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A novel flapless approach versus minimally invasive surgery in periodontal regeneration with enamel matrix derivative proteins: a 24-month randomized controlled clinical trial.

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Abstract

Objectives: This investigation was designed to compare the effectiveness of enamel matrix derivative (EMD) proteins in combination with flapless or flap procedure in periodontal regeneration of deep intrabony defects.

Materials and Methods: Thirty chronic periodontitis patients who had at least one residual periodontal defect with an intrabony component of ≥3 mm were consecutively enrolled. Defects were randomly assigned to test or control treatments which both consisted of the use of EMD to reach periodontal regeneration. Test sites (n = 15) were treated according to a novel flapless approach, whereas control sites (n = 15) by means of minimally invasive surgery (MIST). Clinical and radiographic parameters were recorded at baseline, 12 and 24 months postoperatively.

Results: Both therapeutic modalities yielded similar probing depth (PD) reduction and clinical attachment level (CAL) gain at 24 months. In Flapless-treated sites, a mean PD reduction of 3.6 ± 1.0 mm and a CAL gain of 3.2 ± 1.1 mm were observed. In the MIST group they were 3.7 ± 0.6 mm and 3.6 ± 0.9 mm. The operative chair-time was twice as long in the MIST compared to the flapless group, whereas comparable patient-oriented outcomes were observed.

Conclusion: The flapless procedure may be successfully applied in the regenerative treatment of deep intrabony defects reaching clinical outcomes comparable with those of minimally invasive surgical approaches and may present important advantages in terms of reduction of operative chair-time.

Clinical relevance: The use of EMD as an adjunct to non-surgical periodontal treatment may be considered a suitable option to treat defects mainly in the anterior sextants.

Keywords: biological factors; enamel matrix proteins; minimally invasive surgical procedures; periodontal debridement; periodontal pocket; regenerative medicine.

Introduction

In the last years, enamel matrix derivative (EMD) proteins have received great attention as a possible tool to enhance periodontal regeneration [1,2]. Local application of EMD in intrabony periodontal defects has been shown to result in clinical improvements in terms of clinical attachment gain and pocket reduction, greater than the open flap debridement alone and comparable to other more technically demanding regenerative procedures, such as guided tissue regeneration [3-5]. Moreover, findings from histological studies performed in animals and humans have provided evidence for periodontal regeneration following EMD treatment [6-10].

In certain clinical situations, however, the regenerative potential of EMD appears to be limited by the space-making potential of the material due to its gel-like consistency that may not provide sufficient soft tissue/flap support mainly in non-contained periodontal defects [11,12]. Space provision and clot stability during the early healing phase are key elements for successful and predictable regeneration in intrabony defects [13-15]. The blood clot stability prevents the apical migration of the epithelial cells during the first days of healing and the fibrin clot contains growth factors involved in the periodontal regenerative process [16,17]. Thus, EMD-based regenerative procedures with minimally invasive papilla preservation flaps may provide better clinical results by enhancing blood clot stability while achieving and maintaining primary soft tissue closure [11,18-22]

In the last years, due to the clinical widening of magnification systems and to the availability of micro-surgical devices, the minimally invasive approach has been applied even to the non-surgical periodontal treatment [23]. A recent study reported that minimally invasive non-surgical and surgical approaches were equally effective in the treatment of deep intrabony defects [24].

The application of agents able to promote periodontal regeneration following scaling and root planing (SRP) performed using advanced and minimally invasive technology may further improve clinical and radiographic outcomes in deep intrabony defects. Periodontal tissues regeneration was observed in periodontal pockets treated by EMD and SRP [25]. Based on the biological properties of EMD and hypothesizing that the flapless approach should optimize clot stability it is possible that this novel procedure could lead to clinical improvement comparable to

minimally invasive surgery.

The aim of the present investigation was to compare radiographic and clinical effectiveness of EMD combined with flapless procedure and minimally invasive surgery (MIST) in the treatment of deep intrabony defects.

Material and Methods

Experimental Design

This trial was designed as a single-center, randomized-controlled, parallel group study of 24 months duration. All the experimental sites were treated with the application of EMD (Emdogain, Institute Straumann, Basel, Switzerland). The test sites received the regenerative material at a completion of a closed surgical periodontal treatment (flapless procedure). In the control sites, EMD was applied to the debrided root surfaces accessed with a MIST procedure. A single intrabony defect was treated in each subject.

The study protocol was in accordance with the declaration of Helsinki and was approved by the Institutional Ethics Committee (no. 0108140). All patients gave written consent prior to the beginning of the study. The first procedure was carried out in May 2013. All 24-month follow-up visits were completed in September 2015. All data were entered and statistical analyses were performed at the completion of the 24-month study visits.

Study Population Screening

Periodontitis patients undergoing treatment at the Section of Periodontology, C.I.R. Dental School, Department of Surgical Sciences, University of Turin, (Italy) were consecutively screened for inclusion. Study inclusion criteria were as follows: 1) diagnosis of generalized chronic periodontitis [26], 2) non-smoker status, 3) presence of at least one tooth with probing depth (PD) of ≥ 6 mm associated with a radiographic intrabony defect of ≥3 mm 6 months after the completion of non-surgical therapy, and 4) a full-mouth plaque score (FMPS) and full-mouth bleeding score (FMBS) <15% at the time of the experimental procedure. Exclusion criteria included the following: 1) relevant medical disorders that were considered contraindication to periodontal surgery or detrimental to periodontal healing, 2) consumption of drugs known to affect periodontal status, 3) pregnancy and lactation, and 4) third molars, teeth with furcation

involvement or inadequate endodontic treatment and/or prosthetic restoration.

All patients completed etiological periodontal treatment consisting of instructions and motivation to perform home oral hygiene procedures, supra- and sub-gingival SRP by using ultrasonic (Cavitron Select, DENTSPLY, York, USA) and hand instruments (Gracey curets, Hu-Friedy, Chicago, IL). Great attention was made to avoid marginal and interproximal soft tissue damage. Evaluations of compliance to oral home care were performed monthly. Six months after the etiological therapy patients who fulfilled the inclusion criteria were enrolled into the study and baseline measurements were made.

Sample size and randomization

The radiographic bone fill was set as the primary outcome. A sample size of 11 patients per group was calculated to detect a minimum difference of 1.0 mm in intrabony defect depth between test and control treatment procedures at 2-year follow-up with an expected standard deviation (SD) of 0.7 mm, a two-sided alpha error of 0.05 and a power of 80% [18]. For compensation of possible dropouts, 30 individuals were recruited.

After enrollment, each patient was given a number and was randomly assigned to one of the two treatment regimens. A balance random permuted block approach was used to prepare the randomization tables in order to avoid unequal balance between the two treatment groups. To conceal assignment, forms with the treatment modality were put into identical and opaque envelopes with the patient corresponding number on the outside. The sealed envelopes were placed into the custody of a clinician who was not involved in diagnosis or treatment delivery. He opened the envelope just prior to the treatment delivery and informed the clinician which treatment was to be performed.

The examiners who performed the clinical and the radiographic measurements were different from the clinician who provided the treatment and were not involved in the maintenance care. The treatment codes of the study were not available to the clinician and to the examiners until the data were analysed by the statistician.

Experimental treatment of intrabony defects

All procedures were performed by the same experienced clinician (M.A.) by using an operating

microscope (Zeiss S7, Feldbach, Switzerland). Prophylactic antibiotic therapy (amoxicillin and clavulanic acid 2 g) was administered 1 h prior to the flapless/MIST procedure. In presence of a mobility >1, a splinting procedure was performed.

Flapless group. Experimental sites designated to receive closed surgical treatment were submitted to careful debridement with a combined use of minicurets (Hu-Friedy, Chicago, IL) and ultrasonic instruments with thin and delicate tips (UI25KSF10S, Hu-Friedy, Chicago, IL). Teeth were instrumented until no residual calculus could be detected. A visualization of the root surface was accomplished under magnification of 12.5x by using a gingival retractor and a microsurgical dental mirror to gain access to the periodontal pocket (Hu-Friedy, Chiacgo, IL). Caution was taken to avoid soft tissue trauma. The root surface was conditioned for 2 min with 24% EDTA (Prefgel, Institute Straumann, Basel Switzerland) and thoroughly rinsed with saline solution. EMD was immediately applied on the dried root surface. Great care was taken to preserve the stability of soft tissues with a gentle compression of the gingival margin by means of sterile wetting gauzes until pocket marginal closure was attained.

MIS group. Defects were accessed either with the single flap approach (SFA) [27] or the modified minimally invasive surgical technique (M-MIST) [11]. Vertical-releasing incisions were avoided, and the full-thickness flap was minimally raised. Granulation tissue was removed from the defects and the root thoroughly scaled using minicurets and ultrasonic device with specific tips. The root surfaces were conditioned by a 2-min treatment with EDTA gel and carefully rinsed with sterile saline. Finally, EMD was applied on the dried root surfaces. The flaps were repositioned, and passive internal mattress sutures (Gore-tex, WL Gore & Associated, Flagstaff, AZ, USA) were used to obtain primary closure.

Post-Therapy and Maintenance Care

All patients received antibiotics (amoxicillin and clavulanic acid 1g to be taken 12 h after the flapless/MIST procedure), analgesic medication (ibuprofen 600 mg, every 8 hours for 3 days, only if they experienced pain), and 0.12% chlorhexidine digluconate mouthrinse for 1 min (3 times /day for 4 weeks). In the MIST group, sutures were removed after 10 to 14 days post-surgery. During the postoperative period, patients were prescribed to avoid toothbrushing and

flossing in the treated area for the first 2 weeks. After that, they were instructed to use modified oral hygiene procedures with a soft toothbrush and to perform supragingival interdental cleansing. After 4 weeks, subjects discontinued chlorexhidine mouthrinse and resumed conventional hygiene practices with medium toothbrush and interdental devices. Strict recall appointments for both groups were scheduled weekly during the first month postoperatively, every 2 months during the first year and every 3 months for the remainder of the observational period. The recall appointments consisted of reinforcement of oral hygiene measures, polishing, full-mouth SRP and occlusal adjustment when needed.

Clinical and radiographic measurements

Clinical measurements at experimental sites were taken at baseline (1 week before treatment) and at the 12- and 24-month follow-up visits by the same experienced examiner (F.F.). To perform the intra-examiner calibration, 10 non-study patients presenting with intrabony defects were evaluated by the examiner on two separate occasions within 48 h. The examiner was judged to be reproducible after fulfilling the predetermined success criteria (the percentage of agreement within 1 mm between repeated measurements of PD and CAL had to be ≥ 90%). The intra-class correlation coefficient was calculated as resulting in > 94% reproducibility.

Measurements were taken at the deepest point of the selected defects by using a manual 1-mm graduated periodontal probe (PCP-UNC 15, Hu-Friedy, Chicago, IL) and were rounded up to the nearest millimeter. The following clinical parameters were assessed: presence/absence of bacterial plaque (PI), presence/absence of bleeding on probing (BoP), PD, gingival recession (REC), and clinical attachment level (CAL). The FMPS and FMBS were calculated as the percentage of gingival units (six sites per tooth) that revealed the presence of plaque or bleeding.

Periapical radiographs were taken at baseline, 12 and 24 months postoperatively using the long-cone paralleling technique. An individual customized film holder (RINN XCP Film Holding Instruments, DENTSPLY, York, USA) was fabricated for each patient to allow reproducible positioning during subsequent radiographs. The radiographs were digitized and analyzed using the Image J software, an imaging software package developed by the Polytechnic of the University of Turin [28]. The radiographic reference points were the cemento-enamel junction

(CEJ), the bone crest (BC) level, and the bottom of the bony defect (BD), where the periodontal ligament space was considered as having a normal width [29]. The CEJ position was identified according to Schei et al. [29]. The intrabony defect depth (IBD) was measured as the distance between BC and BD. The baseline defect angle (BDA) between the tooth axis and the wall of the intrabony defect was calculated and expressed in degrees. The radiographic bone fill was calculated by subtracting the IBD measurement recorded at the 12- or 24-month postoperative examination from the baseline IBD. The differences in measurements that existed between the baseline, 12 and 24 months were corrected for distortion.

All measurements were performed by the same blinded investigator (G.M.M.) after an intra-examiner calibration previously done by examining 12 non-study related radiographs twice between 24 hours. The intraclass correlation demonstrated 97% reproducibility for the IBD and 93% for the BDA.

Experimental procedure and patient outcomes

Chair time was measured with a chronograph, starting at the delivery of local anesthesia through the completion of the flapless/MIST procedure. At the end of the experimental procedure patients received a 10-cm horizontal visual analog scale (VAS) to record the discomfort/pain experienced during therapy and during the first postoperative week [30]. The anchors for each end of the scales were designated as none and extreme. In addition, root hypersensitivity, interference with daily activities, and adverse events were recorded by the examiner during the post-operative first month follow-up visits.

Statistical analysis

The primary outcome measurement of the study was the radiographic bone fill. Secondary outcome measurements included 1) residual PD, 2) CAL gain, 3) position of the gingival margin, and 4) patient-centered outcomes. Data were expressed as mean ± standard deviation. No data points were missing. The statistical unit was the patient.

To test whether the data were normally distributed the Kolmogorov-Smirnov and Shapiro-Wilk tests were done. The homogeneity of groups at baseline was tested using the unpaired *t*-test (PD, CAL, BDA) and the Mann-Whitney *U* test (FMBS, FMPS, REC, IBD). Repeated-measures

ANOVA and the Friedman's test were used to detect intragroup differences in clinical and radiographic parameters over time. Multiple comparisons were conducted with the post-hoc tests (Newman-Keuls test and Dunn test). Subsequently, intergroup differences in PD and CAL were statistically explored using the unpaired student t-test and differences in FMPS, FMBS, REC and radiographic parameters with the Mann-Whitney *U* test. The Bonferroni correction was applied for multiple comparisons. The Mann-Whitney *U* test was used to evaluate VAS questionnaires regarding patient perceptions and satisfaction. An experimental level of significance was determined at 5% for all statistical analyses. Statistical analyses were conducted using commercially available software (SAS version 9.2, SAS Institute, Inc, Cary, NC).

Results

Figure 1 summarizes the flow chart of the study. Briefly, 52 individuals were assessed for eligibility. A total of 14 were excluded because of not meeting inclusion criteria. Thirty-eight patients were submitted to the cause-related therapy. After 6 months, 30 patients were enrolled in the study and randomly assigned to the test or control experimental procedures. All 30 participants (15 (flapless) and 15 (MIST)) received the allocated procedure and were included in the statistical analyses.

Patient characteristics at baseline were not significantly different ($p > 0.05$) between groups (Table 1). The distributions of intrabony defects according to teeth were as follows: 26.7% incisive, 26.7% premolar, and 46.6% molar for the flapless group and 33.3% incisive, 26.7% premolar, and 40% molar, for the MIST group. As reported in Table 2, no statistically significant difference was detected for any of the baseline defect characteristics between test and control defect sites.

Post-operative course and patient-reported outcomes

Data concerning chair time and patient-reported outcomes are described in Table 3. A statistically higher chair time was observed in the MIST group than in the flapless group (54.9 ± 7.1 min versus 23.5 ± 2.8 min, $p < 0.001$).

Primary closure was obtained and maintained in all the control sites. During the first postoperative month, no healing complications occurred in both the experimental groups. No edema, hematoma or suppuration was noted in any of the treated sites. Based on a horizontal

VAS, it was observed that the degree of discomfort/pain perceived by the patients during therapy was very discreet and statistically similar between groups *(p* >0.05). Pain-related VAS values collected during the first postoperative week were higher in the test group compared to the control one but the difference was not statistically significant ($p > 0.05$).

Clinical and radiographic outcomes

All patients in both groups attended all supportive periodontal visits. As reported in Table 4, FMPS and FMBS remained below 15% throughout the study, and no statistically significant differences were observed between groups at any time point. Both therapies led to a statistically significant decrease in mean PD and CAL at 12 and 24 months compared to baseline ($p < 0.001$). The greatest reduction occurred during the first 12 months after treatment, whereas no further significant changes were observed within the treatment groups between 12 and 24 months ($p > 0.05$). After 24 months the mean PD reduction and the mean clinical attachment gain amounted to 3.6 ± 1.0 mm and 3.2 ± 1.1 mm, respectively, in the test group and to 3.7 ± 0.6 mm and 3.6 ± 0.9 mm, respectively, in the control group. The differences between treatment modalities were not statistically significant at any assessment time.

The frequency distributions of residual PDs and CAL changes at 12 and 24 months are summarized in Table 5. At 12-month follow-up visit , residual PDs of 4-5 mm were observed at a frequency of 66.67% in flapless-treated sites and 53.33% at MIST sites. No pockets with PD of ≥ 6 mm were observed in either group. A CAL gain ≥ 3 mm was measured in 60 and 80% of test and control defects, respectively. At 24 months the percentage of residual PDs 4-5 mm decreased to 60% in the flapless group and to 40% in the MIST group. The percentage of sites that yielded CAL gain ≥ 3 mm increased to 86.67% at sites treated with MIST compared to 66.67% at flapless-treated sites. However, when considering only test sites located in anterior region all experienced complete pocket closure (PD ≤3 mm).

A slight but not statistically significant increase in REC values was observed after 12 and 24 months in the test group compared to baseline ($p > 0.05$). However, differences between experimental groups did not reach statistical significance

With regards to the radiographic defect fill, in the test group the mean IBD reduction at 12 and 24

months when compared to baseline was 2.1 ± 1.5 mm ($p = 0.003$) and 2.6 ± 1.6 mm ($p = 0.001$). In the control group, it amounted to 3.4 ± 1.4 mm ($p < 0.001$) and 3.8 ± 1.3 mm ($p < 0.001$) at the same time points. The defect fill was statistically significantly higher in the MIST when compared to the flapless group at either 12 or 24 months ($p \leq 0.002$). When molar teeth were excluded from the analysis a comparable defect fill was obtained in test and control group (3.5 ± 1.1 mm versus 3.7 ± 1.3 mm). In Figs. 2 and 3, two cases are presented to illustrate magnitudes of clinical and radiographic changes observed in test and control groups.

Discussion

Although previous studies demonstrated positive clinical outcomes after the application of EMD in combination to minimally invasive surgical techniques [18-21,31], as far as we know, this is the first randomized controlled clinical trial comparing the effectiveness of EMD combined with flapless procedure or MIST in the regenerative treatment of deep intrabony defects.

The results of the present study show that both treatment modalities may lead to substantial clinical improvements which were maintained over a period of 24 months. Previous systematic reviews reported that in initially deep pockets (PD >6 mm) surgical therapy resulted in greater attachment level gain and PD reduction than non-surgical treatment [32,33]. In this study, in which baseline PD measurements were 7.5 ± 0.9 mm and 7.3 ± 0.8 mm for flapless and MIST groups, respectively, clinical evaluations demonstrated that similar mean PD reductions and CAL gains were obtained in both groups at the 12- and 24-month evaluations. The current findings are consistent with those by Ribeiro et al. [24] who observed a mean PD reduction of 3.5 ± 0.9 mm and a mean CAL gain of 2.9 ± 1.2 mm at MIST sites compared to 3.1 ± 0.7 and 2.6 ± 1.1 mm, respectively, at control sites treated by a minimally invasive non-surgical approach. However, they did not apply EMD in the intrabony defects and reported only 6-month clinical data. No radiographic assessment was carried out.

In this regard, it is worth noting that in the present trial the MIST group experienced a statistically significant greater radiographic defect fill compared to the flapless group. One aspect that could be considered when comparing clinical and radiographic data was the different healing response in anterior and posterior sextants. Because of the limited number of patients enrolled in this study,

defects were not limited to any specific site, and no stratification was made with regard to defect location. However, when looking at test sites located on anterior teeth all experienced complete pocket closure and showed bone gain values comparable to the MIST-treated sites. The extent of radiographic bone fill was lower on molar teeth in which the flapless approach was less predictable. However, present data are in line with those of a recent meta-analysis that reported a weighted bone gain of 2.34 ± 0.17 mm when using EMD in a surgical approach [3]. Of note, taking into consideration the 24-month results, additional improvements in radiographic bone fill were observed in both the flapless and MIST groups with respect to 12-month time point. Similarly, Heijl et al. [34] observed radiographically a bone gain of 0.9 mm in EMD-treated defects after 8 months that increased to 2.2 mm after 16 months and to 2.6 mm after 3 years. These changes may suggest that when using EMD additional bone regeneration may occur along with the longitudinal assessment.

The results of the present trial differ markedly from previous studies in which EMD was used in conjunction with conventional non-surgical technique. Gutierrez et al. reported a mean PD reduction of 2.0 ± 0.3 mm and a CAL gain of 1.4 ± 0.3 mm at 3-month follow-up [35]. Sculean et al. [36] and Mombelli et al. [37] presented mean values of 2.0 ± 0.7 mm and 0.5 mm for CAL gain at 6 months and 12 months, respectively. In addition, in these studies EMD application following a single SRP session did not achieve any significant added benefit when compared to SRP alone. No radiological data were reported.

It is possible to hypothesize that the greater PD reduction and CAL gain from the present data may be related to the enhanced bacterial decontamination of the root surface. This is a relevant aspect in periodontal regeneration. As widely demonstrated, traditional SRP rarely eliminates all subgingival plaque and calculus and decreased effectiveness has been associated with increasing PD [38]. Periodontal pockets with 4-6 mm PD still had 15-38% of the root surface covered with deposits and those deeper than 6 mm 19-66% [39]. Additionally, traditional SRP is limited by the operator's inability to detect residual calculus accurately by visualization [40] or tactile sensation [41]. Previous studies demostrated that even with surgical access complete calculus removal is uncommon [42,43]. In agreement with Ribeiro et al. [24] and Nibali et al. [44] the use of high-

level magnification systems combined with coaxial lighting may have improved calculus detection and removal. Inherent difficulties exist when incorporating the operating microscope in the non-surgical therapy. Root anatomy and tooth position in the dental arch influence the degree of visualization of the root surface. In order to facilitate better access to the subgingival structures we employed commercially available devices but they have not been appositely developed to retract gingival tissues. This has impaired visual acuity on molar teeth. New instruments particularly suited to assist in visualizing root surface are needed.

In vitro, EMD has been shown to induce the synthesis of growth factors and alkaline phosphatase by periodontal ligament cells and gingival fibroblasts, to increase collagen and protein production and to stimulate periodontal ligament cells and osteoblast precursor cells to proliferate [2]. In addition, it has been reported that EMD may also promote periodontal renegeration by reducing dental plaque and by selectively restricting growth of periopathogens [3]. However, it remains unclear to what extent the improvement of the clinical and radiographic parameters following the application of EMD represents regeneration of the lost periodontal structures. Data from literature are limited and controversial. Mellonig et al. demonstrated in humans that the use of EMD in conjunction with non-surgical root planing resulted in new bone, ligament and root cementum formation in 3 of the 4 specimens histologically examined [25]. Conversely, in the study by Sculean et al. 8 out of 10 specimens healed by long junctional epithelium and the remaning 2 presented with histologically insignificant amount of new bone and root cementum [36].

Due to its viscous consistency EMD has been considered inadequate to regenerative procedures in relation to its limited space-maintaining potential mainly in the treatment of non-contained defects [12]. In the present study, this aspect was overcome by preserving the soft tissue walls in the non-surgical therapy and by applying the M-MIST and SFA as regenerative flap management techniques. These newly developed flaps result in higher interproximal areas healing in a closed and stable environment and provide space for fibrin clot formation and stabilization [45]. The PD reduction and CAL gain observed in the current study were more than that obtained with conventional access flap surgery [4] but in line with data on EMD combined with minimally invasive surgery (with and without papilla elevation) that showed PD reduction of 2.6 to 5.2 mm

and CAL gain of 1.8 to 4.9 mm [11,18,20,22,46-49]. When considering only findings from clinical trials comparing EMD application with M-MIST or SFA alone, the observed PD reductions ranged from 4.4 to 4.9 mm and CAL gains from 3.8 to 4.5 mm [11,22,49]. The 1-year radiographic bone gain amounted to 3.3 ± 1.2 mm [48]. Interestingly, these clinical trials reported that the additional use of EMD would not seem to further improve the clinical outcomes with M-MIST or SFA surgical approaches [22,49]. These findings raised the hypothesis on the intrinsic healing potential of an intrabony defect when ideal surgical conditions are provided [45].

The results of present study further support the pivotal role of root decontamination and blood clot stability in the regenerative treatment. These aspects may account for the favourable clinical outcomes following the surgical and flapless approach. The adjunct of EMD may have further enhanced periodontal tissue regeneration.

Decision making about the therapeutic approach in treating residual deep intrabony defects should consider defect location and morphology, the ease in performing one technique over another, the patient's perception of costs and benefits and the skill of the operator. In the anterior sextants the use of operating microscope would allow effective root debridement at probing depths beyond depths where closed SRP is reliable. In the posterior sextants the lack of instruments appositely designed to retract gingival tissues and the less visual acuity may affect the regenerative results. These limitations may be overcome by minimally invasive approaches such as M-MIST or SFA. However, they are applicable only to isolated intrabony defects that allow for an appropriate access to root-surface and defect debridement by preserving the interdental papillae and the supracrestal soft tissues.

Other aspects to be considered are the average surgical chair time which was twice as long in the flapless-treated sites and the patient discomfort. Greater but not statistically significant pain-related VAS values were reported by flapless patients compared to MIST patients in the first postoperative week due to dental hypersensitivity. These data are consistent with previous findings in EMD studies [20,45] and compare favourably with data by Ribeiro et al. [24] on minimally invasive non-surgical therapy. We can also hypothesize that the limited intra- and post-operative morbidity may partly explain the good compliance of both flapless and MIST patients

with scheduled visits for supportive periodontal therapy. Finally, all interventions were performed by a single experienced clinician. The flapless procedure should be regarded as technique sensitive. It requires a careful root debridement to eliminate residual calculus under high magnification. This step is very delicate especially at posterior natural teeth. Indeed, these findings may limit the external generalizability of the present findings.

The present study has some limitations. First, the small sample size that limits the interpretation of the observed regenerative effects in light of the defect morphology and, furthermore, the lack of histological analysis. Although the radiographs indicated a possible bony regeneration of the defects, no definitive statement can be made about the qualities of this tissue. Re-entry surgery would have provided more accurate information, but it was not performed in order to avoid a medically unnecessary second procedure.

Conclusions

The clinical outcomes of regenerative periodontal therapy were similar in test and control group, while the flapless approach presented advantages in terms of reduction of operative chair time. Both treatment modalities yielded comparable radiographic bone fill in anterior sextants. Based on the enhanced biological wound stability, the flapless approach with the aid of appositely developed devices may represent an attractive alternative to the MIST in the regenerative treatment of non-contained intrabony defects mainly on anterior teeth. Further studies with a larger database of patients are needed to validate the present findings and to identify the characteristics of defects that may benefit most from either a minimally invasive surgical or non-surgical regenerative strategy.

Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest.

Funding The authors declare that there were no financial support from any external source regarding the current study.

Ethical approval All procedures involving humans were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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Table 1. Characteristics of study subjects at baseline

Variables	Flapless Group (*n*=15)	MIST Group (*n*=15)	*p* value
Age (years; mean ± SD)	44.3 ± 8.1	42.2 ± 6.1	0.429[a]
Females/males (*n*)	5/10	7/8	0.709[b]
FMPS (%; mean ± SD)	11.9 ± 2.0	10.7 ± 2.4	0.148[c]
FMBS (%; mean ± SD)	8.8 ± 2.9	8.3 ± 2.1	0.592[c]

FMPS Full-Mouth Plaque Score, *FMBS* Full-Mouth Bleeding Score, *SD* standard deviation.
[a]Unpaired *t*-test
[b]Chi-square test
[c]Mann-Withney *U*test

Table 2. Baseline characteristics of intrabony defect sites

Variables	Flapless Group (*n*=15)	MIST Group (*n*=15)	*p* value
PD (mm; mean ± SD)	7.5 ± 0.9	7.3 ± 0.8	0.525[a]
CAL (mm; mean ± SD)	9.4 ± 2.0	9.0 ± 1.7	0.559[a]
Radiographic angle (°; mean ± SD)	37.7 ± 4.0	34.5 ± 6.6	0.120[a]
IBD (mm; mean ± SD)	4.9 ± 1.3	5.2 ± 1.4	0.589[b]

PD probing depth, *CAL* clinical attachment level, *IBD* radiographic intrabony defect depth, *SD* standard deviation.
[a]Unpaired *t*-test
[b]Mann-Withney *U* test

Table 3. Patient-related outcomes

Variables		Flapless		MIST		*p* value Flapless vs MIST
		Mean ± SD	Range	Mean ± SD	Range	
Chair time (minutes)		23.5 ± 2.8	21-30	54.9 ± 7.1	44-69	<0.001[a]
Pain/Discomfort (VAS score)	***Procedure day***	0.9 ± 1.1	1-3	0.6 ± 0.9	1-3	NS[b]
	1 week	1.1 ± 1.7	1-5	0.8 ± 1.1	1-4	NS[b]

VAS units visual analogue scale units (with 0=no pain and 10=unbearable pain), *NS* not statistically significant (P>0.05).
[a]Unpaired *t*-test

[b]Mann-Withney *U* test

Table 4. Changes in clinical and radiographic parameters (mean ± SD) over the 24-month experimental period.

Variables	Group	Baseline	12 months	$\Delta_{0\text{-}12\ months}$	24 months	$\Delta_{0\text{-}24\ months}$
FMPS (%)	**Flapless**	11.9 ± 2.0[a]	11.3 ± 2.1	0.6 ± 2.9	11.5 ± 1.6	0.4 ± 2.7
	MIST	10.7 ± 2.4[a]	11.1 ± 2.1	-0.4 ± 3.8	10.9 ± 1.9	-0.2 ± 3.4
Difference between groups		NS[d]	NS[e]		NS[e]	
FMBS (%)	**Flapless**	8.8 ± 2.8[a]	9.3 ± 2.4	-0.5 ± 2.8	9.5 ± 1.6	-0.7 ± 3.8
	MIST	8.3 ± 2.1[a]	8.9 ± 1.8	-0.6 ± 1.9	8.7 ± 1.8	-0.4 ± 3.1
Difference between groups		NS[d]	NS[e]		NS[e]	
PD (mm)	**Flapless**	7.5 ± 0.9[b]	4.1 ± 1.1	3.4 ± 1.2[c]	3.9 ± 0.9	3.6 ± 1.0[c]
	MIST	7.3 ± 0.8[b]	3.7 ± 0.9	3.6 ± 0.8[c]	3.6 ± 0.9	3.7 ± 0.6[c]
Difference between groups		NS[d]	NS[e]		NS[e]	
CAL (mm)	**Flapless**	9.4 ± 2.0[b]	6.3 ± 2.1	3.1 ± 1.2[c]	6.2 ± 2.3	3.2 ± 1.1[c]
	MIST	9.0 ± 1.7[b]	5.5 ± 1.5	3.5 ± 1.0[c]	5.4 ± 1.6	3.6 ± 0.9[c]
Difference between groups		NS[d]	NS[e]		NS[e]	
REC (mm)	**Flapless**	1.9 ± 1.8[a]	2.2 ± 2.0	-0.3 ± 0.6	2.3 ± 2.6	-0.4 ± 0.7
	MIST	1.7 ± 1.2[a]	1.8 ± 1.1	-0.1 ± 0.3	1.8 ± 1.0	-0.1 ± 0.5
Difference between groups		NS[d]	NS[e]		NS[e]	
IBD (mm)	**Flapless**	4.9 ± 1.3[b]	2.8 ± 0.9	2.1 ± 1.5[c]	2.3 ± 0.8	2.6 ± 1.6[c]
	MIST	5.2 ± 1.4[b]	1.8 ± 0.7	3.4 ± 1.4[c]	1.4 ± 0.6	3.8 ± 1.3[c]
Difference between groups		NS[d]	P<0.001[e]		P=0.002[e]	

FMPS Full-Mouth Plaque Score, *FMBS* Full-Mouth Bleeding Score, *PD* probing depth, *CAL* clinical attachment level, *REC* gingival recession, *IBD* radiographic intrabony defect depth *NS* difference between groups is not statistically significant ($p > 0.05$)

[a]$p > 0.05$, p values represent changes among the three time points (ANOVA or Friedman's test)

[b]$p < 0.001$, p values represent changes among the three time points (ANOVA or Friedman's test)

[c]$p \leq 0.001$, p values represent longitudinal changes from baseline (Newman-Keuls test or Dunn test)

[d]Mann-Withney *U* test or unpaired *t*-test

[e]Bonferroni-corrected Mann-Whitney *U* test or Bonferroni-corrected *t*-test

Table 5. Frequency distribution (%) of residual PD and CAL changes at 12 and 24 months.

		RESIDUAL PD (mm)				CAL GAIN (mm)			
		0 to 1	**2 to 3**	**4 to 5**	**≥ 6**	**0 to 1**	**2 to 3**	**4 to 5**	**≥ 6**
12 months	***Flapless* (*n*=15)**	0 % (0)	33.33% (5)	66.67% (10)	0 % (0)	0% (0)	60% (9)	40% (6)	0% (0)
	***MIST* (*n*=15)**	0 % (0)	46.67% (7)	53.33% (8)	0 % (0)	0% (0)	33.33% (5)	66.67% (10)	0% (0)
24 months	***Flapless* (*n*=15)**	0 % (0)	40% (6)	60% (9)	0 % (0)	0% (0)	60% (9)	40% (6)	0% (0)
	***MIST* (*n*=15)**	0 % (0)	60% (9)	40% (6)	0 % (0)	0% (0)	33.33% (5)	60% (9)	6.67% (1)

Figures Legends

Fig. 1 Consort diagram showing the study design

Fig. 2 Test site treated with the flapless approach and enamel matrix derivative (EMD). **a**-**b** Pre-operative clinical and radiographic images of an intra-bony defect on the distal aspect of the mandibular central incisor. **c-d** Two-year clinical and radiographic images.

Fig. 3 Control site treated with modified minimally invasive surgical technique (MIST) and enamel matrix derivative (EMD). **a-b** Pre-operative clinical and radiographic images of a deep intra-bony defect on the distal aspect of the manibular lateral incisor. **c-d** Two-year clinical and radiographic images.

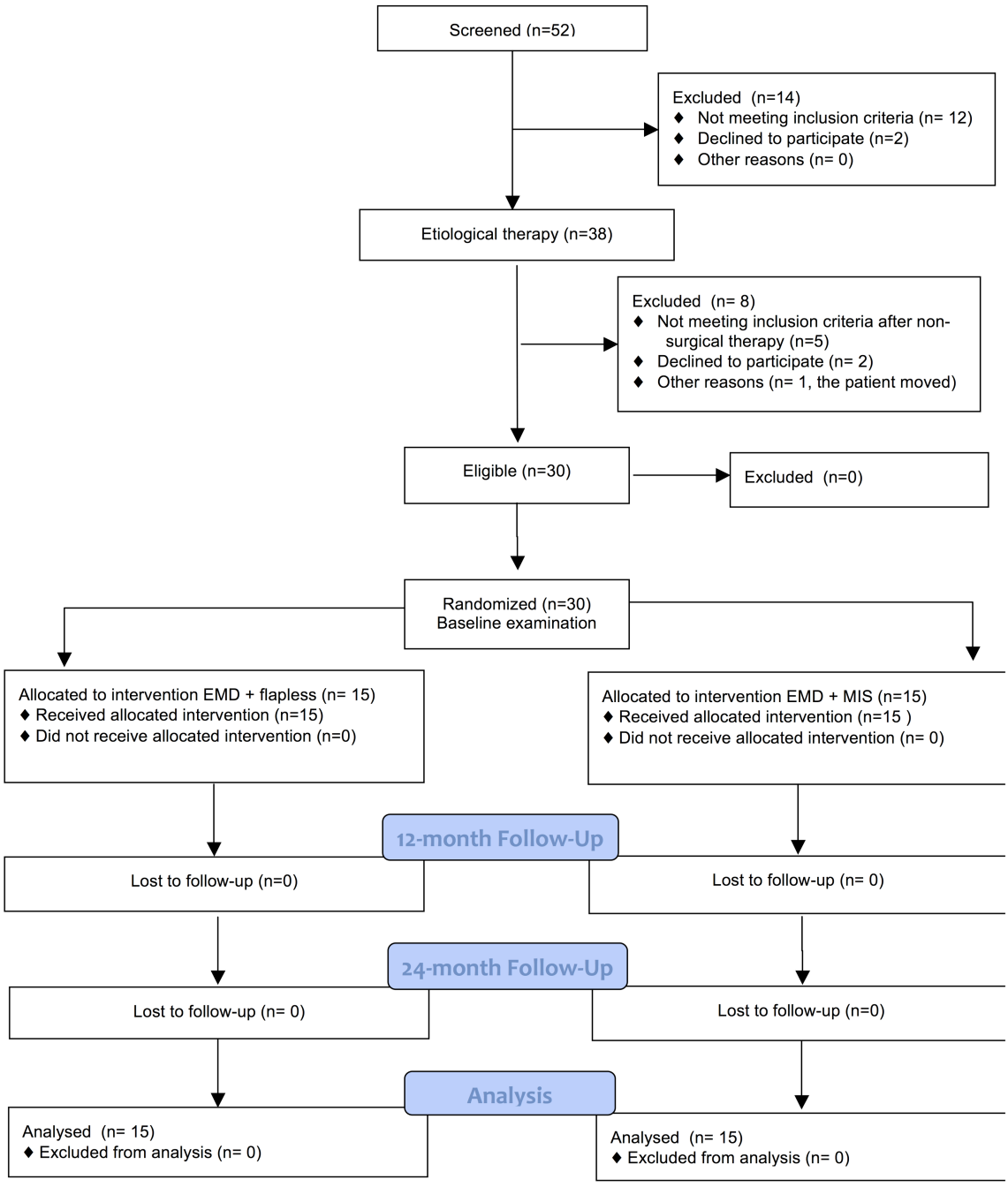
Screened (n=52)
Excluded (n=14)
♦ Not meeting inclusion criteria (n= 12)
♦ Declined to participate (n=2)
♦ Other reasons (n= 0)
Etiological therapy (n=38)
Excluded (n= 8)
♦ Not meeting inclusion criteria after non-surgical therapy (n=5)
♦ Declined to participate (n= 2)
♦ Other reasons (n= 1, the patient moved)
Eligible (n=30)
Excluded (n=0)
Randomized (n=30)
Baseline examination
Allocated to intervention EMD + flapless (n= 15)
♦ Received allocated intervention (n=15)
♦ Did not receive allocated intervention (n=0)
Allocated to intervention EMD + MIS (n=15)
♦ Received allocated intervention (n=15)
♦ Did not receive allocated intervention (n= 0)
12-month Follow-Up
Lost to follow-up (n=0)
Lost to follow-up (n= 0)
24-month Follow-Up
Lost to follow-up (n= 0)
Lost to follow-up (n=0)
Analysis
Analysed (n= 15)
♦ Excluded from analysis (n= 0)
Analysed (n= 15)
♦ Excluded from analysis (n= 0)

Fig. 1

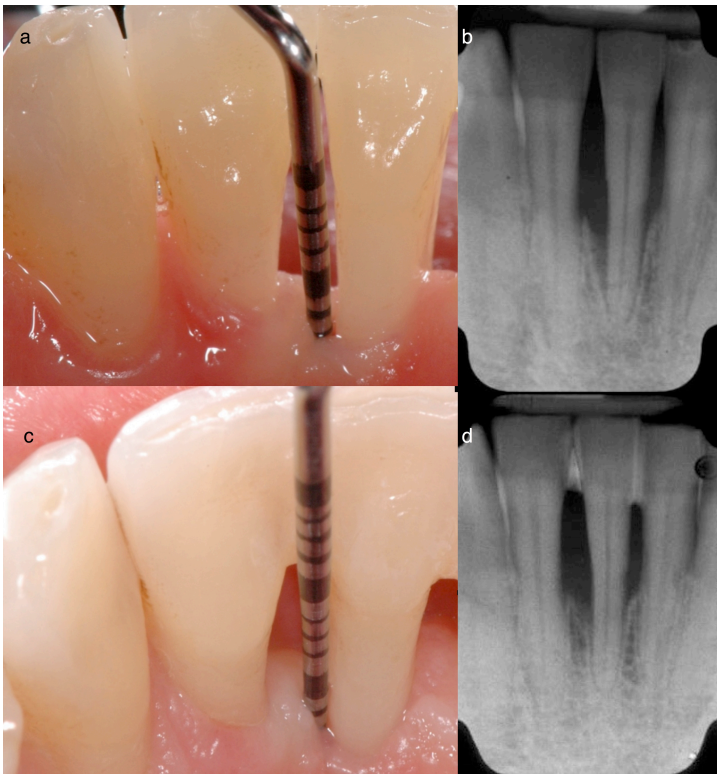

Fig. 2

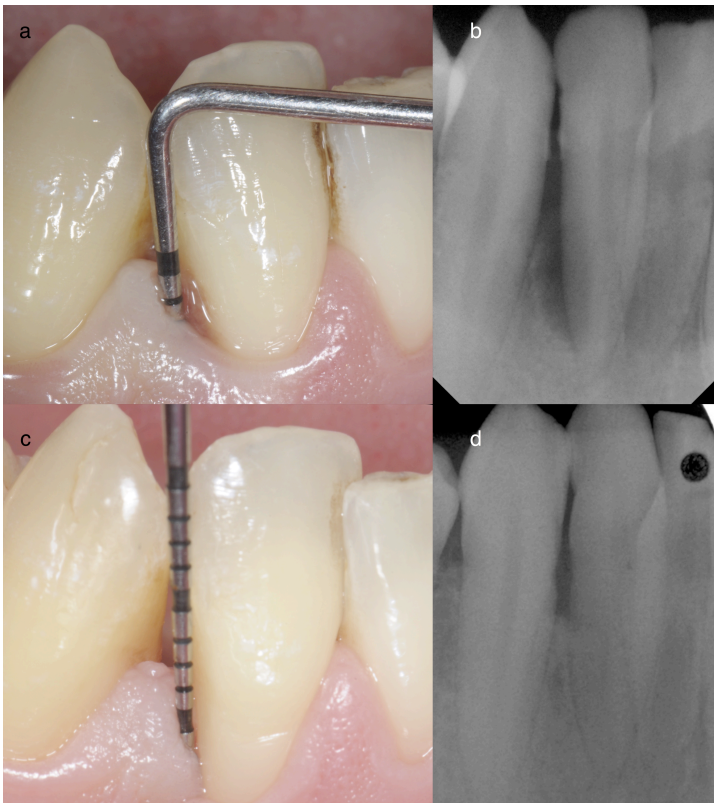

Fig. 3