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ORIGINAL ARTICLE

Clinical and microbiological changes after minimally invasive therapeutic approaches in intrabony defects: a 12-month follow-up

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Abstract
Objectives This 12-month randomized, controlled trial evaluated the clinical effects and microbiological changes of minimally invasive nonsurgical and surgical approaches for the therapy of intrabony defects.
Materials and methods Twenty-nine subjects with intrabony defects in single-rooted tooth were randomly assigned to; (1) minimally invasive nonsurgical technique (MINST) or (2) minimally invasive surgical technique (MIST). Quantities of *Aggregatibacter actinomycetemcomitans*, *Tannerella forsythia*, and *Porphyromonas gingivalis*, determined by using real-time PCR, were evaluated at baseline, 3, 6, and 12 months after the treatments. Clinical recordings—probing depth (PD), position of the gingival margin (PGM), and relative clinical attachment level (RCAL)—were obtained at baseline and 12 months post-therapy. The primary outcome variable of the study was RCAL.
Results Both treatment modalities resulted in an improvement in all clinical recordings, with significant PD reductions ($p<0.05$), RCAL gains ($p<0.05$), and no change in the PGM ($p>0.05$) after 12 months in both MINST and MIST groups. No clinical differences were observed between groups ($p>0.05$). Regarding the microbiological outcomes, at the re-examinations, a significant decrease was observed for *T. forsythia* and *P. gingivalis* when compared with baseline ($p<0.05$) for both treatments. The amount of *A. actinomycetemcomitans* did not reduced decrease throughout the study ($p>0.05$). Intergroup differences in the microbiological assay were not found at any time point ($p>0.05$).
Conclusions Both MINST and MIST provided comparable clinical results and microbiological changes in the treatment of intrabony defects over 12 months follow-up.
Clinical relevance This randomized, controlled, parallel trial revealed that both therapeutic modalities may promote clinical and microbiological benefits at 12 months post-therapy.

Keywords Microbiology · Periodontitis · Microsurgery · Surgical procedures · Minimally invasive · Root planing

Introduction

The presence of one or more pathogenic species in sufficient numbers is required to establish periodontitis. *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Tannerella forsythia* are key pathogens in the initiation and progression of periodontal disease [1]. Thus, the primary objective of periodontal therapy is to reduce the disease-associated pathogens from subgingival biofilm, thus reestablishing periodontal health and colonization by health-compatible microorganisms. However, although several forms of treatment can promote periodontal healing associated with reduction in pathogenic microbiota, some periodontal defects are more challenging in promoting these results. Intrabony lesion, which is related to a higher risk of disease progression and eventually tooth loss [2], is one such condition.

Numerous treatment modalities have been indicated for the therapy of periodontal intrabony defects [3–5]. Among them, nonsurgical debridement and surgical approaches have been performed to resolve periodontal disease in these sites [3, 6, 7]. Nowadays, the trend in therapeutic method in periodontology is adoption of minimally invasive approaches in both surgical and nonsurgical procedures [4, 8–13], producing

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reduced morbidity, a better postoperative period, and higher patient acceptance compared with conventional procedures [12, 14–16].

Studies have demonstrated that minimally invasive nonsurgical subgingival debridement may be effective for achieving satisfactory results in the clinical parameters after therapy of periodontal pockets, whether associated or not with vertical defects [10–13]. The concept of less-invasive procedures has also been surgically employed, mainly in the treatment of intrabony lesions [4, 8, 9, 16–19]. Although these therapeutic modalities have shown successful clinical and patient-related outcomes in the therapy of vertical bony defects [4, 8, 9, 13, 16–19], until this date, no published evidence is available regarding the influence of surgical and nonsurgical procedures, using the principles of minimally invasive approaches, in the microbiological outcomes of treatment of angular lesions. Therefore, the aim of this randomized, controlled trial (RCT) was to compare the 12-month clinical and microbiological performance of minimally invasive surgical and nonsurgical approaches for the therapy of intrabony defects.

Materials and methods

This study was a masked RCT with a parallel design comparing minimally invasive surgical technique (MIST) or minimally invasive nonsurgical technique (MINST) for the therapy of intrabony lesions. The study population and the 6-month clinical and patient-centered outcomes of this RCT have been described in detail previously [13]. All patients included in the 6-month study completed the follow-up at 12 months. The study was approved by the ethics committee of the University of Campinas (Protocol 094/2007). All individuals received a description of the proposed treatment and gave their informed and written consent. Subject recruitment started in June 2008 and was completed in February 2009. The first procedure was carried out in October 2009. All the 12-month follow-up appointments were finalized in December 2010. Data entry of all information and statistical analyses were completed in September 2011.

All individuals were selected from those referred to the Graduate Clinic of the Piracicaba Dental School and received periodontal and radiographic examination. The study inclusion criteria were: diagnosis of chronic periodontitis [20], at least one single-rooted tooth with probing depth (PD) of ≥5 mm with bleeding on probing,clinical attachment level (CAL) at ≥5 mm, radiographic evidence of an isolated intrabony defect with depth of ≥4 mm [4, 9, 13], full-mouth plaque score (FMPS) [21] and full-mouth bleeding score (FMBS) [22] at <20 %, and absence of medical condition that could affect the progression of periodontal disease. Individuals who were pregnant or lactating, required antibiotic premedication, had received antibiotic treatment in the previous 3 months, had received a course of periodontal treatment within the last 6 months, smoked, or whose tooth has presented with signs of mobility and/or traumatic occlusion were excluded from the study.

To compare the depth of the intrabony component between groups, periapical radiographs were taken using the long cone paralleling technique with customized acrylic filmholders. The radiographs were digitized using a scanner at a resolution of 600 dpi. The radiographic measurements of the defects were performed with the assistance of image analysis software, as previously described [13, 18].

All subjects received an initial cause-related therapy by scaling and root planing, motivation sessions, and periodontal supportive therapy. All these procedures were performed by the same operator (MZC). Following 6 months, the subjects who fulfilled the inclusion criteria were incorporated in the study.

A sample size was calculated with a statistical program and included an alpha error of 5 %, 80 % of power value, and standard deviation of 1.0 mm. A difference of 1.0 mm between the groups was considered clinically significant. It was determined that a sample of 12 patients per group would be needed. Considering that some patients might be lost during follow-up, 14 and 15 patients were included in MIST and MINST groups, respectively.

The study employed a blinded examiner (FVR) with a randomized and parallel design. All patients in the study were recruited before the beginning of the randomization to therapeutic approaches. Treatment group assignment was carried out immediately before the beginning of the procedure (MIST or MINST) by a different operator (MAGP) than that responsible for the clinical procedure (MZC) and different from the examiner (FVR).

Operating microscope[1] and microsurgical instruments were employed in all procedures, which were performed by the same operator. The treatment was randomized, and the procedures for specific different groups were chosen:

MINST group Sites designated to receive nonsurgical treatment were carefully scaled and root planed with mini-curettes[2] and an ultrasonic device[3] with specific tips.[4]

MIST group Sites were accessed by the MIST [4], and incisions were performed with preservation techniques [23–25]. Only the defect-associated papilla was accessed, with the full-thickness flap being elevated minimally. The granulation soft tissue was dissected with a microblade and carefully removed with mini-curettes.[2] The visible calculus

[1] DF Vasconcelos, São Paulo, SP, Brazil
[2] Gracey, Hu-Friedy, Chicago, IL, USA
[3] Cavitron, Dentsply, Tulsa, OK, USA
[4] UI25KSF10S, Hu-Friedy, Chicago, IL, USA

was carefully removed with mini-curettes[2] and with an ultrasonic device[3] with specific tips.[4] The flaps were repositioned, and a passive internal mattress sutured.[5]

At the end of procedures, all patients received analgesic medication (paracetamol)[6] and were instructed to take the medication every 6 h for 2 days if they experienced pain. All patients were instructed to rinse with 0.12 % chlorhexidine (twice a day for 15 days). In the MIST group, sutures were removed at 10 days postsurgery.

Re-assessment visits occurred every 15 days during the first month and monthly until the 12th month. At the end of the appointment, supragingival prophylaxis was performed.

Clinical parameters

This study shows the clinical parameters evaluated at baseline and 12-month follow-up visit. The following clinical measurements were performed using an individually manufactured acrylic stent and a PCP-15 periodontal Probe[7] at six sites per tooth: PGM, measured from the stent to the gingival margin, and relative CAL (RCAL), from the stent to the bottom of the periodontal pocket. PD was calculated by deducting PGM from RCAL. FMPS and FMBS were measured calculating the percentage of sites that revealed the presence of plaque or bleeding.

The same examiner who was masked with respect to experimental procedures carried out all measurements of clinical evaluation. To perform the intra-examiner calibration, 12 non-study subjects presenting intrabony defects were selected. The designated examiner measured the RCAL, the primary outcome variable, of all patients twice within 24 h. The examiner was judged to be reproducible after fulfilling the predetermined success criteria (the percentage of agreement within ±1 mm between repeated measurements had to be at least 90 %). The intraclass correlation resulted in 96 % reproducibility.

Microbiological evaluation

Sample collection to PCR analyses were performed at baseline and at the 3-, 6- and 12-month re-evaluations. Following a removal of the supragingival biofilm, the areas corresponding to intrabony defects were washed with water spray, isolated with cotton rolls, and dried. A sterile paper point[8] was inserted into the bottom of the periodontal pocket for 30 s. The paper points were placed in sterile tubes containing 300 μL of Tris–EDTA 0.1 mM and immediately stored at −20 °C. One examiner (FVR) collected all microbial samples.

Microbiological assay was performed as described previously [26, 27]. Briefly, bacterial DNA was extracted from subgingival biofilm. Reaction efficiency was optimized, and final primer concentrations of 0.5 mM for Pg and Aa and 0.3 mM for Tf were chosen.

Real-time PCR was performed with a PCR master mix kit.[9] For each run, water was used as the negative control. Primers and reactions templates were the same as previously described [26, 27]. Absolute quantification of target bacteria in clinical samples was performed using Pg (American Type Culture Collection (ATCC) 33277), Tf (ATCC 43037), and Aa (JP2) as controls. Standard curves were used to convert cycle threshold scores into the number of bacterial cells, using controls with known amounts of bacteria-specific DNA. The level of detection was set at 103 bacteria/plaque sample for all target bacteria.

Data management and statistical analysis

SAS 9.01 program was used. The primary outcome measurement of the study was RCAL. Secondary outcomes included (1) PD, (2) PGM, (3) FMPS and FMBS, and (4) microbiological outcomes. ANOVA and Tukey were used to detect intra- and intergroup differences in microbiological data and clinical parameters (PGM, PD, and RCAL). The Wilcoxon test was used to detect intragroup differences, and Mann–Whitney *U* test was used to detect intergroup differences in FMPS and FMBS. An experimental level of significance was determined at 5 %.

Results

In all, 987 subjects were assessed for eligibility. Among them, 938 individuals did not meet the inclusion criteria and thus were excluded. Forty-nine patients were submitted to initial therapy and maintained in periodontal supportive therapy. After 6 months, 28 patients were recruited at the beginning of the study. The participants were randomly assigned and received the allocated procedure. Two patients presenting one intrabony defect each were lost later during follow-up due to the administration of antibiotic medication for medical reasons or due to address change. The rest of the 27 subjects were included in the statistical analyses.

Patients' characteristics at baseline

The characteristics of the intrabony defects and patient sample that completed the study are summarized in Table 1. Statistical analysis revealed no differences between the experimental groups at the baseline examination for all parameters evaluated ($p>0.05$) (Table 1).

[5] 6.0 polygalactin-A; Vicryl, Johnson & Johnson, São Paulo, SP, Brazil
[6] Tylenol®—750 mg, Janssen-Cilag Farmacêutica Ltda, São Paulo, SP, Brazil
[7] Hu Friedy do Brasil, Rio de Janeiro, RJ, Brazil
[8] Dentsply, Ribeirão Preto, SP, Brazil
[9] SYBR Green kit, Roche Diagnostic Co., Indianapolis, IN, USA

Table 1 Patient and defect characteristics for MIST and MINST groups at baseline

	MIST	MINST
Number	14	13
Age (years)	45.43±6.79	45.31±7.57
Gender (% female)	57.14	69.23
FMPS (%)	16.20±6.05	13.64±5.57
FMBS (%)	9.62±5.46	9.37±3.59
PD (mm)	7.07±1.13	6.35±0.92
RCAL (mm)	10.73±1.56	11.25±2.11
Depth of the intrabony component (mm)	4.33±1.98	4.52±1.63

No significant intergroup differences were observed at baseline ($p<0.05$)

FMPS full-mouth plaque score, *FMBS* full-mouth bleeding score, *PD* probing depth, *RCAL* relative clinical attachment level

Clinical parameters

FMPS and FMBS remained significantly lower than 20 % over 12 months follow-up showing optimal compliance with clinical procedures. No statistically significant differences were observed between groups at evaluated periods ($p>0.05$) (Table 2).

The values of the clinical variables PGM, PD, and RCAL are shown in Table 3. Distribution of PD and RCAL values at each site of MIST and MINST group at baseline and 12 months after therapy are demonstrated in Fig. 1. Regarding PGM, no significant differences were observed after 12 months in either MIST or MINST group compared with baseline measurements ($p>0.05$). Additionally, intergroup analysis did not show any statistically significant differences in the PGM ($p>0.05$). Intragroup analysis demonstrated statistically significant PD reductions at 12-month post-therapy from baseline ($p<0.05$) in both therapies. No significant differences between MIST and MINST groups were observed in this parameter after 12 months ($p>0.05$). Regarding RCAL, intragroup analysis demonstrated significant improvement in this measurement following both therapies ($p<0.05$). No significant intergroup differences have detected between therapies regarding RCAL ($p>0.05$).

Figures 2 and 3 represent the clinical and radiographic pre- and postoperative images of each therapeutic group.

Table 2 Percentages (means±SD) of FMPS and FMBS at the different assessment times

Parameter	Group	Baseline	12 months
FMPS	MIST	16.20±6.05 a	12.43±4.20 b
	MINST	13.64±5.57 a	12.08±2.73 b
FMBS	MIST	9.62±5.46 a	7.84±3.74 b
	MINST	9.37±3.59 a	5.97±2.25 b

Means followed by different letters in a line represent significant intragroup differences by Wilcoxon test ($p<0.05$). No significant intergroup differences were observed by Mann–Whitney *U* test ($p<0.05$)

SD standard deviation, *FMPS* full-mouth plaque score, *FMBS* full-mouth bleeding score

Microbiological assays

The results for all investigated species are summarized in Table 4. Real-time PCR analysis revealed no significant intergroup differences in the number of *A. actinomycetemcomitans*, *P. gingivalis*, and *T. forsythia* at any point in time ($p>0.05$). No significant differences in the quantities of *A. actinomycetemcomitans* were observed following 3, 6, and 12 months in either experimental group compared with baseline measurements ($p>0.05$). Regarding amounts of *P. gingivalis*, statistically significant reductions were achieved after 3-, 6- and 12-month evaluations from baseline ($p<0.05$) in both MIST and MINST groups. In the MIST group, the *T. forsythia* log concentration was statistically reduced at 3 and 12 months ($p<0.05$), and in the MINST group, this reduction was observed at all periods post-therapy ($p<0.05$).

Discussion

The role of microorganisms in the development of periodontal diseases has been the subject of innumerous studies in periodontology. Although the oral cavity is colonized by a variety of pathogens, *P. gingivalis*, *A. actinomycetemcomitans*, and *T. forsythia* have been strongly associated with the progression of periodontitis [28, 29]. With this concept in mind and considering that the management of periodontal intrabony defects represents a relevant challenge in periodontics, the microbiological effects after therapeutic approaches employed to treat these lesions is important. To date, no information was available concerning the impact of minimally invasive non- and surgical therapies on the amounts of periodontal pathogens in sites presenting angular bony lesions. Thus, the present prospective RCT with 12 month's follow-up was designed to compare the clinical and microbiological outcomes associated with two treatment modalities, MIST or MINST, for intrabony defect-associated pockets. In general, the findings of this investigation revealed that both approaches promoted similar benefits in terms of pathogen reduction and clinical parameters 12 months after therapies. It is important to note

Table 3 Means (±SD) of PGM, PD, and RCAL (in millimeters) at baseline and 12 months

Parameter	Group	Baseline	12 months	0–12 months difference
PGM	MIST	3.74±1.09 a	4.32±1.34 a	0.59±0.60
	MINST	4.96±1.66 a	5.55±1.30 a	0.58±0.83
PD	MIST	7.07±1.13 a	3.57±0.76 b	3.50±0.87
	MINST	6.35±0.92 a	3.15±0.66 b	3.19±0.71
RCAL	MIST	10.73±1.56 a	7.93±1.52 b	2.80±1.14
	MINST	11.25±2.11 a	8.67±1.79 b	2.58±1.13

Means followed by different letters in a line represent significant intragroup differences by ANOVA/Tukey ($p<0.05$). No significant intergroup differences were observed by ANOVA ($p<0.05$)

SD standard deviation, *PGM* position of the gingival margin, *PD* probing depth, *RCAL* relative clinical attachment level

that the present study was the first to analyze the microbiological outcomes following minimally invasive procedures at angular lesions, using quantitative analyses (real-time PCR) to determine the actual impact of these treatment protocols on the bacterial load.

Regarding *A. actinomycetemcomitans* amounts, microbiological assays of the current investigation demonstrated no reduction in the levels of these bacteria throughout 12 months following the therapy in either group. The presence of *A. actinomycetemcomitans* is commonly associated with sites with periodontal disease [30–32], including sites presenting intrabony defects [33], and the control of this bacteria in periodontal pockets remains a challenge.

An earlier study, Del Peloso Ribeiro et al. [26], agreed with this finding, as it also did not achieve a significant decrease in *A. actinomycetemcomitans* levels when mechanical debridement alone was applied. The difficulty of controlling the amounts of *A. actinomycetemcomitans* with this mechanical periodontal therapy is in line with other investigations that evaluated the effect of nonsurgical therapy on the levels of key pathogens associated with periodontal disease [37, 38]. In fact, only mechanical periodontal treatment was not effective in reducing the presence of *A. actinomycetemcomitans* once this pathogen invaded the soft tissues and periodontal cells [35, 36], requiring the use of antimicrobial agents to significantly decrease the levels of this species. Indeed, other studies observed that when antimicrobials were associated with mechanical therapy, *A. actinomycetemcomitans* amounts were significantly reduced [34].

Moreover, it should be stated that although *A. actinomycetemcomitans* is a recognized periodontal pathogen, its frequency on periodontal pockets of chronic periodontitis patients is low, ranging from 30 to 45 % of moderate and deep pockets. Its presence is strongly enrolled in aggressive periodontitis ethiopathogenesis and less in chronic disease [27–39].

Concerning the red complex species—*P. gingivalis* and *T. forsythia*—both therapies evaluated in the present study promoted reductions in the levels of these microorganisms from baseline to 3 months, and these reductions were maintained throughout 12 months. These findings are in line with other investigations that point out that major changes in counts of subgingival species after mechanical periodontal treatment, with or without antimicrobials, were more pronounced in the first 3 months after therapy [26, 40–42]. Since these

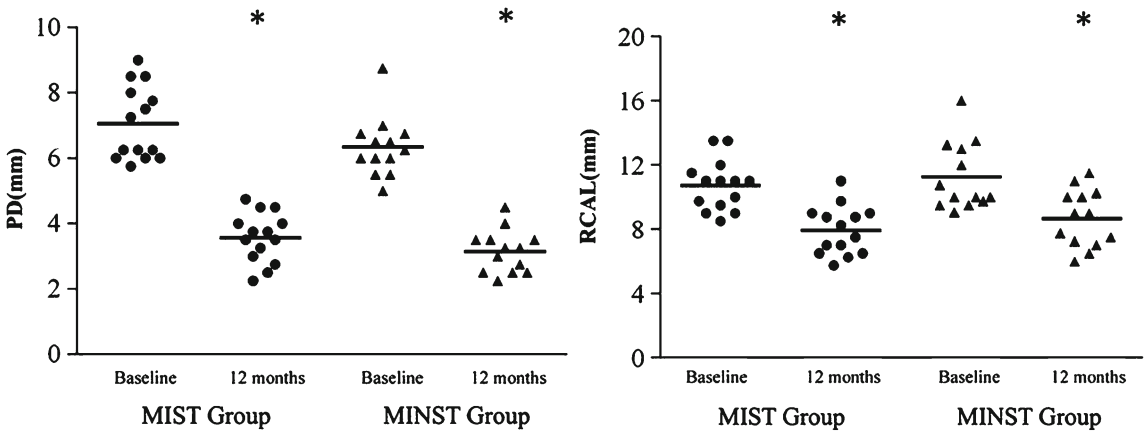

Fig. 1 Distribution of PD and RCAL values from sites of MIST and MINST groups at baseline and 12 months after therapy. The *horizontal bars* show the mean values. The *individual symbols* represent the PD and RCAL values at each site. *$p<0.05$, intragroup differences by ANOVA/Tukey. No significant intergroup differences were observed by ANOVA ($p<0.05$)

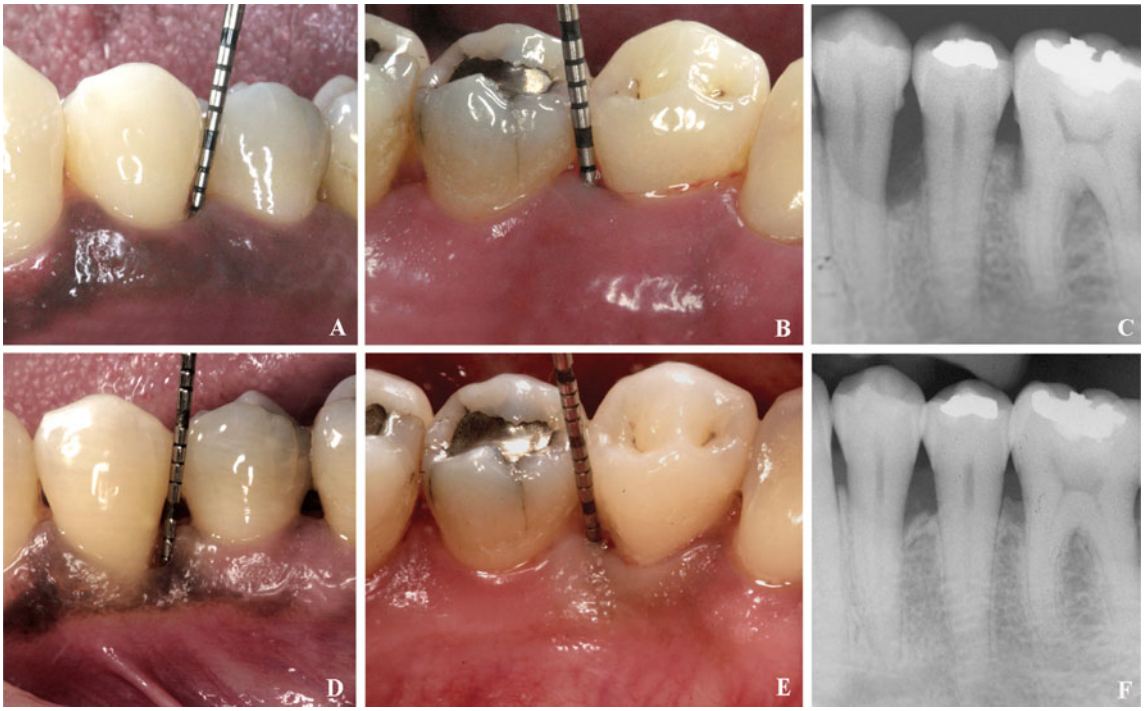

Fig. 2 Minimally invasive nonsurgical technique: preoperative clinical (**a**, **b**) and radiographic view (**c**) of intrabony defect on the distal aspect of pre molar. Clinical (**d**, **e**) and radiographic (**f**) aspect at 12 months after therapy

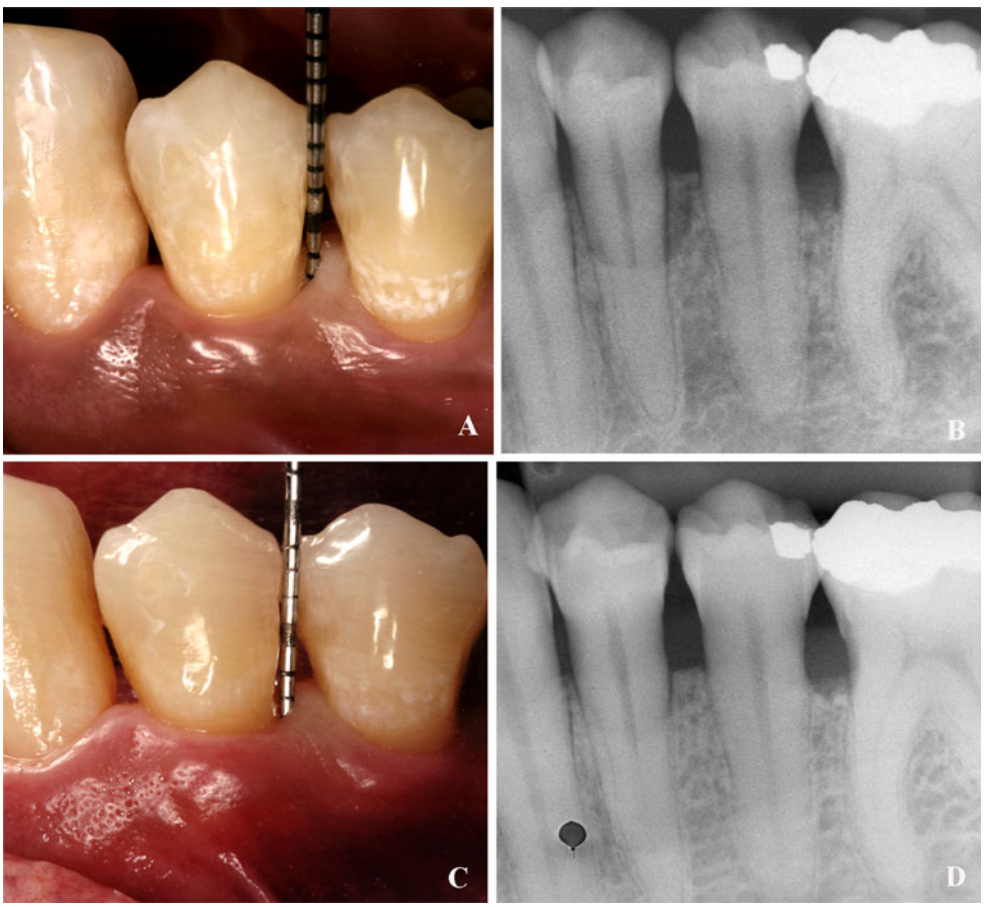

Fig. 3 Minimally invasive surgical technique: preoperative clinical (**a**) and radiographic view (**b**) of intrabony defect on the distal aspect of pre molar. Clinical (**c**) and radiographic (**d**) aspect at 12 months follow-up

Table 4 Amounts (log_{10}±SEM) of *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Tannerella forsythia* at baseline, 3, 6, and 12 months

Bacteria	Group	Baseline	3 months	6 months	12 months
Aa	MIST	1.97±1.77 a	0.76±1.33 a	1.73±1.77 a	0.81±0.98 a
	MINST	1.35±2.11 a	0.63±1.47 a	1.59±1.44 a	1.60±1.90 a
Pg	MIST	2.31±2.88 a	0.56±1.37 b	0.00±0.00 b	0.58±1.41 b
	MINST	2.46±3.07 a	0.26±0.93 b	0.31±1.10 b	0.28±1.00 b
Tf	MIST	4.30±3.03 a	2.73±3.08 b	4.17±2.92 a, b	2.21±2.98 b
	MINST	4.47±3.13 a	3.19±3.14 b	3.25±3.15 b	3.15±3.05 b

Means followed by different letters in the line represent significant intragroup differences by ANOVA/Tukey ($p<0.05$). No significant intergroup differences were observed by ANOVA ($p<0.05$)

periodontal microorganisms are confined to the pocket area, they can be markedly suppressed by thorough mechanical periodontal therapy [37]. These data agree with previous investigations that demonstrated the positive impact of subgingival debridement in significantly decreasing the levels of pathogenic species such as *P. gingivalis* and *T. forsythia*, which in combination with resulted in PD reduction [26–46].

It is important to remember that complete eradication of periodontal pathogens following therapy does not commonly occurs, as also was observed in the present study, and it is not necessary since successful periodontal treatment should lead to a shift in proportions or levels from a pathogenic to a host-compatible periodontal microbiota that should be sustained over time [47, 48].

Clinical measurements of the present study showed a significant mean reduction in PD of 3.50 mm and a mean CAL gain of 2.80 mm for the MIST group, whereas for the MINST group, the respective numbers were 3.19 and 2.58 mm, without differences between therapies. Accordingly, evidence has demonstrated that both nonsurgical and surgical treatments may lead to comparable clinical responses in terms of periodontal health [49, 50]. Nevertheless, data are lacking comparing the effects of nonsurgical and surgical debridement by using minimally invasive techniques, as was carried out in this investigation, especially in treating intrabony lesions, which are considered risk sites for periodontal disease and tooth loss [2]. The motivation for evaluating less-invasive approaches includes decline of trans- and postoperative patient morbidity and maintenance of the initial gingival architecture, which favor comfort and aesthetics [14, 16].

Essentially, the response following periodontal therapies is dependent on the baseline PD [49–51]. Cobb [51] summarized the outcomes of scaling and root planing based on initial PD and showed that in PD corresponding to 4–6 mm, a decrease in the probing of 0.7–1.25 mm and a gain in CAL of 0.25–0.80 mm can be expected, whereas at pockets around 7 mm or higher, the changes may be superior, with a reduction in PD of 1.2–2.9 mm on average and a gain in CAL of 0.5–1.6 mm. Systematic reviewers have also indicated that in initially deep pockets with PD higher than 6 or 7 mm, there was a greater CAL gain and PD reduction following surgical and nonsurgical therapies [49, 50, 52]. The initial means of PD in the present trial were 7.07 and 6.35 mm for MIST and MINST groups, respectively, which may explain, at least in part, the significant PD reductions and CAL gains observed from baseline in the intrabony defects treated by both therapeutic approaches. Besides the greatest baseline PD, two- or three-walled intrabony defects may encourage a better likelihood of reducing PD and gaining clinical attachment [53].

When evaluating the clinical data of this 12-month RCT, it is essential to note that the promising outcomes in terms of CAL gain and PD reduction could be credited by the strict supportive periodontal therapy performed at intervals of 1 month. Previous investigations are in line with this supposition, showing that surgical and nonsurgical therapies may improve CAL gain and PD reduction, even in association with periodontal osseous defects, whether stringent and adequate professional support and acceptable plaque control were maintained [54, 55].

Changes in the gingival recession represent one of the main complaints by patients following subgingival interventions, particularly in areas with high aesthetic demands. Minimally invasive approaches, mainly when surgical procedures are performed, may reduce the risk for gingival recession, favoring patients' satisfaction in terms of aesthetics after the therapy [13]. In the present study, it was observed that after 12 months, no significant changes in the position of gingival margin were observed in either group compared with baseline measurements. Indeed, no differences were noted between groups for PGM alteration (0.59 and 0.58 mm of gingival recession for MIST and MINST groups, respectively). From the aesthetic point of view, the outcomes in the current study in terms of PGM may be considered an enthusiastic success since earlier trials that performed surgical therapies in treating intrabony lesions demonstrated increases in gingival recession of about 2 mm [56–59].

Indeed, as a result of nonsurgical debridement for periodontal sites with moderate or deep probing pocket depth at baseline, the mean values for gingival recession vary between 1.2 and 1.9 mm [60–62]. This highly successful outcome was probably related to the use of less-invasive procedures designed to conserve the integrity of the soft tissues, maintain the stability of the gingival margin, and preserve the blood supply, especially at the anterior segments of the mouth where sites are aesthetically relevant [63, 64]. It is important to note that the absence of changes in gingival margin observed in the present study was not associated with maintenance of periodontal pockets. Instead, it was related to increases in attachment levels and decreases in PD, which may have favored the reduction and, subsequently, the maintenance of low levels of red complex pathogens at 12-month post-therapy.

Overall, considering that this is the first investigation to determine levels of periodontal pathogens at intrabony lesions after minimally invasive procedures, it is difficult to compare the outcomes obtained in this trial and other studies, particularly since it considered the technique for microbial sampling and analysis. Heitz-Mayfield et al. [42] assessed microbial colonization of the intrabony defect-associated pocket using a DNA-DNA checkerboard analysis following surgical or regenerative treatment. Twelve months after the therapies, the authors revealed the presence of high loads of periodontal pathogen in the sites associated with intrabony defects. It was also verified that the presence of these bacteria, especially those of red complex, had a negative impact on the 1-year outcome of surgical/regenerative treatment. Contradictorily, the present study demonstrated positive outcomes in terms of CAL gain and PD reduction in previously infected intrabony defects. This may be attributed to the patients' enrollment in a maintenance program and a meticulous post-therapy regimen to control the dental biofilm. Furthermore, this difference in results may be related to patient factors, such as smoking, and defect characteristics. Finally, the periodontal therapeutic technique used may be suggested as another critical factor that may cause variations in the outcomes.

Although a minimally invasive procedure can be accomplished by utilizing magnification with an endoscopic visualization or operative microscope, as performed in the present trial, the type of magnification does not define the procedure as minimally invasive [14]. Rather, the maintenance of the preoperative position of the gingival margin, a minimal wound, and gentle handling of the tissues are the defining aspects in classifying a technique as minimally invasive [14]. Indeed, it may be assumed that periodontal procedures based on minimally invasive approaches may improve the predictability of the therapies, provide cosmetic outcomes, and contribute to a higher patient comfort level than do traditional periodontal surgical techniques [4, 8, 9, 13]. It is important to highlight that the use of an operative microscope presents limitations, especially for providing visualization of apical portions of defect sites during nonsurgical therapy. Furthermore, this technology is expensive and requires professional ability to execute the procedures.

The current investigation supports the advantages associated with minimally invasive techniques, demonstrating that both nonsurgical and surgical approaches based on this concept may promote successful 1-year clinical effects accompanied by similar microbiological outcomes. Among the improvements observed in this study following both therapies, the reduction of pocket depth may drastically change the prognosis of the affected teeth since this parameter has been associated with long-term tooth survival [65].

Interestingly, this trial showed that minimally invasive nonsurgically performed scaling and root planing achieved very satisfactory outcomes since higher improvement on the attachment level and PD were obtained compare with data from previous research that verified the effect of nonsurgical scaling and root planing in treating intrabony lesions [7, 66, 67]. These aspects support earlier studies that have identified nonsurgical subgingival debridement as an effective therapy to achieve and maintain periodontal health, even in treating vertical defects-related sites [6, 7, 51].

In addition, some favorable aspects have been associated with this therapeutic modality compared with surgical procedures. First, approaches involving surgical procedures are frequently more expensive, and they require considerably more chair-time compared with the nonsurgical treatment [13]. Moreover, Konig et al. [68] revealed that in residual pockets initially treated by subgingival scaling, repeated nonsurgical scaling can reduce the need for periodontal surgery, thus supporting this approach. However, it is difficult to establish whether and how all these factors could influence decisions about treatment of intrabony defects. Evidently, the professional's experience in executing one technique over another and the patient's profile need to be considered in choosing a surgical versus nonsurgical approach.

Importantly, data from earlier longitudinal investigations with monitoring of 5 years or more suggested that failure sites demanding repeated therapy or new treatment were more commonly observed in periodontal deep pockets treated by nonsurgical therapy than those treated surgically [3, 6]. Thus, further investigations with longer follow-ups are required to confirm whether the outcomes promoted by these treatments may be sustained over time.

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Conflict of interest The authors report no conflicts of interest related to this study.

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