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# Levels of platelet activating factor in gingival crevice fluid following periodontal surgical therapy

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*Background and Objective:* Elevated levels of platelet activating factor (PAF), a potent inflammatory phospholipid mediator, have been previously detected in gingival tissues and gingival crevice fluid (GCF) in periodontal disease. However, the role of this mediator during wound healing after periodontal surgery remains unclear. The hypothesis, a relationship between PAF levels and periodontal healing, was tested by measuring PAF levels in GCF samples collected from sites that had undergone guided tissue regeneration (GTR) or flap surgery.

*Material and Methods:* Using a split-mouth design, 30 intrabony defects were randomly assigned to treatment with GTR (group 1) or to flap surgery (group 2). GCF was sampled pre-operatively and at 6-, 12- and 24-wk follow-up evaluation visits. PAF levels in GCF were analyzed by high-performance liquid chromatography (HPLC).

*Results:* Both treatment modalities significantly reduced the probing pocket depth and improved the clinical attachment level ($p < 0.01$). Compared with pre-operative values, the GCF volume and PAF levels were significantly decreased at postoperative weeks 6, 12 and 24 in both groups ($p < 0.01$). There were also significant differences in GCF volume and PAF levels at all time points up to 24 wks in both groups ($p < 0.01$). No statistically significant differences were observed in any of the parameters investigated between the two groups ($p > 0.05$).

*Conclusion:* PAF is detectable in GCF by HPLC and showed a continuous decrease at all the time points monitored following periodontal surgical therapy. This suggests that changes in the levels of this mediator in GCF might be useful for monitoring the progress of periodontal repair and regeneration.

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Periodontal disease, a chronic and frequent inflammatory disease of the supporting tissues of the teeth, causes loss of connective tissues and resorption of the alveolar bone (1). Periodontal regeneration is defined as the reconstruction of the damaged periodontium in the formation of alveolar bone, periodontal ligament and cementum to their original levels before they were lost as a result of periodontal disease (2,3). One of the most widely used regenerative treatments is guided tissue regeneration (GTR), which selectively promotes the repopulation of periodontal ligament and bone cells in periodontal defects using barrier membranes (4). The interaction between the hard and soft connective tissues, as well as epithelium, makes periodontal wound healing a complex process. Significant progress in understanding periodontal wound healing has been made, but

although complete regeneration of the periodontium is possible, it is still not predictable (3,5). The precise understanding of cellular components and biological events in periodontal wound healing could be useful for investigating more effective treatment modalities.

Platelet activating factor (PAF) is a potent phospholipid mediator and synthesized by thrombocytes, neutrophils, macrophages, eosinophils and epithelial cells (6–12). This mediator is linked to many inflammatory and immune responses, including platelet stimulation, neutrophil and monocyte activation, increased vascular permeability, smooth muscle contraction and bone resorption with osteoclasts (8,11–14). Elevated levels of PAF in gingival tissue (6,12), gingival crevicular fluid (GCF) (12,15), blood (8) in periodontal disease, and higher concentrations of PAF in gingival tissue in peri-implantitis (14), have been previously detected. A significant decrease in PAF levels in whole mixed saliva in subjects with chronic periodontitis after initial periodontal therapy has been demonstrated (16), but the precise role of PAF during wound healing after periodontal surgery still remains unclear.

GCF is generally considered to reflect inflammation, turnover of connective tissue and resorption of alveolar bone in the surrounding periodontal tissues (17–21). Some components of GCF are closely associated with the periodontal regeneration process, so analysis of the constituents of GCF has suggested that wound healing mediators are indeed present (18–21). In the light of these observations, transforming growth factor-β1, tissue inhibitor of matrix metalloproteinases-1, matrix metalloproteinase-1 and -8, interleukin-1α, interleukin-1β and interleukin-1 receptor antagonists have been analyzed in GCF following either GTR or flap surgery (21–23). However, the role of PAF has not yet been revealed in GCF during periodontal repair and regeneration. The present study was undertaken to test the hypothesis that there is a significant relationship between PAF levels in GCF and healing of periodontium following surgical therapies. We determined, for the first time, the levels of PAF in GCF samples collected from sites that had undergone GTR compared with flap surgery.

## Material and methods

### Study population

Fifteen systemically healthy patients (six men and nine women) with a mean age of 42.27 ± 1.65 yrs (range 31–52 yrs) and exhibiting radiographic evidence of bone loss, were recruited for the study. The criteria needed for inclusion consisted of patients having paired vertical interproximal osseous defects. The exclusion criteria were systemic diseases, a compromised immune system, pregnancy, lactation, allergy or sensitivity to any drug, and smoking. The subjects had no history of drug therapy for the 6 mo before recruitment to the study.

Initial periodontal therapy, which consisted of oral hygiene instruction, full-mouth scaling and root planing, and occlusal adjustment when indicated, was performed on all the patients. Four to six weeks following completion of initial periodontal therapy, a periodontal re-evaluation was performed. Using a split-mouth design, 30 paired interproximal intrabony defects with pocket depth ≥6 mm were randomly treated with either GTR (group 1) or flap surgery (group 2). Randomization was performed before surgery, according to the flip of a coin.

After receiving information on the study, the patients signed a consent form indicating their agreement to participate. The study protocol and consent form were approved by the University Institutional Review Board.

### Clinical measurements and surgical procedure

Probing pocket depth (PPD) and clinical attachment level (CAL) were measured by a Florida Probe (Florida Probe Corp., Gainesville, FL, USA) on the day of surgery and at 6 mo postoperatively. The plaque index (24) and gingival index (25) were assessed immediately before surgery and at 6, 12 and 24 wks after surgery. Clinical measurements were performed by the same calibrated examiner (intra-examiner calibration) who was blinded to the treatment modality (author BOC).

All the operative procedures were performed under local anesthesia. Following buccal and lingual sulcular incisions, full-thickness mucoperiosteal flaps were raised. All the granulation tissue was removed from the defects, and scaling and root planing were performed on the roots using hand and ultrasonic instruments.

Atrisorb (Atrix Laboratories, Inc., Fort Collins, CO, USA), an absorbable polylactide membrane, was used for GTR. Atrisorb was prepared according to the manufacturer's instructions and placed over the defects in group 1. Application of Atrisorb was not performed in sites of group 2. Flaps were replaced and secured with silk sutures. Sutures were removed 1 wk after surgery. Recall appointments for supragingival professional tooth cleaning and oral hygiene reinforcement were scheduled every second week during the first 2 mo after surgery and once a month for the rest of the observation period.

### GCF sampling

GCF samples of group 1 and group 2 were collected immediately before surgery and at 6, 12 and 24 wks after surgery. A GCF sample was taken at each experimental site. Before GCF sampling, the sites were isolated with cotton rolls, saliva was removed and the supragingival plaque, if present, was also removed using a sterile curet. GCF was sampled with filter paper (Periopaper; ProFlow, Inc., Amityville, NY, USA). Paper strips were placed in the crevice until mild resistance was felt, and left in position for 30 s. Strips with visible signs of saliva or blood contamination were discarded. The GCF volume of each strip was determined by electronic impedance (Periotron 8000; ProFlow Inc.). Samples were placed in a sterile polypropylene tube containing 0.5 ml of methanol-buffered solution and stored at −70°C until analysis.

### Analysis of PAF in GCF samples

The derivatization of PAF to coumarin carbamoyl derivatives was performed as previously described (26). The fluorescent compound, 7-diethylaminocoumarin-3-carbonylazide (DEACZ) (Molecular Probes Inc., Eugene, OR, USA) was used for the derivatization of PAF. By the derivatization, synthetic PAFs [1-0-hexadecyl-2-0-acetyl-*sn*-glycero-3-phosphocholine (PAF $C_{16}$); D-alpha-phosphatidylcholine, beta-acetyl-gamma-*O*-hexadecyl; 1,2-didecanoyl-*sn*-glycero-3-phosphocholine; 1,2-Di-*O*-hexadecyl-*sn*-glycero-phosphocoline; 1,2-didocosanoyl-*sn*-glycero-3-phosphocholine] (Sigma-Aldrich Chemical Company, St Louis, MO, USA) were converted into their corresponding carbamoyl derivatives. Briefly, onto standard samples containing synthetic PAFs in the organic phase (mobile phase), 100 µl of a 1-mg/ml solution of DEACZ in anhydrous toluene (derivatization solution) was added and then heated at 80°C. After 3 h of derivatization, the reaction vials were cooled, the florescent derivatives were directly analyzed by reverse-phase high-performance liquid chromatography (HPLC) and then quantified by fluorimetric detection. The procedure described above was applied directly to the GCF samples in a 100-µl volume.

The separation of coumarin carbamoyl derivatives of PAF was performed on a reverse-phase C-8 Nova-Pack column by a model HPLC, Perkin Elmer-series 3 (Optimize Technologies, Inc., Oregon City, OR, USA) with an isocratic run of a triplet solvent mixture (methanol/chloroform/water; 75 : 20 : 5, v/v) containing 250 mg/l tetramethylammonium chloride (Fluka Chemie GmbH, Buchs, Switzerland). The fluorescence detection was performed at an excitation wavelength of 400 nm and an emission wavelength of 480 nm. The described procedure is suitable for the study of picomolar levels of PAF in GCF. A typical chromatogram of coumarin carbamoyl derivatives, obtained under the experimental conditions described above, is shown in Fig. 1. The coumarin carbamoyl derivatives of the PAF are well separated as sharp peaks using the isocratic elution. The quantification of PAF was made by measuring the peak height of the derivative on the chromatogram. The laboratory personnel working with the PAF analysis were blinded to the treatment modality.

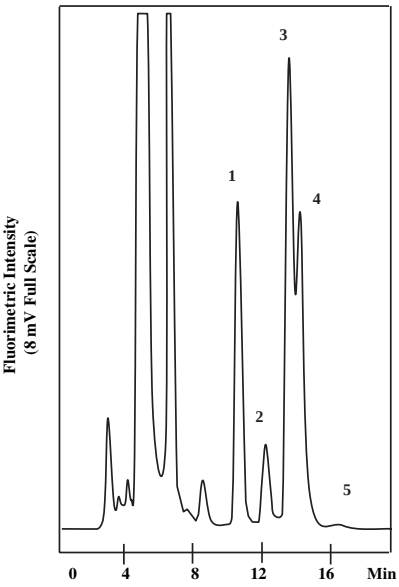


*Fig. 1.* Reverse-phase high-performance liquid chromatography (HPLC) of the standard mixture containing 0.5 mg/l of each compound except for the platelet activating factor (PAF) $C_{16}$, which was present at 0.2 mg/l. Peak 1, 1-0-hexadecyl-2-0-acetyl-*sn*-glycero-3-phosphocholine (PAF $C_{16}$); peak 2, D-alpha-phosphatidylcholine, beta-acetyl-gamma-*O*-hexadecyl; peak 3, 1,2-didecanoyl-*sn*-glycero-3-phosphocholine; peak 4, 1,2-Di-*O*-hexadecyl-*sn*-glycero-phosphocoline; and peak 5, 1,2-didocosanoyl-*sn*-glycero-3-phosphocholine.

### Statistical analysis

Statistical analysis was performed using a commercially available software program (SPSS 12.0; SPSS Inc., Chicago, IL, USA). The Shapiro Wilk test was used to investigate whether or not the data were normally distributed.

For the statistical analysis of PPD and CAL, only the recordings representing the deepest clinical site in each defect were used (27). A paired T parametric test was used for statistical comparisons between pre-operative and 6-mo postoperative measurements in each of the treatment modalities. Intergroup differences in PPD and CAL between group 1 and group 2 were statistically evaluated by the Student T parametric test.

The statistical evaluation for plaque index, gingival index, GCF volume and PAF levels between pre-operative samples and those taken at postoperative weeks 6, 12 and 24 were performed by repeated-measures analysis of variance. Post hoc tests were not additionally performed as there were two groups in the study. The Student T parametric test was used to compare different time points.

The Pearson correlation test was used to test the relationship between PAF levels and clinical parameters. Normally distributed data are shown as means ± standard error of the mean (SEM). Significance levels were calculated at $p < 0.05$.

## Results

### Clinical findings

Intragroup comparisons showed that the PPD decreased and the CAL improved significantly at 6 mo postoperatively, compared with the pre-operative data ($p < 0.01$). No significant difference was observed between the pre-operative values in both groups. The changes in PPD were 4.5 ± 0.26 in group 1 and 4.7 ± 0.19 in group 2. The pre-operative CAL was found to be improved by an average of 3.8 ± 0.22 mm in group 1 and by 3.9 ± 0.23 mm in group 2. The scores of plaque and gingival indices pre-operatively and at all the postoperative intervals are presented in Table 1. No statistically significant difference was observed between the groups ($p > 0.05$).

### Changes in GCF volume and PAF levels

GCF volume during wound healing after periodontal regenerative surgery in both study groups is reported in Table 2. Intragroup analysis showed significant decreases in GCF volume for both groups at postoperative weeks 6, 12 and 24 compared with the pre-operative values ($p < 0.01$). Intergroup analysis demonstrated no

*Table 1* . Plaque index and gingival index scores pre-operatively and following therapy, at the postoperative time points shown

| | | Postoperative intervals | | |
|---|---|---|---|---|
| | Pre-operatively | 6 wks | 12 wks | 24 wks |
| Plaque index | | | | |
| Group 1[ae] | 0.65 ± 0.03 | 0.66 ± 0.03 | 0.63 ± 0.02 | 0.63 ± 0.02 |
| Group 2[ae] | 0.63 ± 0.03 | 0.63 ± 0.02 | 0.61 ± 0.03 | 0.61 ± 0.02 |
| Gingival index | | | | |
| Group 1[e] | 1.41 ± 0.04 | 0.90 ± 0.05[b] | 0.57 ± 0.04[bc] | 0.29 ± 0.04[bcd] |
| Group 2[e] | 1.33 ± 0.05 | 0.82 ± 0.05[b] | 0.49 ± 0.04[bc] | 0.26 ± 0.03[bcd] |

Data were analyzed by repeated-measures analysis of variance and the Student's *T*-test, and are expressed as the mean ± standard error of the mean.
[a]No significant difference pre-operatively and at all the postoperative intervals ($p > 0.05$).
[b]Significantly different from the pre-operative values ($p < 0.001$).
[c]Significantly different from the values at 6 wks ($p < 0.001$).
[d]Significantly different from the values at 12 wks ($p < 0.001$).
[e]No significant difference between the values of the groups ($p > 0.05$).

*Table 2* . Gingival crevice fluid (GCF) volume (μl) pre-operatively and following therapy, at the postoperative time points shown

| | | Postoperative intervals | | |
|---|---|---|---|---|
| Group | Pre-operatively | 6 wks | 12 wks | 24 wks |
| Group 1[d] | 0.337 ± 0.017 | 0.257 ± 0.008[a] | 0.185 ± 0.008[ab] | 0.126 ± 0.005[abc] |
| Group 2[d] | 0.330 ± 0.015 | 0.244 ± 0.005[a] | 0.188 ± 0.007[ab] | 0.137 ± 0.008[abc] |

Data were analyzed by repeated-measures analysis of variance and the Student's *T*-test, and are expressed as the mean ± standard error of the mean.
[a]Significantly different from the pre-operative values ($p < 0.001$).
[b]Significantly different from the values at 6 wks ($p < 0.001$).
[c]Significantly different from the values at 12 wks ($p < 0.001$).
[d]No significant difference between the values of the groups ($p > 0.05$).

significant differences in GCF volume between study groups pre-operatively and at 6, 12 and 24 wks post-operatively ($p > 0.05$).

PAF levels in GCF of group 1 and group 2 during periodontal healing are shown in Table 3. Intragroup analysis showed significant decreases in PAF levels for both groups at 6, 12 and 24 wks postoperatively compared with the pre-operative values ($p < 0.01$). Intergroup analysis demonstrated no significant differences in PAF levels between the study groups pre-operatively and at 6, 12 and 24 wks post-operatively ($p > 0.05$).

*Table 3* . Platelet activating facor (PAF) levels (ng/ml) in gingival crevice fluid (GCF) pre-operatively and following therapy, at the postoperative time points shown

| | | Postoperative intervals | | |
|---|---|---|---|---|
| Group | Pre-operatively | 6 wks | 12 wks | 24 wks |
| Group 1[d] | 473.7 ± 35.48 | 137.3 ± 11.61[a] | 83.3 ± 5.58[ab] | 52.3 ± 4.50[abc] |
| Group 2[d] | 466.0 ± 28.06 | 140.3 ± 9.64[a] | 86.3 ± 6.39[ab] | 56.7 ± 5.45[abc] |

Data were analyzed by repeated-measures analysis of variance and the Student's *T*-test, and are expressed as the mean ± standard error of the mean.
[a]Significantly different from the pre-operative values ($p < 0.001$).
[b]Significantly different from the values at 6 wks ($p < 0.001$).
[c]Significantly different from the values at 12 wks ($p < 0.001$).
[d]No significant difference between the values of the groups ($p > 0.05$).

No correlation between PAF levels and clinical parameters was found ($p > 0.05$).

## Discussion

In the present study, PAF levels, which are considered to be associated with the pathogenesis of periodontal disease and peri-implantitis, were evaluated in GCF just before periodontal surgery and at postoperative intervals up to 24 wks. The clinical findings of this study showed that the treatment of intrabony periodontal defects with both GTR and flap surgery might lead to significant PPD reduction and CAL gain. No significant differences in any of the clinical parameters were observed between the groups. Most of the studies comparing GTR and flap surgery have reported conflicting results: some authors have observed a greater amount of clinical healing in the GTR groups (28,29), while others have found no statistically significant differences between sites treated with GTR and flap surgery (30,31), which is in agreement with our findings. The present data have also shown that there was a significant decrease in the volume of GCF at all the postoperative intervals compared with that of the pre-operative values in both groups. It has been reported that the change in GCF volume is the most sensitive and least subjective indicator of alterations in gingival inflammation (22,32,33). Based on GCF volume, our findings suggest that both GTR and flap surgery treatments may lead to a decrease in inflammation after 6 wks.

It has been demonstrated that the GCF volume decreases at 4 wks following treatment with enamel matrix derivative (22). However, in another study (21), it was shown that after 6 wks the GCF volume at the sites treated with conventional flap surgery decreased to levels that were not significantly different from their pre-operative values. In contrast, the GCF volume at the sites treated with GTR (nonabsorbable membrane) decreased to the pre-operative levels by 12 and 26 wks. The nonabsorbable membrane was removed at 6 wks, and the GCF volume started to decrease at 7 wks

(21). In the present study, a significant decrease in GCF volume was observed at 6, 12 and 24 wks postoperatively, and there were also significant differences between the values of all the intervals up to 24 wks in both groups. The resorption process of absorbable polylactide membrane, which is the solid polymer of polylactic acid, is programmed to ensure barrier function for a minimum of 6 wks, after which it slowly resorbs (34). It is known that during the resorption of the membrane, the inflammatory process may temporarily increase. However, this increase is probably not sufficient to affect the GCF volume, PAF levels in GCF, and other clinical parameters at any intervals in our study.

PAF has been discovered to be a soluble mediator and is found in a variety of body fluids, so it is conceivable that some cells secrete this mediator after synthesis (7). In the periodontology literature, PAF has been analyzed in blood (8), GCF (11,12,15), and saliva (16,35–37). It is considered that GCF reflects cellular activities in the surrounding periodontal tissues and the constituents of GCF are involved in tissue formation and remodeling (18,21,38). Despite the presence of studies analyzing salivary PAF levels (16,35–37), there are few studies related to PAF levels in GCF (11,12,15). A phospholipid molecule with PAF-like activity has been isolated from the GCF of patients with chronic periodontitis (15). Moreover, significantly elevated PAF levels have been found in GCF in cyclosporine A-induced gingival overgrowth sites compared with healthy sites from healthy controls (11).

PAF has also been analyzed in gingival tissues (6,12,14). It has been shown that PAF levels in both GCF and gingival tissue were significantly elevated in periodontitis and gingivitis groups compared with the healthy controls (12). In addition, data indicating significantly higher levels of PAF in inflamed gingiva from periodontitis patients than in healthy gingiva has suggested that the higher levels of PAF in the inflamed gingival tissue are caused by an increase in PAF production (6). The application of omega-3 fatty acid (39), and a combination of selective cyclooxygenase-2 inhibitor and omega-3 fatty acid administration (40), has significantly reduced the gingival tissue levels of PAF in endotoxin-induced periodontitis in rats.

HPLC has mainly been used for purification of the PAF molecule in the literature (8,11,12,15,39,40). In addition, thin-layer chromatography has been used for the analysis of PAF (6,16). However, as PAF is usually present in small amounts in biological systems (e.g. GCF) its detection requires the use of highly sensitive methods, based on HPLC, which is not an inexpensive and convenient method for regular clinical use. A sensitive method for the determination of PAF activity at picomolar levels in human serum, using HPLC with fluorimetric detection, has been described (26). In the present study, a similar method was performed for the first time to measure PAF levels in the GCF samples.

All reports on PAF are generally related to the pathogenesis of periodontal disease and peri-implantitis (6,8,12,14). In the light of these observations, PAF is likely to play a crucial role in the occurrence and maintenance of periodontal disease. However, the role of PAF in healing after periodontal surgery is not fully understood. In the present study, there was a significant decrease in the levels of PAF in GCF at all the postoperative intervals, relative to the pre-operative levels, in both groups. It has been demonstrated that PAF, a potent mediator of inflammatory responses, stimulates osteoclastic resorption (13). Moreover, PAF is considered to be an important mediator of alveolar bone and connective tissue destruction in periodontitis (39). Based on these observations, our study suggests that the decrease in PAF levels following periodontal surgical therapies might be related to the repair and regeneration of the connective and bone tissue. Within the limits of the present study, it can be concluded that both the regenerative and conventional flap surgeries have similar effects on the decrease of PAF levels, which is associated with healing of periodontium.

Overall, in the present study, two different treatment modalities showed similar clinical findings as well as no significantly different GCF volume and PAF levels pre-operatively and at all the postoperative intervals. As flap surgery showed similarly favorable findings with GTR, it is relevant to assume that flap surgery might be as effective as GTR with regard to the parameters of our study. To the best of our knowledge, this is the first report investigating PAF levels in GCF following periodontal surgery. The present findings suggest that PAF is readily detectable in GCF by HPLC and decreases continuously at all time points after surgery; therefore, analysis of PAF in GCF might be useful for monitoring periodontal repair and regeneration. This information is important to the clinician because changes in the PAF levels would be a prognostic marker of periodontal healing to evaluate the success of surgical therapies. Additional studies should be undertaken to elucidate further the role of PAF on wound-healing mechanisms following periodontal regenerative therapies.

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