables optical pH measurement between 6.8 and 8.4. In the present study, an optical measurement system has been designed to measure pH of the medium adjacent to contracting cultured myotubes *in vitro*. In the present study, simultaneous measurement system has been designed for local medium pH and for movement of contracting myotubes *in vitro*.

2. METHODS

Measurement System

The designed measurement system consists of three subsystems: a pH measurement system, a movement measurement system, and a microscope system (Fig. 1). The whole system is located in a dark room.

The optical pH measurement system consists of a light source, concave lenses, convex lenses, a light path with aluminum

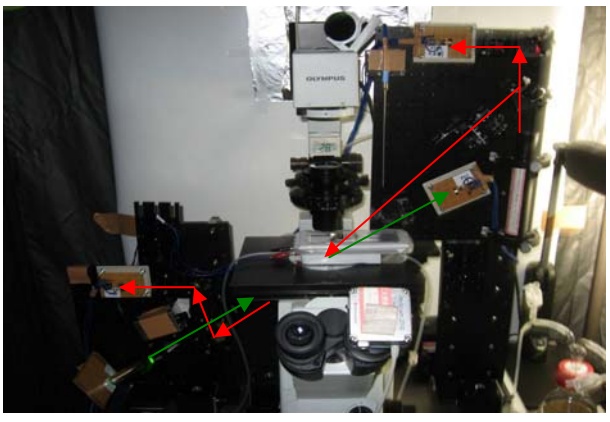

Fig. 1(a): Measurement system.

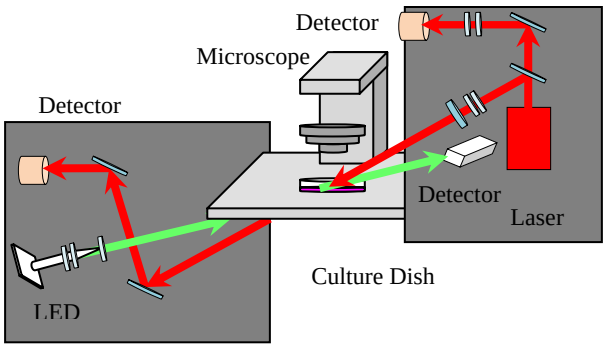

Fig. 1(b): Measurement system.

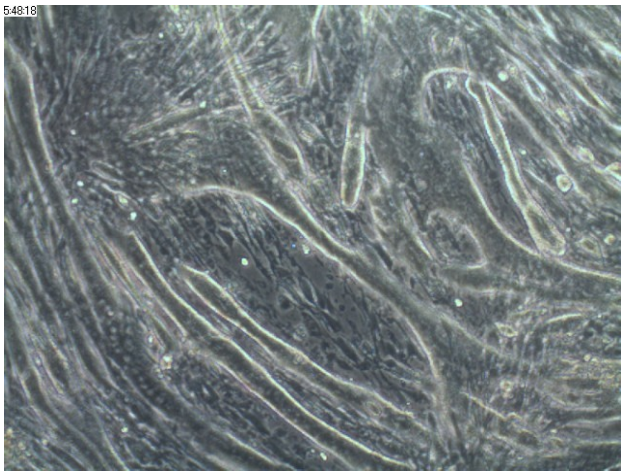

Fig. 2: Myotubes. Distance between left and right is 0.8 mm in the figure.

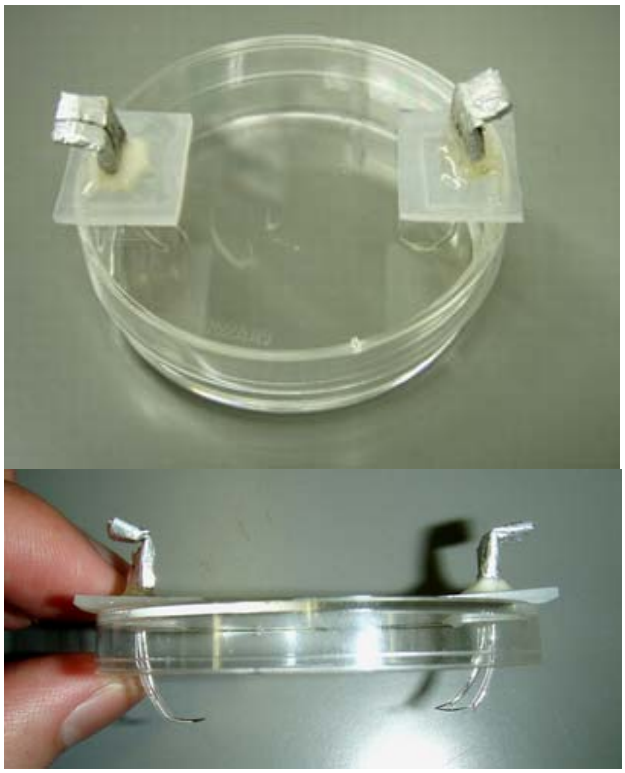

Fig. 3: A pair of electrodes on the cap of culture dish.

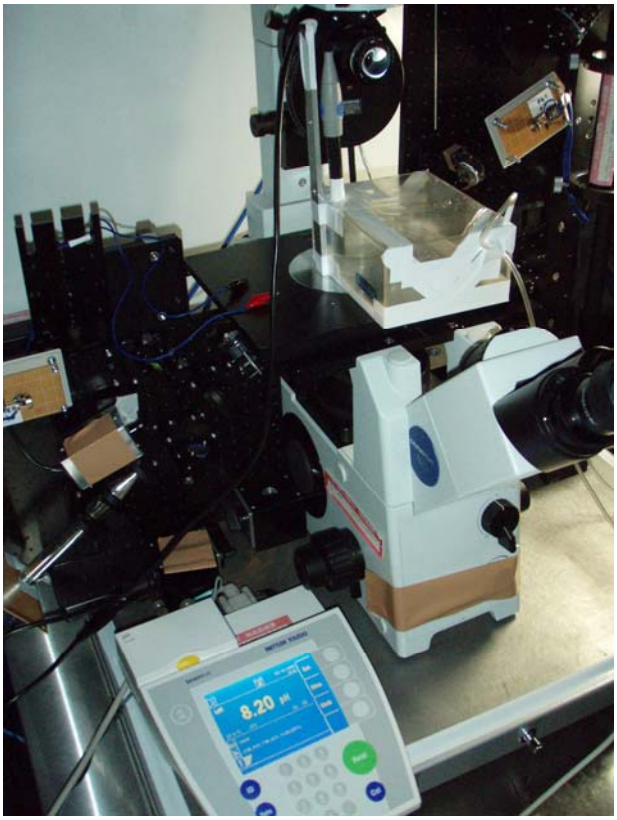

Fig. 4: Measurement of pH.

cylinder, and a light-sensitive element. The light source is made of nineteen green (558 nm wavelength [3]) light emitting diode (LED) elements, which are connected each other in parallel electric circuit, and operated with an electric current of 300 mA (2.05 V) in total. The light-sensitive element is made of a PIN photodiode, which converts light into an electric current. The electric current is converted into an electric potential with an amplifier. Data are collected with a data logger. The light beam from LED does not have directivity. The beam is concentrated with the lenses and cylindrical light path. The beam of 3 mm diameter is irradiated to the culture dish. The intensity of the transmitted LED beam through the culture medium was detected with the photodiode.

The movement measurement system consists of a light source, a plate beam splitter, a total reflection mirror, a deflection plate, two absorptive neutral density filters, two convex lenses and two photodiode detectors. A helium neon laser head with a wavelength of 632.8 nm is used for the light source. The beam is split with the plate beam splitter to check the light source. The intensity of the light decreases through the absorptive neutral density filters. The beam is irradiated in the counter direction of the beam of pH measurement system to avoid interference between two light beams (Fig. 1). Fluctuating intensity of the transmitted laser beam through the periodically contracting myotubes was detected with the photodiode.

The microscope system consists of a phase contrast inverted microscope and a charge-coupled device (CCD) camera. The movement of myotube was observed and recorded with the microscope and with the camera at room temperature.

Myotubes

C2C12 (mouse myoblast cell line) cells were cultured with a high-glucose Dulbecco's Modified Eagle's Medium (D-MEM) on a dish of 52 mm internal diameter to make myotubes in an

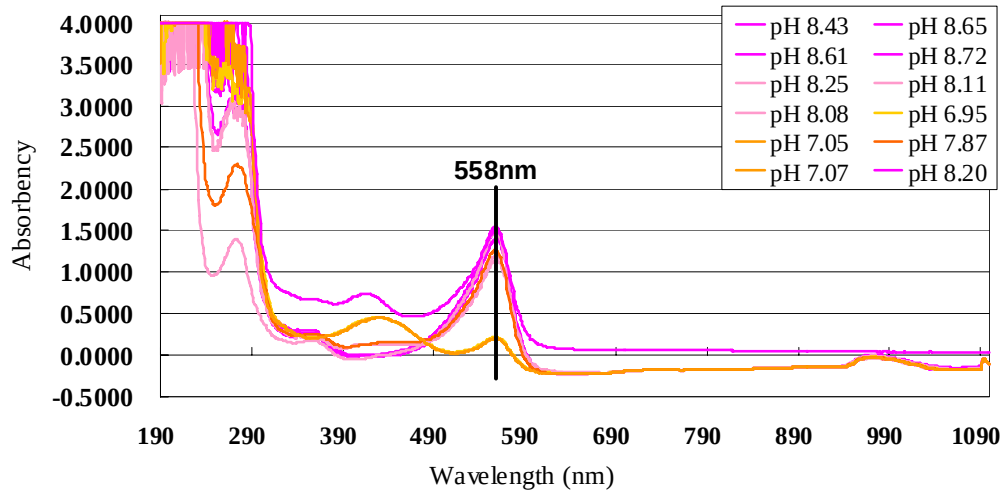

Fig. 5: Relation between absorbency and wavelength for media of various pH.

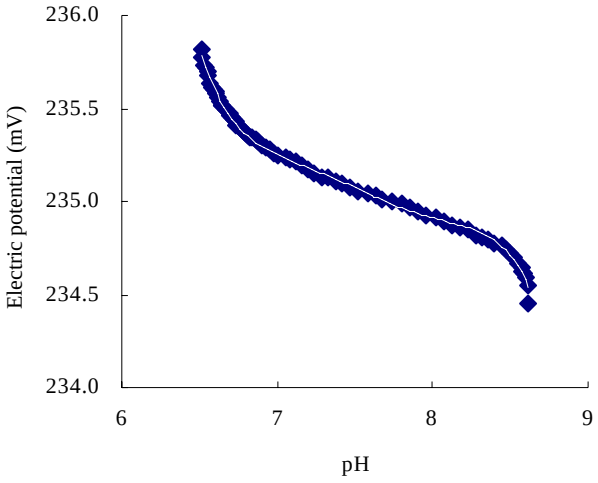

Fig. 6: Relation between pH and output voltage of the measurement system.

incubator. The D-MEM includes phenol red to indicate pH between 6.8 and 8.4. The medium was replaced every two days. In the first term, fetal bovine serum (FBS) was added to the medium at a volume rate with 10 percent of FBS and 90 percent of D-MEM to accelerate proliferation. In the subsequent second term, FBS was switched to horse serum (HS) to induce differentiation (Fig. 2), before cells were proliferated to a sub-confluent state on the culture dish. The second medium consists of seven volume percent of HS and 93 volume percent of D-MEM.

Electric Stimulation

Electric pulses were applied to the culture medium to control the cyclic contractile movement of myotubes. Two electrodes are made of a platinum wire of 0.2 mm diameter (Fig. 3). To fix the position of the tips of the electrode, the wire is inserted through a curved glass pipe of 0.6 mm inside diameter. An electronic stimulator is used to generate periodical rectangular pulses. Repetitive contractions of myotubes were induced with electric pulses of one millisecond width. Variation was made in the period (between 0.2 s and 2 s) and the amplitude (between 40 V and 60 V) of the cyclic electric pulses.

Measurement of pH

Variation was made on medium pH in the culture dish with carbon dioxide gas supply (Fig. 4). The culture dish with the culture medium (without cells) was put in a chamber connected to a compressed gas cylinder of carbon dioxide. The value of pH is confirmed with a conventional pH meter (Seven Multi, Metler Toledo, Switzerland), which has an electrode. The temperature around the dish was measured with a thermoelectric couple.

To select the wavelength for the optical pH measurement system, the transmitted light intensity between 190 nm and 1100 nm was measured on the medium. Several samples of medium of various pH were poured into the micro-cell, which was placed in a spectrophotometer (UVmini1240, Shimadzu, Kyoto, Japan).

The output voltage of the pH measurement system was measured, while the movement of myotubes were measured with the laser system and observed with the microscope.

3. RESULTS

The muscle cells fused and differentiated to the myotubes, after the FBS in the medium was replaced with HS (Fig. 2).

Fig. 5 shows the relation between absorbency and wavelength for media of various pH. The peak of absorbency at the wavelength of 558 nm corresponds to the color displayed with the phenol red.

The light from LED can be successfully concentrated into the spot of 3 mm diameter at a measurement position with the lenses. Through the designed light path with the lenses and with the aluminum cylinder, enough intensity of light arrives at the detector.

The temperature around the culture dish tends to increase with time under lights: LED, laser and halogen lamp of the microscope. When the temperature increases from 17 to 26 degrees centigrade, the output voltage of pH measurement system decreases from 0.255 V to 0.235 V. The shifted value with temperature is able to be compensated with the relation between output voltage and temperature.

Fig. 6 shows the relation between pH and output voltage of the measurement system after compensation about temperature.

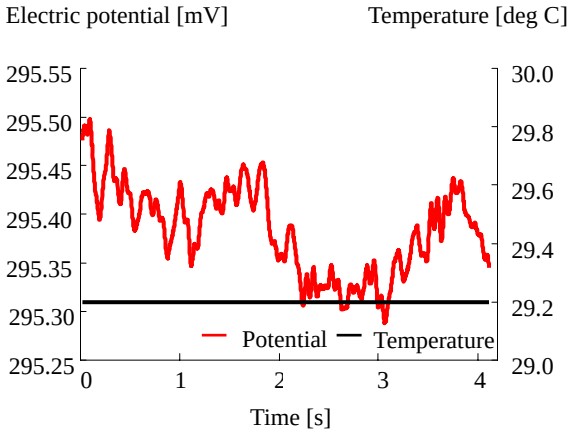

Fig. 7(a): Voltage tracings. Period: 2 s.

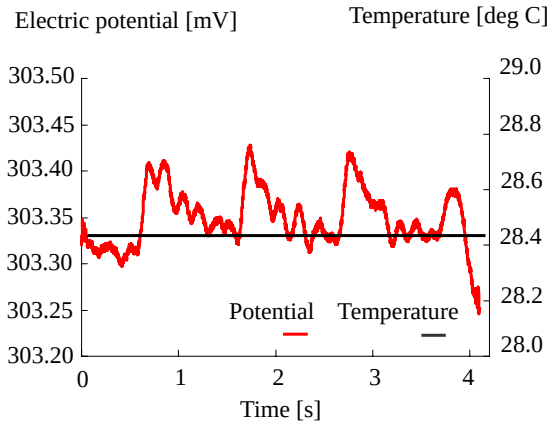

Fig. 7(b): Voltage tracings. Period: 1 s.

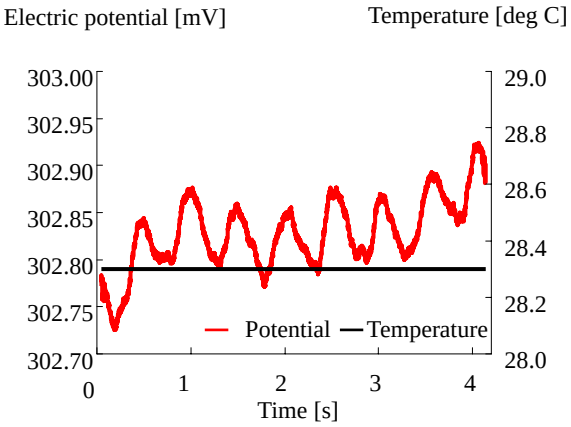

Fig. 7(c): Voltage tracings. Period: 0.5 s.

The figure shows that electric potential varies linearly with pH at the ratio of 0.33 mV/pH in the pH range between 6.8 and 8.4, which is the color variation range for phenol red and is experienced in the normal muscle cell culture medium.

The experimental results on electric stimulation to myotubes show that the cyclic contractive movement of myotubes can be synchronously controlled with cyclic electric pulses conducted through the culture medium. The cyclic movement was confirmed with the phase contrast inverted microscope.

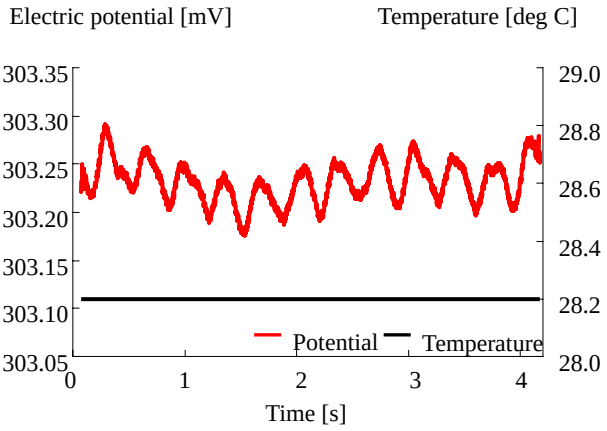

Fig. 7(d): Voltage tracings. Period: 0.33 s.

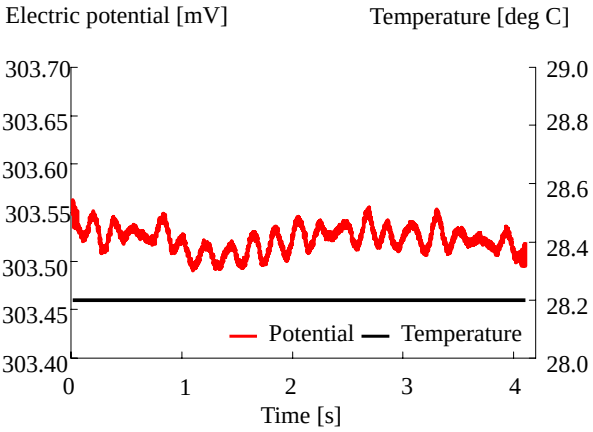

Fig. 7(e): Voltage tracings. Period: 0.2 s.

Fluctuating intensity of the transmitted laser beam was traced through the periodically contracting myotubes. The electric signals of the photodiode were recorded in a computer with a sampling frequency of one millisecond. In Fig. 7, the tracings are displayed after filtration, which cut off the higher frequency than 100 Hz. The spectrum was analyzed on the data in three terms of the period of the corresponding electric stimulation pulses.

Fig. 7 exemplifies output voltage tracings, when the period of cyclic stimulation pulses is one second and the amplitude is 50 V. The peak spectrum was measured at 1 Hz, which coincides with the stimulation period of one second (Fig. 8). The spectrum of the higher frequency corresponds to the fast rising slope of the output voltage tracings. The signal corresponds to faster contraction than sinusoid, where relaxation is slower in the myotubes. Same kinds of data for contracting myotubes were detected with the present experimental system, when variations were made in the period (between 0.2 s and 2 s) and the amplitude (between 40 V and 60 V) of the cyclic electric pulses.

The change of pH was traced with electric potential of the pH measurement system, while the contractile movement of myotubes was traced with the laser system. Figs. 7 and 8 shows data of temperature, which are simultaneously measured with the optical pH measurement system.

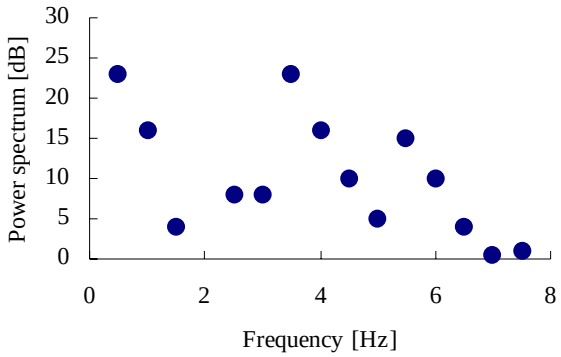

Fig. 8(a): Relation between power spectrum and frequency of electric potential in Fig. 7(a).

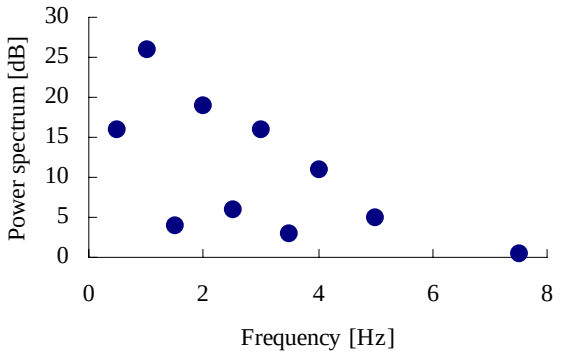

Fig. 8(b): Relation between power spectrum and frequency of electric potential in Fig. 7(b).

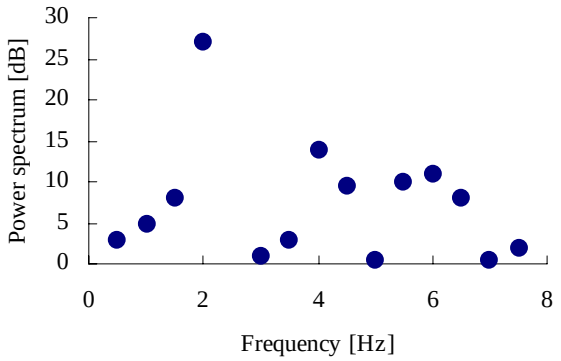

Fig. 8(c): Relation between power spectrum and frequency of electric potential in Fig. 7(c).

After injection of some amount of the carbon dioxide into the chamber, the cyclic contraction of myotubes in the chamber stopped and the voltage signal of the transmitted light of LED increased. The increase of the voltage indicates the decrease of pH in the designed measurement system. When the partial pressure of carbon dioxide decreased in the chamber in the

successive test, the cyclic contraction of myotubes started again and the voltage signal of the transmitted light of LED decreased. The decrease of the voltage indicates the increase of pH.

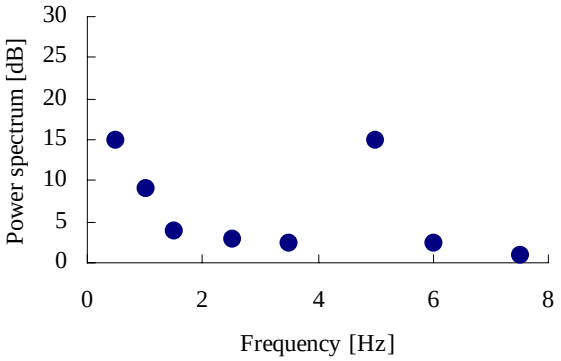

Fig. 8(d): Relation between power spectrum and frequency of electric potential in Fig. 7(d).

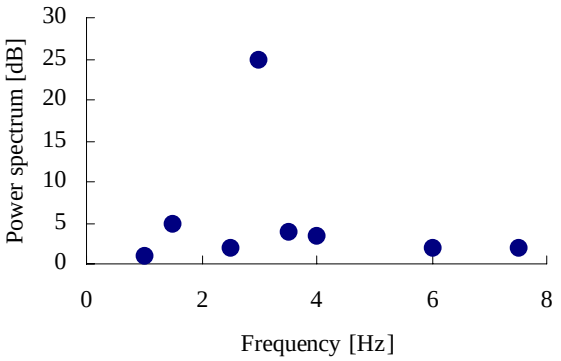

Fig. 8(e): Relation between power spectrum and frequency of electric potential in Fig. 7(e).

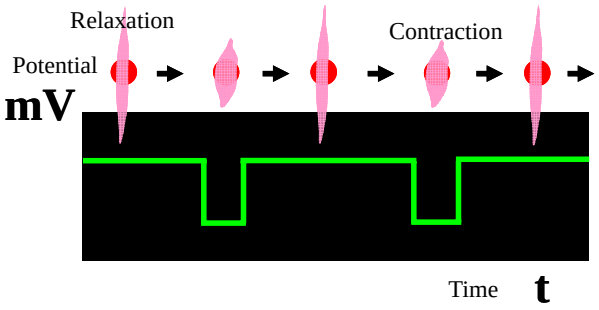

Fig. 9: Myotube movement (upper) and electric potential.

4. DISCUSSION

Two different wavelengths were applied to distinguish signal of pH from that of movement at the simultaneous detection. The two signals were successfully distinguished in the designed system with two separated wavelengths: 558 and 632.8 nm. The counter light path between pH and movement is effective to distinguish two transmitted signals.

Phenol red is contained in the conventional culture medium. The color of the medium changes to yellow, when pH of the medium decreases. The color change enables the optical measurement of pH in the medium. The designed optical

measurement system is useful to trace pH value of the culture medium.

The intensity of the transmitted light depends on the depth of medium in the dish, which demands calibration to calculate absolute value of pH in the optical sensor system.

An optical sensor is non-invasive. It has an advantage against the conventional pH probe, because the conventional dipping probe can cause contamination in the medium. The designed optical measurement system also has a potential to realize measurement for local pH at a micro scale.

The local contractive movement has been analyzed to explain that of the tissue level [4]. The measurement system with laser has a potential to be applied to local measurement for muscle tissue. Cyclic contractive movement of myotubes might cause cyclic fluctuation on the intensity of transmitted laser beam, which enables measurement of movement (Fig. 9).

The optical pH measurement system can detect critical pH value in the medium, when the cyclic contraction of myotubes in the chamber stopped during the test for variation of surrounding partial pressure of carbon dioxide.

The system will contribute to control environment around myotubes to maintain contractile movement [3, 5]. The designed experimental system will contribute to develop a mock-system for biological test instead of animal tests.

5. CONCLUSION

A system has been designed to measure movement of contracting myotubes and to measure local pH of medium adjacent to the myotubes, simultaneously. The designed system is effective to measure pH adjacent to contracting cultured myotubes *in vitro*. The system will contribute to control environment around myotubes to maintain contractile movement.

6. ACKNOWLEDGMENT

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REFERENCES

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[1] M. Okada, S. Hashimoto, J. Takase, M. Ohsuga, K. Nakamura, K. Akazawa, S. Mochizuki, H. Kobayashi, T. Fujisato, T. Kawai, S. Uto, K. Tsujita, E. Yamada, H. Kondo, H. Otani, K. Yoshinaka, T. Yamaoka, “Measurement of periodical contraction of cultured muscle tube with laser”, **Proc. World Multi-Conference on Systemics Cybernetics and Informatics**, Vol. 2, 2008, pp 110–114

[2] Q. Chen and D. R. Anderson, ”Effect of CO2 on Intracellular pH and Contraction of Retinal Capillary Pericytes”, **Investigative Opthalmology & Visual Science**, Vol. 38, No. 3, 1997, pp. 643-651.

[3] Yamada E, Hashimoto S, Inoue D, Kondo H, Mochizuki S, Yamasaki K, Fujisato T, Okada M, Nakaoka H, Ohsuga M (2008) “Medium control system for muscle cell culture”, **Proc. World Multi-Conference on Systemics Cybernetics and Informatics**, Vol. 2, 2008, pp 120–123.

[4] M. L. Armstrong, A. K. Dua and C. L. Murrant, “Time Course of Vasodilation at the Onset of Repetitive Skeletal Muscle Contractions”, **Am J Physiol Regul Integr Comp Physiol**, Vol. 292, 2007, pp. R505–R515.

[5] S. Hashimoto, S. Mochizuki, Y. Morita, H. Tsutsui, M. Yoshiura, K. Akazawa, M. Ohsuga, S. Uto, H. Otani and T. Fujisato, “Environmental Design for Muscle Cell Culture with Magnetic Field”, **Proc. 2007 Inaugural IEEE International Conference on Digital Ecosystems and Technologies (IEEE-DEST 2007)**, 2007, pp. 468-472