Penetration depth of laser Doppler flowmetry beam in teeth

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Objective. The aim of this study was to determine the penetration depth of laser light in teeth for contact and noncontact probe tip.

Study design. Fifty-one freshly extracted human mature maxillary and mandibular single rooted teeth were selected for the study. A laser Doppler flowmeter was used as a laser source. A digital camera was used to take pictures (with "night shot" mode in total darkness). When probe tip was in contact and when it was 1 mm away from the tooth, the depths of the areas illuminated in high and low density were measured.

Results. In contact probe, root was illuminated to 4.28 ± 0.14 mm depth with high density and 13.27 ± 0.27 mm depth with low density. In noncontact probe, 4.36 ± 0.16 mm with high density and 13.28 ± 0.30 mm with low density of illumination were seen on the root. Between contact and noncontact probe situations the difference was not statistically significant in the depth of illumination. On the other hand, in both groups the difference between areas illuminated in high and low density was statistically significant ($P < .05$).

Conclusion. Contact or noncontact of the probe on tooth surface did not show any significant difference in light penetration. Besides, we think that, for the present, it is impossible to eliminate the contamination from periodontal tissues completely even if the necessary precautions (periodontal paste etc.) are taken.

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The laser Doppler flowmeter (LDF) is widely used in pulpal blood flow measurements. In vivo studies have addressed different questions: the estimation of pulpal vitality,[1,2] monitoring revascularization of replanted teeth,[3] the monitoring of tooth vitality after trauma,[4,5] reactions to local pharmacological agents,[6] electrical stimulation,[7] thermal stimulation,[8] and the pulpal reaction to orthodontic[9] and orthognathic procedures.[10,11]

It is reported that the LDF technique is reliable for measuring human pulpal blood flow to determine pulp vitality.[2] However, it has also been claimed that signals obtained from human teeth do not necessarily indicate pulpal blood flow and could be confounded by a signal obtained from nearby gingival tissues, suggesting that the periodontium and other neighboring tissues can contribute to the signal.[12] Likewise, studies[13-17] suggest that 45% to 82% of the blood flow recorded from human teeth may not be from the pulp. Ikawa et al[12] have shown that laser light applied to teeth can reach the periodontal tissues and return to the probe again. The same study indicates that laser light can reach the 6 mm depth of the root, but did not investigate farther depth.

The aim of the study was to determine the penetration depth of laser light in teeth for contact and noncontact probe tip.

MATERIAL AND METHODS

Fifty-one freshly extracted human mature maxillary and mandibular single rooted teeth free of caries, restorations, abrasion cavities, and injury from forceps or fractures were used in this study. The mesiodistal and buccolingual dimension of the crowns and the cervico-apical length of the roots of the teeth were similar for each teeth type. All teeth were radiographed in a buccal direction to check for a similar dentin thickness and pulp size. The teeth had been extracted for periodontic or orthodontic reasons and stored in distilled water until assessment (maximum 1 day).

Before the test, the teeth were immersed in 5% sodium hypochlorite for approximately 15 minutes to remove organic debris from the root surfaces. Any remaining tissue was mechanically removed using a curette with great attention not to damage the root surface.

A Periflux 4001 Master LDF (Perimed, Stockholm, Sweden) was used in this study. The LDF emitted light with a 780 nm wavelength. Bandwidth was 20 Hz−20 kHz and sampling frequency was 32 Hz. The diameter of the probe was 1 mm (415-159; Perimed, Järfälla, Sweden). Calibration (zeroing was done automatically at calibration) was carried out with the PF 1000

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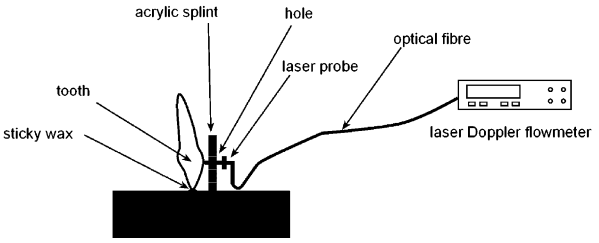


Fig 1. Diagram of preparation.

Calibration Device (Perimed, Järfälla, Sweden) before measurement.

The buccolingual dimension of the crowns was measured with a caliper (VIS, Warsaw, Poland) at 0.1 mm sensitivity. Then each tooth was placed on the prepared experiment mechanism (Fig 1). In order to fix the tooth, sticky wax (772; Giordano Vannini, Florence, Italy) was used.

The LDF probe was placed labially on the cervical third of the tooth crown. The camera (DSC-F717 Cybershot Digital Style Camera, Sony Corporation, Tokyo, Japan) was fixed 10 cm away from the tooth. A photograph was taken when the environment was luminous (Fig 2). The camera's own infrared light source was fastened with black thick tape to eliminate the camera's own light sources. Then the environment was darkened completely. The camera was adjusted to "night shot" mode (to make infrared imaging, removing the charge coupled device's infrared cut filter). Other settings of the camera are shown in Table I.

One photograph was taken for each situation, that is, when dental probe was in contact with the tooth (Fig 3) and when it was 1 mm away from the tooth (Fig 4). After that, these photos were transferred to Photoshop (Version 5.5; Adobe Systems, San Jose, Calif). The width of the tooth was measured on the photo, which was taken in the luminous environment, and this value was divided by the real measurement value of tooth. One factor value was obtained. Thus, all the measurements, which were made on the photo, were converted to mm. On the luminous image according to the cementoenamel junction, used as buccal reference point, the farthest point of root apex was measured by using the "measure tool" and it was noted as "root length." Later, the image taken in the darkness when the probe was contacted was copied and pasted on the image taken in luminous environment. As image sizes and photography techniques were standard, images were placed together from the reference point; the farthest point from which the root could be observed was measured and noted by the name "illumination depth." After that, the "trace contour" command, set at 200 as level value, was used, using the crown region border of densely illuminated area as standard. The length between the points of the

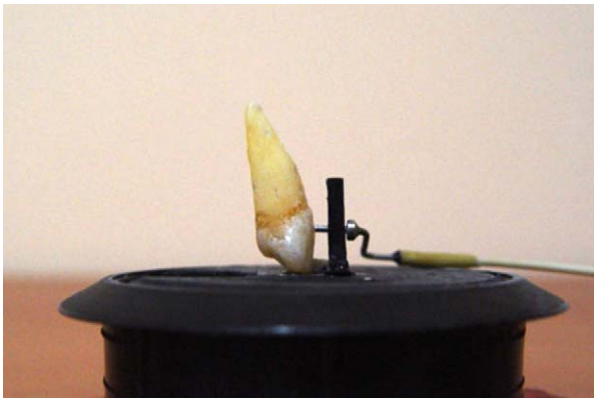


Fig 2. Tooth placed on the experiment mechanism.

Table I. Camera settings

Exposure time (Auto)	0.033-0.05 seconds
ISO speed (Auto)	Max. 800
Exposure bias value	−2
Aperture	f2.0
Flash mode	Off
Focal length	9.7 mm
Pixel X dimension	640
Pixel Y dimension	480
Image mode	JPEG(fine)
Macro mode	On

intersection of buccal border and the border obtained by this command and the reference points were recorded as "dense illumination depth." The image was then changed to the first position (the photo that was taken in luminous environment) by the "revert" command. The same procedures were repeated for the image that was obtained when the probe was 1 mm away.

Statistical analysis was performed using SPSS (Version 10.0; SPSS, Chicago, Ill). Student t test was used for the comparison of mean values of illumination depth between groups and Kruskal-Wallis test between tooth types. The level of statistical significance was set at $P = .05$.

RESULTS

In the groups named "contact probe" and "noncontact probe," when the dense illumination depth measurement values were compared, statistically no significant difference was found between them. In the same way, in the groups named "contact probe" and "noncontact probe," when the illumination depth measurement values were compared, statistically no significant difference was found between them (Table II). However, in both groups statistically significant differences were observed between illumination depth and dense illumination depth measurement values ($P < .05$).



Fig 3. Tooth with contact probe.



Fig 4. Tooth with noncontact probe.

Table II. Illumination depth (mm)

	Contact probe, n = 51 (mean ± SE)	*Noncontact probe, n = 51 (mean ± SE)*	
Dense illumination depth	4.28 ± 0.14	4.36 ± 0.16	T = 0.41, $P > .05$
Illumination depth	13.27 ± 0.27	13.28 ± 0.30	T = 0.02, $P > .05$
	T = 37.97, $P < .05$	T = 33.41, $P < .05$	

Statistically no significant difference was observed between the depth values compared according to tooth types (Table III).

DISCUSSION

After tooth extraction, optical properties of dentin and pulp change, and such changes will affect the amount of light transmitted to tissues outside the tooth.[18,19] For this reason, in this study the measurements of extracted teeth were completed in 24 hours.

The reason for using the Sony camera in the present study is that it has an infrared filter that is preferentially removed (with "night shot" mode). Thus, with this camera it has become possible for us to image the laser wavelength that we used. By having this property, its own infrared light sources work automatically as well. We have eliminated these light sources in order to image only the infrared laser that we used. A fully dark environment has been prepared in order to prevent artifact originating from peripheral light sources.

Night shot mode was active only in automatic mode. In this mode exposure bias value was fixed at −2. Exposure time was adjusted automatically between 0.033-0.05 seconds. ISO speed was also adjusted automatically (the highest value of the instrument was 800). These values could not be adjusted manually in night shot mode.

Vongsavan and Matthews[18] attributed the LDF technique capable of making measurements in skin and mucosa up to an approximate depth of 1-2 mm and passing over the 2-3.5 mm thick enamel and dentin of teeth to measure blood flow to dental tissues, which are more transparent and tubular than dentin and act as light guides. They speculated that the periodontium and other adjacent tissues could contribute to the signal.

Odor et al[20] subsequently showed that enamel prisms and dentin tubules could transmit light rays coming from the tooth surface and distribute them in teeth.

Altshuler and Grisimov[21] cut plane-parallel cross-sectional slices of human teeth and illuminated them with a small laser beam incident on the original outer surface. The angle of incidence of the laser beam with respect to the surface normal was varied. They observed wave guiding of light by the enamel prisms and by the dentin tubules.

The wavelength of the light will affect the extent to which the signal is affected by tissues outside the tooth. This is because of the light of long wavelength such as infrared penetrates tissues to a greater depth than shorter wavelengths.[18,19]

Light pulses move easily down the fiber-optic line because of a principle known as total internal reflection. This principle of total internal reflection states that when the angle of incidence exceeds a critical value, light can not get out of the glass; instead, the light bounces back in.[22,23] In order to have total internal reflection, refractive index of center should be greater than peripheral.[22] The refractive index of dentin tubules is less than that of peritubular dentin (1.33 and 1.59, respectively).[24] Thus, it is seen that dentin tubules do not behave exactly like a fiber and total internal reflection principle is not valid in dentin tubule. Zijp and ten Bosch[24] stated that the tubules are the most important scatterers in dentin, the collagen fibrils play only a minor role, and the mineral crystals do not contribute significantly. For these reasons, we believe that light is

Table III. Illumination depth by teeth types (mm)

Teeth	*Dense illumination (contact probe)*	*Illumination (contact probe)*	*Dense illumination (noncontact probe)*	*Illumination (noncontact probe)*
Upper 1, $n = 6$	3.85 ± 0.29	11.97 ± 0.61	3.72 ± 0.23	11.93 ± 0.61
Upper 2, $n = 4$	3.75 ± 0.48	13.95 ± 1.70	4.28 ± 0.50	14.25 ± 1.63
Upper 3, $n = 5$	4.02 ± 0.26	13.30 ± 0.91	3.84 ± 0.40	13.18 ± 1.08
Upper 5, $n = 9$	4.64 ± 0.40	13.06 ± 0.60	4.62 ± 0.46	13.41 ± 0.60
Lower 1, $n = 2$	4.00 ± 0.50	11.85 ± 0.85	3.25 ± 0.85	11.85 ± 0.85
Lower 2, $n = 5$	3.88 ± 0.33	12.46 ± 0.30	3.78 ± 0.41	12.14 ± 0.76
Lower 3, $n = 5$	4.24 ± 0.07	14.68 ± 0.80	4.42 ± 0.36	14.50 ± 1.33
Lower 4, $n = 13$	4.68 ± 0.31	14.00 ± 0.42	5.04 ± 0.32	13.87 ± 0.47
Lower 5, $n = 2$	4.30 ± 1.50	11.90 ± 1.90	4.60 ± 1.40	12.40 ± 2.70
Kruskal-Wallis	8.83	12.27	12.03	8.34
P value	.265	.092	.100	.303

transmitted principally not linearly but by random scatterings. Also Fried et al[25] show that dentin scatters strongly in the visible and near infrared wavelength and absorbs weakly.

The refractive index of prisms of enamel is 1.619, and that of interprismatic substance is 1.573.[26] Total internal reflection principle is valid for enamel prisms. Here basic transmission technique resembles fiber, but scatterings also occur. Moreover, Fried et al[25] revealed that enamel weakly scatters light between 543-nm and 1.06-μm wavelengths and absorption is negligible. The prisms are somewhat curved and not exactly aligned.[26] The absorption and scattering coefficients of enamel have been measured; its hydroxyapatite crystals are mainly responsible for the scattering processes in this material.[27]

The presence of imperfections in the enamel, such as discolorations, cracks, tufts, and differences in the rate of mineralization of enamel and dentin, can also modify the trajectory of the light, offering preferential lines for its conduction and sources of reflection.[17] The presence of light-absorbing pigments must also be considered.[28]

Ikawa et al[12] showed that laser light could reach to the 6 mm depth of the root. Ikawa et al did not evaluate root surface measurement in the areas deeper than 6 mm. In addition, the signal value in 6 mm depth is not zero, either. They did not evaluate measurement from labial surface. In this study, we observed that laser light achieved approximately 4 mm depth of root densely and 13 mm depth less densely. The most densely illuminated area in this study is vestibule surface of the root. Although the techniques are different, our results are similar to those of Ikawa et al.

That laser light reaches to deep root may be explained by many scatterings. The existence of many scatterings will increase the possibility of multiple collidions of light waves. Thus, frequency shift of signal will increase and the rate of the signal that contributes to the measurement will decrease. We observed that lighting was achieved to the apex in the teeth with short roots.

The densely illuminated area is the vestibule surface where the laser is applied. That is, the contribution of the tissues on this area to the signal is much more than the others. This situation has been stated by Akpınar et al.[29] In our study, it is shown that laser light reaches approximately 4 mm deeper from the cementoenamel junction on vestibule root surface. Application of periodontal paste or rubber dam blocks only the contribution of gingiva. It is not possible to eliminate the contribution to the signal of other periodontal tissues adjacent to root surface by noninvasive methods (especially with periodontally sound teeth).

CONCLUSION

The probe being in contact or not on the tooth surface did not result in any significant difference in light penetration.

The results of this study have brought up again the importance of knowing the region in which the applied laser light scatters and taking precautions (periodontal paste etc.) to deal with this problem in order to reduce the artifacts to the minimum in the measurements made by LDF. However, we think that, for the present, it is impossible to eliminate the contamination from periodontal tissues completely even if the necessary precautions are taken.

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