Gingival Crevicular Fluid Release Profile of Vascular Endothelial Cell Growth Factor and Platelet-Derived Growth Factors – BB Following Minimally Invasive Flap Reflection during Treatment of Intrabony Defects: A Randomized Clinical Trial

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Abstract

Objectives: As a primary objective, we examined the gingival crevicular fluid (GCF) levels of the endogenously released platelet-derived growth factor (PDGF-BB) and vascular endothelial growth factor (VEGF) following the use of minimally invasive surgical flap reflection (MIS) and compared them with those in traditional open flap debridement (OFD). The secondary objective was to determine if any correlation exists between the expression of growth factors (GF), indicated by their levels, and associated clinical outcomes.

Methods: Twenty-eight non-smoking individuals with severe chronic periodontitis were recruited in this prospective, randomized and single-blinded trial. Each person presented one interproximal defect that was randomly assigned to either the experimental MIS group (14 sites) or the open flap debridement (OFD) control group (14 sites). Plaque index, gingival index, probing depth (PD), clinical attachment level (CAL) and the intrabony depth of the defect (IBD) were measured at baseline for the patient's enrollment. Gingival crevicular fluid (GCF) samples were collected on days 1, 3, 7, 14, 21 and 30 after therapy. Clinical follow-ups were scheduled at 6 and 9 months following the therapy.

Results: In both groups, the highest levels of VEGF and PDGF-BB concentrations were found in the GCF during the period from 1 to 14 days. During the early stages of healing (1, 3, 7 and 14 days), the mean VEGF and PDGF-BB levels at sites treated with MIS were significantly higher than those at the OFD-treated sites. Growth factor levels decreased sharply in the samples obtained at days 21 and 30 in both groups, with non-significant differences between the patient and control groups. MIS-treated sites reported significantly more PD reduction and attachment gain 9 months following therapy. No significant difference in intrabony defect reduction was reported between the patient and control groups.

Conclusions: Within the limits of the present study, we can surmise that MIS treatment of periodontal defects is associated with initially higher GCF levels of the studied growth factors. These increased GF levels are well correlated with the improved soft-tissue parameters of the periodontal defects.

Key words: Periodontal regeneration, guided tissue membranes, bone morphogenetic protein, growth factors, periodontal pockets

Introduction

The main objective of periodontal therapy is to arrest and control periodontal infection or to regenerate previously lost periodontal structures (Wang and Greenwell, 2001).

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Full regeneration of the periodontium after periodontal treatment procedures has faced many challenges because of differences in healing abilities among periodontal components (Cho *et al.,* 1995). Down-growth of the junctional epithelium along the exposed root surface is one of the most important factors limiting the achievement of predictable amounts of regeneration (Wirthlin, 1981; Egelberg, 1987). Systematic reviews have indicated that regenerative treatment of intrabony defects leads to significant improvements in clinical outcomes compared with those achieved by access flap surgery alone (Needleman *et al.,* 2002; Trombelli *et al.,* 2002; Murphy and Gunsolley, 2003). Nevertheless, as investigators attempted to understand tissue regeneration in a more mechanistic way, primary flap closure and interproximal tissue maintenance were often reported as important factors in wound healing and improved treatment outcomes (Cortellini *et al.,* 1995; Cortellini *et al.,* 1999; Zucchelli *et al.,* 2003; Trombelli *et al.,* 2005).

In order to get access for many regenerative therapies, open flap debridement is usually employed as an access flap surgery with full reflection of a mucoperiosteal flap. Such an approach cannot guarantee primary interproximal flap closure, which may allow bacteria to contaminate the regenerative material, mediators' proteolysis and rapid root-surface epithelialization. One of the regenerative treatment techniques, the use of microsurgery for primary flap closure, aimed at maximum tissue preservation, was described by Cortellini and Tonetti (2001). A minimally invasive surgical technique for periodontal regeneration has been proposed in order to maintain gingival architecture and to create a minimal wound. In addition, this approach was performed with the use of operating microscopes that may promote less invasive surgery and a more comfortable post-operative period (Tonetti *et al.,* 2004; Cortellini *et al.,* 2009). Wachtel *et al.* (2003) reported significantly improved clinical outcomes using microsurgical access flap compared with primary flap closure according to the principle of the modified papilla preservation technique in enamel matrix derivatives (EMD) treatment of intrabony defects (Cortellini *et al.,* 2009). Minimally invasive surgery allows for decreased tissue manipulation and trauma to surgical sites, and facilitates faster healing with decreased short- and long-term morbidity (Wachtel *et al.,* 2003).

A minimally invasive procedure for periodontal regeneration, termed "minimally invasive surgery" (MIS), was discussed in many studies from 1995 until 2005 (Fitzpatrick and Wickham, 1990; Harrel and Rees, 1995; Harrel, 1999; Harrel, 2000). A flap procedure termed the "minimally invasive surgery technique" (MIST) was described later (Harrel, 2005; Cortellini and Tonetti, 2007). Both MIS and MIST showed no significant differences in outcomes, which were superior to results reported for traditional regenerative periodontal surgeries. In general, the results showed significant improvements in both probing depths and attachment levels. These procedures are performed with small incisions and are often limited only to the facial or lingual aspect. Thermal sensitivity after MIS is markedly reduced because incisions are limited to the area of interest and are not extended to adjacent unaffected teeth. Minimal post-operative gingival recession has been reported in many studies (Cortellini and Tonetti, 2007; Cortellini *et al.,* 2008). Moreover, it has been reported that there is no tendency for deeper probing depths to recur over 6 or more years post-operatively (Harrel *et al.,* 2010).

In the present study, it is suggested that minimally invasive reflection of the periodontal tissues could be a modality that may allow for physiologic containment of the endogenous growth factors released post-surgically. This could have an impact on the clinical outcomes of many applied regenerative therapies. The relatively open and contaminated nature of periodontal defects following traditional open flap debridement could be a source of enzymatic degradation and continuous diffusion of growth factors away from the defect area. To test this assumption, this study was designed to evaluate levels of two important angiogenic growth factors, platelet-derived growth factor–BB (PDGF-BB) and vascular endothelial cell growth factor (VEGF), in GCF during the early stages of healing for sites treated with MIS and OFD, and to correlate growth factor levels with clinical findings. To the author's knowledge, this study is the first to investigate these growth factor levels in the GCF following MIS treatment of periodontal defects.

Materials and methods

Patient selection

This prospective study was designed as a randomized and single-blinded trial. Participants were recruited consecutively from the list of patients seeking periodontal treatment in the School of Dentistry at Umm Alqura University and private clinic of the author, in Jeddah, Saudi Arabia, between January 2015 and April 2016. The criteria implemented for patient inclusion were: 1) no systemic diseases that could affect the outcome of therapy; 2) good compliance with plaque control measures following initial therapy; 3) teeth involved were all vital, with no degree of mobility; 4) each participant contributed a single 3- or 2-wall intrabony interproximal defect around premolar or molar teeth with no furcation involvement; 5) selected 2- or 3-wall intrabony defects (IBDs) measured from the alveolar crest to the defect base in periapical radiographs of $\geq$ 3 mm; 6) selected probing depth (PD) $\geq$ 6 mm and clinical attachment loss (CAL) $\geq$ 5 mm at the site of intraosseous defects three weeks following initial therapy; 7) availability for the maintenance program; 8) absence of periodontal treatment during the preceding 6 months; 9) absence of systemic medications or antibiotic treatment during the preceding 6 months, which could affect healing; and 10) absence of occlusal interference or open interproximal contacts. Pregnant females were excluded from the study.

The research protocol was explained to all patients, and they agreed to participate and signed the appropriate informed consent document. The study was performed according to the Declaration of Helsinki protocol as revised in 1983. IRB approval was obtained from Umm Alqura University, School of Dentistry # -25a-15 in January 2015.

Pre-surgical therapy and grouping

Initial therapy consisted of thorough full-mouth scaling and root planing performed in quadrants with the patient under local anesthesia. This procedure was performed with a combination of hand **(**Gracey curettes 1/2 and 7/8; Hu-Friedy, Chicago, IL, USA**)** and ultrasonic (Cavitron, Long Island City, NY, USA) instrumentation with a P10 tip. Patients were recalled every 4 days for three weeks and received detailed mechanical plaque control instructions. Three weeks following initial therapy, all patients were recalled for reevaluation to confirm the need for periodontal surgery. Criteria used to confirm surgery included the persistence of an interproximal site with PD ≥ 6 mm, CAL ≥ 5 mm and interproximal IBD of ≥ 3 mm. Baseline periodontal disease status of the selected sites was determined by clinical periodontal assessments including plaque index (PI; Silness and Löe, 1964), gingival index (GI; Löe and Silness, 1963), probing depth (PD; Polson *et al.,* 1980) and clinical attachment level (CAL; Ramfjord, 1967) as the distance from the bottom of the pocket to the gingival margin and the cemento-enamel junction (CEJ), respectively. The deepest points of baseline defects were included in the calculations. Routine diagnostic periapical views with intraoral size 2 dental films (Kodak Extraspeed, Eastman Kodak, Rochester, NY, USA) were recorded by the long cone paralleling technique and holders (Rinn Centering Device, DENTSPLY, Weybridge, UK) in an x-ray unit (Heliodent 70, Siemens, Bensheim, Germany). The linear distances from the CEJ to the base of the bony defect, representing the relative intrabony component level (rIBD), were measured. Initial therapy and clinical measurements were performed by a single calibrated clinical assistant who was not involved in the study in any other way (Alaa Atieh - Associate Professor at Umm Alqura University).

Patients were randomly assigned to one of two groups (14 patients each): conventional OFD (control group of open flap debridement) or MIS (minimally invasive surgical debridement test group). Computer-assessed randomization was performed with a computer software package (NCSS-PASS®, Number Cruncher Statistical Systems, Kaysville, UT, USA) immediately before surgery.

Surgical procedures

All surgeries were performed by the same operator (the author). A surgical treatment phase was initiated only if the patient had a full-mouth dental plaque score of less than 1 and a site plaque score of 0. For those in the open flap debridement group, under local anesthesia, a mucoperiosteal flap extending one tooth on either side of the defect was elevated to the mucogingival junction to allow for passive closure. Two vertical incisions were performed on either side of the defect. Debridement of all inflammatory granulation tissue from the intrabony defect was performed by means of Gracey 7/8 metal curettes. Teeth were thoroughly root-planed, combining the use of metal curettes and power-driven instrumentation. The mucoperiosteal flap was repositioned and sutured with 5-0 non-resorbable suture (BioMend Extend, Zimmer Dental, Carlsbad, CA, USA). No periodontal dressing was applied. For the minimally invasive sites, a soft tissue flap was reflected either by the simplified papilla preservation technique (Cortellini *et al.,* 1999; Zucchelli *et al.,* 2003) or according to the modified papilla preservation technique (Cortellini *et al.,* 1995; Wachtel *et al*., 2003). The simplified technique was selected if the width of the inter-dental space was ≥ 2 mm, while the modified one was performed if the inter-dental space was ≥ 2 mm and gingival thickness adequate. In cases of narrow inter-dental papillae, an oblique incision, according to the original technique of Cortellini *et al.* (1999), and buccal and lingual intrasulcular incisions were made with microsurgical blades. In cases of wide inter-dental papillae, a horizontal incision at the base of the papilla was performed following the original technique (Cortellini *et al.,* 1995), and buccal and lingual intrasulcular incisions were performed with microsurgical blades. After the flap was elevated and the papilla flap pushed palatally with a fine papillary elevator, the granulation tissue was removed with curettes and from the inner side of the flap by means of microsurgical scissors. The root surfaces were cleaned and planed by use of manual and ultrasound instruments. After the flap was re-positioned, the buccal and palatal portions were adapted to each other without tension by horizontal mattress sutures using 5-0 non-resorbable suture.

All patients received oral and written post-operative plaque control instructions. Patients were prescribed amoxicillin (500 mg) (Amoxicillin, October Pharmaceutical, Cairo, Egypt) every 8 hours for one week. Those with allergies to amoxicillin or its derivatives were prescribed clindamycin (300 mg) every 8 hours. Plaque control was supplemented by the use of a chlorhexidine rinse (0.2% chlorhexidine gluconate; Hexidine Mouthwash, Health Tree, Mohali, Punjab, India) for two minutes three times daily for 2 weeks. Patients were instructed to stop toothbrushing and inter-dental cleaning at the surgical areas during this time. Sutures were removed 14 days post-operatively, and recall appointments for observation of any adverse tissue reactions and oral hygiene instructions were performed every second week during the first 3 months after surgery. One month after surgery, all patients were instructed to resume their normal mechanical oral hygiene measures of soft toothbrushing with a roll technique and flossing. Clinical and radiographic measurements were reassessed by a blinded calibrated investigator at 6 and 9 months after surgery (Ahmed Dardier - Assistant Professor at Umm Alqura University).

Quantitative measurement of VEGF and PDGF-BB in the GCF samples

The VEGF and PDGF-BB in the GCF samples were measured by means of an enzyme-linked immunosorbent assay kit (ELISA; RayBiotech, Inc., Norcross, GA). Samples were obtained 1 day following surgery and after individuals had fasted overnight from 9:00 p.m. to 10:00 a.m. Micropipettes (5 μL) were used for the collection of two GCF samples from the same site (Cimasoni *et al.*, 1969) by a single examiner who was masked to the attribution of the sites to groups (Ayman Abuelneen-Professor at Umm Alqura University). Following isolation of the selected site with cotton rolls, a Fisher brand disposable micropipette (Sigma-Aldrich, St. Louis, MO) was placed intrasulcularly at the mesiolabial line angle of the selected site to a maximum depth of 2 mm below the gingival margin. Micropipettes were left in place until a 5 μL quantity of fluid was collected. GCF samples were collected at days 1, 3, 7, 14, 21 and 30 after therapy and diluted in saline solution (50 μL) for evaluation of VEGF and PDGF-BB levels. Samples were carried in a dark container, labeled and kept at -25°C until used.

The primary outcome variable for the study was gingival crevicular fluid levels of VEGF and PDGF-BB of both groups collected at 1, 3, 7, 14, 21 and 30 days. Secondary outcome parameters included clinical and radiographic measurements of the treatment outcomes at 6 and 9 months after surgery. According to the available gingival crevicular fluid, GF measurements in the literature and using an α level of 0.05 (5%) and β level of 0.20 (20%), i.e., power = 80%; the predicted minimum sample size (n) was a total of eighteen cases, i.e., 9 cases in each group. However, regarding the correlation with clinical parameters and to obtain a larger sample size; another calculation was performed based upon the changes in clinical attachment level measurements obtained from the available literature and using an α level of 0.05 (5%) and β level of 0.20 (20%), i.e., power = 80%; the predicted minimum sample size (n) was a total of 18 cases, i.e., nine cases in each group. Considering that some patients may not continue during follow-up, 14 patients were included in both the MIS and OFD groups. Numerical data were presented as mean and standard deviation (SD) values. Data were explored for normality by the Kolmogorov-Smirnov and Shapiro-Wilk tests. The Mann-Whitney U test was used for pair-wise comparisons. The Wilcoxon signed-rank test was used to study the changes after treatment within each group. The significance level was set at $p \leq 0.05$. Statistical analysis was performed with the IBM Statistics Version 20 for Windows (IBM Corporation, New York, NY) and SPSS (SPSS, Inc., an IBM Company).

Results

Twenty-eight non-smoking individuals (19 males and 9 females) who ranged in age from 28 to 43 years at the time of the baseline examination (mean age 33.5 ± 6.1 years) and with severe chronic periodontitis (Armitage, 1999) were recruited. During the course of the study, all patients experienced uneventful post-operative healing in both the experimental and control defects. All patients who completed the study tolerated the surgical procedures, and no site had to be eliminated. One patient did not continue his follow-up visits for sample collections in the control OFD group. In total, 8 intrabony defects were excluded during the surgical procedure upon discovery that the defect morphology did not meet the inclusion criteria (2 for G1 and 6 for G2; *Figure 1*). Bony wall-treated defects were distributed as follows: OFD, 10 predominantly 3-wall and 3 predominantly 2-wall; and MIS, 12 predominantly 3-wall and 2 predominantly 2-wall defects (*Table 1*). A summary of the defect characteristics 3 weeks following cause-related therapy for the two groups is provided in *Table 4*. No statistically significant differences were found pre-surgery between groups with respect to soft- and hard-tissue measurements ($p > 0.05$). All GI and PI scores were within clinically healthy parameters. The defects had deep PDs and CAL and were associated with IBD of ≥ 3 mm.

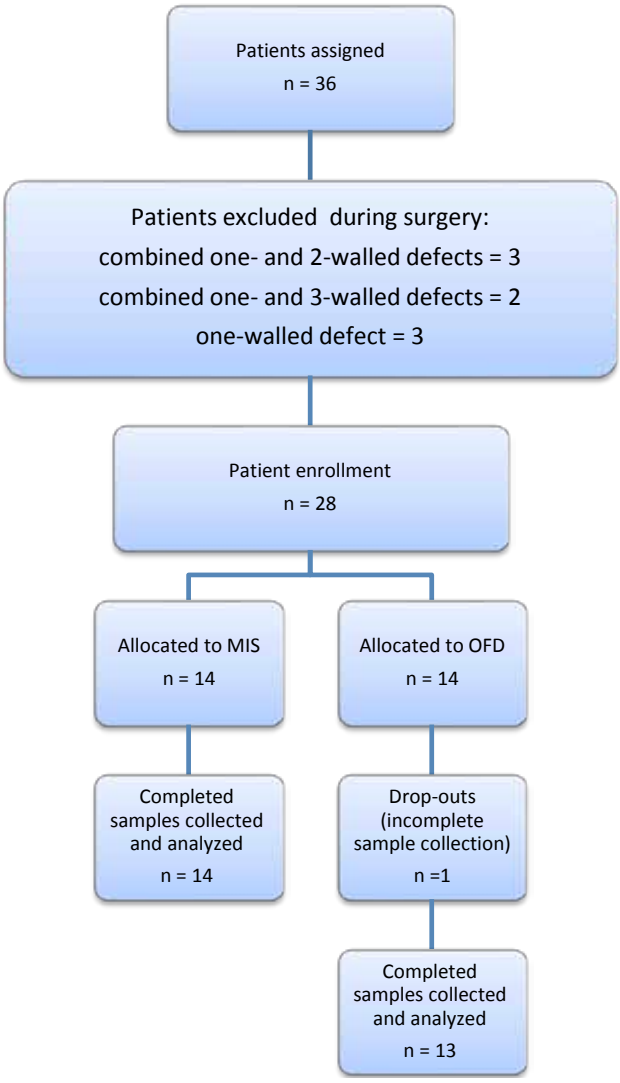

Figure 1. Study profile and patient disposition.

Table 1. Demographic characteristics of the study participants

	Number	Gender		Age	Intrabony defect type	
		Male	Female		3 walls	2 walls
MIS	14	8	6	37.2 ± 4.2	10	3
OFD	13	8	5	31.5 ± 5.3	12	2

Table 2. Means, standard deviation (SD) values and results of Mann-Whitney U tests for the comparison between vascular endothelial growth factor (VEGF) concentrations (pg/mL) in the two groups.

Group	OFD n = 13		MIS n = 14		*p*-value
Period	Mean	SD	Mean	SD	
1 day	1621.1[a]	55.6	1748.5[a]	159.9	0.127
3 days	656.3[b]	122.2	1666.8[a]	148.3	0.027*
7 days	721.6[b]	56.4	1456.2[a]	73.3	0.031*
14 days	643.6[b]	88.8	1344.4[a]	18.8	0.026*
21 days	293.2[c]	43.7	406.6[b]	43.9	0.061
30 day	302.3[c]	23.2	456.1[b]	51.6	0.131

*Significant at $p \leq 0.05$. Different letters indicate significant differences in the same group. MIS, minimally invasive surgical flap reflection; OFD, open flap debridement.

Table 3. The means, standard deviation (SD) values and results of Mann-Whitney U tests for the comparison between platelet-derived growth factor (PDGF-BB) concentrations (pg/mL) in the two groups.

Group	OFD n = 13		MIS n = 14		*p*-value
Period	Mean	SD	Mean	SD	
1 day	1034.1[a]	234.4	1298.2[a]	122.1	0.121
3 days	1022.8[a]	283.2	1934.4[b]	68.2	0.021*
7 days	867.8[a]	150.5	1564.8[a]	55.4	0.032*
14 days	823.5[a]	108.4	1512.6[a]	41.8	0.032*
21 days	344.3[b]	204.3	512.6[c]	25.7	0.733
30 day	233.3[b]	15.1	245.2[c]	23.6	0.516

*$p \leq 0.05$. Different letters indicate significant differences in the same group. MIS, minimally invasive surgical flap reflection; OFD, open flap debridement.

Table 2 illustrates the means, standard deviation (SD) values and results of Mann-Whitney U tests for the comparison between GCF-VEGF concentrations in the two groups at different sampling times. Initially, one day following surgery, both groups showed the highest VEGF level, with no significant inter-group difference. Both groups showed significantly higher levels of growth factor at 1, 3, 7 and 14 days when compared with the later periods at 21 and 30 days. MIS samples showed significantly higher VEGF levels at 3, 7 and 14 days when compared with OFD-treated sites. Levels were significantly reduced in both groups between each time interval. VEGF levels were significantly reduced in the samples obtained at 21 and 30 days in both groups, with no significant differences between the two time intervals. No significant differences were found at 21 and 30 days between the two groups.

Table 3 shows the means, standard deviation values and results of Mann-Whitney U tests for the comparison between PDGF-BB concentrations in the two groups. Parallel to the peaked VEGF levels during the early stages of healing (1, 3, 7 and 14 days), PDGF-BB appeared to record the highest levels during these time periods in both MIS and OFD groups, with significant differences in comparison with the later observation periods at 21 and 30 days. Levels of GF in sites treated with MIS were significantly higher than those of OFD-treated sites during these early time periods (1, 3, 7 and

14 days; $p \leq 0.05$). Levels were significantly reduced in both groups between each time interval. Unexpectedly, the PDGF-BB level in samples collected at day 3 was significantly higher than that of those collected at day 1.

Table 4 shows the mean defect characteristics of both groups initially and during different observation periods. The mean PI and GI were initially low; they remained unchanged at 6 and 9 months for both groups. There were no statistically significant differences between the initial values of all parameters in the two tested groups ($p > 0.05$). At the end of the study, both groups showed statistically significant improvement in PD reduction, CAL gain and IBD fill compared with baseline data ($p < 0.05$). MIS showed statistically significant PD reduction and attachment gain compared with OFD 9 months following therapy. No significant differences in IBC values were reported between the two groups during both observation periods.

Discussion

In the present study, the dynamics of VEGF and PDGF-BB as key mediators for vascular development were evaluated following the use of MIS, which could be a modality that enhances the levels of these growth factors in the defect area. Minimally invasive primary flap closure was considered in this study to improve growth factor containment within the defect area. Because angiogenesis plays a key role in bone formation, growth factors involved in vascular development have become one of the most important mediators involved in periodontal regeneration and were thus evaluated in the present study. PDGF is a potential mitogen and chemotactic factor that mainly regulates the maturation and remodeling of newly formed blood vessels. The PDGF-BB phenotype is reported to be the most active of the PDGF phenotypes in stimulating fibroblast proliferation and adhesion (Gamal *et al.,* 1998; Gamal and Mailhot, 2000). Boyan *et al.* (1994) studied the mitogenic responses of human periodontal ligament cells to recombinant human PDGF-AA, -AB and -BB. They reported that the addition of each PDGF isoform enhanced periodontal ligament cell proliferation in a dose-dependent manner. They also reported PDGF-BB as the most potent mitogen. It was also found that certain levels of PDGFs and their receptors are important for normal tissue repair. Impaired wound healing in aged mice has been associated with a delay in appearance or disturbed levels of PDGF A and B isoforms and α- and β-receptors (Rutkowski *et al.,* 2010). The second important angiogenic growth factor essential for periodontal regeneration is VEGF, which has been reported to enhance osteogenic differentiation of mesenchymal stem cells (MSCs) through an intracrine mechanism (Zaman *et al.,* 1999). It has been reported that the application of neutralizing VEGF-A antibodies that disturb VEGF levels induced a marked reduction in wound angiogenesis in a pig wound model (Arpornmaeklong *et al.,* 2004). Furthermore, the angiogenic potentials in human wound fluid after injury have been shown to be inhibited by VEGF neutralization (Alissa *et al.,* 2010; Cenni *et al.,* 2010).

Table 4. Mean clinical parameter values and results of the Kruskal-Wallis test for the two groups, initially and at 6 and 9 months after treatment.

Parameters	Group		*p*-value
	MIS	OFD	
IBD (mm)			
Baseline	3.8 ± 0.2	4.2 ± 0.2	0.43
6 M	2.5 ± 0.4	2.9 ± 0.4	0.145
9M	2.1 ± 0.3	2.4 ± 0.2	0.222
PD (mm)			
Baseline	6.3 ± 0.4	6.6 ± 0.2	0.41
6 M	2.8 ± 0.4*	3.8 ± 0.3	0.035
9M	2.4 ± 0.2*	3.5 ± 0.4	0.012
CAL (mm)			
Baseline	4.3 ± 0.3	4.5 ± 0.3	0.23
6 M	2.3± 0.4*	3.4 ± 0.3	0.016
9M	2.1± 0.3*	2.8 ± 0.3	0.012
GI			
Baseline	0.3 ± 0.1	0.4 ± 0.02	0.41
6 M	0.3 ± 0.4	0.2 ± 0.3	0.34
9M	0.5 ± 0.3	0.7 ± 0.5	0.23
PI			
Baseline	0.4 ± 0.1	0.3 ± 0.01	0.41
6 M	0.4 ± 0.3	0.4 ± 0.5	0.41
9M	0.3 ± 0.3	0.3 ± 0.4	0.21

*$p < 0.05$. MIS, minimally invasive surgical flap reflection; OFD, open flap debridement; intrabony defect; PD, probing depth; CAL, clinical attachment level; GI, gingival index; PI, plaque index.

Gingival crevicular fluid flow, with its physical protective effects from flushing the pocket, is considered an excellent undispersed medium for evaluation of the PDGF-BB and VEGF released at different time periods. The selection of the 3- and 2-walled intrabony defect types is another factor that helps in maintaining PDGF-BB and VEGF undispersed for accurate evaluation for its availability and release pattern. We decided to start GCF collection a day after surgery because samples collected immediately after surgery are usually blood-contaminated.

Analysis of the GCF revealed that PDGF-BB and VEGF levels were significantly higher at 1, 3, 7 and 14 days after surgery in the MIS groups compared with the later periods of evaluation. These initially high growth factor levels following surgery were confirmed by a series of studies suggesting that, upon injury, PDGF is released in large amounts from degranulating platelets (Matsuoka and Grotendorst, 1989) and is present in wound fluid early after injury (Ono *et al.,* 1995). In addition, it was reported that high levels of receptors for VEGF were detected in blood vessels of granulation tissue in wound healing (Hughes *et al.,* 2006). These initial significantly higher growth factor levels of the MIS-treated group compared with the OFD-treated group could reflect the continuous diluting effect of the non-contained wide soft tissue surgical flap in OFD compared with the restricted contained flap in MIS. It could also be interpreted as a direct effect of the more superior uninterrupted interproximal papillae protection of MIS compared with OFD. Among the many defect factors that influence the outcome of periodontal therapies, defect stability and protection appear to be the most important. Many studies have indicated that contained defects (i.e., 3- or 2-wall defects) may provide a better environment for enhancing blood-clot stability compared with non-contained defects (those with missing bony walls or supra-alveolar defects), thus significantly influencing the outcomes (Cortellini and Tonetti, 2005; Sculean *et al.,* 2008). Growth factor levels were markedly reduced at 21 and 30 days in both groups, with non-significantly higher levels for the MIS-treated group with the OPD-treated group.

The non-significant difference in GF levels between both groups at 21 and 30 days could be a reflection of the wound transfer to normal physiologic levels of GF in both groups. At 3 days, PDGF-BB showed significantly higher levels compared with the initial day 1 GF level in the MIS group. Such an enhanced initial PDGF level could be a factor that favor more cell chemoattraction in the MIS compared with the OFD treated group..

The present study showed significant clinical improvement in probing depth reduction and clinical attachment level gain after treatment with MIS as compared with OFD. This finding suggests that the higher GF level of the MIS-treated group compared with the OFD-treated group could be clinically relevant. It also supports previous findings of more enhanced clinical outcomes associated with the use of MIS, either alone or with other regenerative therapies (Cortellini *et al.,* 1995; Cortellini *et al.,* 1999; Zucchelli *et al.,* 2003; Trombelli *et al.,* 2005). Intrabony defect changes appeared to differ insignificantly between the two groups, which could be attributed to the use of MIS or OFD alone with no supportive regenerative modalities for the selected cases of advanced periodontal destruction.

Conclusions

Within the limits of the present study, since it was based on a small sample size, one can conclude that MIS treatment of periodontal defects could be a modality that significantly contains growth factors within the defect area. Its use was usually associated with initial higher GCF levels of angiogenic growth factors that could improve the clinical outcomes of regenerative surgery. These increased GF levels correlated with improved soft-tissue parameters of the periodontal defects. Hard-tissue improvement may require additional regenerative therapy along with the MIS.

Acknowledgement and conflict of interest

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