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Clinical outcomes after treatment of non-contained intrabony defects with enamel matrix derivative (EMD) and deproteinized bovine bone mineral (DBBM) or guided tissue regeneration (GTR). A 12-month randomized controlled clinical trial

Il trattamento dei difetti infraossei non-contenitivi mediante l'utilizzo di amelogenina (EMD) ed osso bovino deproteinizzato (DBBM) vs rigenerazione tissutale guidata (GTR). Studio clinico controllato randomizzato a un anno

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Summary

To compare the healing of deep non-contained intrabony defects (i.e. with at least a 70% 1-wall component) treated with either a combination of enamel matrix derivative (EMD) and deproteinized bovine bone mineral (DBBM) or DBBM and a collagen membrane (GTR). In this randomized controlled clinical trial, 40 subjects with 1 intrabony defect each were treated. After 12 months the mean PD reduction and mean CAL gain at sites treated with EMD+DBBM was not statistically significantly different compared with that at sites treated with GTR (PD reduction 4.6±1.9 mm vs 4.4±1.7 mm and CAL gain 3.8±1.5 mm vs. 3.7±1.2 mm). *Conclusion:* The application of EMD+DBBM appeared to yield comparable PD reduction and CAL gain compared with GTR therapy in the treatment of deep non-contained intrabony defects.

Riassunto

Paragonare la guarigione di difetti infraossei non contenitivi (almeno 70% ad una parete) trattati con una combinazione di amelogenina ed osso bovino deproteinizzato (DBBM) vs una combinazione di membrana riassorbibile in collagene supportata da osso bovino deproteinizzato. Nel presente studio clinico controllato randomizzato, 40 pazienti con un difetto infraosseo ciascuno sono stati trattati con una delle due metodiche rigenerative. Dopo 12 mesi di follow-up la metodica EMD+DBBM non ha mostrato una differenza statisticamente significativa, in termini di riduzione della profondità di tasca (PD) e di guadagno di attacco clinico (CAL), rispetto alla metodica GTR (riduzione della profondità di tasca 4.6±1.9 mm vs 4.4±1.7 mm, guadagno di attacco clinico 3.8±1.5 mm vs 3.7±1.2 mm). In conclusione, nel trattamento dei difetti infraossei non contenitivi, l'associazione di EMD+DBBM ha dato risultati analoghi alla GTR nella riduzione della profondità di tasca e nel guadagno di attacco clinico.

Introduction

Regenerative periodontal therapy aims at predictably restoring the tooth's supporting periodontal

tissues (i.e new periodontal ligament, new cementum with inserting periodontal ligament fibres and new alveolar bone) lost due to periodontal disease. In several studies (Tonetti et al.1993, Silvestri et al 2003, Tonetti et al. 2004, Sculean et al. 2008a) it was recognized that the morphology of the osseous defect plays an important role in the healing capacity of the defect itself. In the presence of non-contained intrabony defects, the use of a non-resorbable titanium-reinforced membrane or the combination of a resorbable membrane with a grafting material has been advocated (Cortellini & Tonetti 2000). In recent years the combination of resorbable membranes and a grafting material was proposed for periodontal regeneration of intrabony defects. Outcomes from human histological studies indicated that this combination therapy resulted in periodontal regeneration (Camelo et al.1998, 2001, Mellonig 2000, Nevins et al. 2003). These human histological observations were confirmed in subsequent clinical investigations reporting higher amounts of CAL gain compared with open flap debridement alone (Stravropoulos et al. 2003). The results of a randomized controlled clinical trial showed that intrabony defects treated with a combination of DBBM and a collagen membrane gained on average 3.3 ± 1.7 mm of CAL, while control defects treated with open flap debridement alone yielded a significantly smaller mean CAL gain of 2.5 ± 1.5 mm (Tonetti et al. 2004). Mean reductions in probing depths were also significantly higher in the GTR group (3.7±1.8 mm) compared with those in the access flap group (3.2 ± 1.5 mm). However, both contained and non-contained intrabony defects were included in the study by Tonetti et al. 2004. The application of some biomaterials without space-making properties such as EMD may not be indicated for the treatment of deep non-contained intrabony defects. In fact, the results of a clinical study using EMD alone for the treatment of intrabony defects (Tonetti et al 2002) showed that three-walls defects yielded a 2.7x higher probability of gaining at least 3 mm of CAL compared with that of one-wall defects. A comparative study investigated the use of EMD alone or in combination with autogenous cortical bone particles for the treatment of intrabony defects (Yilmaz et al. 2010). In this study, the results have indicated less gingival recession and increased proportion of sites with^3 6 mm of CAL compared with the application of EMD alone following the combination approach.

In order to reduce patient morbidity, additional studies propagated the use of EMD in combination with deproteinized bovine bone mineral for the treatment of intrabony defects (Lekovic et al 2000, Camargo et al 2001, Scheyer et al 2002, Sculean et al 2002, 2003, 2008b, Zucchelli et al. 2003). For example, contained and non-contained intrabony defects treated with a combination of EMD and DBBM yielded statistically significantly greater mean CAL gain after 12 months when compared with that of defects treated with EMD alone (5.8 ± 1.1 mm vs. 4.9 ± 1.0 mm) (Zucchelli et al 2003).

The aim of this randomized controlled clinical trial was to compare the healing of non-contained intrabony defects (i.e. with at least a 70% 1-wall component) treated with a combination of EMD and DBBM with that of comparable defects treated with a resorbable collagen membrane supported by DBBM after an observation period of 12 months.

Materials and Methods

Experimental design

A randomized controlled clinical trial was designed. The null hypothesis of no statistically significant differences between two modalities for the regeneration of non-contained intrabony defects was tested. The defects were treated either by means of a combination of EMD and DBBM or using a resorbable porcine-derived collagen membrane supported by DBBM . A single defect was treated in each subject.

Subject sample

In 40 subjects, a total of 40 non-contained intrabony defects were selected. The subjects were recruited from the patient pool of the Department of Periodontology, University of Naples

"Federico II", Naples, Italy. The study protocol was submitted to and approved by the Ethical Committee of the University of Naples "Federico II", Naples, Italy (protocol Nr. 227/10). Written informed consent was obtained and the study was conducted according to the principles of the Declaration of Helsinki on experimentation involving human subjects. The following inclusion criteria were applied: males and females aged ≥ 18 years, diagnosis of chronic periodontitis previously treated with non-surgical mechanical debridement, Full-Mouth Plaque Score (FMPS) ≤ 20 % at baseline, Full-Mouth Bleeding Score (FMBS) ≤ 20 % at baseline, sites with PD ≥6mm, presence of a non-contained osseous defect with an intrabony component ≥ 3 mm located in the interproximal area of single-rooted teeth including first maxillary premolars and flat surfaces of molar teeth, presence of ≥ 2 mm of keratinized tissue to allow flap management. Subjects were excluded on the basis of: relevant medical conditions contraindicating surgical interventions, pregnancy or lactation, tobacco smoking, interproximal intrabony defects extending into the furcation area of molar teeth.

Randomization

The periodontal defects were randomly assigned to one of the two experimental procedures. The allocation was carried out using a commercially available computer software package. Treatment allocation was performed at time of surgery after debridement of the intrabony defect by opening an envelope containing the information test (i.e. EMD + DBBM) or control (i.e. GTR) procedure, respectively.

Experimental procedures

Clinical measurements

The clinical parameters FMPS and FMBS were recorded at four sites per tooth (i.e. buccal, mesial, oral and distal). The parameters PD and CAL represented the measurements at the deepest site of the intrabony defect of each tooth. The clinical parameters were assessed using a graduated manual periodontal probe: FMPS,FMBS,PD,CAL,REC.

Intrasurgical measurements

The following intrasurgical measurements were recorded: CEJ-BD: the vertical distance from the cemento enamel junction (CEJ) to the bottom of the defect (BD), INTRA: the vertical distance from the alveolar bone crest to the bottom of the defect, WIDTH: the horizontal distance from the root surface to the alveolar bone crest.

Surgical procedures

Depending on the mesio-distal width of the interproximal space, two different incision techniques were selected to access the intrabony defect area (modified papilla preservation technique or simplified papilla preservation technique) A mucoperiosteal flap was elevated and extended at least one tooth mesially and distally of the intrabony defect.

Following flap elevation, the granulation tissue was removed from the intrabony defect by means of metal curettes. Osseous defects with at least a 70% one-wall component and a residual 2- to 3-wall component in the most apical part were selected. At this time point, scaling and root-planing were performed combining the use of metal curettes and power-driven instrumentation. The intrabony defects were randomly assigned to one of the two experimental procedures by opening a sealed envelope. At test sites, an enamel matrix derivative (EMD) was applied following root conditioning for 2 min with a 24% EDTA gel and rinsing with sterile saline solution according to manufacturer's instructions. Thereafter, deproteinized bovine bone mineral (DBBM) particles with a size of 0.25-1.0 mm soaked in EMD were applied into the intrabony defect up to the alveolar crest margin. At control sites, the procedure included the selection and adaptation of a porcine-derived collagen membrane supported by DBBM particles with a size of 0.25-1.0 mm applied into the intrabony component of the defect. After flap repositioning, a tension-free primary closure of the interdental papillae was achieved using 5-0 non-resorbable suturing material. Pain and edema were controlled with 600 mg ibuprofen immediately before the surgical

intervention and after 4 hours. In cases of contraindications to non-steroidal anti-inflammatory drugs (NSAIDs), 500 mg acetaminophen immediately before the surgical intervention and after 6 hours was prescribed. Subjects were instructed to rinse twice daily with 0.12 % chlorhexidine digluconate for the first two weeks and to use modified oral hygiene procedures in the treated areas for the first four post-operative weeks. No systemic antibiotics were prescribed. After four weeks, subjects were instructed to resume regular self-performed oral hygiene procedures. Sutures were removed after 7-10 days and all subjects were recalled after 2 and 4 weeks and after 3, 6, 9 and 12 months for professional maintenance care. After 12 months, a follow-up examination was performed.

Statistical analysis

The heterogeneity of the subject population with respect to test and control procedures was verified using the Fisher test. The comparison of proportions of males and females was based on a Chi-square test. The heterogeneity of the distribution of the intrabony defects with respect to test and control procedures was verified using the Kolmogorov-Smirnov test. The clinical parameters PPD, CAL and REC were expressed in millimeters (mm) whereas the FMPS and FMBS scores were expressed in percentages (%). Descriptive statistics (e.g. means) ± standard deviations (SD) were used to present the subject sample.Within group comparisons were performed using the paired *t*-test, while comparisons between test and control procedures were performed using the unpaired *t*-test.

Results

Baseline and intrasurgical characteristics

The demographic characteristics of the subject sample at baseline are presented in Table 1. A total of 40 subjects fulfilling the inclusion criteria and presenting with one intrabony defect with at least a 70% one-wall component and a 2- to 3-wall component in the most apical part were enrolled. No statistically significant differences ($p>0.05$) were observed with respect to mean age, gender, mean FMPS and FMBS scores when comparing subjects treated either with EMD + DBBM or GTR (Table 1).

Table 1. Demographic characteristics of the study sample at baseline

Parameter	EMD+DBBM Group (N=20)	GTR Group (N=20)	*P*
Age (years mean ± SD)	44.5 ± 5.5	44.3 ± 4.7	0.88
Female/male(n)	9/11	13/7	0.34
FMPS (%)	17.1 ± 2.5	17.8 ± 2.0	0.37
FMBS (%)	17.5 ± 7.9	14.8 ± 2.6	0.17

FMPS: Full-Mouth Plaque Score
FMBS: Full-Mouth Bleeding Score
SD: Standard Deviation
EMD: Enamel Matrix Derivative
DBBM: Deproteinized Bovine Bone Mineral
GTR: Guided Tissue Regeneration

Table 2 summarizes the baseline and intrasurgical characteristics of the intrabony defects. Mean pocket probing depths (PD) at the defect sites were 8.2 ± 1.9 mm for test and 8.1 ± 2.1 mm for control sites, respectively. Mean clinical attachment levels (CAL) amounted to 9.3 ± 2.4 mm and 9.3 ± 2.5 mm at test and control sites, respectively. The gingival recession was 1.2±1.6 mm and 1.1±1.4 mm at test and control sites. The mean distances from the cement-enamel junction to the bottom of the defect (i.e. CEJ-BD) measured at time of surgery were 10.5 ± 2.4 mm for test

and 10.9 ± 2.5 mm for control defects with an intrabony component (i.e. INTRA) of 6.1 ± 1.9 mm at test and 6.0 ± 2.0 mm at control sites, respectively. The mean horizontal width of the defects from the root surface to the alveolar bone at the crestal level (i.e. WIDTH) amounted to 4.2 ± 0.7 mm at test and 3.8 ± 1.0 mm at control sites, respectively. At baseline and at time of surgery, no statistically significant differences ($p > 0.05$) were observed for any of the defect characteristics between test and control sites, respectively (Table 2)

Table 2. Baseline and intrasurgical characteristics of the intrabony defect sites treated with EMD + DBBM and GTR, respectively

Parameter	EMD+DBBM Group (N=20)	GTR Group (N=20)	*P*
PPD (mm)	8.2 ± 1.9	8.1 ± 2.1	0.94
CAL (mm)	9.3 ± 2.4	9.3 ± 2.5	0.95
REC (mm)	1.2 ± 1.6	1.1 ± 1.4	0.92
CEJ-BD (mm)	10.5 ± 2.4	10.9 ± 2.5	0.61
INTRA (mm)	6.1 ± 1.9	6.0 ± 2.0	0.81
WIDTH (mm)	4.2 ± 0.7	3.8 ± 1.0	0.15

PPD: Pocket Probing Depth
PAL: Probing Attachment Level
REC: Soft tissue recession from the Cemento Enamel Junction (CEJ) to the gingival margin
CEJ-BD: Vertical linear distance from the CEJ to the bottom of the intrabony defect
INTRA: Vertical linear distance of the intraosseous component from the alveolar bony crest to the bottom of the defect
WIDTH: Horizontal linear distance from the root surface to the marginal bone at the level of the alveolar crest
EMD: Enamel Matrix Derivative
DBBM: Deproteinized Bovine Bone Mineral
GTR: Guided Tissue Regeneration

Surgical outcomes

No post-surgical healing complications were noted in the EMD+DBBM group. However, 4 (20%) collagen membrane exposures occurred in the GTR group one week after the surgical intervention. In these cases the exposed membranes were treated with additional topical application of 1% chlorexidine gel two times per day for 2 weeks.

Outcomes after 12 months

After 12 months, the mean change in FMPS in the EMD+DBBM group (0.4±1.6%) was not statistically significantly different (p=0.92) compared with that in the GTR group (0.3±1.3%). The mean change in FMBS in the EMD+DBBM group (4.3±8.4%) was not statistically significantly different (p=0.21) compared with that in the GTR group (1.8±0.9%) (Table3). After one year, the mean PD change at sites treated with EMD+DBBM was not statistically significantly different (p=0.79) compared with that at sites treated with GTR (4.6±1.9 mm vs. 4.4±1.7 mm). Similarly, the mean CAL change at sites treated with EMD+DBBM was not statistically significantly different (p=0.82) compared with that at sites treated with GTR (3.8±1.6 mm vs. 3.7±1.2 mm). The mean change in gingival recession (REC) in the EMD+DBBM group (0.6±1.0 mm) did not differ statistically significantly (p=0.74) from that observed in the GTR group (0.7±0.9 mm) (Table 4).

Table 3. Mean changes ± standard deviation (SD) in Full-Mouth Plaque Scores (FMPS) and Full-Mouth Bleeding Scores (FMBS) between baseline and the one-year follow-up in subjects treated with EMD+DBBM and GTR, respectively

Parameter	Baseline	12-month follow-up	Change	*P*
FMPS (%)				
EMD+DBBM (n=20)	17.1 ± 2.5	16.7 ± 2.6	0.4 ±1.6	0.28
GTR (n=20)	17.8 ± 2.0	17.5 ± 1.9	0.3 ± 1.3	0.26
P	0.37	0.34	0.92	
FMBS (%)				
EMD+DBBM (n=20)	17.4 ± 7.9	13.1 ± 2.7	4.3 ± 8.4	0.03
GTR (n=20)	14.8 ± 2.6	12.9 ± 2.1	1.9 ± 0.9	< 0.001
P	0.17	0.80	0.21	

FMPS: Full-Mouth Plaque Score
FMBS: Full-Mouth Bleeding Score
SD: Standard Deviation
EMD: Enamel Matrix Derivative
DBBM: Deproteinized Bovine Bone Mineral
GTR: Guided Tissue Regeneration

Table 4. Mean changes ± standard deviation (SD) in pocket probing depth (PPD), probing attachment level (CAL) and marginal soft tissue recession (REC) between baseline and the one-year follow-up at sites treated with EMD+DBBM and GTR, respectively

Parameter	Baseline	12-month follow-up	Change	*P*
PPD (mm)				
EMD+DBBM (n=20)	8.2 ± 1.9	3.6 ± 1.1	4.6 ± 1.9	< 0.001
GTR (n=20)	8.1 ± 2.1	3.7 ± 1.3	4.4 ± 1.7	< 0.001
P	0.94	0.79	0.79	
PAL (mm)				
EMD+DBBM (n=20)	9.3 ± 2.4	5.5 ± 2.7	3.8 ± 1.6	< 0.001
GTR (n=20)	9.3 ± 2.5	5.6 ± 2.3	3.7 ± 1.2	< 0.001
P	0.95	0.85	0.82	
REC (mm)				
EMD+DBBM (n=20)	1.2 ± 1.6	1.8 ± 2.0	0.6 ± 1.0	0.01
GTR (n=20)	1.1 ± 1.4	1.8 ± 1.8	0.7 ± 0.9	0.002
P	0.92	0.93	0.74	

SD: Standard Deviation
EMD: Enamel Matrix Derivative
DBBM: Deproteinized Bovine Bone Mineral
GTR: Guided Tissue Regeneration
PPD: Pocket Probing Depth

PAL: Probing Attachment Level
REC: Soft tissue recession from the Cemento Enamel Junction (CEJ) to the gingival margin

Conclusion

In conclusion, the combined application of EMD+DBBM yielded comparable clinical outcomes in terms of CAL gain and PD reduction in the management of deep non-contained intrabony defects compared with the application of DBBM and a collagen barrier membrane.

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