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Single minimally invasive surgical technique with an enamel matrix derivative to treat multiple adjacent intra-bony defects: clinical outcomes and patient morbidity

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Abstract

Aims: This case cohort study was designed to evaluate the clinical performance and the intra-operative and post-operative morbidity of the ‘‘minimally invasive surgical technique’’ (MIST) associated with the application of an enamel matrix derivative (EMD) in the treatment of multiple deep intra-bony defects in a single surgical procedure.

Material and Methods: Forty-four deep intra-bony defects in 20 patients ($\geqslant$2 defects/patient) were surgically accessed with the MIST. This technique was designed to limit the mesio-distal flap extension and the apical flap reflection in order to reduce the surgical trauma and increase flap stability. The incision of the defect-associated papilla was performed according to the principles of the papilla preservation techniques. EMD was applied on the debrided and dried root surfaces. Stable primary closure of the flaps was obtained with modified internal mattress sutures. Surgery was performed with the aid of an operating microscope and microsurgical instruments. Clinical outcomes were collected at baseline and at 1 year. Intra-operative and post-operative morbidity was evaluated with questionnaires.

Results: The 1-year clinical attachment level (CAL) gain was 4.4 ± 1.4 mm ($p < 0.0001$ compared with baseline). Seventy-three per cent of defects showed CAL improvements $\geqslant$4 mm. This corresponded to an 83 ± 20% resolution of the defect (15 defects were completely filled). Residual probing pocket depths (PDs) were 2.5 ± 0.6 mm. A minimal increase of 0.2 ± 0.6 mm in gingival recession between baseline and 1 year was recorded. Twelve patients reported a mild perception of the hardship of the surgical procedure. Primary closure was obtained and maintained in all treated sites over time. Only six subjects reported moderate post-operative pain that lasted for 21 ± 5 h.

Conclusions: These data indicate that a MIST in combination with EMD can be applied successfully for the treatment of multiple deep intra-bony defects in the same surgical procedure with excellent clinical outcomes and very limited patient morbidity.

Key words: clinical trial; microsurgery; osseous defects; periodontal diseases; periodontal regeneration

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Conflict of interest and source of funding statement

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Minimally invasive surgical approaches have been developed recently and proposed for periodontal regeneration (Harrel & Rees 1995, Harrel & Nunn 2001, Harrel et al. 2005, Cortellini & Tonetti 2007a, b).

Cortellini & Tonetti (2007a) proposed the minimally invasive surgical technique (MIST) blending the concepts of the minimally invasive surgery (MIS; Harrel & Rees 1995, Harrel et al. 2005) with the application of the papilla preservation techniques and with the use of passive internal mattress sutures (Cortellini et al. 1995, 1999). The clinical rationale for the development of the MIST includes the following: (1) reduction of surgical trauma, (2) increase in flap/wound stability, (3) improvement of primary closure of the wound, (4) reduction of surgical chair time, and (5) minimization of intra-operative and post-operative patient discomfort and morbidity.

The clinical application of the MIST requires the adoption of an operating microscope (or high-power magnification loops) and microsurgical instruments (Cortellini & Tonetti 2001, 2005) in order to increase the visual acuity and allow for a precise and careful flap management and thorough root/defect instrumentation in spite of minimal flap reflection.

A preliminary case cohort on the application of MIST and EMD in isolated intra-bony defects resulted in a clinical attachment level (CAL) gain of 4.8 ± 1.9 mm and an $88.7 \pm 20.7\%$ CAL fill of the intra-bony component of the defect at 1 year. A second case cohort study on 40 isolated intra-bony defects resulted in a CAL gain of 4.9 ± 1.7 mm with a $77.6 \pm 21.9\%$ 1-year resolution of the defect. In both studies, besides a very short surgical time, limited patient discomfort during the surgical session and very limited patient morbidity in the post-operative period were reported.

Although most of the regenerative procedures are reportedly described for treatment of isolated deep intra-bony defects (for a review, see Murphy & Gunsolley 2003, Esposito et al. 2005, Needleman et al. 2006), a recent case report from Harrel et al. (2005) showed 1-year CAL gains of 3.6 ± 1.7 mm associated with residual probing depths of 3.1 ± 0.7 mm following application of MIS and EMD in 16 patients presenting multiple sites with deep pockets associated with different defect morphologies, including furcation involvements.

The aims of the present case cohort study were to evaluate the clinical performance and the intra-operative and post-operative morbidity of the MIST associated with the application of an enamel matrix derivative (EMD) in the treatment of multiple adjacent deep intra-bony defects.

Material and Methods

Study population and experimental design

Patients with advanced periodontal disease, in general good health, presenting with at least two neighbouring teeth with deep intra-bony defects were enrolled for this study. Before entering the study patients completed a protocol of cause-related therapy consisting of scaling and root planing, motivation, and oral hygiene instructions. Flap surgery for pocket elimination was performed, when indicated, in the remaining portions of the dentition of each patient before the regenerative treatment. All subjects gave written informed consent.

Inclusion criteria were the following:

1. *Absence of relevant medical conditions:* Patients with uncontrolled or poorly controlled diabetes, unstable or life-threatening conditions, or requiring antibiotic prophylaxis were excluded.
2. *Smoking status:* Only light smokers were included (<10 cigarettes/day).
3. *Defect anatomy:* Presence of at least two neighbouring teeth with probing pocket depth (PD) and CAL loss of at least 5 mm associated with intra-bony defects of at least 3 mm.
4. *Good oral hygiene:* Full-mouth plaque score (FMPS) $\leqslant 25\%$.
5. *Low levels of residual infection:* Full-mouth bleeding score (FMBS) $\leqslant 25\%$.
6. *Compliance:* Only patients with optimal compliance, as assessed during the cause-related phase of therapy, were selected.
7. *Endodontic status:* Teeth had to be vital or properly treated with root canal therapy.

Forty-four intra-bony defects in 20 subjects (mean age 49.7 ± 8.3, range 35–63 years, six males, and no smokers), which met the inclusion criteria, were consecutively included in this case cohort. The study was conducted in a private practice (PC) setting in an 8-month period between November 2005 and June 2006.

Three months after completion of cause-related periodontal therapy – and needed flap surgery – baseline clinical measurements were recorded. The experimental sites were accessed with the MIST and carefully debrided. Measurements were taken during surgery to characterize the defect anatomy (Cortellini et al. 1993a, b). EDTA and EMD (Emdogain, Institute Straumann AG, Basel, Switzerland) were applied on the instrumented and dried root surfaces and flaps were sutured with modified internal mattress sutures (Tonetti et al. 2002). A questionnaire was given to the patients at the end of surgery, with questions about the subjective perception of the surgical procedure as described previously (Tonetti et al. 2004, Cortellini & Tonetti 2007b). At the 1-week follow-up visit, a second questionnaire was given to patients with questions about the first post-operative week. Patients were enrolled in a stringent post-operative supportive care programme with weekly recalls for 6 weeks and were thereafter included in a 3-month periodontal supportive care programme for 1 year. Outcome measures were taken at 1 year.

Clinical measurements at baseline and at 1-year follow-up visit

The following clinical variables were evaluated at baseline before regenerative therapy and at the 1-year follow-up visit by an independent clinician. FMPS were recorded as the percentage of total surfaces (four aspects per tooth), which revealed the presence of plaque (O'Leary et al. 1972). Bleeding on probing (BOP) was assessed dichotomously and FMBS were then calculated (Cortellini et al. 1993a).

PD and recession of the gingival margin (REC) were recorded to the nearest millimetre at the deepest location of the selected inter-proximal site. All PD measurements and BOP were taken with a pressure-sensitive manual periodontal probe at 0.3 N (Brodontic probe equipped with a PCP-UNC 15 tip, Hu-Friedy, Chicago, IL, USA). CALs were calculated as the sum of PD and REC. The radiographic defect angle of each defect was measured on a periapical radiograph, as described previously (Tonetti et al. 1993a).

Clinical characterization of the intra-bony defects

Defect morphology was characterized intra-surgically in terms of distance between the cemento-enamel junction and the bottom of the defect (CEJ – BD) and total depth of the infra-bony component of the defect (INFRA), essentially as described previously (Cortellini et al. 1993b). The depths of the three-, two-, and one-wall subcomponents, as well as the extension of the buccal and lingual/palatal bone dehiscences, were also measured. Corticalization of the defects was classified as markedly corticalized, regularly cribriform, or very cancellous. Bleeding tendency of the defects was dichotomously recorded, as present or absent after completion of defect debridement.

Surgical and post-surgical variables

The time required for each surgical procedure was calculated, from injection of local anaesthesia to completion of sutures. The number of teeth and the number of inter-proximal spaces involved in the procedure were recorded as well as the use of vertical releasing incision(s) and their position (mesial/distal buccal/lingual). Primary closure of the flaps was evaluated at completion of surgery and at weekly recalls for a period of 6 weeks, along with the presence/absence of root sensitivity, oedema, haematoma, suppuration, granulation tissue, and any other post-surgical complication.

Surgical approach (MIST)

The surgical approach required few modifications with respect to the technique described previously (Cortellini & Tonetti 2007a) for application in isolated intra-bony defects. The flap was extended to include the defect-associated inter-dental papillae of the two neighbouring teeth (Fig. 1) that were accessed with either the simplified papilla preservation flap (SPPF, Cortellini et al. 1999) or the modified papilla preservation technique (MPPT; Cortellini et al. 1995) according to indications. When a defect was associated with an edentulous ridge, a crestal incision was performed (Fig. 2). The mesio-distal extension of the flap was kept to a minimum to allow the reflection of a very small full-thickness flap to expose 1–2 mm of the defect-associated residual bone crest. When possible, only the defect-associated papillae were accessed and vertical-releasing incisions were avoided. However, when the position of the residual buccal/lingual bony wall(s) was (were) deep and difficult or impossible to reach with a minimal incision of the defect-associated inter-dental spaces, the flap(s) was (were) further extended mesially or distally involving one extra inter-dental space. Vertical-releasing incisions were performed when flap reflection caused tension at the extremities of the flap(s). To minimize invasivity, the vertical-releasing incisions were always kept very short and within the attached gingiva, never involving the muco-gingival junction. Periosteal incisions were never performed. If possible, the body of the papilla(e) not associated with the defect(s) was not elevated.

Defect debridement and EMD application

The defects were debrided with a combined use of mini curettes (Gracey, Hu-Friedy) and power-driven instruments (Soniflex Lux, Kavo, Germany) and the roots were carefully planed. During the instrumentation, the flaps were slightly reflected, carefully protected with periosteal elevators, and frequently irrigated with saline. At the end of instrumentation, an EDTA gel (Institute Straumann AG) was applied on the instrumented and dried root surfaces for 2 min. After that, the defects and root surfaces were carefully rinsed with saline and finally EMD (Institute Straumann AG) was applied on the dried root surface. When the defect(s) showed bleeding tendency, care was taken to reduce bleeding in order to be able to apply EMD on dried root/bone surfaces. This was accomplished by compacting a wet sterile gauze into the defect(s) for 3–4 min. After EMD application the flaps were re-positioned.

Flap suturing technique

The suturing approach in most of the instances consisted of two modified internal mattress sutures applied at the defect-associated inter-dental sites to reach primary closure of the preserved papillae in the absence of any tension (Cortellini & Tonetti 2001, 2005). When more inter-dental spaces were accessed, the same suturing technique was used to obtain primary closure in these sites. Vertical-releasing incisions were sutured with interrupted passing sutures. The buccal and lingual flaps were re-positioned at their original level, without any coronal displacement to avoid any additional tension in the healing area.

The surgical procedures were performed with the aid of an operating microscope (Global Protege, St. Louis, MO, USA) at a magnification of $\times$ 4– $\times$ 16 (Cortellini & Tonetti 2001, 2005). Microsurgical instruments were utilized, whenever needed, as a complement to the normal periodontal set of instruments. Incisions were carried out using delaminating microsurgical blades (M6900, Advanced Surgical Technologies, Sacramento, CA, USA). 6-0 e-PTFE (Gore-tex, WL Gore & Associates, Flagstaff, AZ, USA) sutures were preferred to obtain primary closure of the inter-dental tissues.

Control of tooth mobility

Teeth that were found to be hypermobile at baseline were splinted either before or immediately after the surgical procedure (Cortellini et al. 2001). Splinting was carried out between the experimental tooth and the neighbouring teeth with the aid of composite resin. Splinting was performed before surgery for teeth that were severely hypermobile (Miller grade II or greater). In other instances, mobility was re-evaluated at the end of surgery and the splint was applied when mobility was clearly increased with respect to the baseline value.

Post-operative period

A protocol for the control of bacterial contamination consisting of doxicycline (100 mg bid for 1 week), 0.12% chlorhexidine mouth rinsing three times per day, and weekly dental prophylaxis with a chlorhexidine gel was prescribed (Tonetti et al. 2002). Patients were requested to avoid brushing, flossing, and chewing in the treated area for periods of 3–4 weeks. At the end of this period patients resumed full oral hygiene. At the end of the ''early healing phase'', patients were placed on a 3-month recall system for 1 year.

Evaluation of intra-operative and post-operative morbidity

Patient perceptions of intra-operative and post-operative morbidity were evaluated with a questionnaire administered upon completion of the procedure (hardship of the procedure and pain) and at suture removal (pain, discomfort, use of pain killers, interferences with daily activities, and adverse events). Responses were quantified with a visual analogue scale (VAS) of 100 mm as described previously (Cortellini et al. 2001, Tonetti et al. 2004).

Data analysis

The statistical unit of this study was the patient for patient-related variables and the site for site-related variables. A multilevel modelling was utilized to determine the hierarchical structure of this experimental population and understand where and how the effects are occurring. Data were expressed as means ± standard deviation of 44

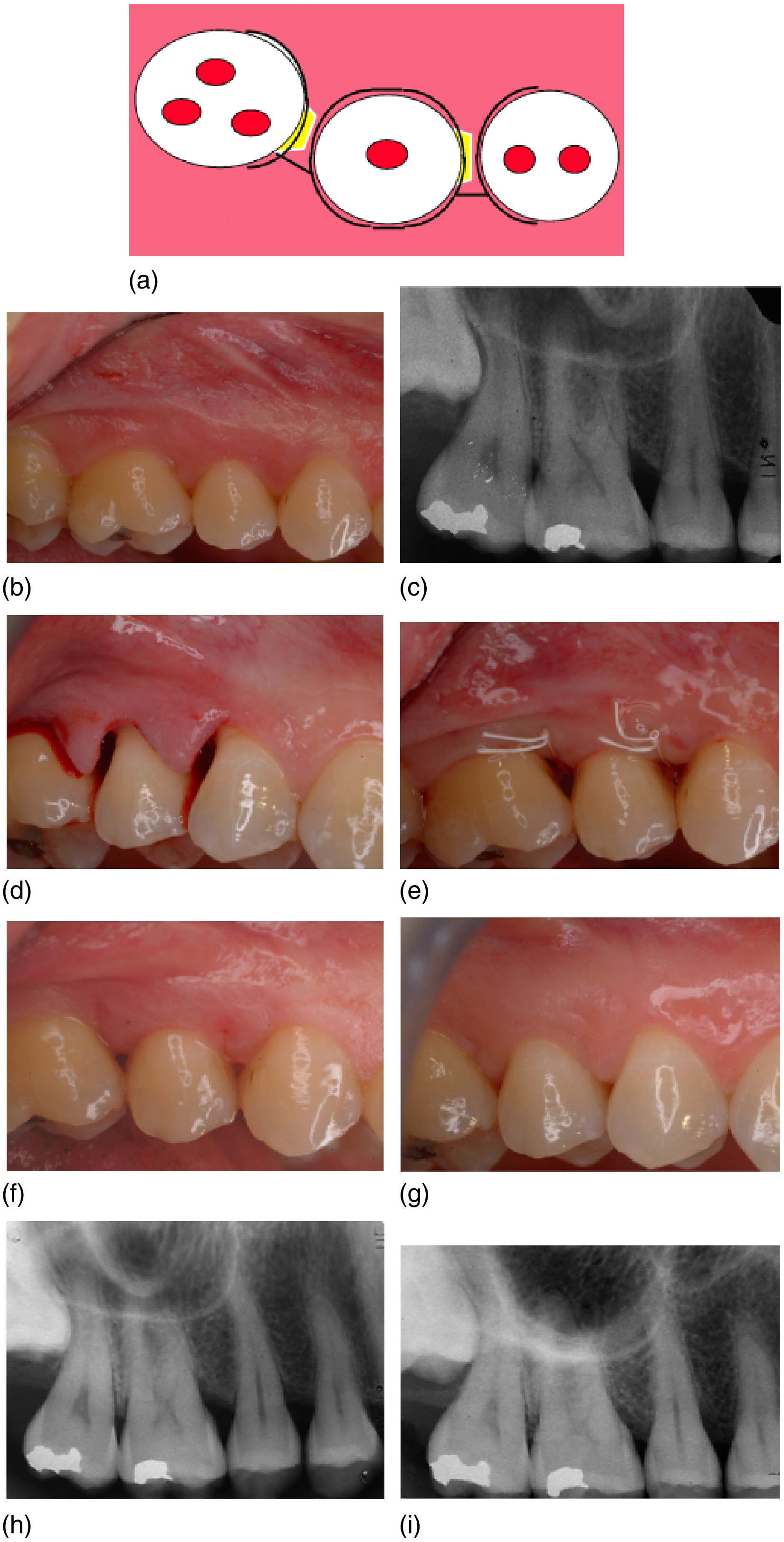
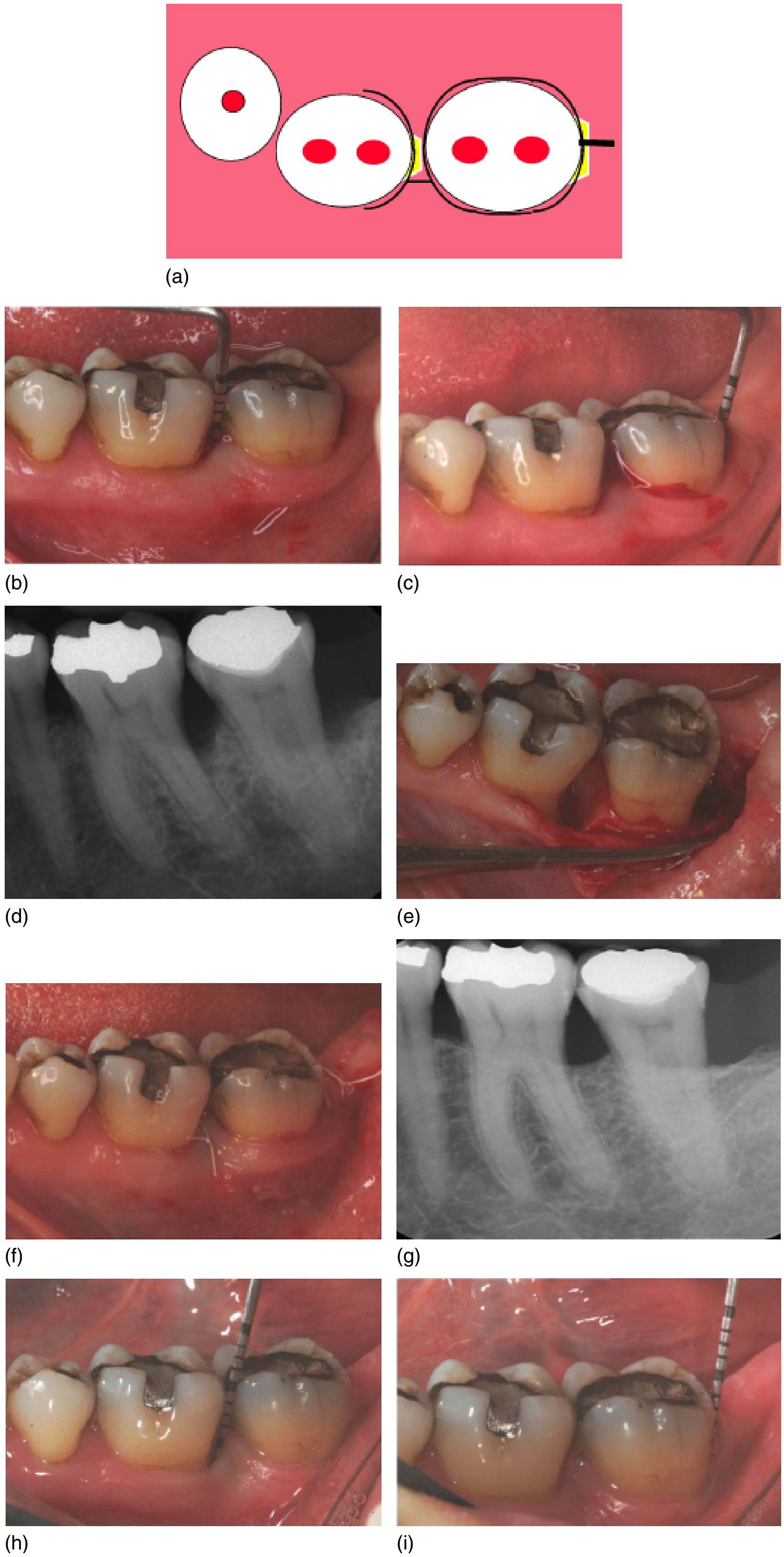


Fig. 1. (a) Drawing of the application of the minimally invasive surgical technique (MIST) in intra-bony defects involving two adjacent teeth. The diagram shows the extent of the incision performed according to the principles of the MIST and the simplified papilla preservation flap (two narrow inter-dental spaces). Mesio-distal extension of the flap is limited to the two inter-dental papillae associated with the defects and reaches the approximal line angle of the two adjacent teeth. (b, c) After successful initial, cause-related therapy, two defects are present on the mesial of the first molar and the second pre-molar. The defects involve the coronal third of the root surfaces and present with a wide radiographic angle. Simplified papilla preservation flaps are used to access the defects. (d) Incisions are stopped at the disto-buccal line angle of the first pre-molar and at the mesio-buccal of the first molar. Root debridement is followed by the application of EDTA and enamel matrix proteins in gel form. (e) Primary closure of the flap that was obtained with two modified internal vertical mattress sutures. (f) Excellent early healing with preservation of the inter-dental soft tissues is evident at 1 week after suture removal. (g) At the 1-year follow-up, absence of inflammation, shallow probing depths, and resolution of the defects are evident. (h, i) Periapical radiographs at 6 and 12 months showing the progressive resolution of the intra-bony component of the defects.

defects in 20 patients. No data points were missing. Comparisons between baseline and 1-year data of the primary outcome variables (CAL, PD, and REC) were calculated with a multilevel approach (patient, site, and time). Percentage fill of the baseline intra-bony component of the defect was calculated as: CAL% = (CAL gains)/INFRA × 100. A multilevel linear regression analysis was carried out at two levels (patient and site) to predict changes in CAL, 1 year after the application of MIST and EMD. The model includes only variables related to defect morphology. A variable indicative of defect severity (CEJ – BD), the depth of the three-wall subcomponent, and the radiographic defect angle as descriptors of the defect morphology were included in the model along with the bleeding tendency of the defect. Patient-related variables were not considered because the ones that could influence the outcomes had been controlled during the cause-related therapy, and the severe entry criteria excluded the enrolment of patients with poor oral hygiene and high levels of residual infection. In addition, none of the patients was a smoker. R^2 was calculated at the site level (Gelman & Hill 2007). All calculations were performed with JMP® 7.0 2007 (SAS Institute Inc.) and MLwiN 1.00 1998 (©Multilevel Project Institute of Education).



Results

Patient and defect characteristics at baseline

FMPS and FMBS at baseline were 14.7 ± 2.3% and 9.9 ± 3.6%, respectively

Fig. 2. (a) Drawing of the application of the minimally invasive surgical technique (MIST) in intra-bony defects involving one inter-proximal space and an edentolous ridge. The diagram shows the incision performed according to the principles of the MIST and the modified papilla preservation flap at the wide inter-dental space and the linear crestal incision performed on the edentolous ridge. Mesial extension of the flap is limited to the mid-buccal and mid-lingual area of the first molar; the distal crestal incision is extended for about 5–6 mm. (a, b, c) Following cause-related therapy, a 9 mm pocket distal to the first lower left molar and a 7 mm pocket distal to the second lower left molar are evident. The pockets are associated with deep intra-bony defects. (e) Minimal flap elevation to expose the residual bone is followed by debridement and EDTA and EMD application. (f) Primary closure of the flap is obtained with modified internal mattress sutures. (g) The 1-year radiograph shows the complete resolution of the defects. (h, i) At 1 year, the residual probing depth is 3 mm at both the treated sites.

(Table 1). CALs of 8.7 ± 1.7 mm and PDs of 7.1 ± 1.4 mm on average were recorded (Table 1). The radiographic defect angle was 33.7 ± 6.4°. The distance from the cemento-enamel junction to the bottom of the defect was 9.9 ± 2.1 mm and the INFRA was 5.5 ± 1.8 mm (Table 1). Seventeen teeth were found to be hyper-mobile (16 presenting with Miller degree 1 and one with degree 2). Only six teeth were splinted (in two patients) at baseline, before surgery.

Table 1. Baseline patient ($N = 20$) and defect characteristics ($N = 44$)

Variables	Mean ± SD	Minimum	Maximum
FMPS (%)	14.7 ± 2.3	11	18
FMBS (%)	9.9 ± 3.6	4	21
PD (mm)	7.1 ± 1.4	5	10
REC (mm)	1.6 ± 1.0	0	4
CAL (mm)	8.7 ± 1.7	6	12
CEJ – BD (mm)	9.9 ± 2.1	6	14
INFRA (mm)	5.5 ± 1.8	3	9
3 wall (mm)	2.5 ± 1.6	0	6
2 wall (mm)	2.1 ± 1.4	0	5
1 wall (mm)	0.9 ± 1.2	0	5
X-ray angle (deg.)	33.7 ± 6.4	20	47

FMPS, full-mouth plaque score; FMBS, full-mouth bleeding score; PD, probing pocket depth; REC, recession of the gingival margin; CAL, clinical attachment level; CEJ – BD, distance between the cemento-enamel junction and the bottom of the defect; INFRA, infrabony component of the defect.

Surgical variables (Table 2)

The SPPF was used in 12 sites; two sites were accessed with a crestal incision and all the others with the MPPT. An incision restricted to the defect-associated inter-dental papillae was performed in 11 out of 20 cases. The flap was further extended to one more inter-proximal site in seven cases and in two cases it involved two more inter-proximal spaces. A vertical-releasing incision was performed in seven patients to aid flap reflection. The average surgical chair-time was 68.0 ± 11.2 min. (range 45–89 min.).

Table 2. Surgical variables ($N = 20$)

Variables	Mean ± SD	*N* (%)	Minimum–maximum
No. of teeth involved in surgery	3.4 ± 1.1	–	2–6
No. of inter-dental spaces involved	2.5 ± 1.1	–	2–5
Periosteal incision	–	0	–
Vertical-releasing incision	–	7 (35%)	–
Surgical time (min.)	68.0 ± 11.2	–	45–89
Inter-dental papilla closure at the end of surgery	–	20 (100%)	–

Evaluation of the surgical procedure and the post-operative period

Table 3. Subject experience in terms of post-operative pain ($N = 20$)

	No. of subjects	Mean ± SD	Minimum–maximum
Subjects reporting pain	6 (30%)	–	–
Intensity of pain (VAS units)	6	19 ± 9	11–31
Duration of pain (h)	6	21 ± 5	12–24
Intake of analgesic tablets	11	1.6 ± 0.7	1–3

VAS units, visual analogue scale units (with 0 = no pain and 100 = unbearable pain).

No patients experienced intra-operative pain, while 12 reported a moderate perception of the hardship of the surgical procedure (24 ± 12 VAS units, on average, range 4–43 with 0 = easy to cope with and 100 = difficult to cope with). In all treated sites, primary closure was obtained at completion of the surgical procedure and maintained in the six following weeks (100% full inter-dental closure). A slight oedema was recorded in six of the cases at week 1. The oedema was fully resolved at week 2 in all the sites. No post-surgical haematoma, suppuration, flap dehiscence, presence of granulation tissue, or other complications were noted in any of the treated sites. Root sensitivity was reported at week 1 by 11 patients and decreased in the following weeks. At week 6, only five patients still reported some root sensitivity.

The prevalence and extent of post-operative pain is presented in Table 3. Fourteen patients did not experience any post-operative pain. The six subjects reporting pain described it as being mild in intensity (VAS 19 ± 9, with 0 = no pain and 100 = unbearable pain). In these patients, pain lasted for 21 ± 5 h, on average. Home consumption of painkillers was 0.9 ± 1.0 on average among the 20 patients (range 0–3). Nine patients did not use any analgesic in addition to the first two compulsory tablets that were taken in the practice immediately after the surgery and 6 h later.

Ten patients experienced mild discomfort (VAS 21 ± 10, with 0 = no discomfort and 100 = unbearable discomfort) that lasted 20 ± 9 h, on average.

Only four patients reported some interference with daily activities (work and sport activities) for 1–3 days.

One-year clinical outcomes

The 20 patients presented at the 1-year follow-up visit with FMPS and FMBS of 11.5 ± 2.1% (range 8–15%) and 7.2 ± 2.3% (range 4–14%), respectively.

The 1-year CAL was 4.3 ± 1.1 mm with a CAL gain of 4.4 ± 1.4 mm (range 2–7 mm, Table 4). Differences in CAL between baseline and 1 year were clinically and statistically highly significant ($p < 0.0001$).

To characterize the distribution of the observed CAL gain among the different sites, data were stratified into four classes of CAL gain (Table 5). The stratified data show that no sites lost attachment, and only two sites gained 2 mm of attachment, while 73% of sites gained 4 mm or more.

The 1-year percentage resolution of the defect was 83 ± 20% (range 50–133%), and reached at least 100% of the baseline intra-bony component in 15 sites. A percentage defect resolution

Table 4. Clinical outcomes at baseline and 1 year after treatment ($N = 44$)

Variables	Baseline	1 year	Difference	p-value*
PD (mm)	7.1 ± 1.4	2.5 ± 0.6	4.6 ± 1.3	< 0.0001
REC (mm)	1.6 ± 1.0	1.8 ± 1.0	− 0.2 ± 0.6	0.0480
CAL (mm)	8.7 ± 1.7	4.3 ± 1.1	4.4 ± 1.4	< 0.0001

*Multilevel test.

PD, probing pocket depth; REC, recession of the gingival margin; CAL, clinical attachment level.

Table 5. Frequency distribution of clinical attachment level gains at 1 year ($N = 44$)

	< 2 mm	2–3 mm	4–5 mm	⩾6 mm
N (%)	0	12 (27%)	22 (50%)	10 (23%)

ranging from 50% to 99% was observed in the other sites.

Residual probing depths were 2.5 ± 0.6 mm (range 1–3 mm) with an average PD reduction of 4.6 ± 1.3 mm (Table 4). Differences between baseline and 1-year probing depths were clinically and statistically highly significant ($p < 0.0001$).

A small increase of 0.2 ± 0.6 mm in gingival recession between baseline and 1 year was recorded (Table 4). This difference was statistically significant ($p = 0.0480$).

Multilevel regression analysis

The multilevel regression analysis carried out to predict changes in CAL, 1 year after the application of MIST and EMD, generated a highly statistically significant model ($p < 0.0001$) that explains 70% of the observed variability in CAL gains at the site level (Table 6). CAL gain was significantly associated with the baseline distance between the cemento-enamel junction and the bottom of the defect (CEJ – BD) and with the baseline radiographic defect angle, while the depth of the three-wall intrabony subcomponent and the intra-surgical bleeding tendency of the defect did not reach statistical significance. In the multilevel regression analysis, no significant intra-patient correlation was found in terms of 1-year CAL gain at the multiple defects ($\sigma_u^2 = 0$).

Table 6. Multilevel regression analysis to predict changes in clinical attachment level, 1 year after the application of MIST and EMD

Term	Estimate	Standard error	p-value
Intercept	1.35	1.01	0.2197
CEJ – BD	0.46	0.06	< 0.0001
3-wall	0.13	0.08	0.1351
X-ray angle	− 0.05	0.02	0.0038
Bleeding	0.17	0.29	0.5736

The model includes only variables related to defect morphology: distance between the cemento-enamel junction and the bottom of the defect (CEJ – BD, mm), depth of the three-wall subcomponent (mm), radiographic defect angle (deg.), and bleeding tendency of the defect (positive bleeding = 1).
$R^2 = 0.70$.
$\sigma_u^2 = 0.00$ (patient-level variance).
$\sigma_e^2 = 0.54$ (defect-level variance).
MIST, minimally invasive surgical technique; EMD, enamel matrix derivative.

Discussion

The present case cohort study was designed to assess the clinical performance and the intra-operative and post-operative morbidity of a MIST in combination with an EMD in the regenerative treatment of multiple adjacent deep intra-bony defects. Most of the procedures proposed so far in the literature, in facts, are designed for treatment of isolated infra-bony defects (for a review, see Murphy & Gunsolley 2003, Esposito et al. 2005, Needleman et al. 2006). However, the frequent occurrence of multiple infra-bony defects in adjacent sites/teeth in the same patient suggests the adoption of a technique designed to successfully approach the defects simultaneously in the same surgical session.

The 44 deep intra-bony defects in 20 patients included in this case cohort resulted in 1-year CAL gains of 4.4 ± 1.4 mm, associated with 2.5 ± 0.6 mm of residual probing depth and a 0.2 ± 0.6 mm increase of gingival recession (Table 4). The evaluation of the clinical improvements in terms of CAL gain shows that no sites lost attachment and only two sites gained 2 mm, while the majority of sites (73%) gained 4 mm or more at 1 year and 23% gained 6 mm or more (Table 5). In addition, using the Ellegaard & Loe criteria (1971) resolution of the intra-bony component of the defects was either complete (34% of the treated sites showed 100% defect resolution) or satisfactory (defect resolution ⩾50%) with an average 83 ± 20 CAL% fill.

The reported clinical outcomes clearly demonstrate the potential of the proposed clinical approach that changed deep pockets associated with multiple deep intra-bony defects into shallow crevices while preventing a clinically relevant apical shift of the gingival margin. This healing pattern favourably compares with that of the most successful regenerative approaches published in the last decade (Cortellini & Tonetti 2000, Needleman et al. 2002, Murphy & Gunsolley 2003) and with the outcomes of a MIST in combination with EMD applied in isolated intra-bony defects (Cortellini & Tonetti 2007b). Similarly, positive clinical outcomes have been reported by Harrel et al. (2005) after treatment of 160 sites in 16 patients with the MIS in combination with EMD. They reported a mean CAL gain of 3.6 ± 1.7 mm associated with a residual probing depth of 3.1 ± 0.7 mm at 1 year.

The outcomes reported in this case cohort were obtained in a patient population, where the patient-associated factors, such as bacterial plaque, residual periodontal infection, and smoke, had been controlled through the delivery of optimal non-surgical periodontal therapy and smoking cessation protocols, in order to reduce the negative impact of the cited factors (Tonetti et al. 1993b, 1995, 1996). Interestingly, no significant intra-patient correlation of 1-year CAL gain observed at the multiple sites was detected. This may be due to the small sample size of this study and/or the fact that, given the level of control of patient factors through pre-treatment phase and the choice of tight inclusion criteria, patient factors did not play a strong role in determining regenerative outcomes. The multilevel analysis explains the majority (70%) of the observed variability in CAL gains in terms of defect anatomy.

Defect-associated factors (Table 6) like the baseline amount of bone loss (CEJ – BD) and the baseline defect radiographic angle influence the 1-year CAL gain. Interestingly, in the present defect population the depth of the three-wall subcomponent was not significantly associated with the final outcome. This is different from the observation of an influence reported in the treatment of isolated intra-bony defects with MIST and EMD (Cortellini & Tonetti 2007b) but can be explained by the small sample size of these reports. The present case cohort does not clearly indicate whether there are differences between non-contained and contained intra-bony defects. Larger sample size studies are required to properly evaluate the healing potential in defects with different morphology. The proposed MIST, however, allows for the use of a combination of grafting material with EMD, given the potential advantage of such an approach in non-contained defects (Palmer & Cortellini 2008).

The surgical technique used in the present study on multiple deep intra-bony defects was designed to minimize flap elevation and reflection in order to reduce intra-operative and post-operative flap mobility and to improve the stability of the healing area (Hiatt et al. 1968, Wikesjo & Nilveus 1990, Haney et al. 1993). Approaching multiple defects required a greater mesio-distal extension of the flap than MIST in isolated defects (Cortellini & Tonetti 2007a, b). In fact, in the latter, the number of teeth and interdental spaces involved were 2.4 ± 0.7 and 1.6 ± 0.6, respectively; this compares with 3.4 ± 1.1 and 2.5 ± 1.1 in the present study (Table 2). Nonetheless, flap reflection could be kept to a minimum with the aim of exposing just the coronal bone crest delimiting the defects. In addition, no periosteal incisions and very few vertical-releasing incisions were performed. This surgical approach resulted in a re-positioned, tension-free, and very stable flap that was passively sealed with modified internal mattress sutures.

The limited surgical trauma and the stability of the flaps also accounted for the absence of a significant post-surgical local side effect. In fact, no post-surgical haematoma, suppuration, flap dehiscence, presence of granulation tissue, or other complications were noted in any of the treated sites. In only six patients with 12 treated sites a slight oedema was recorded at week 1. The oedema was fully resolved at week 2 in all the sites. Root sensitivity was reported at week 1 by 11 patients (55%) and rapidly decreased in the following weeks. At week 6, only five patients still reported some root sensitivity. The very limited post-operative root sensitivity could be explained with the minimal increase in gingival recession observed in the treated sites.

The use of an operating microscope and microsurgical instruments contributed to the gentle soft tissue handling, the careful defect/root debridement, EMD application, and precise suturing to primarily close the wound (Cortellini & Tonetti 2001, 2005). The augmented visual acuity and the optimal field illumination provided by the microscope, along with the skill and experience of the surgeon, are probably key factors for the very limited surgical trauma and optimal healing pattern of the treated cases.

The surgical chair-time was extremely short (68.0 ± 11.2 min. on average, range 45–89 min.). In three surgeries, chair-time was shorter than 60 min., and only eight reached 70 min. This could be compared with 55.7 ± 8.8 min. reported with the use of MIST in isolated intra-bony defects (Cortellini & Tonetti 2007b). The short surgical time and the limited surgical trauma probably could explain, at least in part, the limited intra-operative and post-operative patient morbidity observed in this study (Table 3) that compares with the one reported in a previous study in which MIST and EMD were applied in isolated intra-bony defects (Cortellini & Tonetti 2007b).

In conclusion, this case cohort indicates that the MIST in combination with EMD is an effective and low-morbidity surgical approach for the treatment of multiple adjacent intra-bony defects. Controlled clinical trials should be performed to address the potential advantage of this approach compared with more conventional and well-known techniques.

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References

Cortellini, P., Pini-Prato, G. P. & Tonetti, M. S. (1993a) Periodontal regeneration of human infrabony defects. I. clinical measures. *Journal of Periodontology* **64**, 254–260.

Cortellini, P., Pini-Prato, G. P. & Tonetti, M. S. (1993b) Periodontal regeneration of human infrabony defects. II. Re-entry procedures and bone measures. *Journal of Periodontology* **64**, 261–268.

Cortellini, P., Pini Prato, G. & Tonetti, M. S. (1995) The modified papilla preservation technique. A new surgical approach for interproximal regenerative procedures. *Journal of Periodontology* **66**, 261–266.

Cortellini, P., Pini Prato, G. & Tonetti, M. S. (1999) The simplified papilla preservation flap. A novel surgical approach for the management of soft tissues in regenerative procedures. *International Journal of Periodontics & Restorative Dentistry* **19**, 589–599.

Cortellini, P. & Tonetti, M. S. (2000) Focus on intrabony defects: guided tissue regeneration. *Periodontology 2000* **22**, 104–132.

Cortellini, P. & Tonetti, M. S. (2001) Microsurgical approach to periodontal regeneration. Initial evaluation in a case cohort. *Journal of Periodontology* **72**, 559–569.

Cortellini, P. & Tonetti, M. S. (2005) Clinical performance of a regenerative strategy for intrabony defects. Scientific evidence and clinical experience. *Journal of Periodontology* **76**, 341–350.

Cortellini, P. & Tonetti, M. S. (2007a) A minimally invasive surgical technique (MIST) with enamel matrix derivate in the regenerative treatment of intrabony defects: a novel approach to limit morbidity. *Journal of Clinical Periodontology* **34**, 87–93.

Cortellini, P. & Tonetti, M. S. (2007b) Minimally invasive surgical technique (MIST) and enamel matrix derivative (EMD) in intrabony defects. (I) Clinical outcomes and morbidity. *Journal of Clinical Periodontology* **34**, 1082–1088.

Cortellini, P., Tonetti, M. S., Lang, N. P., Suvan, J. E., Zucchelli, G., Vangsted, T., Silvestri, M., Rossi, R., McClain, P., Fonzar, A., Dubravec, D. & Adriaens, P. (2001) The simplified papilla preservation flap in the regenerative treatment of deep intrabony defects: clinical outcomes and postoperative morbidity. *Journal of Periodontology* **72**, 1702–1712.

Ellegaard, B. & Loe, H. (1971) New attachment of periodontal tissues after treatment of intrabony lesions. *Journal of Periodontology* **42**, 648–652.

Esposito, M., Grusovin, M. G., Coulthard, P. & Worthington, H. V. Enamel matrix derivative (Emdogain) for periodontal tissue regeneration in intrabony defects. Cochrane Database Systematic Reviews, 19 October 2005 (4): CD003875 (review).

Gelman, A. & Hill, J. (2007) *Data Analysis Using Regression and Multilevel/Hierarchical Models*, pp. 473–477. Cambridge: Cambridge University press.

Haney, J. M., Nilveus, R. E., McMillan, P. J. & Wikesjo, U. M. (1993) Periodontal repair in dogs: expanded polytetrafluorethylene barrier membrane support wound stabilisation and enhance bone regeneration. *Journal of Periodontology* **64**, 883–890.

Harrel, S. K. & Nunn, M. E. (2001) Longitudinal comparison of the periodontal status of patients with moderate to severe periodontal disease receiving no treatment, non-surgical treatment, and surgical treatment utilizing individual sites for analysis. *Journal of Periodontology* **72**, 1509–1519.

Harrel, S. K. & Rees, T. D. (1995) Granulation tissue removal in routine and minimally invasive surgical procedures. *Compendium of Continuing Education Dentistry* **16**, 960–967.

Harrel, S. K., Wilson, T. G, Jr. & Nunn, M. E. (2005) Prospective assessment of the use of enamel matrix proteins with minimally invasive surgery. *Journal of Periodontology* **76**, 380–384.

Hiatt, W. H., Stallard, R. E., Butler, E. D. & Badget, B. (1968) Repair following mucoperiosteal flap surgery with full gingival retention. *Journal of Periodontology* **39**, 11–16.

Murphy, K. G. & Gunsolley, J. C. (2003) Guided tissue regeneration for the treatment of periodontal intrabony and furcation defects. A systematic review. *Annals of Periodontology* **8**, 266–302.

Needleman, I., Tucker, R., Giedrys-Leeper, E. & Worthington, H. (2002) A systematic review of guided tissue regeneration for periodontal infrabony defects. *Journal of Periodontal Research* **37**, 380–388.

Needleman, I. G., Worthington, H. V., Giedrys-Leeper, E. & Tucker, R. J. Guided tissue regeneration for periodontal infra-bony defects. Cochrane Database Systematic Reviews, 19 April 2006 (2): CD001724 (review).

O'Leary, T. J., Drake, R. B. & Naylor, J. E. (1972) The plaque control record. *Journal of Periodontology* **43**, 38.

Palmer, R. M. & Cortellini, P. (2008) Periodontal tissue engineering and regeneration. *Journal of Clinical Periodontology* (in press).

Tonetti, M., Pini-Prato, G. & Cortellini, P. (1993b) Periodontal regeneration of human infrabony defects. IV. Determinants of the healing response. *Journal of Periodontology* **64**, 934–940.

Tonetti, M., Pini-Prato, G. & Cortellini, P. (1995) Effect of cigarette smoking on periodontal healing following GTR in infrabony defects. A preliminary retrospective study. *Journal of Clinical Periodontology* **22**, 229–234.

Tonetti, M., Pini-Prato, G. & Cortellini, P. (1996) Factors affecting the healing response of intrabony defects following guided tissue regeneration and access flap surgery. *Journal of Clinical Periodontology* **23**, 548–556.

Tonetti, M. S., Fourmousis, I., Suvan, J., Cortellini, P., Bragger, U. & Lang, N. P. (2004) Healing, post-operative morbidity and patient perception of outcomes following regenerative therapy of deep intrabony defects. *Journal of Clinical Periodontology* **31**, 1092–1098.

Tonetti, M. S., Lang, N. P., Cortellini, P., Suvan, J. E., Adriaens, P., Dubravec, D., Fonzar, A., Fourmousis, J., Mayfield, L., Rossi, R., Silvestri, M., Tiedemann, C., Topoll, H., Vangsted, T. & Walkamm, B. (2002) Enamel matrix proteins I the regenerative therapy of deep intrabony defects. A multicentre randomized controlled clinical trial. *Journal of Clinical Periodontology* **29**, 317–325.

Tonetti, M. S., Pini-Prato, G. P., Williams, R. C. & Cortellini, P. (1993a) Periodontal regeneration of human infrabony defects. III. Diagnostic strategies to detect bone gain. *Journal of Periodontology* **64**, 269–277.

Wikesjo, U. M. & Nilveus, R. (1990) Periodontal repair in dogs: effect of wound stabilisation on healing. *Journal of Periodontology* **61**, 719–724.

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Clinical Relevance

Scientific rationale for the study: MIST was found to be an effective and low-morbidity approach in the treatment of isolated intra-bony defects. The occurrence of neighbouring periodontal defects in some patients suggests the need to develop surgical approaches for the simultaneous treatment of multiple adjacent intra-bony defects to favour regeneration and to limit intra-operative and post-operative patient morbidity.

Principal findings: Application of MIST and EMD for the treatment of multiple adjacent defects resulted in significant clinical improvements: complete resolution of 15 of the 44 treated intra-bony defects, shallow probing depths, and minimal gingival recession. Only a few patients reported minimal post-operative pain and discomfort.

Practical implications: MIST in combination with EMD shows potential for the treatment of multiple adjacent intra-bony defects and results in a high probability of clinical success and minimal patient morbidity.