

Local Gingival Blood Flow at Healthy and Inflamed Sites Measured by Laser Doppler Flowmetry

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Background: This investigation aimed to 1) develop a method to obtain reproducible laser Doppler flow readings (LDFRs) at the gingiva of the maxillary front teeth; 2) evaluate regional gingival blood flow (GBF) in healthy gingiva by laser Doppler flowmetry; 3) compare hand-held LDFR (H-LDFR) with splint LDFR (S-LDFR); and 4) monitor changes in GBF in experimental gingivitis (EG) and chronic gingivitis (CG).

Methods: The LDFR, gingival index (GI), and plaque index (PI) were measured at 13 gingival sites (teeth #6 to #11) in 10 healthy volunteers (five males and five females), 23 to 34 years of age, over a period of 12.5 ± 3.27 days employing a partial-mouth EG model and in 11 patients (three males and eight females), 20 to 63 years of age, with CG. LDFRs were obtained by S-LDFR or H-LDFR.

Results: H-LDFRs were significantly higher than S-LDFRs ($P < 0.05$). All EG subjects developed gingivitis (PI: 2.77 ± 0.23 ; GI: 1.5 ± 0.53). EG-LDFRs at diseased sites increased slightly but not significantly over the study period. All CG-patients had high plaque and inflammation scores (PI: 2.8 ± 0.2 ; GI: 1.63 ± 0.78). CG-LDFRs at sites with GI > 1 were significantly higher than LDFRs at healthy sites ($P < 0.05$). CG-LDFRs were significantly higher than EG-LDFRs at sites with a comparable GI ($P < 0.05$).

Conclusions: LDFRs are positively correlated with the degree of gingival inflammation. GBF demonstrated significant differences in EG and CG. Modifications of the probe are needed to enhance its clinical applicability in clinical research of periodontal diseases. *J Periodontol* 2006;77:■■■■-■■■■.

KEY WORDS

Gingivitis/diagnosis; gingivitis/pathology; laser Doppler flowmetry.

Although the bacterial etiology of periodontal diseases is universally accepted, the exact mechanisms of disease progression are still unclear. Investigations of the pathogenesis of periodontitis focus on the initiation and progression of the disease process, such as the progression from health to gingivitis, from acute to chronic inflammation, from gingivitis to periodontitis, and from remission to activity. A classical experiment published by Löe et al.¹ demonstrated that gingivitis develops when oral hygiene measures are suspended and that this process is reversible when they are resumed. In the following years, the experimental gingivitis (EG) model has repeatedly been used to study macroscopic and microscopic changes in gingival inflammation.

One of the earliest signs of any inflammatory process is changes in the vascular architecture and microvasculature. This is also true for gingivitis. Healthy gingiva is characterized by a subepithelial vascular plexus consisting of a capillary network with loops arching toward the epithelium.² Gingival inflammation results in increased vascularity with more capillary loops,³ larger vessel size and slowed blood flow,⁴ and a restriction of the afferent blood vessels,⁵ all recorded in the gingiva of experimental animals. Capillary units in the crestal gingiva are among the first vessels affected by inflammation.⁶ If changes of the vascular

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morphology in inflammation are related to blood flow changes, these changes may be the first sign to predict the onset of pathological events in gingival tissue. Thus, gingival blood flow (GBF) may serve as a prognostic marker.

Gingival capillary microcirculation has evaded exact evaluation for a long time due to methodological difficulties. Various methods, such as impedance plethysmography or the implantation of microspheres, have been employed to study GBF,⁷⁻¹³ most of them being invasive or inapplicable to humans.

The laser Doppler flowmeter evaluates changes in blood flow non-invasively and has been used to monitor blood flow in living tissues in numerous applications. Introduced by Holloway and Watson,¹⁴ laser Doppler flow measurement (LDFM) enables direct and continuous assessment of blood flow in tissue microcirculation based on the Doppler shift of back-scattered laser light.¹⁵ Laser Doppler flowmetry (LDF) has been used to assess blood flow in intact microvascular systems such as the retina, gut mesentery, renal cortex, the skin, and mucous membranes.^{14,16,17} Dental applications include LDFM of pulpal blood vessels,^{18,19} periodontal ligament,²⁰ gingival or sulcular blood flow in health and disease,²¹⁻²⁵ the effect of orthodontic treatment,²⁶ or the injection of vasoconstrictive anesthetics²⁷ on blood flow.

Although LDF has proved valuable for a variety of clinical applications, there are some limitations to its use in oral medicine. A major drawback is that LDF can only detect net red blood cell movement in a small volume of tissue ($\sim 1 \text{ mm}^3$); thus, variables such as flow in individual microvessels, the number of vessels with active flow, and changes in vessel diameter cannot be analyzed.²⁴ The small measuring area may also influence the reproducibility of the results because a minimal displacement of the probe would lead to a change in the investigated area due to the density of the vascular network of the gum.²⁶ Another source of error in LDF measurements is artifacts caused by tissue motion in relation to the probe. Furthermore, oral LDF readings (LDFRs) have demonstrated considerable intra- and interindividual variability.^{25,28,29}

This might explain why LDF data published on the relationship of blood perfusion and gingivitis are conflicting,^{24,30} but data obtained by other methods appear to be similarly inconclusive,^{4,11,13} indicating the perplexity of the issue. Although LDF has been employed in investigations of EG, to our knowledge, no study evaluated LDFRs in subjects with a history of chronic gingivitis (CG) and compared them to LDFRs of healthy sites or sites exhibiting EG.

The present investigation aimed to evaluate different aspects of the use of LDF in monitoring various stages of gingival inflammation: 1) a suitable method to obtain reliable LDFR was to be developed: 2) the

microcirculation of healthy gingiva by LDF was to be evaluated, and the intra- and interindividual variability of laser Doppler measurements at various locations of the marginal gingiva was to be examined; of special interest was the comparison of hand-held probe measurements with stabilized probe measurements; and 3) LDF was to be used to monitor changes of GBF in EG and CG and to correlate the LDFR to clinical signs of gingival disease.

MATERIALS AND METHODS

Study Design and Population

The study was divided into three parts: 1) evaluation of LDFRs in healthy subjects; 2) monitoring of LDFRs during experimental (early) gingivitis; and 3) evaluation of LDFRs in patients with established (chronic) gingivitis.

Ten dental students and staff members from the Dental School, University of Mainz (five males and five females; aged 23 to 34 years) volunteered to participate in parts I and II of the study, which took place between March and June, 1996. Eleven patients (eight females and three males; aged 20 to 63 years) with a history of CG (repeated recordings of gingival inflammation in the dental chart; gingival index [GI] at more than four test teeth >0) seeking treatment in a private practice between January and March 1998 participated in part III of the study. All patients were systemically healthy, non-smokers, with a complete permanent dentition and without a history or clinical signs of periodontitis. Exclusion criteria were current pregnancy, general diseases, long-term medication (except for contraceptives), the use of antibacterial or anti-inflammatory medication within 1 month before screening, fillings of the test teeth, orthodontic bands or appliances, probing depths $>2 \text{ mm}$, and clinical attachment loss $>1 \text{ mm}$ (non-inflamed gingival recession was accepted). Additional excluding criteria for parts I and II were changes of gingival color or texture and gingival bleeding.

The study protocol was in accordance with the Helsinki Declaration of 1975, as revised in 2000. All patients agreed to participate in the study and gave their written informed consent on an institutional review board consent form.

Laser Doppler Flow Monitoring

Local GBF was always determined prior to the gingival examination at the same time (8:00 am) in an air-conditioned room using a laser flow blood perfusion monitor[†] with a 0.8-mm needle-shaped probe.[§] Each subject was seated in an upright comfortable position in a chair. The gingival sites selected for observation

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§ P-433-2, TSI.

were situated at the labial gingiva of the upper front teeth (from canine to canine), at the tip of the interdental papillae (papilla tip), and the lowest, central part of the facial gingival margin (gingival margin; Fig. 1).

An individual silicone rubber splint^{||} covering the maxillary area between the second premolars was manufactured for each subject. It ensured a reproducible position of the laser Doppler probe at the gingival site under study. Extension of the splint into the oral vestibulum and a minimal thickness of 0.7 cm were required. Small holes were drilled into the silicone material and filled with plastic tubes that had the same internal diameter as the Doppler probe's external diameter (0.8 mm). The plastic tube also prohibited the contamination of the probe with small silicone particles rubbed off when the probe was inserted into the hole. All plastic tubes had the same length of 0.7 cm to guarantee a reproducible placement of the probe tip close to the gingiva without touching it. The laser Doppler probe was marked at 0.7 cm with a rubber stop as an outside control.

Two measuring procedures were performed using the silicone splint, followed by a third measurement by hand. The probe was positioned at the measuring site by one experienced examiner (SP) who could not see the laser Doppler screen. At his signal, a second examiner (JHG) started the computer software recording the flow data. Laser Doppler monitoring included blood flow, volume, and velocity. The measurement period at each measuring site was 15 seconds for the splint measurements and 1 second for the hand measurement. During the splint measurements, the laser Doppler continuously measured GBF, which was averaged every 10 seconds. At the end of the measurement period, the flow readings were recorded as numbers directly from the laser Doppler screen display by the second examiner. The data were also registered by special computer software.[¶] GBF was expressed in laser Doppler units (LDUs), which are not arbitrary but have a low biologic zero (0 to 1 LDUs) and are one-point calibrated with latex beads at 25°C in a teflon vial.

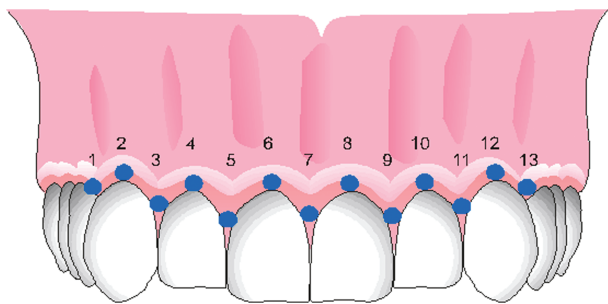


Figure 1.

Localization of the laser Doppler flow measuring sites (1 through 13).

While the probe was moved to the next measuring site, the computer program recording the flow data was interrupted to avoid the recording of fluctuating values caused by the manipulation of the probe during the moving procedure. Between the two splint measurements, the splint was removed for 3 minutes. The second splint measurement took place in the same manner as described above.

Three minutes after the second splint measurement, LDFRs were performed by hand. The LDF values were averaged over 1 second and directly recorded as numbers from the laser Doppler screen display by the second examiner when the first examiner gave a signal, again at the same time being registered by the computer software. The examiner aimed to hold the probe as steady as possible by supporting his hand on the subject's teeth.

Influence of Toothbrushing on LDFRs

In an antecedent experiment, the effect of toothbrushing on LDFR in two male dental students aged 27 and 29 years was determined. The students had healthy gingiva (GI = 0) and no fillings of the upper front teeth. LDF baseline values were determined as described above, in one splint and one hand measurement. The subjects were then advised to brush their teeth in the usual manner; the next LDF measurements by splint and by hand were performed immediately after brushing. Further LDF measurements took place after 10 and 60 minutes.

Clinical Examination

The GI,³¹ plaque index (PI),³² probing depth, and clinical attachment loss were determined at the 13 measuring sites after laser Doppler monitoring procedures. Probing depth and clinical attachment loss were measured using a periodontal probe[#] color-coded at 3, 6, 9, and 12 mm, and read to 1 mm. To determine the GI and the PI at the papilla tips, the disto-labial and mesio-labial sites forming the papilla were examined, and the highest value was recorded. The clinical examinations were carried out by one calibrated examiner (SP).

LDFRs in Healthy Gingiva and EG

A detailed medical history was obtained at the first appointment. Afterwards, the silicone impression to be used as individual splint and a maxillary impression for the fabrication of a splint to cover the teeth and gingiva during brushing in the EG period were taken. At the subsequent appointments, microvascular and clinical data were collected, always at the same time of the day, LDFRs prior to the clinical parameters. Subjects were instructed not to eat, drink, or brush

^{||} Panasil Soft Putty, Kettenbach, Eschenburg, Germany.

[¶] Interactive Laser Doppler Datenaufnahme, Version 7.0, O. Kempfski and L. Kopacz, University of Mainz.

[#] PCP 12, Hu-Friedy, Leimen, Germany.

their teeth for at least 1 hour prior to each appointment.

At the next visit (beginning of study period), baseline data were recorded. The study subjects then refrained from oral hygiene procedures in a proscribed area employing a partial mouth EG model. They were given a custom-made acrylic splint covering teeth and gingiva from teeth #5 to #9 or from #8 to #12 and advised to cover the selected teeth with the splint before performing oral hygiene measures to allow the development of gingivitis as described by Matheny et al.³³ They were also advised to use a regular NaF-containing toothpaste and to refrain from using mouthrinses or dental irrigators.

Gingival health and LDFR were monitored daily over a minimum period of 8 days. The EG period was terminated when at least one measuring site exhibited a GI of 2 and then when the subject decided to end the experimental period (8 to 20 days). On the last day of the study, the experimental teeth were scaled and polished, and dental hygiene was reinstated.

LDFR in CG

In patients with CG, the clinical examination and the LDFMs were performed once in exactly the same manner as described above.

Statistical Analysis

Data analysis was performed on a personal computer using statistical software. ** Data are given as means \pm SD. The differences between clinical indices or LDFRs recorded at the gingival margin and the papilla tips and differences of LDFRs within the EG period and between LDFRs of splint and hand measurements were analyzed using the Student paired *t* test or the Wilcoxon signed-rank test when appropriate. For testing the reliability of the splint measurements, one-way intraclass correlation coefficients were calculated. Differences between subjects with experimental and CG were compared by means of the Mann-Whitney U test. Statistical significance was assigned when $P < 0.05$.

RESULTS

Effect of Toothbrushing on LDFR

Directly after toothbrushing, the splint and hand LDFRs rose. They returned to baseline values within 60 minutes (Fig. 2). As a consequence for the main study, all subjects were advised not to brush their teeth for at least 1 hour prior to each of the appointments to obtain reliable readings.

LDFR in Subjects With Healthy Gingiva

Data analysis revealed significantly higher LDFR at the papilla tips compared to the base of the gingival margin in all measurement procedures. LDFR

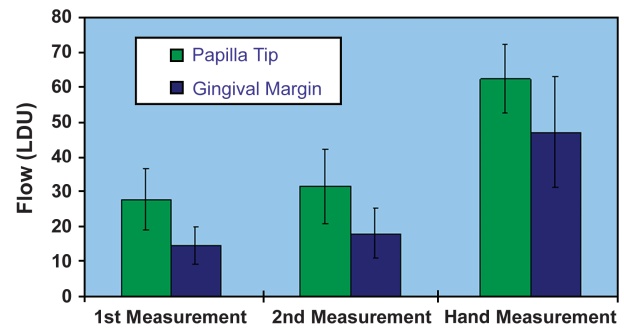


Figure 2.

Effect of toothbrushing on flow values. Directly after toothbrushing, the values of all measurements rose. They returned to baseline values within 60 minutes. Data are given as means \pm SD.

obtained by hand were significantly higher than those recorded with the help of the splint ($P \leq 0.0002$; Fig. 3).

No characteristic pattern was noted for volume. The velocity values showed a pattern similar to the flow values with higher readings and statistically significant differences between the mean splint measurement and the hand measurement at the papilla tips ($P < 0.01$; Table 1).

LDFR in Subjects With EG

All EG subjects developed gingivitis (PI: 2.77 ± 0.23 ; GI: 1.5 ± 0.53) and were monitored over a mean period of 12.5 ± 3.27 days (range: 8 to 20 days). The mean GI at the measuring sites with gingivitis increased significantly over the experimental period and was significantly higher than the mean GI of the control (healthy) sites at the last monitoring session (Fig. 4). LDFRs at diseased sites increased over the study period compared to the control sites; however, this change was not statistically significant (Fig. 5).

Reliability of LDF Measurements

In healthy subjects, the two splint measurements 3 minutes apart showed small differences and a good test-retest reliability (intraclass correlation coefficient $r_I = 0.575$; $P < 0.0001$). The second splint measurement gave higher mean LDFRs than the first measurement at all locations.

The standard deviation as a measure for the intra-individual variation of splint-LDFRs was higher at the papilla tips (8.76) than at the gingival margin (5.47). The standard error of the mean as a measure for the interindividual variation of splint-LDFRs was 9.47 for LDFRs at the papilla tips and 5.71 for LDFRs at the gingival margin.

Table 2 shows means, standard deviations, and intraclass correlation coefficients for splint-LDFRs of (clinically healthy) sites at different time points during

** SPSS, SPSS, Chicago, IL.

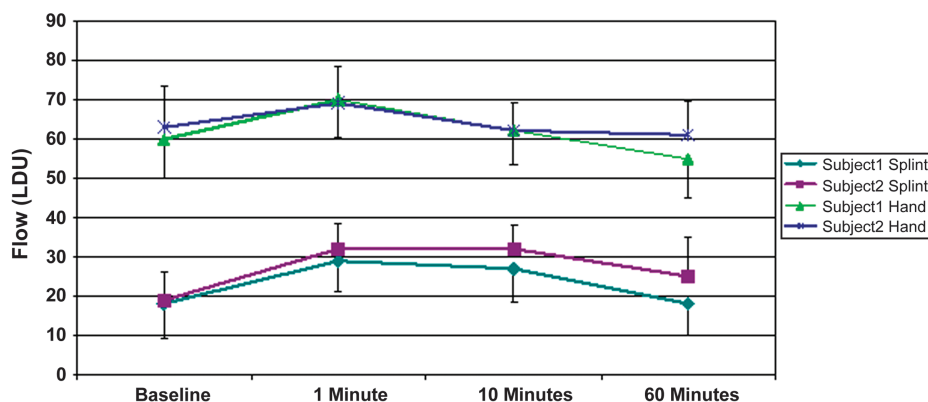


Figure 3.

Mean flow values at the papilla tips were higher than those at the gingival margin in all three measurements; this was statistically significant ($P < 0.05$). There was a statistically significant difference between the two splint measurements at the papilla tips ($P = 0.03$) and between splint and hand measurements at both locations ($P < 0.001$). Data are given as means \pm SD.

the EG period. Although intra-class correlation coefficients of the two splint measurements indicating short time reproducibility ranged between 0.561 and 0.775, the intraclass correlation coefficients of the first or second splint measurement at the baseline visit and the first or second splint measurement at subsequent visits as a measure of stability over time ranged between 0.836 and 0.861 or 0.593 and 0.773, respectively.

Hand measurements were significantly higher, more variable, and less reproducible than those obtained by splint (Fig. 3). The standard deviation

was 9.82 (papilla tips) and 15.82 (gingival margin).

Table 1.

Volume and Velocity Readings at the Papilla Tips and the Base of the Gingival Margin

	Papilla Tips	Base of Gingival Margin
Volume (mL _{LD} /mm ³)		
First measurement (splint)	6.06 \pm 1.44	6.22 \pm 2.00
Second measurement (splint)	6.27 \pm 1.10	6.21 \pm 0.97
Third measurement (hand)	5.41 \pm 1.33	5.71 \pm 1.61
Velocity (mm/s)		
First measurement (splint)	2.49 \pm 0.55	2.02 \pm 0.42
Second measurement (splint)	2.78 \pm 0.61	2.75 \pm 1.52
Third measurement (hand)	4.56 \pm 1.51	2.82 \pm 0.58

LDFRs in Subjects With CG

All CG patients had high plaque and gingival inflammation scores (PI: 2.8 \pm 0.2; GI: 1.63 \pm 0.78). A positive correlation was observed between LDFRs and clinical signs of inflammation as measured by the GI. The regional pattern already observed under healthy gingival conditions persisted in chronic inflammation: while rising flow values were observed at all locations, the rise at the papilla tips was more pronounced than at the gingival margin. When grouped by GI grades, the flow differences between the GI groups were statistically significant except for the difference of LDFRs between GI = 0 and GI = 1 (Table 3; $P < 0.05$).

The comparison of splint-LDFR in EG with splint-LDFR in CG showed significantly higher flow values in the CG group, at the papilla tips, and at the gingival margin (Figs. 6 and 7).

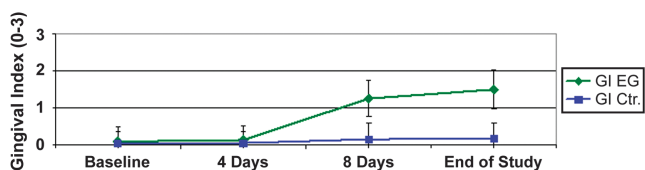


Figure 4.

The mean GI at the EG teeth (GI EG) was steadily rising during the study period, whereas the GI of the control teeth (GI Ctr.) remained at a significantly lower level ($P < 0.0001$). Data are given as means \pm SD.

DISCUSSION

Regardless of being determined by splint or hand, oral LDFMs have demonstrated considerable intra- and interindividual variation,^{23,25,30,34,35} questioning the reliability of LDF measurements and leading to a controversial discussion whether stabilization is required for meaningful measurements.^{26,28} Therefore, one of the major aims of this study was the development of a splint to obtain reliable LDFR and their comparison with hand-held probe measurements, especially because antecedent experiments to this study had demonstrated that movement artifacts or contact of the probe with the gingiva resulted in considerable deviations. Although several other authors have employed

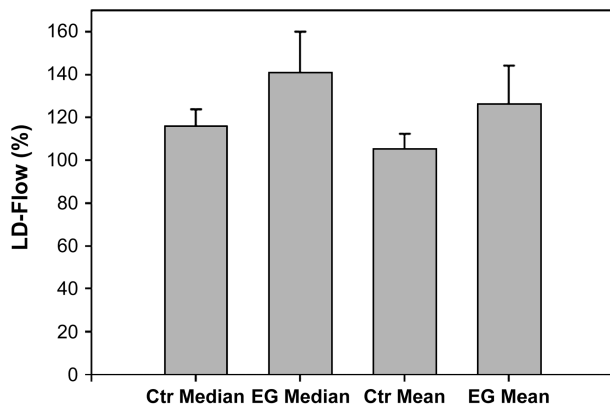


Figure 5.

Laser Doppler flow values increased at the EG teeth (EG) compared to the healthy control sites (Ctr). Data are given as mean and median change between the first and the last measurements (%) \pm SD.

individual acrylic^{21,36} or silicone^{37,38} splints, this study is the first known to the authors to embed plastic tubes matched to the outer diameter of the laser Doppler probe into impression material to ensure the maximal stability of the probe. As a result, LDFRs obtained by splint showed a good reliability over time, which is in accordance with previous reports.^{21,25,35} However, LDFRs obtained shortly after the first measurement were consistently higher than the baseline measurement, probably due to a slight compression of the gingiva by the impression material followed by reactive hyperemia. Contrary to another report,²⁸ this study demonstrated that hand measurements were significantly higher, more variable, and less reproducible than those obtained by splint, most probably due to movement artifacts and the impossibility of exactly repositioning the probe by hand. Therefore, the use of values obtained by splint measurements is superior

Table 2.

Means, SDs, and Intraclass Correlation Coefficients (rI) for Splint-LDFRs Measured at (healthy) Control Sites (N = 70) on Selected Days of the EG Period

	LDFR S1	LDFR S2	rI (S1/S2)*	Correlation of	rI (S1)†	rI (S2)‡
Day 1	23 \pm 19.45	25.9 \pm 17.26	0.730§	Days 1 and 4	0.861§	0.773§
Day 4	22.12 \pm 18.01	24.7 \pm 17.61	0.616§	Day 1 and study end	0.837§	0.593§
Study end	21.18 \pm 17.08	24.85 \pm 18.9	0.775§	Days 1 and 4 and study end	0.836§	0.657§

* rI (S1/S2) = correlation of the two splint measurements at a given time point.

† rI (S1) = correlation of the first splint measurements at two given time points.

‡ rI (S2) = correlation of the second splint measurements at two given time points.

§ $P < 0.0001$.

Table 3.

LDFRs in CG in Relation to the Severity of Gingival Inflammation (GI)

Flow (LDU)	GI 0	GI 1	GI 2	GI 3
Papilla tips				
N sites	9	15	46	7
First splint measurement	23 \pm 6.0	40.7 \pm 9.1	91.7 \pm 28.6	193 \pm 38.1
Second splint measurement	26 \pm 5.3	40.8 \pm 15.6	91.5 \pm 29.4	184 \pm 35.7
Hand measurement	113 \pm 46	104 \pm 40.6	125 \pm 36.7	134 \pm 31.9
Gingival margin				
N sites	7	17	38	4
First splint measurement	14.5 \pm 4.8	27.5 \pm 8.1	54.6 \pm 19.9	138 \pm 31.6
Second splint measurement	16.8 \pm 4.3	30.41 \pm 8.1	58.6 \pm 24.6	124 \pm 35.2
Hand measurement	77.3 \pm 15	88.4 \pm 29.6	109 \pm 45.8	164 \pm 43.3

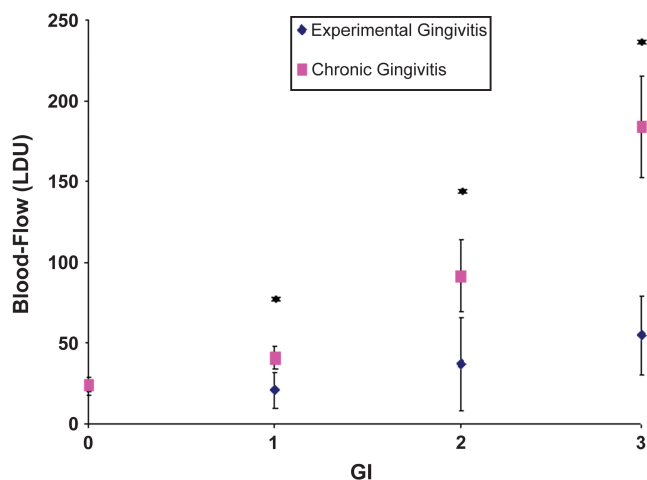


Figure 6.

Correlation of splint-LDFRs and GI at the papilla tips. The CG-LDFRs were significantly higher than the EG-LDFRs in GI > 0 (*P < 0.001). Data are given as means ± SD.

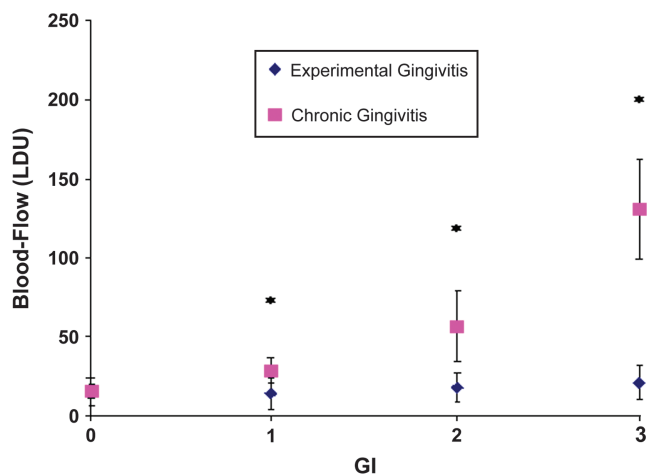


Figure 7.

Correlation of splint-LDFRs and GI at the marginal gingiva. The CG-LDFRs were significantly higher than the EG-LDFRs in GI > 0 (*P < 0.004). Data are given as means ± SD.

to hand measurements and is recommended for clinical research of LDF in inflammatory periodontal diseases.

A serious limitation of LDF is that data obtained with laser Doppler flowmeters of various manufacturers cannot be directly compared to other studies due to different calibration methods, resulting in their expression as arbitrary perfusion units or as a percentage of change to a baseline value. Whether laser Doppler flow values provide an absolute or relative quantification of capillary perfusion has been controversially discussed.^{26,28,29} Although studies by Heimann et al.³⁹ and Kempfski et al.⁴⁰ have shown that

the biologic zero in the system used in this study is very low, i.e., 0 to 2 LDUs, and that repeated measurements yield similar median blood flow readings, GBF was expressed in LDUs taking this controversy into consideration. The numerical values of this study are supported by data on GBF as determined by other methods, e.g., 34.4 ml/minute/100 g in attached cat gingiva⁴¹ and 51.1 ml/minute/100 g in human healthy gingiva.⁴² The comparison of LDFRs is further hampered by varying study protocols. For instance, LDFRs are averaged over varying time periods lasting from 30 to 120 seconds.^{24,37,43,44} In this study, splint-LDFR were averaged over 15 seconds.

The high individual variability of LDFRs is in agreement with the majority of earlier reports.^{23,34,38,45} Smoking and the scattering of the surrounding tissue, and also morphological circumstances such as gingival thickness, might influence LDFR variability because LDFRs at the palatal papillae show considerably higher variation than LDFRs at buccal gingival sites.⁴⁴ A differentiation of LDFRs according to periodontal biotypes seems to be an aspect worth pursuing in further studies.

One of the main reasons for the high variability of LDF is its high spatial resolution of ~1 mm,³ especially at adjacent sites in tissue with a high spatial heterogeneity,⁴⁶ which also applies to human gingival tissue. This can be overcome by scanning techniques yielding multiple location measurements that reflect regional tissue perfusion more precisely than single point assessments³⁹ and also allow for comparisons with successive measurements in the same subject and with data from other subjects.⁴⁷ Therefore, the variability of gingival LDFRs could be reduced by including more measurement locations into LDF monitoring. It has been shown for other tissue that an acceptable precision estimate may require up to 15 measurements,^{48,49} but only a few studies on GBF recorded data at more than 10 sites.^{21,30,43} Because scanning techniques employing a micromanipulator as described by Soehle et al.⁴⁷ are not applicable in the human mouth, the present study aimed to improve the reliability of LDF measurements by including as many as 13 well-defined locations at the upper front teeth into the calculation of regional GBF. Hoke et al.,²⁸ using the same laser Doppler flowmeter used in the present study, reported LDUs of 10 ± 3 and 13 ± 4 for the attached maxillary gingiva and mean LDFRs of approximately 30 LDUs at three not clearly defined sites within the attached gingiva, which compares well with LDFRs determined in the present study. Furthermore, LDFRs in gingival health were comparable with LDFRs of control sites in EG and clinically healthy sites in subjects with CG, confirming the reliability of these measurements for the facial gingiva of the upper front teeth. Further studies are

needed to investigate regional GBF at mandibular and at premolar and molar sites because it has been shown to differ from GBF at the front teeth.²⁸

Reports on changes of GBF in EG show conflicting results. Though animal studies^{13,22,50} and one study in humans³⁰ have demonstrated that blood flow in inflamed gingiva is higher than in healthy gingiva, Matheny et al.,³³ combining LDF and videomicroscopy, reported decreasing LDFRs and an increasing number of superficial vessels. Other clinical studies and a case report found a positive correlation between LDFRs and gingival inflammation or bleeding on probing.^{23,43,51} In the present study, we observed a slight, not significant, rise of LDFRs with an increase of the GI in EG, whereas we found a significant increase of regional GBF in patients with established gingivitis, which corresponded to the severity of clinical inflammation. It is possible that the effect on blood flow may have been missed due to small subject numbers or because of limitations in the oral measurement procedure. Here, the laser Doppler flowmeter in its present form presented some shortcomings. The straight needle form did not allow the examination of posterior sites. Furthermore, the size of the probe was too large to measure blood flow within the gingival crevice where early inflammatory changes might be detected first. Instead, blood flow was measured in the superficial capillary loops near the oral epithelium. Although this area may provide some information regarding the inflammatory state, our results let us assume that it may be less responsive to inflammatory stimuli than the gingival crevice. Possibly, intrasulcular LDFRs at multiple locations may yield more information about a relationship between GBF and early inflammatory vascular changes and need to be investigated in further longitudinal studies, especially because a method for obtaining crevicular LDFRs has already been described by Hinrichs et al.^{25,43}

To our knowledge, no study has yet compared LDFRs in experimental, early gingivitis developing over a relatively short time span with LDFRs in CG. The higher LDFRs at sites exhibiting chronic disease give room for the speculation that changes of the gingival microvasculature, although taking place early in the inflammatory process, may continue for a longer period of time, whose end still remains to be established. Whether LDFRs in EG would have increased further with time was not determined in the present study for ethical reasons.

A limitation to the interpretation of the data is the age difference between the two subject groups, the CG group being older than the EG group, which might have influenced the results. One previous study demonstrated a slight decrease of LDFRs with age,²⁴ whereas our data revealed no significant differences between the baseline LDFRs of the CG and EG groups.

Morphometric studies found fewer vascular structures but higher clinical inflammation scores in gingivitis lesions of old people compared to young subjects.⁵² Accordingly, it seems reasonable to expect the difference between LDFRs of healthy and diseased sites to be even greater had the subjects in the CG group been younger. Further investigations are needed to elucidate the effect of age on LDF measurements at inflamed gingival sites.

The present study demonstrated regional differences in gingival microcirculation at the papilla tips and the central gingival margin in healthy and diseased sites, which, although having been described in a case report,⁵¹ have not been reported for a larger subject group. On the contrary, based on a study using low-power stereomicroscopy demonstrating a lower vessel density in the papillary than the basal marginal gingiva,⁵³ one would expect lower LDFRs at papilla tips. An explanation of our findings could be the fact that LDF also measures subepithelial blood flow, which is not detected by videomicroscopy.

CONCLUSIONS

Although LDF is a valuable, non-invasive method for clinical research of gingival microcirculation, the results of this study show that meaningful LDF measurements require the use of a stabilizing splint because hand measurements do not yield reliable measurements. Multiple location measurements increase the reliability of the LDFR. Furthermore, we have been able to show a significant increase of regional GBF in patients with established gingivitis that corresponds to the severity of clinical inflammation. Modifications of the probe are needed to improve its clinical applicability in studies of gingival and periodontal pathology.

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