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Treatment of infrabony periodontal defects using a resorbable biopolymer of hyaluronic acid: A randomized clinical trial

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Objective: This randomized clinical study examined the use of hyaluronic acid to treat infrabony periodontal defects over a period of 24 months. **Method and Materials:** Forty subjects with a two-wall infrabony defect (probing depth [PD] ≥ 7 mm; clinical attachment level [CAL] ≥ 7 mm) were selected. The defects were randomly divided into two groups: sites treated with hyaluronic acid (test group) and those treated with open flap debridement (control group). **Results:** The 12- and 24-month evaluations were based on clinical and radiographic parameters. The primary outcome variable was CAL. Test defects shows a mean CAL gain of 1.9 ± 1.8 mm, while the control defects yielded a significantly lower gain of 1.1 ± 0.7 mm. PD reduction was also significantly higher in the test group (1.6 ± 1.2 mm) than in the control group (0.8 ± 0.5 mm). Frequency distribution analysis of the study outcomes indicated that hyaluronic acid increased the predictability of clinically significant results (CAL gains ≥ 2 mm and PD reduction ≥ 2 mm) in the test group compared with the controls. **Conclusions:** The treatment of infrabony defects with hyaluronic acid offered an additional benefit in terms of CAL gain, PD reduction, and predictability compared to treatment with open flap debridement. *(Quintessence Int 2013;44:231–240; doi: 10.3290/j.qi.a29054)*

Key words: hyaluronic acid, infrabony periodontal defect, periodontal disease, periodontal regeneration, randomized clinical trial

Successful tissue regeneration requires not only reparative cells with the potential to differentiate between the phenotypes needed to restore the damaged site, but also a microenvironment that supports the proliferation and differentiation of those cells.[1] Over the past decade, considerable progress has been made in the biomaterials and techniques available for simple and predictable periodontal tissue regeneration. The ultimate goal of periodontal therapy is the regeneration of structures lost to disease. Regeneration requires both clinical improvements and knowledge of the underlying biology; treatment decisions must be made using a sound biologic rationale and histologic evidence.[2] Regenerative procedures have been shown to support substantial improvements in clinical parameters compared to open flap debridement (OFD) in the treatment of infrabony defects.[3] The grafting of biomaterials and application of biologic agents have been used with varying degrees of success to reconstruct lost attachment in deep intraosseous defects. The predictability of periodontal regeneration seems to be influenced by multiple factors related to the patient, defect morphology, and surgical procedure.[4] Previous studies have investigated the regenerative treatment of periodontal infrabony defects, and a variety of treatment approaches to

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restore lost periodontal structures have been suggested.[5,6] Controlled clinical trials and meta-analyses have demonstrated that the treatment of deep infrabony defects with alloplastic grafts results in favorable clinical benefits in terms of gains in clinical attachment level (CAL) and reduction of probing depths (PD) compared with OFD alone.[3,7] Currently, autogenous grafts,[8] demineralized freeze-dried bone allografts, biologic mediators alone[9] or with demineralized freeze-dried bone allografts,[10] enamel matrix proteins,[11,12] hydroxyapatite,[13] bioactive glass (PLGA),[14,15] composite alloplast,[16] calcium sulfate,[17] bovine bone xenografts,[18] and growth factors[19] have been used as bone substitutes with regenerative potential.

Hyaluronic acid (HA) is a glycosaminoglycans with a saccharidic component and a molecular weight of more than 10^6 Da.[20] The main action of HA is wound healing as a growth factor and tissue regenerator.[21] The action of HA cooperating in cell migration and differentiation was observed in histologic studies performed in vitro.[22] The initial response to tissue damage is the formation of a temporary matrix that is extremely rich in fibrin and HA and induces the migration of fibroblasts and endothelial cells into the area of the lesion due to its hydrophilic nature.[23] With its hygroscopic nature, HA also possesses a viscosity that seems to delay the penetration of viruses and bacteria.[24] HA plays several roles during the treatment sequence. In the initial stage of inflammation, HA bonds with the fibrin clot; activates inflammatory cells such as leukocytes and macrophages, stimulating migration toward the lesion and clearing of pathogens; and stimulates production of cells such as cytokines, keratinocytes, ameloblasts, fibroblasts, and osteoblasts, which promote inflammation. Next, HA is degraded by an enzyme, the hyaluronidase, to produce low-molecular-weight HA, which in turn promotes local angiogenesis.[25,26]

The use of an ester of HA could represent an interesting supplement to OFD or even an alternative treatment. HA is a biomaterial that is easy to handle and offers biotolerability and biocompatibility. HA can be fully absorbed by the organism and therefore does not need to be removed from the graft site. It also has an osteoconductive action.[27]

The primary aim of this 24-month randomized controlled clinical trial was to compare the clinical and radiographic effectiveness of a composite graft consisting of treatment with esterified HA (test group) or OFD alone (control group) in the surgical treatment of human periodontal infrabony defects.

METHOD AND MATERIALS

Study design and population

This study analyzed 90 patients (54 women and 46 men aged between 38 and 68 years; mean age, 45.1 years) who were referred for treatment of moderate to severe chronic periodontitis. The protocol was approved by the Ethics and Research Committee of University of Messina, Messina, Italy, and the experimental procedures were applied in accordance with the Declaration of Helsinki of 1975, as revised in 2000. All patients signed an informed consent form. The inclusion criteria were as follows: absence of systemic disease, negative history for pregnancy, no regular use of medication or drugs during the previous 6 months, nonsmoking, advanced generalized chronic periodontitis, Plaque Index[28] (PI) < 1, presence of an angular two-wall infrabony defect in the interproximal area (PD ≥ 7 mm; CAL ≥ 7 mm), absence of furcation involvement or angular defects extending into the furcation area of the target tooth, absence of caries or overflowing restorations, and absence of periapical injuries. The sample size required to detect a true difference of 0.5 mm between the test and control groups with 90% power and an alpha error of .05 was estimated as described previously[29] using CAL changes as the primary outcome variable. Based on previous estimates of outcome variability[30] and subject attrition rates observed in previous clinical trials of similar design by this group, 90 subjects with complete data were required.

Control of periodontal infection was achieved prior to the experimental phase by an initial treatment consisting of patient motivation, oral hygiene instruction, and scaling and root planing. When indicated, the clinicians supplemented mechanical debridement with antiseptics.

Table 1 Characteristics of the study groups

	Control (n = 20)	Test (n = 20)	Total (n = 40)
Age (mean ± SD) (y)	42.3 ± 8.4	47.7 ± 8.1	45.0 ± 8.2
Age range (y)	39–68	38–63	38–68
Male (n [%])	10 (50.0)	8 (37.5)	18 (43.75)
Female (n [%])	10 (50.0)	12 (62.5)	22 (56.25)
Defect position			
Maxillary posterior (n [%])	10 (50.0)	10 (50.0)	20 (50.0)
Maxillary anterior (n [%])	2 (12.5)	3 (18.75)	5 (15.6)
Maxillary anterior (n [%])	7 (37.5)	4 (18.75)	11 (28.1)
Mandibular anterior (n [%])	1 (6.25)	1 (6.25)	2 (6.25)

SD, standard deviation.

After the maintenance phase, 50 patients were excluded because they were not in line with the inclusion criteria of the study (mean PI > 1).[28] The final number of patients analyzed in the study was reduced to a total of 40 (Table 1).

All patients showed a deep infrabony defect (≥ 5 mm) in the interproximal region. After the maintenance period, the patients were immediately planned for surgery. For each patient, only one infrabony defect (the deepest one) was chosen for study. The defects, which were a consequence of chronic periodontitis, were randomly included in one of two groups: 20 defects were treated with HA (test group) and 20 cases with OFD alone (control group).

In the test group, the treated sites were filled with the biomaterial (Hyaloss matrix, Meta G.C.M) after the surgical access and cleaning phases were carried out. A similar procedure was performed for the control group, only without the application of the biopolymer.

Clinical measurements

The following clinical parameters were recorded immediately prior to the surgery and repeated 12 and 24 months later: PI, bleeding on probing (BoP), PD, and CAL.

All measurements were obtained using a standard periodontal probe (no. 15, Hu-Friedy) at the mesiobuccal, buccal, distobuccal, distolingual, lingual, and mesiolingual surfaces of each tooth. Measurements were rounded up to the nearest millimeter. All measurements were recorded by one calibrated examiner (GI) who was blinded to the surgical procedure. To ensure acceptable intraexaminer reliability, the examiner was calibrated to show > 90% agreement within 1 mm by duplicate measurements of PD and CAL of 30 randomly selected teeth.

Both the depth and size of the defect were measured during the intraoperative phase. The depth was measured in terms of the distance (mm) between the base of the defect (BD) and the most coronal portion of the residual bone ridge (FC) as well as the distance between the cementoenamel junction (CEJ) and the base of the defect (BD). The defect size was measured as the distance between the CEJ and the residual ridge bone (CR).

Radiographs were taken preoperatively and after 6, 12, and 24 months using a periapical radiograph with a Rinn alignment system (Dentsply Rinn) to measure the radiographic depth of the infrabony component.

The test material used in this study was an ester of HA in a fiber form that, on contact with the patient's blood, could instantaneously form a gel. With the addition of bone in granules, this gel forms an easy-to-handle paste that makes the application of the graft easier and allows perfect adaptation to the morphology of the defect.

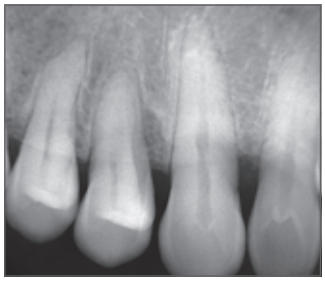


Fig 1 Preoperative radiograph of an infrabony defect in the test group.

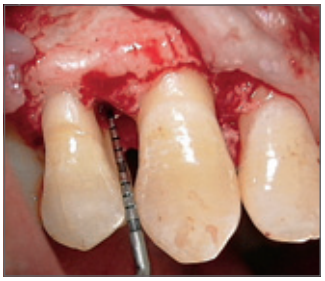


Fig 2 Intraoperative view of a periodontal defect in the test group. Clinical measurements were taken of the bone levels.

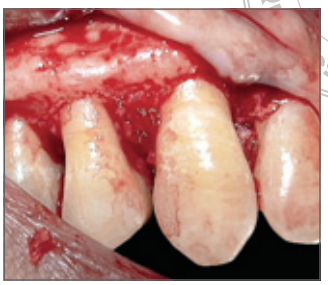


Fig 3 Application of the HA-based biomaterial to the defect.

Double-blinding and randomization

The dentists who performed the measurements were different from those who performed the surgery; therefore, the examiner was blinded during the entire duration of the study. Intraexaminer reproducibility was evaluated as the standard deviation of the difference of triplicate measurements. All investigators reached the target standard deviation (< 0.4 mm) for attachment levels. Interexaminer variability was evaluated as the standard deviation of the difference from the gold standard represented by the first and second authors. The computed value for attachment level was less than 0.5 mm for all clinicians. Every clinical measurement was recorded to the nearest millimeter with a manual pressure–sensitive probe by one trained investigator (GI) at the deepest location of the selected site.

At baseline, the 40 patients were randomly placed into either the test or control group. A number from 0 to 40 was assigned to each patient. All clinical data for each patient were provided by a surgeon (EB) in an envelope labeled with the corresponding number. Every envelope was sealed by another examiner not involved in the diagnosis or treatment of patients. Shortly before each surgery session, after the defect was degranulated, EB entered the operating theater, opened the envelope bearing the number by which the patient would subsequently be identified, and informed the surgeon, who then randomly assigned the patient to one of the two treatments. EB then left the operating theater immediately. Neither the patients nor the examiners were informed about the type of treatment for the duration of the study, thus avoiding prejudices in the data evaluation.

Surgical technique

All treatments were performed by the same surgeon. After local anesthesia, the operation was carried out using simplified papilla preservation flaps[31] to maintain the greatest amount of soft tissue for primary closure and obtain complete access to the defect (Figs 1 and 2). The exposed defects were carefully scaled and root planed to remove residual mineralized deposits but not necessarily the root cementum. A combination of sonic, ultrasonic, and/or hand instrumentation was used for this purpose.

The clinical measurements of bone levels (CEJ-BD and FC-BD) were then made. The site was washed with physiologic sterile solution, and root conditioning with 24% ethylenediaminetetraacetic acid (EDTA) was carried out for 3 minutes. The surgical area was rinsed with sterile saline to remove any residual EDTA. At this stage, the treatment proceeded with either the test or control protocols. For each patient of the test group, HA fibers were packed directly into the defect (Fig 3). For patients in the control group, the procedure was identical to the test surgery except for the omission of HA application with OFD (Figs 4 to 6). Finally, the tension-free flaps were

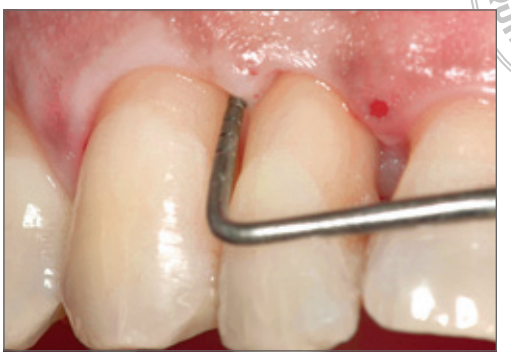


Fig 4 Preoperative view of an infrabony defect in the control group.

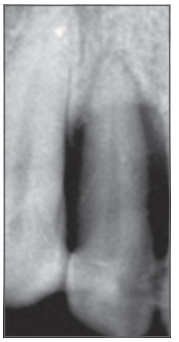


Fig 5 The radiographic depth of the infrabony component was checked before surgery.

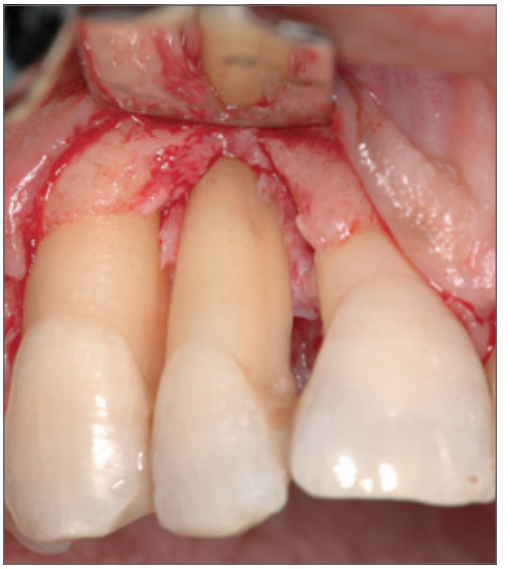
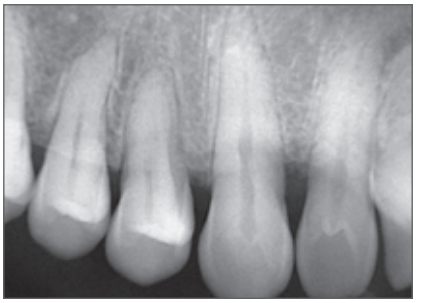


Fig 6 Intraoperative view of a periodontal defect in the control group.



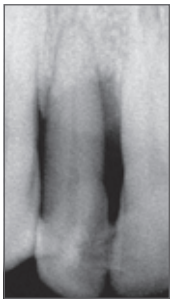


Fig 7 *(left)* Radiograph showing bone regrowth in the test group.

Fig 8 *(right)* Radiograph showing bone healing in the control group.

repositioned at the presurgical level using 4.0 interrupted interproximal sutures (Vicryl, Ethicon), which were left in situ for 14 days without surgical packing. During this period, the patients suspended home hygiene procedures in the treated area to minimize mechanical trauma. The patients were checked weekly during the first month after surgery, once a month for the next 6 months, and quarterly thereafter (Figs 7 and 8). During follow-up sessions, supportive therapy consisting of supragingival scaling, polishing, and reinforcement of oral hygiene instructions was provided.

Table 2 Clinical parameters at baseline (mean ± SD)

	Control group	Test group
CAL (mm)	8.3 ± 1.2	7.2 ± 1.5
PD (mm)	8.0 ± 0.7	8.6 ± 1.1
BD-FC (mm)	5.4 ± 0.6	5.1 ± 1.8
CEJ-CR (mm)	6.0 ± 1.3	5.7 ± 1.2
CEJ-BD (mm)	7.7 ± 1.9	8.4 ± 1.6
PI	2	1
BoP	3	3

SD, standard deviation; CAL, clinical attachment level; PD, pocket depth; PI, Plaque Index; BoP, bleeding on probing; BD-FC, distance between the base of the defect and the most coronal portion of the residual bone ridge; CEJ-CR, distance between the cementoenamel junction and the residual ridge bone;
CEJ-BD = distance between the CEJ and the base of the defect.

Table 3 Clinical parameters at baseline and at 12 and 24 months (mean ± SD)

Group	Clinical index	Baseline	12 mo	24 mo
Control				
	PD (mm)	8.0 ± 0.7	7.1 ± 1.3	7.2 ± 0.5*
	CAL (mm)	8.3 ± 1.2	6.9 ± 1.8	7.2 ± 0.7**
	PI	2	1	1
	BoP	3	2	1
Test				
	PD (mm)	8.6 ± 1.5	7.4 ± 0.6	7.0 ± 1.2**
	CAL (mm)	7.2 ± 1.5	6.5 ± 0.9	5.3 ± 1.8**
	PI	1	1	1
	BoP	3	2	1

SD, standard deviation; CAL, clinical attachment level; PD, pocket depth; PI, Plaque Index; BoP, bleeding on probing. *Statistically significant ($P < .05$); **highly significant ($P < .001$).

Statistical analysis

The two groups were assessed for comparison at baseline and after 12 and 24 months using the Student *t* test and chi-square test. SPSS 15 (IBM) was used for all analyses. Analysis of variance (ANOVA) was used to test variations in CAL and PD, and the Cochran test and ANOVA were used to analyze changes in BoP and PI at baseline and 12 and 24 months. A change in CAL was specified as the primary outcome variable used to check the significance of differences between the study groups.

RESULTS

There were no significant differences between the two groups in terms of age, sex, and distribution of treated sites in the maxilla and mandible. The BD-FC, CEJ-CR, and CEJ-BD measurements also showed no significant differences between the two groups at baseline (*t* test, $P < .05$) (Table 2). Differences were found regarding CAL values, which were higher in the control group than in the test group (*t* test, $P > .05$). Using the Fisher exact test, no significant differences were found regarding BoP or PI after 12 and 24 months, but there was a reduction of 18.75% in both groups. BoP decreased at 12 and 24 months in both the test group and the control group (Table 3).

Complete healing was found in 94.6% of the 40 patients who successfully completed the study. In two cases (6.4%), the lack of healing was attributed to the loss of patient compliance in an advanced stage of the study. Improvements of PD, CAL, and BoP were found at 12 and 24 months, with a significant improvement ($P < .05$) of the CAL and PD values in both groups (Table 3).

The values of PD and CAL at baseline and at 12 and 24 months changed significantly (ANOVA, $P < .001$). The changes in CAL values ($P = .003$) were statistically significant in the test group. A significant difference with regard to the PD values (ANOVA, $P = .025$) was shown.

A slight decrease in PD and CAL was found only in the test group between 12 and 24 months, which was statistically significant in terms of the CAL ($P < .001$).

The frequency distribution of various CAL gains at test and control sites is shown in Table 4. Almost double the number of sites with clinically relevant CAL gains of 2 mm or more (86.7%) were found in test sites compared with controls (68.5%). Further, sites with no or very small gains

were more frequent in the control group (28.9%) than in the test group (12.1%). Of the few sites that lost attachment as a result of treatment, more than twice as many were in the control group (2.6%) than in the test group (1.2%). The frequency distribution of PD reduction (Table 5) showed similar results between test and control groups, with a reduction of 1 mm or more evident in more of the test sites (77%) than in controls (27.5%). Small reductions in PD (0 to 1 mm) occurred more in the control group (69.9%) than in the test group (22.1%).

Bone gain was found in both sites with radiographic assessment, especially in the test sites from baseline to 24 months (see Figs 1 and 4). The mean CAL gain at 12 months was 0.7 ± 0.9 mm in the test group and 1.4 ± 1.8 mm in the control group (Table 3). At 24 months, the mean CAL gain was 1.9 ± 1.8 mm in the test group and 1.1 ± 0.7 mm in the control group. One year after therapy, the mean PD reduction was 1.2 ± 0.6 mm in the test group and 0.9 ± 1.3 mm in the control group. Two years after the treatment, mean PD reductions of 1.6 ± 1.2 mm for the test group and 0.8 ± 0.5 mm for the control group were observed. This difference was statistically significant for the control group (ANOVA, $P = .025$) and highly statistically significant for the test group.

Table 4 Frequency distribution of CAL gain

	CAL gain (mm)				
Group	Loss	0–1	1–2	2–3	≥ 4
Test	1.2%	12.1%	68.4%	13.5%	4.8%
Control	2.6%	28.9%	58.4%	8.9%	1.2%

CAL, clinical attachment level.

Table 5 Frequency distribution of PD reduction

	PD reduction (mm)				
Group	Loss	0–1	1–2	2–3	≥ 4
Test	0.9%	22.1%	66.2%	9.5%	1.3%
Control	2.6%	69.9%	20.4%	6.1%	1%

PD, pocket depth.

DISCUSSION

The results showed that the use of HA led to significant improvements in PD and CAL at 24 months compared to baseline. Further, a significantly greater improvement in PD was observed in the test subjects compared to controls (Fig 9 and Table 3). No statistically significant differences in PI or BoP were found between the groups at any point during the study. No differences in recession of the gingival margin were observed between test and control patients. The mean CAL gain at 24 months was 1.1 ± 0.7 mm in the control group and 1.9 ± 1.8 mm in the test group (Fig 10). The rationale for this discrepancy may be related to the biologic property of the material used in the test group. A previous clinical study reported a mean CAL gain of 3.8 mm.[32]

Few studies have dealt with the use of HA in dentistry. Pistorius et al[33] studied 40 patients with gingivitis treated with a topical application of HA. The results showed that HA reduced gingival bleeding and acted as a cofactor in the reduction of inflammation in gingivitis. The use of HA in periodontal defects was also investigated by Engström et al,[34] who investigated the antiinflammatory effect and effect on bone regeneration of HA in surgical and nonsurgical groups. They observed that the difference in bone height between the test and control sites in the surgical group after 12 months was less than 1 mm, which was only detectable in radiographs. The present study also demonstrated that a significant reduction in PI was observed in both groups. This observation is in accordance with previous reports by Mesa et al[35] and Jentsch et al[36] on improved gingival health after the supragingival application of various HA formulations in subjects with gingivitis. However, other investigators found no beneficial effect of HA on periodontal health. Xu et al[37] studied the use of HA associated with scaling and root planing in patients with chronic periodontitis and found no differences between the HA site and control site relative to BoP and PD.

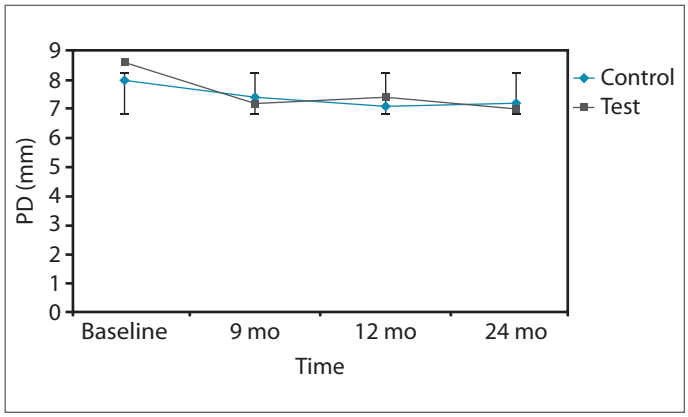


Fig 9 PD evaluation at baseline and after 9, 12, and 24 months in the control and test groups.

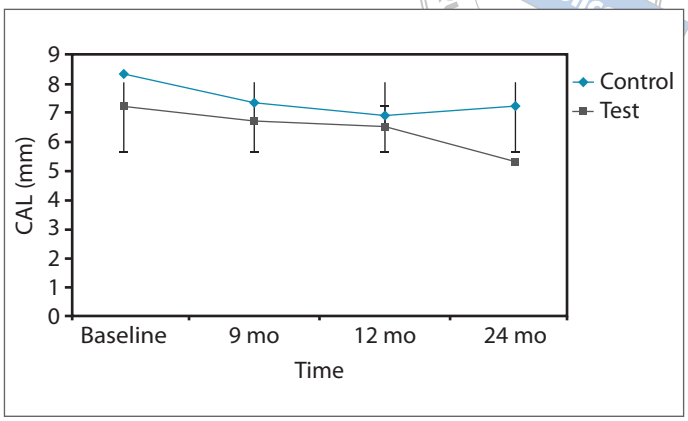


Fig 10 CAL evaluation at baseline and after 9, 12, and 24 months in the control and test groups.

Only sulcus fluid flow rate improved in the HA group relative to controls. The HA gel was administered six times: at baseline and then every week up to week six. Differences in treatment protocols and measurements may explain the different outcomes of the present study and Xu et al's. Mendes et al[38] evaluated the effects of sodium hyaluronate in the healing process of the tooth sockets of rats in an immunohistochemistry study. The right sockets were treated with 1% HA gel, while the left sockets were used as controls (blood clot). The data showed that HA treatment induced earlier trabecular bone deposition, resulting in a more organized bone matrix at 7 and 21 days after tooth extraction. HA also stimulated the expression of osteogenic proteins such as bone morphogenetic protein 2 (BMP-2) and osteopontin. Smith et al[39] and Wikesjö et al[40] studied the effect of HA (absorbable collagen sponge) as a carrier for BMP-2 and growth factors such as platelet-derived growth factor BB (PDGF-BB). These studies showed that absorbable collagen sponge appears to be a suitable candidate carrier for BMP-2 to stimulate alveolar bone and cementum formation in periodontal defects in dogs and in infrabony defects. In the control groups, the radiographic investigation confirmed that the defects were filled. Patients in our study also have not shown significant increases in the level of the bone crest. The best results were obtained in cases in which the bony defects were limited (6 to 7 mm) and with a favorable conformation.

The present study demonstrated the biocompatibility, ease of use, and osteoconductive capacity of HA. Moreover, HA could be used as a carrier for growth factors such as PDGF-BB[41] and BMP-2 for periodontal regeneration. HA's undeniable biocompatibility, ease of handling, extent of healing, radiographically documented bone regrowth, and absence of complications make it worthy of further study. Along with clinical and radiographic studies, histologic studies should be performed to verify the extent of the material's osteoconductive capacity.

CONCLUSION

This study showed that HA appears to effectively support periodontal wound healing and can be adaptable to different anatomical morphologies. The use of HA could provide an additional benefit in terms of CAL gain and PD reduction compared with open flap debridement. Defects with a nonsupporting anatomy may be at risk for failure and advanced midfacial recession.

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