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ORIGINAL ARTICLE

Clinical evaluation of anorganic bovine-derived hydroxyapatite matrix/cell-binding peptide (P-15) in the treatment of human infrabony defects

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Abstract The purpose of the present study was to compare the clinical outcomes of infrabony periodontal defects following treatment with an anorganic bovine-derived hydroxyapatite matrix/cell-binding peptide (ABM/P-15) flow to open flap debridement. Twenty-six patients, each displaying one infrabony defect with probing depth ≥6 mm and vertical radiographic bone loss ≥3 mm participated in the present study. Patients were allocated randomly to be treated with ABM/P-15 flow (test group) or open flap debridement (control group). At baseline and at 12 months after surgery, the following clinical parameters were recorded by a blinded examiner: plaque index, gingival index, probing depth (PD), clinical attachment level (CAL), and gingival recession. Both treatments resulted in significant improvements between baseline and 12 months, in terms of PD reduction and CAL gain ($p<0.001$). At 12 months following therapy, the test group showed a reduction in mean PD from 7.8±1.6 mm to 3.5±1.0 mm and a change in mean CAL from 8.5±2.1 mm to 4.6±1.2 mm, whereas in the control group the mean PD decreased from 7.5±0.8 mm to 4.9±0.7 mm and mean CAL from 8.2±1.2 mm to 6.4±1.4 mm. The test group demonstrated significantly greater PD reductions ($p=0.002$) and CAL gains ($p=0.001$) compared to the control group. In conclusion, treatment of infrabony periodontal defects with ABM/P-15 flow significantly improved clinical outcomes compared to open flap debridement.

Keywords Hydroxyapatite/therapeutic use · Grafts · Infrabony defects · Periodontal regeneration · Periodontal diseases

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Introduction

A complete and predictable regeneration of tooth supporting structures which have been destroyed due to periodontal disease is the main goal of periodontal therapy. A variety of treatment modalities including the use of bone replacement grafts [21, 45], root surface conditioning [4], guided tissue regeneration (GTR) with the use of barrier membranes [12, 22], and growth factors [18] have been proposed to promote the regeneration of periodontal tissues. Although GTR is one of the best documented regenerative approaches, the clinical application is often difficult and the outcome of regenerative therapy appears to be affected by several confounding factors [38, 40]. Thus, the bacterial contamination of exposed barrier membranes and the underlying healing tissues have been associated with reduced clinical outcomes [7, 34]. In order to overcome these issues, alloplastic materials which are synthetic, inorganic, biocompatible bone graft substitutes represent a possible alternative in the treatment of infrabony defects. Findings from clinical studies have indicated that the treatment of deep infrabony defects with alloplastic grafts may provide additional clinical benefits in terms of clinical attachment level (CAL) gains and probing depth (PD) reductions as compared to open flap debridement alone [20, 24, 41]. However, although these materials have demonstrated clinical effectiveness, functional periodontal

repair, and apparent defect fill, a predictable high rate of success in terms of regeneration has not yet been achieved [28, 44]. Thus, histologic studies demonstrated that healing often occurred with encapsulation of these graft materials in connective tissue without or just minimal bone formation and that the healing was mostly characterized by formation of a long junctional epithelium [10, 31, 36]. More recently, a new combination of a natural anorganic bovine-derived hydroxyapatite matrix (ABM) with a synthetic cell-binding peptide (P-15; PepGen® P-15, Dentsply Friadent, Mannheim, Germany) has been introduced as a bone graft material for regenerative periodontal surgery. ABM is a natural, microporous, xenoplastic bone mineral occurring in a particulate form. The peptide component is a synthetic analogue of the 15 amino acid sequence (GTPGPQGIAGQRGVV) of type I collagen that has been demonstrated to be involved in the binding of cells, particularly fibroblasts and osteoblasts [2]. The ABM/P-15 bone replacement graft material has been shown in vitro to enhance cell attachment and periodontal ligament fibroblast promotion [3, 35]. Furthermore, ABM/P-15 enhanced expression of alkaline phosphatase, as well as increased nucleic acid and protein synthesis in dermal fibroblasts grown on ABM/P-15 compared to cells grown on ABM alone [3, 26]. Clinical evaluation using the ABM/P-15 in its particulate form demonstrated better clinical results in infrabony defect fill compared to decalcified freeze-dried bone (DFDBA), open flap debridement and ABM alone [46, 47]. However, the particulate form of ABM/P-15 may be difficult to deliver to the surgical site and can be displaced or reduced in amount due to flap manipulation, which is mainly attributed to the lack of particle adhesion. In order to overcome these handling technical difficulties, ABM/P-15 flow was developed as a new formulation combining the original particles with a biocompatible inert carrier containing water, glycerol, and sodium carboxymethylcellulose. This gel consistency should facilitate handling and placement into defects. So far, there are still limited data from controlled clinical studies evaluating the healing of infrabony defects following treatment with ABM/P-15 in its flow formulation [1, 19].

Therefore, the purposes of the present study were to evaluate and compare the treatment of infrabony periodontal defects with a novel ABM/P-15 flow bone replacement graft (test group) with open flap debridement alone (control group).

Materials and methods

Experimental design and study population

A parallel group, randomized, controlled clinical trial was designed to test the efficacy of ABM/P-15 flow (PepGen® P-15 flow, Dentsply Friadent, Mannheim, Germany) in infrabony periodontal defects, as compared to conventional open flap debridement alone. Twenty-six generally healthy patients (14 males and 12 females, aged 32–65 years, mean age 51.7±7.7 years) seeking care for moderate to advanced periodontal disease were selected to participate in the present trial. Informed consent was obtained after informing patients verbally and in writing of the investigational nature of the test material and the need for increased documentation. The study was performed according to the Helsinki Declaration of 1975, as revised in 2000. The criteria for inclusion in the study were as follows: (1) absence of relevant medical conditions that contraindicated periodontal surgery; (2) no use of antibiotics during the previous 6 months; (3) no pregnancy or lactation; (4) optimal compliance (no missed treatment appointments), as assessed during cause-related therapy; (5) good oral hygiene standards, defined as a whole-mouth plaque index (PI)<1 [17]; and (6) presence of one infrabony defect with a PD≥6 mm and a radiographic depth of the defect ≥3 mm. Patients with teeth exhibiting furcation involvement, inadequate endodontic treatment, or overhanging margins were excluded from the study. None of the patients was a smoker [39]. Each patient first received cause-related therapy consisting of scaling and root planing, motivation, and oral hygiene instructions at least 3 months prior to the start of the study.

Clinical parameters

The following clinical parameters were evaluated at baseline before regenerative therapy and 1 year after surgery by a calibrated examiner. The examiner who performed the measurements was not the surgeon who provided the treatment and did not know which treatment was provided.

Clinical parameters measured included: plaque index, gingival index (GI) [17], probing depth, clinical attachment level, and soft tissue recession (REC). All measurements were recorded at six aspects per tooth (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual, disto-lingual) by using a standard periodontal probe (PCP 15, Hu-Friedy, Chicago, IL, USA). The cemento-enamel junction (CEJ) was considered as the reference point. Intraoral radiographs were taken before surgery and at the 1-year follow-up visit using the long cone paralleling technique.

Intra-examiner reproducibility

A calibration exercise was performed to obtain acceptable intra-examiner reproducibility for probing depth and recession of the gingival margin. Five patients, each with ten teeth (single and multirooted) with probing depth >6 mm on at least one aspect of each tooth, were used to calibrate

the examiner. The examiner evaluated the patients on two separate occasions, 48 h apart. Calibration was accepted if measurements at baseline and at 48 h were within a millimeter at >90% of the time.

Surgical procedure

All surgical procedures were performed under local anesthesia by the same surgeon (AK). The surgical procedure consisted of using sulcular incisions followed by the reflection of full-thickness mucoperiosteal flaps bucally and lingually. Vertical releasing incisions were placed only if they were considered necessary for a better access and/or to achieve a better closure of the surgical site. The defects were cleared of granulation tissue, and the exposed root surfaces were thoroughly scaled and root planed using ultrasonic and hand instruments. The surgical area was then rinsed with sterile saline. In no cases osteoplasty/ostectomy was carried out. Following complete debridement of the surgical sites, the following measurements were performed: (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 interproximal crest (CEJ-BC); and (3) bone crest to the bottom of the bony defect (INFRA). The measurements were recorded to the nearest millimeter at the deepest interproximal point of the defect. The defects were also classified according to the number of osseous walls remaining as 1, 2, or 3 walls based on the classification by Goldman and Cohen [11]. The defects were randomly assigned at the time of each surgical procedure to receive one of the two treatment procedures by the toss of a coin after all defect and root preparation had been completed. In the test sites, bleeding into the defects was reduced to a minimum and the sites were subsequently filled with ABM/P-15 flow, starting from the bottom of the defect. Care was taken in order to obtain direct contact between ABM/P-15 flow and the adjacent alveolar bone. Finally, the flaps were repositioned coronally and sutured tightly with vertical mattress sutures using non-resorbable # 5-0 suturing material (Premilene®, Braun Aesculap, Tuttlingen, Germany). No surgical dressing was used. The control sites were treated in the same way without graft placement.

Post-surgical infection control

The patients rinsed twice a day with a 0.2% chlorhexidine digluconate solution for 4 weeks. Postoperative pain was controlled with 600 mg ibuprofen (every 8 h as necessary). No additional antibiotic therapy was administered postoperatively. Patients were requested to avoid brushing, flossing, and chewing in the surgical area for a period of 4 weeks. Sutures were removed after 14 days. During the first 2 months, the patients were recalled every 2 weeks for oral hygiene control and professional tooth cleaning. After 2 months, recall visits occurred every 4 weeks. No attempt at probing or deep scaling was made before the 1-year follow-up visit.

Statistical analysis

All statistical analyses were performed using statistical software (SPSS, 12.0 for Windows, Chicago, IL, USA). Mean and standard deviation (SD) were calculated for each clinical parameter. In the calculations, the deepest site per tooth was included. For the statistical evaluation of the differences between the groups, the Wilcoxon–Mann–Whitney test was used. The Wilcoxon signed rank test was used to evaluate changes from baseline to 12 months for each treatment group. In all test procedures, a significance level of $p<0.05$ was considered statistically significant.

Results

A total of 26 patients (test group $n=13$, control group $n=13$) completed the 12-month follow-up period. The patient and defect characteristics of the test and control groups yielded no significant differences between any of the patient-associated variables (Table 1). In all treated sites, primary closure was obtained at completion of the surgical procedure. The postoperative healing was uneventful in all cases, and no complications or infections were observed throughout the study period.

The PI and GI values for both groups at baseline and after 12 months are summarized in Table 2. Following treatment, PI and GI mean values improved throughout the study period in both groups. At 12 months following therapy, the mean PI and GI was 0.5±0.5 in both study

Table 1 Baseline patient and osseous defect characteristics

Variables	Test ($N=13$)	Control ($N=13$)	p value
Age	50.7±9.2	52.7±6.1	–
PI	0.6±0.5	0.8±0.4	0.673
GI	0.9±0.6	1.0±0.6	0.844
PD (mm)	7.8±1.6	7.5±0.8	0.672
REC (mm)	0.7±0.8	0.8±1.0	0.999
CAL (mm)	8.5±2.1	8.2±1.2	0.888
CEJ-BD (mm)	7.8±2.1	6.9±1.1	0.285
CEJ-BC (mm)	4.0±1.9	3.3±1.4	0.476
INFRA (mm)	3.8±0.9	3.6±0.8	0.540
1 wall (%)	30.8	23.1	0.795
2 walls (%)	46.2	46.2	0.795
3 walls (%)	23.1	30.8	0.795

Table 2 Mean ± SD plaque and gingival index scores at baseline and the 12-month evaluation (N=13 patients in each group)

Variables	Baseline	12 months	Difference	p value
PI				
Test	0.6±0.5	0.5±0.5	−0.1±0.4	0.500
Control	0.8±0.4	0.5±0.5	−0.2±0.6	0.375
p value	0.673	1.000	0.645	
GI				
Test	0.9±0.6	0.5±0.5	−0.5±0.7	0.063
Control	1.0±0.6	0.5±0.5	−0.7±0.7	0.063
p value	0.844	1.000	1.000	

groups. These values indicated a trend toward decreasing PI and GI between baseline and 12 months in both groups, but the differences were not statistically significant (p>0.05).

CEJ-BD distance was 7.8±2.1 mm (range 5–12 mm) for the test defects compared to 6.9±1.1 mm (range 6–9 mm) for the control defects. The infrabony (INFRA) mean values for the test and control groups from the deepest site were 3.8±0.9 mm (range 3–6 mm) and 3.6±0.8 mm (range 3–5 mm) in depth, respectively (p=0.540). There were no significant differences for any of the defect characteristics between the test and control sites at baseline (Table 1).

The clinical results (PD, CAL, REC) at baseline and 12 months after surgery are summarized in Table 3. In comparison with the baseline data, both the test and control groups showed statistically significant reductions of PD and CAL (p<0.001). At 12 months after therapy, the test group showed a reduction in the mean PD from 7.8±1.6 mm to 3.5±1.0 mm (p<0.001) and a change in mean CAL from 8.5±2.1 mm to 4.6±1.2 mm (p<0.001). In the control group, the mean PD decreased from 7.5±0.8 mm to 4.9± 0.7 mm (p<0.001) and the mean CAL changed from 8.2± 1.2 mm to 6.4±1.4 mm (p<0.001). The PD (p=0.002) reductions and CAL (p=0.001) gains in the test sites were significantly higher than in the control sites.

The frequency distribution of various CAL gains in the test and control group is given in Fig. 1. Six sites (46.2%) in the test group presented CAL gain ≥4 mm, while this was seen only in one site (7.7%) in the control group. In the test group, a CAL gain of 1 and 2 mm occurred in three sites (23.1%) as opposed to ten sites (77.0%) in the control group. A CAL gain of 3 mm was measured in four sites (30.8%) in the test group, while this was seen in two sites (15.4%) in the control group.

An average change of 0.3±0.6 mm in the position of the gingival margin between baseline and 12 months was observed in the test group and 0.7±0.7 mm in the control group (Table 3). In both groups, the REC showed tendency to increase, but there was no statistically significant difference between the groups with respect to pre- and post-surgical recession (p>0.05).

Table 3 Clinical parameters at baseline and 12 months for the test and control groups

Variables	Baseline	12 months	Difference	p value
PD				
Test	7.8±1.6	3.5±1.0	−4.3±1.3	<0.001
Control	7.5±0.8	4.9±0.7	−2.5±1.1	<0.001
p value	0.672	0.001	0.002	
CAL				
Test	8.5±2.1	4.6±1.2	−3.9±1.7	<0.001
Control	8.2±1.2	6.4±1.4	−1.8±1.0	<0.001
p value	0.888	0.002	0.001	
REC				
Test	0.7±0.8	1.0±1.1	0.3±0.6	0.219
Control	0.8±1.0	1.5±1.3	0.7±0.7	0.016
p value	0.999	0.443	0.271	

Discussion

The aim of the present controlled clinical trial was to evaluate the adjunctive effect of filling the infrabony defect with ABM/P-15 in its "flow" presentation compared to open flap debridement alone. The two treatments in the present study resulted in clinically significant improvements between baseline and 1 year, in terms of PD and CAL. However, the test treatment showed statistically significant greater PD reductions and CAL gains than the control treatment. The clinical superiority of the test treatment compared to the control treatment can also be gathered from the frequency distribution data which indicated that 46.2% of sites treated with ABM/P-15 flow exhibited ≥4 mm gain in CAL, compared with 7.7% of sites from the group treated with open flap debridement alone.

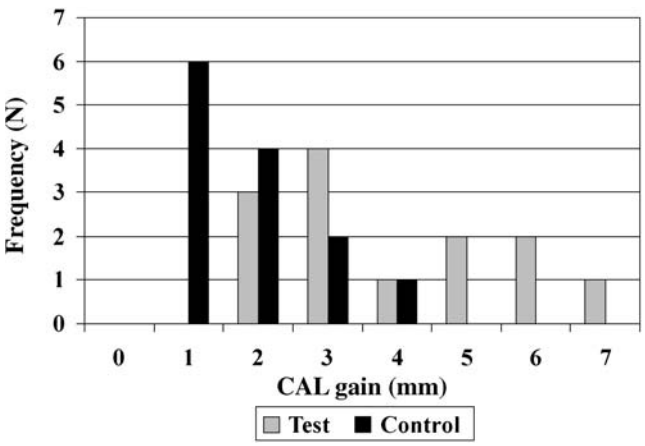

Fig. 1 Frequency distribution of clinical attachment gain in the test and control groups

At 12 months, the results for the ABM/P-15 flow group demonstrated an average CAL gain of 3.9±1.7 mm and an average probing depth reduction of 4.3±1.3 mm. The magnitude of the CAL gain (3.9 mm) observed in the test treatment group (ABM/P-15 flow) compares favorably with the best results obtained (ranging from 1.3 to 4.0 mm) in previous clinical trials [1, 15, 29, 33, 47], using bovine-derived xenogenic grafts alone or combined with barrier membranes or enamel matrix proteins in the treatment of infrabony defects. In previous studies, the ABM/P-15 combination demonstrated to be clinically effective and superior to ABM alone, compared to open flap debridement alone and to decalcified, freeze-dried bone [46, 47]. Yukna et al. [46] reported a mean CAL gain of 1.3±1.9 mm in an ABM/P-15 group compared to 0.5±1.8 mm in a group treated with decalcified, freeze-dried bovine allograft, while a reduction of only 0.1±2.4 mm was obtained with open flap debridement alone. However, ABM/P-15 demonstrated a decided clinical advantage over DFDBA only in terms of bone fill, while for the soft tissue variables no significant differences were found between the treatment procedures except for greater clinical attachment level gain with ABM/P-15 compared to open flap debridement. Later, Yukna et al. [47] observed a mean CAL gain of 2.2±2.0 mm in ABM/P-15-treated sites versus 2.1±1.8 mm in ABM-treated sites at the 6-month follow-up visit. While the soft tissue variable measurements yielded very similar results, the ABM/P-15 produced 73% bone fill versus 51% for ABM alone in infrabony defects. Furthermore, these studies supported adding a biologic mediator to a bone replacement graft. The differences in CAL gain reported in the different studies can be attributed to known prognostic factors in the outcome of regenerative periodontal surgery, such as the patients plaque and infection control, smoking status, surgical technique, surgeons variability as well as the initial depth of the defects [6]. Indeed, clinical studies have demonstrated that the CAL gain following regenerative periodontal surgery is strongly dependent on the initial defect depth and configuration [13, 16, 37, 42]. The greater the initial defect depth, the greater the amount of CAL gain. Deeper defects demonstrated to have greater potential for bone healing due to their improved source of osteogenic cells from the periodontal ligament and bone [12]. On the other hand, it should be remembered that placement of a graft material into a defect may modify gingival tissue consistency and, therefore, limit penetration of the probe without necessarily having induced any gain in clinical attachment [5]. The clinical attachment gain observed in the control sites of the present study was 1.8 mm and is comparable with most of the reported results [14, 25, 27]. The results demonstrated that open flap debridement provided also clinically and statistically significant improvements in PD and CAL measurements compared to baseline values. It is interesting to note that in the present study, no treated site either in the test or control group demonstrated a clinical loss of attachment. Moreover, all sites in the two treated groups showed a positive CAL gain. The reasons for the positive results seen in the present patient population may include the level of plaque control and the frequency of professional maintenance which, in previous studies utilizing open flap debridement, was shown to directly correlate with CAL gain [9, 23, 30].

When the change in free gingival margin levels was examined, no statistically significant difference between groups was observed at any time points. The gingival recession of 1.0 mm in the ABM/P-15 flow group is parallel to those of Yukna et al. [46]. In the present study, ABM/P-15 flow seemed to enhance flap adaptation and stability in the postoperative healing period. The clinical handling of the ABM/P-15 flow formulation demonstrated to be very good and since the material is supplied in prefilled syringes, there was no need for prehydration. The hydrogel formulation of the present bone graft material used in this study contains less mineral particles per volume providing better interparticulate spacing that should facilitate more rapid vascularization and resorption in comparison to the particulate form of ABM/P-15. Although the gel consistency of this formula could be considered as not ideal to maintain space for regeneration, good clinical results were obtained in all of the defects treated. In a comparative study in critical-sized fenestration defects in dogs [43], a putty formulation of ABM/P-15 resulted in significantly greater bone formation when compared to ABM/P-15 particulate. In contrast, Scarano et al. [32] demonstrated in cortical bone defects in rabbits a statistically significantly greater regeneration of new bone in defects treated with the ABM/P-15 particulate graft in comparison to the hydrogel formulation. Matos et al. [19] compared in a clinical study the ABM/P-15 particulate with the hydrogel form. The results of the study failed to demonstrate the superiority of one form of the graft material over the other.

Since all defects as previously mentioned were intra-osseous-type defects, extrapolation of these results to other defect types (i.e., furcations, horizontal bone loss) would be inappropriate. No significant differences compared to open flap debridement were found following the use of ABM/P-15 flow in the treatment of class II furcation defects [8].

A limitation of clinical regeneration studies, such as the present one, is the inability to assess the histologic characteristics of the repaired tissues. Studies to examine the histologic nature of the interface of a treated bony defect and the root surface are difficult to conduct for ethical reasons. In an isolated histologic case report, ABM/P-15 demonstrated to have the potential to promote true periodontal regeneration [49], but its predictability in achieving results on a large scale needs to be determined.

The time frame of 12 months in the present study may be considered adequate to evaluate the effect of regenerative periodontal therapy, as it is in agreement with the data provided by Yukna et al. [48]. In this 36-month follow-up of 25 patients treated with ABM/P-15, the majority of the clinical changes were primarily observed at the time of 6 to 7 months, with only minor changes between 6 to 7 months and 3 years. This observation may also suggest that it is possible to longitudinally maintain the stability of the results achieved following treatment with ABM/P-15 bone replacement graft.

In conclusion, results from this clinical trial demonstrated that 12 months after surgery both treatments resulted in significant PD reductions and CAL gains and that treatment with ABM/P-15 in its flow formulation produced enhanced clinical results in terms of PD reductions and CAL gains when compared to open flap debridement alone. Further studies including human histologic evidence are required to claim true periodontal regeneration when using ABM/P-15 flow in human infrabony periodontal defects.

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