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Simultaneous determination of hemolysis and hematocrit in extracorporeal circulation by plasma surface reflectance spectroscopy

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Abstract— **To achieve quantitative non-invasive optical diagnosis of blood abnormalities during extracorporeal circulation therapies, plasma surface reflectance spectroscopy was developed by implementing oblique-incidence optical fiber reflectometry on the surface of circulating blood. The reflected light in the wavelength range from 450 to 600 nm changed with respect to the plasma free hemoglobin level and could be used to quantify the free hemoglobin at an accuracy of 5.7±3.5 mg/dL. In contrast, the spectrum did not changed by varying the hematocrit. In the wavelength range from 600 to 800 nm, the obtained spectrum was affected by both the hematocrit change and hemolysis. The linear correlation between the hematocrit value and the spectrum was confirmed at R^2 = 0.99. The feasibility of determining of the hematocrit of arbitrary hemolyzed blood was confirmed. The developed system permits the extraction of the optical characteristics of both plasma and red blood cells without centrifugation. The study establishes non-invasive optical diagnostics capable of analyzing the optical properties of both plasma and red blood cells.**

I. Introduction

For extracorporeal circulation therapies, such as those using a percutaneous cardiopulmonary support system, extracorporeal membrane oxygenation system, left ventricular assist device, and hemodialyser, the blood condition, including bleeding, oxygen utilization, hemolysis, thrombosis, and alteration in the chemical components should be continuously monitored to prevent anemia, infection, infarction, and to detect any early malfunction of the cardiopulmonary devices. To achieve this objective, a non-invasive and continuous diagnosis system using visible or near-infrared light is a valuable tool. To date, various optical blood monitoring systems have been proposed. These approaches include multiple scattering light spectroscopy [1,2], photoacoustic spectroscopy [3], and Raman spectroscopy [4-6]. However, to develop long-term quantitative and reliable systems for blood diagnosis, understand the optical properties of blood is necessary.

The optical properties of blood are mainly affected by hematocrit (HCT) because the light is scattered mainly by red blood cells (RBCs). In terms of the clinical aspects, the alteration in the HCT level indicates important symptoms such as bleeding and thrombosis [7,8]. Therefore achieving accurate quantification of HCT is of clinical value and provides significant benefits for the development of various optical diagnostics of blood. However, the conventionally proposed optical measurements of HCT are not adaptable for the alteration in the refractive index of plasma, which consequently results in the need for calibration [1,2,11]. Especially, hemolysis frequently occurs in extracorporeal circulation therapies due to the nonphysiological shear flow and osmotic pressure in the devices. Excessive hemolysis results in anemia, and the plasma free hemoglobin (fHb) is nephrotoxic [9], sometimes causing multiple organ failure. Hemolys is also affects the optical blood monitors because fHb dominantly changes in both the real and imaginary parts of the refractive index of plasma and results in changes in the scattering and absorption of light in blood [10-13].

Therefore, we have proposed plasma surface reflectance spectroscopy (PSRS) by implementing oblique-incidence optical fiber reflectometry on the blood surface to extract only the optical characteristics of plasma in flowing blood [14]. PSRS extracts the optical characteristics of plasma non-invasively and continuously without centrifugation. The objective of the present study is to investigate the feasibility of the simultaneous determination of HCT and fHb levels using PSRS to establish non-invasive optical diagnostics capable of analyzing the optical properties of both plasma and RBCs.

II. Materials and Methods

A. Experimental set-up of PSRS in extracorporeal circuit

The experimental set-up is shown in Fig. 1. An in vitro closed loop circuit was constructed using a rotary blood pump (BPX-80 BIO-Pump Plus, Medtronic, Inc., MN, USA), Oxygenator (Baby-RX, Terumo corp., Tokyo, Japan), a reservoir pack, and 1/4 inch transparent Tygon tubing. The experiments were conducted using bovine blood (Funakoshi Co., Ltd., Tokyo, Japan) obtained with 24 hours storage period. The blood was anticoagulated with trisodium citrate (final concentration: 0.45 weight/volume%) immediately after the blood was harvested. The continuous blood flow was generated by the rotary blood pump at 2 L/min. The temperature of the circulating blood was maintained at 37 °C during the experiments. The oxygen saturation was maintained at 100%. A halogen lamp (wavelength range, 400 to 800 nm) was used for the light source. A developed glass optical flow cell was attached to the outlet tubing of the oxygenator. The halogen light was guided into an incident optical fiber. The light emitted by the fiber was collimated and

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emitted to the flat surface of the flow cell at the incident angle of 45°. To eliminate the reflection of light on the outer surface of the glass cell, liquid paraffin (Wako Pure Chemical Industries, Ltd., Osaka, Japan), which has a refractive index (1.48 at 589.3 nm) nearly equal to that of the glass was added to the incident area space, as shown in Fig. 1. As a result, only the light reflected on the plasma surface was detected by the detection fiber. The detected light spectrum was analyzed by a spectrophotometer (BLUE-Wave Miniature Fiber Optic Spectrometers, StellarNet-Inc., FL, USA).

The concept of PSRS is based on the axial accumulation of RBCs in tube flow and the plasma skimming effect [14, 15]. The effect reduce the HCT in the near-wall area, which results in the formation of an extremely-thin plasma layer. Therefore, because the reflected light on the surface of the flowing blood hardly irradiates RBCs so that the spectrum derived from plasma can be extracted accurately.

B. *Alteration in hemolysis and hematocrit levels in the PSRS experiment*

To investigate the spectral changes with respect to the HCT and fHb levels, the blood HCT was altered by adding autologous plasma that had been prepared beforehand by centrifugation of the blood. The sample of hemoglobin solution was prepared by osmotic hemolysis, and the electrolytic concentration was subsequently adjusted to coincide with that of saline. The resultant hemoglobin solution was added to the autologous plasma to control the fHb level of the blood. HCT and fHb levels were directly measured by the HCT tube method and cyanmethemoglobin method respectively.

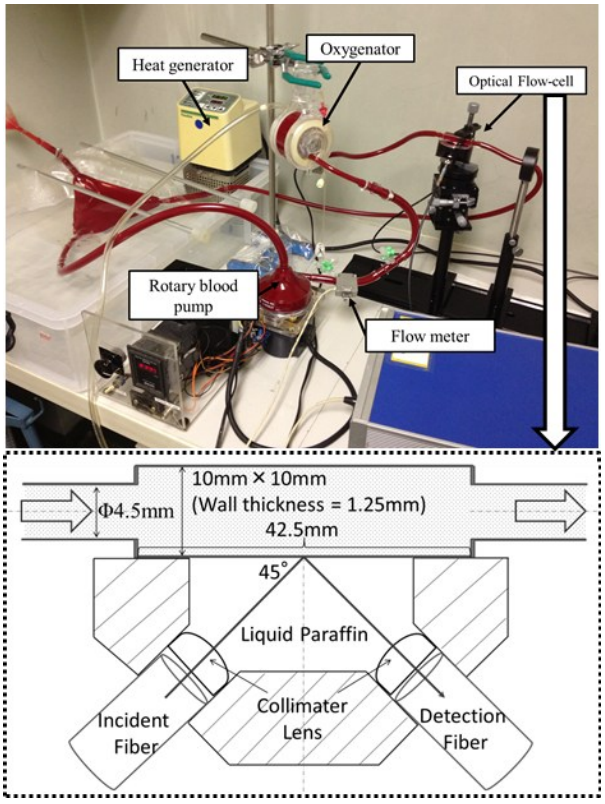

Figure 1. **Experimental set-up**

III. RESULTS

A. *Changes in PSRS signal with respect to hemolysis and hematocrit levels*

The optical density (OD) was calculated by following equation:

$$OD(\lambda) = -\log_{10}\frac{I_{blood}(\lambda)}{I_{saline}(\lambda)}, \quad (1)$$

where, I_{blood} is the detected light intensity in blood at the wavelength λ, I_{saline} is the detected light intensity when saline is pumped through the circuit. The typical result is shown in Fig. 2. In spite of the HCT and fHb levels, at a wavelength of 600 nm, the OD was 0 because the detected light intensity of blood was equal to that of saline. To show the alteration in the optical density with respect to the HCT and fHb respectively, equation (1) was differentiated in terms of HCT and fHb, as shown in the following equations:

$$\frac{\partial OD}{\partial fHb} = \frac{OD(fHb) - OD(fHb_0)}{OD(fHb_0)} \quad (2)$$

and

$$\frac{\partial OD}{\partial HCT} = \frac{OD(HCT) - OD(HCT_0)}{OD(HCT_0)}, \quad (3)$$

where, fHb_0 in equation (2) is 31.2 mg/dL, and HCT_0 in equation (3) is 30 %. These results are shown in Fig. 3a and Fig. 3b respectively. The differential spectrum diverged at 600 nm because there was no distinction between the blood and saline. At wavelengths < 600 nm, there was no significant differences in the HCT changes, but the distinction between the blood and saline was shown by the fHb changes. At wavelengths > 600 nm, the differential spectrum was decreased when the fHb level was increased and the HCT level was decreased.

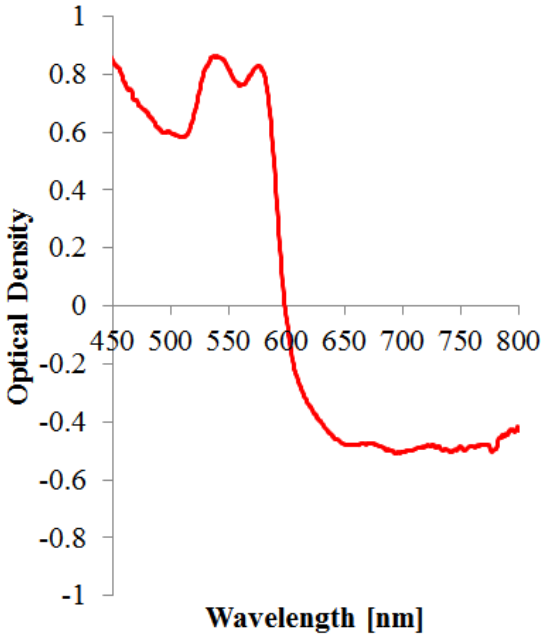

Figure 2. **Spectrum obtained by PSRS**

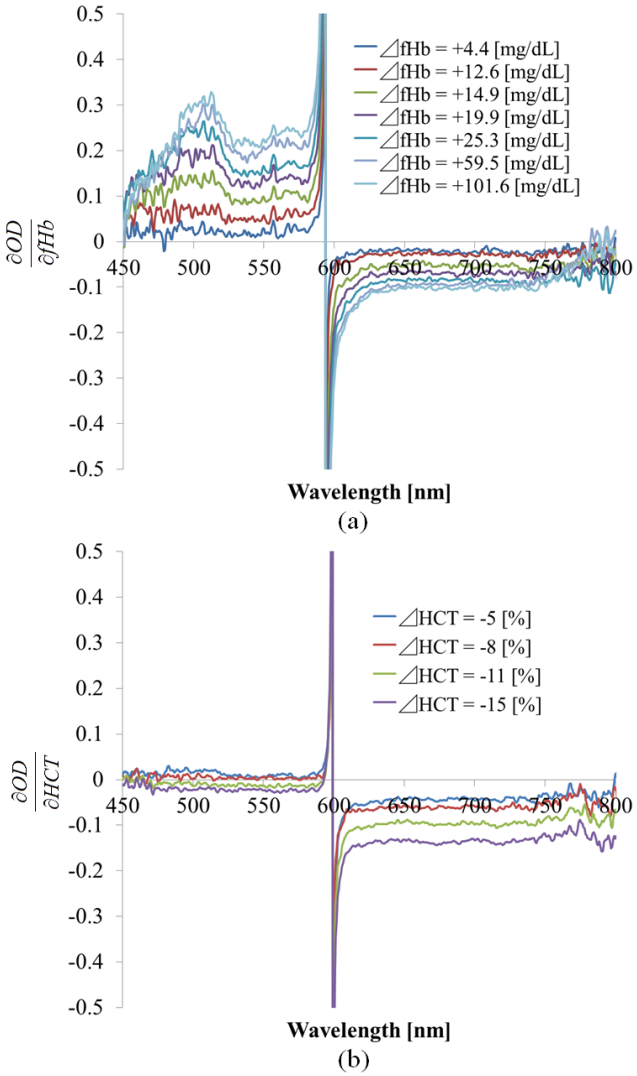

Figure 3. **Aitation in optical density by fHb and HCT changes**

B. *Prediction of plasma free hemoglobin level by PSRS*

Considering Fig. 3, the feasibility of the quantification of fHb was investigated at wavelengths < 600 nm. The fHb prediction formula was defined by the following equation:

$$\Delta OD = OD(\lambda 1) - OD(\lambda 2), \quad (4)$$

where $OD(\lambda 1)$ and $OD(\lambda 2)$ are the mean ODs in the wavelength range from 540 to 545 nm and from 500 to 515 nm, respectively. The correlation between ΔOD and the measured fHb is shown in Fig. 4. The ΔOD was linearly correlated with the fHb at R^2 = 0.9573, and the mean error between the predicted fHb value by PSRS and the measured value was 5.7±3.5 mg/dL.

C. *Determination of hematocrit by PSRS*

The mean OD in the wavelength range from 650 to 700 nm was calculated: the results are shown in Fig. 5. The value was linearly decreased by varying HCT at R^2 = 0.99. In addition the gradient was constant in spite of the fHb level at -0.0047±0.0005 [$OD(\lambda)$/HCT]. However, the parallel translation of the mean $OD(\lambda)$ occurred between fHb = 16.9±0.6 mg/dL and 37.4±0.2 mg/dL. At a much higher fHb level, the shift hardly occurred.

IV. Discussion

Experimental results showed that the optical properties of blood were altered with respect to HCT and fHb levels due to the changes in the number of RBCs and the changes of the refractive index of plasma. With conventional visible and near-infrared spectroscopy, it is difficult to distinguish one from the other. However, PSRS could extract the information of hemolysis without depending on the varying HCT by using the obtained spectrum at wavelengths < 600 nm, as shown in Fig. 3. The results indicate that PSRS is able to accurately and simultaneously diagnose both RBCs and plasma disorders.

According to the data shown in Fig. 3, the spectrum was shifted by varying fHb, and it was not dependent on varying HCT at wavelengths < 600 nm. In contrast, at wavelengths > 600 nm, the spectrum changed by varying both fHb and HCT. This is caused by the absorption spectrum of a hemoglobin molecule. The absorption by oxy-hemoglobin is rapidly

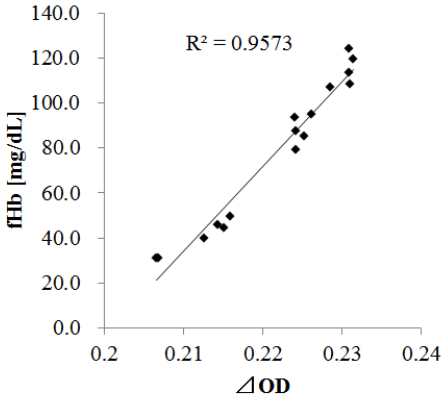

Figure 4. **Correlation between fHb and ΔOD**

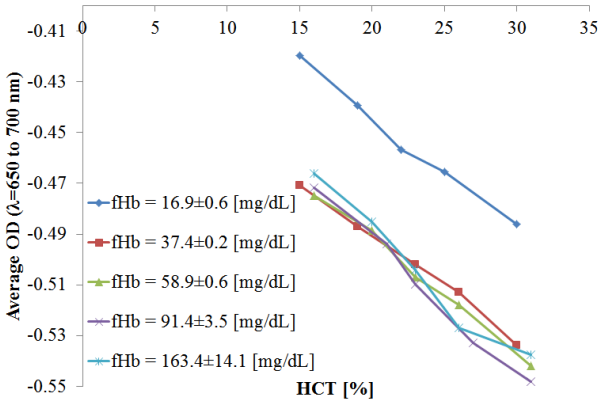

Figure 5. **HCT and fHb responce of mean OD in the wavelength range from 650 to 700 nm**

decreased at > 600 nm [16]. In the experiment, the incident angle of PSRS was 45°, so the light transmitted into the blood. Light scattering subsequently occurred when the transmitted light hit RBCs. The scattered light was then detected by the detection fiber. Therefore, the spectrum at > 600 nm was changed by varying HCT. The detected light intensity was increased by increasing HCT because the backward scattering is increased with respect to the increase in the number of RBCs. However, at < 600 nm, most of the transmitted light is absorbed when the light hit the RBCs due to the extremely higher absorption by the intracellular hemoglobin. Hence only the reflected light on the plasma surface could be detected, and the reflected light would scarcely hit RBCs because of the axial migration of RBCs and the plasma skimming effect. Consequently, the developed PSRS could detect the fHb without depending on RBCs.

Although the freasibility of determining HCT by using light wavelenghs > 600 nm was confirmed, as shown in Fig. 5, the spectrum was also affected by the fHb level, especially at fHb = 37.4 mg/dL Hemolysis at this fHb level is possible in extracoporeal circulation therapies. The problem with most of the optical blood monitors is the correction for uncontrolled changes of other non-measured blood parameters that exert an optical influence [11]. Although the current measurement monitors do not adjust for these effects, PSRS is able to respond to the changes by extracting the plasma information.

In the past study, the optical properties of circulating blood in extracorporeal circulation have been investigated by other researchers using inverse Monte Carlo simulations [10]. Our group also newly developed the photon-cell interactive Monte Carlo simulation that directly describes the influence of the blood parameters such as HCT, RBC volume, the intracellular hemoglobin, and the orientation of these optical properties [17,18]. These optical measurement systems combining theoretical models contribute to the quantitative diagnosis of various blood components. However, these simulators require the relative refractive index between RBCs and plasma to calculate the scattering. PSRS provides the information and can contribute to the development of the non-invasive optical diagnosis of the multiple component of blood.

V. Conclusion

Plasma surface reflectance spectroscopy of circulating blood in the extracorporeal circulation can non-invasively and continuously extract the plasma free hemoglobin without centrifugation. The technique would be useful for the highly accurate quantification of the hematocrit. Plasma surface reflectance spectroscopy provides information on the optical properties of both red blood cells and plasma.

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