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A novel procedure for evaluating gingival perfusion status using laser-Doppler flowmetry

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Abstract

Objectives: To systematize a procedure that allows one to characterize the perfusion response pattern of attached gingiva to the topical and transitory compression of alveolar mucose, using laser-Doppler flowmetry.

Material and methods: A cross-sectional study was carried out, in 20 healthy adult subjects of either sex, with teeth in antero-mandibular sextant but without periodontitis at the lower left lateral incisor (LLLI). Sample was selected by convenience non-probability sampling. Gingival perfusion was evaluated at labial LLLI attached gingiva using a specially designed gingival tray. Two perfusion recordings were carried out 5 min. apart, each one consisting of a 40 s control phase, a 22 s compression phase and a 40 s post-compression phase. During compression phase, LLLI alveolar mucose was compressed with a wood-mounted cotton swab until the perfusion decreased to about 1/5 of its control perfusion value.

Results: Integrated primary basal flow (IPBF) during control phase was of 14,210 ± 1075 perfusion units (PU), whereas integrated flow during compression phase was of 1651 ± 202 PU ($p < 0.05$). After compression was released, integrated total secondary real flow was 13,322 ± 1513 PU ($p < 0.05$) which represented a 91.3 ± 3.8% of IPBF. Gingival compression propitiated an induced flow debt (IFD) of 6478 ± 781 PU, which increased in 980 ± 482 PU after compression was released, representing 18% of the IFD (Debt index).

Conclusions: A hypoaemic response in reaction to topical and transitory LLLI alveolar mucose compression was observed. Debt index and the ITSRF% are reproducible indices of microvascular perfusion response whose validation under pathological circumstances remains to be evaluated.

Key words: gingival perfusion evaluation; laser-Doppler flowmetry

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Laser-Doppler flowmetry (LDF) emerged almost 30 years ago in the absence of any other method to fulfil the clinical requirements of a sensitive, continuous, frequency responsive, non-invasive and real-time method for perfusion measurements in undisturbed microcirculation (Stern 1975). Although laser-Doppler methodology made it possible to demonstrate that blood flow wave patterns differ consistently among gingival tissue types (Baab et al. 1986) and that there are no within-subject differences over time in laser-Doppler readings (Boutault et al. 1989, Hinrichs et al. 1995), one of the major drawbacks of this methodology is that it gives the average blood cell flux (cells/m^2/s) linear (Nilsson 1984) read-out in relative perfusion units (PU) (Leahy et al. 1999). It has been documented in cat gingiva (Sasano et al. 1995), as well as in human skin (Johnson et al. 1984) that relative PU read-outs correlate linearly and significantly with absolute flow values (ml/min./100 g of tissue mass) measured by result-discrete methods; however, the slope of the regression line for each animal differs significantly from every other. This has been attributed to (1) spatial heterogeneity of blood flow within the tissue itself, (2) a small sampled volume, (3) optical properties of sample volume and (4) variations in microvascular haematocrit (Salerud et al. 1983, Johnson et al. 1984, Sasano et al. 1995; Leahy et al. 1999). Whatever the reason, the critical point is that, so far, there is no average calibration factor able to convert relative PU to absolute flow values.

The consequences of the above are multiple: (1) it contributes to high be-

tween-subjects variability in laser-Doppler readings (Baab et al. 1986); (2) it is not possible to compare individual relative PU values between subjects, although it is possible to compare between-groups relative PU means (Kerdvongbundit et al. 2003) and (3) it is not possible to know whether in an individual a given read-out value of relative PU represents much or little perfusion; individual assessment of sufficiency of perfusion must, therefore, be based on its variation in response to an appropriate stimulus, each subject serving as own control.

Although several stimuli have been proposed to induce microvascular gingival blood flow variations (heat, cold, localized pressure on sampled gingiva, occlusal force, vasoconstrictors, tooth brushing, periosteal stimulation, etc.) and evaluated (Baab et al. 1986, 1990, Atkins & Tuncay 1993, Ambrosini et al. 2002), with none of these calibrated stimuli have the haemodynamic tissue repercussions, which result from their application, been controlled and much less quantified.

Because we are interested in having a way of evaluating in vivo sufficiency of perfusion, the aim of the present study was to systematize, in healthy subjects without periodontitis at the lower left lateral incisor (LLLI), an operative and analytical procedure that allows us to characterize perfusion response pattern at labial LLLI attached gingiva to topical and transitory compression of LLLI alveolar mucose, using LDF. The following principles dictated our operative design: (a) the procedure should be simple; (b) the stimulus should be tolerable, topic, transitory, controllable and reproducible; (c) the laser-Doppler probe should be spatially insulated from sampled gingiva, and this should be a site different from the site of gingival compression in order to assure mechanical and geometric stability before, during and after stimulus and finally (d) the technique should preserve the microenvironment humidity in attached gingiva. To possess a procedure that allows us to evaluate clinically, individually and quantitatively the sufficiency of perfusion constitutes a tool that will improve the quality of periodontological attention, while the technology gives us the possibility of measuring absolute gingival blood flow.

Material and Methods

A cross-sectional study was carried out from August 2002 to July 2003, with subjects attending the Clínica de Medicina Estomatológica, Facultad de Odontología, UASLP, México. The Ethics Committee of the school approved the study in accordance with international guidelines for the protection of human beings. Forty subjects participated in two appointments in our facilities. The first appointment was used for obtaining a complete clinical and oral history, laboratory tests and complete periodontal evaluation and making an mandibular impression. The second appointment was used for obtaining vital signs and performing gingival vascular response, for which, the subjects were not allowed to eat for at least 2 h before the start of the study. By convenience non-probability sampling, 20 subjects were selected, who fulfilled the following criteria: (a) inclusion criteria: either sex, absence of systemic diseases, preprandial and 2 h post-prandial glucose concentration <120 and <140 mg/dl, respectively, body mass index $<27\,kg/m^2$ (Casillas & Vargas 1980), without arterial hypertension or first-grade diabetes mellitus antecedent, presence of teeth in antero-mandibular sextant without periodontitis at LLLI and informed written consent to participate in the study; (b) exclusion criteria: pregnancy, evident genetic diseases, use of drugs that affect blood glucose, have anticholynergic action or inhibit angiotensin converting enzyme; current treatment of periodontal disease; epilepsy; edentulous persons; smokers and persons with renal transplantation; (c) elimination criteria: (1) technical impossibility to reach, during gingival compression, a perfusion value of about 1/5 of its control value in two consecutive recordings and/or (2) differences greater than 30% in perfusion responses to gingival compression between two consecutive recordings and/or (3) differences greater than 15% in total backscatter signal between two consecutive recordings.

Periodontal evaluation

The periodontal evaluation was performed in LLLI. The same 6-months pre-calibrated examiner conducted all clinical examinations. The presence or absence of gingival inflammation was evaluated and graded (Löe 1967) in mild (0.1–1.0 GI score), moderate (1.1–2.0 GI score) or severe (2.1–3.0 GI score). The probing depth and attachment level were registered with a calibrated periodontal probe graded at 1, 2, 3, 5, 7, 8, 9, and 10 mm (Hu-Friedy, Chicago, IL, USA). The probing depth was evaluated from gingival margin to the base of the gingival pocket, considering a healthy sulcus as <3 mm. The attachment level was evaluated from the cemento-enamel junction to the base of sulcus, considering a healthy sulcus as <2 mm. The criteria to consider the presence of periodontitis at LLLI were probing depth >3 mm and attachment level >2 mm.

A dental cast was made and a blocking out resin spacer (LC Block-Out, Ultradent, South Jordan, UT, USA) of 0.5 mm was transiently applied over LLLI attached gingiva labial aspect of the model and a probe miniholder (PH 07-5, Perimed AB, Jarfalla, Sweden) superposed to it in order to keep constant the distance between the small straight probe and attached gingival (Probe 407-1, Perimed AB), and to keep the tray from contacting the LLLI tissues. A heat/vacuum tray-forming machine (Sta Vac, Buffalo Dental, Syosset, NY, USA) and 0.20-in hard-tray (Splint Sheets, Ultradent) were used to fabricate the horseshoe-shaped tray extended from the second left premolar to the second right premolar. The lowest margin of labial aspect of the tray was trimmed at the level of the inferior edge of the probe miniholder.

Gingival vascular response evaluation

Each subject was placed in a decubitus-supino comfortable position on a dental chair, the head placed with discrete neck hyperextension, using a travel pillow. Moreover, the head was kept motionless by using a semicircular frontal metallic arc fixed to the dental chair and then packed with low-compression pillows, to avoid lateral head movements. All subjects were given a full explanation of the procedure and were asked to keep relaxed during the procedure, not to gesticulate, swallow or speak and to breathe normally and smoothly. After 20 min. of stabilization, a soft-tissue retractor (Morita Co., Osaka, Japan) was placed in the oral cavity and both gingival tray and laser-Doppler probe (Probe 407-1, Perimed AB) were installed (Fig. 1). Before starting gingival perfusion recordings, vital signs were taken.

Gingival perfusion recordings were obtained using a Periflux System (Perimed AB) consisting of a PF 5001 main unit, a PF 5010 LDPM unit (Class 1

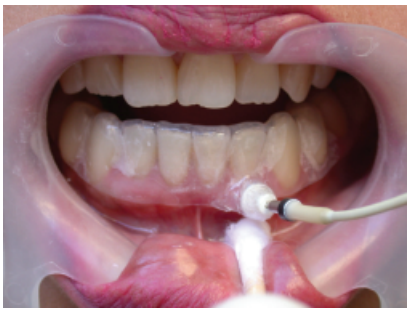

Fig. 1. Oral cavity with soft-tissue retractor, gingival tray, miniholder (Perimed, PH 07-5) and laser-Doppler probe (Perimed, Probe 407-1) installed. The site where the wood-mounted cotton swab was placed is shown.

laser, 780 nm, near-infrared laser diode, power = 1 mW, time constant = 0.2 s, bandwidth = 20 Hz–20 kHz, with validated electronic linearizer (Nilsson 1984), a Probe 407-1 (5 mm diameter, 0.25 mm fibre separation) and a PF1000 calibration device. With the equipment calibrated, two continuous perfusion recordings were carried out 5 min. apart. Each one consisted of a 40 s control phase, a compression phase of 22 and a 40 s post-compression phase. During compression phase, the LLLI alveolar mucose below the tray margin was quickly ($\sim$ 2.5 s) and progressively compressed with a wood-mounted cotton swab (Prodema, Bogotá, Colombia) until the perfusion decreased to about 1/5 of its control perfusion value. Once there, compression was borne for 20 s, after which, came the post-compression phase. Both the perfusion signal and the total backscatter signal were captured to a frequency of 32 Hz and analysed by PSW Perisoft 1.30 V software, which allows one to obtain the area under the curve (AUC) as well as spectral analysis directly.

Panels A–D in Fig. 2 describe the perfusion parameters evaluated in each perfusion recording; the terms "primary" and "secondary" in this context are synonymous of control phase and post-compression phase, respectively. Preliminary recordings performed under control phase conditions gave evidence that if gingiva was not disturbed, control perfusion values were stable for at least 3 min. Thus, the term "basal" means under undisturbed flow conditions, so that the term "integrated secondary basal flow" speaks of the integral of flow that would exist if gingival perfusion had not been disturbed (i.e., undisturbed), and it was proportionally derived from integrated primary basal flow, whereas the term "real" means the measured flow. Finally, flows and flow debts are expressed in relative perfusion units (PU) and time durations in seconds (s).

Data analysis

Before starting the study, the examiner was calibrated with an expert periodontist and with herself in all periodontal variables to be measured. For this, the agreement rates weighted κ and Cohen's κ with their 95% confidence bounds and bands were computed in 30 study-independent subjects. The perfusion parameter (see Fig. 1) values as well as the power spectral density values (dB) for every frequency in the two consecutive perfusion recordings of each subject were averaged. From these 20 averaged values, mean, SE and coefficient of variation (CV,%) were obtained, the latter taken as index of between-subject variability (Zar 1974). The degree of absolute agreement between perfusion measurements obtained in the two consecutive recordings was evaluated, using intra-class correlation coefficient (ICC, one way, single measurement, randomly chosen subjects, two observations, one rater, $n = 40$, Model ICC1; McGraw & Wong 1996, calculating their 95% confidence bounds and bands). To evaluate κ and ICC, Landis and Koch criteria (Kramer & Feinstein 1981) were used. The time course of perfusion was analysed by one-way repeated measurements (subject as block) before Box–Cox data transformation to adhere to assumptions of parametric statistics (Winner et al. 1991); $p < 0.05$ was considered statistically significant. All the data were analysed by using both JMP V.4 (SAS Institute) and R 1.8.0.beta packages.

Results

Twenty healthy subjects, 13 females and seven males (age 30–55 years) with body mass index of 24.5 ± 0.4 kg/m^2, mean arterial pressure of 92 ± 1 mmHg, heart rate of 80 ± 2 bpm, pre-prandial glucose of 89 ± 2 mg/dl and 2 h post-prandial glucose of 100 ± 4 mg/dl constituted the group selected. From the remaining 20 eliminated subjects, three (15%) were eliminated by criterion 1, nine (45%) by criteria 1+2, five (25%) by criteria 1+3 and three (15%) by criteria 1+2+3. The inter- and intra-observer reproducibility of LLLI periodontal indices reached by examiner showed concordance indices above 0.80. Their κ 95% confidence bands were from 0.20 to 0.55. The labial LLLI periodontal diagnostic in the selected group was as follows: no gingivitis 25%, mild gingivitis 0%, moderate gingivitis 70% and severe gingivitis 5% of the cases. No subject showed periodontitis at LLLI.

Figure 3 shows the four different gingival perfusion patterns seen in response to LLLI alveolar mucose compression. From 40 recordings obtained, pattern 1 was seen in 40% of them, pattern 2 in 10%, pattern 3 in 5% and pattern 4 in 45%. The consistency in gingival perfusion response pattern between two consecutive recordings was 80%. Figure 4 shows the average time course of gingival perfusion during the three phases. As can be seen, the compression significantly reduced gingival perfusion to almost 1/5 of its control value, showing a recuperation to control phase levels 9 s after compression was released. A pattern of reactive hypoemia was observed in 80% of the subjects; the remaining showed a pattern of reactive hyperaemia.

The perfusion parameters evaluated in the selected group are shown in Table 1. Primary basal flow during control phase was of 357 ± 39 PU, which, in a lapse of 2.4 ± 0.2 s, was reduced to give a compression flow of 60 ± 8 PU. The integrated primary basal flow was $14{,}210 \pm 1075$ PU (IPBF), whereas the integrated flow during compression phase was 1651 ± 202 PU, which represented a $12.4 \pm 1.3\%$ of IPBF. After compression was released, integrated total secondary real flow (ITSRF) was $13{,}322 \pm 1513$ PU, which represented a $91.3 \pm 3.8\%$ of IPBF (ITSRF% = ITSRF/IPBF $\times$ 100). The transitory gingival compression induced a debt of flow (IFD) of 6478 ± 781 PU, which, after compression was released, increased early (903 ± 321 PU) and late (77 ± 208 PU), generating a total secondary flow debt (TSFD) of 980 ± 482 PU, which represented 18% of the debtinduced during the compression phase (debt index = TSFD/IFD). Debt index and ITSRF% showed between them a negative ($r = -0.9926$) and significant ($p < 0.0001$) correlation. On the other hand, there was a light but significant correlation between labial LLLI gingival index and ITSRF% ($\rho = 0.4620$, $p = 0.040$) as also with

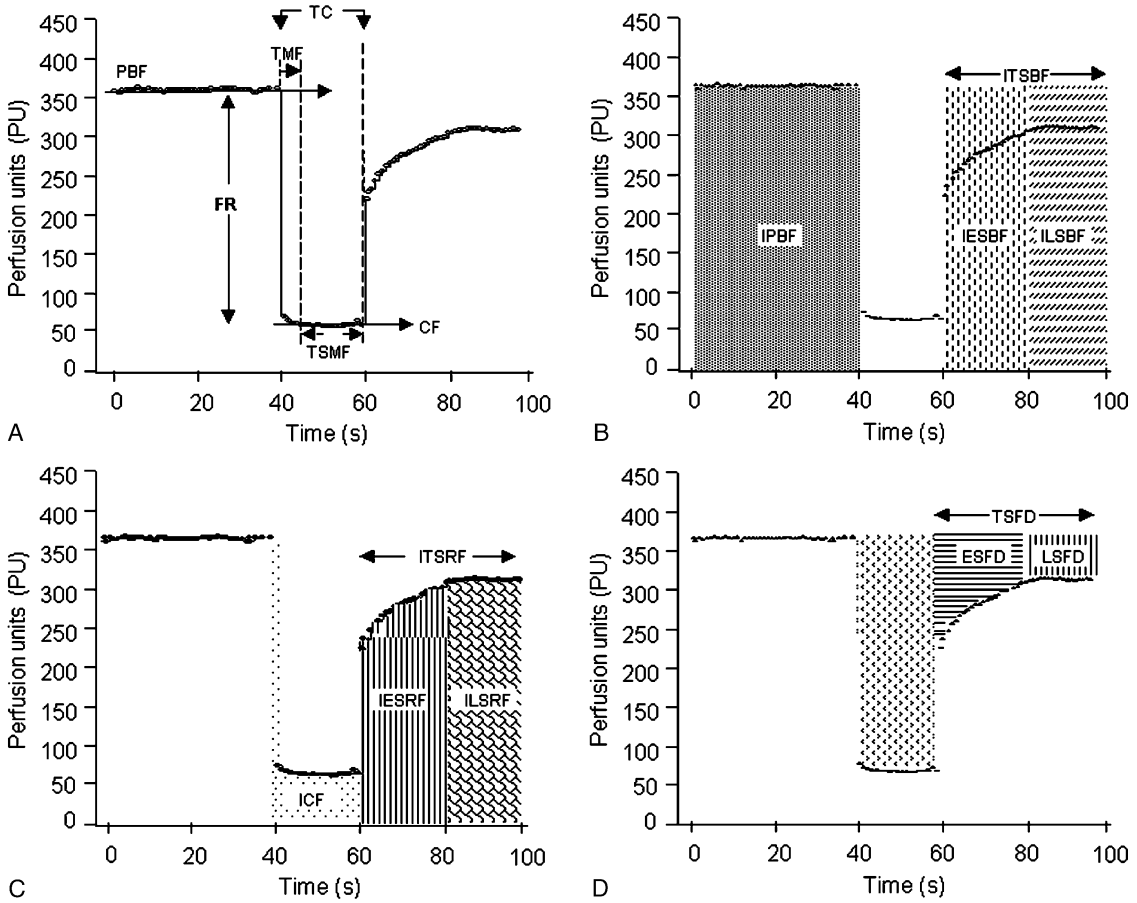

Fig. 2. Perfusion parameters schematically represented. Panel (A) basic parameters: primary basal flow (PBF, average perfusion during control phase); compression flow (CF, average perfusion during maximal and sustained gingival compression); flow reduction (FR = PBF – CF); time for minimal flow (TMF, time required for reaching the start of CF); time of compression (TC, duration total of compression); time of stable minimal flow (TSMF, time of maximal and sustained gingival compression); Panel (B) integrated basal parameters: integrated primary basal flow (IPBF, area under the curve (AUC) from t_0 to $t_{40\,s}$); integrated early secondary basal flow (IESBF = IPBF × duration in s of compression phase/40 s); integrated late secondary basal flow (ILSBF = IPBF × 40 s–duration in s of compression phase/40 s); integrated total secondary basal flow (ITSBF = IESBF+ILSBF); Panel (C) integrated real parameters: integrated compression flow (ICF, AUC of real flow during the compression phase); integrated early secondary real flow (IESRF, AUC of real flow after compression phase but with its duration); integrated late secondary real flow (ILSRF, AUC of real flow after compression with duration = 40 s-duration in s of compression phase); integrated total secondary real flow (ITSRF = IESRF+ILSRF); Panel (D) calculated flow debts: induced flow debt (IFD = IPBF with duration of compression phase – ICF); early secondary flow debt (ESFD = IESBF – IESRF); late secondary flow debt (LSFD = ILSBF – ILSRF); total secondary flow debt (TSFD = ESFD+LSFD). Debt index = total secondary flow debt/induced flow debt ratio = TSFD/IFD ratio (positive = reactive hypoaemia, negative = reactive hyperaemia).

debt index ($\rho = -0.4531$, $p = 0.045$) but not with primary basal flow.

Between-subjects variability in perfusion parameters (excluding times) measured directly (basic, primary basal, compression and secondary real), or calculated (secondary basal and induced flow debt) until just before releasing the gingival compression had similar and relatively elevated coefficient of variation (CV = 52% on average, Table 1). In contrast, calculated secondary debts and debt index showed dramatic increases in their CV (e.g. debt index CV = 212.2%). When integrated compression flow (ITC) and ITSRF were expressed as percentage of integrated primary basal flow (ITC% and ITSRF%, respectively), their CV decreased to 45.4% and 18.8%, respectively.

Perfusion parameter absolute agreement between two consecutive perfusion recordings (within-subject variability) was, on the other hand, very good (Table 1). With the exception of the times and secondary flow debts, the remaining perfusion parameter ICCs were >0.82 with ICC 95% confidence bands <0.29. Even though times during compression phase ICCs were lower and confidence bands wider, both integrated compression flow and IFD showed almost-perfect ICCs with narrow bands. The same happened with integrated secondary real flows (early, late and total); however, when these were expressed as percentage of IPBF (e.g. ITSRF%), their ICCs decreased on average to 0.76 and their confidence bands increased on average to 0.43. The debt index ICC was substantial (0.82) with a 95% confidence band relatively narrow (0.29).

The real-time wave pattern of attached gingiva perfusion was characterized by a heart-synchronous flow, with a superimposed slower and gradual pattern. Mean power spectral density analysis (Fig. 5) corroborated it, showing that the highest power density (8.0 ± 1.7 dB) was seen at about heart

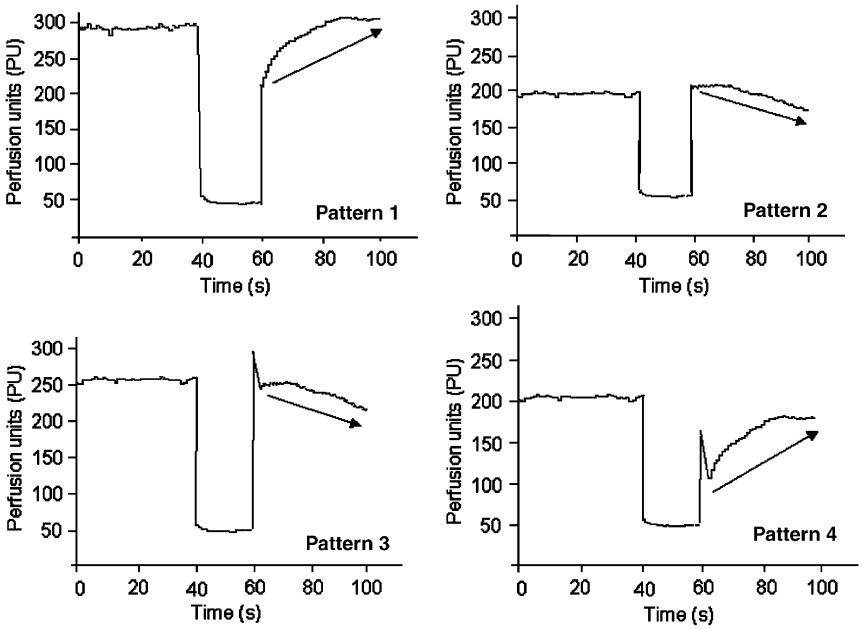

Fig. 3. Schematic representation of LLII gingival perfusion patterns seen in response to the topical (LLII alveolar mucosa) and transitory gingival compression. Pattern no. 1: There was no initial post-compression peak and post-compression response went up. Pattern no. 3: There was an initial post-compression peak that could or could not exceed control phase value and post-compression response went down. Pattern no. 2: There was no initial post-compression peak and post-compression response went down. Pattern no. 4: There was an initial post-compression peak that could or could not exceed control phase value and post-compression response went up.

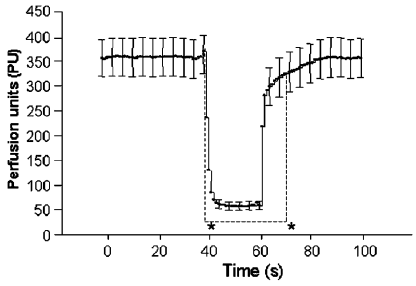

Fig. 4. LLII gingival perfusion time course in the group of subjects studied during the three phases (control, compression and post-compression). Values are mean ± SE ✻- - -✻ $p < 0.05$ *versus* average control phase value, $n = 20$.

frequency (~ 1.13 Hz); however, there were two additional small peaks at 1.07 and 1.32 Hz. An important power density peak (6.8 ± 0.9 dB) was also seen at a much lower frequency (0.13 Hz), which corresponded to vasomotion frequency (8/min).

Discussion

Laser-Doppler flowmetry (LDF) was developed (Stern 1975) as a plausible alternative to former invasive and non-invasive methods (Bishop & Dorman 1968; Baab et al. 1986, Leahy et al. 1999) to assess blood flow in intact microvascular systems, and it has been applied to study oral tissue blood flow. One of the major drawbacks of this methodology is the absence of an average calibration factor capable of converting relative perfusion units (PU) to absolute blood flow units. Although the consequences of this are multiple, a very important one is that it is impossible to know whether in an individual a given relative PU value represents much or little perfusion. So, the aim of the present study was to systematize an operative and analytical procedure that permits characterization of perfusion response pattern at attached gingiva to the compression of alveolar mucose, using LDF as a mean to assess the sufficiency of gingival perfusion individually.

In our operative design, it was necessary first to decide about the nature, intensity, site of application and duration of gingival stimulus. From the multiple stimuli reported and evaluated so far (Baab et al. 1986, 1990, Atkins & Tuncay 1993, Ambrosini et al. 2002), the mechanical compression was chosen because it is topic, tolerable and selective and it can be transitory and sharply applied, allowing one to evaluate the short-term gingival perfusion recuperation. Moreover, the haemodynamic tissue repercussion, which results from its application, can be controlled, reproduced and quantified. In order to assure that stimulus intensity was proportional to baseline gingival perfusion, this was reduced to about 20% of its control value instead of (a) reducing it to a fixed positive value (e.g. two or 10 relative PU), which would represent the different extent of gingival ischaemia in each subject or (b) applying a calibrated standard force, which does not guarantee that perfusion decreases in approximately the same proportion in all the subjects. The LLLI alveolar mucosa was selected as the compression site because (a) small arteries from which facial mucosal-supra-periostical arterioles come are accessible here and are easily compressible (Nuki & Hock 1974, Itois & Carranza 2002), (b) dental loss is more frequent in antero-mandibular and molar teeth (Drury et al. 1996), (c) when there is interest in studying gingival arteriolar dynamics, it is not desirable to compress these attached gingiva-localized structures and (d) mechanical and geometric stability of laser-Doppler probe before, during and after stimulus application is essential (Leahy et al. 1999). Therefore, the sites of gingival compression and perfusion recording (facial LLII attached gingiva) were separated. Finally, about 20 s of compression duration were chosen on the basis of prior gingival hyperaemia descriptions (Baab et al. 1986, Omori et al. 2002) as well as our preliminary gingival compression-tolerance assays.

The results indicate that the procedure here reported allowed us to measure perfusion parameters with a reproducibility intra-observer, within-subject almost-perfect, corroborating that there are no within-subject differences over time in laser-Doppler readings (Boutault et al. 1989, Hinrichs et al. 1995). That integrated compression flow had an almost-perfect ICC when ischaemia-times had moderate to substantial ICC, suggests that: (1) the extent of ischaemia reached is a greater determinant of compression flow than the little within-subject differences in ischaemia times, and (2) that these ischaemia-time differences had minimal influence on total secondary real flow because this had almost-perfect ICC. The similar CV among IPBF, integrated compression

Table 1. Basic, integrated (basal and real) and debt values of perfusion parameters with indices of between-subjects variability and intra-observer agreement

	Mean	SE	CV	ICC	95% Band
Basic parameters					
Primary **b**asal **f**low*	357	39	48.3	0.97	0.04
Compression **f**low*	60	8	63.0	0.94	0.09
Flow **r**eduction*	298	35	52.3	0.97	0.04
Time for **m**inimal **f**low*	2.4	0.2	36.2	0.55	0.62
Time of **c**ompression*	22.9	0.4	7.2	0.70	0.46
Time of **s**table **m**inimal **f**low*	20.6	0.3	7.5	0.66	0.51
Integrated basal parameters					
Integrated **p**rimary **b**asal **f**low*	14,210	1075	48.3	0.97	0.04
Integrated **e**arly **s**econdary **b**asal **f**low	8140	899	49.4	0.97	0.05
Integrated **l**ate **s**econdary **b**asal **f**low	6162	663	48.1	0.96	0.06
Integrated **t**otal **s**econdary **b**asal **f**low	14,302	1545	48.3	0.97	0.04
Integrated real parameters					
Integrated **c**ompression **f**low*	1651	202	54.9	0.94	0.10
Integrated **e**arly **s**econdary **r**eal **f**low*	7237	865	53.5	0.93	0.11
Integrated **l**ate **s**econdary **r**eal **f**low*	6085	677	49.8	0.93	0.12
Integrated **t**otal **s**econdary **r**eal **f**low*	13322	1513	50.8	0.95	0.08
Debts					
Induced **f**low **d**ebt	6478	781	54.0	0.98	0.04
Early **s**econdary **f**low **d**ebt	903	322	159.3	0.63	0.54
Late **s**econdary **f**low **d**ebt	77	208	1203.3	0.60	0.57
Total **s**econdary **f**low **d**ebt	980	482	220.0	0.66	0.50
Debt **i**ndex	0.18	0.08	212.2	0.82	0.29

Boldface letters constitute the acronym of perfusion parameter. Parameters with asterisk were measured; otherwise parameters were calculated. Flows and flow debts in relative perfusion units (PU), times in seconds (s) and debt index adimensional. Values are mean ± SE of 20 healthy subjects without periodontitis. Coefficient of variation (CV in %) was taken as index of between-subjects variability ($n = 20$). Intraclass correlation coefficient (ICC), with its 95% confidence band, was taken as index of intra-observer within-subject absolute agreement ($n = 40$). Perfusion parameters as in Fig. 1 legend.

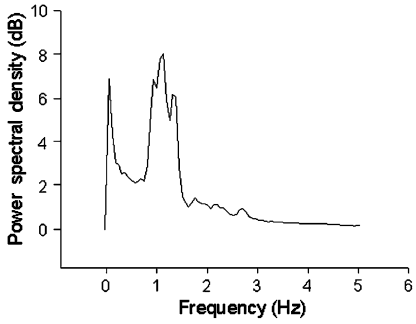

Fig. 5. Mean power spectral density (dB) analysis of LLII perfusion recordings during the control phase in the group of subjects studied. Frequency (Hz), $n = 20$.

flow and integrated secondary real flow indicate that between-subjects variability, although high, existed before, during and after gingival compression. In addition to spatial heterogeneity of blood flow, size and optical properties of sampled volume (which depends, among other things, on gingiva thickness), subject and site-specific variations in microvascular haematocrit, individual differences in basal neurogenic sympathetic activity could also contribute to between-subjects variability (Sasano et al. 1995).

The flow debt concept comes from circulatory physiology (Gregg & Fisher 1963) and describes the difference between undisturbed and real territorial blood flow (measured at nutritious vessel) when nutritious vessel is partial or totally occluded. Depending on vascular territory handled, this flow debt can be variably overpaid, underpaid or in some cases increased after the occlusion is released (Gregg & Fisher 1963). We transferred this concept to gingival microcirculation, with the reservation that perfusion measurement was carried out on the microcirculatory bed supplied by the nutritious vessel but not in the nutritious vessels per se. The results indicate that topical and transitory compression of facial LLLI alveolar mucosa induced a debt of flow (IFD) that increased (TSFD) after compression was released. This response, on average, can be characterized as reactive hypoaemia with a debt index (TSFD/IFD) of +18%. The last one reflects how the post-compression perfusion response is (direction and magnitude), in function of the debt contracted. Although ICC debt index was substantial, its CV was almost four times greater than IFD CV (54% *versus* 212.2%). This CV increment not only happened with debt index but also with secondary flow debts. Because positive (hypoaemia) and negative (hyperaemia) flow debts coexisted within the same group (reducing the mean and increasing standard deviation and therefore the CV), a calculated parameter that reflects the same phenomenon, without however, containing positive and negative values (therefore with a much lower CV) was looked for. It was found that when ITSRF is expressed as the percentage of IPBF (ITSRF%), its CV decreased from 51% to 19%. This reparameterization is consonant with debt index because values $<100\%$ translate reactive hypoemia (+debt index), whereas values $>100\%$ translate reactive hyperaemia (− debt index). ICC ITSRF% decreased from almost-perfect to substantial and increased the 95% confidence band from 0.10 to 0.39.

Baab et al (1986) reported that application of localized pressure on inter-dental, free or attached gingiva and on alveolar mucose caused ischaemia followed by reactive hyperaemia in all tissue types studied. As our results show, on average, a hypoaemic response in reaction to topical and transitory LLLI alveolar mucose compression does not invalidate our findings, because both the stimuli applied (site intensity and duration) and the way of recording the perfusion was totally different. In contrast to our study, Baab's group pushed the laser-Doppler flow probe against the tissues with a calibrated force ($1.5\,N/\sim 150\,g$) for 60 s and then they observed the perfusion response in the same site after releasing the force. On the one hand, this procedure does not assure mechanical or geometric stability between the probe and tissue before, during or after gingival compression, thereby introducing some noise to the laser-Doppler signal. On the other hand, it is likely that such a stimulus affects primarily meta-arteriolar and capillary structures of the microcirculatory unit; therefore, the microcirculatory response reflects the interaction of at least two components that are known to control tissular perfusion: (a) tissue vasodilator metabolites such as CO_2, adenosine, K^+, etc. and (b) endothelium-derived vasodilator/vasoconstrictor metabolites (Pohl &

de Wit 1999, Omori et al. 2001). The stimulus in the present work, in contrast, affected primarily the small arteries and it is likely that the microcirculatory response (reactive hypoaemia) reflects the interaction of at least a third component, in addition to the two above mentioned, which would consist of the miogenic vasoconstrictor response of arterioles when they are faced with normal blood pressure after the compression is released (De Wit et al. 1997). So far, however, the existence of this third component in gingival circulation has not been documented.

Finding a light but significant correlation between labial LLLI gingival index and ITSRF% ($\rho = 0.4620$, $p = 0.040$) as also with Debt index ($\rho = -0.4531$, $p = 0.045$) suggests that to greater gingival inflammation there is a greater trend to show reactive hyperaemia. The biological significance of this cannot be ascertained from the present study, but it invites us to suggest that capillary and venular conductance is permanently augmented in gingival inflammation. The lack of association between facial LLLI gingival index and primary basal flow does not support this contention but does not invalidate it either, because it is difficult to show such an association when there exists a high between-subjects variability in primary basal flow. Recently, Kerdvongbundit et al (2003) reported that, as group, patients with moderate gingivitis have greater gingival perfusion than healthy gingival patients and that gingival perfusion was reduced to normal levels after the inflammation subsided.

In summary, by systematizing an operative-analytical procedure (simple, tolerable, controllable and reproducible), we characterized the perfusion response pattern of labial LLII attached gingiva microvasculature to the topical and transitory compression of LLII alveolar mucose as being one of reactive hypoaemia. Debt index (ITSRF/ICF) and the ITSRF% (ITSRF/IPBF $\times$ 100) are reproducible indices of microvascular perfusion response, whose validation under pathological circumstances remains to be evaluated.

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References

Ambrosini, P., Cherene, S., Miller, N., Weissenbach, M. & Penaud, J. (2002) A laser Doppler study of gingival blood flow variations following periosteal stimulation. *Journal of Clinical Periodontology* **29**, 103–107.

Atkins, S. E. & Tuncay, O. C. (1993) Gingival blood flow. *Mississippi Dental Association Journal* **49**, 27–29.

Baab, D. A., Öberg, P. A. & Holloway, G. A. (1986) Gingival blood flow measured with a laser Doppler flowmeter. *Journal of Periodontal Research* **21**, 73–85.

Baab, D. A., Oberg, A. & Lundstrom, A. (1990) Gingival blood flow and temperature changes in young humans with a history of periodontitis. *Archives of Oral Biology* **35**, 95–101.

Bishop, J. G. & Dorman, H. L. (1968) Control of blood circulation in oral tissues. *Advances in Oral Biology* **3**, 1–44.

Boutault, F., Cadenat, H. & Hibert, P. J. (1989) Evaluation of gingival micro-circulation by a laser-Doppler flowmeter. Preliminary results. *Journal of Craniomaxillofacial Surgery* **17**, 105–109.

Casillas, L. & Vargas, L. (1980) Cuadros de peso y talla para adultos mexicanos. *Archivos de Investigación Medica* **11**, 157–175.

De Wit, C., Jahrbeck, B., Schäfer, C., Bolz, S. S. & Pohl, U. (1997) Nitric oxide opposes myogenic pressure responses predominantly in large arterioles in vivo. *Hypertension* **31**, 787–794.

Drury, T., Brown, L. & Zion, G. (1996) Tooth relation and tooth loss in the permanent dentition of adults, 1988–1991. *Journal of Dental Research* **75**, 684–695.

Gregg, D. E. & Fisher, L. C. (1963) Blood supply to the heart. In: Hamilton, W. F. (ed). *Handbook of Physiology, Section 2, Circulation*, Vol. II, pp. 1517–1584. Washington, DC: American Physiological Society.

Hinrichs, J. E., LaBelle, L. L. & Aeppli, D. (1995) An evaluation of laser Doppler readings obtained from human gingival sulci. *Journal of Periodontology* **66**, 171–176.

Itois, M. E. & Carranza, F. A. (2002) The Gingiva. In: Newman, M. G., Takei, H. H. & Carranza, F. A. (eds). *Clinical Periodontology*, 9th edition, pp. 16–35. New York: W.B. Saunders.

Johnson, J. M., Taylor, W. F., Sheperd, A. O. & Park, M. K. (1984) Laser-Doppler measurement of skin blood flow: comparison with plethysmography. *Journal Applied Physiology* **56**, 798–803.

Kramer, M. S. & Feinstein, A. R. (1981) Clinical biostatistics LIV. The biostatistics of concordance. *Clinical Pharmacology and Therapeutics* **29**, 111–123.

Kerdvongbundit, V., Vongsavan, N., Soo-Ampon, S. & Hasegawa, A. (2003) Microcirculation and micromorphology of healthy and inflamed gingivae. *Odontology* **91**, 19–25.

Leahy, M. J., deMul, F. F. M., Nilsson, G. E. & Maniewski, R. (1999) Principles and practice of the laser-Doppler perfusion technique. *Technology and Health Care* **7**, 143–162.

Löe, H. (1967) The gingival index, the plaque index and the retention index systems. *Journal of Periodontol* **38**, 610–616.

McGRaw, K. O. & Wong, S. P. (1996) Forming inferences about some intraclass correlation coefficients. *Pyschological Methods* **1**, 30–46.

Nilsson, G. E. (1984) Signal processor for laser Doppler tissue flowmeters. *Medical and Biological Engineering and Computing* **22**, 343–348.

Nuki, K. & Hock, J. (1974) The organization of the gingival vasculature. *Journal of Periodontal Research* **9**, 305–313.

Omori, Y., Takahashi, S. & Todoki, K. (2002) Role of nitric oxide in post-ischemic gingival hyperemia in anesthetized dogs. *Redox Rep* **7**, 300–303.

Pohl, U. & de Wit, C. (1999) A unique role of NO in the control of blood flow. *News in Physiologycal Sciences* **14**, 74–79.

Sasano, T., Shoji, N., Kuriwada, S. & Sanjo, D. (1995) Calibration of laser-Doppler flowmetry for measurement of gingival blood flow. *Journal of Periodontal Research* **30**, 298–301.

Salerud, E. G., Tenland, T., Nilsson, G. E. & Oberg, P. A. (1983) Rythmical variations in human skin blood flow. *International Journal of Microcirculation: Clinical & Experimental* **2**, 91–102.

Stern, M. D. (1975) In vivo evaluation of microcirculation by coherent light scattering. *Nature* **254**, 56–58.

Winner, B. J., Brown, D. R. & Michels, K. M. (1991) Single-factor experiments having repeated measures on the same elements. In: *Statistical Principles in Experimental Design*, eds. Chiappetta, M. E., Vaicunas, J., Morris, J. M., 3rd edition, pp. 220–283. New York: McGraw-Hill, Inc.

Zar, J. H. (1974) Measures of dispersion and variability. In: *Biostatistical Analysis*, 1st edition, eds. McElroy, W. D., Swanson, C. P., pp. 29–40. Englewood Cliffs, NJ: Prentice-Hall Inc.

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