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Development of Cell Culture Monitoring System and Novel Non-Contact pH Measurement

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***Abstract*—This paper describes a cell culture monitoring system for regenerative medicine. To realize this monitoring system, a new culture vessel and a removable measurement unit were proposed. The measurement unit was installed in the culture vessel and it was used to measure important cell culture parameters (e.g., temperature, CO_2 level, and pH). Thus, the status of the culture could be monitored. In addition, we developed a novel noninvasive method based on spectrophotometry for measuring pH. This method is a non-contact method that permits noninvasive and contamination-free pH measurement. The spectroscopic pH measurements agreed well with pH measurements using an electrode. The error was within 0.02; thus, the new pH measurement method is sufficiently accurate for cell culture. This new system is expected to contribute to advances in tissue engineering and regenerative medicine.**

I. Introduction

Regenerative medicine is considered to be one of the most effective ways for treating damaged tissue. Recent advances in tissue engineering offer promising strategies for reconstructing and repairing damaged tissue [1]–[4]. There have been several successful clinical trials of tissue engineering techniques [5] [6]. Although regenerative medicine develops dramatically, most operations in the regenerative medicine are manual manipulations. The quality of cultured cells normally depends on the skills of those who culture cells. Therefore, further development and spread of regenerative medicine technology requires quality control and stabilization [7] [8].

This paper describes a new cell culture monitoring system for regenerative medicine. This system can monitor the status of a culture. In addition, a novel method for noninvasively measuring pH that is based on spectrophotometry is developed. This is a non-contact method and thus permits the pH be measured noninvasively and without introducing contamination.

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II. Materials and Method

A. Monitoring system for regenerative medicine

Fig. 1 schematically depicts the proposed monitoring system for regenerative medicine. The system consists of four components: newly developed culture vessels, monitors, a central monitor, and an alarm system.

The culture vessels contain a measurement unit that can measure important cell culture parameters (e.g., temperature, CO_2 levels, and pH), allowing the status of the culture to be monitored. Measured data are transmitted to the monitor by a telemetry system. The central monitor manages all the data. When the culture status is abnormal, a notification is transmitted to a portable device such as a cellular phone.

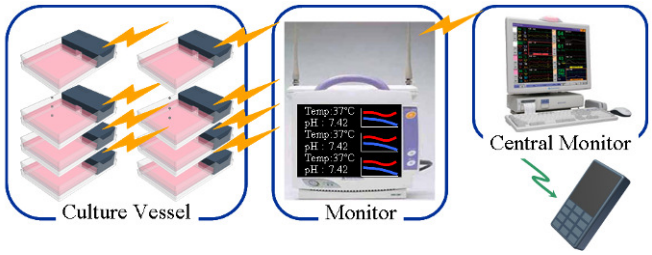

Fig. 1 Monitoring system for regenerative medicine

B. Measurement unit

Figs. 2 and 3 show the developed novel culture vessel. Its dimensions are 85 mm × 128 mm × 28 mm (the vessel thus conforms with SBS standards). The culture vessel consists of a measurement area and a cultivation area. A measurement unit is installed in the measurement area. The unit consists of a sensing region, the main board, a battery, and a wireless communication system. There is no need for an external connector because the unit contains a wireless energy transfer module and a wireless communication system. The unit is thus completely enclosed. In addition, the measurement unit is removable (see Fig. 2) and can be sterilized several times using hydrogen peroxide or ethylene oxide gas. Consequently, it is reusable.

Because the unit also contains a modified TG-970P (Nihon Kohden Co., Tokyo, Japan) and temperature sensor, CO2 levels and temperature can be measured. In addition, we developed new non-contact pH sensor, pH can be measured.

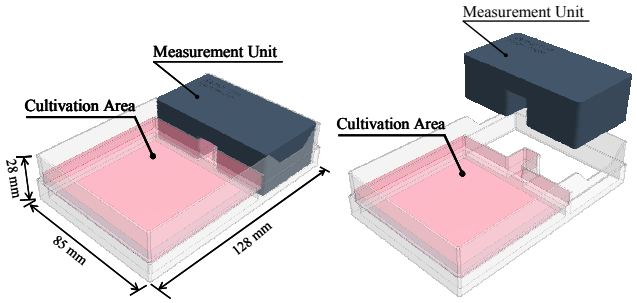

Fig. 2 Novel culture vessel. The measurement unit is removable and can be sterilized

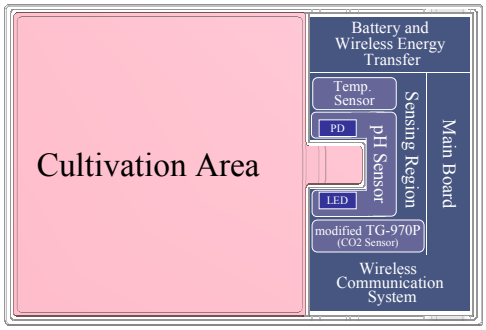

Fig. 3 Measurement unit

C. *Novel noninvasive pH measurement*

The pH is one of the most important culture parameters. During cell growth, cells absorb and metabolize glucose and generate lactic acid as a metabolite, causing the pH of the culture medium to decrease. Therefore, the pH reflects the cellular metabolic activity and the status of the culture. However, the pH of a culture should not be measured using pH electrodes since they may introduce contamination and result in cellular invasiveness. The pH is generally determined from the color of a pH indicator added to the culture medium. The pH indicator is included in almost all of culture media. However, the color depends on the medium's composition. Consequently, two media with different compositions can have different colors even when they have the same pH. This makes it difficult to accurately measure the pH.

To overcome this problem, we developed a novel non-contact pH measurement method based on spectrophotometry. This new pH measurement method employs a multi-wavelength LED. The color of a culture medium is mainly determined from three parameters: pH, pH indicator concentration, and fetal bovine serum (FBS) concentration. Phenol red (PR) is usually used as the pH indicator. Fig. 4 shows the absorption spectra of FBS and the pH indicator. With decreasing pH decreases, the absorbance of PR at about 558 nm increases and its absorbance at about 430 nm decreases. The absorbance of PR has isosbestic points at 367 and 479 nm.

The absorbance $A_{(\lambda)}$ is given by

$$A_{(\lambda)} = \alpha_{FBS(\lambda)} \cdot C_{FBS} \cdot D + \alpha_{PR(\lambda,pH)} \cdot C_{PR} \cdot D + S_{(\lambda)} \cdot A_{\text{offset}}, \quad (1)$$

where λ is the wavelength and $\alpha_{FBS(\lambda)}$ and $\alpha_{PR(\lambda,pH)}$ are the absorption coefficients of FBS and PR, respectively. $\alpha_{FBS(\lambda)}$ is a function of λ while $\alpha_{PR(\lambda,pH)}$ is a function of both λ and pH. C_{FBS} and C_{PR} are the concentrations of FBS and PR respectively, D is the length of the light path, $S_{(\lambda)}$ is the scattering coefficient, and A_{offset} is the offset due to scattering.

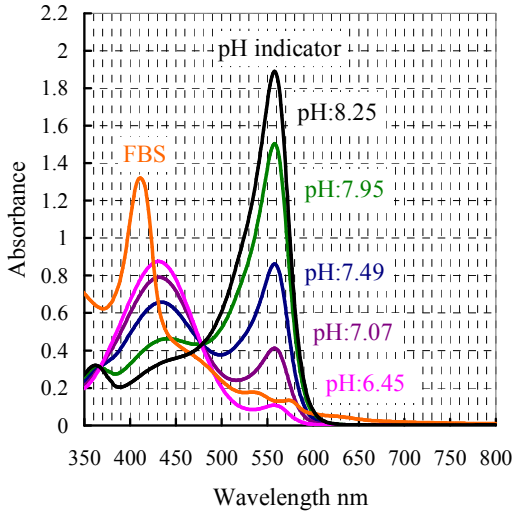

Fig. 4 Absorbance spectra of FBS and PR (pH indicator)

To realize noninvasive pH measurement by spectrophotometry, wavelengths of 367, 430, 558, and 700 nm were selected.

Because neither FBS nor PR absorb at a wavelength of 700 nm, the absorbance at that wavelength is described by

$$A_{(700)} = S_{(700)} \cdot A_{\text{offset}} \quad (2)$$

At a wavelength of 367 nm, because it is an isosbestic point of PR, $\alpha_{PR(367,pH)} = B_{1\_367}$, where B_{1_367} is a constant. Therefore, the absorbance is given by

$$A_{(367)} = \alpha_{FBS(367)} \cdot C_{FBS} \cdot D + B_{1\_367} \cdot C_{PR} \cdot D + S_{(367)} \cdot A_{\text{offset}} \quad (3)$$

Fig. 5 shows the relationship between the absorption coefficients at 430 nm ($\alpha_{PR(430,pH)}$) and 558 nm ($\alpha_{PR(558,pH)}$). Therefore, the absorption coefficient at 430 nm ($\alpha_{PR(430,pH)}$) can be approximated by a linear function of the absorption coefficient at 558 nm ($\alpha_{PR(558,pH)}$)

$$\alpha_{PR(430,pH)} = B_{0\_430} \cdot \alpha_{PR(558,pH)} + B_{1\_430}, \quad (4)$$

where B_{0_430} is the slope and B_{1_430} is the intercept. Therefore, the absorbances at 430 and 558 nm are respectively described by

$$A_{(558)} = \alpha_{FBS(558)} \cdot C_{FBS} \cdot D + \alpha_{PR(558,pH)} \cdot C_{PR} \cdot D + S_{(558)} \cdot A_{\text{offset}} \quad (5)$$

$$A_{(430)} = \alpha_{FBS(430)} \cdot C_{FBS} \cdot D + \left(B_{0\_430nm} \cdot \alpha_{PR(558,pH)} + B_{1\_430nm}\right) \cdot C_{PR} \cdot D + S_{(430)} \cdot A_{\text{offset}}. \quad (6)$$

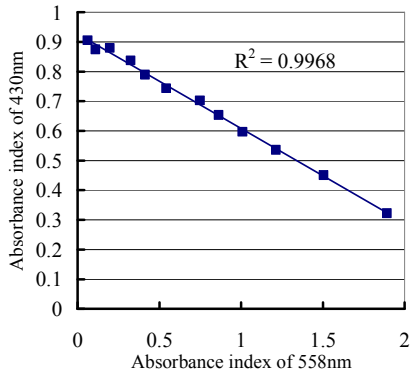

Fig. 5 Relationship between absorption coefficients at 430 nm ($\alpha_{PR(430,pH)}$) and at 558 nm ($\alpha_{PR(558,pH)}$)

Combining equations (2), (3), (5), and (6), we obtain the following matrix equation:

$$\begin{pmatrix} C_{FBS} \\ \alpha_{PR(558,pH)} \cdot C_{PR} \\ C_{PR} \\ A_{offset} \end{pmatrix} = \begin{pmatrix} \alpha_{FBS(367)} \cdot D & 0 & B_{1\_367} \cdot D & S_{(367)} \\ \alpha_{FBS(430)} \cdot D & B_{0\_430} \cdot D & B_{1\_430} \cdot D & S_{(430)} \\ \alpha_{FBS(558)} \cdot D & D & 0 & S_{(558)} \\ 0 & 0 & 0 & S_{(700)} \end{pmatrix}^{-1} \cdot \begin{pmatrix} A_{(367)} \\ A_{(430)} \\ A_{(558)} \\ A_{(700)} \end{pmatrix} \quad (7)$$

The matrix on the left-hand side of equation (7) is unknown. The matrixes on the right-hand side consist of absorption coefficients and other known constants and the absorbance, which can be measured. Using equation (7), the FBS concentration (C_{FBS}), the PR concentration (C_{PR}), and the absorption coefficient at 558 nm ($\alpha_{PR(558,pH)}$) can be obtained.

Fig. 6 shows the relationship between the pH and the absorption coefficient at 558 nm ($\alpha_{PR(558,pH)}$). This relationship can be used to determine the pH.

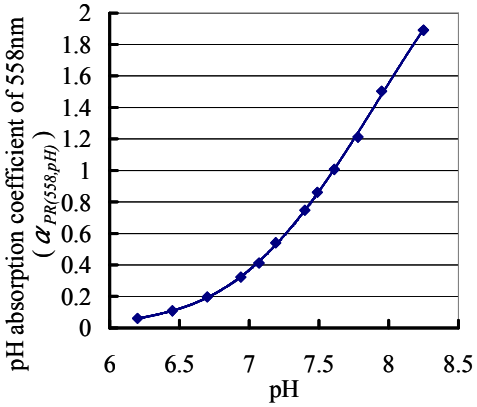

Fig. 6 Relationship between pH and absorption coefficient at 558 nm ($\alpha_{PR(558,pH)}$)

D. *Evaluation Model*

We developed a system for evaluating the new pH measurement method (see Fig. 7). A pH electrode is inserted in the culture vessel. Because an optical cell is also attached to the vessel, this spectroscopic pH measurement system can be installed at the optical cell.

The pH can be controlled to be any value using an acid solution (0.1 M HCl), an alkaline solution (0.1 M NaOH), and the pH controller. Tube pumps are used to drip these acid or alkaline solutions into the culture medium based on the amounts determined by the pH controller. To ensure that the pH of the medium is uniform throughout the vessel, the medium is stirred by a stirrer and circulated using a tube pump. The culture vessel is set in an incubator whose temperature is controlled at 37°C.

Fig. 8 and Fig. 9 show a block diagram and photographs of the spectroscopic pH measurement probe. A Si photodiode (PD) was used as the detector. Because the multi-wavelength LED pulse alternately, the each absorbance can be measured.

This evaluation system could be used to compare measurements by the pH electrode and the spectroscopic pH measurement system.

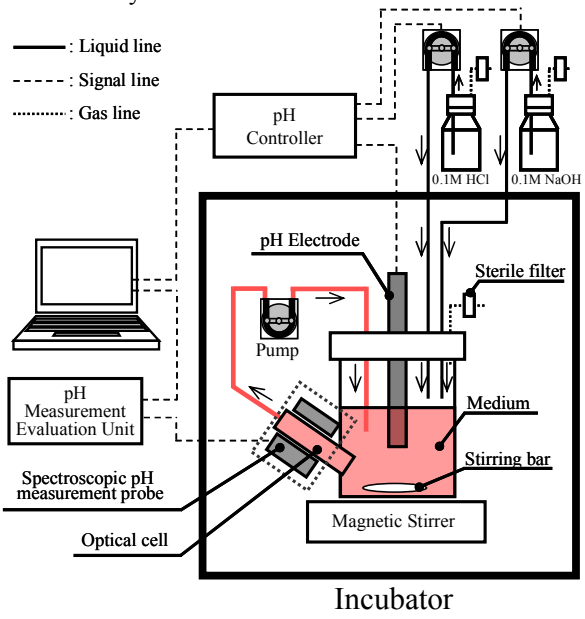

Fig. 7 System for evaluating spectroscopic pH measurements. This evaluation system can be used to compare measurements by the pH electrode and the spectroscopic pH measurement system.

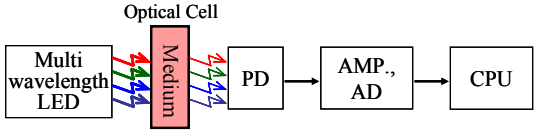

Fig. 8 Block diagram of the spectroscopic pH measurement system. The multi-wavelength LED pulse alternately, and the each absorbance can be measured.

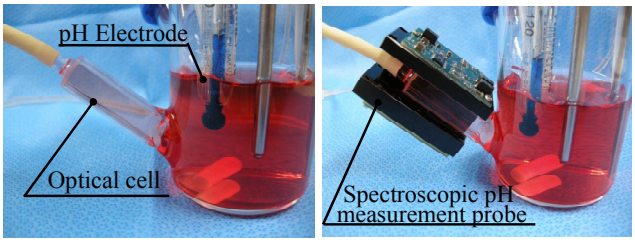

Fig. 9 Photographs of spectroscopic pH measurement probe. A multi-wavelength LED and PD are installed in the spectroscopic pH measurement probe. This new pH measurement method enables noncontact pH measurements.

III. Results

Figs. 10 and 11 compare pH spectroscopic measurements with pH electrode measurements. The pH of the medium was controlled to be 7.1, 7.3, 7.5, 7.7, and 7.9 using the pH controller. In addition, the PR concentration is shown in Fig. 10. In the process of the pH control, the PR concentration was gradually decreased because the acid solution and the alkaline solution were dripped into the culture medium.

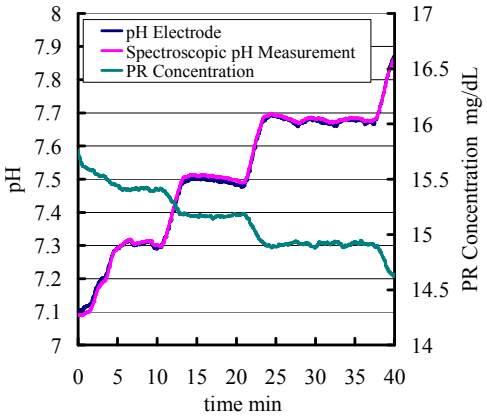

Fig. 10 Comparison of pH electrode and spectroscopic pH measurements

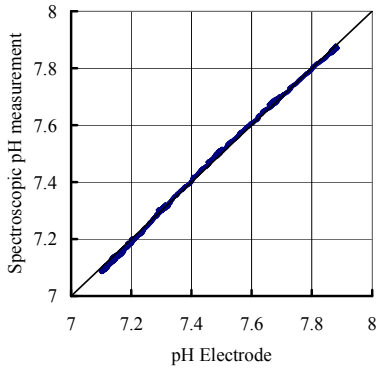

Fig. 11 Relationship between pH measurement and spectroscopic pH measurements

IV. Discussion

Fig. 10 reveals that the pH electrode and spectroscopic pH measurements exhibited similar responses. The spectroscopic pH measurement system can measure the pH in a few milliseconds. This measurement time is sufficiently short for the developed system to be applied to cultures that undergo rapid reactions. And Fig. 10 shows that PR concentration also could be measured and was decreased because of the pH control.

Fig. 11 shows that there is good agreement between the spectroscopic pH measurements and the pH electrode measurements. The error is within 0.02, which indicates that the new pH measurement method is sufficiently accurate for cell culture. And even though the PR concentration was changed, the pH could be measured with a high accuracy. Therefore, the proposed method can perform non-contact and contamination-free pH measurements.

V. Conclusion

A monitoring system for regenerative medicine was proposed. To realize this monitoring system, a new culture vessel and removable measurement unit were developed. The measurement unit was installed in the culture vessel and important cell culture parameters (e.g., temperature, CO_2 level, and pH) were measured using the unit. The status of the culture could thus be monitored.

In addition, we developed a novel pH measurement method based on spectrophotometry. This new pH measurement method enables non-contact pH measurements to be performed. Thus, the method can perform noninvasive and contamination-free pH measurements. The new pH measurement method is sufficiently accurate for cell culture.

Monitoring systems for regenerative medicine are necessary to enhance quality control and quality stabilization. The proposed monitoring system and the new pH measurement method are effective and powerful tools for tissue engineering and regenerative medicine.

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