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Effect of Gingiva on Laser Doppler Pulpal Blood Flow Measurements

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The purpose of this study was to evaluate the effect of labial and palatinal gingiva on laser Doppler pulpal blood flow measurements. Twenty upper central incisor teeth determined to be vital from 20 volunteers were included into the study. To stabilize the probe, a laboratory-made acrylic splint positioned the probe labially on the cervical third of the tooth crown. We collected measurements without any gingival covering, and under three conditions: after application of an opaque paste on the labial gingiva, the palatal gingiva, or both the labial and palatal gingiva. The measurement values decreased 46% when there was opaque paste on labial gingiva, 10% on palatinal gingiva, and 63% on both. The decrease in the values when there was an opaque paste on palatinal gingiva was not statistically significant ($p > 0.05$). The differences between all other groups were statistically significant ($p < 0.05$). In these measurements, the contribution of labial gingiva to laser Doppler pulpal blood flow measurements is more than that of palatinal gingiva.

The laser Doppler flowmeter (LDF) evaluates dynamic changes in blood flow through noninvasive measurement methods (1, 2) by detecting blood cell movement in a small volume of tissue (approximately 1 mm^3). The flow in individual microvessels, the number of vessels with active flow, and the vessel diameter cannot be analyzed (3), but total flow can be monitored throughout the tissue. Its use in human teeth was first described by Gazelius et al. (4).

In the orofacial region, the perfusion of gingiva (1–3), bone (5), and masticatory muscles (6) and the pulpal blood flow (PBF) have been explored. In vivo studies have addressed different questions: the estimation of pulpal vitality (4, 7, 8), the monitoring of tooth vitality after trauma (9), reactions to local and systemic pharmacologic agents (1), electrical stimulation (10), thermal stimulation (11), and the pulpal reaction to orthodontic (12) and orthognathic procedures (13, 14).

It is reported that the LDF technique is reliable for measuring human PBF to determine pulp vitality (7, 15, 16). Vongsavan and Matthews (17) report that the LDF technique may detect blood flow in skin and mucosa to an approximate depth of 1 to 2 mm and to 2 to 3.5 mm of enamel and dentin of teeth. The basis for detection of PBF through enamel and dentin is thought to be the transparent and tubular nature of dentin, acting as a light guide.

However, it has also been claimed that signals obtained from human teeth do not necessarily indicate PBF and could be confounded by a signal obtained from nearby gingival tissues (16). These authors speculate that the periodontium and other neighboring tissues can contribute to the signal. Additionally, it has been reported that laser light applied to teeth can reach the periodontal tissues and return to the probe (16).

Taken together, these studies indicate that the gingiva may contribute to PBF measurements made by LDF (18, 19). However, to date, no study has evaluated the relative contributions of labial and palatinal gingiva to these measurements. The purpose of this study was to evaluate the relative effect of labial and palatinal gingiva on laser Doppler PBF measurements.

MATERIALS AND METHODS

The study was performed at Cumhuriyet University, School of Dentistry Hospital. The protocol was approved by the Ethics Committee of Cumhuriyet University, School of Medicine. The volunteers were informed in writing about the purpose of the study and the procedures involved.

The following were excluded from the study:

(a) Nonvital teeth
(b) Caries
(c) Restored teeth
(d) Orthodontic treatment or treatments for any previous traumatic injuries
(e) Gingivitis or periodontitis
(f) Smokers
(g) Cardiovascular diseases

Tooth responsiveness to external stimuli was evaluated by conventional electrical and thermal tests. Radiographs were taken to determine that the pulp chamber of the tooth was visible and that the periapical status of the tooth was normal. The tip of the pulp was above the bone level for each tooth evaluated.

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In this study, all subjects had clinically healthy gingiva. Gingiva was defined as clinically healthy when sulcular depth was 3 mm or less and there was no evidence of bleeding on probing and no attachment loss or tooth mobility. Periodontal health was evaluated by the qualitative plaque and gingival indexes (20), probing depth, clinical attachment level, and tooth mobility. Pocket depth and clinical attachment level (the distance from the cemento-enamel junction to the base of the pocket) were measured using a Hu Friedy periodontal probe. All clinical measurements were performed on six sites per tooth (mesial and distal sections on the buccal and palatal sides). Tooth mobility was evaluated by using Periotest system (Siemens AG, Germany).

Twenty upper central incisor teeth determined to be vital from 20 volunteers (nine females and 11 males) were included into the study. The average of the patients was 23.10 yr.

A Periflux 4001 Master LDF (Perimed, Sweden) was used to measure PBF. The LDF emitted light with a 780-nm wavelength. Band width was 20 Hz to 20 kHz, and sampling frequency was 32 Hz. The diameter of the probe was 1 mm (415-159, Perimed, Sweden). Calibration was performed with the PF 1000 Calibration Device (Perimed) before each measurement.

To stabilize the probe, a laboratory-made acrylic splint positioned the probe labially on the cervical third of the tooth crown. An opaque periodontal paste (Peripac Paste, De Trey/Dentsply, Germany) was used to isolate gingiva in the following measurement processes. Peripac Paste is an opaque paste that consists of calcium sulphate, zinc sulphate, zinc oxide, polymethyl methacrylate, dimethoxy tetraethyleneglycol, ascorbic acid, flavor, and iron oxide pigment. This periodontal paste belongs to the no-eugenol paste group. While the paste was positioned, attention was paid to filling the gingival sulcus.

Volunteers were kept resting for at least 15 min before the processes. All treatments were performed in an environment with constant temperature and by the same dentist. All of the patient treatments were performed in standard position.

Laser Doppler flowmeter measurements were taken at the following phases:

(a) Only acrylic splint
(b) Acrylic splint plus periodontal paste (labial gingiva)
(c) Acrylic splint plus periodontal paste (palatinal gingiva)
(d) Acrylic splint plus periodontal paste (labial and palatinal gingiva)

Perfusion units (PU), concentration of moving blood cells, velocity, and total backscatter were recorded using the flowmeter and monitored using Perisoft software (Version 5.1, Perimed, Stockholm, Sweden). Having observed that the signal was stable and demonstrated a pulsatile nature, we determined the mean PU values (PU is an arbitrary measurement unit) using at least 10 s of recording.

Statistical tests were performed using SPSS (Version 9.0, SPSS Inc., Chicago, IL). Repeated measures analysis of variance was used while assessing the variation within the group. Post hoc comparisons were made with the Tukey test. The level of statistical significance was set at $p = 0.05$.

RESULTS

The mean PBF values under the four treatment conditions are presented in Table 1. Analysis of variance indicated a highly significant treatment effect ($F = 99.69$; $p < 0.05$). Post hoc evaluation indicated that all groups significantly ($p < 0.05$) differed from one another, with the exception of comparison between the acrylic splint control and the group receiving the acrylic splint and paste applied to the palatal gingiva.

TABLE 1. Mean pulpal blood flow values in perfusion unit

	Mean ± SEM
Only acrylic splint	5.40 ± 0.39
Acrylic splint + periodontal paste (labial gingiva)	2.94 ± 0.26
Acrylic splint + periodontal paste (palatinal gingiva)	4.88 ± 0.40
Acrylic splint + periodontal paste (labial and palatinal gingiva)	1.98 ± 0.18

N = 20.

DISCUSSION

Previous LDF studies have demonstrated that several factors influence PBF measurements, including the temperature of the environment, the position of the patient, the resting status of the patient, and the position of the probe (21–23). Therefore, in this study, all of the patients were kept resting for at least 15 min, their position was kept fixed, the experiments were performed in the same room, and the acrylic splint and probe were kept fixed in the same position and without motion.

Studies have reported placing the LDF probe 1 to 1.5 mm (24), 2 mm (25), 2 to 3 mm (26), approximately 3 mm (27), and 4 to 5 mm (14) coronal to the gingival margin. In our study, we placed the probe on the cervical third of tooth crown, taking the crown sizes of the teeth into account (9). One reason for this sampling site is that the output signal for transmitted laser light is minimal because of the thickness of the tooth in this region (28), or because of pulpal backscattering. Likewise, Ikawa et al. (16) showed that laser light could reach even to the 6-mm depth of the root.

We selected a 15-min resting period based on previous studies evaluating 5 min (14), 10 min (27), and 15 min (29). At the end of this resting period, the PBF signal was stable and demonstrated normal pulsatile values suitable for recording mean levels over a 10-s period. Both continuous and periodical measures can be collected by LDF. The momentary value displayed on the LDF screen represents a 3-s rolling mean value. After obtaining a signal of pulsatile nature and stable rate, we obtained a mean PBF value by collecting the maximal value over at least a 10-s period (24). It is known that laser Doppler probe can detect even small movement artifacts, and therefore, false signals may occur (22). To reduce this negative effect to the minimum, we used a rigid acrylic splint that provided stable values over time.

According to Vongsavan and Matthews (17), the LDF technique is capable of making measurements in skin and mucosa to an approximate depth of 1 to 2 mm and passing over the 2- to 3.5-mm thick enamel and dentin of teeth and measuring blood flow because dental tissues are more transparent and the tubular structure of dentin acts as a light guide. They speculate that the periodontium and other neighboring tissues can contribute to the signal. This may occur because of enamel prisms and dentin tubules transmitting light from the tooth surface (30). Studying pig teeth, Vongsavan and Matthews (31) claim that the effect of tissues other than the pulp is very small (7% to 14.9%). It may be that the discrepancy between these results is caused by species differences in the

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optical properties of the dentin and enamel that cause a greater proportion of the incident light to be transmitted to the tooth-supporting tissues in humans. In support of this point, other studies have demonstrated that when the probe position approaches the gingival aspect of the crown, there is an increase in blood flow values (21). This increase may represent the light from the flowmeter sampling a larger volume of pulp tissues, but contributions from tissues other than the pulp may also be increasing. Moreover, recent studies suggest that 45% to 82% of PBF values collected from an intact human incisor may not be from the pulp (8, 18).

Hartmann et al. (19), investigating vital teeth using a polyurethane splint and rubber dam combination, showed that blood flow measurement values decreased by 69% compared with controls in which only a polyurethane splint was used. In our study, the measurement values obtained after covering only the labial gingiva and covering the labial and palatinal gingiva decreased statistically to a significant degree (46% versus 63%, respectively). Only the isolation of palatinal gingiva did not affect the measurement value statistically to a significant degree (approximately 10% decrease). Our findings are similar to the findings of Amess et al. (18) and Hartmann et al. (19). The rigid splint and opaque paste combination may be proposed as an alternative to the rigid splint and rubber dam practice of previous studies.

In the PBF measurements, the contribution of labial gingiva to signal is more than that of palatial gingiva. Furthermore, the use of an opaque periodontal paste that is easy to apply, in combination with a rigid splint, increases the accuracy of PBF recordings.

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