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ORIGINAL ARTICLE

Clinical effects of nanocrystalline hydroxyapatite paste in the treatment of intrabony periodontal defects: a randomized controlled clinical study

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Abstract The purpose of the present randomized controlled clinical study was to compare the clinical outcomes of papilla preservation flap surgery with or without the application of a novel nanocrystalline hydroxyapatite (nano-HA) bone graft substitute. Fourteen patients with paired intrabony periodontal defects of ≥4 mm participated in this split-mouth design study. The defects in each subject were randomly selected to receive nano-HA paste in conjunction with papilla preservation flaps or papilla preservation flaps alone. Probing bone levels (PBL) from a customized acrylic stent and probing pocket depths (PPD) were measured at baseline and again 6 months following surgery. No differences in any of the investigated parameters were observed at baseline between the two groups. Healing was uneventful in all patients. Both treatments resulted in significant improvements between baseline and 6 months ($p<0.05$). At 6 months after therapy, the sites treated with nano-HA paste showed a reduction in mean PPD from 8.3±1.2 to 4.0±1.1 mm and a gain in PBL of 4.3±1.4 mm, whereas in the control group, the mean PPD changed from 7.9±1.2 mm to 5.0±1.2 mm and PBL gain was 2.6±1.4 mm. Results demonstrated statistically greater PPD reduction and PBL gain ($p<0.05$) in the test group as compared with the control group. In conclusion, after 6 months, the treatment of intrabony periodontal defects with a nano-HA paste leads to significantly improved clinical outcomes when compared with papilla preservation flap surgery alone.

Keywords Nanostructured hydroxyapatite · Grafts · Intrabony defects · Periodontal regeneration · Periodontal diseases

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Introduction

The ultimate goal of periodontal therapy is the regeneration of periodontal tissues which have been destroyed due to periodontal disease. Periodontal regeneration is the reconstruction of the lost tissues as evidenced histologically in the formation of new cementum, new alveolar bone, and a functionally oriented periodontal ligament. Different modalities have been proposed to obtain regeneration of periodontal tissues employing various bone grafts, bone substitute materials, guided tissue regeneration (GTR), combination of bone grafts or bone substitutes with GTR, and growth factors [1–6]. In recent years, various alloplastic synthetic bone graft materials have gained clinical acceptance in the treatment of human periodontal osseous defects. These materials have been extensively used in periodontal regenerative surgery with the primary objective of reducing patient morbidity. However, while clinical results of using alloplast grafts to treat periodontal defects appear promising [7], histologically, the grafts tend to heal almost exclusively by connective tissue encapsulation with minimal or no bone formation and not by true periodontal regeneration [8].

Hydroxyapatites (HAs) represent a family of bone grafting materials with a high degree of biocompatibility which is largely attributable to its presence in natural calcified tissue. HA, $Ca_{10}(PO_4)_6(OH)_2$, is a calcium phosphate-based bioceramic material which makes up the majority of the inorganic components of human bones and teeth. The compound has a Ca/P mole ratio of 1.67 and is formed by precipitation of calcium nitrate and ammonium dihydrogen phosphate. When HA is implanted into a bony site, it has been shown that it is slowly resorbed, providing calcium and phosphate that is then available for the process of biomineralization and new bone formation [9]. However, one of the major drawbacks was that HA-based biomaterials required high-temperature and high-pressure processing, which resulted in higher density and decreased porosity. Therefore, the HA bone-grafting materials exhibited decreased osteoconductivity and poor degradation characteristics [10]. Thus, even though HA grafts demonstrated effectiveness in clinical resolution of periodontal defects [11], the healing after treatments with these graft materials evidenced a long junctional epithelium with only a limited regenerative potential [7, 12]. In pursuit of improving these shortcomings, a novel fully synthetic nanocrystalline hydroxyapatite (nano-HA) paste (Ostim®; Heraeus Kulzer, Hanau, Germany) containing 65% water and 35% of nanostructured apatite particles has been introduced for augmentation procedures in osseous defects [13, 14]. The nano-HA paste has already been used for the treatment of various types of metaphyseal fractures such as the calcaneus and tibia in orthopedic surgery [15], as well as for tooth perforations [16], jaw cysts [17], and peri-implantitis lesions [18]. So far, there are still limited data evaluating the healing of intrabony periodontal defects following treatment with nano-HA paste [19, 20].

The purpose of the present study was to evaluate the clinical outcomes obtained following treatment of intrabony periodontal defects with papilla preservation surgery with or without application of nano-HA paste.

Materials and methods

Experimental design and study population

A split-mouth, randomized, controlled clinical trial was designed to test the efficacy of two treatment modalities in intrabony periodontal defects. Change in probing bone level was the primary outcome parameter. Assuming a standard deviation of 1.5 mm for the difference in bone gain between test and control treatment, a sample size thirteen was calculated to be able to detect a difference in bone gain of 1.5 mm with a power of at least 90%. For the compensation of possible dropouts, a total of fifteen systemically healthy patients (eight females and seven males), aged 38 to 50 years, seeking care for moderate to advanced periodontal disease were recruited for the study.

Inclusion criteria consisted of two similar periodontal intrabony defects with an intraosseous component ≥4 mm as evidenced by intraoral radiographs taken during the screening phase and confirmed during surgery. The subjects exhibited a total of 28 predominantly two-wall defects. The patient exclusion criteria consisted of severe acute or chronic systemic diseases, compromised immune system, pregnancy and/or lactation, and medications known to affect the gingival status. The study included four current smokers and ten nonsmokers. Patients were informed as to the character and purpose of the study and were required to sign an informed consent. The study was performed according to the Helsinki Declaration of 1975, as revised in 1983.

Initial periodontal therapy consisted of oral hygiene instructions, full-mouth supra- and subgingival scaling and root planning, and occlusal adjustment when indicated. Teeth with mobility of greater than class one were splinted to adjacent teeth with light-cured resin. At least 6 weeks elapsed from the completion of the initial treatment phase before the baseline examination. Full-mouth plaque scores and full-mouth bleeding scores of ≤20% were required for all patients before proceeding with the surgical phase of therapy.

Clinical measurements

All baseline clinical parameters were recorded at the day of surgery and 6 months later. Prior to surgery, a customized acrylic occlusal stent was fabricated for each patient on a cast model obtained from an alginate impression. Grooves were placed on the stents to ensure the same position and angulation of measuring probes postsurgery. Probing bone level (PBL) included the distance from the stent to the bottom of the bone defect and was measured under local anesthesia. Probing pocket depth (PPD) was measured from the gingival margin to the base of the pocket. All measurements were performed by a single-blinded calibrated examiner at six aspects per tooth (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual, and disto-lingual) using a standard periodontal probe (PCP 15; Hu-Friedy, Chicago, IL, USA). The site with the deepest measurements in each defect at baseline was considered as the observation site. Intraoral radiographs were taken before surgery and at the 6-month follow-up visit using the long-cone paralleling technique.

Surgical procedures

Two selected sites in each patient were operated simultaneously employing papilla preservation flaps to gain access

to the bony defects. In the narrow interproximal spaces (≤2 mm), incision with the preservation of the buccal papilla according to the simplified papilla preservation technique [21] was applied, whereas in the wide interdental spaces (>2 mm), the modified papilla preservation technique was used [22]. Full-thickness mucoperiosteal flaps were raised to gain complete access to the defect; granulomatous tissue was debrided from the osseous defect and the root surfaces were carefully scaled and root planed by ultrasonic and hand instruments. In no cases was osteoplasty/ostectomy carried out. The surgical area was then rinsed with sterile saline. In each patient, the first defect was allocated to one of the two treatment regimens according to a randomization list. In the test sites, nano-HA paste was placed within the defect up to the existing level of the alveolar crest and care was taken not to overfill the defect. The mucoperiosteal flaps were repositioned and secured in place using nonresorbable # 6-0-suturing material (Prolene®; Ethicon, Hamburg, Germany). Vertical or horizontal mattress sutures and additional interrupted single sutures were performed to obtain primary closure of the interdental space. The selection of the suturing technique was based upon the dimension of the interdental space and the thickness and height of the interdental tissues. No surgical dressing was used. The surgical procedure for control sites was identical to the test sites except for the omission of the placement of the nano-HA paste into the defects.

Postsurgical care and infection control

Postoperative pain was controlled with 600 mg ibuprofen, as necessary. All patients were instructed to rinse with 0.2% chlorhexidine solution twice a day for 4 weeks with additional application of 1% chlorhexidine gel to the wound area. In order to minimize traumatic injury to the marginal tissues, patients were instructed to avoid mechanical oral hygiene procedures in the treated area for 4 weeks. No interdental cleaning was allowed in the treated area for the first six postoperative weeks. Smokers were asked to limit and possibly avoid smoking. Sutures were removed 10–14 days following surgery. All patients received professional supragingival tooth cleaning together with oral hygiene reinforcement at 6 weeks, 12 weeks, and 6 months.

Statistical analysis

The design of the study was a split-mouth design so that each subject served as his own control. All statistical analyses were performed using statistical software (SPSS 12.0 for windows; Chicago, IL, USA). Mean and standard deviation (SD) were calculated for PPD and PBL at baseline and at 6 months following surgery in both groups. The baseline and 6 months values were then compared for changes that took place over time. The paired t test was utilized to evaluate differences between the treatment groups. The level of significance was set at $p<0.05$.

Results

All patients tolerated the surgical procedures well, experienced no postoperative wound-healing problems or inflammatory reactions, complied with the study protocol, and 14 patients completed the 6-month follow-up. One patient was lost to follow-up. No statistically significant differences were found between the test and control sites for any of the investigated clinical parameters at baseline.

Full-mouth plaque scores and full-mouth bleeding scores were maintained at ≤20% throughout the study period. Table 1 summarizes the changes in clinical measurements throughout the study period. In comparison with the baseline data, both the test and control sites demonstrated statistically significant reduction of PPD and gain of BL ($p<0.05$). The average probing bone gain was 4.3±1.4 mm after nano-HA paste application (test) as compared with 2.6±1.4 mm after access flap surgery alone (control). The probing bone gain in the test sites was significantly higher than in the control sites ($p<0.05$). At 6 months after therapy, the test sites showed a reduction in mean PPD from 8.3±1.2 mm to 4.0±1.1 mm and from 7.9±1.2 mm to 5.0±1.2 mm in the control sites. The PPD reductions in the test sites were significantly higher than in the control sites ($p<0.05$). Representative clinical cases are shown in Figs. 1 and 2.

Discussion

The present study evaluated the clinical outcomes obtained following treatment of intrabony periodontal defects with papilla preservation surgery with or without application of a nano-HA paste. It was designed as a split-mouth investigation to facilitate the comparison of two treatment procedures by eliminating patient-specific characteristics which might have impact on the results of regenerative surgeries [1, 23]. The split-mouth design has been considered adequate for evaluating regenerative procedures in a recent systematic review [24].

PPDs and clinical attachment levels are commonly used as primary outcome measures in clinical trials and for routine clinical assessment of periodontal therapies. However, current probing methods are also subject to a multitude of errors due to variations in probing force, probe design, and to the degree of tissue inflammation [25, 26]. In the present study, probing bone level measurements (bone-sounding measurements) under local anesthesia were used as a primary outcome

Table 1 Probing pocket depth (PPD) and probing bone level (PBL) values at baseline and the 6 months evaluation (millimeter) following treatment of intrabony defects with nano-HA paste (test) or papilla preservation flap surgery alone (control)

Patient	Tooth number	Treatment group	PPD			PBL		
			Baseline	6 months	Change	Baseline	6 months	Change
1	14	AF	7	5	2	9	7	2
1	34	Nano-HA	9	3	6	12	5	7
2	46	AF	9	6	3	13	12	1
2	36	Nano-HA	10	5	5	15	11	4
3	33	AF	8	4	4	14	12	2
3	44	Nano-HA	8	3	5	13	8	5
4	34	AF	9	6	3	16	12	4
4	44	Nano-HA	9	5	4	15	11	4
5	14	AF	7	4	3	16	13	3
5	24	Nano-HA	7	4	3	15	13	2
6	23	AF	7	5	2	10	9	1
6	34	Nano-HA	9	3	6	12	7	5
7	36	AF	8	4	4	14	12	2
7	46	Nano-HA	9	6	3	16	11	5
8	35	AF	7	5	2	9	9	0
8	26	Nano-HA	8	6	2	10	6	4
9	36	AF	8	5	3	9	7	2
9	24	Nano-HA	6	4	2	9	5	4
10	14	AF	7	5	2	15	12	3
10	34	Nano-HA	9	3	6	16	12	4
11	36	AF	9	5	4	12	9	3
11	13	Nano-HA	9	4	5	12	6	6
12	26	AF	8	3	5	14	9	5
12	35	Nano-HA	8	2	6	14	10	4
13	25	AF	10	7	3	16	12	4
13	14	Nano-HA	10	4	6	16	11	5
14	33	AF	7	6	1	12	9	3
14	44	Nano-HA	6	4	2	10	9	1
Mean±SD		Nano-HA	8.3±1.2	4.0±1.1	4.3±1.6	13.2±2.5	8.9±2.7	4.3±1.4
Mean±SD		AF	7.9±1.2	5.0±1.2	2.9±1.1	12.8±2.6	10.2±2.1	2.6±1.4
P value			n.s.		<0.05	n.s.		<0.05

AF access flap, *nano-HA* nanocrystalline hydroxyapatite, *n.s.* non significant

measure. Although surgical reentry is the most accurate method to assess hard-tissue changes, it may cause discomfort to the patient and possibly damage the regenerated tissue. Therefore, probing bone level measurements have been introduced and found to be an accurate method of assessing bone level making reentry procedures in clinical trials unnecessary [27].

Overall, the results of this study indicate that treatment of periodontal defects with nano-HA paste is clinically superior to treatment without nano-HA for each clinical parameter measured. PBL and PPD changes were shown to be superior in the nano-HA paste sites to a level which was not only statistically, but also clinically, significant. Despite utilizing a different study design, the added benefit observed in this study compares favorably with a previous randomized controlled clinical trial, where 3.9±1.2 mm for PPD reductions were reported after application of nano-HA paste to intrabony defects [19]. The test group of this study, therefore, performed as expected based on the previous evidence. Slight differences in the reported results may, at least in part, be explained by patient characteristics and differences in the employed surgical technique. In the present study, nano-HA was used in combination with surgical techniques based on interdental soft-tissue preservation. Data from previous clinical studies strongly suggest that the supracrestal preservation of soft tissues may have a

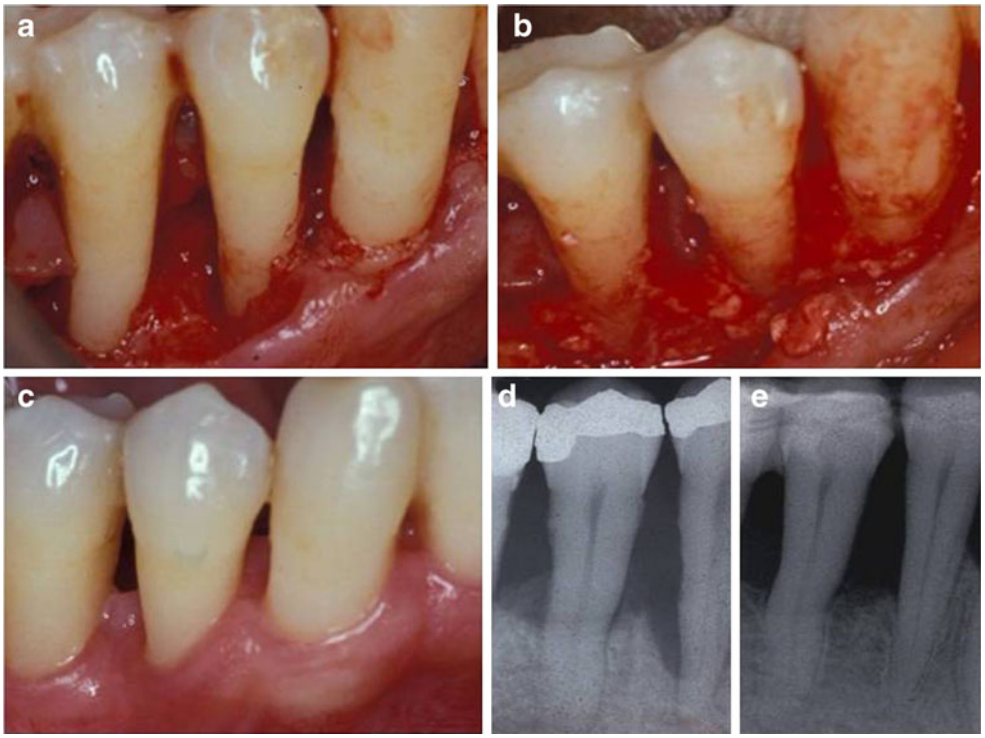

Fig. 1 Surgical treatment, test site. **a** The intraoperative clinical image shows a deep intrabony defect distal of the lower right first premolar. **b** Nano-HA paste placed. **c** Clinical situation 6-month postoperative. **d** Presurgical radiograph. **e** 6-month postoperative radiograph

significant influence upon postoperative clinical outcomes [22, 28]. The presence of thick and high/wide interdental soft tissue facilitates flap management and suturing technique, improves the possibility to achieve and maintain primary closure in the interdental area, and reduces the risk of soft-tissue collapse into the defect area [29]. Thus, in the present study in all treated sites, primary closure of the interdental sites was obtained at completion of the surgical

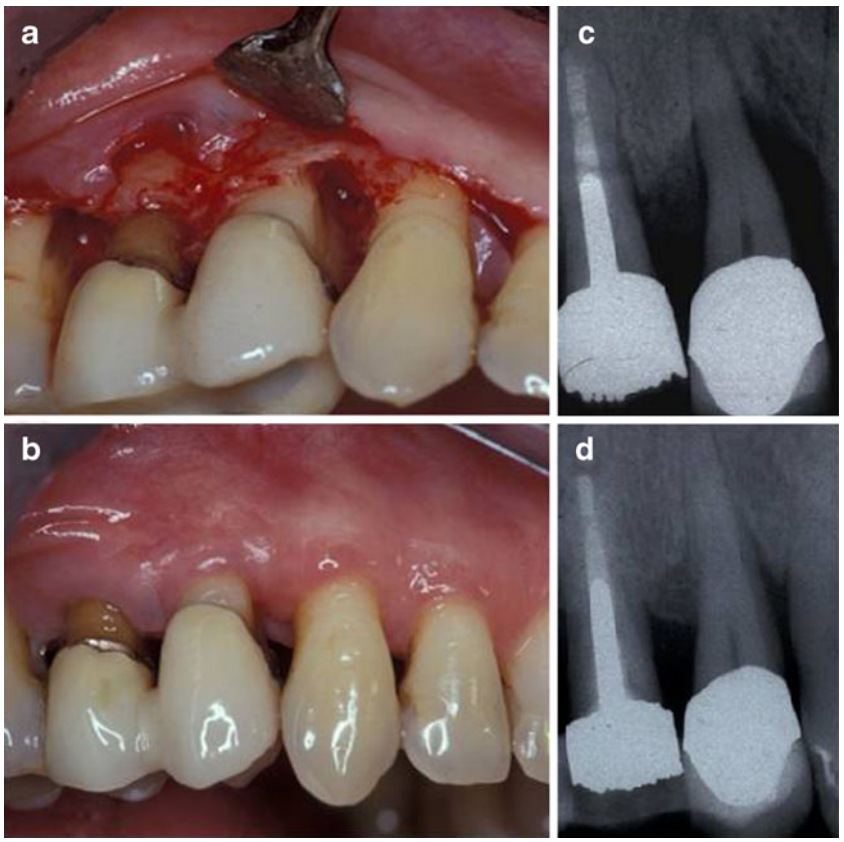

Fig. 2 Surgical treatment, control site. **a** The intraoperative clinical image shows a deep intrabony defect mesial of the upper right first premolar. **b** Clinical situation 6-month postoperative. **c** Presurgical radiograph. **d** 6-month postoperative radiograph

procedure. Under these circumstances, it is reasonable to assume that wound healing occurred in a sealed environment with minimal levels of bacterial contamination, which is a prerequisite to allow optimal retention and biological activity of the applied material. The clinical handling of the nano-HA paste demonstrated to be very good, and since the material is supplied in prefilled syringes, there was no need for prehydration. Zuev et al. [20] reported in a comparative study that complications after treatment of periodontal defects with nano-HA paste occurred only in 1.5% cases, as compared with a complication rate of 3.6% in the group treated by transplanted bone matrix.

The exact biologic mechanism of action of nano-HA paste has not yet been elucidated. A recent in vitro study has demonstrated that when nano-HA is added to cultures of periodontal fibroblasts, cell proliferation and adhesion are enhanced [30]. It should also be emphasized that, within the context of regenerative outcomes, interpretation of improvements in clinical parameters is incomplete without consideration of wound healing on a histological level. Therefore, it is not possible to make any comment regarding the regeneration of a functional periodontal apparatus. As yet, essentially, all available data indicate that alloplastic grafts function primarily as biocompatible space fillers and support periodontal repair rather than regeneration [31]. Thus, despite of good clinical results following treatment of intrabony defects with HA-based grafts [32, 33], human histologic studies in the past have failed to demonstrate periodontal regeneration following implantation of HA-based biomaterials [7, 34]. However, differences in the physicochemical and structural characteristics between the novel nano- HA paste and the various forms of HA used in the past may lead to differences in the regenerative/osteoconductive properties as well as in resorption characteristics. Material properties, like for example, porosity, surface geometry, and surface chemistry play a determinant role in osteoconductive capacities of a graft [35]. The results of a recent histologic case series suggested that some regeneration may be possible with nano-HA paste [36]. The amount of new cementum and new bone varied from 0 to 0.86 mm and from 0 to 1.33 mm, respectively. Furthermore, the histologic analysis revealed an almost complete resorption of the bone graft material after 7 months. The graft is composed only of nanostructured apatite particles (35%) with a mean size of approximately <100 nm and water (65%); these features, in conjunction with the reported resorption characteristics, suggest that the improvements in clinical parameters demonstrated in the present study, cannot be attributed to the presence of residual particles modifying gingival tissue consistency and therefore impeding penetration of the periodontal probe. However, future studies including histological evidence are required to determine the nature of wound healing when using nano-HA paste. A special feature of the nanostructured materials is the existence of an extremely high number of molecules on the surface in comparison to bulk material. The results of a recent in vitro study demonstrated better compatibility and dissolvability of nanometer HA as compared with dense HA [37]. When the nano-HA paste was used as a bone graft substitute, rapid healing of critical size defects was observed in animal experiments and in human applications [13, 38]. A complete resorption of the material was observed within 12 weeks [39]. Schnettler et al. [40] found that nanocrystalline HA binds to the bone and stimulates bone healing by stimulation of osteoblast activity.

In conclusion, results from the present study indicate that at 6 months after surgery, the treatment of intrabony defects with an interdental soft-tissue preservation flap and nano-HA application leads to enhanced clinical results for probing bone gain and probing pocket depth reductions when compared with papilla preservation flaps alone.

Conflict of interest The authors declare that they have no conflict of interest.

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