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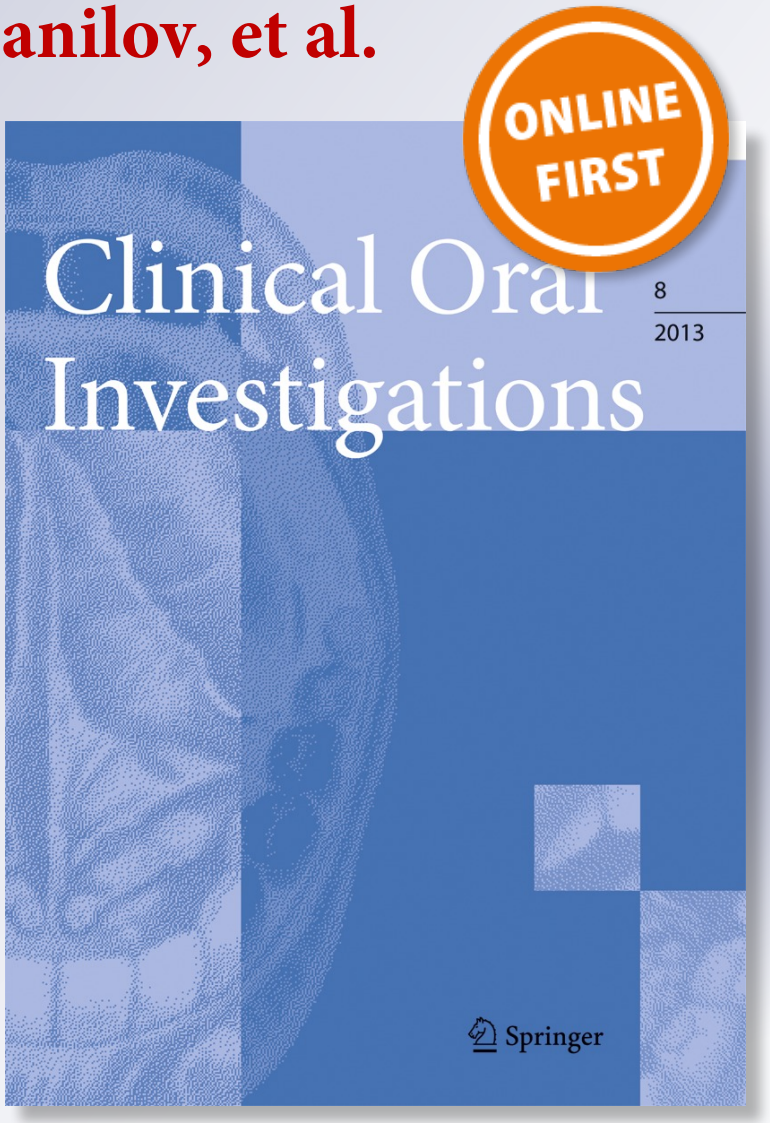

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ORIGINAL ARTICLE

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Abstract
Objective Noninvasive optical methods such as photoplethysmography, established for blood pulse detection in organs, have been proposed for vitality testing of human dental pulp. However, no information is available on the mechanism of action in a closed pulp chamber and on the impairing influence of other than pulpal blood flow sources. Therefore, the aim of the present in vitro study was to develop a device for the optical detection of pulpal blood pulse and to investigate the influence of different parameters (including gingival blood flow [GBF] simulation) on the derived signals.
Materials and methods Air, Millipore water, human erythrocyte suspensions (HES), non-particulate hemoglobin suspension (NPHS), and lysed hemoglobin suspension (LHES) were pulsed through a flexible (silicone) or a rigid (glass) tube placed within an extracted human molar in a tooth–gingiva model. HES was additionally pulsed through a rigid tube around the tooth, simulating GBF alone or combined with the flow through the tooth by two separate peristaltic pumps. Light from high-power light-emitting diodes (625 nm (red) and 940 nm (infrared [IR]); Golden Dragon, Osram, Germany) was introduced to the coronal/buccal part of the tooth, and the signal amplitude [ΔU, in volts] of transmitted light was detected by a sensor at the opposite side of the tooth. Signal processing was carried out by means of a newly developed blood pulse detector. Finally, experiments were repeated with the application of rubber dam (blue, purple, pink, and black), aluminum foil, and black antistatic plastic foil. Nonparametric statistical analysis was applied ($n=5$; $\alpha=0.05$).
Results Signals were obtained for HES and LHES, but not with air, Millipore water, or NPHS. Using a flexible tube, signals for HES were higher for IR compared to red light, whereas for the rigid tube, the signals were significantly higher for red light than for IR. In general, significantly less signal amplitude was recorded for HES with the rigid glass tube than with the flexible tube, but it was still enough to be detected. ΔU from gingiva compared to tooth was significantly lower for red light and higher for IR. Shielding the gingiva was effective for 940 nm light and negligible for 625 nm light.
Conclusions Pulpal blood pulse can be optically detected in a rigid environment such as a pulp chamber, but GBF may interfere with the signal and the shielding effect of the rubber dam depends on the light wavelength used.
Clinical relevance The optically based recording of blood pulse may be a suitable method for pulp vitality testing, if improvements in the differentiation between different sources of blood pulse are possible.

Keywords Dental pulp · Vitality · Blood flow · Laser Doppler flowmetry · Photoplethysmography · Red light · Infrared light · Transmission · Spectrum

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Introduction

In daily clinical practice, dental pulp vitality is assessed by applying hot or cold stimuli or electric current. In doubtful cases, even exploratory trepanation of the respective tooth without anesthesia has been recommended [1]. Notably, Mullaney et al. [2] showed that even such exploring trepanations as a last resort may lead to erroneous results because, in their study, trepanation of "non-vital" teeth proved to be painful in certain cases even though the nerve fibers were already degenerated [2]. Also, in patients with trauma, contused nerves may not respond to the previously mentioned testing methods, despite functional blood circulation [3]. Furthermore, in special clinical situations (e.g., treatment of handicapped patients, of children, or under general anesthesia), patients cannot report on nerve responses. As a result, presently available pulp vitality tests, which rely on nerve response, may be regarded unreliable and in need of significant improvement [4].

Blood flow is a commonly used indicator for tissue vitality [5]. Several methods have been proposed to assess dental pulp vitality in patients by measuring the pulpal blood flow (PBF) including laser Doppler flowmetry (LDF) [6], photoplethysmography (PPG) [7], and pulse oximetry (PO) [8]. LDF is based on the amount of moving blood cells and their velocity [6], and it has been advocated for pulp blood flow measurement because it is noninvasive and the technology is commonly used in clinical routine for several non-dental applications [6, 9–12]. However, it was also shown that scattering of light in the tooth [13] and even small movements of the patient [14, 15] lead to disturbances, making it difficult to distinguish between the blood flow through the dental pulp and that through the surrounding tissues [16]. The use of black rubber dam shielding lead to a 70 % reduction of the LDF signal and the authors hypothesized that the remaining part of the signal originated from the dental pulp [17]. Nevertheless, other studies suggest that the human tooth could function as a light guide refracting the laser light down into the alveolar bone, which might lead to signals originating from the surrounding oral tissues [13, 14, 16–19]. Consequently, the clinical reliability of LDF systems was regarded questionable [19]. Ultrasound Doppler imaging was also used in vivo to compare the PBF in vital and root canal filled teeth. In vital teeth, the authors observed pulsating signals, characteristic for arterioles, whereas the signals derived from the root canal filled teeth had a rather linear, non-pulsating form [20]. However, the test device is not generally available in the market.

PPG is a noninvasive optical method to detect blood pulse and blood oxygenation in organs via the expansion of the blood vessels synchronous to the cardiac cycle. It is used in finger and ear lab sensors in daily medical routine [21]. For dental pulp vitality testing, Daley et al. [4] used PPG in combination with elaborate signal processing and verified in vivo up to 70 % of the tested vital teeth as vital, whereas without signal processing, only 20 % could be identified as vital [4]. Using a 576-nm light-emitting diode (LED), Diaz-Arnold et al. [7] showed in vitro that light transmission through the tooth changed according to the erythrocyte concentration of the test media, whereas blood flow velocity did not have any effect at this particular wavelength [7]. These authors also observed a decrease in the signal-to-noise ratio with increasing tooth thickness. Because the pulp is encapsulated in dental hard tissue, they found it to be unclear whether PPG signals derived from the dental pulp resulted from the periodic expansion of blood vessels or not. The authors rather suggested that the signals resulted from pulsatile changes in blood cell numbers inside the dental pulp [7]. No data were found concerning the effect of gingival blood flow (GBF) on tooth PPG measurements.

PO is also used in finger and ear lab sensors (see the previous paragraph), being closely related to PPG. PO is based on absorption measurements of oxyhemoglobin and deoxyhemoglobin and takes advantage of the fact that the absorption of red and infrared (IR) light changes according to the oxygenation grade of hemoglobin [22]. With this method, Schnettler et al. [8] could distinguish between vital and endodontically treated teeth. Nevertheless, further investigations on the reliability of this method were warranted by the authors [8].

Recently, we could show that, depending on the wavelength of the transmitting light, blood flow velocity may indeed have an influence upon light transmission through the tooth and that, at certain wavelengths, the dental hard tissue became rather translucent, whereas blood adsorbed this light [23]. Based on these data, we hypothesized that optical blood pulse detection has the potential to be used for dental pulp vitality testing. Necessary conditions to be fulfilled are, among others, (a) that the signal-to-noise ratio can be optimized by constructing a potent data acquisition system and that blood pulse signals derived from fluids containing corpuscular structures can be detected not only in a flexible environment (in analogy to the classical PPG), but also in the "rigid" pulpal chamber which is surrounded by dental hard tissues; (b) that an optimal wavelength is found; (c) that blood pulse signals derived from surrounding tissues like the gingiva/periodontium do not interfere with signals derived from the pulp; or (d) that adequate measures may be taken to prevent interference. Therefore, the aim of the present study was to develop an optical device with emitting light on one side of a tooth and recording light intensity on the other side, together with a low noise preamplifier and a data recorder for detecting pluses. This device was used to detect in vitro signals after pulsated perfusion of a tooth–gingiva model (TGM) under different conditions: (a) with different liquids containing particles or not, (b) using both rigid and flexible tubes inside a tooth simulating the pulp in the TGM, (c) using two different wavelengths (red and IR), (d) with or without

simultaneous simulation of GBF, and (e) applying different shielding devices.

Materials and methods

Experimental configuration

The experimental apparatus is shown in Figs. 1 and 2. It consisted of:

- Two peristaltic pumps (Ecoline VC-280; Ismatec, Glattbrugg, Switzerland);
- A beaker with the test liquid (Millipore water or red blood cell suspension in different conditions);
- The optical recording device including light source and sensor;
- The blood pulse measuring device including the amplifying electronics and readout;
- The TGM, consisting of a human tooth in a resin base.

A cylindrical hole was cut in the occlusal–apical direction into the center of a freshly extracted and cleaned human molar. To simulate the PBF, a (rigid) glass tube (outer/inner diameter, 2.0/1.0 mm) or a (flexible) silicone tube (outer/inner diameter, 2.0/1.0 mm) was placed through the hole and connected to one of the two peristaltic pumps. For simulating GBF, an acrylic resin (Paladur clear; Heraeus Kulzer, Hanau, Germany) base was constructed, which contained the root of the tooth, and around the root, a circular channel of 3 mm diameter was fabricated inside the resin (Fig. 2c) using the lost wax technique. This channel was connected via standard tube connectors to the second peristaltic pump. Both pumps were adjusted to deliver a flow of 25 ml/min at a frequency of 1 Hz. Fluid pressure [P, in millimeters of mercury] was measured by pressure sensors (40PC015G1A, Honeywell, Morristown, NJ, USA) for pulpal and gingival circulatory systems, and average pressures of 43 mmHg for the PBF and 17 mmHg for the GBF were recorded by a sensor interface (ScienceWorkshop 500 Interface, PASCO Scientific, Roseville, CA, USA) connected to a laboratory PC.

The optical unit consisted of high-power LEDs (Nr. 6656189, 625 nm (red) and Nr. 389378, 940 nm (IR); Golden Dragon, Osram, Germany), a sensor (SFH229, Siemens, Munich, Germany), and a multi-fiber optical cable (LEONI Fiber Optics GmbH, Berlin, Germany). The LEDs were connected via the multi-fiber optical cable to a custom-made sensor holder (Fig. 2b). The sensor was directly integrated into the sensor holder (Fig. 2a). Light from the LEDs was, thus, introduced via optical cable onto the buccal surface of the molar crown, and transmitted light was recorded by the sensor at the opposite side.

An individually designed pulse detector/amplifier was developed [24] based on a cascade connection of three passive low-pass filters (RC networks) and inverting amplifiers. Signal amplitude [ΔU, in volts] was derived from visualized voltage recordings (see Figs. 3 and 4) using a sensor interface (ScienceWorkshop 500 Interface, PASCO Scientific) connected to a laboratory PC.

For pulsed perfusion, air, Millipore water (Type 08.2217; TKA Wasseraufbereitungssysteme GmbH, Niederelbert, Germany), or human erythrocyte suspensions (HES) in different conditions were used. The HES was obtained from the blood bank (University Hospital of Regensburg, Regensburg, Germany) in the form of a blood conserve. Additional to the standard anticoagulant ingredients of blood conserves, Synperonik (Synperonik F68; SERVA Electrophoresis GmbH, Heidelberg, Germany) diluted with physiological saline solution was added (600 μl 10 % Synperonik/NaCl solution+59.4 ml HES) for all in vitro experiments. For determining the influence of particles, HES was lysed by ultrasound (Elmasonic S30H; Elma, Singen, Germany) treatment for 1 h and centrifugation at 9,000 rpm for 30 min. The resulting supernatant was used (1) as non-particulate hemoglobin suspension (NPHS) containing no cell fragments and (2) after agitation of the supernatant and the cell fragments as lysed hemoglobin suspension (LHES). Furthermore, HES, NPHS, and LHES were submitted for scanning electron microscopy (SEM) in low vacuum mode (FEI Quanta 400F; FEI, Eindhoven, Netherlands). To prevent the hemoglobin suspensions from crystallizing, no Synperonik was added. Fresh human blood served as a control and was derived using a standard EDTA blood collection tube (Monovette 2.7 ml, Lot 1094705; SARSTEDT, Nümbrecht, Germany).

Experimental procedures

(a) Pulsed perfusions of HES, LHES, NPHS, air, and Millipore water were performed with rigid or flexible tubes at the two wavelengths in the TGM.
(b) HES, LHES, NPHS, and controls were morphologically visualized in the scanning electron microscope.
(c) HES was pulsed through the glass tube (pulp simulation) and the circular channel (gingiva simulation) in the TGM simultaneously, but with a 90° phase shift in three different ways: (1) tooth alone (TA), (2) gingiva alone (GA), and (3) tooth and gingiva (TAG).
(d) Shielding procedures: Gingival shielding was provided by rubber dam (blue (0.21 mm), purple (0.193 mm), pink (0.206 mm) (Coltene/Whaledent GmbH, Langenau, Germany) and black (0.079 mm) (GLYDE Health Pty Ltd, Lindfield, Australia)). The black rubber dam was also used in two layers. Furthermore, aluminum foil (standard, 0.015 mm) and black antistatic plastic foil (standard, 0.077 mm) were used. Aluminum foil and antistatic plastic foil were fixed to the TGM by clear double-faced adhesive tape (standard, 0.098 mm), whereas the rubber

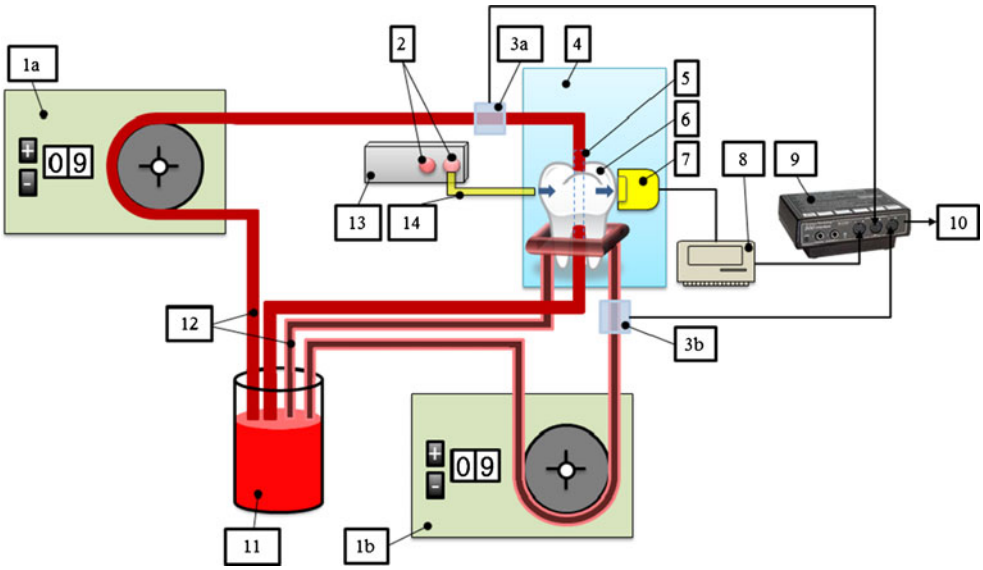

Fig. 1 Schematic drawing of the experimental setup: peristaltic pumps (*1a*/*b*), high-power LEDs (*2*), pressure sensors (*3a*/*b*), sensor holder (*4*), rigid or flexible tube (*5*), human tooth (molar) with PBF simulation and additional blood flow around the root (TGM) (*6*), photodetector (*7*), photoplethysmographic measuring and amplifier device (*8*), sensor interface (*9*), laboratory PC (*10*), test liquid (*11*), silicone tubes (*12*), light source (*13*), multi-fiber optical cable (*14*)

dam was punched and applied to the TGM without any further fixing device.

(e) The HES suspension was changed after every second measurement.

Data treatment

Resulting voltage signals were graphically displayed (Fig. 3) using the Data Studio software (volume 1.9.8; PASCO Scientific). For each experiment, signals were recorded for 5 s and signal amplitude [$\Delta U = U_{max} - U_{min}$, in volts] was calculated for every second signal amplitude by subtracting U_{min} from U_{max} (Fig. 4). The median was used as the representative result for this experiment. Five independent experiments were performed for each group. All results for ΔU in volts (medians with 25–75 % percentiles) were statistically analyzed using the SPSS software (SPSS Statistics 20, IBM, Ehningen, Germany) and presented as bar charts. Nonparametric Mann–Whitney *U* test and error rates method were applied on the 0.05 level of significance.

Results

Rigid vs. flexible tubes

Signal amplitude (ΔU) values for the flexible silicone tubes and the rigid glass tubes are shown in Fig. 5 for the two wavelengths measured and for the different preparations used. For HES, signal amplitude was significantly lower for the rigid tube compared to the flexible silicone tube at both wavelengths. ΔU for IR light was significantly higher than that for red light using the flexible tube, whereas for the rigid glass tube, ΔU was significantly higher for red light. Nevertheless, a signal could be detected for HES in all cases.

HES vs. LHES, NPHS, air, and water

For LHES compared to HES, ΔU values were significantly higher for both tube materials when IR light was used, whereas for red light, ΔU was significantly lower (Fig. 5). For all conditions, only very low signals could be derived for NPHS and they were statistically different from HES and LHES. No

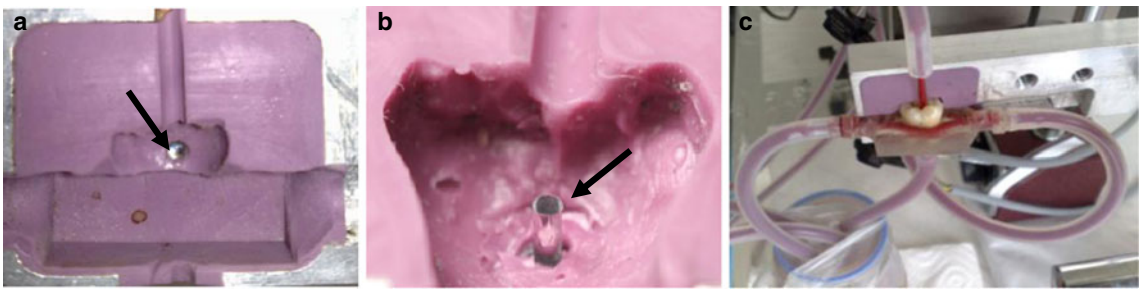

Fig. 2 Sensor holder with **a** photodetector (*arrow*) and **b** multi-fiber optical cable (*arrow*). **c** Sensor holder with TGM—silicone tubes connected to a glass tube (pulp) and a rigid plastic channel (gingiva) for dental blood flow simulation

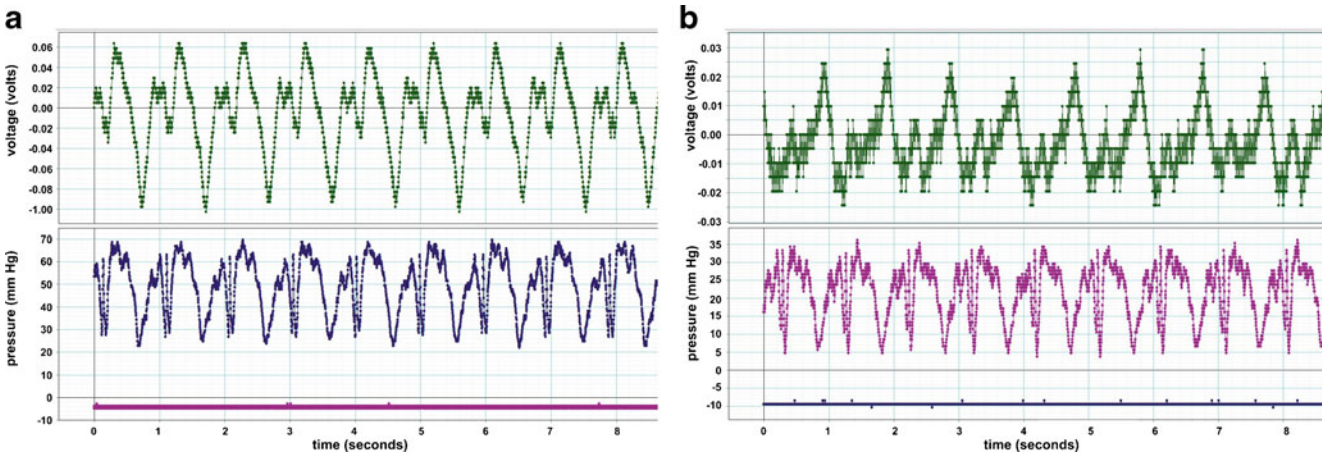

Fig. 3 Signals graphically displayed (abscissa: time [in seconds], ordinate: voltage [in volts] or pressure [in millimeters of mercury]) using the Data Studio software: HES pulsed through TA (**a**) or GA (**b**), measured at 625 nm; blood pulse signals (*upper plots*), corresponding blood pressure signals (*middle plots*), and blood pressure signals of the corresponding non-perfused component of the TGM (*straight lines*, *lower plots*)

signals were obtained using air and Millipore water (data not shown). SEM showed that for both LHES and lysed fresh human blood, only deformed and damaged red blood cells could be seen. The supernatants after centrifugation were free of particles (data not shown).

Perfusion of pulp and gingiva

Data for pulpal and gingival perfusion are shown in Fig. 6a for red light and in Fig. 6b for IR light. Generally, for IR light (940 nm), ΔU was significantly higher than for red light (625 nm). Using red light, ΔU values of TA (perfusion of TA) were not significantly different from TAG (perfusion of TAG) and ΔU values of GA (perfusion of GA) were significantly lower. This indicates that signals from the gingival parts of the model had no or only a small influence on the tooth signal. In contrast, for IR light, GA signals were significantly higher than TA signals and corresponded with TAG signals.

Shielding procedures

The influence of different shielding procedures on the derived signal (ΔU) is shown in Fig. 7a (red light) and b (IR light). For red light, measured signals after different shielding procedures were in the same order of magnitude for TA and TAG, but in the gingiva group, lower signals were measured. Considering shielding procedures, the error rates method revealed no statistically significant difference among TA and TAG results, whereas GA signals were, by tendency, lower than both TA and TAG signals. The shielding procedure had no statistically

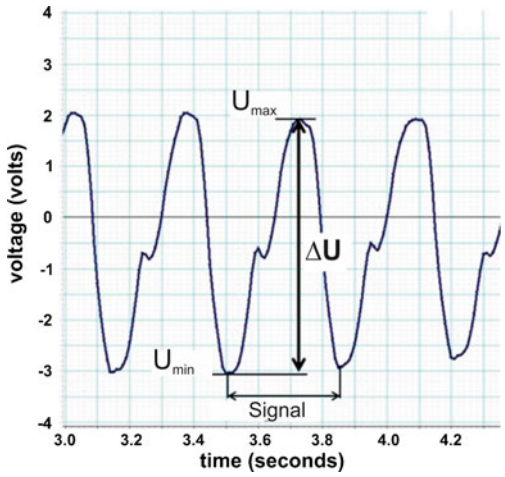

Fig. 4 Signal amplitude [ΔU, in volts] calculated for every second signal amplitude by calculating $U_{max} - U_{min}$ from graphs displayed (abscissa: time [in seconds], ordinate: voltage [in volts]) using the Data Studio software

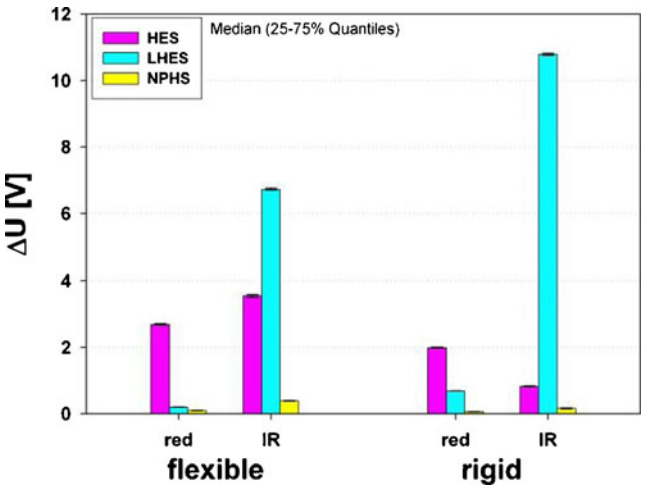

Fig. 5 Signal amplitude [ΔU, in volts] of transmitted light applying human erythrocyte suspensions (*HES*), lysed hemoglobin suspension (*LHES*), and non-particulate hemoglobin suspension (*NPHS*) in flexible and rigid tubes measured with red and IR light

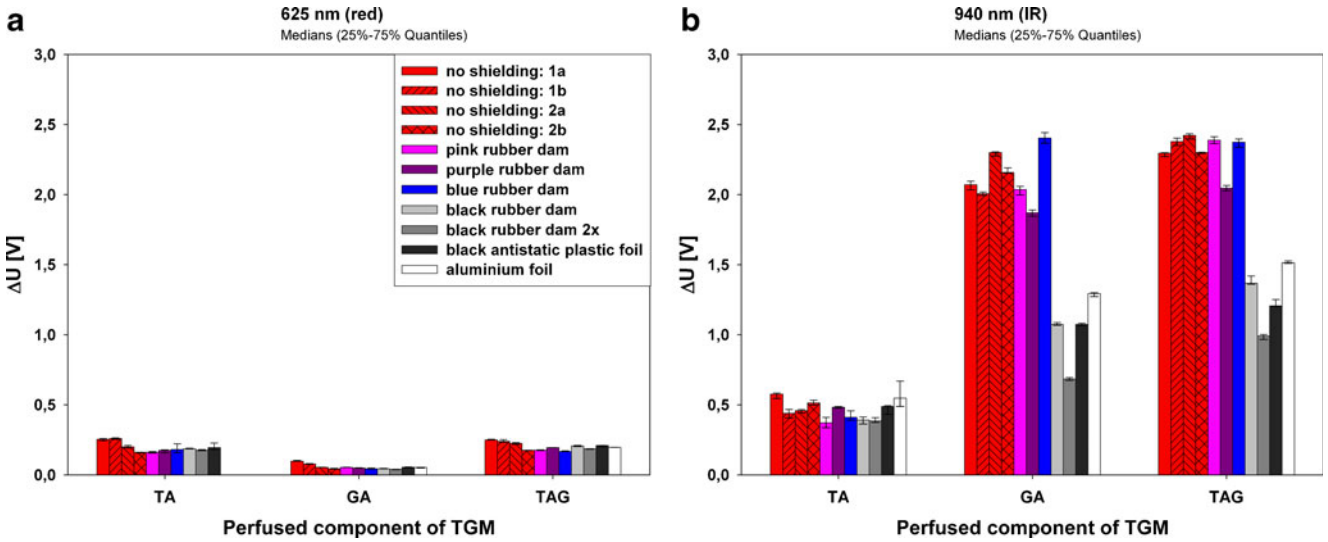

Fig. 6 Signal amplitude [ΔU, in volts] of transmitted light from the shielding experiments at 625 nm (**a**) and at 940 nm (**b**) for the perfused components TA, GA, and TAG of the TGM

significant influence for all of the perfused components TA, GA, and TAG. However, for IR light, signal amplitude of GA signals was significantly higher than the signal amplitude of TA signals and shielding procedures had a significant effect for certain materials: Compared to no shielding, shielding with purple and black rubber dam, aluminum foil, and black plastic foil significantly reduced the GA and TAG derived signals. The highest shielding effect could be seen with a double layer of black rubber dam.

Discussion

Tooth–gingiva model

To gain reproducible measurements, a durable fixation of the tooth in relation to the light source and the sensor was ensured by the custom-made sensor holder. Because the TGM was completely encapsulated by the sensor holder, almost no external light could influence the measurements, even though the tooth socket was translucent. In the present study, the tooth was drilled in the occlusal–apical direction, straight through the center of the crown. This contrasts deliberately with another study where a tube was placed in the roots, running through the pulpal chamber [25]. As the main goal of the present study was to test a new technique for the detection of blood pulsation on an in vitro tooth model, it was necessary to exclude in a first step as many potential interference sources as possible. Turbulence in the blood flow might be also considered as a source of interference. By using straight tubes, we tried to achieve a laminar blood flow throughout the whole experimental setup. The disadvantage of this method is that a tube with constant lumen was used, while the dental pulp is known to consist of a broad variety of arteries, veins, arterioles, venules, and capillaries [26]. However, for these in vitro experiments, it is necessary to use tubes because, otherwise, the erythrocytes could infiltrate the dentin tubules and change the optical properties of the tooth [27].

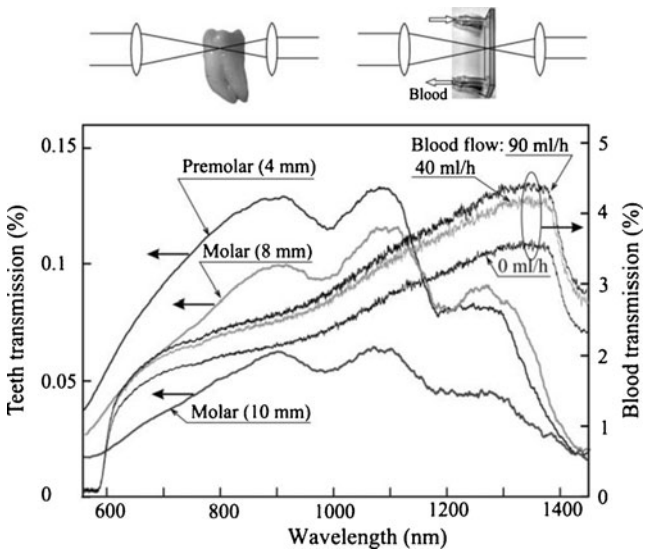

Fig. 7 Typical transmission spectra for different kinds of human teeth (*left axis*) and flowing blood (*right axis*) measured using Fourier transform infrared (FTIR) spectrometer (Bruker Vertex 80v). The *numbers in brackets* indicate the thickness of investigated teeth. The blood data shown here were obtained for blood pumped with different flow rates through the 0.2-mm thick quartz cuvette (Starna type 48). We also measured different cuvette thicknesses (0.1–0.5 mm) and blood flow rates, varying from 0 (lentic blood) up to 99 ml/h [23]. *Top panels* show schematically the experimental geometry used for FTIR measurements of teeth (*left*) and blood (*right*)

Blood flow simulation

In accordance with the resting heart rate in humans, a pump frequency of 1 Hz was chosen. In combination with the lumen of the tube, a laminar flow of 25 ml/min and a pressure of 43 mmHg resulted in the pulpal part of the TGM, at a room temperature of 22 °C. PBF in young dogs is about 40–50 ml/min per 100 g tissue weight [26, 28] and, therefore, far beyond the values used in the TGM. As one of the main goals of the present study was to reduce interfering signals to a minimum in order to investigate solely the optical properties of pulsating blood inside the human tooth in vitro, a rather high blood flow rate was chosen. Only when the optical behavior of blood inside human teeth is completely understood in the in vitro model, the measuring devices can be configured for the in vivo situation. As for cats, an arterial pulpal blood pressure of 43 mmHg is reported in the literature [26, 28]; the TGM was adjusted to that value. Diaz-Arnold et al. used an infusion pump for blood flow simulation [7] and other authors combined it with gas bubbles [25]. Because a defined and constant blood pulse signal was needed in the present study, the bubble technique was not applicable. In the present study, the test liquid was changed after every second test run. Signal amplitude measurements before (Fig. 6, bar "no shielding: 1a") and after (Fig. 6, bar "no shielding: 1b") two test runs proved that a short pumping process had no influence on the signal amplitude. However, preliminary tests showed that the quality of the signal amplitude is reduced, if more than 10 test runs were performed with the same red blood cell suspension. By comparing the first (Fig. 6, bar "no shielding: 1a") and the last (Fig. 6, bar "no shielding: 2b") test run of one test day, any influence of wear of the pump hoses on the signal amplitude could be excluded.

To simulate the GBF, test liquids were pumped through the gingival part of the TGM at a frequency of 1 Hz. Due to the bigger lumen of the circular channel, the laminar flow of 23.4 ml/min caused a pressure of 17 mmHg. As an average gingival blood pressure of 18.7 mmHg is specified in the literature [28], the gingival part of the TGM can be considered as a realistic model to simulate gingival blood pressure. Nevertheless, the acrylic base was designed using rigid, clear material and may not have the same optical properties as the human alveolar process. Further improvements must be made on the TGM by identifying more appropriate materials to simulate the optical as well as the anatomical properties of gingiva and alveolar bone.

Wavelength

In the present study, we focus on the two spectral areas of 625 and 940 nm which, according to our preliminary results obtained for the wide spectral range from visible to terahertz, have been proven to be the most suitable for the method discussed here [23]. Figure 7 shows the IR transmission spectra of different types of human teeth (premolars and molars), together with the blood spectra obtained for lentic and flowing blood (HES). As an important result, the data show that the intensity of near IR light transmitted through the teeth is decreased to a fraction of a percent. In spite of this relatively strong light attenuation, this range can be successfully implemented for all-optical detection of tooth vitality. Indeed, this attenuation does not prevent the achievement of large signal-to-noise ratios, particularly in an optical configuration consisting of a semiconductor laser and a sensitive detector placed immediately behind the tooth. Figure 7 also indicates that, for almost all wavelengths in this spectral range, the light transmission depends on the blood flow rate. Consequently, the difference between the lentic and flowing blood light transmission can be used as a tool for the in vivo determination and characterization of blood pulsation in the dental pulp. Using a light which can be recorded after passing through a tooth and whose transmission is sensitive to the pulsation of blood flowing through the dental pulp is a prerequisite for the realization of an all-optical clinical diagnosis of dental pulp vitality. In particular, the signal modulation by flowing blood makes the application of lock-in modulation techniques possible using the human pulse as a reference. Analysis of the data in Fig. 7 shows that the best contrast of tooth transparency and blood absorption with respect to its flow rate can be obtained at wavelengths between ~0.85 and ~1.38 μm. It could be observed that signal amplitude strongly depends on the wavelength. As we could show in the experiments with LHES (Fig. 5), IR light seemed to be more sensitive for small particles in the suspension, resulting in higher ΔU readings. We could also show that, for IR light, the gingival blood pulse had a clear impairing influence on the joint TAG signal (Fig. 6b). In contrast to that, this influence was almost negligible for red light. As our previous investigation showed, the human tooth has a higher optical transparency for IR light than for red light (Fig. 7) and also the difference in light transmission between lentic and flowing blood was greater for longer wavelengths [23]. Therefore, all of these results correspond with the literature, where longer wavelengths are proposed for PBF detection [7]. However, in our recent study, we added the GBF as another interfering factor and surprisingly found the signal-to-noise ratio being significantly better with red light than with IR. Indeed, one reason for these findings could be that the transmission of the tooth is quite high for IR light. This fact might lead to more scattering inside the tooth and facilitate the transmission of gingival signals through the root into the coronal part of the tooth, where the sensor was placed. As red light scatters less, due to a higher absorption rate, the gingival artifacts might be suppressed. In summary, it can be said that, for identifying an ideal wavelength for optical blood pulse detection in teeth, the interference signal of the oral tissues, particularly the gingiva, should be considered. Therefore, more research should be

done on this issue because the best wavelength for the detection of light modulation in the dental pulp may not be the ideal one for the method as a whole.

Sensor sensitivity

The sensor diode SFH229 [29] used in this study shows the typical sensitivity variations of semiconductor silicone for different wavelengths. Beginning at a minimum level of 20 % for 400 nm, sensitivity increases almost linear to 100 % for 850 nm and then decreases again. For 950 nm, as well as for 650 nm, the sensitivity is reduced to about 80 %. Consequently, all measurements could be easily compared.

Rigid vs. flexible tubes

In the present study, we could detect a pulse-derived signal with flexible and rigid tubes, although signal amplitudes for HES were lower with rigid glass tubes. Blood in motion has different optical properties, compared to lentic blood. This depends as well on the wavelength used [23] as on the blood flow velocity [23, 30, 31]. As the shape and the accumulation behavior of red blood cells are influenced by the shear rate, resulting in different light absorption properties [31], one can assume that, in this study, pulse signals recorded for rigid tubes did not result from pulse-dependent volume changes. A conclusion regarding wavelength is not possible because of the different optical properties of the tubing materials. However, as for both wavelengths a pulse signal in the rigid tube could be detected, the method can basically be used in a rigid environment like the dental pulp chamber for detecting blood pulses.

Particulate vs. non-particulate fluids

In the present study, we found that signal generation depended on the presence of particles because, with air, water, and cell-free HES lysate, no signal could be recorded. LHES and lysed fresh human blood only consisted of cell fragments and plasma. Despite mechanical lysis of the red blood cells by ultrasound treatment, particles (cell remnants) were still present. The difference in morphology between HES cells and cell remnants was reflected in the size of the recorded signal. This indicates that the morphology of the particles influences the signal. Nevertheless, it is to be expected that cell destruction even increased the optical surface in the suspension. This is in accordance with the fact that shape modifications of red blood cells, caused by changes in blood flow velocity, induce different light transmission properties [31]. This leads to the idea that signal amplitude directly correlates with particle deformation during one pulsation cycle.

Effects of shielding

As red light signals originating from the gingival parts of the model had no significant influence on the amplitude of the tooth signal, one can assume that shielding the gingiva has no significant effect for 625 nm in the in vitro model. However, for light with 940 nm, the amplitudes of GA signals were significantly higher than those of TA signals and shielding procedures had a significant effect for certain materials. The fact that rubber dam shielding of blue and pink colors did not show a reduction of the signal derived from GA for 940 nm compared to not shielding could be explained by the optical properties of these materials, being transparent for IR light. Shielding with purple and black rubber dam, aluminum foil, and black plastic foil reduced significantly the gingiva-derived signals compared to not shielding. These findings also correspond with the optical transparency of these materials in the respective IR spectral area.

Conclusions

1. The present in vitro study provides evidence that blood pulse in a rigid environment can be optically detected through dental hard tissues, probably due to the deformation of red blood cells.
2. The detected signal is dependent on the wavelength and the tubing materials.
3. The pulp-derived signal may be impaired by signals from surrounding tissues, more so at 940 nm than at 625 nm.
4. Shielding measures like rubber dam are not able to totally prevent this effect, especially for 625 nm.
5. The presented optical method for blood pulse detection has the potential for being used for pulp vitality testing, if a local resolution for the signal acquisition can be provided.

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Conflict of interest The authors declare that they have no conflict of interest.

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