Enamel Matrix Proteins and Bovine Porous Bone Mineral in the Treatment of Intrabony Defects: A Comparative Controlled Clinical Trial

G. Zucchelli,* C. Amore,* L. Montebugnoli,* and M. De Sanctis*

Background: Various clinical studies have demonstrated that applying commercially available enamel matrix proteins (EMP) on the instrumented root surface during access flap surgery promotes clinically significant gains of clinical attachment and bone in intrabony defects. The aim of the present controlled clinical trial was to evaluate the adjunctive effect of filling the intrabony lesion with bovine porous bone mineral (BPBM) to a simplified papilla preservation (SPP) flap and EMP surgical procedure.

Methods: Sixty deep interproximal intrabony lesions in 60 patients with chronic periodontitis were treated with the SPP flap and EMP. In the 30 test defects, the intrabony component was filled with BPBM particles previously reconstituted with the EMP gel. A stringent infection control program was adopted for 1 year. The clinical and radiographical reevaluation was made 1 year after surgery.

Results: Both techniques resulted in clinically and statistically significant improvements between baseline and 1 year, in terms of clinical attachment level (CAL) gain, probing depth (PD) reduction, and radiographic bone fill; however, the BPBM test treatment showed statistically significantly greater CAL (5.8 ± 1.1 versus 4.9 ± 1.0) and radiographic bone (DEPTH) level gains (5.3 ± 1.1 versus 4.3 ± 1.5), and less increase in gingival recession (0.4 ± 0.6 versus 0.9 ± 0.5) than the control surgical procedure.

Conclusion: The present study data supported the hypothesis that the adjunctive use of BPBM in grafting intrabony defects has the ability to improve clinical and radiographical outcomes achievable with EMP alone. *J Periodontol 2003;74:1725-1735.*

KEY WORDS

Bone reconstruction; clinical trials, controlled; furcation/surgery; furcation/therapy; proteins, enamel matrix; soft tissue/surgery; surgical flaps.

* Department of Stomatologic Science, Bologna University, Bologna, Italy.

The ultimate goal in periodontal therapy is the regeneration of tooth-supporting apparatus which have been destroyed due to periodontal disease.[1] Several treatment procedures including the use of various bone grafts,[2-4] bone substitute materials,[5] guided tissue regeneration (GTR),[6,7] combination of bone grafts or bone substitutes and GTR,[8-10] and growth factors have been suggested and utilized with varying degrees of success to achieve this goal.[11-16]

The ideal graft material is the one that is osteoinductive, absorbable, easy to handle, and available in large quantities.[17] Autogenous bone meets the first three requisites, but obtaining the needed amount from an intraoral site is often problematic and, even if possible, is not well accepted by the patient. Allografts and alloplastic materials have been extensively used in periodontal regenerative surgery with the primary objective of reducing patient morbidity. Allografts have shown positive results in terms of promoting periodontal regeneration[11] but their osteinductive capabilities have been shown to be quite variable.[18,19] Alloplasts, even though effective in clinical resolution of periodontal defects,[20] have been shown to work as bone filler and the histologic evaluation indicates that these grafts heal almost exclusively with connective tissue encapsulation and not with true periodontal regeneration.[21-23] More recently, a bovine derived xenograft has been introduced into periodontics. This material has an unlimited supply and proven safety.[24-26] It is prepared by protein extraction of bovine bone, but maintains the natural structure of bone.[27] When evaluating parameters such as inner surface area, porosity, crystalline size, and calcium-to-phosphorous ratio, bovine porous bone mineral (BPBM) most closely resembles human cancellous bone as compared to other allografts or synthetic hydroxylapatite materials.[28]

Several studies documented the ability of BPBM to enhance bone formation in sinus elevation procedures,[29] around implants,[30] and in critical-sized osseous defects.[31] In periodontal regeneration, BPBM has been shown to improve clinical attachment levels and reduce probing depth when used alone[32,33] or in combination with absorbable membranes.[17,32,34] True histologic regeneration has been demonstrated in humans when using BPBM alone[35,36] but more complete and more predictable histologic periodontal regeneration was achieved when BPBM was used in combination with bioabsorbable membranes[35] and even more with autogenous bone and resorbable membranes.[37]

Another way to address periodontal regeneration is to mimic the process that takes place during the development of the nascent root and periodontal tissues. The discovery of the presence of the enamel matrix layer between the peripheral dentin and the developing cementum, together with the capability of enamel matrix proteins to induce acellular cementum, periodontal ligament, and alveolar bone formation, has provided the fundamental concept for enamel matrix derivative-supported tissue engineering in regenerative periodontal therapy.[38] Findings from various clinical studies[39-44] indicated that topical application on the disease root surface of commercially available enamel matrix proteins (EMP) during access flap surgery promoted clinically significant gains of clinical attachment and bone in intrabony defects.[40,43,44] Furthermore, prospective controlled clinical trials[39,42,45] have demonstrated that these gains are significantly greater than those expected from access flap alone and close to those achievable with self-supporting, non-resorbable membranes when specifically designed flaps to preserve interdental soft tissues are performed.[45] New attachment formation following treatment of intrabony periodontal defects with EMP has been demonstrated in both animal[38,46] and human[47-49] studies.

Combining osseous grafting with EMP has the potential to result in a synergistic effect of both materials. This assumption is based upon the fact that two distinct wound healing processes may take place together in a given vertical bony defect: while the graft material may act as osteoinductive and/or osteoconductive together with maintaining defect space, EMP can work at the root level, promoting new cementum and new attachment apparatus formation. This hypothesis was partially tested in a clinical study on the treatment of deep vertical bony defects in which EMP, combined with BPBM, resulted in statistically and clinically more favorable results than access flap surgery.[50]

In order to demonstrate that a synergetic effect does exist when combining EMP and BPBM, it is necessary to evaluate whether the additional application of one of the two materials improves the results achieved when a single material is used. In particular, since the primary objective in periodontal regenerative therapy is the restoration of the lost attachment apparatus, is it worthwhile to evaluate whether the adjunctive use of BPBM to EMP therapy can be of some clinical benefit with respect to the use of EMP alone.

The aim of the present prospective randomized controlled clinical trial was to compare the clinical efficacy in the treatment of deep intrabony periodontal defects (in terms of CAL gain, PD reduction, and radiographic bone fill) of a surgical procedure comprised of EMP, BPBM, and the simplified papilla preservation (SPP)[51] flap with that observed using the same flap design (SPP) and EMP alone.

MATERIALS AND METHODS

Experimental Design

Two different approaches for the treatment of deep intrabony defects were compared in a randomized controlled clinical trial. The same surgical access to the bony defect (simplified papilla preservation flap, SPP)[51] and topical application of EMP[†] on the root surface were performed in both patient groups. The only difference between test and control groups was that the test defects were filled with BPBM.[‡]

Clinical outcomes were longitudinally followed for 1 year. To avoid randomization imbalances, vertical bony defects were assigned to the two treatment groups after controlling for two prognostic factors: depth of the intrabony component (INFRA) and clinical attachment level (CAL).[52-54]

Study Population

The participants were selected among patients seeking care for moderate to advanced periodontal disease at the department of Periodontology, University of Bologna and a private practice in Florence. Patients with systemic disease, who smoked >20 cigarettes/day, received antibiotics in the 6 months preceding the study, or with a full-mouth plaque score (FMPS) and full-mouth bleeding score (FMBS) >25% after initial preparation therapy were excluded. Following initial preparation which consisted of oral hygiene instruction and scaling and root planing, 60 patients (26 males and 34 females, 34 to 62 years of age; mean age 46.2 ± 8.4) with severe chronic periodontitis[55] (clinical attachment loss >5 mm)

† Emdogain, Biora AB, Malmö, Sweden.
‡ BioOss, Geistlich Pharma AG, Wolhusen, Switzerland.

were enrolled, on a consecutive basis, in this clinical study. The study was approved by the Ethics Committee of Bologna University, and all patients gave informed consent. One tooth per patient, located in the interproximal area, associated with an angular bony defect (radiographic intrabony component >3 mm) and a probing attachment loss >6 mm was identified. Defects did not extend into a furcation. The 60 teeth consisted of 26 incisors, 18 cuspids, 10 bicuspids, and 6 molars. Forty teeth were located in the maxillary arch.

Baseline FMPS was 11.3 ± 2.4 and FMBS was 11.2 ± 1.8. Twenty patients smoked >10 cigarette a day; the other 40 patients did not smoke.

Investigator Calibration

A calibration exercise was performed to obtain acceptable intraexaminer reproducibility for probing depth, recession of the gingival margin, and radiographic depth of the defect. Intraexaminer reproducibility was evaluated as the standard deviation of the difference of triplicate measurements. The investigators reached the target of a standard deviation <0.4 mm.

Radiographic Measurements

A commercially available film holder device[§] and customized acrylic-made bite blocks were used to take standardized radiographs of all teeth included in the study immediately before surgery and at the 1-year follow-up visit.

The following linear radiographic measurements were taken:

1. Depth of the intrabony component of the defect (DEPTH) measured as the vertical distance in mm from the bone crest and the most apical extension of the defect where the periodontal ligament space was considered as having a normal width.[56]
2. Vertical width of the defect (ANGLE) determined (in degrees) as the angle defined by the root surface and the vertical bony wall of the defect.[40]

Radiographic measurements were performed by a single calibrated examiner.

Clinical Characterization of Patients and Selected Sites

Full-mouth plaque score (FMPS) was recorded as the percentage of total surfaces (four aspects per tooth) that revealed the presence of plaque.[57] Full-mouth bleeding score (FMBS) was recorded as the percentage of total surfaces (four aspects per tooth) that revealed the presence of bleeding upon probing, assessed dichotomously at a force of 0.3 N with a manual pressure-sensitive probe.

The following clinical measurements were taken 1 week before the surgery and at 1 year follow-up: Clinical attachment level (CAL) measured from the cemento-enamel junction (CEJ); probing depth (PD) measured from the gingival margin; and marginal gingival recession (REC) measured from the CEJ to the gingival margin. A single investigator masked to the treatments performed all clinical measurements.

Measurements were performed at six sites around all teeth; the study, however, reports only local measurements at the deepest interproximal point of the selected defects. All measurements were performed by means of a manual pressure sensitive probe and were rounded up to the nearest millimeter.

Clinical Measurements at Baseline

The following clinical measurements were taken at the time of the surgery immediately after defect debridement:[54] 1) distance from the CEJ to the bottom of the defect (CEJ-BD); 2) distance from the CEJ to the most coronal extension of the bone crest (CEJ-BC). The intraosseous component of the defects was defined as INFRA = (CEJ-BD) – (CEJ-BC).

Randomization

Before surgery, assignment to the two treatment regimens (30 patients/group) was performed using a custom made program based on balanced permuted blocks.[41] Blocking to control for the effects of the prognostic variables INFRA and CAL was used to decrease outcome variability.[38,39,52,58,59] These two variables were categorized to make blocks as follows: CAL < or ≥10 mm, INFRA ≤ or >6 mm. For randomization purposes INFRA was estimated before surgery on radiographs and by performing transgingival bone sounding. Furthermore, to reduce the chance of unfavorable splits between test and control groups in terms of key prognostic factors, the randomization process balanced smoking status and location of the defect at the upper first premolars in the test and control groups.[41]

Surgical Procedures (Figs. 1 and 2)

The access to the bony defects of all patient's groups was achieved with the simplified papilla preservation technique previously described.[51] Following careful scaling, root planing, and debridement of the bony defect, the exposed root surface was conditioned with a 24% EDTA gel for 2 minutes in order to remove the smear layer. The root was subsequently rinsed with saline. The EMP gel in 0.7 ml syringes was gently applied on the root surface in the apical-coronal direction and left in place for 2 minutes during which bleeding from the adjacent areas was controlled with the use of gauzes.

In the test group (Fig. 1), the EMP solution remaining in the syringe was used to reconstitute the cancellous BPBM material which had been prepared by pouring the particles into a sterile dappen dish. The resulting grafting material was extremely easy to handle because the gel acted as sealing material. The graft was gently packed into the defects using an amalgam

§ Rinn Centering Device, Dentsply Ltd., Weybridge, U.K.

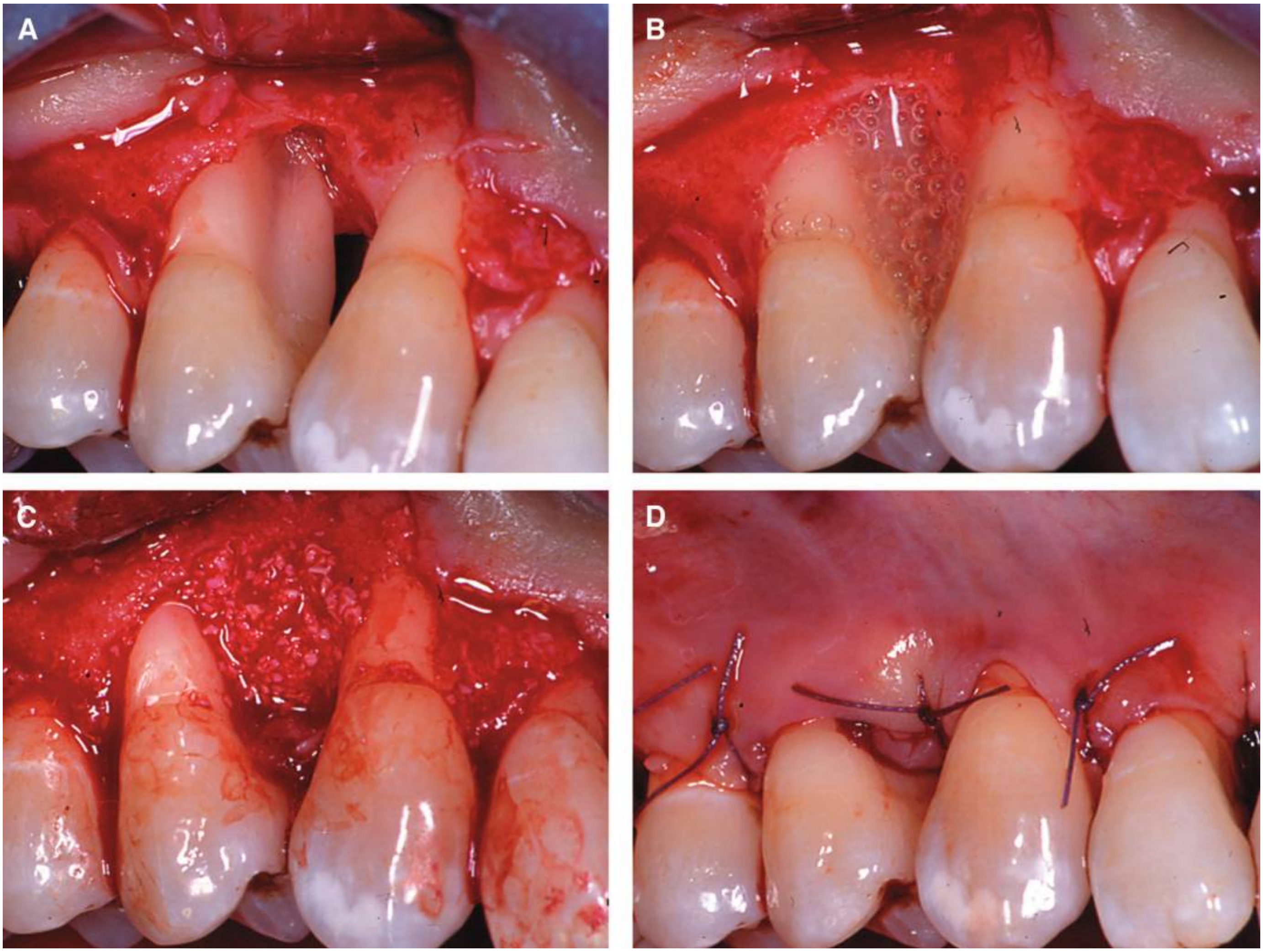

Figure 1.
*Surgical treatment, test site. **A)** The intraoperative clinical image shows a deep intrabony defect mesial to tooth #14. **B)** EMP was applied to the root surface facing the defect. **C)** BPBM was gently packed into the defects. Note the presence of supracrestal soft tissue above the graft material; this permitted soft tissue closure above the BPBM material. **D)** Complete closure of the interdental soft tissues was achieved.*

condenser. Experimental defects were filled up to the most coronal levels of the defect walls.

Great care was taken to obtain complete closure of the interdental soft tissues above the treated defects in both patient groups: flaps (buccal and lingual/palatal) were positioned slightly coronal with respect to the presurgical level in order to achieve primary closure of the interdental area without any tension. This was done by performing blunt dissection of the vestibular lying mucosa which permitted coronal displacement of the buccal flap. Flaps were sutured using resorbable #5-0 and #6-0 suturing material. The selection of the suturing technique for the interdental tissues covering the defect, internal offset vertical mattress suture,[51] interrupted single suture, or both, was based upon the dimension of the interdental space and the thickness and height of the interdental soft tissues.

Infection Control

Patients were given amoxicillin plus clavulanic acid[i] 1 g per day starting the day before surgery and for the next 6 days. Sutures were removed 14 days following surgery.

All patients were instructed to rinse with a 0.12% solution of chlorhexidine twice a day for 11 weeks; during this period, they were recalled once a week for professional tooth cleaning.

Plaque Control

When chlorhexidine was discontinued, full mechanical interproximal cleaning in the surgically treated area was reinstituted. Patients were recalled for professional tooth cleaning and reinforcement of self-performed oral hygiene measures at 1-month intervals up to the 1-year

i Augmentin, Smith Kline Beecham, S.p.a., Milan, Italy.

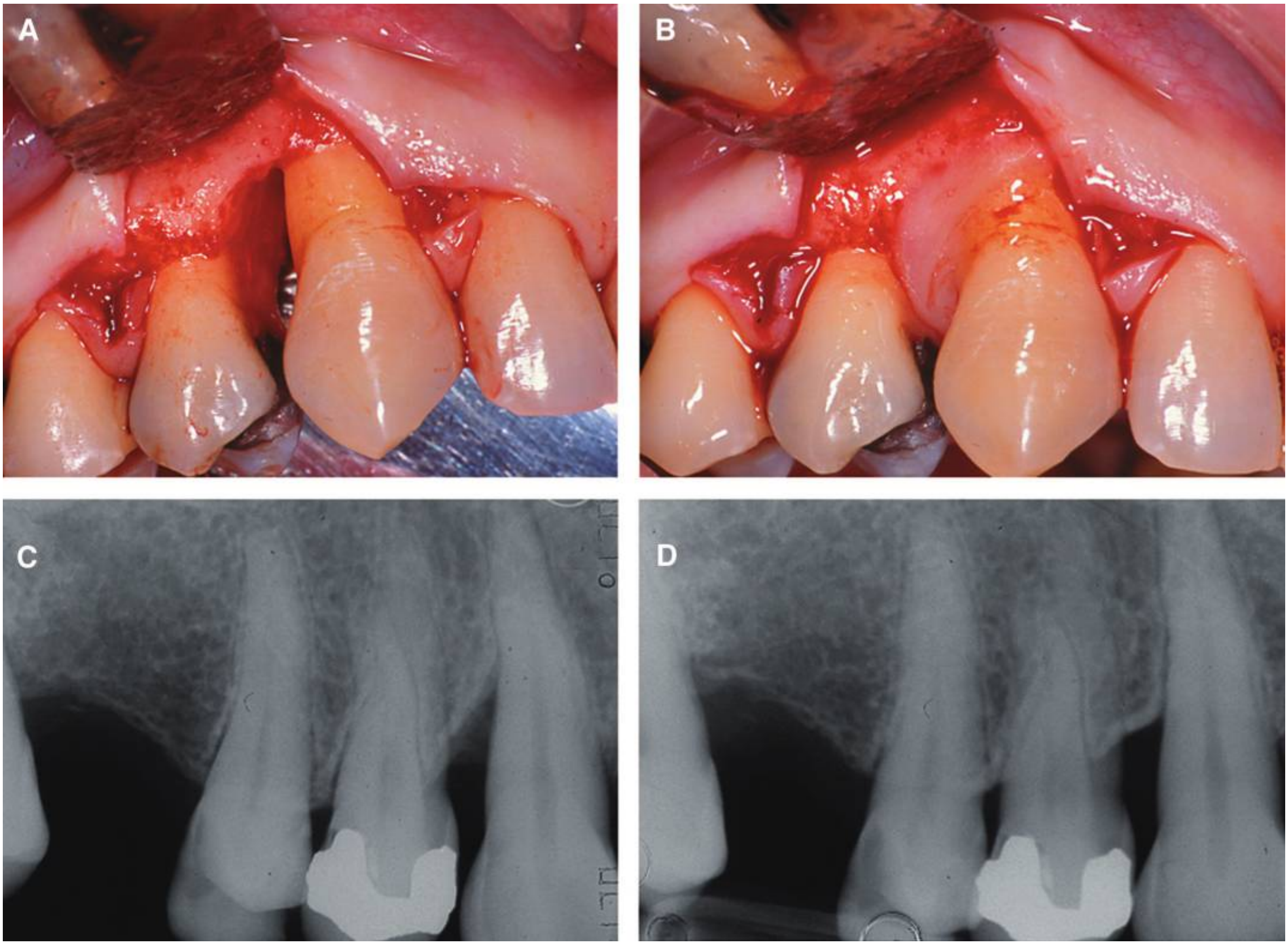

Figure 2.
Surgical treatment, control site. ***A)*** *The intraoperative clinical image shows a deep intrabony defect distal to tooth #13.* ***B)*** *EMP was applied to the root surface facing the defect.* ***C)*** *Preoperative radiograph.* ***D)*** *One-year radiograph shows almost complete bone fill. Interdental bone crest (highly radio-opaque) maintains an oblique outline; this may be ascribed to interdental soft tissue collapse into the intrabony defect.*

reevaluation. No attempt at probing or deep scaling was made before this follow-up.

Data Analysis

Statistical software¶ was used for the statistical analysis. Data were expressed as mean values ± standard deviation. A power analysis indicated that a minimum of 48 unpaired defects (24 in each group) would be needed in this study to demonstrate statistical significance at the $P<0.05$ level with a power of 0.85. The following outcome and predictor variables were defined as: 1) CAL gain = baseline CAL – 1 year CAL; 2) DEPTH gain = baseline DEPTH – 1 year DEPTH; 3) PD reduction = baseline PD – 1 year PD; and REC increase = 1 year REC – baseline REC.

The normality assumption was verified and the presence of any randomization imbalance between the two experimental groups was tested by one-way analysis of variance (ANOVA) and chi-square analysis.

A new variable, representing a surrogate measurement of the amount of supracrestal soft tissues (SUPRA), was calculated as follows: SUPRA = (CEJ-BC) – REC.[56]

General linear models were fitted relating 1 year CAL gain, DEPTH gain, REC increase, and PD reduction to three categorical (technique, smoking status, and tooth type, anterior versus posterior) and six continuous (FMPS, FMBS, CAL, INFRA, SUPRA, ANGLE) factors as covariates (ANCOVA). Factors affecting 1-year CAL gain were also evaluated for each surgical approach adopted in the present study by fitting a general linear model. For both techniques two categorical (smoking status and tooth type) and six continuous (FMPS, FMBS,

¶ SAS, Version 6.09, SAS Institute, Cary, NC.

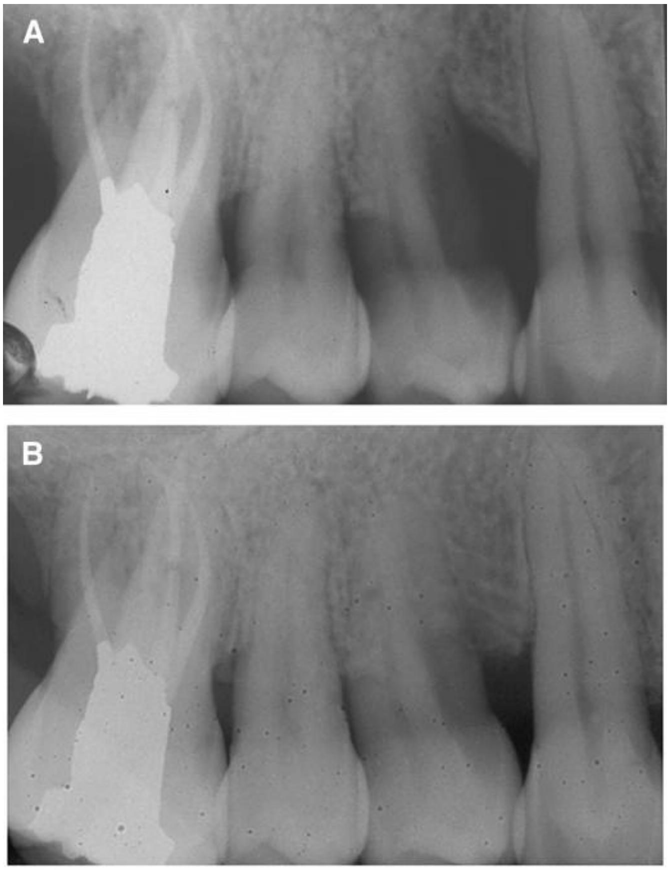

Figure 3.
*Preoperative **(A)** and 1-year radiographs **(B)** in a control patient. Note that complete bone fill was achieved. Very few graft residuals can be recognized in the most coronal portion of bone filling the defect.*

CAL, INFRA, SUPRA, ANGLE) covariates were included. Unpaired Student *t* test was applied to compare clinical outcomes in terms of CAL gain, DEPTH gain, REC increase, and PD reduction achieved in patients with and without BPBM residuals in the 1-year radiographs.

RESULTS

Study Population

Mean age in the test and control groups was 47.2 ± 5.3 and 45.8 ± 6.8, respectively. There were 18 female patients in the test group and 16 in the control group. Sixteen incisors, eight cuspids, four bicuspids and two molars were treated in each group. There were 10 smokers in each group. None of the patients exited the study before the 1-year examination.

Baseline Oral Hygiene and Defect Characteristics (Table 1)

No statistically significant difference was observed among the two matched experimental groups in any of the considered clinical parameters, indicating that the randomization process was effective.

Baseline FMPS in the test group was 12.8 ± 2.4, and 11.8 ± 2.8 in the control group. FMBS was 11.6 ± 2.2 and 10.8 ± 1.6 for the test and control groups, respectively. Baseline CAL was 10.3 ± 1.5 in the test group and 10.1 ± 1.4 in the control group. The depth of the infrabony component (INFRA) of the defects was 6.7 ± 1.0 and 6.8 ± 0.8 in the test and control groups, respectively.

Early Healing Events

All sites healed uneventfully. No wound edge necrosis or flap dehiscence was observed in any patient.

Oral Hygiene at 1 Year

No significant difference between the two groups was found in the FMPS and FMBS mean values recorded at 1 year. FMPS was 10.2 ± 1.2 in the test and 10.8 ± 1.6 in the control group. FMBS was 9.9 ± 1.2 in the test group and 10.2 ± 1.4 in the control group. A comparison between the individual mean plaque and bleeding scores calculated from the baseline and the 12-month follow-up revealed that no marked change had occurred in the oral hygiene status in any patient.

Clinical Changes at 1 Year (Table 2)

The significance of factors affecting 1-year CAL gain, DEPTH gain, PD reduction, and REC increase was evaluated by adopting general linear models.

CAL gain. Sum of squares, degrees of freedom, mean square, F value, and *P* level for each variable entering the model are reported in Table 3.

The R-squared statistic indicates that the model as fitted is highly significant and explains 85.78% of the variability in CAL gain.

One of the most significant variables entering the model and affecting the CAL gain at 1 year was the periodontal procedure (F = 27.92). In particular, a CAL gain of 5.8 ± 1.1 (range 4 to 9 mm) was obtained in the test group and a CAL gain of 4.9 ± 1.0 (range 3 to 7 mm) was achieved in the control group.

Other significant variables entering the model and affecting CAL gain were SUPRA, SMOKE, and INFRA. A greater amount of CAL gain was expected in non-smoking patients and in cases with deeper infrabony component and with higher amount of supracrestal soft tissue at baseline.

SUPRA was a significant factors affecting 1 year CAL gain even when test and control surgical techniques were considered separately (respectively: F = 24.36 and F = 4.33), while defect ANGLE (F = 17.41) was demonstrated to be a significant variable only for the control procedure. In the EMP-control surgical approach, better results in terms of CAL gain were achieved in vertical bony defect with a lower degree of defect angle measured at baseline.

Table 1.

Baseline Oral Hygiene and Defect Characteristics (means ± SD)

	EMP (control) (n = 30)	EMP + BPBM (test) (n = 30)	*P*
FMPS (%)	11.8 ± 2.8	12.8 ± 2.4	NS
FMBS (%)	10.8 ± 1.6	11.6 ± 2.2	NS
CAL (mm)	10.1 ± 1.4	10.3 ± 1.5	NS
PD (mm)	9.2 ± 1.1	9.4 ± 1.1	NS
REC (mm)	0.9 ± 0.9	0.9 ± 0.7	NS
CEJ-BD (mm)	11.4 ± 1.7	11.7 ± 1.7	NS
INFRA (mm)	6.8 ± 0.9	6.7 ± 1.0	NS
DEPTH (mm)	6.1 ± 1.3	6.1 ± 1.2	NS
ANGLE (degrees)	33.1 ± 6.0	34.4 ± 5.3	NS
SUPRA (mm)	3.6 ± 1.0	4.0 ± 0.9	NS

Table 2.

Clinical Changes at 1 Year (means ± SD; in mm)

	EMP (control) (n = 30)	EMP + BPBM (test) (n = 30)	*P*
CAL gain	4.9 ± 1.0	5.8 ± 1.1	<0.01
PD reduction	5.8 ± 0.8	6.2 ± 0.4	NS
REC increase	0.9 ± 0.5	0.4 ± 0.6	<0.01
DEPTH gain	4.3 ± 1.5	5.3 ± 1.1	<0.01

Table 3.

Analysis of Variance for CAL Gain

Source	Sum of Squares	Df	Mean Square	F Ratio	*P* Value
Model	63.4223	9	7.04692	33.52	0.0000
Residual	10.5111	50	0.210221		
Total (Corr.)	73.9333	59			

Source	F Ratio	*P* Value
TECHNIQUE	27.92	0.0000
SMOKE	8.07	0.0065
TOOTH	2.59	0.1140
FMBS	1.49	0.2285
FMPS	0.01	0.9066
CAL	1.22	0.2740
INFRA	19.62	0.0001
SUPRA	51.20	0.0000
ANGLE	2.20	0.1439

DEPTH gain. The R-squared statistic indicates that the model as fitted is highly significant and explains 89.86% of the variability in DEPTH gain.

The surgical approach was one of the most significant variables entering the model and affecting the DEPTH gain at 1 year (F = 53.21; *P* <0.01). A DEPTH gain of 5.3 ± 1.1 (range 4 to 8 mm) was obtained in the test group and a DEPTH gain of 4.3 ± 1.5 (range 2 to 8 mm) was achieved in the control group (Fig. 3).

Other significant variables entering the model and affecting DEPTH gain were SUPRA (F = 32.77) and DEPTH (F = 164.71). In other words, a greater amount of bone gain was expected in defects with a greater infrabony component, measured in the radiographs, and with higher amount of supracrestal soft tissue at baseline.

REC increase. A significant difference in REC increase was found between the two techniques (F = 11.38; *P* <0.01). In particular, the mean increase in gingival recession was 0.4 ± 0.6 in the test group (range 0 to 1 mm) and 0.9 ± 0.5 in the control group (range 0 to 2 mm).

The significant variables entering the model and affecting REC increase were SMOKE (F = 3.89), SUPRA (F = 10.57), and INFRA (F = 4.07). A lower increase in gingival recession was observed in nonsmoking patients and in cases with deeper infrabony component and with greater amount of supracrestal soft tissue at baseline.

PD reduction. The mean PD reduction was 6.2 ± 0.4 in the test group (range 5 to 9 mm) and 5.8 ± 0.8 in the control group (range 4 to 7 mm). No significant difference in PD reduction was found between the two techniques (F = 1.86).

No significant differences were found between patients with (n = 17) or without (n = 13) BPBM residuals at the 1-year radiographs in terms of CAL gain (5.9 ± 1.3 versus 5.7 ± 0.6; t = 0.47), DEPTH gain (5.4 ± 1.2 versus 5.3 ± 0.9; t = 0.26), REC increase (0.4 ± 0.5 versus 0.5 ± 0.7; t = 0.51), and PD reduction (6.2 ± 1.1 versus 6.2 ± 0.7; t = 0.23).

Table 4 displays the frequency distribution of CAL gain for the two treatment groups. In the EMP + BPBM test group, 47% of sites gained ≥6 mm of CAL. This

Table 4.

Frequency Distribution of CAL Gain at 1 Year

CAL Gain (mm)	EMP	EMP + BPBM
<2	0%	0%
≤2 to >4	6%	0%
≤4 to >6	67%	53%
≥6	27%	47%

Table 5.

Frequency Distribution of DEPTH Gain at 1 Year

DEPTH Gain (mm)	EMP	EMP + BPBM
<2	0%	0%
≤2 to >4	36%	0%
≤4 to >6	47%	57%
≥6	17%	43%

compares favorably with 27% of sites from EMP alone group (chi-square 13.15; $P<0.05$).

Table 5 displays the frequency distribution of DEPTH gain for the two experimental groups. All sites treated with EMP + BPBM gained at least 4 mm of bone. This compares favorably with 64% of sites from the group treated with EMP alone (chi-square 15.4; $P<0.05$). Furthermore about half (43%) of the test sites showed a ≥6 mm gain in radiographic bone.

DISCUSSION

The aim of the present controlled clinical trial was to evaluate the adjunctive effect of filling the intrabony defect with BPBM to the same surgical procedure, interdental soft tissue preservation flap and topical application of EMP to the exposed root surface, without BPBM, in 60 deep interproximal intrabony lesions.

Control treatment consisting of open flap debridement was not performed since numerous clinical studies have documented the superiority of EMP treatment to access flap surgery.[39-45,56] Furthermore, a recent clinical study[50] has shown that combining EMP and BPBM as a regenerative procedure for intraosseous defects results in more favorable clinical and radiographic outcomes than open flap debridment surgery. In this study, however, it was not possible to determine which of the materials utilized, EMP or BPBM, was responsible for the better results. The selection of identical EMP-based surgical techniques in test and control patient groups permitted us to attribute differences observed in the test sites to the bone substitute material being implanted, and not to the surgical procedure employed.

The two treatments in the present study resulted in clinically significant improvements between baseline and 1 year, in terms of CAL, PD, and radiographic bone level.

The test treatment, however, showed statistically significant greater CAL gain, radiographic bone level gain (DEPTH gain), and lower increase in gingival recession. The clinical superiority of the test compared to the control treatment can also be gathered from the frequency distribution data which indicated that all sites treated with EMP + BPBM gained at least 4 mm of bone, comparing favorably with 64% of sites from the group treated with EMP alone. Furthermore a 6 mm or more gain in CAL and radiographic bone were obtained in a higher percentage of the EMP + BPBM test cases compared to the EMP control treated cases. Therefore, the hypothesis that osseous grafting with BPBM used in combination with EMP has the ability to improve clinical outcomes achievable with EMP alone was supported by present study data.

The observed CAL gain (4.9 mm) and radiographic bone level gain (4.3 mm) in the EMP control group compares well with the most recent literature[45,56] in which EMP was used in combination with surgical techniques based on interdental soft tissue preservation, while they differ considerably from other previous reports[39-44] where intrabony defects were treated with more conventional surgical access and EMP application.

The importance of supracrestal soft tissues preservation in regenerative procedures has been demonstrated both for membrane-supported[45,51,58] and EMP-based[45,56] surgical procedures and it was further confirmed both by Trombelli et al.[56] and the present data, which indicated a significant positive correlation between CAL and DEPTH gains and the amount of interdental supracrestal soft tissues (SUPRA).

The presence of thick and high/wide interdental soft tissues facilitates flap management and suturing technique, improves the possibility to achieve and maintain primary closure in the interdental area, and reduces the risk of soft tissue collapse into the bone defect, thus optimizing available space for regeneration.[56]

It can be speculated that the better results (greater CAL and bone level gains and lower increase in gingival recession) obtained in the test defects of the present study may be attributed, at least in part, to the space-maintenance properties of BPBM. The BPBM graft, in fact, may have acted as a rigid barrier avoiding the collapse of the interdental soft tissues into the intrabony defect, before being integrated and subsequently substituted by new bone. This might have reduced the recession of the soft tissue margin and increased the amount of hard tissue regeneration. This role of BPBM in maintaining space can be of particular importance when treating wide and less supportive bone defects in which the risk of collapse of the interdental soft tissues (even though entirely preserved during the surgery) into the vertical defect is greater. This hypothesis was supported by present study data, which indicated a negative influence of defect angle on the amount of CAL gain in the control non-grafted and not in the test-grafted sites. The space-maintenance capability of BPBM has been previously demonstrated in ridge augmentation,[60] sinus elevation,[29] and periodontal regeneration[34] studies.

On the other hand, caution should be exercised when interpreting the significance of postoperative CAL and radiographic bone fill measurements. In fact, it cannot be excluded that placement of a graft material into the defect may modify gingival tissue consistency and, therefore, limit periodontal probe penetration without necessarily having induced any gain in tissue attachment.[50] Furthermore, the remaining graft material may prevent adequate soft tissue (CAL) measurements.[34] Most of the 1-year radiographs of the present study clearly demonstrated the presence of recognizable BPBM particles within the repaired bone filling the intrabony defects. Since the resorption rate of BPBM is very slow, "bone fill" of the present defect probably consisted of a combination of bovine bone particles and regenerating vital human bone.[34] This hypothesis was supported by both animal[30] and human[35] histological studies on the osteoinductive capability of BPBM. These reports showed that bovine bone mineral particles were still present 6[30] and 9[35] months after implantation, although well incorporated by ingrowth of new lamellar bone, and were progressively replaced by new bone.[29]

The speed by which BPBM graft particles were replaced by new bone seemed to vary among patients in the present study: only 43% of the patients did not show visible graft residuals in the 1-year radiographs. The lack of histological and re-entry data did not allow us to determine if graft residuals were also present but not recognizable in the radiographs. In patients with recognizable graft particles, clinical changes (CAL gain, DEPTH gain, PD reduction, and REC increase) were not statistically different from those in patients not showing BPBM residuals. Further studies with a longer follow-up period are needed to evaluate the clinical significance and the fate of persistent graft material.

The amount of CAL and radiographic bone level gain achieved in the test group of the present study are greater than those reported by Camargo et al.[50] Differences between these studies may, at least in part, be explained by patient (number of smokers) and defect (depth of the intrabony component) characteristics, the maintenance phase for infection control, surgical technique, and follow-up period.

A limitation of clinical periodontal regeneration studies, such as the present one, is the inability to assess the histologic characteristics of the repaired tissues including the nature of the attachment between the newly formed bone and the previously diseased root surface. Histologic new attachment formation has been demonstrated with either EMP[39,47,48] or BPBM,[35,37] but the predictability of such an outcome, especially when the two "regenerative" materials are combined, is still to be investigated.

In conclusion, results from the present study confirm that a regenerative procedure based on an interdental soft tissue preservation flap and EMP application leads to clinically and statistically significant gains in clinical attachment and radiographic bone fill and suggest the positive effect of the adjunctive implantation of BPBM into the intrabony component of the defects in improving both hard and soft tissue condition of deep angular periodontal defects.

Further studies should be performed in order to determine: 1) the type of periodontal healing that occurs in human intrabony periodontal defects when using a combination of EMP and BPBM and 2) the fate of bovine bone particles still recognizable in the 1-year radiographs.

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Correspondence: Dr. Giovanni Zucchelli, Dipartimento di Scienze Odontostomatologiche, Bologna University, Via S. Vitale 59, 40125 Bologna, Italy. Fax: 39-051-225208; e-mail: giovanzu@tin.it.

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