Optical Measurement System for pH in Medium around Contracting Myotubes in Vitro

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ABSTRACT

An optical measurement system has been designed to measure local pH of the medium around contracting myotubes *in vitro*. Variation was made for the light source to be applied to the culture medium: a light-emitting diode and a filtered mercury lamp. The medium was placed on the stage of an inverted phase contrast microscope, with which contracting myotubes were observed. The intensity of the transmitted light through the medium from the light source was measured with a photodiode to detect pH in the medium, which includes phenol red. For calibration test, variation was made on pH in the medium with injection of carbon dioxide gas. The optically detected signal was referred to pH, which was measured with a conventional pH meter. The results show that the designed system was effective to measure local medium pH around contracting myotubes *in vitro*.

Keywords: Biomedical Engineering, Cell Culture, Optics and pH

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 [1]. 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 [2].

The value of pH is one of the useful indicators to control the content of the medium for cell culture [3, 4]. 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.

Most of medium, on the other hand, contains phenol red for pH indicator, which enables optical pH measurement. In the previous study, an optical measurement system *in vitro* has been designed to measure repetitive contracting movement of myotube and local pH between 6.8 and 8.4 around myotube simultaneously [5]. In the present study, the optical measurement system for pH in the medium has been modified to improve signal to noise ratio.

Fig. 1: Green light emitting diode.

2. METHODS

Optical property of medium
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 with various pH were poured into the micro-cell, which was placed in a spectrophotometer (UVmini1240, Shimadzu, Kyoto, Japan).

Measurement System
The optical pH measurement system consists of a light source, a light path, and a light-sensitive element. Variation was made for the light source to be applied to the culture medium: a light-emitting diode and a filtered mercury lamp.

In the first system, the light source is made of a green (wavelength of 522 nm) light emitting diode (LED, LPF-05HGR-300N, Kilala-Factory, Japan), which is operated with a direct electric current of 20 mA at 4.5 V (Fig. 1). The optical cable (POC-15B) was applied to the light path to restrict the light beam (Fig. 2). The light-sensitive element is made of

Fig. 2: Optical cable.

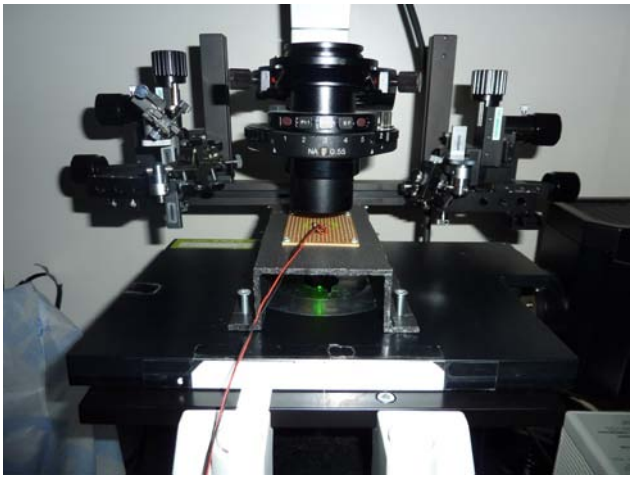

Fig. 3: Measurement system with LED.

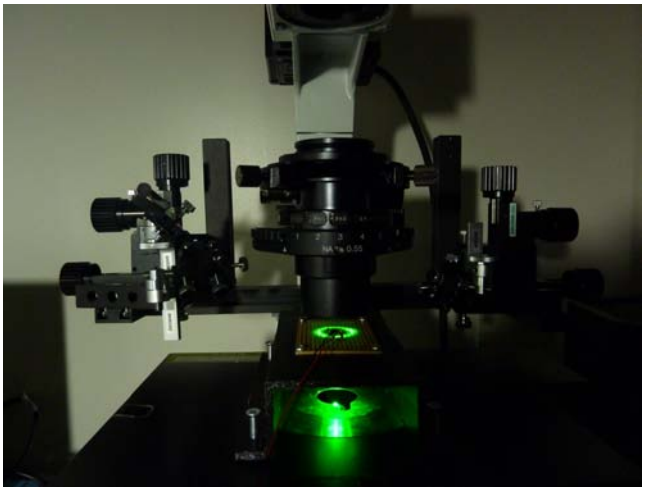

Fig. 4: Measurement system with filtered mercury lamp.

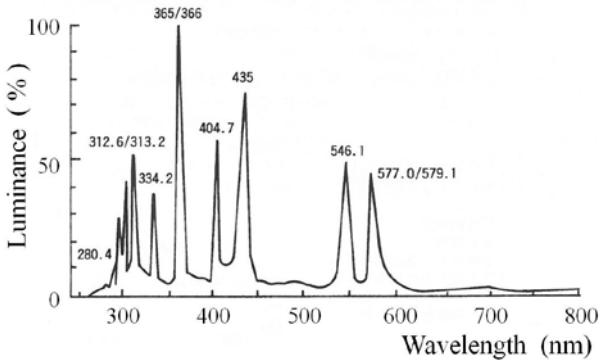

Fig.5: Optical characteristics of the super high-pressure mercury lamp

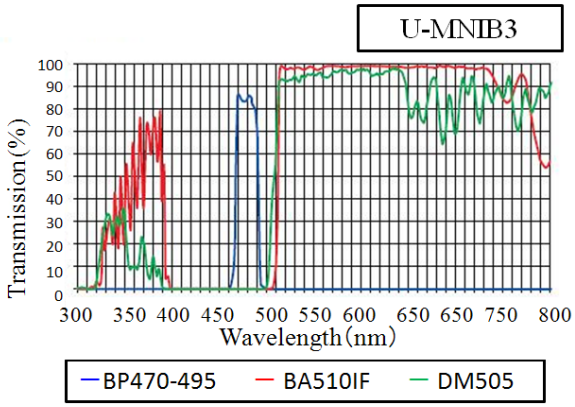

Fig. 6: Optical characteristics of U-MNIB3 filter.

a Si photodiode, which converts light into an electric current. The intensity of the transmitted LED beam through the culture medium was detected with the photodiode (Fig. 3). The variation of electric characteristic of the photodiode was detected, and the relation between the variation and pH was examined. The beam of 3 mm diameter is irradiated to the culture dish.

In the second system, a super high-pressure mercury lamp was used for the light source. The optical characteristics of the super high-pressure mercury lamp are illustrated in Fig. 4. Before introduced to the medium, the light beam was filtered with U-MNIB3 (Blue excitation, Fluorescence Mirror Unit, Olympus Corporation, Japan) or with U-DM-FI/TR2 (Green excitation, Fluorescence Mirror Unit, Olympus Corporation, Japan) (Fig. 4). Fig. 5 shows the optical characteristics of the super high-pressure mercury lamp. Fig. 6 shows the optical characteristics of U-MNIB3 filter. Fig. 7 shows the optical characteristics of U-DM-FI/TR2 filter. The light sensitive element is the same as that in the first system.

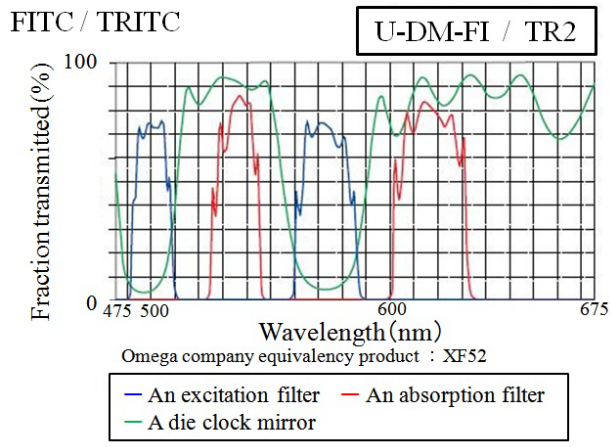

Fig. 7: Optical characteristics of U-DM-FI/TR2 filter.

Measurement of pH

Variation was made on pH (6.5-8.5) of medium in the culture dish with carbon dioxide gas supply. The carbon dioxide gas was produced by a chemical reaction with acetic acid and sodium bicarbonate liquid. The value of pH is checked with a conventional pH meter (Seven Multi, Mettler Toledo, Switzerland), which has an electrode (Fig. 8). The experiment was performed at the room temperature of 25 degrees Celsius.

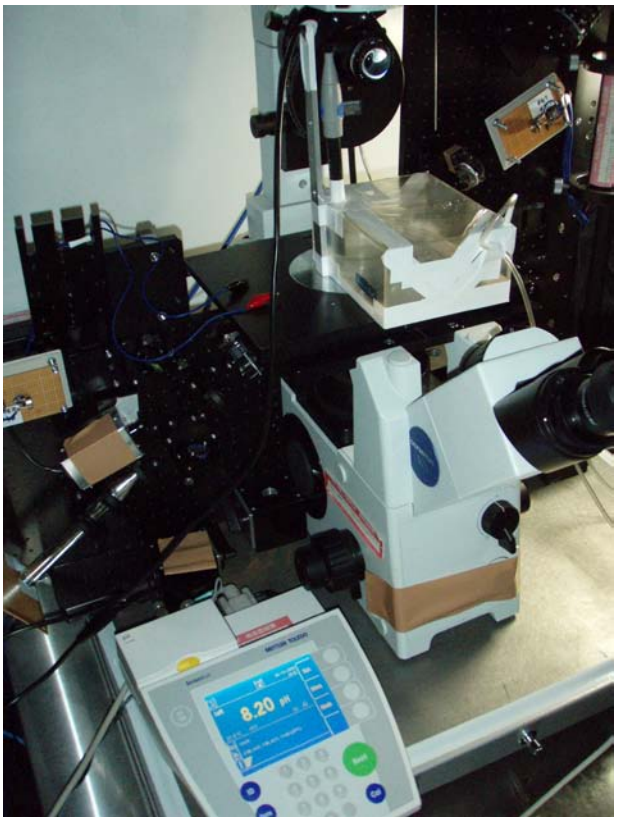

Fig. 8: Measurement of pH.

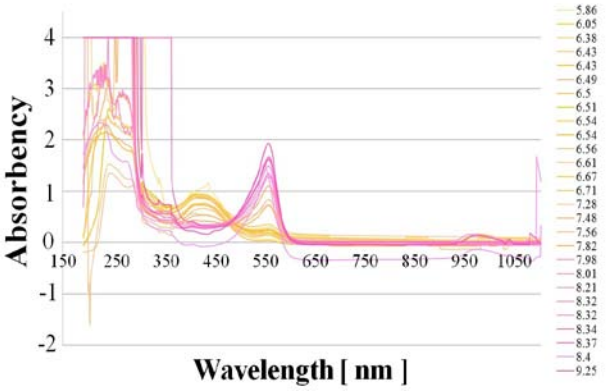

Fig. 9: Absorbency of medium in relation to wavelength with the variation of pH.

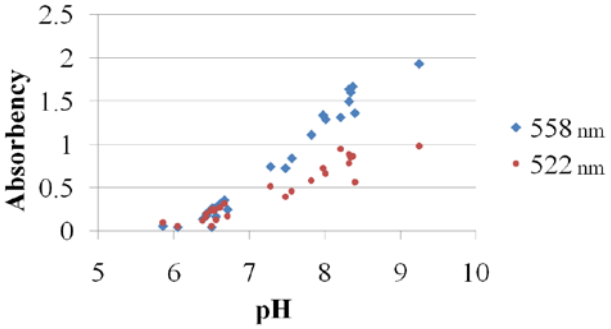

Fig. 10: Absorbency of medium in relation to pH at two wavelengths.

3. RESULTS

Fig. 9 shows the relation between absorbency of the medium and wavelength with the variation of pH. The peak of absorbency at the wavelength of 558 nm corresponds to the color displayed with the phenol red. The result shows that absorbency also increases with pH at the wavelength of 522 nm (Fig. 10).

The light from LED can be successfully irradiated at a measurement position with the optical cable. Fig. 11 shows the relation between pH and electric resistance of the detector at the LED test. The figure shows that electric property varies linearly with pH between 6.3 and 7.8, which is the color variation range for phenol red. The range includes pH value of the normal muscle cell culture medium.

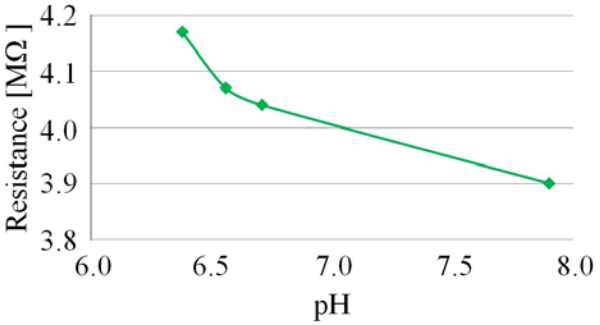

Fig. 11: Detected signal in relation to pH at LED.

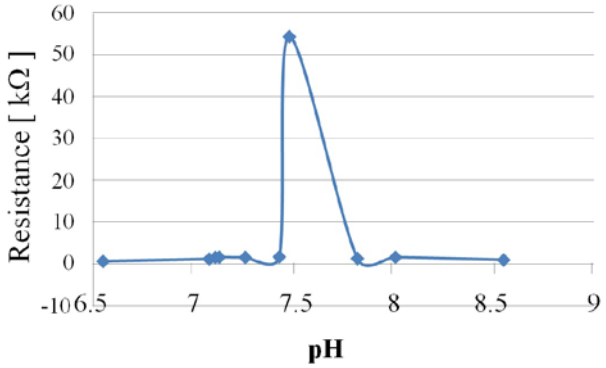

Fig. 12: Detected signal in relation to pH at mercury lamp filtered with U-MNIB3.

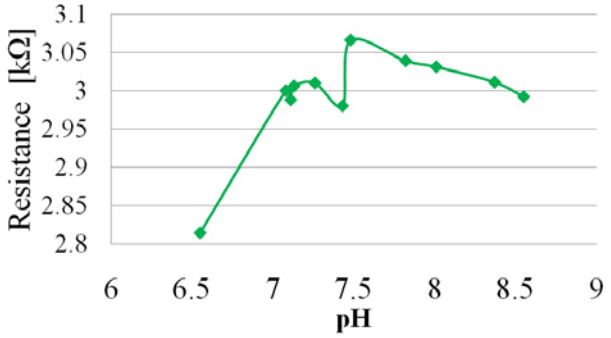

Fig. 13: Detected signal in relation to pH at mercury lamp filtered with U-DM-FI/TR2.

Fig. 12 and Fig. 13 show the relation between pH and electric resistance of the detector at the filtered mercury lamp test. Fig. 12 shows the detected signal at the filtered with U-MNIB3. Fig. 13 shows the detected signal at the filtered with U-DM-FI/TR2. Fig. 13 shows that that electric property varies linearly with pH between 7.5 and 8.5.

4. DISCUSSION

The light source and path have been improved to increase the signal to noise ratio.

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. Several perfusion systems have been developed for tissue culture [6-9]. The present measurement system has a potential to be applied to the bioreactor system.

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.

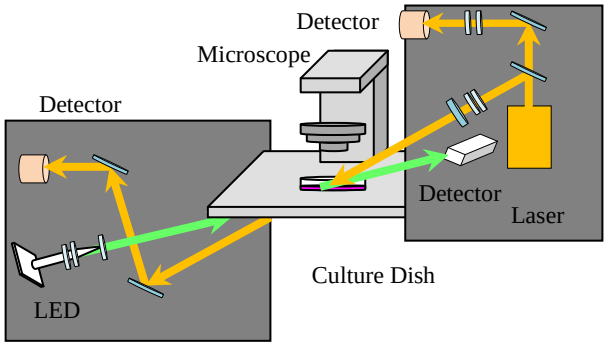

Fig. 14: Simultaneous optical measurement system.

The optical pH measurement system, for example, can detect critical pH value in the medium, when the cyclic contraction of myotubes in the chamber stopped during the test with variation of surrounding partial pressure of carbon dioxide [3]. The optical system can be applied to the simultaneous measurement system for pH and movement of cell (Fig. 14) [5].

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. Those optical systems have also a potential to study muscle fibers or cells in detail [10, 11].

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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