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Hemoglobin assay in anemic patients with a photothermal spectral-domain optical coherence reflectometric sensor

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ABSTRACT

Background: We recently introduced an optical sensor, termed photothermal spectral-domain optical coherence reflectometer (PT SD-OCR), capable of direct measurement of hemoglobin concentration ([Hb]) without chemicals and pre-processing of blood. PT SD-OCR measures [Hb] by quantifying refractive index changes of blood samples due to photothermal effect under the irradiation of 532-nm light. We evaluated diagnostic accuracy and precision of PT SD-OCR in anemic patients.
Methods: We used PT SD-OCR to measure [Hb] in 50 anemic patients, and examined its accuracy against a hematology analyzer (ADVIA® 2120i, Siemens AG). Its precision was assessed by examining the SD and CV based on 20 repeated measurements on 3 blood samples. Its performance was also compared with a hemoglobinometer (HemoCue® 201+, HemoCue®).
Results: The PT SD-OCR demonstrated good correspondence with the hematology analyzer with a Pearson's correlation coefficient of 0.992 and a bias of −0.055 g/dl. Standard deviation and CV were measured to be <0.25 g/dl and <2.05%.
Conclusions: Simple and chemical-free operation of PT SD-OCR enabled rapid and accurate measurement of [Hb] in anemic patients. The sensor is expected to facilitate clinical procedures related to blood-related disorders in patient care.

1. Introduction

Hemoglobin (Hb) is the oxygen transport protein that comprises ~97% of red blood cells (RBCs). The mass concentration of Hb ([Hb]) provides a measure of the oxygen-carrying capacity of blood, and can thus be used as an indicator of blood disorders and of possible complications during operation. The reference range of [Hb] for normal blood samples is 12–18 g/dl, while [Hb] values of <5 g/dl or >20 g/dl are considered critical [1]. Anemia, a condition of decreased [Hb], is one of the most common blood-related diseases. Anemia is defined by the World Health Organization as a [Hb] below 12 g/dl for females and below 13 g/dl for males [2,3]. Although anemia is often regarded as a minor medical condition by the general population, it is a significant disorder that healthcare professionals have recognized as negatively affecting mortality and morbidity [4,5]. Anemia strains the cardiopulmonary system, and thus leads to increases in the heart rate and stroke volume to maintain oxygen delivery. It may also be related to fatigue and lethargy [6]. Patients with chronic anemia and [Hb] < 5 g/dl may experience more serious complications such as angina, congestive heart failure, heart attack, and stroke [7].

Owing to its significance, [Hb] is measured as an integral part of blood screening to diagnose anemia [8,9]. Clinical laboratories measure [Hb] with automated hematology analyzers and blood gas analyzers [9]. While these instruments provide accurate determinations of various blood components, they are bulky, expensive to operate, and time-consuming [10]. Several alternative technologies for [Hb] measurement have been developed. The cyanide methemoglobin method uses chemicals such as potassium cyanide (KCN) to destroy the lipid bilayers of erythrocytes. The released hemoglobin then turns into cyanide hemoglobin through cyanization and is exposed to light of a specific wavelength for colorimetric detection [11]. This method has been applied in portable hemoglobinometers such as HemoCue® 201+ detector (HemoCue® AB), which is commonly employed in laboratories and blood donation centers. However, the use of toxic materials such as KCN and dimethyllaurylamine oxide is problematic [12]. Spectrophotometry methods provide a precise measurement of [Hb], but require measurements at multiple wavelengths, large sample volume and reference measurement for accurate determination of absorption at each wavelength [13,14].

Other methods for [Hb] measurement have been investigated. Electrochemistry and immunoassay have been explored, especially for

Abbreviations: PT SD-OCR, photothermal spectral-domain optical coherence reflectometer; DPSS, diode-pumped solid state; PT, photothermal.

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the detection of small traces of biomarkers in the blood [15,16]. Despite their high sensitivity with detection limits of <1 μg/ml, their limited detection ranges (<100 μg/ml) hinder their broad utilities in regular blood tests [16]. In addition, electrochemical and immunoassay methods require sophisticated devices and chemicals such as reagents and labels for operation.

We recently developed a simple and chemical-free optical sensor capable of rapid [Hb] measurement of unprocessed blood samples. The sensor, termed as a photothermal spectral-domain optical coherence reflectometer (PT SD-OCR) [17], measures optical path-length change of a blood-containing chamber upon the irradiation with a 532-nm laser beam. Hemoglobin molecules absorb the 532-nm light energy and convert it into heat in the medium. The resulting temperature change then leads to refractive index alterations of the chamber, which can be measured by the PT SD-OCR sensor. Other components of blood such as leukocytes, thrombocytes, and plasma were found not to contribute to the temperature changes [17]. Our previous study involved the direct measurement of [Hb] of blood samples and examined its detection limit and dynamic range.

2. Materials and methods

2.1. Blood samples

This study was approved by the Institutional Review Board of Severance Hospital (Seoul, Republic of Korea), an affiliated hospital of the Yonsei University Health System with a 2500-bed tertiary care teaching hospital. The samples were the residual blood samples that had been acquired and processed in clinical tests at the institution. We used blood samples collected in tubes with EDTA (BD Vacutainer, Becton Dickinson). Prior to the measurement of [Hb] with the PT SD-OCR sensor, the hematology analyzers (ADVIA® 2120i and HemoCue® Hb 201 + systems) recorded standard hematological parameters along with [Hb]. Fifty blood samples from anemic patients were used to assess the performance of the PT SD-OCR sensor in diagnosing anemia. All of the specific sampling conditions can be found in Table S1.

2.2. PT SD-OCR sensor

The details of the PT SD-OCR operation can be found elsewhere [17]. In brief, the sensor is based on a fiber-based common-path interferometer (Fig. S1). In our setup, an 840-nm superluminescent laser diode with a full width at the half maximum bandwidth of 70 nm (Broadband Light Source Modules, BLM-S-840-B-I-20) was directed to the interferometer. In the sample arm, the probe beam was collimated with a diameter of 2.4 mm, and focused on a blood-containing chamber via an achromatic lens (AC254-035-B, Thorlabs). The reflections from the top and bottom surfaces of the inner chamber were then coupled back to the fiber interferometer, and its interference spectrum was measured by a spectrometer (USB 4000, Ocean Optics, Inc). A 532-nm diode-pumped solid state (DPSS) laser (MGL-III-532, CW and DPSS laser, CNI Laser) was employed as a photothermal (PT) excitation light source, as Hb exhibits high absorption at that wavelength [18,19]. The light absorption of Hb at 532 nm was greater by >2 orders of magnitude than at 840 nm.

Upon the illumination by the PT laser, the Hb molecules in the erythrocytes absorb the PT light energy and produce heat, which subsequently changes the refractive index of the blood. As illustrated in Fig. 1, this change in refractive index leads to an optical path-length change between the top and bottom surfaces of the inner chamber (Supplementary Information). We quantify this alteration by measuring the phase changes of the interference signal between the reflections from the 2 interfaces (Fig. S1).

A commercially available quartz cuvette (49-0.1-Q, Starna Cells) was used as a measurement chamber. The thickness of the inner chamber was estimated to be 100 μm. It should be noted that any chamber made of transparent materials may be used in our method.

For measurement, we employed the intensity-modulation scheme to achieve highly sensitive detection of the phase variation (Fig. 1B). The light intensity of the PT laser was set to 10.5 W/cm^2 and the laser was modulated at 1 Hz. The phase fluctuation at only that frequency was measured, and therefore, the effects of the noise components at other frequencies such as vibration could be avoided. The intensity modulation was achieved using an optical chopper (Optical chopper system with the MC1F2 chopper wheel, Thorlabs) in the PT beam path.

The common-path interferometric configuration in the PT SD-OCR sensor removes common-mode noises, thereby enabling high phase stability. However, as the sensor operates in a low-frequency range, other low-frequency noises, such as large bulk motion, still could affect our measurement. In order to further eliminate the effect of these noise components, we used the probe and PT excitation beams with long depths of focus. The depth of focus of the probe beam, which is defined as twice the Rayleigh range, was set to ~1.82 mm. This is significantly larger than the thickness of the measurement chamber (~100 μm). The depth of focus of the PT excitation laser was much larger (~6.64 mm). Therefore, the effect of relative motion between the optical beams and the chamber on the phase measurement could be markedly reduced.

2.3. Measurement procedures

For the [Hb] measurements, blood samples of 10 μl were injected into the chamber, which provided a sufficiently large volume for the measurement. The sample volume could be further reduced down to 5 μl by using a sample chamber with a smaller volume. The blood-containing chamber was then illuminated by the PT SD-OCR probe beam, followed by the modulated photothermal excitation laser. We recorded the phase fluctuation for 10 s, and evaluated the magnitude of its Fourier transform. The magnitude at the PT laser modulation frequency represents the PT SD-OCR signal (Fig. S3). We set the measurement time to be 10 s to achieve a high-sensitivity [Hb] measurement over a large detection range (0.4 g/dl–20 g/dl) (Sec. 3 in the Supplementary Information). In order to obtain the [Hb], the measured PT SD-OCR signals were converted to corresponding [Hb] values based on the calibration curve at a PT laser intensity of 10.5 W/cm^2 (Fig. S4, Table S2) [17].

2.4. Statistical analysis

We used Minitab® 15 (Minitab, State College) to compute Pearson's correlation coefficient (r) for comparison of the measurement results from PT SD-OCR and HemoCue® 201 + sensors against those from ADVIA® 2120i analyzer. Bland–Altman plots were also examined to visualize the comparison. We assessed the precision of PT SD-OCR sensor by examining the standard deviation (SD) and coefficients of variation (CV) based on the twenty repeated measurements at three different Hb concentrations.

3. Results

3.1. PT SD-OCR measurement of anemic blood samples

Whole blood samples from 50 anemic patients were used to investigate the diagnostic capacity of our sensor for anemia. The measurement results from the PT SD-OCR sensor and the hematology analyzers (ADVIA® 2120i and HemoCue® 201 + systems) are presented in Table 1.