

# Comparison of gingival blood flow during healing of simplified papilla preservation and modified Widman flap surgery: a clinical trial using laser Doppler flowmetry

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#### Abstract

**Aim:** This prospective randomized-controlled clinical trial compared the gingival blood flow responses following simplified papilla preservation (test) *versus* modified Widman flap (control).

**Materials and Methods:** Twenty contra-lateral upper sites with pocket depth  $\ge 5$  mm after initial treatment in 10 chronic periodontitis patients were randomly assigned to either test or control treatment, using a split-mouth design. Laser Doppler flowmetry recordings were performed pre-operatively, following anaesthesia, immediately post-operatively and on days 1, 2, 3, 4, 7, 15, 30 and 60, at nine selected sites per flap. **Results:** Significant ischaemia was observed at all sites following anaesthesia and immediately post-operatively. At the mucosal flap basis, a peak hyperaemic response was observed on day 1, which tended to resolve by day 4 at the test sites, but persisted until day 7 at the control sites. The buccal and palatal papillae blood perfusion presented the maximum increase on day 7 in both groups and returned to baseline by day 15. Both surgical modalities yielded significant pocket depth reduction, recession increase and clinical attachment gain.

**Conclusions:** Periodontal access flaps represent an ischaemia–reperfusion flap model. The simplified papilla preservation flap may be associated with faster recovery of the gingival blood flow post-operatively compared with the modified Widman flap.

Key words: blood flow; laser Doppler flowmetry; periodontal access flap; wound healing

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Periodontal surgical therapy constitutes a key aspect of the treatment of patients

# Conflict of interest and source of funding statement

The authors declare that they have no conflict of interests.

This study was supported by a Clinical Research and Development Committee grant from the Special Trustees, Royal Free and University College Medical School (CRDC Project Grant G125). having periodontal diseases. A variety of surgical techniques have been developed and tested for their potential to restore the periodontal tissues lost due to destructive periodontal disease (Wennström et al. 2003).

The elevation of a mucoperiosteal flap induces an ischaemic response particularly in the coronal parts of the flap, which is indicative of a significant vascular trauma of the operated tissues (McLean et al. 1995). During the early post-operative period following flap surgery, inadequate blood supply and ischaemia–reperfusion injury are critical factors associated with detrimental changes in the operated tissues, which may lead to post-operative complications, such as necrosis, especially at the distant parts of the flap (Carroll & Esclamado 2000, Zhang et al. 2004). Therefore, it may be anticipated that a periodontal flap design that would minimize the damage of the microvasculature of the operated area and would ensure sufficient blood supply to the

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<sup>1</sup>Periodontology Unit, Eastman Dental Institute, University College London, London, UK; <sup>2</sup>European Research Group on Periodontology Network different parts of the periodontium might have an essentially positive effect on the speed and on the quality of the healing process. Furthermore, an improved healing process would be of paramount importance for the final outcome of various regenerative procedures, as it has been shown that the presence of a non-bioresorbable membrane interferes with the re-vascularization of the operated area (Vergara et al. 1997) and in certain cases it might create ischaemia of the flaps (Donos et al. 2002) and partial necrosis of the superficial bone (Grevstad 1993).

The simplified papilla preservation flap (SPPF) has been designed and successfully applied for periodontal surgery (Cortellini et al. 1999). The advantages of this flap include easier application in narrow inter-dental spaces and in the posterior areas, more effective primary closure of the flap leading to improved clinical wound healing and maintenance of the original morphology of the inter-dental tissues. However, this flap design has not been quantitatively compared with other established surgical flaps.

Laser Doppler flowmetry (LDF) is a non-invasive method extensively used to study the skin microcirculation. Several studies have shown a high correlation between LDF and other currently available techniques for blood perfusion measurements (Choi & Bennett 2003), and an intra-individual coefficient of variation of 25% has been reported for LDF measurements of the skin perfusion, which is considered to be acceptable for the physiological variable skin blood flow (Abbink et al. 2001). The LDF technique has been widely used in the field of plastic surgery for monitoring the microvascular blood flow in skin transplants and flaps, in order to detect early signs of impaired circulation and thus predict and possibly prevent surgical complications (Svensson et al. 1985, Yuen & Feng 2000). In the field of dentistry, the LDF has been used, among other applications, in order to evaluate the effect of periodontal disease, smoking and periosteal stimulation on gingival blood flow (Baab et al. 1986, Meekin et al. 2000, Ambrosini et al. 2002). The results of a recent pilot study from our research team have indicated that LDF presents clinical applicability, in order to assess the gingival blood flow changes following periodontal access flap surgery (Donos et al. 2005).

The aim of the present randomizedcontrolled clinical trial was to compare the microvascular gingival blood flow changes of the alveolar mucosa and the buccal and palatal inter-dental papillae, during the post-operative healing period, following SPPF *versus* modified Widman flap (MWF) in patients with chronic periodontitis, using LDF.

#### Material and Methods Subject population

This was a randomized-controlled single-blinded split-mouth clinical trial with a 2 month follow-up.

The experimental protocol for this study was approved by the Eastman Dental Institute and Hospital Joint Research and Ethics Committee. Ten patients (six females, four males, mean age  $40.5 \pm 6.5$  years) referred to the Department of Periodontology, Eastman Dental Institute, for the treatment of chronic generalized periodontitis participated in the study. Further inclusion criteria were as follows:

- the presence of contra-lateral sites of the upper jaw with a probing pocket depth of 5 mm or more and bleeding on probing at the re-evaluation appointment 3 months following completion of the initial periodontal treatment;
- non-smokers;
- good systemic health;
- age between 35 and 65 years;
- lack of previous treatment of periodontal disease; and
- no systemic antibiotics intake for at least 6 months before the start of the study.

Signed informed consent was obtained from all subjects.

#### Clinical measures

The following clinical parameters were assessed at the baseline visit and 2 months following the surgical procedure:

- Percentage of total surfaces (six aspects per tooth) that revealed the presence of plaque.
- Percentage of total surfaces (six aspects per tooth) that presented bleeding on probing from the base of the pocket.
- pocket probing depth (PPD), gingival recession (REC) and clinical

attachment level (CAL) in six aspects per tooth were recorded to the nearest millimetre with a standard manual periodontal probe (PCP 12, Hu-Friedy, Chicago, IL, USA).

All the clinical measurements were performed by the same calibrated examiner (M. R.) (% sites within 2 mm agreement for PPD = 98.9%; for REC = 97.8%; for CAL = 95.4%).

#### Power analysis

Based on our previous study, which showed that at the end of the first postoperative week the LDF measurements at the papillary and mucosal sites presented increased by 52.6-61.6 perfusion units (PU) (Retzepi et al. 2007), the aim of the present study was to detect a true difference of at least 50 PU between the test and control surgical procedures. Based on the assumptions of normal distributions of the LDF measurements and a standard deviation of 114, power analysis confirmed that a sample size of 10 subjects, each conferring three sites to the control and three sites to the test group in a split-mouth intra-individual design, would provide sufficient power (0.9) to detect significant differences between the two treatment groups at the two-sided 5% level.

### Randomization, surgical procedures and post-operative care

A split-mouth design was applied. In each patient, each contra-lateral area of the upper jaw, presenting with PPD of 5 mm or more and bleeding on probing following the initial phase of periodontal therapy, was randomly assigned to periodontal surgical treatment with MWF (control group) or SPPF (test group). All operations were performed by the same operator (M. R.). A computer-generated randomization list was drawn up by the research co-ordinator and given to the operator. The treatment code was revealed following performance of local anaesthesia and immediately before the surgical procedure.

Each flap design included three consecutive inter-proximal papillae. Local anaesthesia was performed buccally and palatally with delivery of 3.6 and 1.8 ml, respectively, of lidocaine 2% with epinephrine 1:80,000 (Xylocain, Astra, Mölndal, Sweden). *MWF* was performed on the assigned to the control group, as described previously (Ramfjord & Nissle 1974). In brief, the initial incision was placed 0.5-1 mm away from the free gingival margin in areas with PPD  $\geq 5 \text{ mm}$ , whereas in areas with shallow pockets the incision was intra-crevicular. Vertical incisions were not performed. Buccal and lingual mucoperiosteal flaps were raised and the exposed defects were carefully scaled and root planed using a combination of mechanical and hand instrumentation. The flaps were then repositioned and single inter-dental sutures were placed using resorbable 5.0 sutures (Vicryl Rapide, Ethicon, Norderstedt, Germany).

SPPF was performed on the areas assigned to the test group, as described previously (Cortellini et al. 1999). More specifically, buccal and lingual intracrevicular incisions were performed, combined with a single inter-proximal incision. Buccal and lingual mucoperiosteal flaps were raised and the exposed defects were carefully scaled and root planed using a combination of mechanical and hand instrumentation. The flaps were then repositioned and closed using a two-layered suturing technique: deep horizontal mattress resorbable 5.0 sutures (Vicryl, Ethicon, Germany) with the aim of removing residual tension from the flap margins, in combination with single inter-dental resorbable 6.0 sutures (Vicryl Rapide, Ethicon) with the aim of achieving passive primary closure of the wound margins in the area of the interdental papilla.

The patients were asked to refrain from oral hygiene for the first 7 days following the operation. The sutures were removed on day 7 following the surgical procedure. The patients were instructed to rinse with 0.2% chlorhexidine digluconate twice per day throughout the 2-month follow-up period. The patients received professional tooth polishing at days 15, 30 and 60 following the operation.

#### LDF measurements

#### Equipment

The LDF technique is based on the Doppler principle. Specifically, a laser beam is emitted by an optical fibre to the tissue to be studied. The light hitting moving erythrocytes is scattered back in shifted frequency (Doppler effect) and is captured by one or more optical fibres. The light signals are then converted into electric signals and the resulting photocurrent is processed to provide a recording of the blood flow (Stern et al. 1977). Although the multiple scattering events that determine the propagation of light in tissue prevent absolute velocity measurements when used in vivo, relative blood flow measurements can be obtained. Therefore, the term used to describe blood flow is flux - a quantity proportional to the average speed of the blood cells and their concentration. This is expressed in arbitrary PU, which are linearly related to flux. A commercially available laser Doppler flowmeter (5010 Periflux, Perimed, Jarfalla, Sweden) with wavelength 780 nm equipped with a standard probe (PF416 with outside diameter 1.0 mm and fibre separation 0.25 mm) was used for all measurements. The flowmetre time constant was 0.2 s, with an upper bandwidth at 20 kHz and 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. The signals were recorded in arbitrary PU and monitored using the Perisoft software (Version 2.10. Perimed AB).

#### LDF measurements

The LDF measurements were performed as described previously (Donos et al. 2005). In brief, a 2 min. LDF recording was performed in each of nine selected measurement sites per flap, including:

- three sites located on the mucosal flap basis (one centrally located, one close to the mesial and one close to the distal flap edge);
- three buccal papillary sites, one at each of three adjacent inter-dental papillae included in the flap design (distal, centre and mesial); and
- three corresponding palatal papillary sites.

The LDF measurements at the test and control group were performed on the day of the surgery before the injection of the local anaesthesia (baseline), 5 min. following local anaesthesia induction, immediately following completion of the surgical procedure and on postoperative days 1, 2, 3, 4, 7, 15, 30 and 60.

## Standardization and reproducibility of the LDF recordings

In order to standardize the position (location and angulation) of the LDF

probe in relation to the gingival tissue at all observation time points, the LDF measurements were performed with the tip of the fibre-optic probe inserted into the holes of an individual acrylic stent prepared on dental casts as described previously (Donos et al. 2005). Thus, the LDF probe was placed at a standardized location perpendicular to the tissues and at a distance of 0.5 mm from the gingivae and remained motionless during repetitive LDF measurements.

During all LDF measurements, the subjects were comfortably seated and relaxed in a standardized semi-reclined position on the same dental chair, in a quiet room with a constantly stable temperature. All the LDF measurements were performed by the same previously calibrated examiner (M. R.) (Intra-class correlation coefficient = 0.68, 95% confidence interval = 0.50-0.80). The reproducibility of the LDF measurements was tested before the start of the study on seven periodontally healthy volunteers, on whom two sets of LDF measurements were performed at nine gingival areas. A paired-samples *t*-test was performed and revealed no significant differences between the two sets of recordings.

#### Statistical analysis

All recording periods impaired by the artefacts caused by the relative motion of the probe were excluded. An average of the 2-min. period of each individual recording was calculated by the Perisoft computer programme (Version 2.10, Perimed, Stockholm, Sweden). All values were transferred to the Microsoft Excel program for further calculations. Changes of blood flow values in the alveolar mucosa, palate and papillae were expressed as the difference  $(\Delta PU)$  between the PU value at a specific site at a specific observation time point  $(PU_t)$  and the individual baseline value of the same site (PU<sub>0</sub>):  $\Delta PU =$  $PU_t - PU_0$ 

Statistical analysis was performed using the SPSS statistical software (SPSS 11.0, Chicago, IL, USA). The  $\Delta$ PU values in the alveolar mucosa, palate and buccal papillae were analysed using the general linear model (GLM) univariate test, after the assumptions of homogeneity of variance and normality of the residuals distribution were checked. The surgical treatment type and the observation time point were modelled as fixed factors and the patient as a random factor, with the  $\Delta PU$  as the dependent variable. The least significant differences test was used, in order to determine the differences between baseline and subsequent time points within each treatment group.

The significance of differences in PPD, REC and CAL from baseline to 2 months post-therapy between the test and control groups was evaluated using one-way analysis of variance, with the patient modelled as a random factor and the baseline PPD as a covariate. The significance of the differences between baseline and 2 months post-therapy was evaluated with the paired-samples *t*-test for PPD, REC and CAL within each treatment group and with Wilcoxon sign-rank test for the percentage-based measures of FMPS and FMBS.

Data are presented as mean  $\pm$  standard error unless otherwise indicated. Statistical significance was accepted at p < 0.05.

#### Results LDF measurements

#### Alveolar mucosal areas

The GLM revealed a significant effect of time (p < 0.001) and treatment type (p = 0.049) on the  $\Delta$ PU values recorded in the mucosal areas.

Both treatment groups presented overall similar patterns of change in PU following anaesthesia and during the post-operative healing period. On the first post-operative day, a hyperaemic response was indicated by a maximum increase of the blood flow compared with the baseline, in both the MWF and the SPPF. The microcirculatory blood perfusion remained significantly increased until post-operative day 7 in the control group, whereas in the test group the perfusion values remained significantly increased above baseline only until day 3. Moreover, on post-operative day 4, the increase of the PU values above baseline was significantly higher in the control compared with the test group (p = 0.025) (Fig. 1).

#### Buccal papillary areas

The GLM revealed a significant effect of time (p < 0.001) and treatment type (p = 0.048) on the  $\Delta$ PU values recorded in the buccal papillary areas.

Both treatment groups presented overall similar patterns of change in



*Fig. 1.* Plot of the time course of the gingival blood flow changes at the alveolar mucosal areas of the simplified papilla preservation flap (SPPF) and the modified Widman flap (MWF), expressed as difference in perfusion units from baseline ( $\Delta$ PU). Plotted points include measurements taken preoperatively (baseline), following anaesthesia, immediately post-operatively and on post-operative days 1, 2, 3, 4, 7, 15, 30 and 60. Statistically significant differences in perfusion units between treatments are indicated by \*. Error bars = SEM (p < 0.05).



*Fig.* 2. Plot of the time course of the gingival blood flow changes at the buccal papillary areas of the simplified papilla preservation flap (SPPF) and the modified Widman flap (MWF), expressed as difference in perfusion units from baseline ( $\Delta$ PU). Plotted points include measurements taken preoperatively (baseline), following anaesthesia, immediately post-operatively and on post-operative days 1, 2, 3, 4, 7, 15, 30 and 60. Statistically significant differences in perfusion units between treatments are indicated by \*. Error bars = SEM (p < 0.05).

PU following anaesthesia and during the early post-operative period. The PU values at the buccal papillary sites remained at baseline levels during the first four post-operative days, in both treatment groups. A significant increase above the baseline was observed on day 7, in the microcirculatory perfusion at both the MWF and the SPPF sites. Notably, the PU values at the papillary sites where SPPF had been performed were significantly decreased compared

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*Fig. 3.* Plot of the time course of the gingival blood flow changes at the palatal papillary areas of the simplified papilla preservation flap (SPPF) and the modified Widman flap (MWF), expressed as difference in perfusion units from baseline ( $\Delta$ PU). Plotted points include measurements taken preoperatively (baseline), following anaesthesia, immediately post-operatively and on post-operative days 1, 2, 3, 4, 7, 15, 30 and 60. Statistically significant differences in perfusion units between treatments are indicated by \*. Error bars = SEM (p < 0.05).

with the baseline at post-operative days 30 and 60 (Fig. 2).

#### Palatal papillary areas

The GLM revealed a significant effect of time (p < 0.001) and treatment type (p = 0.001) on the  $\Delta$ PU values recorded in the palatal papillary areas.

The palatal sites in both treatment groups presented overall a tendency towards a gradual increase in the PU values during the post-operative healing period. A significant peak increase above the baseline was observed on day 7 at the palatal papillae of both treatment groups, which was significantly higher at the SPPF versus the MWF sites (p = 0.024) (Fig. 3).

#### **Clinical outcomes**

Healing was uneventful in all cases (Figs 4 and 5). Table 1 displays the PPD, CAL and REC values at baseline and 2 months post-operatively for sites with PPD  $\geq$  5 mm at baseline, as well as the mean changes of these clinical parameters between baseline and 2 months post-therapy. For sites with initial PPD  $\geq$  5 mm, the MWF yielded a significantly greater PPD reduction compared with the SPPF (p = 0.014). The

differences in REC increase and CAL gain between treatments failed to reach statistical significance (p = 0.097 and p = 0.609, respectively). Table 2 displays the FMPS and FMBS scores at baseline and at the re-evaluation 2 months post-operatively. There were no significant changes in the FMPS median scores at the re-evaluation visit compared with baseline (p = 0.139). Surgical treatment led to a significant reduction in the FMBS median values 2 months post-operatively (p = 0.019).

#### Discussion

The present study compared the temporal pattern of the microvascular blood flow changes of the MWF versus the SPPF, in patients with chronic periodontitis, using LDF. The results indicated that the flap design was associated with temporal variations in the microvascular blood flow responses, which were observed among the treatment groups during the wound-healing period. Furthermore, our observations confirm the results of our pilot study (Donos et al. 2005), indicating that LDF may present clinical applicability in recording the gingival blood flow changes following periodontal surgery.

The injection of a local anaesthetic with a vasoconstrictor induced a de-

crease in the microvascular blood flow compared with the baseline, by 50-72% at the mucosal sites and by 67-72% in the inter-dental papillae in both the MWF and the SPPF flaps. These results are in agreement with similar previous reports (Ketabi & Hirsch 1997, Ambrosini et al. 2002, Donos et al. 2005). The flap ischaemia induced by the injection of a local anaesthetic with a vasoconstrictor to the alveolar mucosa lasted for at least 2h, as evidenced by the significantly decreased perfusion observed following completion of the surgical procedure. Therefore, the periodontal access flap may be considered as an ischaemia-reperfusion flap model (Carroll & Esclamado 2000).

At the mucosal periphery of the flap, where the microvascular architecture was preserved, a peak hyperaemic response was observed on post-operative day 1 in both the MWF and SPPF, which is in agreement with our previous report on post-operative blood flow changes following access flap periodontal surgery (Retzepi et al. 2007). Furthermore, these observations are in accordance with clinical and in vivo studies, which, by means of LDF or imaging, reported an increment of the blood flow in areas proximal to the flap basis, which peaked on the first postoperative day (Marks et al. 1984, Eichhorn et al. 1994, Aydin & Mavili 2003). It may be hypothesized that the observed hyperperfusion is associated with vasodilation occurring as a predominant microvascular response at the undisturbed wound periphery during the very initial healing period (Rendell et al. 1997), which is considered to be principally mediated by the substantial induction of nitric oxide synthetase (Rendell et al. 2002).

During the elevation of a mucoperiosteal flap, the connection of the gingivoperiosteal plexus with the periodontal ligament vascular plexus is severed (Nobuto et al. 2005) and significant vascular trauma is induced, especially in the inter-dental areas (McLean et al. 1995). On the other hand, the preservation of the arterial inflow constitutes the most critical determinant in achieving optimal flap healing (Nakayama et al. 1982, Grace 1994). In the present study, the hyperaemic response observed postoperatively at the mucosal sites tended to resolve by day 4 at the SPPF sites, but persisted until day 7 at the MWF sites. It may therefore be anticipated that the shorter duration of the hyperaemic



*Fig. 4.* Buccal view of experimental control side, where a modified Widman flap was performed; (a) pre-operative view; (b) post-operative view; (c) post-operative day 1; (d) post-operative day 2; (e) post-operative day 4; (f) post-operative day 7; (g) post-operative day 60.

response at the periphery of the SPPF indicates faster recovery of the microcirculation and remission of the increased blood inflow requirements in this flap design.

In contrast to the mucosal sites, the buccal and palatal papillary sites presented a maximum increase of the blood perfusion levels on post-operative day 7 in both flap designs, which is in agreement with our previous report on post-operative blood flow changes observed in the papillary areas following access flap periodontal surgery (Retzepi et al. 2007). These results are in accordance with observations in skin experimental flaps, where blood flow increases at the distal zones of the flaps were observed on post-operative days 3-6 and coincided with resolution of the hyperaemia at the areas located proximal to the flap

periphery (Aydin & Mavili 2003). Furthermore, taking into consideration that the LDF light penetrates the tissues variably to a depth of about 0.6 mm (Fullerton et al. 2002), it may be assumed that the LDF readings from the free gingivae of the papillae reflect only blood perfusion changes occurring at the supraperiosteal gingival plexus, which is a coarse network structure consisting mainly of small arterioles and venules (Nobuto et al. 1989). In this context, our results are in agreement with data from animal experiments reporting a substantial increase of blood flow observed on the seventh day of healing in the centre of wounds created at sites perfused by small capillaries (Rendell et al. 1998), which is similar to the free gingivae. At 4 days, the vascular proliferation in the organizing blood clot is advancing with anastomotic channels connecting the cancellous bone circulation with those of the flap and mucosa and by day 7, the gingival vessels show continuity with those of the periodontal membrane (Caffesse et al. 1981). Therefore, the increase of the blood flow that was observed on post-operative day 7 could be attributed to the formation of granulation tissue with increased vascularization due to angiogenesis, as suggested by histological observations in animal experiments (Nobuto et al. 2003). It is well documented that, during the early postoperative days, the newly formed blood vessels in the provisional granulation tissue will re-establish the microvascular network in the connective tissue and supply nutrients and oxygen to the wound area (Folkman & Shing 1992).



*Fig.* 5. Buccal view of experimental test side, where a simplified papilla preservation flap was performed; (a) pre-operative view; (b) post-operative view; (c) post-operative day 1; (d) post-operative day 2; (e) post-operative day 4; (f) post-operative day 7; (g) post-operative day 15; (h) post-operative day 60.

*Table 1.* Baseline, 2 months post-operatively and difference ( $\Delta$ ) in probing pocket depth, recession of the gingival margin and clinical attachment level at sites with initial PPD $\ge$ 5 mm

Outcome variable	Baseline	2 months	<i>p</i> -value paired <i>t</i> -test	Difference 0–2 months	<i>p</i> -value
PPD					
Control	$5.7 \pm 0.1$	$2.8 \pm 0.1$	< 0.001	$2.8\pm0.2$	0.014
Test	$5.8\pm0.1$	$3.3 \pm 0.1$	< 0.001	$2.5\pm0.2$	
REC					
Control	$1.1 \pm 0.1$	$2.6\pm0.2$	< 0.001	$-1.5\pm0.2$	0.097
Test	$1.1 \pm 0.1$	$2.2 \pm 0.1$	< 0.001	$-1.1\pm0.1$	
CAL					
Control	$6.7\pm0.2$	$5.4 \pm 0.3$	< 0.001	$1.3 \pm 0.3$	0.609
Test	$6.9\pm0.2$	$5.5\pm0.2$	< 0.001	$1.5\pm2.1$	

Data presented in mm; mean  $\pm$  standard error (standard deviation).

PPD, probing pocket depth; REC, recession of the gingival margin; CAL, clinical attachment level. Student's *t*-test for paired observations.

It should be noted, however, that although the palatal and buccal papillary sites presented a similar pattern of postoperative blood flow changes, some differences between the two flap designs were noted in the palatal papillae. More specifically, at the SPPF sites, the hyperaemic response at the palatal papillae was observed as early as the second post-operative day. This difference could be attributed to a better preservation of the supraperiosteal gingival vascular plexus associated with the SPPF design, thus allowing a prompt hyperaemic response during the early post-operative days. Furthermore, the hyperaemic response at the palatal papillae was significantly higher compared with the MWF sites on day 7. Taking into consideration that the blood flow values at the end of the first postoperative week reflect changes in the blood flow of the newly formed granulation tissue (Caffesse et al. 1981, Nobuto et al. 2003), this difference may again reflect the less traumatic nature of the preservative SPPF design, which allows faster formation and organization of the granulation tissue.

In the present study, the microcirculatory perfusion levels of both the buccal and palatal papillae returned back to baseline levels by post-operative day 15,

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*Table 2.* Baseline, 2 months post-operatively and difference ( $\triangle$ ) in full-mouth plaque scores and full-mouth bleeding on probing scores

Outcome variable	Baseline	2 months	<i>p</i> -value
FMPS (%)	22.6 (5.6-40.1)	18.2 (2.2–34.2)	0.139
FMBS (%)	29.4 (15.0-43.8)	18.4 (4.0–32.8)	0.019

Data presented in %: median (interquartile range).

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

Wilcoxon signed-ranks test.

which is in agreement with previous in vivo studies, reporting regression of the newly formed blood vessels in the repaired gingival tissue by the 21st post-operative day (Nobuto et al. 2005).

The presence of gingival inflammation and increased PPD has been shown to affect the LDF recordings obtained from the periodontal tissues (Rodriguez-Martinez et al. 2006). However, our results have shown that, in both treatment groups, resolution of the gingival inflammation, as well as elimination of pockets with PPD>4 mm were achieved.

Conclusively, the results of the present study confirm those of our previous studies (Donos et al. 2005, Retzepi et al. 2007), in that the gingival blood flow presents specific patterns of dynamic changes post-operatively and that LDF may present clinical applicability in recording changes in the microcirculatory blood perfusion following periodontal surgery. Furthermore, our results indicate that the location of the incisions and the management of the SPPF, aiming at preserving the papillary aspects, may have a positive effect on the recovery of the gingival blood flow post-operatively.

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#### References

- Abbink, E. J., Wollersheim, H., Netten, P. M. & Smits, P. (2001) Reproducibility of skin microcirculatory measurements in humans, with special emphasis on capillaroscopy. *Vascular Medicine* 6, 203–210.
- Ambrosini, P., Cherene, S., Miller, N., Weissenbach, M. & Penaud, J. (2002) A laser Doppler study of gingival blood flow variations following periosteal stimulation. *Jour*nal of Clinical Periodontology 29, 103–107.

- Aydin, M. A. & Mavili, M. E. (2003) Examining microcirculation improves the angiosome theory in explaining the delay phenomenon in a rabbit model. *Journal of Reconstructive Microsurgery* **19**, 187–194.
- Baab, D., Oberg, P. & Holloway, G. (1986) Gingival blood flow measured with a laser Doppler flowmeter. *Journal of Periodontal Research* 21, 73–85.
- Caffesse, R. G., Castelli, W. A. & Nasjleti, C. E. (1981) Vascular response to modified Widman flap surgery in monkeys. *Journal of Periodontology* 52, 1–7.
- Carroll, W. R. & Esclamado, R. M. (2000) Ischemia/reperfusion injury in microvascular surgery. *Head and Neck* 22, 700–713.
- Choi, C. M. & Bennett, R. G. (2003) Laser Dopplers to determine cutaneous blood flow. *Dermatologic Surgery* 29, 272–280.
- Cortellini, P., Prato, G. P. & Tonetti, M. S. (1999) The simplified papilla preservation flap. A novel surgical approach for the management of soft tissues in regenerative procedures. *International Journal of Periodontics and Restorative Dentistry* 19, 589–599.
- Donos, N., D'Aiuto, F., Retzepi, M. & Tonetti, M. (2005) Evaluation of gingival blood flow by the use of laser Doppler flowmetry following periodontal surgery. A pilot study. *Jour*nal of Periodontal Research 40, 129–137.
- Donos, N., Kostopoulos, L. & Karring, T. (2002) Alveolar ridge augmentation by combining autogenous mandibular bone grafts and non-resorbable membrane-like barriers. An experimental study in the rat. *Clinical Oral Implants Research* 13, 185–191.
- Eichhorn, W., Auer, T., Voy, E. D. & Hoffmann, K. (1994) Laser Doppler imaging of axial and random pattern flaps in the maxillofacial area. A preliminary report. *Journal of Craniomaxillofacial Surgery* 22, 301–306.
- Folkman, J. & Shing, T. (1992) Angiogenesis. Journal of Biological Chemistry 267, 10931– 10934.
- Fullerton, A., Stücker, M., Wilhelm, K. P., Wårdell, K., Anderson, C., Fischer, T., Nilsson, G. E. & Serup, J. (2002) Guidelines for visualization of cutaneous blood flow by laser Doppler perfusion imaging. *Contact Dermatitis* 46, 129–140.
- Grace, P. A. (1994) Ischaemia–reperfusion injury. *The British Journal of Surgery* 81, 637–647.
- Grevstad, H. J. (1993) Effect of subperiosteally implanted polytetrafluoroethylene (PTFE) material on alveolar bone in the rat.

Scandinavian Journal of Dental Research **101**, 224–228.

- Ketabi, M. & Hirsch, R. S. (1997) The effects of local anesthetic containing adrenaline on gingival blood flow in smokers and nonsmokers. *Journal of Clinical Periodontology* 24, 888–892.
- Marks, N. J., Trachy, R. E. & Cummings, C. W. (1984) Dynamic variations in blood flow as measured by laser Doppler velocimetry: a study in rat skin flaps. *Plastic and Reconstructive Surgery* **73**, 804–810.
- McLean, T. N., Smith, B. A., Morrison, E. C., Nasjleti, C. E. & Caffesse, R. G. (1995) Vascular changes following mucoperiosteal flap surgery: a fluorescein angiography study in dogs. *Journal of Periodontology* 66, 205– 210.
- Meekin, T. N., Wilson, R., Scott, D., Ide, M. & Palmer, R. M. (2000) Laser Doppler flowmeter measurement of relative gingival and forehead skin blood flow in light and heavy smokers during and after smoking. *Journal of Clinical Periodontology* 27, 236–242.
- Nakayama, Y., Soeda, S. & Kasai, Y. (1982) The importance of arterial inflow in the distal side of a flap: an experimental investigation. *Plastic and Reconstructive Surgery* 69, 61–67.
- Nobuto, T., Imai, H., Suwa, F., Kono, T., Suga, H., Jyoshi, K. & Obayashi, K. (2003) Microvascular response in the periodontal ligament following mucoperiosteal flap surgery. *Journal of Periodontology* 74, 521–528.
- Nobuto, T., Suwa, F., Kono, T., Hatakeyama, Y., Honjou, N., Shirai, T., Mitsuyama, M. & Imai, H. (2005) Microvascular response in the periosteum following mucoperiosteal flap surgery in dogs: 3-dimensional observation of an angiogenic process. *Journal of Periodontology* **76**, 1339–1345.
- Nobuto, T., Yanagihara, K., Teranishi, Y., Minamibayashi, S., Imai, H. & Yamaoka, A. (1989) Periosteal microvasculature in the dog alveolar process. *Journal of Periodontology* 60, 709–715.
- Ramfjord, S. P. & Nissle, R. R. (1974) The modified Widman flap. *Journal of Periodontology* 45, 601–607.
- Rendell, M. S., Johnson, M. L., Smith, D., Finney, D., Capp, C., Lammers, R. & Lancaster, S. (2002) Skin blood flow response in the rat model of wound healing: expression of vasoactive factors. *Journal of Surgical Research* 107, 18–26.
- Rendell, M. S., Milliken, B. K., Finnegan, M. F., Finney, D. A. & Healy, J. C. (1997) The skin blood flow response in wound healing. *Microvascular Research* 53, 222–234.
- Rendell, M. S., Milliken, B. K., Finnegan, M. F., Finney, D. E., Healy, J. C. & Bonner, R. F. (1998) The microvascular composition of the healing wound compared at skin sites with nutritive versus arteriovenous perfusion. *Journal of Surgical Research* 80, 373–379.
- Retzepi, M., Tonetti, M. & Donos, N. (2007) Gingival blood flow changes following periodontal access flap surgery using laser Doppler flowmetry. *Journal of Clinical Periodontology* 34, 437–443.

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- Rodriguez-Martinez, M., Patino-Marin, N., Loyola-Rodriguez, J. P. & Brito-Orta, M. D. (2006) Gingivitis and periodontitis as antagonistic modulators of gingival perfusion. *Journal of Periodontology* **77**, 1643–1650.
- Stern, M.D, Lappe, D. L. & Bowen, P. D. (1977) Continuous measurement of tissue blood flow by laser Doppler spectroscopy. *American Journal of Physiology* 28, 1746–1751.
- Svensson, H., Pettersson, H. & Svedman, P. (1985) Laser Doppler flowmetry and laser photometry for monitoring free flaps. *Scandinavian Journal of Plastic and Reconstructive Surgery* 19, 245–249.

#### **Clinical Relevance**

*Scientific rationale for the study:* The design and handling of periodontal flaps affect the extent of the damage of the microvasculature in the operated area and the temporal pattern of the reperfusion.

- Vergara, J., Quinones, C., Nasjleti, C. & Caffesse, R. (1997) Vascular response to guided tissue regeneration procedures using nonresorbable and bioresorbable membranes in dogs. *Journal of Periodontology* 48, 217–224.
- Wennström, J., Heijl, L. & Lindhe, J. (2003) Periodontal therapy: access therapy. In: *Clinical Periodontology and Implant Dentistry*, 4th edition, pp. 519–560. Copenhagen: Blackwell Munksgaard.
- Yuen, J. & Feng, Z. (2000) Monitoring free flaps using the laser Doppler flowmeter: five-year experience. *Plastic and Reconstructive Surgery* **105**, 55–61.

*Principal findings:* The hyperaemic response observed post-operatively at the mucosal periphery of the SPPF was of a shorter duration compared with the MWF. This finding was associated with faster reperfusion of the papillary microvascular network.

Zhang, F., Waller, W. & Lineaweaver, W. C. (2004) Growth factors and flap survival. *Microsurgery* 24, 162–167.

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*Practical implications:* The location of the incisions and the management of the periodontal flap in order to preserve the papillary aspects may have an essentially positive effect on the post-operative recovery of the blood flow. This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.