

Optical Detection of Pulpal Blood

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An optical system to assess blood within the pulpal cavity was developed. The system included a light-emitting diode source, human incisor teeth, and a silicon photodiode detector. An automated syringe pump was used to flow bovine blood lengthwise through the teeth. Transmitted light intensities were recorded at a collection frequency of 20 readings/min and apparent absorbance units were calculated. The effects of blood concentration, blood flow rate, and pulp chamber size were investigated. It was found that changes in blood flow rate did not cause significant changes in transmitted light intensity. Increases in blood concentration and pulp chamber size resulted in larger absorbance values. These results suggest that photoplethysmography measurements may be sensitive to the amount of blood in the pulp chamber as it reflects the pulsatile-related expansion and contraction of capillaries within the pulp tissue.

Photoplethysmography has been suggested as a potential non-invasive method to detect vascularity within the dental pulp (1, 2). Recent optical techniques demonstrate distinct, reproducible intensity changes due to blood volume within the pulp chamber (3). The concept of the measurement is to pass a selected band of light through the tooth while continuously monitoring the intensity of the transmitted light. In vivo experiments with both human (4, 5) and animal (6, 7) subjects clearly reveal pulsatile variations in intensity-time plots during photoplethysmographic experiments. The correct interpretation of pulsatile-related intensity variations requires a detailed knowledge of the influence of critical physiological parameters on the measured optical signal. The shape and frequency of these features suggest that they are caused by the dynamics of blood associated with vascularity within the pulp chamber. Still, the exact nature of these intensity-time features is unclear.

The purpose of this study was to establish the physical parameters of pulpal vascularity that affect the intensity-time plots of photoplethysmography. An optical system was designed and a series of in vitro experiments was performed to systematically investigate the influence of blood concentration, blood flow rate, and pulp chamber size on the intensity-time profile.

MATERIALS AND METHODS

An optical system using solid-state components was designed. The system included a rectangular (5-mm × 3-mm × 1-mm) light-emitting diode (LED) powered by a 3-V battery as the light source. The light emission characteristics of this LED were analyzed with a SPF-500C spectrofluorometer (SLM-Aminco, Urbana, IL). The LED had a peak output at 576 nm with a 36-nm bandwidth at half the peak intensity. A silicon photodiode detector (S2386-18K; Hamamatsu Corp., Bridgewater, NJ) with a spectral range of 400 to 1100 nm was used in a photovoltaic mode that required no external power supply. The detector output was interfaced directly to an 8088-based computer.

A flow-through system was designed whereby autoclaved human teeth were used as cuvettes. A coronal access to the pulp space was made and the pulpal tissue was extirpated. Clear polyethylene tubing (Butterfly infusion set tubing; Abbott Laboratories, North Chicago, IL) was sealed to the coronal pulp access and connected to a syringe infusion pump (model 55-2219; Harvard Apparatus, South Natick, MA). The pump was capable of producing desired flow rates of solutions lengthwise through the teeth. The LED and detector were positioned directly on opposite surfaces of a tooth. Custom made acrylic holders assured reproducible positions. The experimental set up was housed within optical carriers and holders on an optical rail (Melles Griot, Irvine, CA) to rigidly position and align all components. To control light conditions, ambient light was eliminated during data collection. A schematic of the optical arrangement is shown in Fig. 1.

Unless specified otherwise, the following parameters and procedures were used throughout. Transmitted light intensities were collected as a function of time at a collection

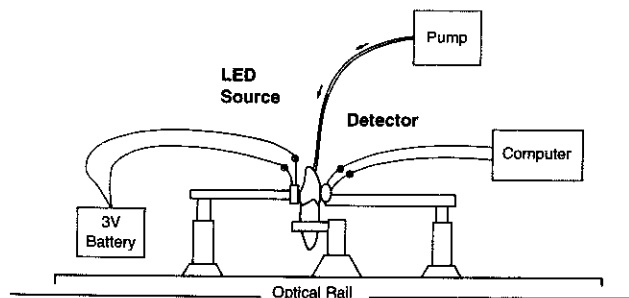


FIG 1. Optical arrangement.

frequency of 20 readings/min. Typically, the intensity values were collected over a period of 1 min. The solution flow rate was maintained at 0.1 ml/min. Fresh bovine blood was treated with 1.5 g/ml of EDTA as an anticoagulant and was stored at 4°C until use. Apparent absorbance values were calculated according to the equation ($A = -\text{Log}(I/I_0)$), where I and I_0 correspond to the measured light intensities with blood and saline (0.15 M NaCl), respectively, in the pulpal chamber.

Part 1. System Reproducibility: Method and Results

The reproducibility of the experimental system was established by monitoring the detector output while cycling saline and bovine whole blood through the chamber of a mandibular central incisor. The results from this experiment are presented graphically in Fig. 2. Transmitted intensities for the air-filled chamber were recorded. Saline and whole blood solutions were then intermittently flowed through the tooth 10 times for 1 min each. Between solutions, data collection was discontinued as the pulp space was thoroughly flushed with distilled water. The mean intensity values across the 10 trials for saline and whole blood were 1.163 and 0.334, respectively, and the corresponding pooled standard deviations were 0.016 and 0.016, respectively.

Part 2. Blood Concentration: Method and Results

The effect of blood concentration was then assessed by sequentially passing solutions with different amounts of bovine blood through the incisor tooth. A series of blood solutions was prepared by serial dilution of the stock bovine blood with saline. Whole blood and blood-saline solutions of 1:1, 1:2, 1:4, 1:8, and 1:16 were pumped through the incisor tooth in order of ascending concentration. The pulp space was not irrigated between solutions but data collection was interrupted long enough to change solutions. When the pump was restarted, data collection was resumed over the period of 1 min for each solution. Representative intensity-time data for one experimental trial are presented in Fig. 3. An increase in the number of blood cells in the optical path caused a decrease in the intensity of detected light.

A plot of apparent absorbance versus relative blood concentration for two experimental trials is presented in Fig. 4. The relative concentrations were calculated based on the blood to saline ratio. Thus, a whole blood solution represents a blood relative concentration of one, the 1:1 solution equals a relative concentration of 0.5, a 1:2 solution equals 0.25, and so on. Finally, a pure saline solution would represent a blood relative concentration of zero. Figure 4 reveals a nonlinear relationship where higher relative blood concentrations result in larger apparent absorbances corresponding to lower light transmittance.

Part 3. Flow Rate: Method and Results

The effect of flow rate was investigated for the bovine whole blood and the 1:16 blood-saline solution. In this experiment, the pump flow rate was varied to include rates of 0, 0.001, 0.01, 0.05, 0.1, 0.2, and 0.5 ml/min. The transmitted light intensities for each flow rate were recorded over 1 min. Data

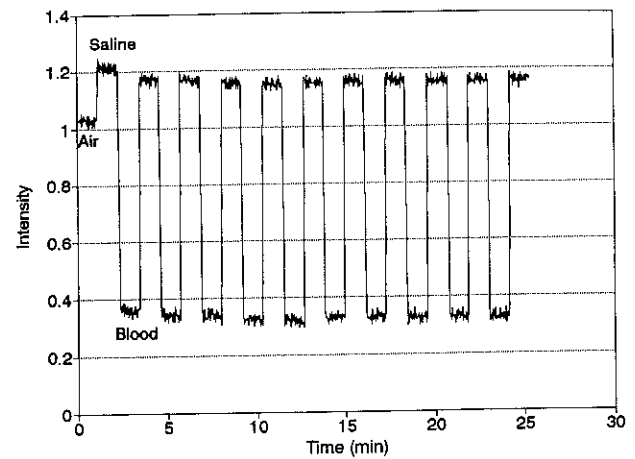


Fig 2. Representative pattern of transmitted light intensities obtained by whole blood and saline solutions through a mandibular incisor.

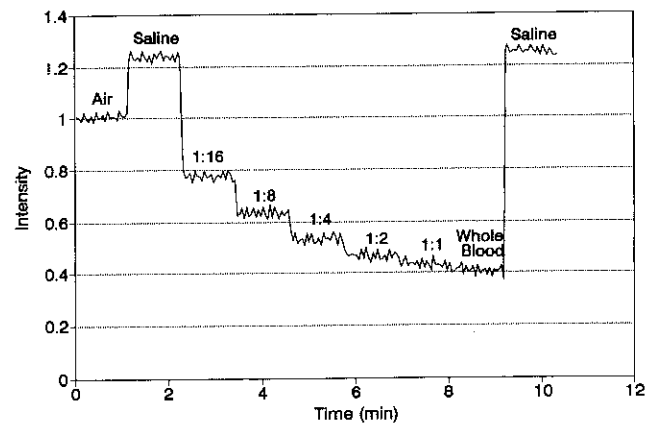


Fig 3. Representative pattern of transmitted light intensities obtained by various blood to saline solutions through a mandibular incisor.

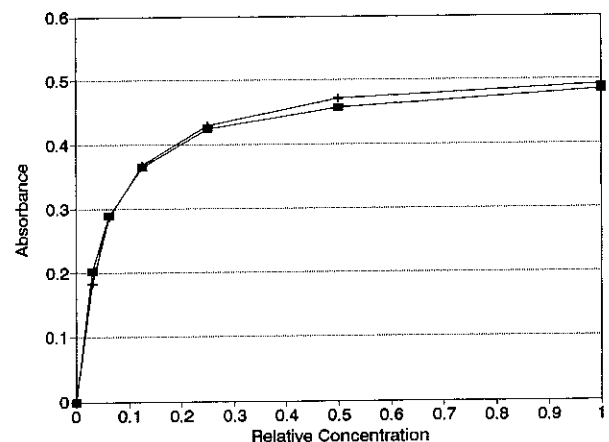


Fig 4. Relative concentration versus absorbance units of two experimental trials of a mandibular incisor.

collection between flow rates was only interrupted long enough to change the flow rate in the pump assembly. Transmitted light intensities for two experimental trials of the whole blood and the 1:16 solution at the various flow rates are

presented in Fig. 5. Changes in blood flow rate did not demonstrate significant changes in transmitted light intensity.

Part 4. Pulp Chamber Size: Method and Results

The effect of pulp chamber size was evaluated in mandibular canine and premolar teeth. As before, whole blood and blood-saline solutions of 1:1, 1:2, 1:4, 1:8, and 1:16 were pumped through the teeth at a rate of 0.1 ml/min and transmitted light intensities were recorded as a function of time. Gates Glidden burs (Union Broach, Long Island City, NY) were used to enlarge the pulp chambers approximately 2-fold, the solutions were again pumped through the teeth, and transmitted light intensities were measured. Radiographs of the teeth were measured to give approximate linear buccolingual (B-L) and mesiodistal (M-D) dimensions of the pulp chamber in the area of light collection. Mean intensity values were used to calculate apparent absorbance values and the corresponding plots of apparent absorbance versus relative blood concentration for two repetitions of the canine tooth are presented in Fig. 6. An identical pattern was obtained for the premolar tooth. Table 1 summarizes the mean absorbance values of two experimental trials for each tooth. Considerably larger absorbances were measured as the volume of the pulp chamber was increased. The percentage of increase in absorbance due to change in canal size was tabulated for each concentration (Table 1). For the canine tooth, a 165% mean increase in light absorption was noted as the pulp chamber volume was increased 181%. The premolar tooth exhibited a mean light absorption increase of 158% with a corresponding 269% increase in pulp chamber volume.

DISCUSSION

The results presented in Fig. 2 show the effect of whole blood on the magnitude of the transmitted light. By replacing saline with whole blood, less light reaches the detector, presumably due to scattering of the incident radiation by the blood cells. The simple optical system used in this study was able to record consistent, reproducible changes in light intensity. The coefficients of variance for the pooled intensity values are only 1.4% and 4.7% for saline and whole blood, respectively. These findings indicate that the experimental protocol is effective at completely filling the chamber and avoiding carry-over between solutions. Additionally, the stability and low noise of the optical arrangement are evident from these results.

Figures 3 and 4 illustrate the relationship between amount of blood in the pulpal chamber and the amount of light transmitted through the tooth. As the relative blood concentration increases, less light is detected because more incident light is scattered by the larger number of blood cells in the optical path. Turbidimetric methods of this nature are typically treated in a fashion analogous to Beer's law where an apparent absorbance is calculated from intensities measured in the presence and absence of the scattering medium ($A = -\log(I/I_0)$). A linear relationship is usually obtained between the apparent absorbance and concentration of the suspended particles (8). A plot of the collected data, however, reveals a sharply nonlinear relationship (Fig. 4). This nonlinear relationship is characteristic of a transmission experiment where

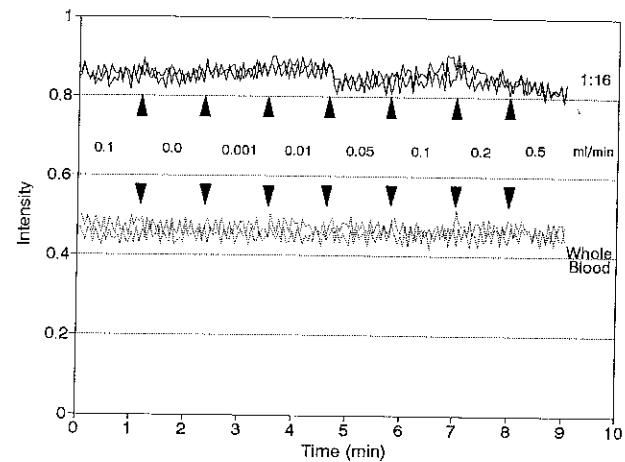


FIG 5. Transmitted light intensities obtained over time at varying flow rates (ml/min) for whole blood and a 1:16 blood to saline solution. Arrowheads denote flow rate change.

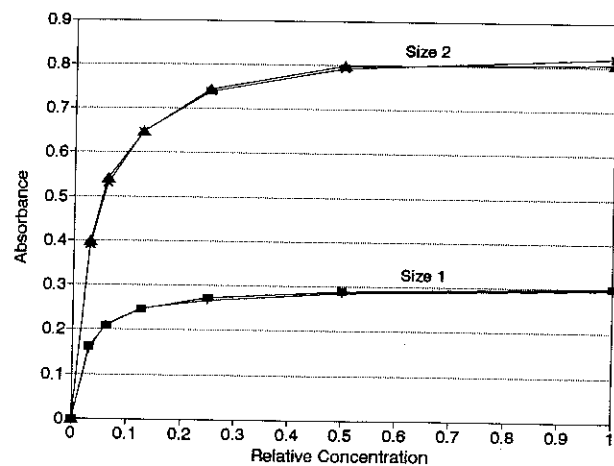


FIG 6. Relative concentration versus absorbance units of two experimental trials of a mandibular canine at two different pulp chamber sizes. Size 1, initial size; Size 2, enlarged size.

only a fraction of the total detected light actually passes through the scattering medium of interest (8). Due to the small size of the pulp chamber, a significant fraction of the detected light passes through the tooth but bypasses the pulpal chamber and is, therefore, not scattered by the blood cells. This "nonpulpal" light provides a constant background intensity that is independent of the relative concentration of blood cells in the pulpal chamber. As the number of blood cells increases, nearly all of the light passing through the pulpal chamber is scattered, thereby resulting in a maximum or limiting apparent absorbance. The data presented in Fig. 3 demonstrate that although the relative blood concentration is doubled between the 1:1 and whole blood samples, the change in detected light intensity is minimal. This result indicates that nearly all of the light entering the pulpal chamber is scattered by the 1:1 solution.

This explanation for the nonlinear Beer's law plots is corroborated by the results presented in Fig. 6. As the pulp chamber is enlarged, a larger fraction of the incident radiation passes through the pulpal chamber, thereby decreasing the intensity of the nonpulpal light. Greater limiting absorbances

TABLE 1. Mean absorbance obtained from canine and premolar teeth initially and after pulp canal space enlargement

Tooth	Blood:Saline Relative Concentration	Mean Absorbance of Initial Size	Mean Absorbance of Enlarged Size	Calculated Absorbance Increase (%)
Mandibular canine*	0.03125	0.1582	0.3945	149
	0.0625	0.2090	0.5348	156
	0.125	0.2466	0.6469	162
	0.25	0.2698	0.7404	174
	0.5	0.2872	0.7954	177
	1	0.2981	0.8116	172
	(whole blood)			Mean 165
Mandibular premolar†	0.03125	0.1252	0.3191	155
	0.0625	0.1674	0.4470	167
	0.125	0.2152	0.5610	161
	0.25	0.2530	0.6629	162
	0.5	0.2893	0.7150	147
	1	0.3055	0.7816	156
	(whole blood)			Mean 158

* Initial size: M-D = 1.2 mm, B-L = 1.5 mm; enlarged size: M-D = 2.2 mm, B-L = 2.3 mm.

† Initial size: M-D = 1.3 mm, B-L = 1.3 mm; enlarged size: M-D = 2.6 mm, B-L = 2.4 mm.

are expected because the scattered light occupies a larger fraction of the total amount of transmitted light. These results indicate the method is applicable to teeth with large or small pulp chambers, although the minimum size is unknown at this time.

The findings presented in Fig. 5 indicate that light transmittance measurements while responsive to blood volume are insensitive to solution flow rates. This feature represents a fundamental difference between light transmittance measurements and Doppler flowmetry. Light transmittance depends on the number of blood cells in the optical path; not the rate at which these cells are moving. The viability of pulpal tissue is dependent on pulpal blood flow, however. Laser Doppler flowmetry has been successful in the detection of blood flow changes (9-11), but this method is unable to quantitate blood volume directly and is adversely affected by movement and saliva contamination (12). In addition, recent studies have estimated that approximately 14% of the area of the pulp is occupied by vessels and that Doppler instruments are linear only if the moving blood cells occupy no more than 1% of the tissue volume (13). In this regard, light transmittance and Doppler flowmetry could provide complementary information.

Previous *in vivo* photoplethysmography studies have reported that the changes in light intensity recorded as a function of time represent variations in blood distribution rather than blood volume pulses (14). Inasmuch as the pulp tissue is encapsulated by rigid walls, it is doubtful that blood pulsations rapidly cause changes in pulp canal size. The *in vitro* experiments performed in this study suggest that light intensity changes are caused by variance in the number of blood cells within the pulp chamber during vascular pulsations. The periodic expansion and contraction of the capillaries that feed the tissue within the rigid pulp chamber are likely responsible for this phenomenon. Capillary expansion results in a larger volume of blood in the optical path which causes an increase in light scattered and lower light transmitted through the tooth.

Enhancements in optical detection are anticipated by further optimizing the wavelength and radiation source specifi-

cations. A previous study reported the optimal wavelength for tooth plethysmography to be 540 to 580 nm (5). The wavelength of 576 nm was used in this study because it is within that range. Indeed, the chosen wavelength was capable of penetrating the human incisor, canine, and premolar teeth without creating interferences due to heat. The positioning apparatus maximized the signal while minimizing noise and drift. Lower signal-to-noise ratios were noted, however, with an increase in tooth thickness as a result of less light passing through enamel and dentin. In the future, the use of longer wavelengths may offer higher optical throughput by providing greater penetration depths into the tooth matrix and brighter incident intensities. Also, less diffuse LED sources may provide considerably greater intensities by funneling the generated light into a narrow beam that can be directed toward the pulp.

The results of this study support the feasibility of optical techniques for the detection of blood variations within pulp chambers of varying sizes. Single wavelength optical measurements, however, will not differentiate between healthy and unhealthy tissue since any material capable of absorbing light will give readings different from those of an empty pulp. Further studies involving two or more wavelengths are needed to provide information pertaining to critical blood components such as total hemoglobin concentration and oxygen saturation levels.

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