Dental Pulp Location and Dentin Thickness Assessment *in Situ* with Diffuse Reflectance Spectroscopy

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Abstract. The modern approach to the treatment of caries requires maximum preservation of tooth tissues and pulp viability, for which it is necessary to know the residual thickness of dentin during its removal. Currently existing methods (Cone-beam Computed Tomography, electrical impedance device, and optical coherence tomography) are not widely used in clinical practice due to the laboriousness of their use or low accuracy. We evaluated the capabilities of the diffuse reflectance spectroscopy (DRS) method for determining dentine thickness *in situ*. Dentin tissues transmit light well in the visible and near-IR range, which makes it possible to detect the optical response of the dental pulp. The pulp contains hemoglobin and water, while dentin contains no hemoglobin, and its water content is less than 10%. Thus, the selection of the contributions of these components allows estimating the thickness of the dentin. Our results show a strong correlation (> 0.9) between dentin thickness and the amplitudes of the water and hemoglobin components. However, hemoglobin content is more susceptible to changes, caused by inflammation or the action of anesthesia. Thus, the most promising approach is use the water component as a proxy. The proposed method can be the basis for the development of a fiber-optic laser probe for clinical dentistry.

Keywords: diffuse reflectance spectroscopy; biophotonics; caries; dentine; dental pulp.

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1 Introduction

Since the minimally-intervention approach was approved by dental specialists, caries early diagnosis and careful removal of carious dentin remain the current issues in routine dental practice [1]. The current concept of caries treatment is focused on preserving pulp vitality, so the criteria for carious dentin removal and following restoration strongly depend on the cavity depth [2]. However, determination of the remaining dentin thickness after cavity preparation is a challenge for dental practitioners as the size of pulp chamber and location of pulp horns may significantly vary between individuals [3]. Cone-beam Computed Tomography (CBCT) is the accurate method for measurement of dentin thickness [4], but it is hardly applicable within the

caries treatment procedure due to radiation dose and inability of chair-side use. Therefore, the chair-side non-radiographic device to determine pulp proximity in carious cavities would be useful for routine dental practice. In recent years, two methods were presented: electrical impedance device [5] and optical coherence tomography [6]. Nevertheless, none of these methods has been widely adopted.

Optical diagnostic systems seem to be a promising tool in dentistry [7–9] due to tooth hard tissues translucency in visible and near-infrared (near-IR) wavelength ranges [10]. The optical properties of tooth hard tissues may significantly change depending on mineral content, for instance, in the case of caries progression or sclerosis [11]. The optical response of the pulp is due to collagen matrix, hemoglobin and water [12]. The detection of optical response of hemoglobin in the light transmission scheme by dopleometry and pulse oximetry was carried out to test the viability of the dental pulp [13–18], but these methods do not allow determining the distance to the dental pulp at the point of interest to the doctor.

Dentin is a dense protein matrix of collagen mineralized with hydroxyapatite crystals. By mass, it consists of hydroxyapatite (70%), organic matter (20%), and water (10%). The absorption spectrum of dentine does not have peaks in the visible and IR regions and falls off exponentially with increasing wavelength [19]. Thus, the dentin transmits light well in the visible and IR range, and scattering does not prevent reaching the pulp at natural thicknesses.

The present work is devoted to the study of the applicability of the diffuse reflection spectroscopy (DRS) method for determining the thickness of dentin. This technique is widely used in biophotonics to determine the composition and thickness of layers of biological tissues such as skin, organ surfaces, etc. [20–24]. Diffuse reflection spectroscopy and imaging were earlier applied for caries detection, dental calculus visualization and investigation of teeth tissues *ex vivo* [25–27]. The principle of its operation is as follows: a broadband light source illuminates the tissue through an optical fiber probe, the reflected light is detected through another fiber and recorded by a spectrometer. Thus, the light scattered and reflected from the tissue characterizes the content of chromophores, which, in turn, can correlate with biologically significant parameters. This fast and minimally invasive method has the potential to be used in clinical practice [20]. In dentistry, it is of interest to develop a method allowing assessment of the state of the dentin tissue using a probe in the form factor of currently used mechanical instruments.

Here, we present an approach for determination of the hemoglobin and water components signal from the tooth DRS spectra. The dental pulp consists of 75–80% water and also contains hemoglobin in the composition of its blood vessels, while hard dentin contains less than 10% of water and does not contain hemoglobin [12]. Therefore, it can be expected, that with a decrease in the thickness of the dentin, an increase in the absorption peak of these chromophores will be observed.

In clinical practice, it is important to determine the thickness of the dentin, regardless of the presence of caries, which affects the absorption of the tooth in the visible spectral region. Therefore, using the near-IR range is a promising and previously unexplored approach. Also, the hemoglobin content in the pulp is affected by the action of anesthesia, and in the case of a non-viable pulp, hemoglobin concentration may be low, however, in this case it is also important to know the distance to prevent infection of the pulp area. Hence, the focus of this paper was investigate the possibility of using water absorption for determination of the dentin layer thickness.

2 Materials and Methods

2.1 Teeth Samples Prepare

For the present study four unrestored non-carious third molars were selected. The teeth were extracted following the orthodontic treatment plan. The teeth were cleaned from periodontal ligament and microbial biofilm with the ultrasonic scaler and antiseptic solution (chlorhexidine 2%), and thoroughly rinsed with water. The roots were separated from the tooth crown with a diamond disk and a low-speed handpiece, the coronal pulp was removed. Then the occlusal surface was prepared with a disk forming the inclined plane. The thickness of the pulp roof was measured by a dental micrometer (minimal size was 1 mm). The prepared samples were stored in saline solution at 5 °C and measured within 1–3 days after removal.

An X-ray of the teeth samples was obtained using a Kodak 2100 intraoral X-ray system.

2.2 Dental Pulp Phantom

The pulp cavity of the extracted teeth was filled with a pulp optical phantom consisting of a collagen sponge (85–88% by weight) and a 1–10% phosphate-buffered saline (PBS) solution of blood (12–15% by weight), which corresponds to the composition and color of a healthy human pulp.

Blood was collected from a finger of a healthy volunteer. To estimate hemoglobin concentration, hemolysis using freeze-thaw procedure was used. Blood was diluted 100 times in water distillate, then suspension was frozen and then thawed to destroy erythrocytes membranes. Obtained solution was centrifuged at 13000 rpm for 5 min. Supernatant part was separated and measured using PerkinElmer Lambda25 spectrophotometer. The value of 130 g/L was obtained from the absorption peak at 540 nm.

2.3 Spectroscopy Setup for Measurements of the Teeth Samples

To measure the transmission spectra of the samples, two optical fibers with 500 μm diameter (IPG Photonics,

Russia) were fixed coaxially at opposite sides of the tooth. The light of a halogen lamp (with a continuous emission spectrum in the range of 400–2000 nm) was directed into one of them, the other was connected to a spectrometer (Maya2000Pro, Ocean Optics, USA) with a spectral resolution of 10 nm. Transmittance coefficient T was calculated as:

$$T = \frac{I_{tr} - I_{dark}}{I_{ref} - I_{dark}}, \quad (1)$$

where I_{tr} is the intensity transmitted by the sample and measured by the spectrometer, I_{ref} is the reference signal intensity, and I_{dark} is the spectrometer dark noise intensity.

The reference signal was measured for LabSphere white standard at a 10 mm distance. The dark noise intensity was measured in a similar way, with the lamp off.

Similar setup was used for measuring DRS. The same fibers were arranged codirectionally with a distance between the centers of the end-faces equal to 1 mm (Fig. 1E). Diffuse reflection coefficient R was calculated as:

$$R = \frac{I - I_{dark}}{I_{ref} - I_{dark}}, \quad (2)$$

where I is the intensity reflected by the sample and measured by the spectrometer.

After that, the optical density was calculated as:

$$OD = -\ln R,\ OD' = -\ln T, \quad (3)$$

for reflectance and transmittance measurements correspondingly.

The water and hemoglobin indexes where estimated as the distance between the peak and background interpolation (see Fig. 1F, G):

$$WI = OD(980) - OD_{bg}(980); \quad (4)$$

$$HbI = OD(575) - OD_{bg}(575), \quad (5)$$

where WI is a water index, $OD_{bg}(980)$ is a background optical density estimated from a straight line connecting points at 920 and 1045 nm, HbI is a hemoglobin index and $OD_{bg}(575)$ is a background optical density estimated from a straight line connecting points at 520 and 610 nm.

To scan the tooth surface, the fiber optic probe was connected to 2D mechanical linear translator (Thorlabs, United States), which made it possible to move the fibers vertically and along the tooth surface in 0.1 mm increments and 0.01 mm accuracy, respectively.

3 Results and Discussion

3.1 Transmission Spectra of the Teeth Samples

Transmission spectra of the teeth were measured in the near-IR range with a water-filled pulp area to determine the effect of water on the spectrum. The optical fibers were fixed opposite to each other, with the tooth placed between them; measurements were performed at points with the minimum and maximum thickness of the dentin (Fig. 2).

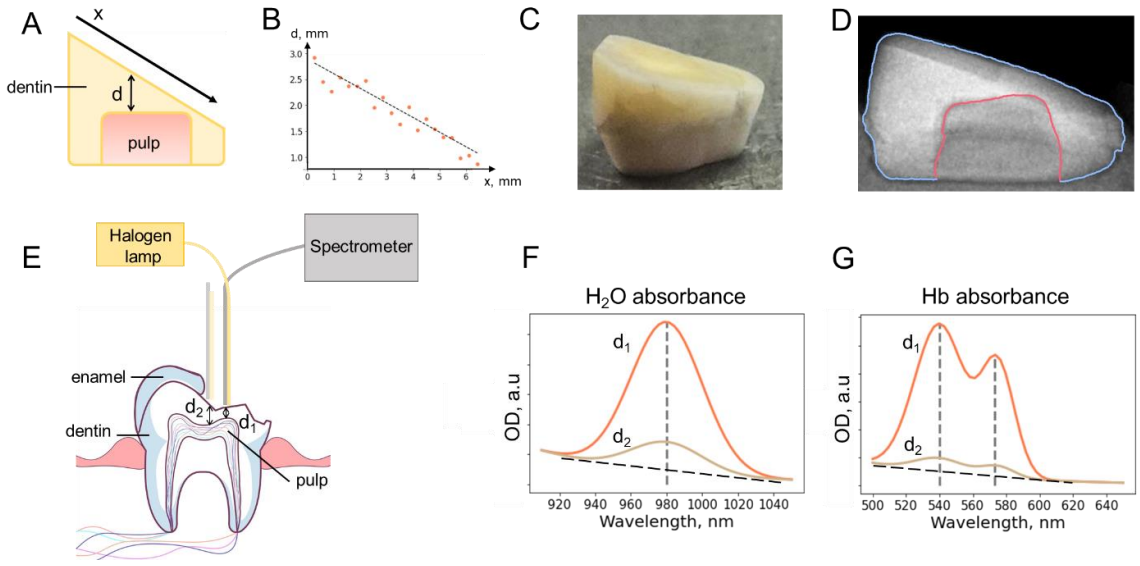

Fig. 1 (A) Schematic images of ground teeth samples. (B) Dependence of the dental thickness on the position of fibers x. (C) Photo of a tooth sample. (D) X-ray image of a tooth sample. The pink line limits cavity of the pulp, the blue one – the contour of the tooth. (E) Optical setup for measuring diffuse reflectance spectra. (F) Schematic representation of optical density in near-IR range (900–1050 nm) and water index calculation. The values of d_1 and d_2 correspond to two positions of the fibers as shown in panel (E). Dashed lines correspond to the maxima of spectra and the background. (G) Schematic representation of optical density in the visible range (500–640 nm) and hemoglobin index calculation. The values of d_1 and d_2 correspond to two positions of the fibers as shown in panel (E). Dashed lines correspond to the maxima of spectra and the background.

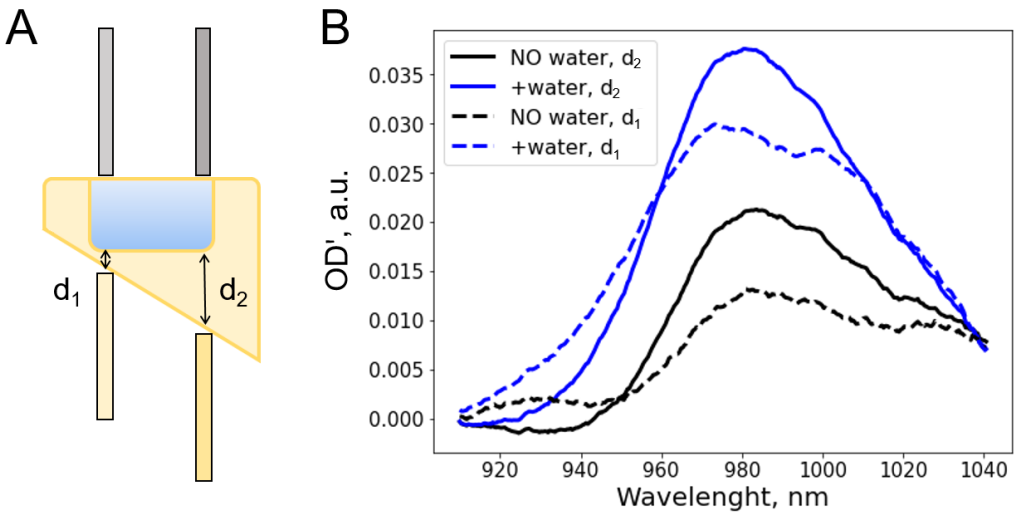

Fig. 2 (A) Schematic images of setup for measuring transmission spectra of tooth sample. d_1 and d_2 are the thickness of the dentin at the measurements points. (B) Optical density spectra of a tooth sample with (blue line) and without (black line) water in thin (d_1, dashed) and thick (d_2, solid) dentin areas (see panel (A)) after subtracting mean value at 905–910 nm.

The detected optical density spectrum in the near-IR range exhibited a peak at 980 nm, corresponding to the water absorption maximum. After filling the pulp with water (blue lines in Fig. 2), a significant increase in the amplitude of this peak was observed, and the magnitude of the changes was similar for points with the minimum and maximum dentin thicknesses. Thus, water in the pulp made a significant contribution to the detected spectrum.

Next, we explored the possibility to assess absorbance of water and hemoglobin in the dental pulp in a reflection scheme using DRS as a proxy of the dentin layer thickness.

3.2 Diffuse Reflectance Spectra of the Teeth Samples

The dependence of the DRS spectra on the thickness of the dentin was studied. For this, scanning was performed with an optical probe along the inclined cut of the tooth with a step of 0.25 mm. During the scan, an increase in the peaks of water and hemoglobin absorption was observed as the thickness of the dentin decreased (Fig. 3A). The dependences of the amplitudes of the peaks of water and hemoglobin on the coordinate on the tooth surface correlated with the profile of the tooth thickness (Fig. 3B–C).

The dependence of peak amplitude can be explained as follows. Typical values of scattering coefficient μ_s and scattering anisotropy g for dentin are 280 cm^{-1} and 0.9, respectively (λ = 633 nm) [28]. Thus, at a depth of about $1/\mu_s' = 1/(\mu_s(1-g)) = 0.35$ mm light from the fiber becomes isotropic. Depending on the thickness of the dentin, the ratio of the response that hits the detector fiber and does not reach the pulp, thus not being absorbed by pulp, increases. If the thickness of the dentin is small, the light propagates in the pulp, as a result, the absorption of water and hemoglobin in the detected signal becomes more pronounced.

Thus, the results shown in Fig. 3 confirm the applicability of the proposed method for determining the dentin thickness.

The content of hemoglobin in the tissues of the dental pulp can vary in a wide range: it increases during inflammation, and decreases as a result of bleeding and anesthesia. To study this effect, we varied the blood concentration in the pulp phantom. A 2-fold decrease in blood concentration in the pulp phantom causes a corresponding 2-fold decrease in the amplitude of the hemoglobin index. With a decrease in blood concentration by an order of magnitude, the hemoglobin index reaches negative values at all points of the scan, meaning that it is impossible to distinguish the contribution of hemoglobin from the background caused by the scattering signal. For the highest hemoglobin concentration, the hemoglobin and water indices correlated with each other (R^2 = 0.88), and with a 2-fold decrease in blood concentration, the correlation dropped to 0.83. Correlation disappeared after reduction of hemoglobin concentration by a factor of 10 ($R^2 < 0.5$).

The water index was practically independent on the hemoglobin concentration and, therefore, is a more robust indicator for its use in clinical practice. Also, the optical properties of dentin in the visible range are more dependent on the mineral composition of the dentin than in the near-IR. To study the relationship between water and hemoglobin indices and dentin thickness, the thickness of dentin tissue was measured using a special caliper with contact points in the form of needles, which made it possible to perform measurements in a narrow pulp cavity.

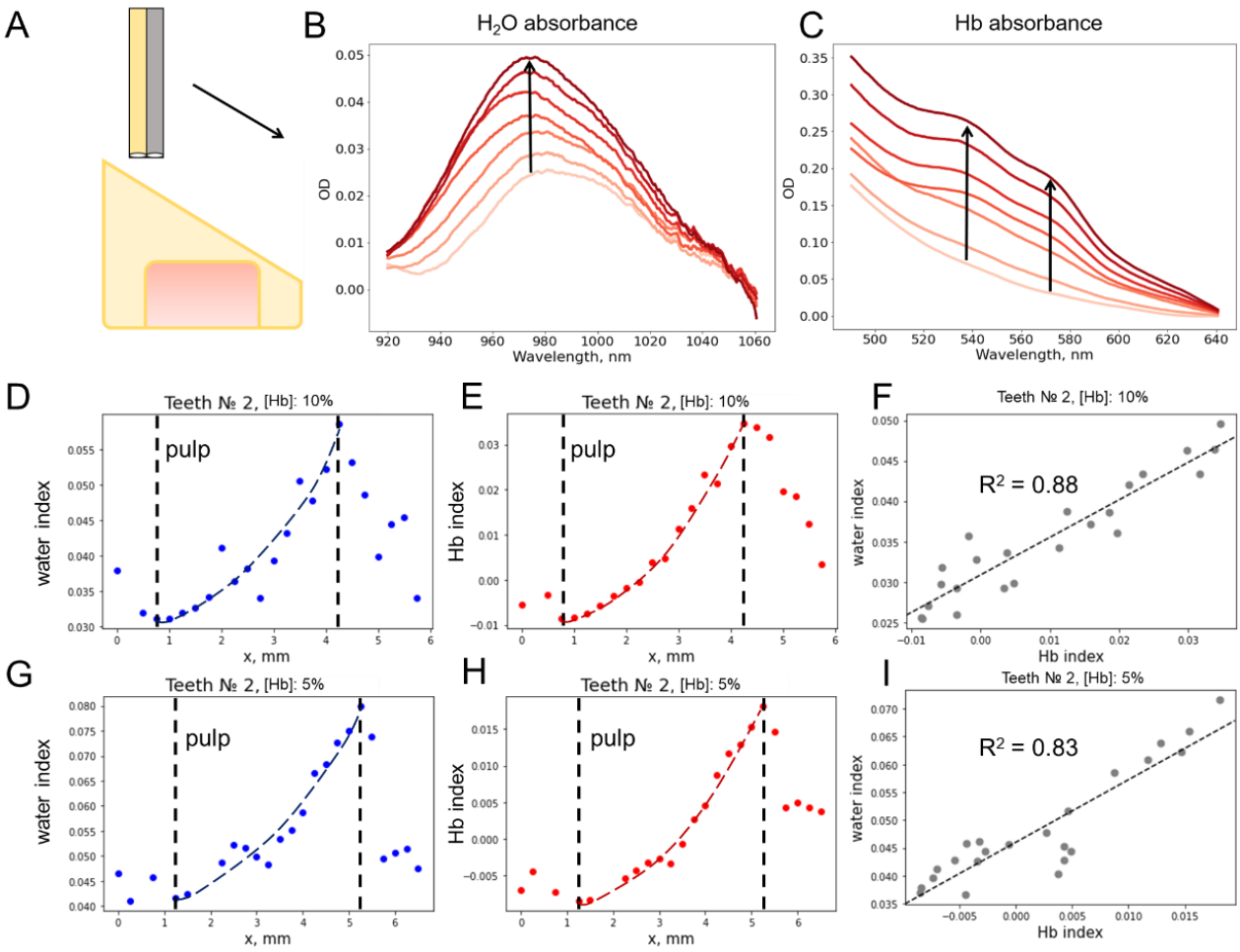

Fig. 3 (A) Schematic images of setup for measuring diffuse reflectance spectra of tooth sample. The arrow shows the scanning direction. (B) Optical density in near-IR range (920–1040 nm) of a tooth sample. The intensity of the spectrum line increases with the scan point. The arrow shows the change in the spectrum when moving along the scan. (C) Optical density in visible range (490–640 nm) of a tooth sample. The intensity of the spectrum line increases with the scan point. The arrow shows the change in the spectrum when moving along the scan. (D) Water index dependence on the coordinate of the scan for highest concentration of blood. (E) Hemoglobin index dependence on the coordinate of the scan for highest concentration of blood. (F) Correlation between water and hemoglobin indexes for highest concentration of blood. (G) Water index dependence on the coordinate of the scan for 2-fold decrease concentration of blood. (H) Hemoglobin index dependence on the coordinate of the scan for 2-fold decrease concentration of blood. (I) Correlation between water and hemoglobin indexes for 2-fold decrease concentration of blood.

A linear relationship was found between water and hemoglobin indices and dentin thickness (Fig. 4) in the pulp area (points between dashed lines in Fig. 3B–C. The correlation coefficients between the indices and the thickness of the dentin were quite large, 0.92–0.96, for high hemoglobin concentrations. When the hemoglobin concentration decreased by a factor of 10 relative to the initial value, the correlation of the hemoglobin index with the thickness became insignificant ($R^2 < 0.5$), thus showing that the water absorption could be preferable compared to hemoglobin absorption for dentin thickness estimation.

4 Conclusions

Determining the thickness of dentin in the process of removing a carious lesion is an urgent clinical task, since at the moment there is no reliable technology. Optical methods allow obtaining information about the tissue quickly and non-invasively. Numerous papers [13–18] describe a method for determining the viability of the pulp based on the detection of the optical signals of hemoglobin in the light transmission scheme. However, these schemes are not suitable for the task of determining the thickness of dentin at the tooth drilling point.

Hemoglobin in the pulp is subject to variations depending on the clinical picture, and absorption in the visible range (where there is a maximum absorption of hemoglobin) is strongly dependent on the mineral composition of the dentin, which changes with caries than in IR. These factors introduce errors in the case of using the hemoglobin signal to determine the thickness of the dentin.

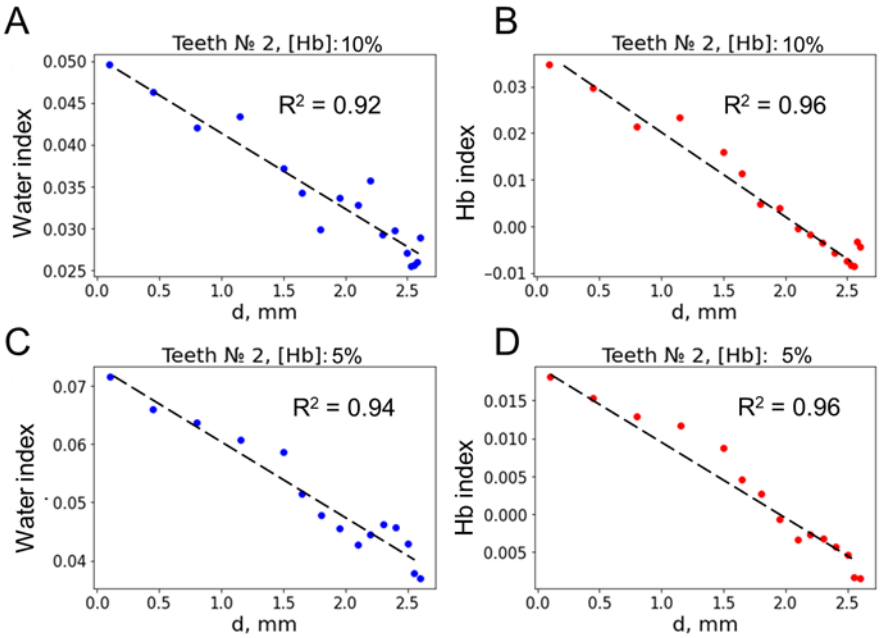

Fig. 4 (A) Correlation between water index and dentin thickness for highest concentration of blood. (B) Correlation between hemoglobin index and dentin thickness for highest concentration of blood. (C) Correlation between water index and dentin thickness for 2-fold decrease concentration of blood. (D) Correlation between hemoglobin index and dentin thickness for 2-fold decrease concentration of blood.

We proposed a new approach to solve this problem using near-IR DRS of water in pulp for dentin thickness estimation. Pulp tissue contains 85–90% of water, while dentin is low in water content, and the inter-patient variability is expected to be less than that for hemoglobin. We observed a linear correlation of water and hemoglobin indices with dentine thickness, as well as their cross-correlation at high hemoglobin concentrations. Thus, measuring the contribution of water, as well as hemoglobin, makes it possible to characterize the thickness of the dentin. It was also shown that with a decrease in the hemoglobin concentration, due to an increase in the error in determining the contribution of hemoglobin, the correlation coefficients of its absorption and thickness decreased. This fact demonstrates the advantages of detecting water in the case of an anesthetized pulp.

Disclosures

The authors declare no competing interests.

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