The effects of Nanohydroxyapatite on bone regeneration in rat calvarial defects

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Introduction

With the unstoppable trend of an increasing aging population in both the developing and developed countries, scientists in the field of regenerative medicine and tissue engineering are continually looking for new ways to apply the principles of cell transplantation, materials science, and bioengineering to construct biological substitutes that will restore and maintain normal functions in diseased and injured tissues[1].

Applications of such technology in dentistry, and periodontics in particular, are no exception as periodontal destruction can be found to increase in prevalence with increasing age[2,3].

The traditional clinical procedures of scaling, root planning and periodontal flap surgery, if followed by an adequate postoperative supportive periodontal care, results, in most cases, in successful management of progressive periodontal diseases[3,4].

More recently, the regenerative treatment of periodontal defects with any agent, or procedure, has attracted enormous defects with any agent, or procedure, has attracted enormous interest from materials scientists and also from both private companies and government organizations because of its considerate economic potential [6] and

scientific significance. One of the emerging areas is tissue engineering that seeks to develop techniques and materials to aid in the formation of new tissues to replace damaged tissues[7].

The necessary strategies for complete regeneration of human tissues should be the ultimate endpoint for the field of regenerative medicine and engineering. However, for many tissues this goal remains elusive[8]. Using natural processes as a guide, substantial advances have been made at the interface of nanomaterials and biology, including the fabrication of nanofiber materials for three dimensional cell culture and tissue engineering[9]. One example is nanohydroxyapatitie. It is well known that bioactive materials can integrate well with living bone tissues by spontaneously forming a biologically active bone-like apatite layer on their surface[10]. Hydroxyapatite (HA) is the main mineral constituent of teeth and bones. HA ceramics do not exhibit any cytotoxic effects. They show excellent biocompatibility with hard tissues, skin and muscle tissues[10]. HA is a useful alternative to autogenous bone grafts in orthopedic, dental and maxillofacial applications, due to its chemical and structural similarity to the mineral component of bone[11].

In this study NHA has been synthesed and sintered by sol-gel combustion method in Shahid Chamirane university in Iran.

The aim of this study was to histologically and histomorphometrically evaluate the bone repair quality of this NHA and putting to comparison with Bio Oss (Geistlich Sons Ltd. Wolhusen, Switzerland) in experimental defects prepared in rat calvaria.

Materials and Methodology

The experimental alloplasts used in the present study were Nano crystalline hydroxyapatite and Bio Oss.

This study included 24 male Sprague-Dawley rats (body weight 200-250g) maintained in plastic cages in room with a 12 hour day/ night cycle and an ambient temperature of 21°C. The rats were allowed free access to water and standard laboratory food pellets. Animal selection, management, surgical Protocol and preparation was approved by Ethical Research committee of Ahvaz Jondishapoor University. The study was perfonmed at physiologic Researches center of Ahwaz Jondishapoor university of medical science.

The rats were placed under general anesthesia by injection of ketamine HCL (10mg/ kg) and xylazine (5mg/ kg). The rats were also given an intramuscular prophylactic dose of penicillin G (25000 u/kg), and the surgical site was shaved and prepared with Betadine (povidine – iodine). the calvarium was exposed by making , 3 cm longitudinal incision in the occipital cranium of the rat. The periosteum was stripped and one 8cm diameter full thickness bony hole was created by removal of a bone disc of similar size using a rotary drill and irrigated with normal saline. The dura mater was carefully protected. After homeostasis was achieved, implantation of materials began. The animals were divided to three groups:

1-NHA group, 2- Bio Oss group, and 3- control group. In NHA Group, the bony hole was filled with NHA powder, then one layer membrane of Bio Guide cover the surgical area and at least 2 mm of intact bone around the hole. In Bio Oss group, Bio Oss powder was implanted in to bony hole and in control group no biomaterial was put in bony hole. In three groups Bio Guide membrane was used. Following placement of the biomaterials and membrane, the soft tissues and skin were closed in layers with interrupted absorbable chromic gut sutures.

The experimental animals were transferred to a room with a constant temperature of 21°C. To control postoperative pain 0.05 ml ketoprofen was administered daily for 3 days.

One group of rats sacrified 4 weeks after the surgery and the other group was sacrified after 8 weeks. The animals were placed under general anesthesia with an injection of ketamine HCl (100 mg/kg) and xylazine (5mg/kg) . Then they were sacrified by dislocation of cervical vertebrae. Samples were then collected from the surgical experimental defects. The calvarium was detached from the scull with a rotary bar under irrigation by normal saline. Then fixed in 10% buffered formalin for 5 days. And were decalcified with EDTA for 2 days, neutralized for 12 h, doucked, dehydrated, soaked in wax and embedded. The wax blocks were sectioned across the material and bone into several slices with thickness of 5 μ m, stained with H & E and observed using a light microscope (Nika ECLIPSE, E 200 pol, Japan).

Results

Wound healing was generally uneventful and appeared similar for all groups. Material exposure and other complications of the surgical sites were not observed.

Mann-whitney analysis

groups / variable	NHA/Bio Oss	NHA/Control	Bio Oss/Contro
RB4	0.029*	0.029*	0.029*
RG4	0.029*	-	-
RB8	0.114**	0.029*	0.029*
RG8	0.114**	-	-

*P<0.05 statically significant
** P>0.05 not statically significant

Kruskal-wallis

Variable	RB4	RG4	RB8	RG8
Between groups	0.007*	0.006*	0.012*	0.011*

* P<0.05 statically significant

At 4 and 8 weeks post-surgery, defects in control group filled with thin, loose connective tissue, with minimal new bone formation originating from the defect margins, were observed.

In Bio Oss and NHA groups at 4 and 8 weeks after surgery new bone formation was observed remained material, connective tissue and foreign body inflammation also seen in these groups, but decreased from 4 weeks to 8 weeks.

For histomorphometric analysis, Digital images (DIGITAL SIGHT Nikon, Japan) were taken of each sample through a light microscope at x20 magnification. In each case, the photographic field was selected from the middle portion of the prepared calvarial opening.

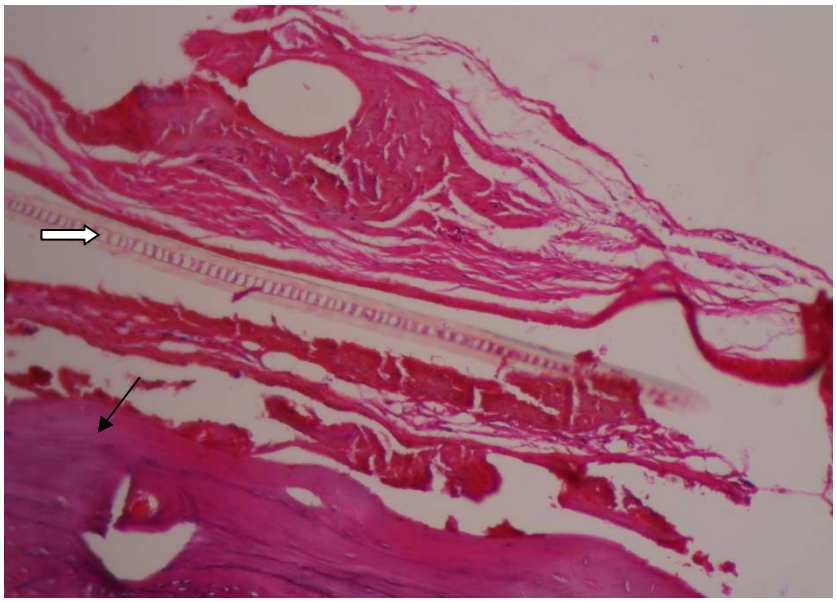
These digital photographs were stored and subsequently analyzed by Sigma Scan Pro Image Analysis software, version 5.0. In this analysis the number of pixel of new bone and remained materials were counted.

Obtained data were analyzed by SPSS version 16 (SPSS, Inc, Chicago, IL, USA). We use Kruskal – Wallis (kW) and Mann Whitney for statically analysis.

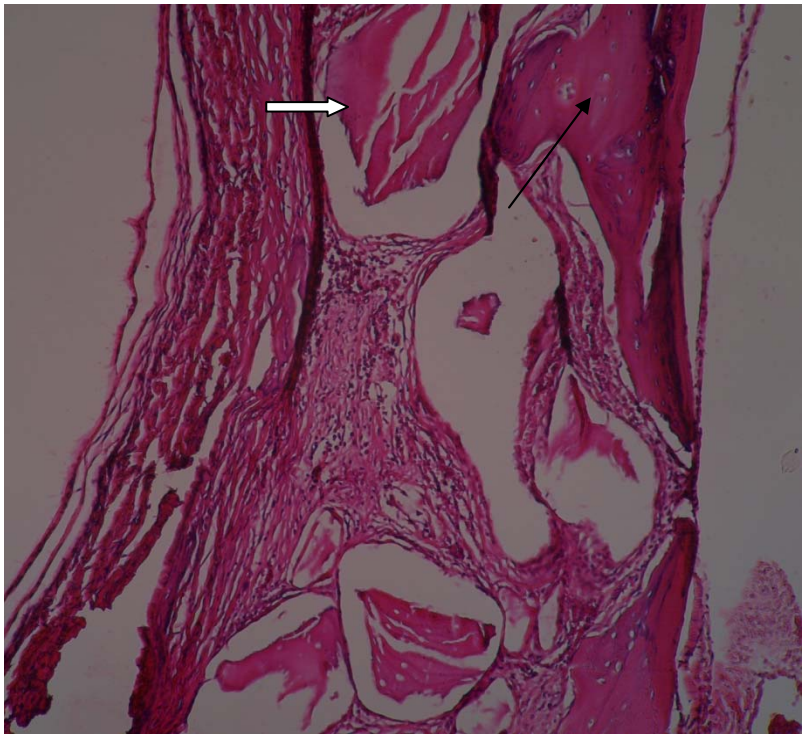
Histomorphometric analysis of bone tissue resulted in mean 19-32% in Bio Oss group, 16.94% in NHA group and 3.7% in control group in 4 weeks. Mean of bone formation was 64.26%, 53.56% and 4.42% in Bio OSS, NHA and control group, respectively in 8 weeks.

Analysis of remained grafts showed 33.63% and 18.96% in Bio OSS and NHA groups respectively in 4 weeks and 17.79% and 12.94% in Bio OSS and NHA groups in 8 weeks respectively. The intergroup analysis (KW) to compare the amounts of bone in the corresponding three groups indicated a statically significant difference between groups in 4 and 8 weeks after surgery.

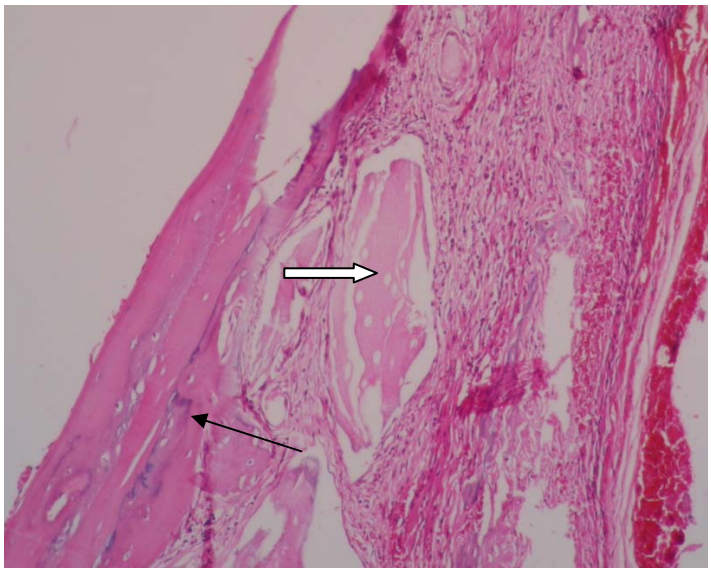
Bio Oss 4week



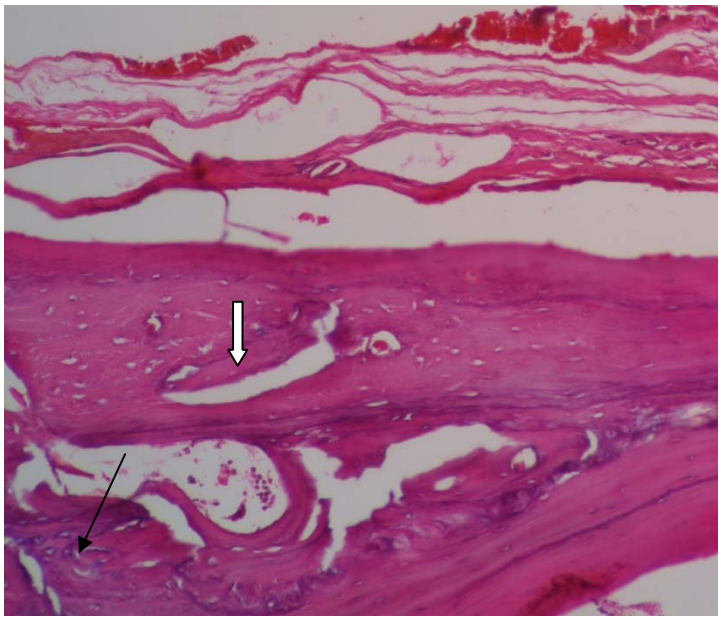
NHA 4week



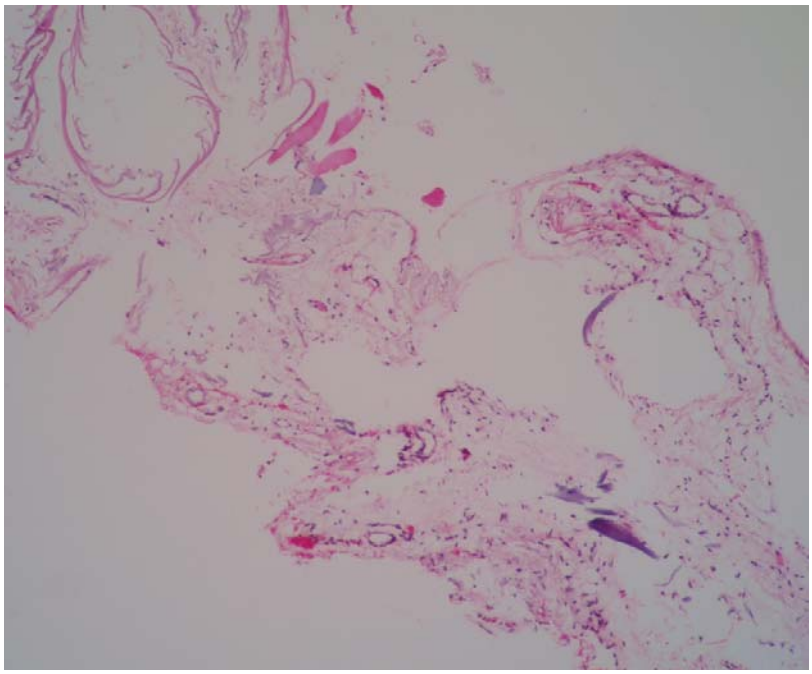
NHA 8week



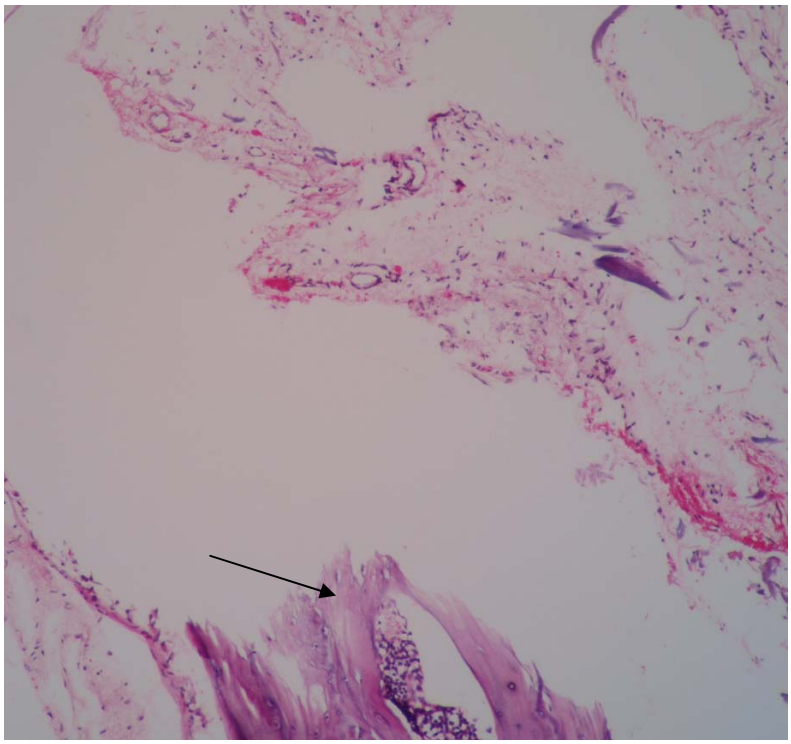
Bio Oss 8week



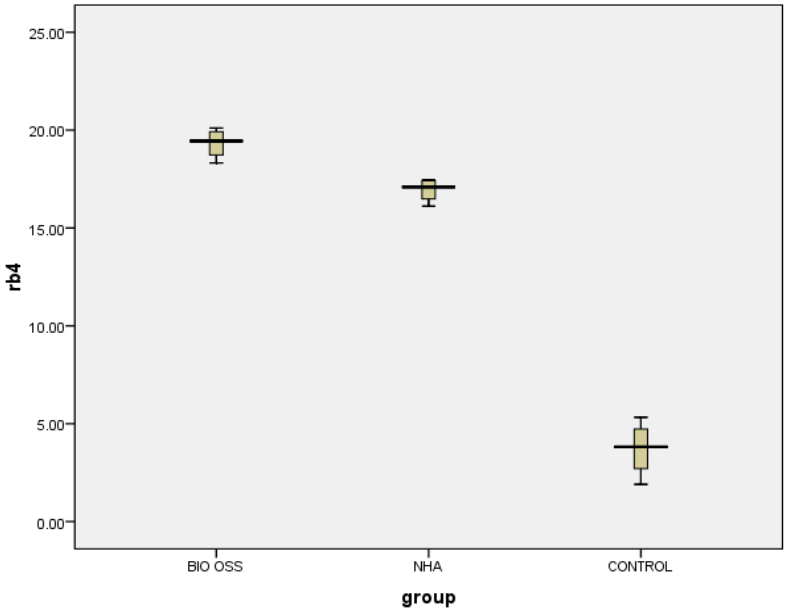
Control 4week



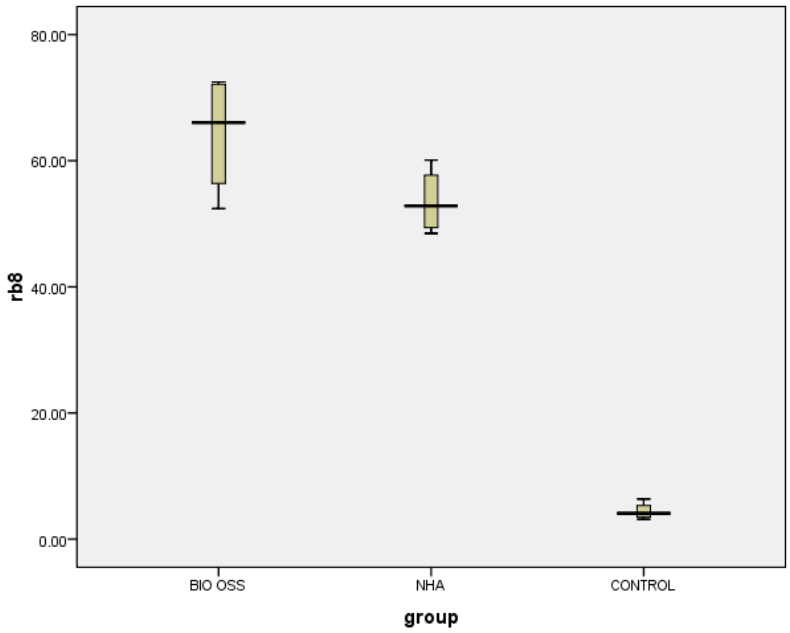
Control 8week



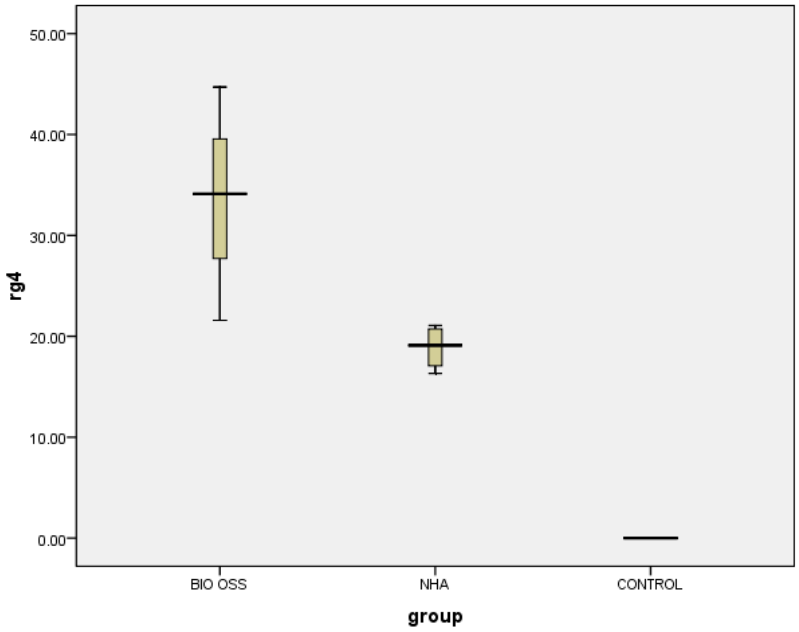
Regenerating bone in 4week



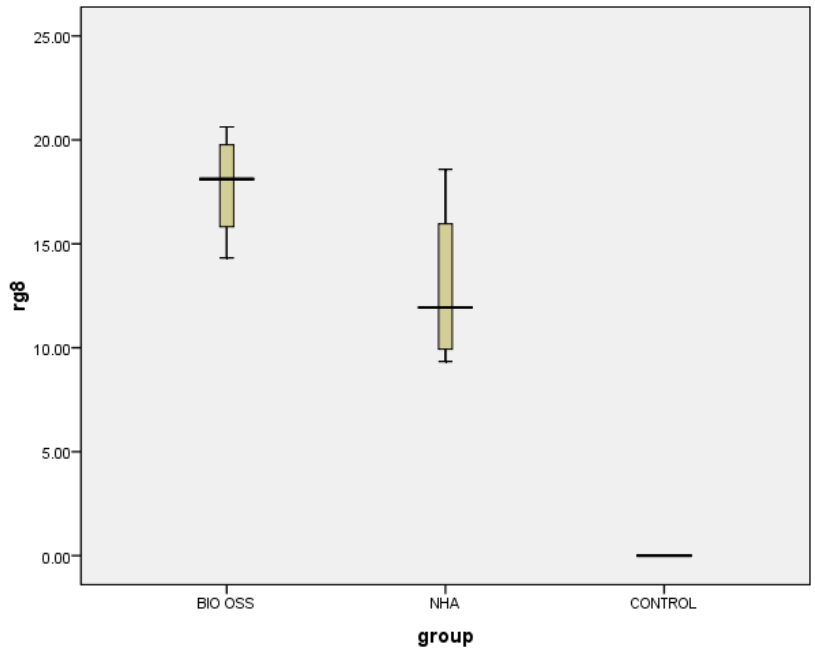
Regenerating bone in 8week



Remained graft in 4week



Remained graft in 8week



Discussion

Nano- sized HA may have other special properties due to its small size and huge specific surface area[12]. Webster et al [13] have demonstrated a significant increase in protein adsorption and osteoblast adhesion on the nano- sized ceramic.

In this study, we attempted to show the clinical efficacy of NHA in a rat model. The experimental model used in this study has been shown to be effective for evaluating the potential for bone formation[14-16]. The rat calvarial defect model is convenient for examining bone regeneration because of its effective accessibility and lack of fixation requirements[12] .Critical Size Defect (CSD) in experimental models are essential for invivo experiments for bone reconstruction. The calvarial CSD of the rat is one of the models for new bone reconstruction and especially a good model for flat bone tissue engineering because the calvaria are suitable for the creation of defects, implantation of grafts and analysis of reconstruction [17,18]. In previous studies, the small defect in 3mm diameter could undergo spontaneous bone regeneration[19]. In present study, we choose 8 mm for defect size.

Hydroxyopatite (HA) is a widely used bone substitute various types of HA are available for the treatment of bone defects [20]. However, the largest available portions of available HA are synthetic, although Bio Oss (Geistich pharma), a bovine porous

bone mineral is one of the most commonly used grafting material[20]. Bio Oss has been widely tested for the treatment of periodontal bone defects[21,22], maxillary sinus elevation procedures[23,24], and bone deficiencies[25]. This bone substitute has osteoconductive properties, and it is capable of producing amounts of bone comparable to that produced by autologous bone chips[26]. Thus we used Bio OSS for positive control in this study.

Based on histomorphometric analysis the amount of bone regeneration in control group was very small in 4 and 8 weeks after surgery and mostly accrue in defect margins. In most of other studies the authors have reported very small or no bone regeneration in critical size defects without any osteoinductive or osteoconductive materials, because the defects in 5 mm diameters is beyond the size of spontaneous bone regeneration[28,29]. Comparisons of two different samples were performed by the Mann Whitney (mw) rank sum test, while comparisons among three different samples were performed by kruskal – wallis (kw) test (the analog of one –way analysis of variance). Mann- whitney analysis showed statistically significant difference in bone forming and remained graft material between Bio Oss and NHA group at 4 weeks ($P<0.05$) , but in 8 week the differences were not statistically significant ($p>0.05$).

This may mean that the rate of bone forming has increased in NHA group after 4 weeks. Park et al (2009) compared the amount of bone formation with Bio Oss and N-HA derived from hen eggshell in critical sized rat calvarial defect. In their research bone formation in N-HA group was statistically significant greater than Bio Oss group in 6 and 12 weeks after surgery[27] .But Bertoldi et al (2012) obtained different results . They evaluated the amount of regenerating bone in Bio Oss and NAH (Ostim, Heraeus kulzer) in distal femur rabbit. Bone forming in Bio Oss group was greater than NHA at 2 and 4 month after surgery [20].

Biomaterials such as those used in this study have been studied in different situations and have led to different and sometimes contrasting conclusions.
Starropouls et al observed that bovine bone may arrest bone formation or produce low bone regeneration [30]. Tamimi et al [31] showed good results, and Schneider et al [32] actually considered BPBM as a “gold standard” (positive control) in graft comparisons. Another material was NHA synethesized by physic group in Chamran

University in Iran. Recent experimental study have shown that nano sized HA (NHA) may represent a promising class of bone graft substitute because of its osteocoductive properties [33,34] .The result of this study showed that this material has osteoconductive properties and can compete with Bio Oss in reconstruction of bone defect.

Conclusion

Nano HA synthesized by combustion sol- Gel method has osteoconductive properties and behaves adequately as grafting material for bone regeneration.

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