Journal of Research in Medical and Dental Sciences
Volume 5, Issue 3, Page No: 69-81

Available Online at: www.jrmds.in
eISSN No. 2347-2367: pISSN No. 2347-2545

Histomorphometric and Histologic Evaluation of Nano-HA with and without PRGF in Bilateral Sinus Lift Augmentation: A Randomized Clinical Trial

Mansour Meimandi[1], Anahita Ashouri Moghaddam[2]*, Gholam Ali Gholami[3], Fatemeh Mashadi Abbass[4] and Mostafa Solati[5]

[1]Associate Professor, Department of periodontics, Dental School, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2]Assistant Professor, Department of periodontics, Dental School, Gilan University of Medical Sciences, Rasht, Iran
[3]Full Professor, Department of periodontics, Dental School, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[4]Assistant Professor, Department of Oral and Maxillofacial Pathology, Dental School, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[5]Assistant Professor, Department of periodontics, Dental School, AJA University of medical sciences, Tehran, Iran

DOI: 10.24896/jrmds.20175312

ABSTRACT

Background and Aim: Positive results were reported after application of plasma-rich in growth factors (PRGF) in sinus elevation augmentations. Furthermore, PRGF products are available in different formulations and using them along with different graft biomaterials possibly induces bone formation and remodeling. This study assessed the histologic and histomorphometric results of NanoBone® biomaterial with and without PRGF in bilateral sinus augmentations. Materials and Methods: In this randomized split-mouth clinical trial, 10 patients who needed sinus floor elevation were selected and activated liquid PRGF was obtained through centrifuge of their blood. The space between alveolar process and sinus floor were filled with NanoBone® + PRGF (test site) or NanoBone® alone (control site) post-surgery. After 6 months, the implants were inserted in the regions and bone specimens were obtained using trephine burs. The sections were prepared by the standard techniques and bone remodeling was examined in both groups. The data were subjected to paired t test. Results: In case sites, the mean new bone formation percentages were 30.29%±8.54 and 30.84% ±6.76 in control sites. The mean remnant particles were 26.16%±10.03 and 26.18%± 10.09 in case and control sites respectively. No significant differences were noted between case and control sites regarding mean new bone formation and remnant particles. Chronic inflammation was noted in all specimens with dominant range of 10-30%. The giant cells were evident and all specimens showed bones of lamellar and woven types. Conclusion: Although no significant differences existed between the sites filling using NanoBone® with and without PRGF in bilateral sinus augmentations, the results are probably related to the lengthy time periods for specimen preparation and the type of bone materials used. Conclusion was that PRGF did not induce bone formation in the aforementioned period of time.

Keywords: Plasma rich in growth factor (PRGF), Bone formation, NanoBone®, Sinus Augmentation

HOW TO CITE THIS ARTICLE: Mansour Meimandi, Anahita Ashouri Moghaddam, Gholam Ali Gholami, Fatemeh Mashadi Abbass, Mostafa Solati, Histomorphometric and Histologic Evaluation of Nano-HA with and without PRGF in Bilateral Sinus Lift Augmentation: A Randomized Clinical Trial, J Res Med Dent Sci, 2017, 5 (3): 69-81, DOI: 10.24896/jrmds.20175312

Corresponding author: Anahita Ashouri Moghaddam
e-mail✉ anahita.a.moghaddam@gmail.com
Received: 06/05/2017
Accepted: 20/07/2017

INTRODUCTION

Implant insertion in the posterior region of the maxilla is a complicated procedure. Two different

studies showed that progressive resorption of horizontal and vertical bone increases the cavity and also reduces the thickness of the maxillary sinus floor [1,2] . The absence of upper molars may accelerate bone resorption, resulting in sinus pneumatization. These limitations may challenge implant insertion and endanger success rate and stability of implants. Studies reported a higher rate of implant failures evident in the upper jaw more than that of other oral regions [3,4].

The maxillary sinus floor augmentation technique is the one generally used in the treatment of resorbed posterior maxilla. According to researches the use of autogenous bone, either as blocks or particles, has been considered as the gold standard among graft materials [5,6]. Based on studies, donor site morbidity, limited availability and the tendency to resorption are most important drawbacks at the time of harvesting autologous bone [7,8]. Therefore, bone substitute materials have been tested in some experimental and clinical studies: Demineralized freeze-dried bone Allograft [9,10], bovine bone matrix [8], resorbable and nonresorbable Hydroxyapatite [11,12], Composite bone graft including Platelet-rich Plasma [13] and Tricalcium Phosphate [14]. NanoBone® [Artoss®, Rostock, Germany) is a newly developed graft material consisting of Nano crystalline hydroxyapatite granules embedded in a silica gel matrix. Due to the open SiOH or SiO groups of polysilicic acid, this nanostructured biomaterial shows an absolutely huge internal surface (about 84 m2/g). The rough granule surface created an interconnecting porous structure which ranged from μm to mm in dimensions [15]. Henkel in 2005 showed higher rates of bone formation when compared to other Hydroxyapatite (HA) and TriCalcium Phosphate (TCP) materials or gelatin sponges and an 8-months complete resorption period post-implantation [16]. Histological and immunohistochemical evaluation again showed osteoconduction, osteoinduction, and early remodeling for this material [17]. Clinical investigation has demonstrated that NanoBone® has osteoconductive and biomimetic properties and is incorporated in the host physiological bone turnover at an early level [18]. Other researches have shown that grafting maxillary sinus floor using a nano-structured hydroxyapatite silica gel as bone filler is a reliable method in sensitive anatomical conditions after the early healing stage [19]. Also bone formation in socket preservation using this new material by Gholami in 2012 showed similar and comparable results to Bio-Oss [20].

Platelet-rich plasma (PRP) is the source of autologous growth factors and was first introduced in 1998 by Marx in addition to autologous bone grafts for the reconstruction of mandibular defects [21]. The contribution of PRP formulations to the bone healing process is considered to be based on the growth factors (GFs). Anitua in 1999 suggested the use of plasma rich in growth factors (PRGF), where the platelets contain growth factors like TGF- ß, VEGF, and IGF. These proteins meddle in functions like directed cell migration (chemotaxis) and in cellular differentiation and proliferation, all of which are key events in repair and regeneration processes [22]. PRGF is an autologous plasma product rich in platelets which enables local release of multiple growth factors and bioactive proteins that modulate the processes of wound healing and tissue engineering after activation with calcium [21,23,24].

Moreover, some of the researchers (Anitua et al. 2007; Anitua, 1999) showed improvement in healing and new bone regeneration after using growth factors. [25,22] However, these results were not reported in other researches [26,27]. The present study investigated the potentials of PRGF technology and its autologous formulations in sinus elevation in ten consecutive patients in which bilateral sinus lift augmentations were carried out. The effects of PRGF combined with Nanobone (one side) were compared with the biomaterial alone (contralateral side) in order to analyze this combination for observing bone regeneration increase in sinus lift augmentation procedure.

MATERIALS AND METHODS

This randomized split mouth clinical trial was carried out in the Department of Periodontics, Shahid Beheshti University of medical sciences (2009-10). The sample consisted of 10 consecutive patients (6 women, 4 men) in the age range of 30 to 60 years with a loss of height in the posterior maxilla which required application of a sinus lift technique to allow rehabilitation by dental implants. The selection of 10 patients with 20 posterior sites was carried out considering that

sizes of both sinuses (lateral and contralateral) were almost equal.

The inclusion criterion was a residual bone crest (distance between sinus floor and bone crest) ranging between 2 to 4mm in height. Buccolingual widths were at least 6mm in CBCT (cone beam computed tomography).

The exclusion criteria were: sites with acute infection, a full mouth plaque score and a full mouth bleeding score more than 25%, acute infections of schneiderian membrane, chronic sinusitis, allergies with respiratory component, smokers with more than 5 cigarettes per day, a history of bisphosphonate therapy, uncontrolled diabetes (HbA1c > 6%, glycemic level > 110 mg/dl), pregnancy and lactating. The protocol was explained to all participants with probable risks and benefits.

All the cases signed the informed consent. Patients underwent a preoperative digital panoramic examination and computerized tomography scan, After peri-oral preparation and disinfection, the operative area was reached by means of a full thickness flap. Access to the cavity was obtained using a periodontal ultrasonic generator (Pizotome, France. Parse etekal .88545400) required to investigate antral anatomy (Figure 1). One week prior to the surgery, full mouth professional prophylaxis appointment was scheduled.

Liquid PRGF

Peripheral blood (20–30 ml) from each patient was taken by venipuncture before the surgery and was put directly into 5-ml tubes (blood collecting tubes®, BTI) which contained 3.8% (wt/vol) sodium citrate anticoagulant. Liquid PRGF was prepared by centrifugation (PRGF system, Vitoria, Spain) at 460g for 8 minutes at room temperature. The 0.5 ml plasma fraction located just above the red cell fraction, excluding the buffy coat, was collected and deposited in a glass dish. PRGF activator® was added to the liquid PRGF preparation (50 ml PRGF activator® per milliliter of preparation) to initiate clotting and formation of a fibrin matrix for the continuous release of growth factors and proteins (Figures 2a, 2b, 2c & 2d).

Surgical Protocol

combined with an independent irrigation system. The osteotomy line was made by cutting and dispersing the osseous table in a controlled and progressive manner (Figure 3).

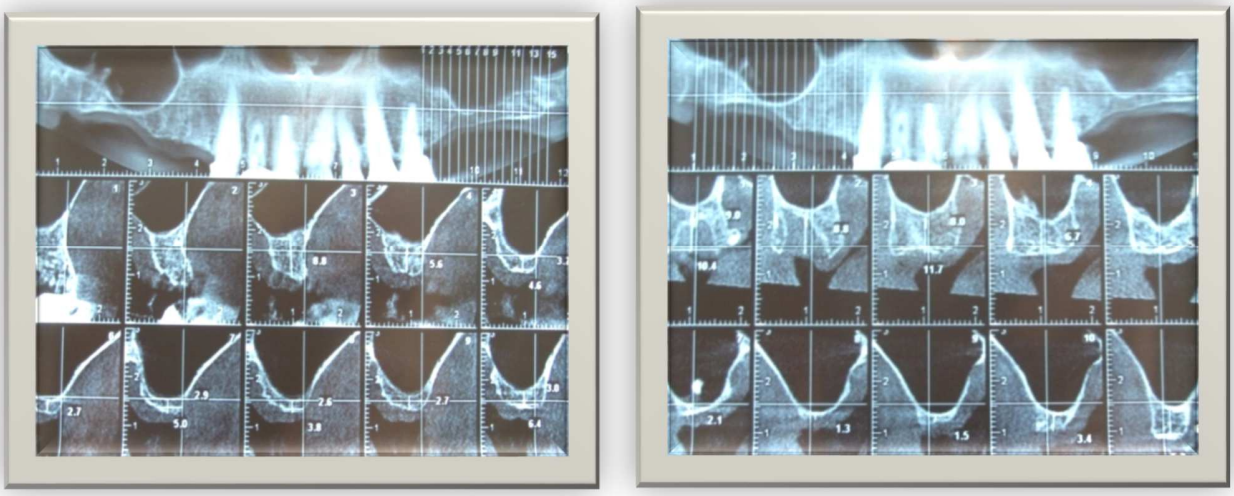


Fig. 1: Pre-surgical radiographs for one of the patients (male, 46). Not enough bones, both sinus cavities almost equal

Fig. 2a: Same patient's blood samples before centrifugation

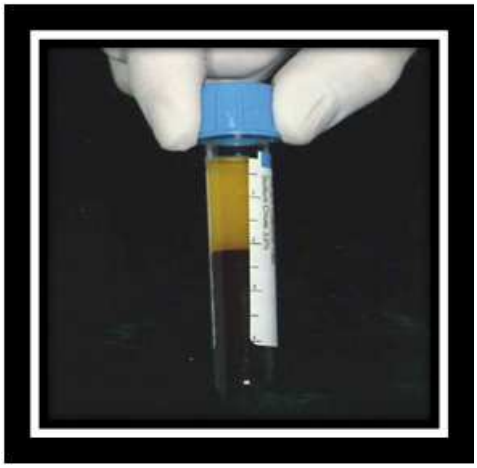


Fig. 2b: One of the blood tubes after centrifugation

Fig. 2c: Schematic view of PRGF after centrifugation

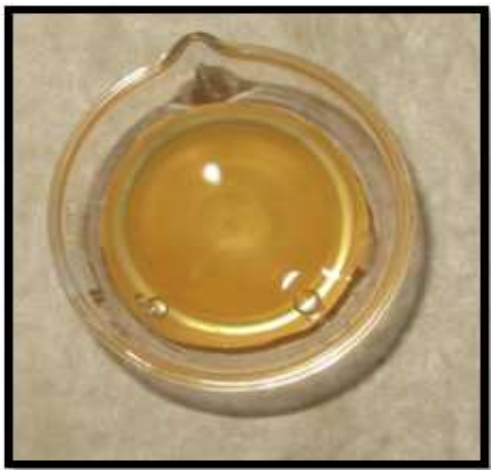


Fig. 2d: After addition of activator to PRGF

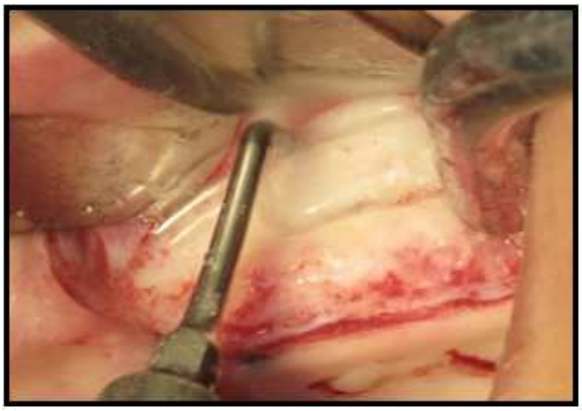


Fig. 3: Pizosurgery for preparing sinus window for the same patient

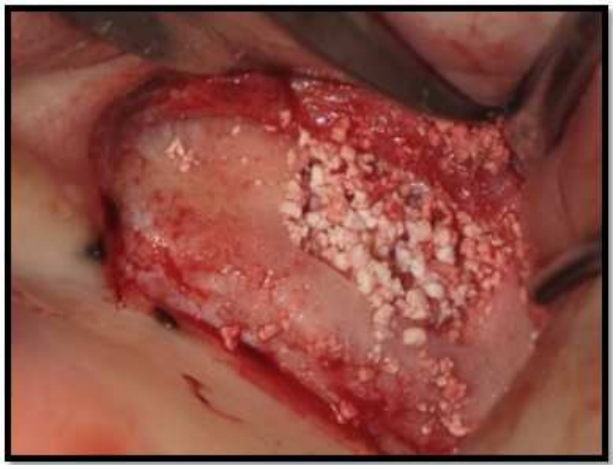


Fig. 4: After Sinus lifting (Trap Door technique) filling the cavity with NanoBone® and with PRGF(test group)

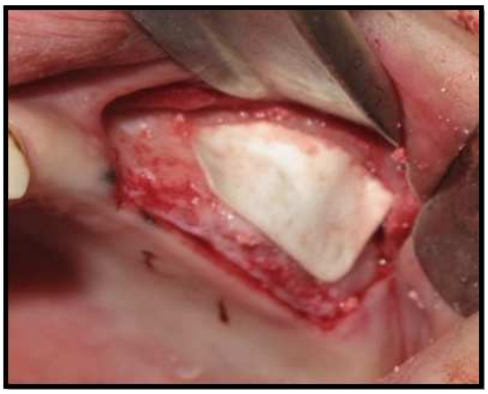


Fig. 5: Placing the membrane on window

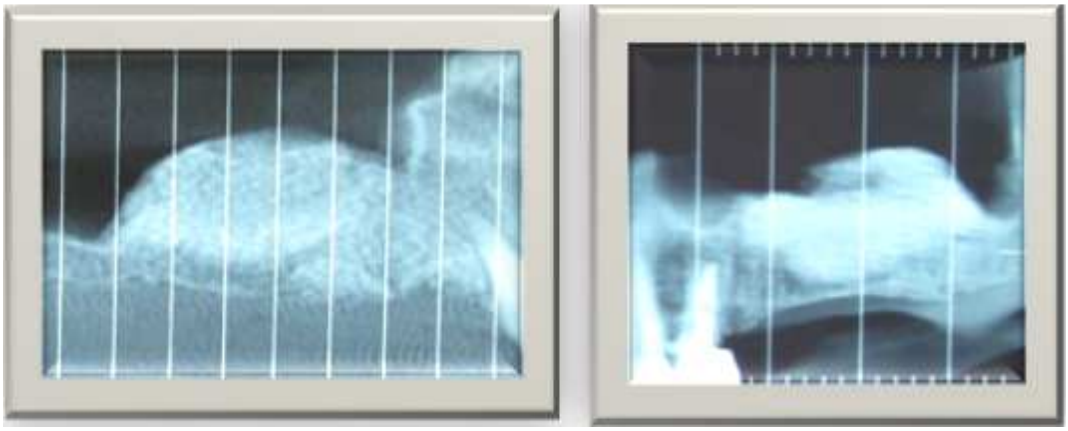


Fig. 6: Post surgery radiography after 6 months

Surgical Re-entry: After 6 months, a panoramic radiography and a CT scan were performed and the re-entry procedure was done (Figure 6). The ultrasonic tip of the device enabled an increased tactile control and avoided soft tissue damage. The bone in the center of the window remained attached to the schneiderian membrane which was carefully elevated within the sinus cavity, leaving it completely free from the original floor of the sinus cavity, anteriorly, posteriorly and medially. Concurrently, the lateral wall was elevated inward to create the new relocated sinus floor (Trap door technique). The superior cortical plate is resistant to resorption and supports bone substitute. After elevation of sinus floor the antral space between alveolar process and sinus floor was grafted (Figure 4).

Sinus sites were randomly grafted in 2 groups:
1- Test group: PRGF + BCG (collagen membrane, Bioteck®) + Nanobone® (1× 2 mm gross particle)
2- Control group: BCG (collagen membrane, Bioteck®) + Nanobone® (1× 2mm gross particle)
The graft material used was NanoBone® (Artoss, Rostock, Germany and 1×2 mm gross particle) with activated liquid PRGF in the case of one sinus, and in the other, saline solution instead. Collagen membrane was used in both groups (Figure 5). Both were sutured with 4-0 silk with primary closure without tension.

Mucoperiosteal flaps were elevated to access to alveolar ridge, a modification of implant site preparation protocol included the use of a trephine with 2 mm internal diameter, 3 mm external and 10 mm in length for histologic and histomorphometric evaluation. Trephine specimens were provided to the number of implants so for analyzing new bones there would be enough specimens in each group. Finally, drilling protocol for each of the implants completed & implants were submerged.

Histologic and Histomorphometric Analysis

Analyses were carried out using the standardized protocol in Shahid Beheshti University of medical sciences and analyzed by an examiner masked to the type of treatment. The bone biopsies were decalcified in formic acid 10% for a period of 2 days, processed, sectioned and prepared for histologic and histomorphometric analysis using hematoxylin and eosin staining. Serial longitudinal sections were cut through the central core of the bone biopsies using a microtome (Jung, Heidelberg, Germany), were coded and were used with a light microscope (Nikon Eclipse, E400, Tokyo, Japan) for histologic examination. Histomorphometric measurements were performed using images (magnification x40) captured by a digital camera (Nikon, E8400). The digital images were analyzed using Iranian Histo Morpho Meter Version 1.0. At least three randomly selected sections of each subjects were used for evaluation of : type of inflammation:1-acute (existence of Neutrophil, Eosinophil) 2-chronic (existence of lymphocyte, plasmacell), inflammation percent (0-10%, 10-30% ,30-50%), existence of Giant cell (+,-), type of connective tissue (normal, fibrous, fibrovascular or granulation tissue), type of bone (Lamellar, Woven or Lamellar/Woven), existence of remnant particles (+,-), Percent of vital bone and Percent of remnant particles (NanoBone®).

In test group the mean of new bone formation was 30.29±8.45% and in control group it was

Statistical Analysis

All the histologic and histomorphometric analyses were done by an oral and maxillofacial pathologist blindly. Quantitative variables (percentage of bone formation and remnant particles) were evaluated with Paired t test and qualitative variables were compared using Wilcoxon Signed Rank test.

RESULTS

10 patients (6 women, 4 men) in the range of 30 to 60 years of age were subjects of the research. Several variables related to bone formation were evaluated in 2 groups. Test group (figure 7) with PRGF and control group (figure 8) without PRGF.

Out of 20 sinus surgery, 2 were perforated ≤3mm. Nevertheless none of the patients had an important symptom except for the first 2 weeks post surgery and for the first week after implant insertion. The symptoms were pain and swelling.

Histomorphometric Analysis

In the test group the mean of remnant particles was 26.16±10.03% and in control group it was 26.18±10.09%. There were no significant differences between the 2 groups (Paired t test p>0.99) (Graph 1)

30.84±6.76%. There were no significant differences between 2 groups (Paired t test p>0.85) (Graph 2)

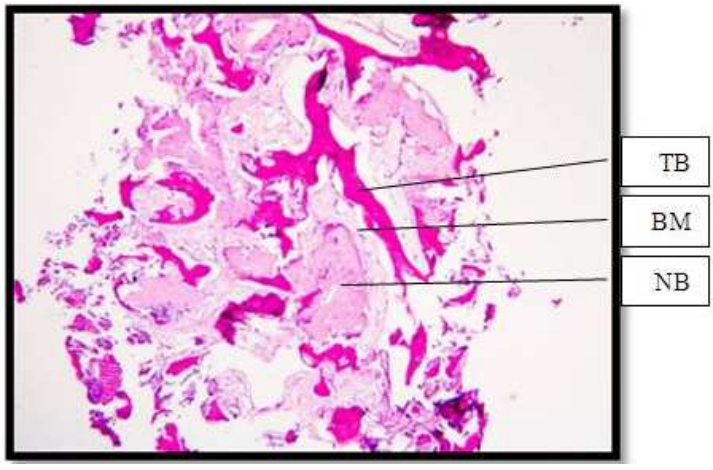


Fig. 7: Test group, focus 40x (NB: NanoBone®, TB: Trabecular Bone and BM: Bone Marrow)

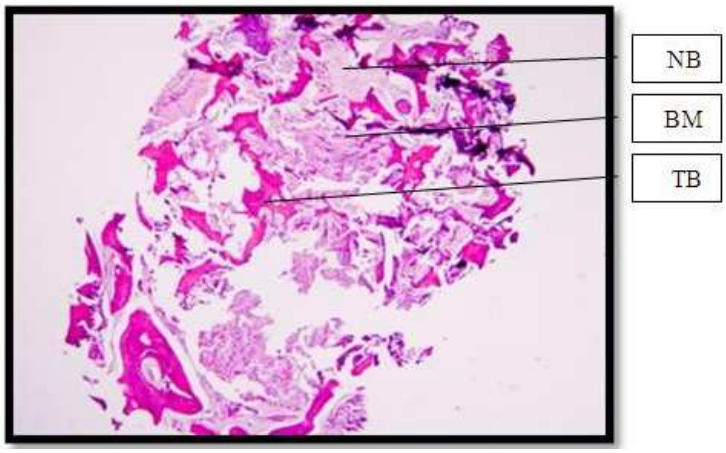
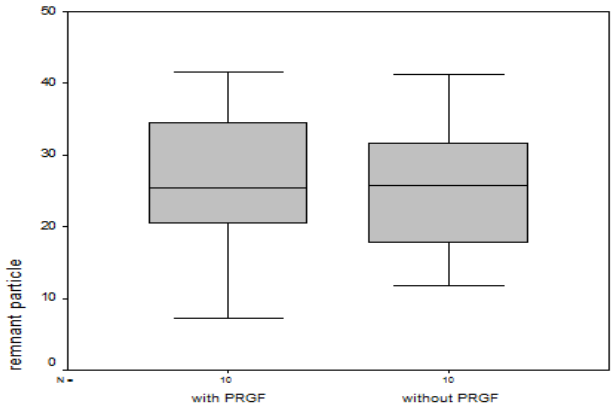
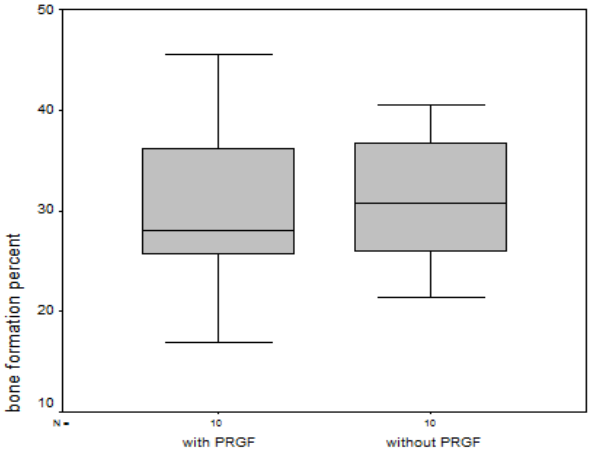


Fig. 8: Control group, focus 40x (NB: NanoBone®, TB: Trabecular Bone and BM: Bone Marrow)



Graph 1: the test group the mean of remnant particles



Graph 2:The test group the mean of new bone formation

Table 1: Wilcoxon Signed Ranks

Parameter	Parameter group	With PRGF	Without PRGF
Remnant particle	+ (existing)	10(100)%	9(90%)
	- (non-existing)	0(0%)	1(10%)
Remnant particle reaction	Giant cell existing	10(100%)	9(90%)
	Giant cell non-existing	0(0%)	1(10%)
Bone type	Lamellar or woven	0(0%)	0(0%)
	Lamellar & woven	10(100%)	10(100%)
	Normal	1(10%)	2(20%)
Connective tissue type	Fibrovascular	8(80%)	7(70%)
	Fibrous	0(0%)	1(10%)
	Granulation tissue	1(10%)	0(0%)
Inflammation type	Chronic	10(100%)	10(100%)
Inflammation percent	≤10%	4(40%)	3(30%)
	10-30%	5(50%)	7(70%)
	30-50%	1(10%)	0(0%)

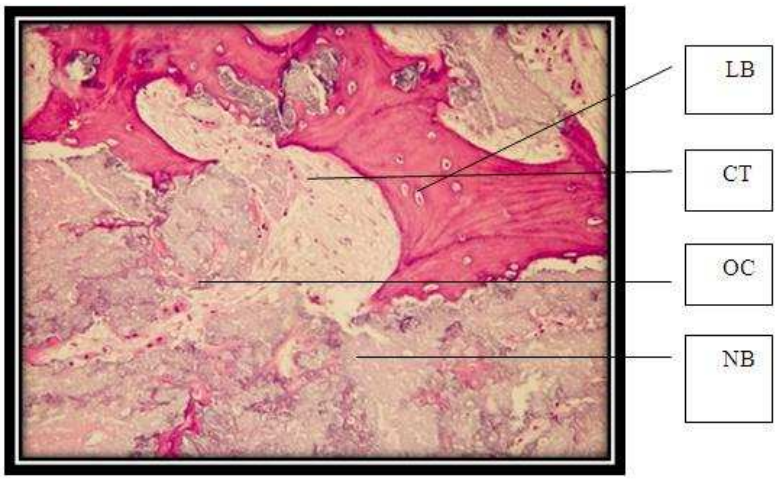


Fig. 9: Lamellar bone (LB), Connective tissue (CT), Osteoclast (OC) and Remnant particles of Nanobone® (NB)

Histologic Analysis

In both groups types of new bones were lamellar and woven, and type of inflammation were chronic. Other qualitative variables evaluated with Wilcoxon Signed Ranks are explained in the charts. (Table 1) There were no significant differences between the two groups.

DISCUSSION

The present study investigated the potential of PRGF application in the lateral approach of sinus floor elevation. No significant differences in new bone percentage between the test & control groups were observed. Type of new bone in two grafted sites were lamellar & woven (figure 9), there were no significant differences in remnant particles between PRGF & non PRGF groups, so, combination of PRGF technique with Nanobone® in bilateral sinus floor elevation did not increase new bone formation.

This result can be related to the preparation time of histologic specimen (6 months) and osteoconductive and remodeling characteristics of Nanobone® that leads to few differences between the groups after 6 months.

Gerard and others realized that PRP increased bone formation and remodeling in the first and second months, though these beneficial effects decreased after third and sixth months [28]. Also, the nanostructured hydroxyapatite investigated in the present study was embedded in a highly porous matrix of silica gel. The Nano crystals produced a large, bioactive surface [110 m2/g) and presented a micro porosity size ranging from 10 to 20 nm. This combination seems to induce

migration, adhesion, and proliferation of osteoblasts inside the pore network and to promote angiogenesis inside [18]. These events also, could explain bone formation at the early stages. Therefore, it might be postulated that if specimen were evaluated in a shorter time after grafting, differences between the 2 groups might have been highlighted. Also, existence of giant cells around Nanobone® indicates rapid remodeling within the bone graft.

These results are comparable with the research of Scarano et al in 2006, that reported bone formation in 16 sinus sites with Nanobone® were 32%, bone marrow spaces were 40% and remnant particles were 34% [29]. The research done by Canullo & Dellavia in 2009, showed that after 6 months, in 16 patients regenerated bone, residual NanoBone®, and bone marrow occupied 48%, 28% and 24% of the grafted volume respectively [19]. In another research by Canullo et al in 2012: on 10 healthy patients, 3 months after maxillary sinus augmentation with NanoBone®, its residuals accounted for the 38.26% ± 8.07% of the bioptical volume, marrow spaces for the 29.23% ± 5.18% and bone for the 32.51% ± 4.96% [30]. The PRGF used in this study was obtained following the protocol described by Anitua in 1999[22]. PRGF was used because the activator is calcium chloride, which takes out the risk of immune reactions and the transmission of diseases related to the use of exogenous bovine thrombin. PRGF can be obtained in a single centrifugation step at 460g for 8 minutes. In contrast, the double centrifugation technique used to obtain PRP requires a greater blood volume [minimum 50 ml). [31] PRGF has some advantages: It allows simultaneous action of multiple growth factors, and is an autologous product. PRGF also increases tissue vascularization. The product is biocompatible, effective, and safe and is reabsorbed by the body in a few days after beginning of regeneration [21,22,23,25], as reported by de Obarrio et al in 2000, considered not only the positive effects of PRP resulting from the release of GFs but also its physical and chemical characteristics[32].

So it would be probable that addition of PRGF with Nanobone® can lead to more bone formation. Another potential advantage of using PRGF is that they have the capability to reduce post-surgical inflammation. It has been observed that platelet products suppress monocyte cytokine release and limit inflammation [33]. In this paper, chronic inflammation in most cases was reported 10-30% and in test group and inflammation was mostly less than 10% but with no significant differences.

As mentioned in the research of Anitua (2010): on five patients who received bilateral sinus floor augmentation, the effects of PRGF combined with bovine anorganic bone (one side) were compared with the biomaterial alone (contralateral side). The effects of using liquid PRGF to maintain the bone window and autologous fibrin membrane to seal the defect were evaluated. After histologic and histomorphometric evaluation, PRGF can have a role in reducing tissue inflammation post-surgery, increasing new bone formation and promoting the vascularization of bone tissue after 5 months [34]. These results were contradicting the present research.

Nowadays, a variety of methods exist for preparation of platelet rich plasma and they can affect the results of research performed in evaluation of bilateral sinus floor elevation. Also, type of the bone graft used can be of importance. In the case of PRP application with non autogenous bone graft, conducted a test on 23 sinus floor elevation patients and investigated whether the combination of Beta TCP with platelet-rich plasma (PRP) enhances bone regeneration, and concluded that the formation of new bone was about 8–10% higher when PRP was applied [35]. Also in another study (Torres , 2009) : the combination of anorganic bovine bone (ABB) with platelet-rich plasma (PRP) were widely used in bone regeneration procedures in five edentulous patients and after 6 months, the amount of augmented bone was more significant in the test group [36]. Although neither of the studies used membrane for the two sites.

In using PRP with autogenous bone graft (Raghoebar, 2005) investigated five edentulous patients in split mouth design, after 3 months the result of histomorphometric evaluation was not significant [37]. Moreover, in the study of Consolo in 2007 on sixteen adults in bilateral sinus floor augmentation, autologous (iliac crest) bone was used on one side and PRP plus autologous bone contra laterally. Implants were inserted randomly 4, 5, 6 and 7 months after surgery in the patients. Histological documents revealed enhanced bone activities in sites treated with PRP 4 months after surgery and reduced bone activity was observed

in both sites 5, 6 and 7 months post-surgery. Bone amount, higher in sites treated with PRP (mean trabecular bone volume), decreased in both sites over time and this shows a certain regenerative potential of PRP when used with autologous bone. But the effect of this enhancement of bone regeneration seemed to be limited to shorter treatment times. A continuous elimination of the PRP effect was recorded after an interval longer than 6 to7 months [38].

However in another research, (Schaaf ,2008) did not report any positive effects of PRP on bone density 4 months post sinus floor augmentation [39]. In 2010, in the systematic review, it was stated that use of PRP does result in early regeneration and reduction in healing time of soft and hard tissues, But there is no human study that documents the advantages of using PRP in sinus augmentation procedures [40]. But in another systematic review,the study supported the use of PRP for bone formation on a sinus bone graft, whereas there was no significant effect on the implant survival and bone-to-implant contact [41]. About the necessity of using membrane, (Wallace, 2003) indicated membrane placement over the lateral window to be an essential element to improve regenerated bone quality [42]. Moreover, in a bilateral randomized controlled trial, reported a vital bone formation of 25.5% if a membrane was used and 11.9% when it was not placed over the lateral window [43]. It was decided that membrane would be used in both sites in this study.

CONCLUSION

The results of this study indicated that there were no significant differences between the two groups (Nanobone® with PRGF & Nanobone® alone). However, considering the favorable effects of PRGF in the first months, the lack of significant differences seems to be due to lapse of time post-surgery (6 months) and probably, special characteristics of Nanobone® prevented expression of the desired characteristics of PRGF growth factors. PRGF could not stimulate bone formation in this study, although further studies are required to make a completely accurate assumption.

Acknowledgements

We would like to thank all the Professors of Periodontology Department of Shahid Beheshti university of medical sciences especially Dr. R. Amid,Dr .M . Talebi and Patholology Department; Ms. M. Shahnazi for assisting in histologic preparation and Ms. S. Bajelan for all her efforts.

REFERENCES

1. Bays R: The pathophysiology and anatomy of edentulous bone loss. Reconstruc Preposthet Oral Maxillofac Surg 1986; 1: 1–17.
2. Hutton JE, et al: Factors related to success and failure rates at 3 years follow up in a multicenter study of overdentures supported by Branemark implants. Int J Oral Maxillofac Implants 1995; 10: 33-42.
3. Adell R, et al: A 15-years study of osseointegrated implants in treatment of the edentulous jaw. Int J Oral Surg 1981; 10: 387–416.
4. Jaffin RA, Berman CL: The excessive loss of Bränemark fixtures in type IV bone. A 5-year analysis. J Periodont 1991; 62:2–4.
5. Boyne PJ, James RA: Grafting of the maxillary sinus floor with autogenous marrow and bone. J Oral Surg 1980; 38:613–616.
6. Cordaro L: Bilateral simultaneous augmentation of the maxillary sinus floor with particulated mandible. Report of technique and preliminary results. Clin Oral Implants Res 2003; 14:201–206.
7. Kalk WW, et al: Morbidity from iliac crest bone harvesting. Int J Oral Maxillofac Surg 1996; 54:1424–1430.
8. Maiorana C, et al: Treatment of maxillary ridge resorption by sinus augmentation with iliac cancellous bone, anorganic bovine bone, and endosseous implants: a clinical and histologic report. Int J Oral Maxillofac Implants 2000; 15:873–878.
9. Cammack GV, et al: Histologic evaluation of mineralized and demineralized freeze-dried bone allograft for ridge and sinus augmentations. Int J Periodontics Restorative Dent 2005; 25:231–237.
10. Motamedian SR, Hosseinpour S, Ahsaie MG, Khojasteh A. Smart scaffolds in bone tissue engineering: A systematic review of literature. World journal of stem cells. 2015;7(3):657.
11. Karabuda C, et al: Histological and clinical evaluation of 3 different grafting materials for sinus lifting procedure based on 8 cases. J Periodontal 2001; 72:1436–1442.
12. Ewers R, et al: Histologic findings at augmented bone areas supplied with two

different bone substitute materials combined with sinus floor lifting. Report of one case. Clinical Oral Implants Res 2004; 15:96–100.
13. Galindo-Moreno P, et al: Evaluation of sinus floor elevation using a composite bone graft mixture. Clin Oral Implants Res 2007; 18: 376–382.
14. Zerbo IR, et al: Histomorphometry of human sinus floor augmentation using a porous beta-tricalcium phosphate: a prospective study. Clin Oral Implants Res 2004; 15:724–7
15. Kruse A, Jung RE: Bone regeneration in the presence of a synthetic hydroxyapatite/silica oxide-based and a xenogenic hydroxyapatite-based bone substitute material. Clin Oral Impl Res 2011 May;22(5):506-11
16. Henkel K-O, et al: Repair of bone defects by applying biometrics with and without autologous osteoblasts. J Carnio-Maxillofacial Surg 2005; 33:45-49.
17. Henkel K-O, et al: Macroscopical, histological, and morphometric studies of porous bone-replacement materials in minipigs 8 months after implantation. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006; 102: 606-613.
18. Goetz W, et al: Immunohistochemical characterization of nanocrystalline hydroxyapatite silica gel (NanoBone®) osteogenesis: a study on biopsies form human jaws. Clin Oral Impl Res 2008; 19: 1016-1026.
19. Canullo L, Dellavia C: Sinus lift using a nanocrystalline hydroxyapatite silica gel in severely resorbed maxillae: histological preliminary study. Clin. Implant. Dent. Relat. Res2009: 11 (Suppl. 1), e7–e13.
20. Gholami GA, et al: Clinical, histologic and histomorphometric evaluation of socket preservation using a synthetic nanocrystalline hydroxyapatite in comparison with a bovine xenograft: a randomized clinicaltrial. Clin Oral Implants Res. 2012 Oct; 23(10):1198-204.
21. Marx RE, et al: Platelet-rich plasma: growth factor enhancement for bone grafts. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998; 85(6):638-646.
22. Anitua E: Plasma rich in growth factors: preliminary results of use in the preparation of future sites for implants. Int J Oral Maxillofac Implants. 1999; 14(4):529-35.
23. Anitua E, et al: New insights into and novel application for platelet-rich fibrin therapies. Trends Biotechnol 2006; 5: 227-234.
24. Uma Shanker Pal, et al : Platelet-rich growth factor in oral and maxillofacial surgery . Natl J Maxillofac Surg. 2012 Jul-Dec; 3(2): 118–123.
25. Anitua E, et al: The potential impact of the preparation rich in growth factors (PRGF) in different medical fields. Biomaterials 2007; 28:4551–4560.
26. Aghaloo TL, et al: Evaluation of platelet-rich plasma in combination with anorganic bovine bone in the rabbit cranium: A pilot study. Int J Oral Maxillofac Implants 2004; 19:59–65.
27. Plachakova AS, et al: The bone regenerative effects of platelet rich plasma in combination with osteoconductive materials in rat cranial defects. Clin Oral Implant Res 2006; 17:305–311.
28. Gerard D, et al: Effects of platelet-rich plasma on the healing of autologous bone grafted mandibular defect in dogs. J Oral Maxillofac Surg. 2006;64(3):443-51.
29. Scarano A, et al: Maxillary sinus augmentation with different biomaterials: a comparative histologic and histomorphometric study in man. Implant Dent 2006; 15:197-207.
30. Canullo L, et al: Maxillary sinus floor augmentation using a nano-crystalline hydroxyapatite silica gel: Case series and 3-month preliminary histological results. Ann Anat. 2012 Mar 20;194(2):174-8
31. Molina-Minano F, et al: Plasma rich in growth factors and bone formation: a radiological and histomorphometric study in New Zealand rabbits. Braz Oral Res 2009; 23(3):275-80
32. de Obarrio JJ, et al: The use of autologous growth factors in periodontal surgical therapy: platelet gel biotechnology - Case reports. Int J Periodontics Restorative Dent. 2000; 20(5):486-97.
33. El-Sharkawy H, et al: Platelet-rich plasma: growth factors and pro- and anti-inflammatory properties. J Periodontol 2007; 78:661-669.
34. Anitua E, et al: Bilateral Sinus Elevation Evaluating Plasma Rich in Growth Factors Technology: A Report of Five Cases. Clin Implant Dent Relat Res 2010 Jul 9.
35. Wiltfang J, Schlegel K: Sinus floor augmentation with $_{\beta}$-tricalciumphosphate ($_{\beta}$ - TCP): Does platelet-rich plasma promote its osseous integration and degradation? Clin Oral Impl Res 2003; 14:213–218.
36. Torres J, et al: Effect of platelet-rich plasma on sinus lifting: a randomized controlled clinical trial. J Clin Periodontol 2009; 36: 677–687.

37. Raghoebar G, et al: Does platelet-rich plasma promote remodeling of autologous bone grafts used for augmentation of the maxillary sinus floor. Clin. Oral Impl. Res. 16, 2005 / 349–356.
38. Consolo U, et al: Platelet-rich plasma activity on maxillary sinus floor augmentation by autologous bone. Clin Oral Implants Res 2007; 18:252-262.
39. Schaaf H, et al: Sinus lift augmentation using autogenous bone grafts and platelet rich plasma: radiographic results. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008; 106:673-678.
40. 40-Arora NS, et al: Platelet-rich plasma in sinus augmentation procedures: a systematic literature review: Part II. Implant Dent. 2010 Apr;19(2):145-57.
41. Ji-Hyun Bae, et al: Effects of Platelet-Rich Plasma on Sinus Bone Graft: Meta-Analysis. J Periodontol 2011;82:660-667.
42. Wallace SS, Froum SJ: Effect of maxillary sinus augmentation on the survival of endousseous dental implants. A systematic review. Ann Periodontol 2003; 8:328-343.
43. Tarnow DP, et al: Histologic and clinical comparison of bilateral sinus floor elevations with and without barrier membrane placement in 12 patients: part 3 of an ongoing prospective study. Int J Periodontics Restorative Dent 2000; 20:117–125.