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Histologic and Histomorphometric Evaluation of Maxillary Sinus Floor Elevation Using Nanobone® and Easy-Graft™ Crystal: A Split-Mouth Clinical Trial

Mansour Meymandi[1], Mostafa Solati[2*], Mohammadreza Talebi Ardakani[1], Fatemeh Mashadi abbas[3] and Anahita Ashouri Moghaddam[4]

[1]Department of periodontics, Dental School, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2]Department of periodontology, Dental School, AJA University of Medical Sciences, Tehran, Iran
[3]Department of Oral and Maxillofacial Pathology, Dental School, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[4]Department of periodontics, Dental School, Gilan University of Medical Sciences, Tehran, Iran

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ABSTRACT
Maxillary sinus floor elevation is an effective method for bone augmentation in the posterior maxilla. Due to the limitations of autogenous bone grafts, bone substitutes are often used for this purpose. This study sought to compare the histologic and histomorphometric results of using NanoBone® and easy-graft™CRYSTAL for maxillary sinus floor elevation. This randomized double-blind split-mouth clinical trial was conducted on nine healthy patients requiring bilateral (n=18) sinus floor augmentation. Dental implants were placed six months after sinus floor elevation. Biopsy samples were taken at the time of implant surgery and analyzed using HistoMorphoMeter Ver.1.0 software. Histomorphometric analysis indicated that NanoBone® and easy-graft™ residues accounted for 32.71±10.39% and 26.61±9.48% of the bioptical volume, respectively. The amount of new bone formation was 25.29±7.29% and 18.69±5.63% in the NanoBone® and easy-graft™ groups, respectively. Paired samples t-test showed significant differences between the two groups in this respect (P=0.0001). Well-mineralized regenerated bone with lamellar parallel-fibered structure and Haversian systems surrounded the particles in both groups. Both tested materials yielded acceptable histological outcomes six months after surgery. NanoBone® caused superior new bone formation. Although longer follow-ups and larger sample size are needed, these preliminary results encourage further research in this respect.

Key words: Bone regeneration, sinus elevation, bone graft, NanoBone®, Easy-Graft™CRYSTAL.

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Corresponding author: Mostafa Solati
e-mail✉Mstf.solati@gmail.com
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INTRODUCTION

Placement of implants in the posterior maxilla has always been challenging. Progressive horizontal and vertical bone resorption in this area increases the size of the sinus cavity and reduces the thickness of bone under maxillary sinus floor [1, 2]. Sinus floor augmentation refers to the use of an internal maxillary sinus graft to increase the thickness and vertical dimension of bone in the posterior maxilla to enhance implant placement in this area. Thus, maxillary sinus floor augmentation is an effective method for bone reconstruction in the posterior maxilla. Due to the limitations associated with the use of autogenous bone grafts, bone substitutes have been used for this purpose with variable results. The most commonly used bone substitutes include demineralized freeze-dried bone allograft (DFDBA), resorbable and non-resorbable hydroxyapatite, biphasic calcium phosphate (BCP) [3-6].

NanoBone® is a new granular bone graft material consisting of nanocrystalline hydroxyapatite in

combination with a silica gel matrix. Application of NanoBone® in bone tissue engineering demonstrated acceptable results in the literature [7, 8]. The characteristics of NanoBone® originate from the free SiO and SiOH groups in the poly silicic acid in the inner surface of this material and the pores in the silica gel, which measure 10 to 20 nm in size and increase its porosity by 60%. Moreover, the surface of granules is very rough, yielding a porous structure in micrometer and millimeter scales. However, NanoBone® has high fracture strength of about 40 μPa [9, 10].

Easy-graft™ CRYSTAL is a completely synthetic and absorbable graft material. This material is a BCP compound consisting of hydroxyapatite (60%) and beta tricalcium phosphate (β-TCP) (40%). This mixture is combined with a microscopic polylactide coating. After the addition of bio-linker to the granules, this material can be directly applied by a syringe and also has the consistency of putty. This material becomes harder after contacting with water in bone defects. It has been shown that hydroxyapatite in BCP has high biocompatibility with bone [11, 12]. Also, studies have shown that β-TCP can be successfully used for sinus floor elevation as a bone substitute [5, 13]. Although β-TCP biodegrades sooner and has a different absorption pattern compared with hydroxyapatite [14], BCP combines the bioactive features of hydroxyapatite with high resorption rate of β-TCP. Moreover, studies have demonstrated its successful application in sinus floor elevation surgery and for treatment of mandibular bone defects [10, 15]. This material has excellent properties including osteoconductivity and biocompatibility. It is associated with an implant success rate of over 90% and the ability to form new bone similar to that by the use of allografts and xenografts [16-18].

The features of NanoBone® and easy-graft™CRYSTAL alone or in comparison with other bone substitutes have been extensively evaluated in the literature. However, these two materials have not been compared in a randomized clinical trial. In order to find the most efficient material for use as a bone substitute in sinus floor elevation, this study aimed to compare the histologic and histomorphometric results of using NanoBone® and easy-graft™CRYSTAL for maxillary sinus floor elevation.

MATERIAL AND METHODS

This randomized double-blind split mouth clinical trial was conducted on nine patients with partial edentulism of the maxilla who were candidates for bilateral implant placement after receiving a bone graft. The patients gave their written informed consent to participate in the study and the study protocol was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences. All patients had been referred to the Department of Periodontics at Shahid Beheshti University of Medical Sciences between 2012 and 2013. After clinical and radiographic examinations, the patients who met the following inclusion criteria were enrolled: Patients who required at least two implants to be placed in the posterior maxilla bilaterally but due to sinus pneumatization and bone resorption they could no undergo one-stage implant placement. Also, the patients had to be nonsmokers, with no pathology of the sinus and no history of chronic sinusitis. The exclusion criteria were full mouth plaque/bleeding score of more than 25%, acute infection of the Schneiderian membrane, chronic sinusitis, allergies, smoking (more than five cigarettes/day), treatment with bisphosphonates, uncontrolled diabetes and pregnancy. Oral hygiene instruction and complete oral prophylaxis including scaling and root planning were performed one week prior to surgery. Panoramic radiographs were also taken before sinus floor elevation, after surgery and before implant installation.

Surgical procedure (stage one)

Since the study had a split-mouth design, the two quadrants of each patient were randomly assigned to the two groups. Local anesthesia was administrated after surgical preparation. An incision was made at the top of the ridge crest with a releasing incision in the mesial to elevate a full thickness flap. After determination of the sinus wall, the surgical site was outlined by piezosurgery under irrigation until the blue zone of sinus membrane appeared. After palpation of the Schneiderian membrane, buccal plate and membrane were elevated using a blunt instrument to create a window. Finally, the bone plate served as the inferior border of the sinus. Cortical bone plate is resistant against resorption and protects the bone graft material. After elevating the sinus floor, a space was created between the alveolar process and sinus floor. The graft material was then applied to fill the prepared space, which had a height of approximately 12-13mm from the

sinus floor to the bone crest. All bone substitutes were applied without using blood or serum. The materials were packed medially, mesially and distally. In both groups, collagen membrane was placed over the window. Then, the incisions were sutured by ceralone® 4-0. In case of perforation during surgery, membranes were placed on both sides of the perforation site.

Post-operative care

For pain control, 400 mg Gelofen® capsules (Ibuprofen) were prescribed for all patients every six hours for three days after surgery. Patients were advised to continue their pain medication for up to six days if they still had pain after three days. Also, 500mg amoxicillin capsules were prescribed for patients every nine hours for one week. Patients were instructed to rinse 0.12% chlorhexidine gluconate (oral rinse) mouthwash at least twice a day for two weeks. After two weeks, the sutures were removed and patients were recalled three weeks after surgery to evaluate the healing process and for professional cleaning. Also, the patients were recalled monthly to control for possible infection, inflammation or membrane exposure.

Surgical procedure (stage two)

During the second stage of surgery for implant installation, which was a six months later, radiograph were taken and biopsy samples were obtained by a trephine Bur at a site with the highest amount of bone. Implants were installed in their respective site, the flap was sutured with 4-0 silk sutures and implants were submerged. The tissue specimens were then evaluated histologically and histomorphometrically. The post-operative instructions given to patients at this stage were similar to those after the first stage of surgery.

Histological and Histomorphometric examination

Tissue samples taken during implant placement had 2mm diameter. They were placed in 10% formalin for fixation for 24 hours. Then, decalcification was performed by immersion of the samples in 10% formic acid for two to eight days. Finally, the tissue was processed using the tissue processor (DS 2080/H, Germany). Then, the specimens were paraffin embedded and sectioned at the center using a microtome (Jung Heidelberg, Germany). The samples were stained with Hematoxylin & Eosin. At least two slides of each sample were prepared and blind coded. Nikon (Eclipse, E400, Japan) microscope equipped with a digital camera was used for histological assessments under ×40 magnification. Degree (percentage) of inflammation (chronic/acute), quantity of inflammation (less than 10%, 10%-30%, more than 30%), presence (percentage) of giant cells (positive/negative), connective tissue condition (normal, fibrovascular, fibrosis, granulation), bone type (woven, lamellar, both), percentage of new bone formation and percentage of remaining material were all calculated in the tissue specimens. To determine the amount of bone, histomorphometric analysis with HistoMorphoMeter ver. 1.0 software (Iran) was performed. All histologic and histomorphometric analyses were performed by an oral and maxillofacial pathologist, who was not aware of the type of treatment and bone substitute used in each quadrant.

Statistical analysis

A nonparametric analysis of variance (Kruskal-Wallis) was used to detect statistically significant differences between the two groups. Following this, the Mann-Whitney test was applied to detect statistically significant differences between treatments within the same group as well as between the groups. $P<0.05$ was considered statistically significant (SPSS 22, SPSS Inc., NY, IL).

RESULTS

In total, 18 sinus elevation procedures with easy-graft™CRYSTAL and NanoBone® (nine per each group) were performed in nine patients (six males and three females). The patients' age at the time of procedure ranged between 42-57 years. In the NanoBone® group, sinus membrane perforation occurred during the surgery in one patient and since it was smaller than 3 mm, it was covered by absorbable collagen membrane; sinus elevation was then performed. All patients had pain and edema for only two weeks after sinus elevation and implant installation and they did not have any noticeable signs or symptoms in their next appointments. In addition, no signs or symptoms of maxillary sinus infection occurred after the surgical procedure.

Histological and histomorphometric results

All biopsy samples contained newly formed mineralized tissue. The histomorphometric areal measurement demonstrated 32.71±10.39% residual graft material and 25.29±7.29% newly formed bone in NanoBone® group and

Table 1: Descriptive results in easy-graft™CRYSTAL group and NanoBone® group

GROUP		Remaining bone substitute	Bone formation
NanoBone®	Mean	32.7178	25.2900
	N	9	9
	Std. Deviation	10.39103	7.29207
	Std. Error of Mean	3.46368	2.43069
	Minimum	16.48	15.14
	Maximum	44.42	35.50
easy-graft™CRYSTAL	Mean	26.6100	18.6944
	N	9	9
	Std. Deviation	9.48456	5.63756
	Std. Error of Mean	3.16152	1.87919
	Minimum	16.52	10.59
	Maximum	41.82	24.07
Total	Mean	29.6639	21.9922
	N	18	18
	Std. Deviation	10.14981	7.17597
	Std. Error of Mean	2.39233	1.69139
	Minimum	16.48	10.59
	Maximum	44.42	35.50

Table 2: Remaining bone substitute and bone formation Comparison Paired Samples Test; Statistical analysis at95% level of significance for the parameters evaluated

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Remaining bone substitute	28.16389	10.32070	2.43261	23.03153	33.29625	11.578	17	.000
Pair 2	Bone formation	20.49222	7.43310	1.75200	16.79583	24.18862	11.696	17	.000

26.61±9.48% residual graft material and 18.6944±5.63% newly formed bone in easy-graft™CRYSTAL group. According to the paired samples t-test, the difference between the two groups in this regard was statistically significant (P<0.05)(Tables 1 and 2).

In all analyzed samples, bone tissue formed predominantly in the apical part of the defects. Bone graft substitute particles, which are identified by their round shape, were mainly detected at the center of the samples (Fig. 1).

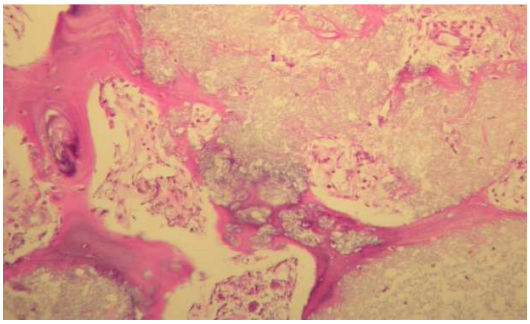

Figure 1: Easygraft™CRYSTAL specimen which includes native and grafted bone (magnification ×200)

In both groups, the graft particles were partially surrounded by trabecular woven bone. Lamellar bone was noted occasionally, suggesting that the formation of osteon-like structures had already started (Figs. 1 and 2).

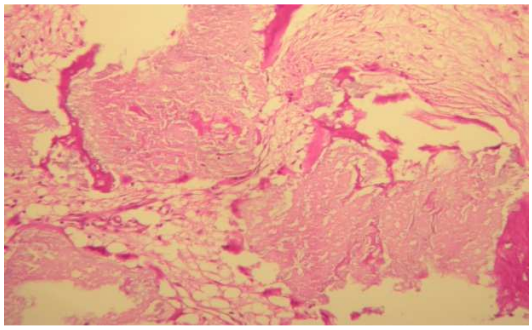

Figure 2: NanoBone® specimen which includes native and grafted bone (magnification ×200)

New lamellar bone formation was observed more in NanoBone® group compared to easy-graft™CRYSTAL group. However, this difference was not statistically significant (Wilcoxon Signed Rank test, P>0.05). Chronic inflammatory cells were seen in all specimens in both groups. In NanoBone® group, three specimens showed inflammation rate less than 10%, three showed 10-30%, and the remaining showed 30-50% inflammation. However, in easy-graft™CRYSTAL

group, five specimens showed inflammation rate less than 10%, two showed 10-30%, and the remaining showed 30-50% inflammation (Wilcoxon Signed Rank test, P> 0.05).

DISCUSSION

In the present study, we compared the histologic and histomorphometric results of using NanoBone® and easy-graft CRYSTAL for bilateral maxillary sinus floor augmentation. Histomorphometric analysis indicated that NanoBone® remnants accounted for 32.71±10.39% of the bioptical volume, while easy-graft™ remnants accounted for 26.61 ± 9.48% of the bioptical volume. The amount of new bone formation was 25.29±7.29% in NanoBone® group and 18.69 ± 5.63% in easy-graft™group. According to the paired samples t-test, this difference between the two groups was statistically significant (P=0.0001). Well-mineralized regenerated bone with lamellar parallel-fibered structure and Haversian systems surrounded the particles in both groups.

The natural bone architecture inspired the scientists to use nanostructured biomaterials for bone regeneration. In fact, bone is a complex nano-composition of organic and inorganic compounds in which, organic phase mainly consists of type I collagen (50-500 nm diameter) [19]. The inorganic phase consists of non-stoichiometric hydroxyapatite with 100 nm length and 20-30 nm width [20, 21]. Therefore, use of nanostructured and biomimetic scaffolds is increasing. Application of nanocrystalline hydroxyapatite by Chitsazi *et al*. showed its acceptability for bone defect healing even in comparison with autogenous bone graft [22]. Moreover, Gotz *et al*, in a study conducted on rabbit calvarial defects indicated fast appearance of bone proteins by immunohistochemical analyses [23]. Kruse *et al.* demonstrated that bony bridging was greater in presence of hydroxyapatite nanoparticles after four weeks in animal models [24].

NanoBone® (Artoos, Rostock, Germany) is a new granular bone substitute consisting of nanocrystalline hydroxyapatite in a silica gel matrix and previous studies showed favorable results of its application for bone regeneration [8, 9]. Presence of SiO and SiOH ending groups in polysilicic acid increases the internal surface of NanoBone® ($84 m^2/g$). On the other hand, the porous nature of silica gel (10-20 nm) increased its porosity by 60%. Roughness of the surface of granules in NanoBone® results in a porous structure in micrometer or millimeter scale. However, NanoBone® has a high fracture strength (40 μPa) [9, 10].

Gerber *et al.* demonstrated silica gel replacement with unstructured organic matrix and its biodegradation by scanning electron microscopy and energy-dispersive X-ray analysis [25]. In natural bone formation, woven bone is initially formed and by the bone maturation process, lamellar bone gradually increases. Our results showed higher rate of new bone formation in NanoBone® group (P=0.0001). On the other hand, NanoBone® specimens showed higher percentage of lamellar bone than easy graft specimens at six months; thus, NanoBone® probably causes faster bone maturation. In long-term, they both may show similar amount of lamellar bone. Therefore, further studies with longer follow ups are required to make an evidence-based decision. It seems that NanoBone® structure can explain these results.

Based on the literature, six to nine months are needed to evaluate osteogenesis in humans [26, 27]; thus, we followed up patients for six months. In several studies, faster turnover was seen in NanoBone® group compared to other bone substitutes [28-30]. It seems that it is because of the presence of silica gel matrix, its biodegradability and its replacement with organic matrix. In addition, nano-pores in hydroxyapatite can improve adhesion of bone matrix proteins and differentiation of precursor cells. These facts can explain greater new bone formation in NanoBone® group, which is in accordance with the results of other studies [23, 25].

Particle size can act as an important factor for particle resorption and bone formation [31]. Previous studies estimated minimum inter-particle space for bone formation and neovascularization to be 100 μm; more space resulted in better bone formation [32-34]. To maintain this inter-particle space, minimum size of particles should be 380 μm in diameter [35]. The particles of both biomaterials used in this study were bigger than the minimum size and were approximately of the same size. Therefore, the inter-particle space was similar in both experimental groups and thus, this factor was ruled out as a confounding factor. According to

our results, NanoBone® can be used as an acceptable bone substitute in sinus elevation surgery (Fig. 3).

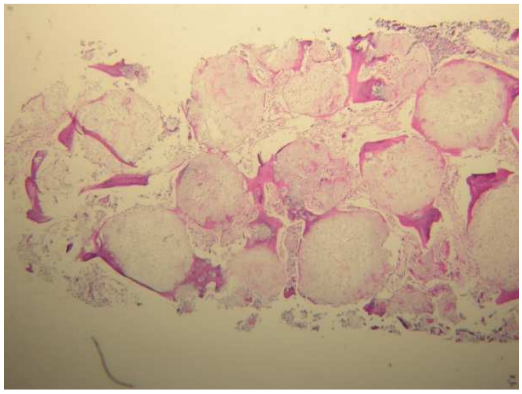

Figure 3: Easygraft™CRYSTAL specimen which includes native and grafted bone (magnification ×40)

Easy-graft™CRYSTAL is a synthetic and absorbable bone substitute. This BCP compound consists of 60% hydroxyapatite and 40% β-TCP. Gacic *et al*. showed that β-TCP was totally absorbed after six months [36]. In addition, easy-graft™CRYSTAL covered microscopic polylactic-*polyglycolic* acid copolymer. Its manufacturer claims that it does not require a collagen membrane. However, in the current study, we used collagen membrane for both materials to match the experimental groups.
Easy-graft™CRYSTAL has easier handling due to its consistency, toughness and injectability; also it hardens after contact with water. Therefore, the risk of contamination will be lower. In addition, it was shown that presence of hydroxyapatite in the composition of BCP increased its biocompatibility [11,12]. Several studies assessed β-TCP and demonstrated promising results due to its application for sinus elevation [5,13]. Theoretically, β-TCP breaks down into Ca^{2+} and PO_4^{3-} while hydroxyapatite remains stable and prevents bone graft resorption [37]. Finally, it seems that β-TCP is gradually resorbed by calcium deficient hydroxyapatite with or without bone matrix replacement [37,38]; therefore, less residual bone substitute was detected. Based on our histological analysis, it seems that new bone formation occurred at the center of the particles and it was clearly seen in the specimens (Fig. 4).

These results were in accordance with those of kury *et al*, who investigated bone regeneration in goat by use of easy-graft™CRYSTAL (39). On the other hand, biodegradation of β-TCP at the center of particles can partly explain this finding. This bone graft is partially resorbable and this observation clearly shows that.

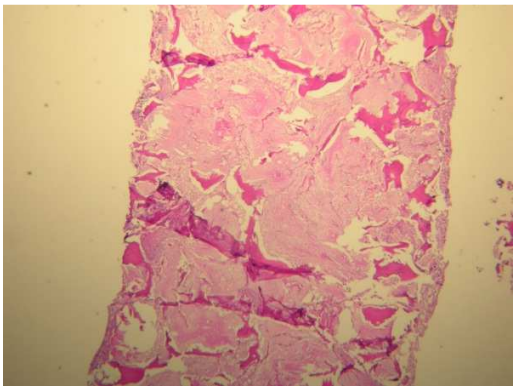

Figure 4: NanoBone® specimen which includes native and grafted bone (magnification ×40)

Foreign body reaction and inflammatory cell infiltration in both groups were in the acceptable range, which confirms the biocompatibility of both bone substitutes used in our study. These findings were in accordance with those of other studies [40-43]. New bone matrix was seen in all specimens in our study, which was in accordance with the results of Pearce *et al,* and Trombolli *et al*, [44, 45]. In addition, histological analysis in our study indicated more lamellar bone formation in NanoBone® group and faster bone maturation in this group compared to easy-graft™CRYSTAL group.

CONCLUSION

In conclusion, both tested materials in the current study showed acceptable histological results six months after surgery. Moreover, NanoBone® showed greater new bone formation while easy graft showed less residual graft material. Although longer follow-ups and larger sample size are required, these preliminary results encourage further research in this respect.

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