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Guided tissue regeneration in intrabony periodontal defects following treatment with two bioabsorbable membranes in combination with bovine bone mineral graft

A clinical and radiographic study

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Abstract

Aim: Comparison of two bioabsorbable barriers (collagen and polylactic acid (PLA) membranes) combined with a bovine bone mineral (BBM) graft, with an access flap procedure (AFP) alone for treating intrabony defects.

Material and Methods: Thirty-four subjects participated in this prospective, controlled clinical trial. Baseline clinical examination (probing depth (PD), clinical attachment level (CAL)) of selected sites was performed 2 months after completion of conservative treatment in conjunction with hard-tissue measurements to ascertain the depth of the defect (cementoenamel junction to the bottom of the defects). After randomly dividing patients into three groups (two membrane groups, one control group), full thickness flaps were elevated and exposed root surfaces planed before filling defects with bone graft and positioning a barrier membrane covering the defect. The control group was treated identically except for the barrier and bone graft placement. Clinical treatment outcomes were finally evaluated 12 months after surgery for changes of PD and CAL. Radiographs at baseline and 12 months were compared using non-standardized digital radiography.

Results: A mean reduction in PD value of 5.08 mm and mean CAL gain of 4.39 mm occurred in the collagen-BBM group. Corresponding values for the PLA-BBM group were 4.72 and 3.71 mm, while acess flap procedure (AFP) sites produced values of 2.50 and 2.43 mm. All improvements in clinical parameters were statistically significant ($p < 0.001$) within groups for all variables. Both membranes produced statistically greater PD reduction and CAL gain compared with AFP treatment ($p < 0.05$). Comparison between barrier groups failed to reveal any statistically significant difference in probing pocket depth reduction ($p = 0.56$) or in CAL gain ($p = 0.34$).

Conclusion: Placement of the two barrier membranes used in the present study in combination with BBM graft significantly improved clinical and radiographic parameters of deep intrabony pockets and proved superior to access flap alone.

Key words: bovine bone mineral; collagen barrier; guided tissue regeneration; intrabony defects; periodontal surgery; polylactic acid barrier

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Over recent decades several treatment modalities, including the use of bone grafting materials (Yukna 1993, Rosen et al. 2000), root surface conditioning (Claffey et al. 1987, Caffesse et al. 1988) and, above all, guided tissue regeneration (GTR) with the use of barrier membranes (Nyman et al. 1982, Gottlow et al. 1986, Karring et al. 1993), have been proposed to promote the regeneration of the periodontal tissues lost due to periodontal disease.

In pursuit of the successful accomplishment of this goal, various biomaterials facilitating periodontal regeneration have been utilized. There is evidence that the principle of GTR based on the use of barrier membranes placed over the periodontal defects and denuded root surfaces, thus allowing progenitor periodontal ligament cells to selectively repopulate the diseased root surfaces, predisposes a successful clinical outcome.

Several studies in human and animals have demonstrated that both non-resorbable expanded polytetrafluoroethylene membranes and bioresorbable barriers can be successfully applied to facilitate periodontal regeneration in mandibular class II furcation lesions and intraosseous defects (Gottlow et al. 1986, Caffesse et al. 1990, Becker & Becker 1993, Cortellini et al. 1993a, b, 1996a, b, Becker et al. 1996, Mattson et al. 1999, Eickholz et al. 2000).

However, the use of bioabsorbable membranes seems to be gaining popularity since it obviates the need for a second surgical procedure for removal, thus reducing the risk of damaging the newly formed granulation tissue.

For the treatment of vertical osseous defects both the GTR procedure and bone grafting techniques have been utilized (Chen et al. 1995, Mellado et al. 1995). The use of bone substitutes in combination with the barrier effect has been reported to enhance the regenerative clinical outcome, by providing a better support to the membrane or by adding the potential osteoinductive properties of the grafting material to the barrier effect.

The comparison of absorbable and non-absorbable barrier materials has demonstrated that they lead to similar clinical results when used in the treatment of intrabony defects (Gottlow 1993, Chen et al. 1995, Christgau et al. 1995, Mattson et al. 1995, Caffesse et al. 1997, Weltman et al. 1997, Pontoriero et al. 1999, Zybutz et al. 2000). The bioabsorbable materials, which have been introduced in the recent years, can be classified into two major categories based on their composition: (1) barriers of collagen origin and (2) those composed of polylactic and polyglycolic acid polymers.

The present study was conducted in order to compare the clinical effect of two different bioabsorbable barrier membranes in combination with anorganic bovine bone as a grafting material in the treatment of intrabony periodontal lesions. For this reason a bi-layer collagen membrane, and a polylactic acid membrane were used.

The collagen membrane is composed of collagen I and III of porcine origin. It has a bi-layer configuration with a compact cell occlusive layer on the outside, facing the soft tissues, and a porous, rough layer with random fibers promoting integration, facing the defect. The polylactic barrier is composed of poly(DL-lactide) (PLA) dissolved in *N*-methyl-2-pyrrolidone (NMP), which exhibits biodegradation. It is flowable and is applied directly over the periodontal defect. As it is bioadhesive it adheres to the surrounding tissues after polymerization and eliminates the need for stabilizing sutures. The bone grafting material is an anorganic bovine bone mineral (BBM) that is highly osteoconductive (Pinholt et al. 1991, Hislope et al. 1993) and was used as a space-maintaining graft material. It is prepared by protein extraction of bovine bone and results in a porous structure similar to human cancellous bone. Over the time this graft seems to undergo physiologic remodeling and becomes incorporated into the surrounding bone.

A number of studies have produced findings after the combined use of collagen as a barrier material and anorganic bone substitute in the treatment of periodontal defects (Clergeau et al. 1996, Trejo et al. 2000, Lekovic et al. 2001). Sites implanted with this combination showed successful regeneration of the surrounding periodontal tissues. To our knowledge there are no studies reporting on the clinical outcome after placement of the polylactic copolymer material applied in the present study with the objective of periodontal regeneration.

Therefore, the purpose of the present clinical trial was to investigate the use of the above two resorbable barrier membranes in combination with the anorganic bovine bone as grafting material and to compare the clinical outcomes with those of conventional periodontal surgery in the treatment of intraosseous periodontal defects.

Material and Methods

Experimental design

This study was designed as a prospective controlled clinical trial. Three different surgical approaches for the treatment of deep angular bony defects were compared in the present trial. The two experimental groups were treated with bioresorbable barrier membranes combined with a BBM graft, while the control group was treated with an access flap procedure (AFP). Clinical and radiographic findings following treatment of the three groups were longitudinally evaluated at 12 months postsurgery.

Patient and site selection

Thirty-four subjects (age range 32–61 years, 11 male and 23 female), referred for treatment of moderate or advanced chronic periodontitis to the Department of Preventive Dentistry, Periodontology and Implant Biology, Dental School, University of Thessaloniki, were included in the present study.

The inclusion criteria considered for a patient to participate in this study were: (i) healthy adults between 18 and 70 years of age, (ii) presence of generalized advanced periodontal tissue destruction, (iii) presence of at least one deep angular bony defect, as defined by x-rays, exhibiting the following characteristics: (a) probing depth (PD) and attachment loss $\geqslant 7$ mm, (b) one-, two- or three osseous walls and (c) not related to a furcation involvement. The exclusion criteria were: (i) known systemic disease and/or drug therapy known to interfere with wound healing, (ii) no periodontal treatment performed during the previous 6 months, (iii) smoking habits, since this appears to influence treatment outcome (Tonetti et al. 1995) and (iv) participation in other dental clinical trials.

There were 14 subjects in the first group treated with a collagen membrane and BBM, 14 subjects in the second group treated with PLA membrane and BBM, and 12 subjects in the third group where an access flap was performed. In the control group, six out of the 12 subjects, those with a bilateral intrabony

lesion treated with the combination of grafting material, were also included in one of the two barrier groups. In order to avoid a bias in statistical analysis, a comparison was performed between the two subgroups: the first subgroup consisted of the six subjects also included in one of the two test groups, while the second subgroup consisted of the remaining six subjects where only an access flap was utilized. No statistical significant difference was found between the two subgroups regarding probing pocket depth (PPD) and clinical attachment level (CAL) values ($p = 0.63$ for PPD measurements, $p = 0.70$ for CAL measurements). This finding allowed the control group to be considered as a homogenous group, removing the possibility of bias in the statistical analysis.

Prior to the start of the study, all 34 subjects received non-surgical periodontal treatment, including oral hygiene instructions as well as comprehensive subgingival scaling and root planing. The decision to perform a surgical procedure was taken at least 2 months after the completion of the presurgical initial phase and was based on clinical and radiographic examination. All participants achieved an acceptable level of plaque scores prior to surgical procedures (hygiene index (HI) $\leqslant 15\%$) (O'Leary et al. 1972). To ensure randomization as well as ''blindness'' on the part of the clinician, the assignment of treatment was conducted as follows: a sealed envelope, with a card indicating the surgical procedure to be applied, was opened by the surgeon at the time of the surgery, immediately following defect debridement.

The participants were informed about the risks and benefits of the procedure and signed an informed consent.

Clinical measurements

The following outcome variables were measured at baseline (prior to the surgical procedure) and at 12 months post-surgery:

1. *Plaque Index* (presence or absence) (O'Leary et al. 1972).
2. *Bleeding index* (presence or absence) (Ainamo & Bay 1975).
3. *PPD*: measured with a calibrated periodontal Michigan probe (diameter = 0.5 mm) to the nearest mm.
4. *CAL*: Determined by using the same probe and the cementoenamel junction (CEJ) as the marginal reference level. If the CEJ was not noticeable because of the presence of a restoration (crown, filling) the margin of this restoration served as a reference point.

Measurements were performed at six sites around all teeth but only sites with PD values $\geqslant 7$ mm adjacent to treated bony defects were considered for further evaluation. Patients to receive either GTR (collagen or PLA barrier) or access flap procedure were assigned to each group by a computer randomization program.

The following clinical measurements were taken during surgery, immediately after debridement of the defects: distance from the CEJ to the bottom of the defect (CEJ-BD); distance from the CEJ to the most coronal extension of the interproximal alveolar crest (CEJ-AC). Those sites encompassing the defects were measured with the periodontal probe oriented parallel to the long axis of the teeth.

All clinical measurements were performed by one of the authors (E.A.) who was calibrated to show more than 90% intraexaminer agreement within $\pm$ 1 mm by duplicate measurements of PPD and CAL of 30 teeth randomly selected. The examiner was not aware of the surgical procedure to be performed.

Radiographic evaluation

Radiographic examination was carried out preoperatively and 12 months postoperatively. Periapical radiographs were taken using the parallel technique and holders. Occlusal stents or other mechanical fixation devices were not used, but we tried to ensure that both pre- and postoperative radiographs had a close projection geometry and similar optical density. All radiographs were digitized using a scanner so that all images were of the same size. The preoperative image of each patient was used as reference. The second image taken 12 months postoperatively was reconstructed according to its reference image by means of the geometric standardization software (Emago/Advanced V. 3.4, Oral Diagnostic Systems, Amsterdam, the Netherlands). For the alignment of the second image, four featured points in the reference image were selected. The corresponding locations in the second image were identified and the computer then used to reconstruct it with approximately the same geometric projection as the first image. In this way, two pairs of images could be formed for each intrabony defect: (reference-second image and reference-reconstructed second image) in order to perform linear measurements (Tsiklakis et al. 1995). The following measurement – distance from the CEJ to the base of the defect – was performed in both radiographs, preoperative and reconstructed postoperative. The investigator (E.A.) who had not participated in the clinical treatment performed the measurements, which were recorded as numbers of picture elements (pixels). The position of the CEJ was identified as described by Schei et al. (1959). The crossing of the silhouette of the AC with the root surface was defined as the bone crest. The most coronal area where the periodontal ligament maintained an even width was identified to measure the furthest apical extension of the intrabony defect (Bjorn et al. 1969). Each measurement was repeated three times by the investigator and the mean value was recorded for the final evaluation. Percentage of change for the linear measurement was calculated according to the following mathematical type and used for further comparison:

$$\left[\frac{\text{preoperative measurement} - \text{postoperative measurement}}{\text{preoperative measurement}}\right] \times 100.$$

Surgical procedures

At the time of the surgical procedure, the patients were assigned at random, as described above, into one of the following treatment groups: (1) BBM graft with collagen membrane, (2) BBM graft with PLA barrier and (3) access flap (control). Two surgeons performed all surgical procedures (I.V. and A.K.).

The surgical procedures were performed using local anesthesia with lidocaine 2% containing epinephrine at a concentration of 1:100.000. Following intracrevicular incisions, mucoperiosteal flaps were elevated on the buccal and lingual/palatal aspects of the teeth taking care to preserve the marginal and the interdental tissues at the maximum possible level. The flaps were extended mesially and distally to provide full

visibility and access of the denuded root surface and associated defect. Vertical releasing incisions extending into the alveolar mucosa were performed as needed to ensure proper access to the defect and to facilitate coronal positioning of the flap at the completion of the procedure. The inner surface of the flaps was carefully curetted to remove granulation tissue. Complete defect debridement as well as scaling and root planing was accomplished with hand curettes and ultrasonic instruments. The surgical area was rinsed with copious amounts of sterile saline.

At this stage, different treatments were used for each of the barrier groups. In the first group, the BBM graft (Bio-Oss® spongiosa, Geistlich Pharma AG, Wolhusen, Switzerland) was placed into the defect. The graft was hydrated with sterile saline solution and tightly packed into the defects to the level of the surrounding bony walls. Care was taken not to overfill the osseous defects. If a collagen barrier (Bio-Gide®, Geistlich Pharma AG) was to be placed, a membrane of proper size was chosen and after trimming, it was placed to completely cover the grafted area and the adjacent 2–3 mm of bone tissue. No sutures were used for stabilization of the membrane as after hydration (either with blood or blood–saline solution) and it was well adapted and appeared to adhere naturally to the bone and root surface. The PLA barrier (Atrisorb® Free Flow™) was applied in a fluid state according to the manufacturer's instructions using a syringe and a sterile canule. Care was taken to ensure that it covered the graft, was in intimate contact with the bone and extended slightly over the adjacent alveolar bone. Then it was sprayed with sterile saline solution for approximately 10–20 s to effect polymerization. Once firm and stable it adhered to the surrounding and underlying tissues thus eliminating the need for stabilizing sutures.

After placement of the biomaterials, the flaps were coronally advanced to accomplish complete coverage of the barrier membrane and care was taken to secure adequate interproximal closure. The flaps were sutured using single interrupted sutures as necessary. Patients were instructed to rinse with 0.12% chlorhexidine gluconate mouthrinse, 2 × day until the 4th postoperative week, while antibiotics were prescribed for 10 days (Augmentin 1 gr, 1 × 2). No periodontal dressing was placed and the flap sutures were removed after 3 weeks.

Mechanical oral hygiene, consisting of brushing and flossing or proxybrushing, was initiated at the end of the 4th postoperative week. Thereafter, gentle brushing on buccal and lingual surfaces with a soft toothbrush was recommended. At 6 weeks postoperatively, each patient was reinstructed in proper oral hygiene measures including sulcular and interproximal brushing.

All patients were examined monthly after the surgery. Postoperative care included reinforcement of oral hygiene and mechanical supragingival plaque removal whenever necessary. No subgingival instrumentation was attempted at any of the postoperative visits.

A case showing an intrabony defect treated with a collagen membrane combined with the bone xenograft is presented in Figs 1a–d. The respective radiographic images are illustrated in Figs 2a–d.

Statistical analysis

Clinical measurements from the test and control groups were statistically analyzed to compare treatment results between and within groups. The single subject was regarded as the unit of statistical analysis (14 patients in the collagen/BBM group, 14 patients in the PLA/BBM group and 12 patients in the AFC group). Comparisons of PDs, PD reductions, attachment levels, attachment level gains and bone height changes (radiograph), were made. The primary outcome variable was CAL. Data for each type of surgical procedure were pooled and analyzed by the use of Student's *t*-test. For all analyses, a p value <0.05 was considered statistically significant.

Patient averages were used for statistical evaluation of PPD and CAL in the three groups of subjects at baseline and after 12 months. The mean difference between baseline and 12 months was also calculated. Statistically significant differences between groups were determined with Student's *t*-test.

Osseous defect characteristics were only descriptively shown and were not statistically analyzed as they were measured only intraoperatively at baseline. Radiographic differences (% of change) between baseline and 12 months post-treatment and between groups were analyzed using Student's *t*-test.

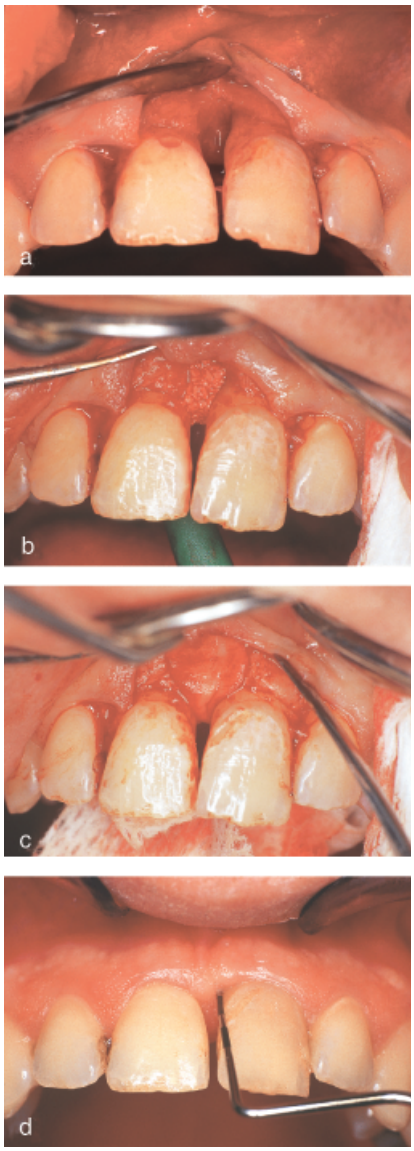


Fig. 1. Clinical appearance of the intrabony defect at tooth 21 at the time of surgery (a). Placement of the bovine bone mineral graft (b) and the collagen barrier (c). Clinical appearance at 12 months posttreatment (d).

Results

Mean PD and attachment level values at baseline and 12 months in each treatment group are shown in Table 1. Comparisons of mean differences in clinical parameters over time for each group are shown in Table 2, while a correlation between the CAL gain reported at 12 months and the initial PPD and CAL is shown in Tables 3 and 4.

At the 12-month examination it was observed that in all study groups and selected sites there was, in comparison with the baseline data, a marked and

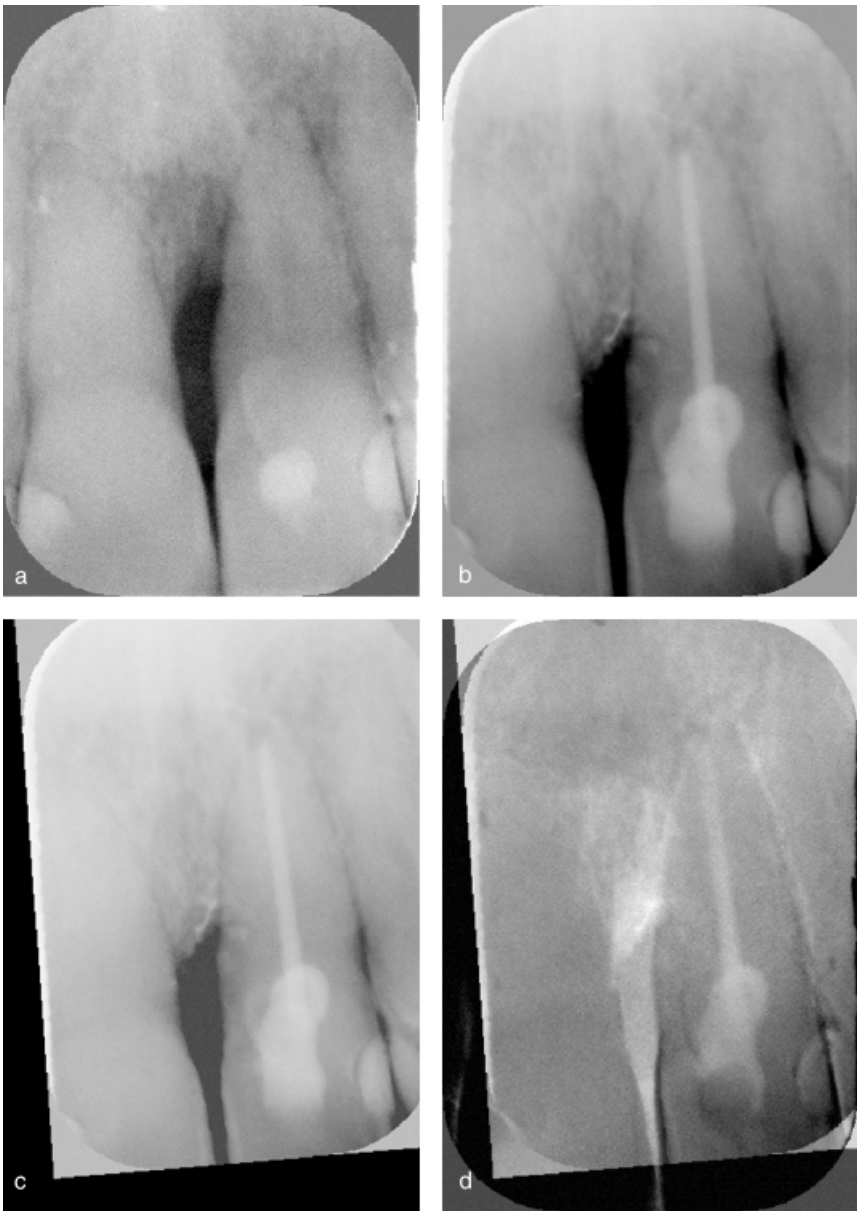


Fig. 2. Standardized radiographs obtained at baseline showing an intrabony defect at tooth 21 (a) and 12 months posttreatment (b). Reconstructed image of the posttreatment radiograph (c). Subtraction image (d). New bone formation is indicated by the white area.

Table 1. Mean values and standard deviation of probing pocket depth (PPD) and clinical attachment level (CAL) between baseline and 12 months achieved for all treatment groups

		N	Baseline	12 months	Significance
collagen- BBM	PPD	14	8.82 ± 1.03	3.73 ± 1.14	$p<0.001$
	CAL	14	10.38 ± 1.77	5.98 ± 1.71	$p<0.001$
PLA- BBM	PPD	14	8.25 ± 1.39	3.53 ± 1.16	$p<0.001$
	CAL	14	9.60 ± 1.21	5.88 ± 1.14	$p<0.001$
AFP	PPD	12	7.72 ± 0.68	5.22 ± 0.84	$p<0.001$
	CAL	12	8.52 ± 0.97	6.09 ± 0.94	$p<0.001$

N, number of defects treated; BBM, bovine bone mineral graft; PLA, poly(DL-lactide) barrier; AFP, access flap procedure. Student's *t*-test.

statistically significant ($p<0.001$) reduction in the PPD values as well as a statistically significant ($p<0.001$) gain of the CAL value (Table 1).

The mean probing depth for the 1st (collagen membrane-BBM graft) and the 2nd (PLA barrier-BBM graft) groups were reduced from 8.82 and 8.25 mm to 3.73 and 3.53 mm respectively, while for the control group the mean values at baseline and at 12 months were 7.72 and 5.22 mm, respectively.

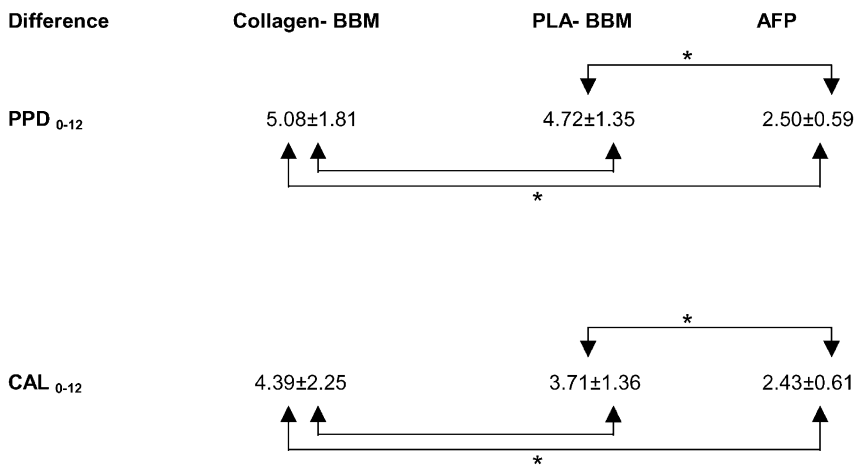
The mean CAL values after 12 months were also improved significantly for all treatment groups. The mean CAL for the 1st (collagen membrane-BBM graft) and the 2nd (PLA barrier-BBM graft) groups were improved from 10.38 and 9.60 mm to 5.98 and 5.88 mm respectively, while for the control group the mean values at baseline and at 12 months were 8.52 and 6.09 mm, respectively.

The mean changes in PD at the end of 12 months were 5.08 mm for the combination of collagen membrane and BBM graft, 4.72 mm for PLA barrier combined with BBM graft and 2.50 mm for access flap. Evaluating the outcomes of therapy produced by each of the combination treatments, it was observed that there were no statistically significant differences with respect to PPD reduction and CAL gain between the two groups (Table 2). The mean reduction of PPD between these groups was of the same magnitude ($p = 0.56$). On the other hand, when each of the membrane group was compared with the control group, the difference in the reduction of mean PD reached statistically significant levels ($p = 0.000$).

The group using collagen membrane combined with BBM graft produced a 4.39 mm gain in clinical attachment while the gains in the PLA barrier-BBM graft and in the control groups were 3.71 and 2.43 mm, respectively. Comparison of attachment level gains between groups revealed that treatment with either barrier presented with significantly greater attachment gain ($p = 0.01$ and 0.00, respectively) at the end of the observation period when compared with sites treated with the access flap. Differences in CAL gain between the two barrier groups showed no statistical significance ($p = 0.34$).

To characterize the variation in the probing attachment gain between baseline and 12 months, the results were stratified into two different classes according to a system originally described by Cortellini et al. (1993a, b). Table 3 reports distribution of patients' averages related to probing attachment gain (<3 mm, $\geqslant 3$ mm) 12 months post-

Table 2. Comparison of differences achieved over time in clinical parameters between treatment groups (mean values and standard deviation)



$PPD_{0–12}$, mean difference in probing pocket depth achieved between baseline and 12 months; $CAL_{0–12}$, mean difference in clinical attachment level achieved between baseline and 12 months; BBM, bovine bone mineral graft; PLA, poly(DL-lactide) barrier; AFP, access flap procedure. Student's *t*-test. Statistical significance at $p<0.05$. (*) Statistically significant. Statistical significance at $p<0.05$.

Table 3. Distribution of patients related to CAL gain (<3 mm, $\geqslant 3$ mm) 12 months post-treatment

	N	CAL <3 mm	CAL $\geqslant 3$ mm
collagen- BBM	14	2	12
PLA- BBM	14	4	10
AFP	12	8	4

N, number of patients treated; CAL, clinical attachment level; BBM, bovine bone mineral graft; PLA, poly(DL-lactide) barrier; AFP, access flap procedure.

Table 4. Baseline defect characteristics related to CAL gain (<3 mm, $\geqslant 3$ mm) 12 months post-treatment (mean $\pm$ standard deviation)

		CAL <3 mm	CAL $\geqslant 3$ mm
Collagen- BBM	PPD	7.83 ± 0.26	8.98 ± 1.03
	CAL	8.83 ± 1.65	10.63 ± 1.72
	CEJ-BC	7 ± 1.41	9.66 ± 2.90
	CEJ-BD	11 ± 1.41	12.33 ± 2.05
PLA- BBM	PPD	7.52 ± 0.65	8.55 ± 1.53
	CAL	9.23 ± 0.63	9.75 ± 1.37
	CEJ-BC	6.13 ± 1.11	7.25 ± 2.04
	CEJ-BD	10.88 ± 0.78	11.05 ± 1.86
AFP-	PPD	7.68 ± 0.59	7.87 ± 0.85
	CAL	8.28 ± 0.81	9.12 ± 1.03
	CEJ-BC	6.5 ± 1.19	6 ± 1.63
	CEJ-BD	9.37 ± 1.06	8.62 ± 2.13

PPD, probing pocket depth; CAL, clinical attachment level; CEJ-BC, cemento-enamel junction to bone crest; CEJ-BD, cementoenamel junction to bone defect; BBM, bovine bone mineral graft; PLA, poly(DL-lactide) barrier; AFP, access flap procedure.

treatment, while Table 4 reports baseline defect characteristics, PPD and CAL values at sites that exhibited a probing attachment gain of <3 mm and $\geqslant 3$ mm, 12 months post-treatment. Specifically, in the first group (collagen membrane and BBM graft combination) two out of 14 sites gained 0–2 mm of attachment while the majority of sites (12 out of 14) gained $\geqslant 3$ mm. In the PLA barrier-BBM group, it was observed that four out of 14 sites gained <3 mm of attachment while the remaining 10 sites gained $\geqslant 3$ mm. In the control group, eight out of 12 sites gained <3 mm of attachment and only few sites (four out of 12) gained $\geqslant 3$ mm.

Radiographic differences (% of change) between the baseline and reconstructed images 12 months post-treatment are also presented in Table 5. The mean improvement in the distance between the CEJ and the bottom of the defect were comparable ($p>0.05$) between the two barrier groups (in the collagen-BBM group $23.68 \pm 11.93\%$, in the PLA-BBM group $20.01 \pm 13.32\%$) but both groups exhibited statistically significant differences ($p<0.05$) in comparison with the improvement in the access flap group ($6.36 \pm 9.24\%$).

Discussion

The findings of this prospective controlled clinical trial showed that the use of both collagen and polylactic acid barrier membranes in conjunction with BBM as a graft material significantly improved the clinical and radiographic parameters 12 months after the surgical treatment of deep intrabony pockets. Additionally, this study indicated that sites treated with the combined GTR-grafting procedures resulted in significantly better clinical outcomes than sites treated with an access flap only. Specifically, the mean CAL gain observed in the collagen-BBM group was 4.39 ± 2.25 mm and the corresponding value for the PLA-BBM group was 3.71 ± 1.36 mm. The difference between those two values was not statistically significant ($p = 0.34$). The group treated with an access flap showed a mean gain of CAL of 2.43 ± 0.61 mm. A statistically significant difference between this value and the values concerning the attachment gain achieved by both GTR procedures was observed (Table 2).

The outcomes obtained are comparable with those of previous studies evaluating either GTR alone, or the combined application of bone grafting with barrier membranes in the treatment of intrabony pockets (Cortellini et al. 1993a, b, 1996a, b, Guillemin et al. 1993, Falk et al. 1997, Pontoriero et al. 1999, Camargo et al. 2000, Trejo et al. 2000, Lekovic et al. 2001, Christgau et al. 2002).

Table 5. Comparison of radiographic changes (%) between preoperative and postoperative measurements (mean ± standard deviation) over time between treatment groups

Difference	Collagen- BBM	PLA- BBM	AFP
CEJ-BD $_{0\text{-}12}$	23.68±11.93	20.01±13.32	6.36±9.24

* (PLA- BBM vs AFP)

* (Collagen- BBM vs AFP)

CEJ-$BD_{0–12}$, mean difference in bone level achieved between baseline and 12 months; BBM, bovine bone mineral graft; PLA, poly(DL-lactide) barrier; AFP, access flap procedure. Student's *t*-test. Statistical significance at $p<0.05$. (*) Statistically significant.

There have been a number of studies evaluating different bioabsorbable membranes in the treatment of periodontal defects. Most have used a nonresorbable membrane (e-PTFE) as the ''gold standard'' to evaluate the results of bioabsorbable barrier materials. As there is an increased interest in the use of absorbable membranes, the purpose of the present investigation was to compare a collagen barrier material with a PGA/PLA membrane in the treatment of one-, two-, or three-walled intrabony defects.

The properties of the collagen barrier as reported by Chen et al. (1995) are the following: (1) It is either incorporated into the healing connective tissues or degraded by macrophages in 6–8 weeks. (2) It is chemotactic to fibroblasts from periodontal ligament and gingivae. (3) It creates a thrombogenic surface that stimulates platelet attachment, producing hemostasis. In addition, collagen materials possess additional advantages including weak immunogenicity, ease of manipulation and the ability to augment tissue thickness by providing a collagenous scaffold (Bunyaratavej & Wang 2001).

The PLA membrane is composed of a synthetic co-polymer of glycolide and lactide and is dissolved in NMP. The polylactide polymer chains of the barrier are cleaved by hydrolysis to form monomeric acids and eliminated from the body through the Krebs cycle as carbon dioxide and water. The period for the biodegradation of this membrane is about 50–60 days. Biodegradable polymers have been used to produce medical devices for many years. In particular, polylactic biomaterials have demonstrated satisfactory biocompatibility, absence of toxicity and no interference with tissue healing (Chen et al. 1995, Hürzeler et al. 1997). Caffesse et al. (1994) histologically tested two bioabsorbable membranes made from a synthetic copolymer of glycolide and lactide for their biocompatibility, resorption characteristics and ability to support periodontal regeneration in the dog model. They reported that hydrolysis of the material is minimal for approximately 6 weeks and is essentially completed by 8 months. The resorption process was not seen to interfere with the healing process. The formation of new connective tissue attachment was favored by the use of these PLA barriers as reported by the authors.

The authors of the present trial chose to use bone grafting materials in combination with barrier membranes because bone grafts aim mainly to provide space maintenance and to recruit cells with regenerative potential. It is believed that these materials not only maintain the space, but also might provide an osteoinductive and/or osteoconductive capacity (Schmitt et al. 1997, Camelo et al. 1998, Stephan et al. 1999, Schwartz et al. 2000). Both membranes tested in this trial lacked rigidity and tended to collapse into the defects thus reducing the space needed for tissue regeneration. This was particularly the case with the PLA barrier material, which is applied in a flowable condition over the intrabony defect, and would probably collapse into the defect without the introduction of a biocompatible filler (BBM graft) to ensure the maintenance of sufficient space.

We have to point out that comparison between individual clinical studies evaluating the combined treatment effect of bone graft and membrane is difficult. The literature contains studies reporting a greater improvement of clinical parameters than that observed in this trial (Schallhorn & McClain 1988, Benqué et al. 1997a, b). Schallhorn & McClain (1988) reported a PD reduction of 5.0 mm and a gain in CAL of 5.3 mm after treating furcation defects and interproximal intrabony pockets with GTR in combination with osseous composite graft. On the other hand, Guillemin et al. (1993) reported a value of 2.3 mm regarding PPD reduction and a value of 3.2 mm for gain in CAL. Becker et al. (1996) reported on the results of a multicenter study evaluating a PLA/PGA membrane differing in structure from that evaluated in the present study. They reported a mean PD reduction of 4.0 mm and a gain in CAL of 2.9 mm, 1 year after treatment in intrabony defects, which is consistent with the findings of this study. More recently, Trejo et al. (2000) used DFDBA as an osseous filler of vertical osseous defects combined with PLA membranes, expecting to enhance the regenerative potential of human bone by the activity of bone morphogenetic proteins believed to be contained in DFDBA. The clinical outcome (PD reduction was 3.37 mm, CAL gain was 2.29 mm) was not found to be superior to the one observed in our study. On the other hand, a recent publication (Lekovic et al. 2001) utilized a combination of a bioabsorbable barrier, BBM graft and enamel matrix proteins as regenerative therapy for intrabony pockets. This treatment modality reduced the PD by 4.74–4.95 mm while the gain in CAL was 3.78–3.89 mm. Of course, it is difficult to draw definite conclusions from studies utilizing such a complex experimental design, as it is not clear how the different components influenced the treatment outcome. Comparisons of data using combined treatment modalities between different studies and interpreting them should be made with caution.

According to the results of a meta-analysis evaluating grafting materials, the use of biological agents in periodontal intrabony defects produces a favorable change in PPD and CAL values when compared with an access flap procedure (Trombelli et al. 2002). Nevertheless, there appeared to be a marked variation in CAL gain and PPD reduction with respect to different biomaterials or even between studies evaluating the same biological agent. General conclusions cannot be drawn concerning the clinical significance and consistency of these procedures, the stability of the periodontal tissues and

tooth survival or the long-term outcome, because of the marked variability in results between studies. The heterogeneity reported between different studies evaluating the same biological agents seems to suggest that other factors may significantly influence the clinical response. It has been documented that there are several prognostic factors that affect the outcome of the regenerative procedures including type of the defect treated (initial PDs and attachment level, width, depth and angle of defects, intrabony wall components), type of barrier membrane (different cross-linking techniques) or graft used (biological and physico-chemical characteristics of bone grafts), operator's experience, surgical variables and methods, measuring techniques, postoperative maintenance and statistical analysis should be recognized as significant influences on outcomes. Among patient characteristics, plaque accumulation and smoking habits have been shown to correlate negatively with clinical attachment gain and bone regrowth following a GBR procedure (Kornman & Robertson 2000).

A significant heterogeneity between studies is also reported in a meta-analysis evaluating studies utilizing GTR in the treatment of intrabony periodontal defects (Needleman et al. 2002). The authors have included studies which show marked differences in their experimental design as described above. The small increase in CAL over open-flap debridement calculated in this meta-analysis, something not consistent with the findings of the majority of studies dealing with GTR, may be due to the difficulty experienced by the authors in finding homogenous and comparable studies in terms of experimental design. The authors took this into account when stating that because of the statistically significant heterogeneity between studies included in this meta-analysis, their findings should be interpreted with caution.

In the present study, the investigated osseous lesions consisted of one-, two- and three- wall intrabony defects. There is a prevailing theory that defect morphology plays an important role in the healing response of GTR procedures in intrabony defects, particularly the number of associated bony walls and overall defect depth. However two investigations, failed to demonstrate a significant association between the number of residual bony walls and the clinical outcomes (Tonetti et al. 1993, 1996). In fact, there was an agreement on the lack of significance of defect configuration and/or number of tooth surfaces involved. Clinical improvements were associated mostly with the depth of the intrabony component of the defect (Selvig et al. 1993), the width of the defect angle, as well as with the gingival thickness, which has been associated with the prevalence and severity of flap dehiscence over the membrane.

Beside these, other factors associated with bacterial contamination, innate wound-healing potential, and the surgical procedure affect primarily the treatment outcome (Kornman & Robertson 2000).

From the clinical point of view both barriers tested in the present trial were proven effective in achieving the objectives of guided periodontal tissue regeneration. They exhibited biocompatibility as witnessed by the uneventful clinical response of the periodontal tissues, were easy to trim and adapt to the tooth, and adhered to the bone surface.

One factor that might negatively influence the treatment outcome is membrane exposure, which can result in bacterial accumulation and contamination (Demolon et al. 1993, Machtei et al. 1994, Nowzari et al. 1995, Zybutz et al. 2000). The incidence of membrane exposure in the present study was very similar between the two test groups (six collagen and eight PLA membranes) and did not seem to affect the post-treatment clinical parameters since the healing response, at the sites where exposure was detected, was uneventful.

Digitized radiographs and subtraction images have been recently introduced in clinical studies in order to assess changes in osseous tissues. To be a useful tool for diagnostic purposes, radiographs have to fulfill the requirements of standardization and reproducibility. The shape of a periodontal lesion in a radiograph is dependent, to a large degree, on the orientation of the radiographic projection. Comparison of radiographs taken with a time interval is possible only when the projection geometry is identical. Recently a software-based method has been developed to perform the reconstruction of an image according to the projection geometry of a reference image, thus producing a set (pair) of images with identical projection geometry (Dunn & Van Der Stelt 1992). The availability of this method imposes less strict requirements on the projection conditions, making digitized subtraction radiography easier to use routinely. Linear measurements on the preoperative and reconstructed postoperative images and subtraction images can be performed without the need for standardized radiographs with mechanical devices (Dunn & Van Der Stelt 1992, Tsiklakis et al. 1995).

The percentage of change of measurements recorded in pixels was evaluated instead of converting the pixel measurements into mm. This assessment was thought to be preferable because although the two images are comparable, elongation or foreshortening results in images are not directly comparable with clinical measurements or radiographic changes expressed in millimeters (Parashis & Tsiklakis 2000). Analysis of radiographically measured bone changes following surgical treatment of intrabony periodontal defects using either a GTR procedure or an open-flap debridement, showed a bone refill. The results revealed that there were no significant differences in mean improvement of bone height between membrane groups, while both barriers resulted in more osseous gain compared with the control group, as was expected. Additionally, bone changes in the present study were comparable with findings by other investigators who evaluated bone changes observed using bioresosbable materials combined with demineralized freeze-dried bone allograft (Parashis et al. 1998).

In conclusion, based on the above data, both regenerative procedures were beneficial for the treatment of intrabony defects. The use of bovine bone graft with resorbable collagen or PLA membranes are comparable in terms of clinically and radiographically assessed periodontal healing.

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