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ORIGINAL ARTICLE

Efficacy of periodontal minimally invasive surgery with and without regenerative materials for treatment of intrabony defect: a randomized clinical trial

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Abstract

Objectives The minimally invasive surgical technique was modified in suture (MISTms) in this study. The trial was to determine the efficacy of MISTms with and without regenerative materials for the treatment of intrabony defect and to identify factors influencing 1-year clinical attachment level (CAL) gain.

Methods Thirty-six patients with interdental intrabony defects were randomly assigned to MISTms (MISTms alone, 18) or MISTms plus deproteinized bovine bone mineral and collagen membrane (MISTms combined, 18). Wound healing was evaluated by early healing index (EHI) at 1, 2, 3, and 6 weeks. Probing depth (PD), CAL, gingival recession, radiographic defect depth, and distance from the base of defect to the cementoenamel junction were recorded at baseline and 1 year postoperatively. A one-year composite outcome measure based on the combination of CAL gain and post-surgery PD was evaluated. Factors influencing 1-year CAL gain were analyzed.

Results Fifteen patients in MISTms-alone and 16 in the MISTms-combined group finished the study. The MISTms-alone group showed significantly better wound healing at 1 week. CAL significantly gained in the MISTms-alone and MISTms-combined group, with 2.53 ± 1.80 mm and 2.00 ± 1.38 mm respectively. The radiographic bone gain was 3.00 ± 1.56 mm and 3.85 ± 1.69 mm respectively. However, there were no significant differences between the two groups about 1-year outcomes. Lower EHI (optimal wound healing) and more baseline CAL positively influenced 1-year CAL gain.

Conclusions MISTms is an effective treatment for intrabony defects. The regenerative materials do not show an additional effect on 1-year outcomes. Early wound healing and baseline CAL are factors influencing 1-year CAL gain.

Clinical relevance MISTms with and without regenerative materials are both effective treatments for intrabony defect.

Trial registration ClinicalTrials.gov Identifier: ChiCTR2100043272

Keywords Minimally invasive surgical technique · Periodontal regeneration · Bone graft(s) · Guided tissue regeneration

Introduction

Periodontitis is initiated by the dysbiosis of the polymicrobial community. The subgingival dysbiosis triggers deleterious inflammatory, which destroys tooth-supporting structures and leads to tooth loss [1]. The disease progression could be arrested by removing and controlling subgingival biofilms [2]. Regeneration of tooth-supporting tissues could obtain in some vertical defects. Barrier membrane, bone grafting, and their combination are usually applied in periodontal regenerative therapy. The efficacy of the combination of collagen membrane and deproteinized bovine bone mineral (DBBM) has been confirmed by several studies in the past few decades. With the combination treatment, new attachment formed on periodontitis-involved root surface [3, 4], and significantly greater clinical attachment level (CAL) gain was achieved than with conventional access flap alone [5, 6].

Flap design was important for periodontal regenerative surgery. It was continually improved by clinicians [7, 8].

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To minimize the size of flaps, reduce trauma, and preserve blood supply, minimally invasive surgery was introduced into periodontal treatment in 1995 [9]. The papilla preservation technique, developed in 1995 and 1999, reduced wound failure rates in the interdental area from 50–100 to 30% [10, 11]. The wound failure rates decreased to < 10% when microscopes and microsurgical instruments were used [12, 13]. Based on these findings, the minimally invasive surgical technique (MIST) was proposed in 2007 for the treatment of isolated intrabony defects. The main principles of MIST were the use of minimized flap, papilla preservation technique, and modified internal mattress suture to achieve no-tension wound closure [14, 15]. In the present study, MIST was modified in suturing technique, and the modified surgical method was named MISTms for short.

It was reported that minimally invasive surgery alone and in combination with regenerative materials both yield favorable outcomes in periodontal treatment [16–18]. However, whether regenerative materials have additional benefits in minimally invasive surgery is not clear now. Ribeiro et al. compared the efficacy of MIST alone and MIST combined with EMD. No significant difference between alone and combined groups was found [17]. Differently, Wachtel et al. reported that clinical results in the combination of microsurgical access flap with the EMD group were superior to that in microsurgical access flap alone [19, 20]. The effect of several other regenerative materials in minimally invasive surgery was evaluated. The materials included hydroxyapatite + collagen membrane, DBBM + EMD, platelet-derived growth factor, and platelet-rich fibrin. Adjunctive effects of these materials were not found in minimally invasive surgery [21–24]. The combination of DBBM and collagen membrane was reported to be effective in the treatment of intrabony defect by conventional regenerative surgery as mentioned above. It has been widely applied in the clinic. However, when minimally invasive surgery was used, whether collagen membrane + DBBM have additional efficacy is still not known and needed to explore. Therefore, the primary objective of this study was to determine the efficacy of MISTms with and without DBBM + collagen membrane in the treatment of periodontal intrabony defects.

The factors associated with outcomes of minimally invasive surgery have been explored in several studies [25–27]. Defect morphology and the bleeding tendency were reported influencing 1-year CAL gain with MIST + EMD [25]. Non-supportive anatomy, frequent plaque, and complication were the risk factors for treatment failure with MIST/modified-MIST + collagen-enriched bovine-derived xenograft [26]. The early healing index (EHI) was evaluated, but no association with 6-month CAL gain was found [27]. The secondary objective of this study was to identify the factors influencing outcomes of MISTms.

Materials and methods

Study design and setting

This randomized controlled, single-blind, clinical trial with a 1:1 allocation ratio was conducted at the Department of Periodontology, Peking University School, and Hospital of Stomatology. This study was prepared according to CONSORT 2010 (Consolidated Standards of Reporting Trial Statement). Prior to patient screening, the study protocol and informed consent temple were approved by Peking University School and Hospital of Stomatology (PKUSSIRB-2012080) in November 2012, in accordance with the Helsinki Declaration of 1975 as revised in 2000. The study was carried out from December 2012 to April 2018. At enrollment, all included participants signed the informed consent. Additionally, the study was registered at www.chictr.org.cn (ChiCTR2100043272).

Study population

From December 2012, patients were screened after completion of 2–3 months of initial therapy, including oral hygiene instruction, scaling and root planning, and occlusal therapy if indicated. They were eligible for inclusion if (1) they were diagnosed with chronic or aggressive periodontitis with good oral hygiene [28]; (2) they were between the ages of 18 and 65 years and in good general health; (3) they had an interdental defect in a single-rooted tooth or in the mesial aspect of a first molar; (4) the tooth was either vital or had received proper root canal therapy; (5) probing depth (PD) and attachment loss were ≥ 5 mm and intrabony defect was ≥ 3 mm; and (6) distance between root apex and defect base was ≥ 3 mm on radiographs. The exclusion criteria were (1) use of medications affecting periodontal status; (2) pregnancy or lactation; (3) smoking more than 10 cigarettes per day; (4) participation in another study within the past 30 days; (5) allergy to regenerative materials; or (6) grade III mobility or class III furcation involvement of the tooth [29].

Sample size calculations

A difference of 1.0 mm in CAL gain between the two groups was assumed to be clinically and statistically significant [17, 30]. The SD was 1.0 mm, based on the CAL gain of 20 patients previously treated with access flap + collagen membrane + DBBM in our center. A sample of 12 patients per group would be sufficient to detect a difference of 1 mm in CAL gain with type 1 error (α) of

0.05 at 80% power. Taking into account the loss of follow-up, 18 patients per group were enrolled.

Randomization and blinding

At enrollment, after written informed consent was obtained, each patient was allotted a code number. The investigator not involved in clinical procedures (JL) performed simple randomization using computer-generated lists. The randomization scheme was kept by another investigator (WL) alone. Then, the patients were randomly assigned to receive either MISTms alone (MISTms-alone group, $n = 18$) or MISTms + DBBM + collagen membrane (MISTms-combined group, $n = 18$). The treatment assignment was disclosed to the periodontal surgeon (XO) only after surgical debridement. The clinical examiner (JK) and radiographic investigator (SZ) remained blinded to the treatment assignment throughout the study.

Clinical parameters

Clinical parameters at baseline and 1 year after surgery were recorded by a trained examiner (JK) using a manual periodontal probe (PCP-UNC-15; Hu-Friedy, Chicago, IL, USA). The following parameters were recorded: (1) plaque index (PLI) [31]; (2) bleeding index (BI) [32]; (3) furcation involvement (FI) [29]; (4) PD, measured from the bottom of the pocket to the gingival margin; (5) CAL, measured from the bottom of the pocket to the cementoenamel junction (CEJ); and (6) gingival recession (GR), measured from the CEJ to the gingival margin. PD, CAL, and GR were measured at the deepest location at each site.

To perform the intraexaminer calibration, 10 non-study patients were evaluated by the examiner on two separate occasions within 48 h. The kappa value for PD and CAL were both > 0.9, indicating good reproducibility.

Composite outcome measure (COM)

At 1 year after surgery, patients were evaluated by composite outcome measure (COM) proposed by Trombelli et al. [33]. The examiner (JK) performed the evaluation. According to 1-year clinical outcomes, the patients were allocated into one of the following categories: (1) CAL gain ≥ 3 mm and post-surgery PD ≤ 4 mm (treatment success); (2) CAL gain ≥ 3 mm and post-surgery PD > 4 mm; (3) CAL gain < 3 mm and post-surgery PD ≤ 4 mm; and (4) CAL gain < 3 mm and post-surgery PD > 4 mm (treatment failure).

Radiographic parameters

Intraoral periapical radiographs of the study sites were obtained at baseline and 1 year after surgery, using the long-cone paralleling technique. At both time points, individualized bite blocks were used to obtain reproducible films. The investigator (SZ) evaluated all radiographs, using image-analysis software (Digimizer@4). The following parameters were measured: (1) radiographic angle, i.e., the angle between the root surface (line from the base of defect (BD) to CEJ) to the defect surface (line from BD to the lateral margin of the defect) (expressed in degrees) [34]; (2) the distance (in mm) between CEJ and BD (CEJ-BD(x)); (3) radiographic defect depth (DD(x)). For measurement of DD(x), a line was first drawn to denote the tooth axis (line 1); then, a second line (line 2) was drawn from the alveolar crest (AC), perpendicular to line 1. The distance from the point where line 2 crossed the root surface to the BD was the DD(x) [35].

Intraexaminer reproducibility was assessed by having the examiner perform measurement on 10 non-study radiographs. The intra-class correlation coefficient of CEJ-BD(x) and DD(x) were 0.996 and 0.998 respectively, indicating good reproducibility.

Intrasurgical clinical measurements

After surgical debridement, a UNC-15 periodontal probe was used to measure the distance between CEJ and BD (CEJ-BD(s)) and the distance between CEJ and AC (CEJ-AC(s)). The total depth of the intrabony component (INFRA) was calculated as the difference between CEJ-BD(s) and CEJ-AC(s). The defects were classified as 1 wall, 2 walls, 3 walls, or combination defects.

Wound healing

Postoperative wound closure in the interproximal area was evaluated at 1, 2, 3, and 6 weeks after surgery. The trained examiner (JK) performed all evaluations. As a secondary outcome, wound healing was graded on a scale of 1 to 5, using the early wound healing index (EHI) [19]; the grading was as follows: 1 = complete flap closure with no fibrin line; 2 = complete flap closure with fine fibrin line; 3 = complete flap closure with fibrin clot; 4 = incomplete flap closure with partial necrosis of tissue; 5 = incomplete flap closure with complete necrosis of tissue.

Surgical procedure

All surgeries were performed under loupe magnification (×3.5) by one periodontal surgeon (XO). The buccal and lingual intracrevicular incisions were designed to conserve as much soft tissue as possible. The incision in the interdental area was made using a simplified papilla preservation technique when the interdental space was ≤ 2-mm wide and a modified papilla preservation technique when it was > 2-mm wide [10, 11]. A mucoperiosteal flap was elevated at both buccal and lingual aspects. The mesio-distal extension of the flap was minimized while the access for intra-surgery debridement was adequate. Root and defect debridement was performed using curettes and ultrasonic instruments. The intrasurgical measurements were made after debridement. In the MISTms-combined group, the defect was filled with DBBM (Bio-Oss®; Geistlich, Wolhusen, Switzerland) and slightly condensed; overfilling was avoided. The graft material was covered with a collagen membrane (Bio-Gide®; Geistli, Wolhusen, Switzerland; Fig. 1). In the MISTms-alone group, regenerative material was omitted (Fig. 2). Wound closure was done by the microsurgical suturing technique, using a 6–0 monofilament polypropylene suture. Notably, the suturing technique was different from that in MIST. A horizontal mattress suture was first placed at the base of the papilla. Then, an interrupted suture was placed between the most coronal portion of the papilla to ensure primary closure of the flap. The teeth with more than grade I mobility were splinted immediately after surgery.

Postoperative care

Postoperatively, patients were advised 0.12% chlorhexidine mouth rinse twice a day. Sutures were removed after 2 weeks. Brushing, flossing, and chewing in the treated area

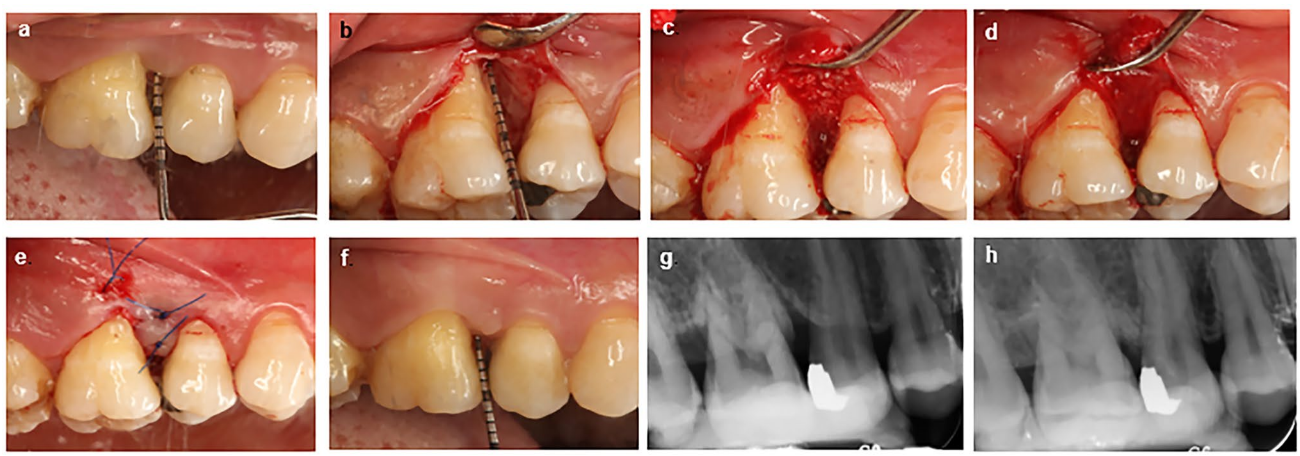

Fig. 1 Intrabony defect mesial to upper right first molar treated with MISTms-combined method. **a** Preoperative probing. **b** Flap elevation and defect debridement. **c** Placement of bovine porous bone mineral. **d** Placement of collagen membrane. **e** Suture. **f** Healing at 1 year after surgery. **g** X-ray at baseline. **h** X-ray at 1 year after surgery

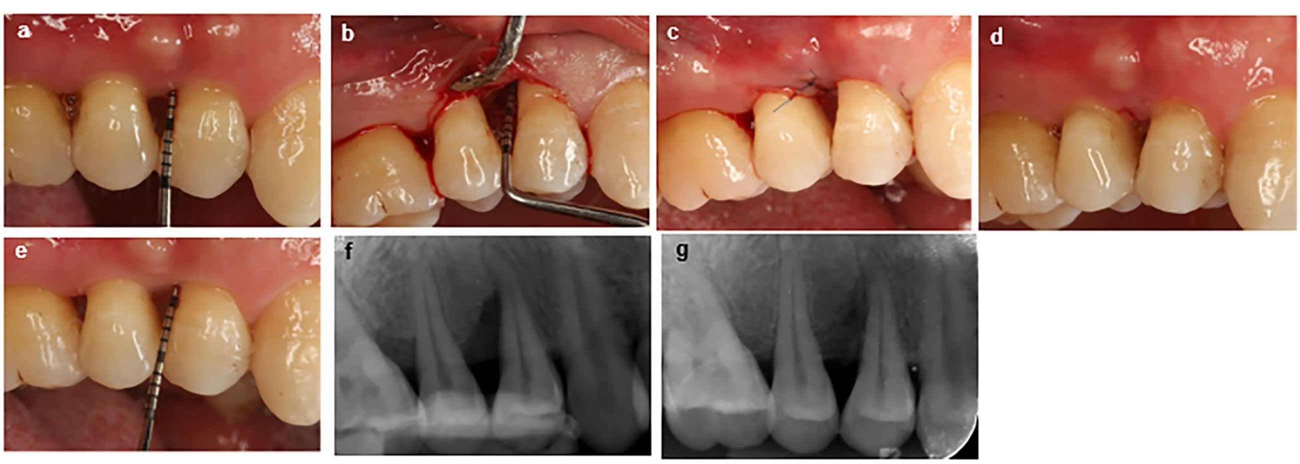

Fig. 2 Intrabony defect distal to upper right first premolar treated with MISTms alone method. **a** Preoperative probing. **b** Flap elevation and defect debridement. **c** Suture. **d** Suture removal at 2 weeks after surgery. **e** Healing at 1 year after surgery. **f** X-ray at baseline. **g** X-ray at 1 year after surgery

were forbidden for 6 weeks. Smokers were requested to minimize their smoking.

All patients requested to return at 1, 2, 3, and 6 weeks after surgery for professional prophylaxis and evaluation of EHI. They then attended a 3-monthly periodontal supportive program, receiving full-mouth professional prophylaxis and calculus removal. Clinical and radiographic parameters were reevaluated 1 year after surgery.

Statistical analysis

Power value was calculated with PASS 15.0 (NASS Corp. USA). Using the sample size and results of this study, the present trial was estimated to have 82% power to detect a difference of 1 mm in CAL gain between two groups.

The patient was the statistical unit for all analyses. Each patient contributed one defect. The normality of the distribution of variables was checked by the Shapiro–Wilk test. Intragroup comparisons between baseline and 1 year after surgery were performed using the paired *t*-test (if normally distributed) or Wilcoxon signed-rank test (if not normally distributed). For intergroup comparisons, an independent *t*-test was performed for normally distributed continuous variables; the Mann–Whitney *U* test was used for non-normally distributed continuous variables and ordinal variables; the Fisher exact test was used for categorical variables.

To identify factors influencing 1-year CAL gain (as a continuous variable), the univariate analysis was first performed. The following parameters were regarded as independent variables: treatment method, smoking status, PLI, BI, FI, mean EHI (mean value of EHI at 1, 2, 3, and 6 weeks after surgery for each patient), baseline CAL, radiographic angle, and defect configuration. Factors influencing CAL gain at $p < 0.05$ were entered into a multivariate linear regression model. Data analysis was performed using SPSS 20 (IBM Corp., Armonk, NY, USA). Statistical significance was at $p < 0.05$).

Results

Study sample

A total of 36 patients between the ages of 24 and 63 years were enrolled. During the 1-year follow-up, three patients in the MISTms-alone group and two in MISTms-combined lost due to moving to other cities or abroad. Thus, for the final analysis, there were 15 patients (6 males, 9 females; mean age, 43.20 ± 9.50 years) in the MISTms-alone group and 16 patients (8 males, 8 females; mean age, 36.63 ± 8.29 years) in the MISTms-combined group (Fig. 3). Patients in the MISTms-combined group were younger than those in the MISTms-alone group. Other baseline characteristics, such as

Fig. 3 Study flowchart

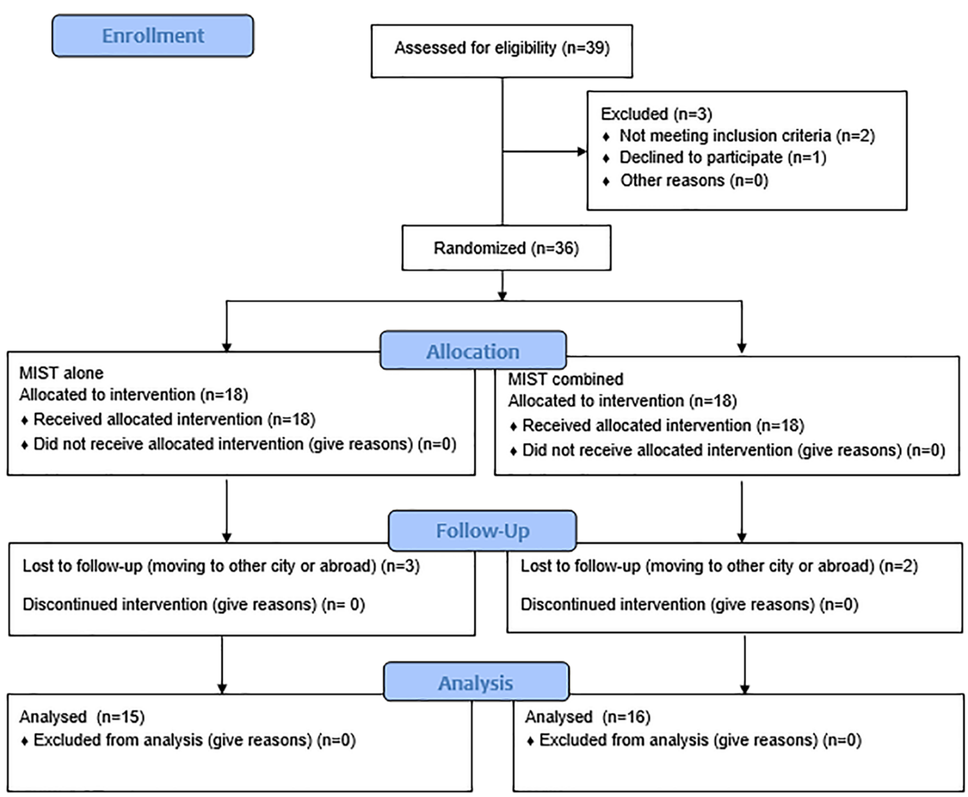

Table 1 Baseline characteristics

Parameters	MISTms-alone group ($n = 15$)	MISTms-combined group ($n = 16$)	p value
Age (years)	43.20 ± 9.50	36.63 ± 8.29	0.049*
Sex (male/female)	6/9	8/8	0.722
Smoking (smoker/nonsmoker)	1/14	2/14	> 0.99
Mandible/maxilla	5/10	6/10	> 0.99
Anterior/posterior	5/10	5/11	> 0.99
PLI	1.40 ± 0.51	1.25 ± 0.58	0.572
BI	2.40 ± 1.06	2.38 ± 0.62	0.892
FI (presence/absence)	3/12	2/14	0.654
CEJ-BD(s) (mm)	9.00 ± 1.36	8.56 ± 1.78	0.450
INFRA (mm)	5.33 ± 1.22	4.72 ± 1.47	0.093
1 wall or 2 walls/3 walls	5/10	3/13	0.433
Radiographic angle (°)	30.24 ± 9.62	31.37 ± 9.22	0.741

*Statistically significant at $p < 0.05$

MISTms, modification of minimally invasive surgical technique in suture; *PLI*, plaque index; *BI*, bleeding index; *CEJ-BD(s)*, intrasurgical distance between cementoenamel junction and base of defect; *INFRA*, total depth of the intrabony component

Table 2 Frequency distribution of EHI

EHI (1/2/3/4)	1 week	2 weeks	3 weeks	6 weeks
MISTms-alone group	6/7/2/0	4/8/3/0	5/7/3/0	9/5/1/0
MISTms-combined group	1/6/7/2	2/7/6/1	2/7/7/0	5/6/5/0
p value	0.006*	0.163	0.129	0.086

*Statistically significant at $p < 0.05$

EHI, early healing index; *MISTms*, modification of minimally invasive surgical technique in a suture

PD, CAL, defect depth, and angle, were comparable between the two groups (Table 1, Table 3).

Wound healing

Table 2 shows the frequency distribution of EHI at 1, 2, 3, and 6 weeks after surgery. At 1 week, wound healing was significantly better in the MISTms-alone group than in the MISTms-combined group. There was complete wound

Table 3 Clinical and radiographic parameters at baseline and 1 year after surgery

Parameter	Group	Baseline	Re-evaluation	Change	p value
PD (mm)	MISTms-alone	6.63 ± 0.83	4.13 ± 0.88	2.50 ± 1.22	< 0.001*
	MISTms-combined	6.63 ± 1.06	4.31 ± 1.50	2.31 ± 1.47	< 0.001*
	p value	NS	NS	NS	
CAL (mm)	MISTms-alone	7.83 ± 1.84	5.30 ± 1.41	2.53 ± 1.80	< 0.001*
	MISTms-combined	7.50 ± 1.61	5.50 ± 2.08	2.00 ± 1.38	< 0.001*
	p value	NS	NS	NS	
GR (mm)	MISTms-alone	1.20 ± 1.79	1.17 ± 1.48	0.03 ± 1.19	0.915
	MISTms-combined	0.88 ± 1.36	1.19 ± 1.31	−0.31 ± 0.93	0.198
	p value	NS	NS	NS	
CEJ-BD(x) (mm)	MISTms-alone	8.12 ± 2.06 (5.03–12.67)	5.11 ± 1.79 (2.91–8.56)	3.00 ± 1.56 (0.76–5.71)	< 0.001*
	MISTms-combined	7.92 ± 1.61 (4.73–10.60)	4.07 ± 1.63 (1.18–7.32)	3.85 ± 1.69 (1.16–8.25)	< 0.001*
	p value	NS	NS	NS	
DD(x) (mm)	MISTms-alone	4.80 ± 0.94 (3.03–6.75)	1.42 ± 1.10 (0.15–4.11)	3.38 ± 1.23 (0.90–5.25)	< 0.001*
	MISTms-combined	4.47 ± 1.52 (3.09–7.81)	1.05 ± 0.60 (0.23–2.62)	3.42 ± 1.49 (0.59–6.53)	< 0.001*
	p value	NS	NS	NS	

*Statistically significant at $p < 0\ .05$; NS, not statistically significant

MISTms, modification of minimally invasive surgical technique in suture; *PD*, probing depth; *CAL*, clinical attachment level; *GR*, gingival recession; *CEJ-BD(x)*, radiographic distance between cementoenamel junction and base of defect; *DD(x)*, radiographic defect depth closure at all sites in the MISTms-alone group, whereas there was partial necrosis of the interproximal tissues (EHI = 4) at 2 of 16 sites in the MISTms-combined group; in both cases, the necrotic tissues were gradually replaced by fibrin clot, and complete closure was achieved at 2 or 3 weeks.

Clinical and radiographic outcomes

At 1 year, both groups presented significant PD reduction, CAL gain, and minor GR (Table 3). Mean reductions in PD were 2.50 ± 1.22 mm and 2.31 ± 1.47 mm in MISTms-alone and MISTms-combined groups, respectively. Mean CAL gains were 2.53 ± 1.80 mm and 2.00 ± 1.38 mm in MISTms-alone and MISTms-combined groups, respectively. Differences between the two groups were not statistically significant.

At 1 year, the radiographic evaluation showed significant reductions in CEJ-BD(x) (i.e., bone gain) and reductions in DD(x) (Table 3). The bone gain was 3.00 ± 1.56 mm in the MISTms-alone group vs. 3.85 ± 1.69 mm in the MISTms-combined group. The reduction of DD(x) was 3.38 ± 1.23 mm in the MISTms-alone group vs. 3.42 ± 1.49 mm in the MISTms-combined group. Differences between the two groups were not statistically significant.

No adverse events were reported throughout the study.

Composite outcome measure (COM)

According to COM, the patients were allocated into four different categories. The distribution of patients in two groups was shown in Table 4. There was no significant difference between the two groups.

Factors influencing 1-year CAL gain

Table 5 shows the factors influencing 1-year CAL gain. Mean EHI and baseline CAL were found to associate with 1-year CAL gain in the multivariate analysis. More baseline CAL resulted in significantly greater 1-year CAL gain (estimate = 0.41). Higher EHI negatively influenced the CAL gain (estimate = −0.91). In other words, lower EHI positively influenced the CAL gain; sites presenting with optimal healing were more likely to obtain greater CAL gain at 1 year.

Table 4 Composite outcome measure (COM) at 1 year

COM	MISTms-alone group ($n = 15$)	MISTms-combined group ($n = 16$)	p value
CAL gain ≥ 3 mm and post-surgery PD ≤ 4 mm	8 (53.33%)	4 (25.00%)	0.093
CAL gain ≥ 3 mm and post-surgery PD > 4 mm	1 (6.67%)	0 (0%)	
CAL gain < 3 mm and post-surgery PD ≤ 4 mm	3 (20.00%)	5 (31.25%)	
CAL gain < 3 mm and post-surgery PD > 4 mm	3 (20.00%)	7 (43.75%)	

COM, composite outcome measure; *MISTms*, modification of minimally invasive surgical technique in suture; *CAL*, clinical attachment level; *PD*, probing depth

Table 5 Univariate and multivariate analysis of CAL gain at 1 year

Variable	Univariate analysis			Multivariate analysis		
	Estimate	SE	*P*	Estimate	SE	*P*
Treatment (combined vs alone)	−0.53	0.57	0.360			
Smoking (smoker vs nonsmoker)	−1.95	0.91	0.042*	−1.24	0.79	0.128
PLI	0.05	0.55	0.931			
BI	0.11	0.35	0.750			
FI (presence vs absence)	−0.55	0.78	0.491			
Mean EHI	−1.06	0.37	0.008*	−0.91	0.34	0.012*
Baseline CAL (mm)	0.40	0.16	0.016*	0.41	0.13	0.004*
Radiographic angle (°)	0.04	0.03	0.211			
Defect configuration (1 or 2 walls vs 3 walls)	−0.77	0.65	0.245			

Multivariate analysis: significance of model $p < 0.05$, adjusted r-square = 0.40. *SE*, standard error; *PLI*, plaque index; *BI*, bleeding index; *FI*, furcation involvement; *EHI*, early healing index; *CAL*, clinical attachment level

Discussion

In the present study, significant attachment gain and bone fill in the intrabony defect were obtained at 1 year in the MISTms-alone group. Normal ligament space was observed between new bone and root surface exposed to the defect previously. The outcomes indicated that new bone and new attachment formed in MISTms alone.

The attachment gain was 2.53 mm and the bone gain was 3.00 mm in the MISTms-alone group in this study. The attachment gain was consistent with that in studies including MIST alone [17–20, 36]. In their studies, the average attachment gain in MIST alone ranged from 1.7 to 2.8 mm. To our knowledge, only one study has reported radiographic outcomes in MIST alone, which resulted in 0.95 mm of bone gain in the intrabony defect [17]. Our result was superior to theirs. The difference between the two studies could be attributed to different baseline levels. This study was presented with a narrower defect at baseline, which positively influenced bone gain in intrabony defect treated by access flap alone [34]. And, different time points of radiographic evaluation might partially explain the different results between the two groups. Besides, we also observed normal ligament space between the new bone and root surface previously exposed to defect. No other study reported the ligament space on radiographs till now. Wider ligament space was always observed when long junctional epithelium healing. Therefore, it was suggested that new attachment may form in MISTms alone in our study.

The traditional methods of periodontal regeneration were challenged by the results of minimally invasive surgery alone. In the traditional view, the source of periodontal regeneration was mainly from the periodontal ligament. It was necessary to use a barrier membrane to block cells originating from the gingiva and also provide space for regeneration. Alternatively, biomaterials that accelerate cell differentiation, such as EMD, could promote periodontal regeneration. Thus, regenerative material was an essential part of regenerative therapy [37]. However, the present study showed that MISTms alone obtained comparable outcomes to the MISTms-combined method. There are several possible explanations for this finding. First, primary healing was achieved in patients receiving MISTms alone, because of the delicate handling of tissue and precise wound closure. The impact of early wound healing on clinical outcome was evident by our 1-year CAL gain which was significantly associated with a lower EHI score. In other words, sites presenting with optimal healing could be expected to show more favorable clinical outcomes. Early exposure to the oral environment leads to bacterial colonization, which negatively affects clinical outcomes [8]. Therefore, early protection by soft tissue is crucial for periodontal regeneration. Second, MISTms improved the stability of blood clots in the interproximal area. Stable adhesion of blood clots to the root surface is a prerequisite for periodontal regeneration. Interference with adhesion or maturation of the blood clot results in the formation of long junctional epithelium in animal studies [38, 39]. Moreover, a stable clot creates a hypoxic environment around the defect, which induces the synthesis of stromal cell-derived factor-1 (SDF-1) [40]. SDF-1 recruits stem cells into the destroyed periodontal tissue and promotes periodontal regeneration [41, 42]. Third, degranulation of platelets in blood clots releases growth factors such as platelet-derived growth factor, transforming growth factor, and basic fibroblast growth factor-2. These growth factors participate in the regulation of proliferation, migration, and differentiation of progenitor cells, which are basic events for tissue regeneration [43, 44]. The role of growth factors in periodontal regeneration has been confirmed in numerous animal studies and clinical trials [45, 46]. Fourth, MISTms preserved interdental soft tissue and, thereby, interdental vascular supply [47]. It is speculated that a sufficient blood supply may provide more stem cells into defect. Several animal studies have shown that stem cells transported via the tail vein could be detected in periodontal bone defects, and the authors concluded that systemic stem cells probably entered periodontal defect via blood flow [48, 49]. Stem cells are not only the origin of endogenous regeneration, but also reduce the inflammatory response and modify wound healing [50–52].

The combination of MISTms with DBBM and collagen membrane also achieved significant attachment gain and bone fill, but not superior to those achieved with MISTms alone. Although the materials possess the capacity of epithelial cell occlusion and space maintenance, they may compensate for the regenerative benefits of MISTms for the following reasons. First, wound healing was poorer when MISTms was combined with DBBM and collagen membrane. Wound dehiscence was found in two sites at 1 week. The materials made no-tension flap closure more difficult and thus impaired wound healing and early protection from soft tissue. Second, the blood supply in our MISTms-combined group might have been influenced by the materials. It was reported that the presence of membrane reduced blood perfusion in mucoperiosteal flap [53]. Therefore, the regenerative benefits of MISTms were compensated by the materials. MISTms and materials did not appear to have a synergistic effect.

In this study, multivariable analysis showed that EHI significantly influenced 1-year CAL gain. Differently, there was no association between 2-week EHI and 6-month CAL gain in the study by Farina et al. 2013 [27]. The inconsistency between the two studies may be accounted for several reasons. First, Farina et al. used several reconstructive technologies, including EMD, hydroxyapatite-based graft, and so on. The different regenerative benefits of materials might be a potential confounding factor, which affected correlation analysis between EHI and CAL gain. Second, Farina et al. applied a single flap approach (SFA), leaving one side supracrestal gingival tissues intact. Compared with MISTms, SFA might better maintain wound stability and facilitate local revascularization. These benefits could compensate for the negative influence of suboptimal wound healing. Third, the 2-week EHI might not be sensible enough to predict the 6-month result. In the presented study, mean EHI was used as an independent variable. It was found that lower mean EHI positively influenced 1-year CAL gain. This result suggested that the assessment of postoperative wound healing should last for a period of time, 6 weeks or even longer, not just limited to one time point. Moreover, the same study by Farina et al. showed that suboptimal healing at 2 weeks was related to the presence of reconstructive devices [27]. Consistently, in the present study, wound healing was worse in the MISTms-combined group than in the MISTms-alone group. These results furthermore indicated that the presence of regenerative devices might result in suboptimal healing, which partly compensated for their regenerative benefits.

On the radiographs, bone fill in the intrabony defects could be detected in both groups. In the MISTms-alone group, radiographic bone gain suggested that bone regeneration occurred, but whether periodontal ligament and cementum newly formed have been never known. In the MISTms-combined group, the new-formed radiopaque area may include new bone and some residual bovine bone. Therefore, the effect of MISTms needs to be verified by further re-entry surgery and histologic examination.

In the present study, five patients dropped out. The loss of follow-up bias could not be excluded. Ages in the two groups were not well matched. Moreover, all surgeries were performed by the same experience periodontal surgeon in one hospital, which may limit the generalizability of the findings in this study.

Conclusions

In conclusion, MISTms appears to be effective for the treatment of intrabony defects. The regenerative materials do not show the additional effect on 1-year outcomes. Early wound healing and baseline CAL are factors influencing 1-year CAL gain.

Author contributions All authors have made substantial contributions to the study. Bei Liu contributed with data analysis, data interpretation, and manuscript drafting. Xiangying Ouyang designed the study and treated patients. Jun Kang performed the clinical evaluation. Shuangying Zhou performed a radiographic evaluation. Chao Suo and Lingqiao Xu contributed to data entry and verification. Jianru Liu performed randomization. Wenyi Liu assisted for study organization.

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Declarations

Ethical approval The Ethics Committee of Peking University School and Hospital of Stomatology approved the study protocol (PKUSSIRB-2012080). The trial was registered at the Chinese Clinical Trial Registry (ChiCTR2100043272). All procedures involving human participants were performed in accordance with the ethical principles of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent All participants included in the study signed the informed consent.

Conflict of interest The authors declare no competing interests.

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