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Evaluation of gingival blood flow by the use of laser Doppler flowmetry following periodontal surgery. A pilot study

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Objectives: The aim of this pilot study was to evaluate the applicability of laser Doppler flowmetry (LDF) in recording the gingival blood flow following periodontal surgery.

Material and methods: Five patients suffering from advanced chronic generalized periodontitis were included in the study. After completion of basic periodontal therapy, full-mouth plaque score, bleeding on probing, gingival recession and clinical attachment level were recorded. The upper anterior areas with a pocket probing depth of 6 mm or more were treated with an access flap. LDF recordings were performed in both the buccal and palatal aspect of the operated areas with the aid of an individual acrylic stent at the day of the surgery, prior to local anaesthesia, 3 min following anaesthesia, immediately after the operation and at days 1, 2, 3, 4, 7, 15, 30 and 60 following operation.

Results: Overall, the blood flow decreased immediately following anaesthesia and remained in lower values compared to baseline immediately following operation. The gingival blood flow presented an overall increase in comparison to baseline values until the 7th day following surgery at the buccal and palatal interdental sites, as well as at the alveolar mucosa sites. By the 15th day, as well as at the following observation periods of 30 and 60 days, the gingival blood flow values at the palatal and alveolar mucosa sites were very similar to baseline. Increased blood flow changes were observed at 30 and 60 days following operation at the buccal interdental sites.

Conclusion: The results of the present pilot study suggest that the LDF might present clinical applicability in recording changes in gingival blood flow following periodontal surgery.

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Periodontal surgical therapy still remains a key aspect of the treatment of patients suffering from periodontal disease. The main objective of periodontal surgery is to preserve the periodontium by facilitating plaque removal and plaque control. This is achieved by creating accessibility for proper professional scaling and root planning and by establishing a gingival morphology that facilitates the patient's self-performed plaque control. A variety of surgical techniques have been developed and tested for their potential to restore the

periodontal tissues lost due to destructive periodontal disease. Most of them were aiming not only at the periodontal 'pocket reduction' but also at the regeneration of the lost periodontal attachment (1). However, the location of the primary and the vertical releasing incisions, as well as the design and the elevation of the different flap designs during periodontal surgery is mainly based on practical principles that facilitate mainly the surgical handling of the tissues.

Successful wound healing following periodontal surgery is strongly influenced by the revascularization rate, as well as by the preservation and the reconstruction of the microvasculature of the gingival tissues. Repair of connective tissue also depends on the development of a new vascular system, which can supply blood and nutrients to the wound area. The nutritional demands of the wound are greater than those of the non-wounded connective tissue (2) and they are greatest at the time when the local circulation is least capable of complying with the demand (3). All the body tissues, including the periodontium, are dependent upon the nutritive and hydrating elements ferried to them via the vasculature, which include the blood capillaries, the venules and the lymphatic channels. The free gingiva receives its blood supply from supraperiosteal blood vessels, blood vessels of the periodontal ligament, and the blood vessels of the alveolar bone (4). However, it has been reported that the blood supply of human gingivae is not homogeneous, and main gingival vessels may be severed in the regions where incisions and flaps are performed (5). In animal experiments, it has been demonstrated that the simple act of raising a mucoperiosteal flap initiates significant vascular trauma. The lack of blood circulation at the most coronal portion of the flaps lasts for at least 3 days, but persists for 7 days at the interproximal surfaces (6). This is in agreement with other investigations in animals, where it was shown that the rate of healing may vary among closely adjacent locations in the oral tissues (7). Therefore, a surgical procedure with properly located incisions and a flap design that would stringently conserve afferent blood supply to the different parts of the periodontium might have an essentially positive effect on the speed and the healing pattern of the operated tissues. It would also be essential at the different regenerative procedures, where it has been shown that the presence of a non-bioresorbable membrane is interfering with the revascularization of the operated area (8) and in certain cases it might create ischaemia of the flaps (9, 10) and partial necrosis of the superficial bone (11). Furthermore, an improved healing process would also imply less postoperative complications such as interdental papilla necrosis, opening of the flaps leading to compromised postoperative morphology of the interdental soft tissues and flap dehiscence exposing the root surfaces of the teeth, as well as improved postoperative comfort for the patient. Therefore, the location of the incision and the design of the flap might be of paramount importance for the final outcome of the periodontal operation, as the amount of the operated tissues, as well as the damage of the microvasculature of the area can be effectively reduced.

The laser Doppler flowmetry technique (LDF) depends on the Doppler principle (12), where a low power light from a monochromatic stable laser is directed via an optical fibre to the tissue to be studied. The light hitting moving blood cells undergoes a change in wavelength/frequency (Doppler shift), whereas light hitting static objects is unchanged. The frequency broadened light, together with laser light scattered from static tissue is photodetected and collected by one or more other optical fibers. The resulting photocurrent is processed to provide a blood flow measurement. The term used to describe blood flow is flux – a

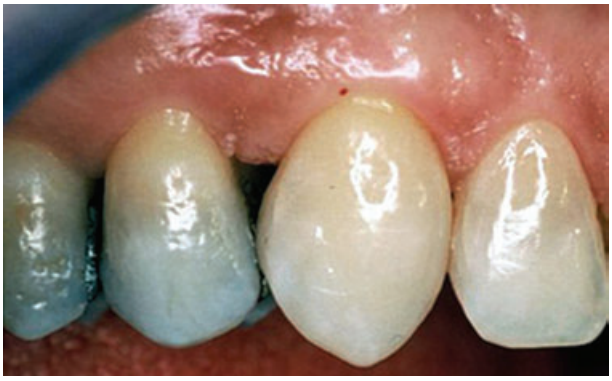

Fig. 1. Pre-operative clinical view.

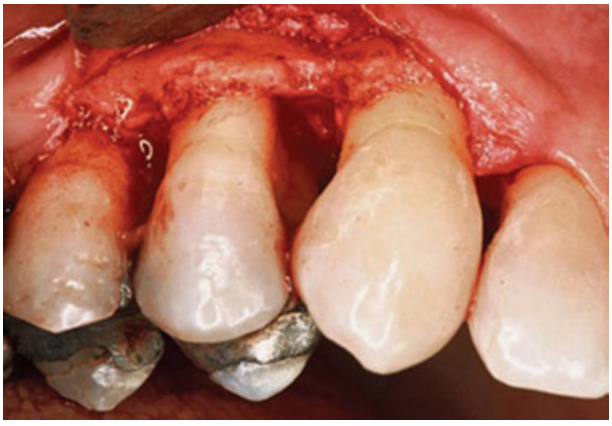

Fig. 2. Following flap elevation.

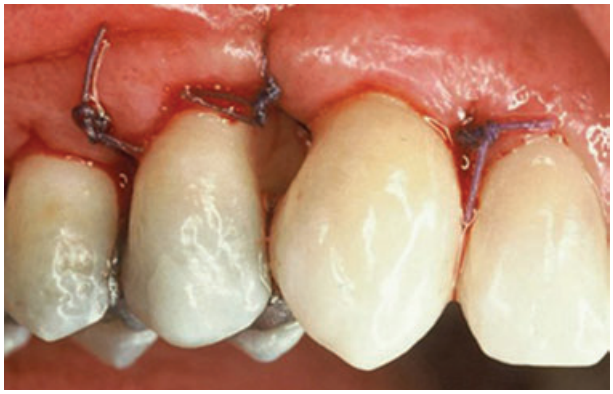

Fig. 3. Following suturing.

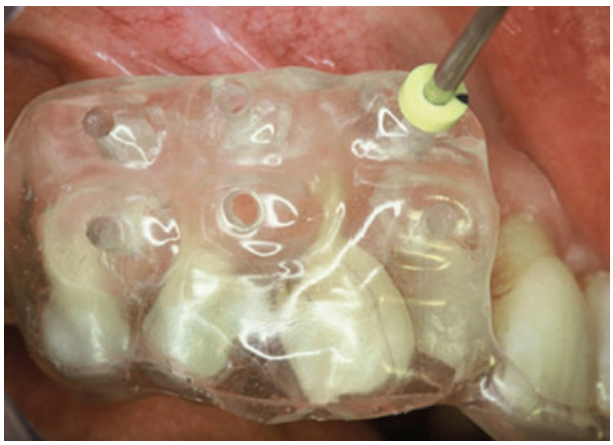

Fig. 4. View of the acrylic stent.

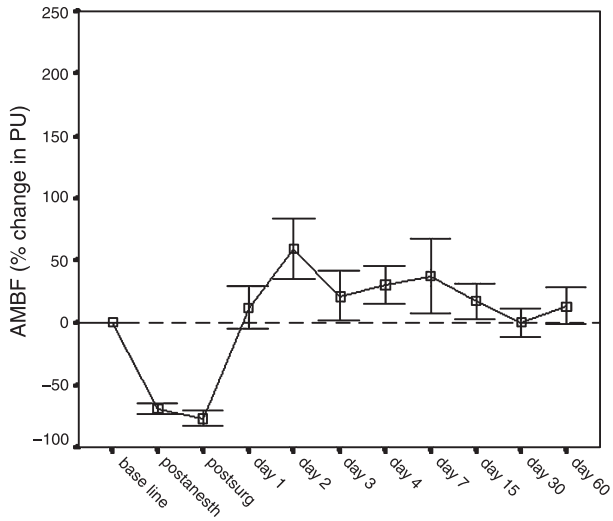

Fig. 5. Percentage of change of the alveolar mucosa blood flow (AMBF) at different time points compared to baseline values indicated by the horizontal line. Values: means ± SEM, $n = 15$ (five patients, 15 observations). PU, perfusion units.

quantity proportional to the average speed of the blood cells and their concentration (often referred to as blood volume). This is expressed in arbitrary perfusion units, which are linearly related to flux.

Today, LDF is an extensively used non-invasive method for measuring predictably the changes in the blood flux. It has been tested with a wide variety of clinical applications in the medical field. Especially in the field of plastic surgery, LDF is used for monitoring the blood perfusion in skin transplants and flaps where early signs of impaired circulation can be detected. It has been suggested that loss of the transplants can be prevented because the LDF device provides reliable and readily accessible information concerning the state of flap blood flow (13, 14). The LDF is also recommended for the postoperative evaluation of tissue after microvascular anastomoses, as it indicates vascular occlusion at an early stage where re-exploration is possible (15). In the field of dentistry, LDF has been successfully used for the measurement of blood flow under different pulpal conditions (16). It has also been tested for assessing the gingival blood flow in healthy situations (17), following tooth brushing (18) and following smoking (19, 20).

The aim of this study was to evaluate the applicability of the LDF in recording the gingival blood flow *in vivo* and to assess the changes of gingival blood flow following periodontal surgery.

Materials and methods

Patient selection

The study was approved by the Eastman Dental Institute and Hospital Joint Research and Ethics Committee. Five patients (two smokers, three non-smokers) referred to the Department of Periodontology, Eastman Dental Institute for the treatment of chronic generalized severe periodontitis were included in this pilot study. Further inclusion criteria were as follows:

- general good health;
- lack of systemic diseases;

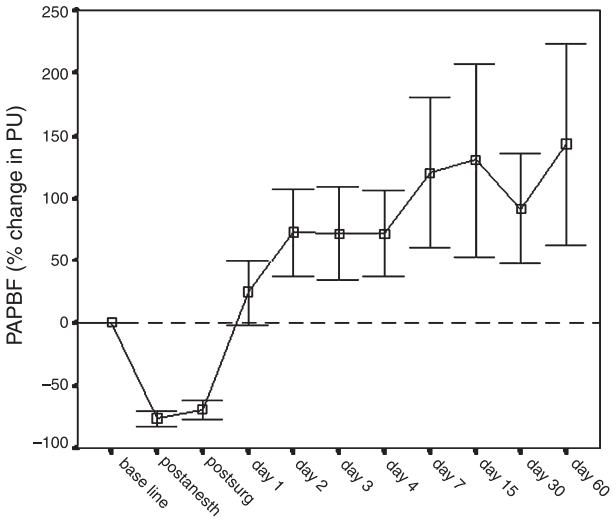

Fig. 6. Percentage of change of the papillary blood flow (PAPBF) at different time points compared to baseline values indicated by the horizontal line. Values: means ± SEM, $n = 15$ (five patients, 15 observations). PU, perfusion units.

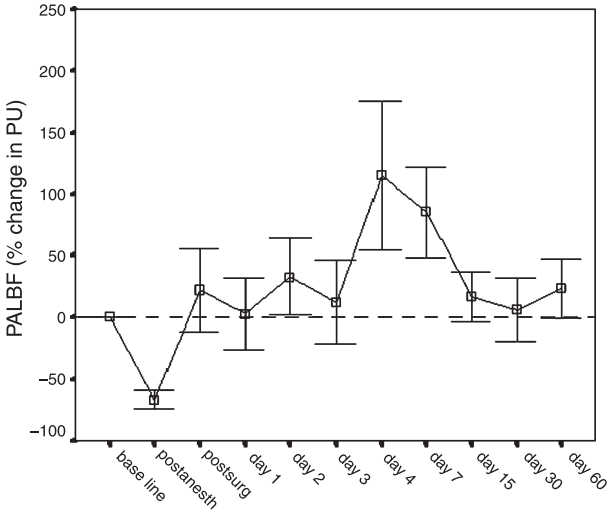

Fig. 7. Percentage of change of the palatal blood flow (PALBF) at different time points compared to baseline values indicated by the horizontal line. Values: means ± SEM, $n = 15$ (five patients, 15 observations). PU, perfusion units.

- lack of previous treatment of periodontal disease;
- no systemic antibiotics or non-steroidal anti-inflammatory drugs for at least 6 months prior to the start of the study.

Signed informed consent was obtained from all subjects.

Clinical procedures and experimental protocol

Following initial examination, detailed periodontal recording, treatment planning and case presentation, each patient was given instructions in proper plaque control measures. In addition, they received four sessions (one for each quadrant) of basic periodontal therapy, i.e. scaling and root planing by an experienced hygienist.

Three months after the completion of basic periodontal therapy, the following clinical assessments (baseline) were performed by the same previously calibrated examiner in six sites per tooth (mesio-buccal, buccal, disto-buccal, linguo-mesial, lingual, linguo-distal):

- full-mouth plaque score;
- full-mouth bleeding on probing to the base of the pockets;
- pocket probing depth, gingival recession and clinical attachment level with a standard manual periodontal probe (PCP 12, Hu-Friedy, Chicago, IL, USA).

Surgical treatment — In order to have direct access for the LDF measurements without other anatomical structures interfering with the procedure, only upper jaw areas between the two contralateral second premolars that after basic periodontal therapy still presented pocket probing depth of 6 mm or more were included for surgical treatment with a modified Widman flap operation (Figs 1–3 (21). The operations were divided and performed into sextants. The patients were asked to refrain from oral hygiene for the first 7 days following operation. The sutures were removed at 7 days following operation. In order to reduce gingival inflammation due to presence of plaque, the patients were instructed to rinse with 0.2% chlorhexidine digluconate twice per day throughout the period of the 2 months healing period. The patients received professional tooth polishing at days 15, 30 and 60 following operation.

All the above-mentioned clinical measurements were also assessed at 2 months following operation.

Standardization of the location for LDF recordings — Individual acrylic stents (4–6 mm thick) covering the whole buccal and lingual surface of the operated area were constructed by a professional dental technician for each patient in order to position the probe of the LDF always in the same position. A wax spacer (0.5 mm) was applied over the gingival area of the stone models and later was removed to prevent the stent from applying pressure on the gingival tissues. In this way, it was also ensured that the LDF probe was always in a distance of 0.5 mm from the gingival tissues.

The recordings of the probe of the LDF were made through holes (in total nine holes per stent), which were

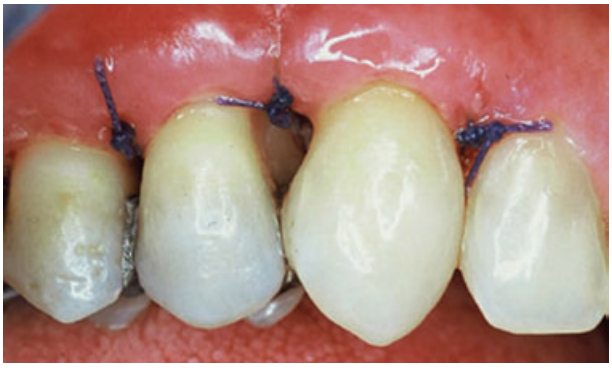

Fig. 8. Clinical healing; day 1.

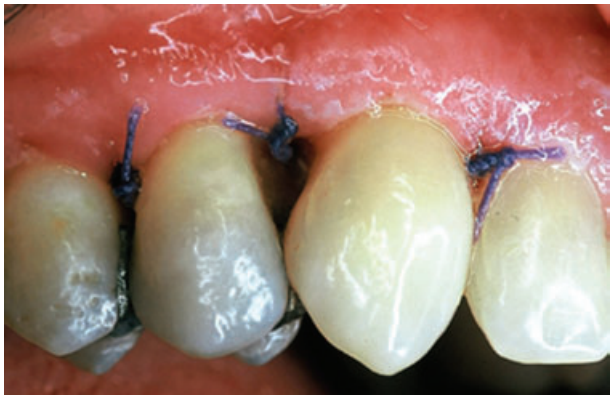

Fig. 9. Clinical healing; day 2.

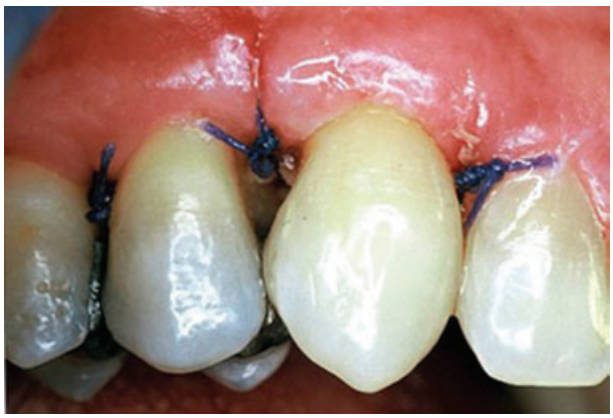

Fig. 10. Clinical healing; day 3.

drilled through the acrylic stent in standardized buccal/palatal locations for each tooth: one in each interdental papilla, and two at the level of the mucogingival junction (one mesial and one distal of the tooth). At the palate, one hole was drilled in each interdental papilla. The thickness of the acrylic stent around the holes was 5 mm (in order to support the LDF probe). The diameter of the holes was exactly the same as the diameter of the LDF probe. This was achieved by having taken an impression of the LDF probe and having cast metal copies of the same dimension, which were positioned with the same direction as the LDF probe over the areas of the LDF recordings during the polymerization of the acrylic stent. In this way, the direction and the angulation of the LDF probe was always the same during all observation periods (Fig. 4).

A commercially available laser Doppler Flowmeter (5010 Periflux, Perimed AB, Jarfalla, Sweden) with wavelength 780 nm and equipped with a standard probe (PF416 with outside diameter 1.0 mm and fibre separation 0.25 mm) was used for all measurements. The flowmeter time constant was 0.2 s, with an upper bandwidth at 20 kHz and a lower bandwidth at 20 Hz. The instruments and fibre-optic probes were calibrated by means of the Perimed PF 1000 Motility Standard according to the manufacturer's specifications before each measurement.

LDF measurements — The reproducibility of the LDF measurements was tested prior the start of the study in healthy gingival conditions using healthy volunteers. LDF measurements were performed at the same gingival areas of the same healthy (no presence of periodontal disease) volunteers with less than 30 min difference between them. A paired samples test was used to detect statistical significant differences between the two recordings. No statistical significance was found.

All the LDF measurements were performed by the same previously calibrated examiner.

During all LDF measurements, the subjects were comfortably seated and relaxed in a semi-reclined position in a dental chair, in a quiet room with a constantly stable temperature. The tip of the fibre optic probe was inserted into the holes of the acrylic stent and thus held motionless relative to the tissues during continuous measurements. All recording periods impaired by the artefacts caused by the relative motion of the probe were excluded. One 120-s measurement was taken in each hole of the acrylic stent in each tooth of the operated area. The signals were recorded in arbitrary perfusion units (PU) and monitored using the Perisoft software (Version 2.10, Perimed AB).

The LDF measurements were performed:

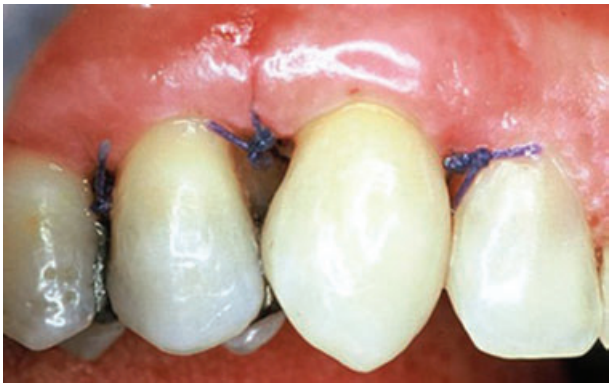

Fig. 11. Clinical healing; day 4.

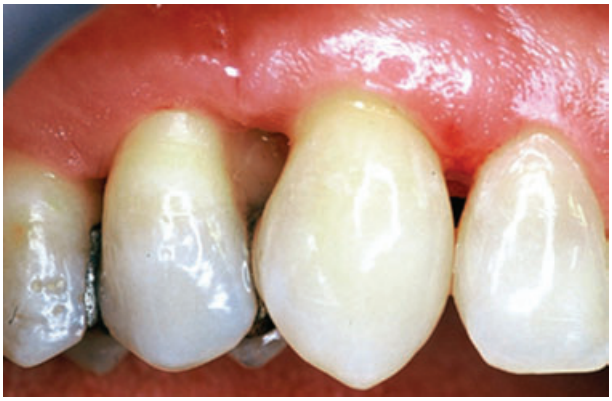

Fig. 12. Clinical healing; day 7.

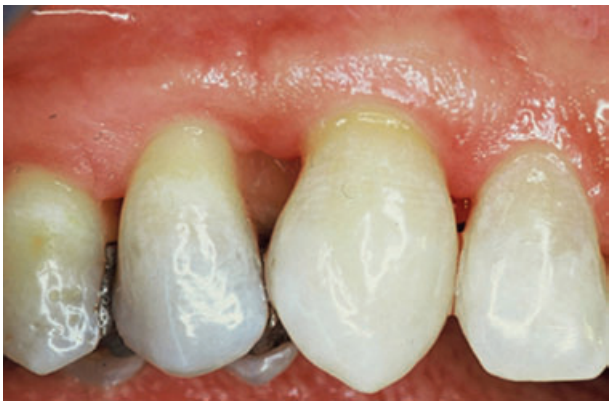

Fig. 13. Clinical healing; day 15.

- at the day of the surgery, prior to the injection of the local anaesthesia;
- 3 to 4 min following local anaesthesia with vasoconstrictor (Xylocain, Astra-Zeneca AB, Södertälje, Sweden);
- immediately after the completion of the operation;
- at days 1, 2, 3, 4, 7, 15, 30 and 60 following operation.

Statistical analysis

All recording periods impaired by the artefacts caused by the relative motion of the probe were excluded. Averages of the 2-min periods of all individual recordings were calculated by means of the Perisoft computer program (Version 2.10, Perimed AB).

All values were transferred to the Microsoft Excel program for further calculations. The LDF measures only relative changes in the flux of blood cells multiplied by their velocity. Consequently, PU recordings can not be used as absolute values. Therefore, changes of blood flow values in alveolar mucosa, palate and papillae were expressed as percentage of the difference between the PU value at a specific site in a specific time point (PU_t) and the individual baseline value of the same site (PU_0):

$$(PU_t - PU_0) \times 100/PU_0$$

Descriptive statistics were performed using the SPSS statistical software (SPSS 11.0, Chicago, IL, USA), in order to summarize data in terms of changes in blood flow in the alveolar mucosa, palate and papilla during time.

Results

Changes in blood flow

The changes in blood flow at the alveolar mucosal, palatal and papillary sites expressed as percentages of PU values to baseline are depicted in Figs 5, 6 and 7.

Alveolar mucosa — Overall, the blood flow decreased immediately following anaesthesia and remained in lower values also immediately following operation. Until the 7th day following surgery, the gingival blood flow presented to be increased in comparison to baseline values. By the 15th day, as well as at the following observation periods of 30 and 60 days the gingival blood flow changes were very similar to baseline (Fig. 5).

Buccal papillae — Following anaesthesia and immediately following surgery, the gingival blood flow presented significant decrease in comparison to baseline values. Progressively increased blood flow changes were observed until the 7th and the 15th day following surgery. Increased blood flow changes were also observed at 30 and 60 days following operation (Fig. 6).

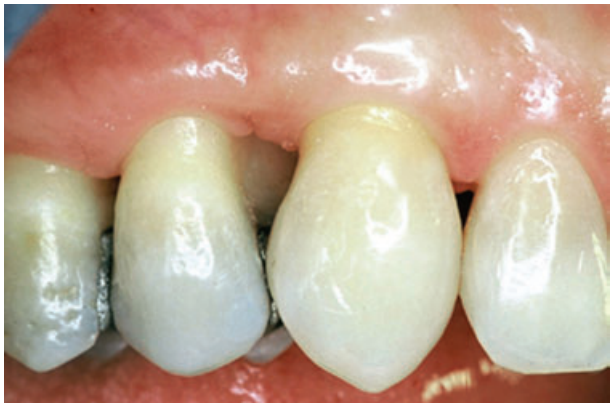

Fig. 14. Clinical healing; day 30.

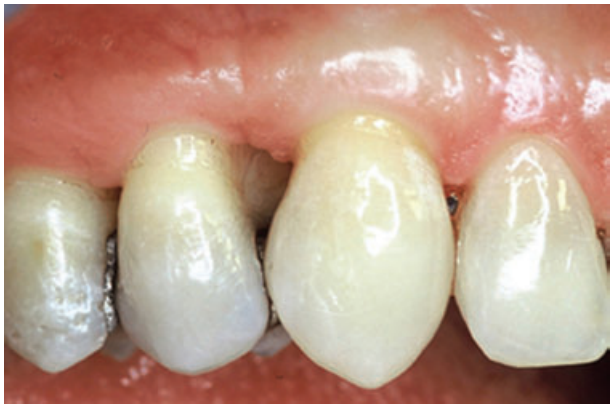

Fig. 15. Clinical healing; day 60.

Palatal papillae — The blood flow changes presented a significant variation at the palatal sites. A significant increase of blood flow was observed at the 4th and 7th day following operation. By the 15th day the blood flow values were similar to the baseline (Fig. 7).

Clinical parameters

Healing was uneventful in all cases (Figs 8–15). At 60 days, all patients presented improvement in terms of pocket reduction, gain of clinical attachment and reduction of full mouth bleeding score (Tables 1 and 2).

Table 1. Full-mouth clinical indices (mean ± SD)

	Baseline ($n = 5$)	Day 60 ($n = 5$)
FMPS	15.59 ± 5.48	15.39 ± 6.75
FMBS	27.56 ± 15.06	18.19 ± 11.95

FMPS, full-mouth plaque score; FMBS, full-mouth bleeding score.

Table 2. Selected-site clinical indices (mean ± SD)

	Baseline ($n = 40$ sites)	Day 60 ($n = 40$ sites)
PPD	5.98 ± 1.025	3.22 ± 1.025
CAL	7.90 ± 1.736	7.30 ± 1.884
REC	1.92 ± 1.575	4.08 ± 1.716

PPD, pocket probing depth; CAL, clinical attachment level; REC, gingival recession.

Discussion

The results of the present pilot study suggest that the LDF might present clinical applicability in recording changes in gingival blood flow following periodontal surgery.

In the present investigation, the LDF measurements were performed in three distinct parts of the periodontal flap: buccal papillae, alveolar mucosa and palatal papillae. In this way, it was attempted to evaluate if there are significant topographical differences within the same periodontal flap in terms of gingival blood flow changes during the early and late wound healing.

Following anaesthesia and irrespective of the flap area, the blood flow presented significant decrease. This observation is in agreement with previous studies where the use of local anaesthesia with vasoconstrictor led to a significant drop of the blood flow (22). In accordance with a recent LDF study (23), at 7 days following operation, increased gingival blood flow was observed in all sites of the flap. In the present investigation, by the 15th day following operation, the gingival blood flow was similar to baseline values except for the sites located at the buccal papillae. These LDF observations could easily be associated with the histologic (24) and revascularization wound healing events following periodontal surgery (25). It is well established that after 1 day following modified Widman flap operation, the vascular network within mucoperiosteal flaps presents hyperemic response, which continues in both buccal and lingual flaps until the 3rd day, with simultaneous vascular proliferation of the blood clot in areas of close flap adaptation (25). At 4 days, the vascular proliferation in the organizing blood clot is advancing, with anastomotic channels connecting the cancellous bone circulation with those of flap and mucosa, and by day 7 the gingival vessels show continuity with those of periodontal membrane. Following a healing period of 21, 30 and 90 days, the vascular network appears to be normal. In the present investigation, the increased gingival blood flow changes that were still observed at 15 days following surgery at the buccal papillae can be explained by the fact that the elevation of a mucoperiosteal flap initiates a significant vascular trauma, and a lack of circulation at the most coronal portion of flaps lasts for at least 3 days, but persists for 7 days at the interproximal regions (6). It has also been observed

that the elevation of a mucoperiosteal flap served as a trigger for angiogenesis of the periodontal ligament vascular plexus, which persisted even 14 days following surgery (26). Therefore, it would be reasonable to assume that the prolonged increased blood flow in the buccal papillae would represent a combination of an increased vascular trauma of the area and possible blood flow contamination from deeper tissue layers (27) such as the periodontal ligament vascular plexus. One of the limitations of the LDF is that flow readings are not only dependent on the blood flow in the measurement volume, but also on the scattering properties of the surrounding tissues. It has been reported that up to 80% of the laser Doppler blood-flow signal recorded from an intact human pulp is of non-pulpal origin (28). The same could be anticipated for the LDF measurements performed on the gingivae.

The significant variation of measurements observed at the palatal sites could be attributed to the morphology and to the increased thickness of the gingival tissues in the palate. Furthermore, taking into consideration that smoking constitutes a confounding factor known to affect the gingival blood flow (19, 20), the observed variability, especially in the palatal sites, could possibly be associated with the fact that two of the patients were smokers. Nevertheless, further investigation with a significantly larger study sample is needed to elucidate this issue.

Another limitation of the LDF technique is that it is lacking an absolute zero measurement, even when red blood cell flow is reduced to nil experimentally or surgically. This is attributed to the Brownian motion of macromolecules in the interstitial compartment (29). As a result we are not able to compare gingival blood flow changes between patients and we can not compare the PU values of different sites, even within the same patient. Therefore, we can only evaluate proportional changes of PU values to individual baseline values following a procedure causing dynamic changes, i.e. the course of wound healing over time following periodontal surgery. Nevertheless, larger sample clinical studies are needed in order to detect a clear pattern of changes of blood flow following periodontal surgery, as well as to evaluate their association to clinical wound healing. This could not be defined by the small sample of the present pilot study, whose aim was to evaluate the applicability of the LDF technique in recording changes in gingival blood flow, as well as to establish the methodological aspects for future clinical trials.

Acknowledgements

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