Bone Repair via Osteon and Bio-Oss: A Comparative Histological and Histomorphometric Animal Study

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ABSTRACT

Background and Aim: Autogenous bone grafts are considered the gold standard although they have several disadvantages, leading to a search for suitable alternative graft biomaterials. This study evaluates the histological and histomorphometric properties of regenerated bone in defects in rabbits following the application of two commercially available xenografts (Bio-Oss and Osteon).

Materials and Methods: This animal study was carried out on 14 New Zealand rabbit calvaria. Four 6.5-mm critical-size defect (CSD) models of bone regeneration were formed in each surgical site. The first defect was filled with Bio-Oss, the second with large Osteon (L-Osteon), the third with small Osteon (S-Osteon), and the last one remained unfilled (the control group). The cases were sacrificed. Bone forming properties (amount of new bone formation, inflammation, and foreign body reaction) were observed at 4- and 8-week intervals through histological and histomorphometric examinations. The Friedman test, Kruskal-Wallis test, and Wilcoxon test for multiple comparisons were used for data analysis. The level of statistical significance was set at 0.05.

Result: There was no statistically significant difference for regenerated bone among the four groups (P>0.05). The L-Osteon site showed more inflammation and foreign body reaction compared to the other groups.

Conclusion: The results of this study showed that Bio-Oss and Osteon appear to be highly biocompatible and osteoconductive and can thus successfully be used as bone substitutes in augmentation procedures.

Keywords: Biocompatible Materials, Bio-Oss, Bone Grafting, Bone Formation, Bone Substitutes, Histology, Osteon

Original Article

Introduction:
Intraoral donor sites for autogenous bone harvesting are limited and do not provide adequate bone. Harvesting autogenous bone may also lead to donor site morbidity.(1) Autogenous bone grafts are considered the gold standard because they are not immunogenic and have osteogenic, osteoinductive, and osteoconductive properties.(2,6)

There are several disadvantages associated with autogenous bone grafts, including donor site morbidity, prolonged healing time, the need for second surgical intervention, the need for general anesthesia and hospitalization, increased cost of treatment, and unpredictable graft resorption.(7)

These disadvantages have led to a search for suitable graft biomaterials that are biocompatible and osteoinductive or at least...
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present with an osteoconductive alternative to autogenous bone augmentation procedures. Today, there are many bone substitutes and various bone grafting materials, which act as a scaffold for bone formation. However, the process of bone regeneration is slow when compared to autogenous bone grafts.8

The alloplastic Osteon (Osteon®, Genoss Co. Ltd., Suwon, Korea) has a hydroxyapatite (HA) surface (70%) coated with Beta-tricalcium phosphate (β-TCP; 30%). The pore size of Osteon is 300-500 µm, and its volumetric porosity is approximately 77%. It is available in two particle sizes: 0.51 mm and 1-2 mm.9 Clinical evaluations of Osteon as a new alloplastic material in sinus bone grafting and its effect on bone healing have been previously reported.10 Kim et al, in 2008, clinically assessed the use of Osteon® as a sinus graft material and measured the effect of healing 4 and 6 months postoperatively.9 Bae et al, in 2010, clinically evaluated the use of Osteon as a sinus bone graft material and measured the loss of sinus bone graft volume and marginal bone loss around dental implants.11 Anorganic bovine bone particles (Bio-Oss; Geistlich Biomaterials, Wolhusen, Switzerland) are one of the most popular grafting materials used today, which consist of the mineral part of bovine bone, acting as a scaffold for osteoprogenitor cell housing.12,13 Kim et al studied differences in the healing process of sinus bone grafting using the following grafting materials: a mixture of autogenous bone and Bio-Oss, a mixture of Bio-Oss and Orthoblast, Bio-Oss only, and synthetic Osteon.10 In 2012, Zhang et al evaluated the osteoconductive effectiveness of bone grafts derived from calcined antler cancellous bone (CACB) through an experimental study of defects formed in the mandible of rabbits.14 Paknejad et al, in 2014, evaluated the efficacy of two types of bone substitutes, Bio-Oss and NuOss, for the repair of bone defects.15 Xuan et al, in 2014, compared the potentials of PRFmixed Bio-Oss and Tisseel-mixed Bio-Oss for bone regeneration enhancement in a canine sinus model.12

This animal study aimed to evaluate and compare the histological and histomorphometric properties of regenerated bone in defects in rabbits following the application of two biomaterials, namely Bio-Oss and Osteon.

Materials and Methods:

Animals:
This study was conducted using 14 New Zealand white (NZW) rabbits (approximately 2.5 kg in weight) with the approval of the Ethics Committee of the Faculty of Dentistry of Islamic Azad University of Medical Sciences, Tehran, Iran.

Test materials:
In this animal study, two different commercially available xenografts, namely Bio-Oss (Geistlich Pharma AG, Wolhusen, Switzerland) and Osteon (Osteon®, Genoss Co. Ltd., Suwon, Korea), in two different sizes (large Osteon: L-Osteon and small Osteon: S-Osteon) were used and compared with one another (Table 1).

Table 1: Comparison of the test materials [BioOss (Geistlich Pharma AG, Wolhusen, Switzerland) and Osteon (Osteon®, Genoss Co. Ltd., Suwon, Korea) in two different sizes (large Osteon: L-Osteon and small Osteon: S-Osteon)]

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Bio-Oss</th>
<th>S-Osteon</th>
<th>L-Osteon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pore size (µm)</td>
<td>200-500</td>
<td>300-500</td>
<td>500-1000</td>
</tr>
<tr>
<td>Porosity (%)</td>
<td>71-4.35</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>Ca/P Ratio</td>
<td>1.47</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Surgical procedures:
The procedure was performed under general anesthesia using intramuscular injection of 2% Xylazine (5 mg/kg; Alfasan, JA Woerden, Netherlands) and 10% Ketamine (35 mg/kg; Alfasan, JA Woerden, Netherlands). Following shaving and aseptic preparation of the surgical site with the application of 7% Betadine for 5 minutes, a craniocaudal linear 10-cm incision was made on the calvaria of the rabbits, and full-thickness flaps were reflected with an elevator. Next, 6.5-mm bone defects were drilled. Four defects (2 defects in the frontal and 2 defects in the parietal bones) were created in each surgical site using a trephine bur under copious irrigation with sterile saline to prevent damage to the meninges. Anatomic landmarks, the occipital protuberance and craniocaudal suture, which separate the parietal bone horizontally, were used to standardize the defect sites.
For each animal, one defect was filled with Bio-Oss, the second one with S-Osteon, the third one with L-Osteon, and the fourth remained unfilled to be used as the control site. To minimize the probable effect of defect sites on the results of the study, the first site was chosen and filled randomly, and the others were filled in a clockwise pattern afterward.

To prevent confusion, each site was numbered and given a code according to their distance from the transverse/sagittal sutures and recorded in a chart. The periosteum was elevated intact, and no membrane was used to cover the materials. Next, the periosteum and the calvarium were respectively sutured with 4.0 vicryl and 3.0 nylon. Intramuscular injections of Ketoprofen (0.1 mL/day for 3 days), penicillin G (60000 daily), gentamycin (5 mg/kg), dexamethasone (0.5 mL), and B-complex (0.5 mL) were given as postoperative medications for 5 days.

Inflammation, broken sutures, secretions, and probable infection present at the sites were evaluated and recorded every day. The animals were sacrificed by marginal intravenous injection of 3% pentobarbital sodium to the ear after healing periods of 4 and 8 weeks (Figure 1). All the ethical guidelines for animal studies have been considered in the present research.

Histological analysis:
The calvarium skin was dissected using a #22 scalpel. The forehead of the calvarium was separated from the other parts (using a surgical saw) for the protection of the superior orbital rim for detecting the anterior and posterior aspects of the specimen. After removing all of the soft tissues, the samples were separately fixed in 10% neutral phosphate buffered formalin for 2 weeks. The specimens were immersed in 10% formic acid for 4 weeks and were put in formalin to fix the decalcified sites again every other day. The specimens were stored in 20% lithium bicarbonate for 5 minutes to be neutralized. Next, based on their chart coding, the defects were divided into two fragments both longitudinally and in an anterior-posterior direction. The borders of the specimens, which were from the mid part of the defect, were coded with Indian ink. The dehydration process was carried out by immersion in 70-100% ethanol for 24 hours, and the specimens were then embedded in paraffin blocks from their coded end and were cut into 4-μm sections using a Leica RM 2025 microtome (Leica Microsystems Inc., Nussloch, Germany). Each paraffin block was cut into five sections (5μm thick) and stained using hematoxylin and eosin (H&E). Finally, the specimens were covered with a thin lamel.

Quantitative evaluations (histological and histomorphometric evaluations) were performed with light microscopy (Olympus-Bx51, Olympus Co., Tokyo, Japan) equipped with a camera (Olympus-Dp12, Olympus Co., Tokyo, Japan) connected to a personal computer (Figure 2).
The amount of new bone formation and the remaining materials were evaluated using the Magic Wand software (Amazon Co., Seattle, USA). The following parameters were assessed:

**Inflammation:**

Inflammation was evaluated according to the number of inflammatory cells present in the high-power field (400×) of the light microscope. The presence and severity of inflammation were evaluated based on the following grading scores:

- Grade 0: No inflammatory cells
- Grade 1: Less than 25 inflammatory cells
- Grade 2: 25-125 inflammatory cells
- Grade 3: More than 125 inflammatory cells. (15)

**Foreign body reaction:**

Foreign body reaction was evaluated by the presence of multinucleated giant cells in the high-power field (400×) of the light microscope. The presence and severity of inflammation were determined based on the following grading criteria:

- Grade 0: No giant cells
- Grade 1: Mild foreign body reaction (presence of giant cells in one high-power field)
- Grade 2: Moderate foreign body reaction (presence of giant cells in 1-3 high-power fields)
- Grade 3: Severe foreign body reaction (presence of giant cells in more than 3 high-power fields). (16)

**Newly formed bone and remaining biomaterial:**

To evaluate the percentage of newly formed bone and remaining biomaterial, digital images were obtained from the histological sections (20×) using the Olympus DP12. Using the histogram of the Magic Wand software, the pixels of the newly formed bone were compared with those of the defects.

- Newly formed bone:
  - Grade 0: None to few (bone center in all high-power fields)
  - Grade 1: Low (bone center in one high-power field)
  - Grade 2: Medium (bone center in 1-3 high-power fields)
  - Grade 3: High (bone center in more than 3 high-power fields). (17)

- Remaining biomaterial:
  - Grade 0: None to few (biomaterial in all high-power fields)
  - Grade 1: Low (biomaterial in one high-power field)
  - Grade 2: Medium (biomaterial in 1-3 high-power fields)
  - Grade 3: High (biomaterial in more than 3 high-power fields).

**Statistical analysis:** SPSS 11.5 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Ordinal variables (inflammation, foreign body reaction, bone formation, and bone type) were analyzed by Friedman and Kruskal-Wallis tests. Quantitative variables (number of inflammatory cells and the amount of new bone) were analyzed by Friedman test. Multiple comparisons were made using Wilcoxon test.

**Results**

**Inflammation:**

Inflammation was consistently observed in all four groups with maximum inflammation seen for the Osteon group four weeks postoperatively. According to Friedman and multiple comparison tests, the level of inflammation was significantly higher for Bio-Oss in comparison with the control group (P=0.008). A significant difference was found between the L-Osteon group and the Bio-Oss group (P=0.023). There was no statistically significant difference between the Osteon groups (S and L; Table 2).

**Table 2: Inflammation rate in each group**

<table>
<thead>
<tr>
<th>Group</th>
<th>Time</th>
<th>Inflammation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bio-Oss</td>
<td>4 weeks</td>
<td>Grade 3</td>
</tr>
<tr>
<td>L-Osteon</td>
<td>4 weeks</td>
<td>Grade 3</td>
</tr>
<tr>
<td>Control</td>
<td>4 weeks</td>
<td>Grade 0</td>
</tr>
</tbody>
</table>

**Foreign body reaction:**

Foreign body reaction was significantly higher for Bio-Oss when compared to the control group (P=0.005). A significant difference was found when comparing the L-Osteon group to the Bio-Oss group (P=0.038). However, there was no significant difference between the other groups (Table 3). Remained bone biomaterials and new bone These indices showed no statistically significant These indices showed no statistically significant differences between the groups (Tables 4 to 6).
Table 3: Foreign body reaction in each group

<table>
<thead>
<tr>
<th>Time</th>
<th>4 weeks</th>
<th>8 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bio-Oss</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>S-Osteon</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>L-Osteon</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 4: Newly formed bone in each group

<table>
<thead>
<tr>
<th>Time</th>
<th>4 weeks</th>
<th>8 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bio-Oss</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>S-Osteon</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>L-Osteon</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Control</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 5: Remained biomaterial in each group

<table>
<thead>
<tr>
<th>Time</th>
<th>4 weeks</th>
<th>8 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bio-Oss</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>S-Osteon</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>L-Osteon</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Control</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 6: The results of statistical analyses

<table>
<thead>
<tr>
<th>Inflammation (P-value)</th>
<th>Foreign Body reaction (P-value)</th>
<th>Newly Formed Bone (P-value)</th>
<th>Remained Biomaterial (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control and Bio-Oss</td>
<td>0.008*</td>
<td>0.085*</td>
<td>0.076</td>
</tr>
<tr>
<td>L-Osteon and Bio-Oss</td>
<td>0.023*</td>
<td>0.038*</td>
<td>0.083</td>
</tr>
<tr>
<td>S-Osteon and Bio-Oss</td>
<td>0.029*</td>
<td>0.062</td>
<td>0.089</td>
</tr>
<tr>
<td>L-Osteon and S-Osteon</td>
<td>0.070</td>
<td>0.083</td>
<td>0.075</td>
</tr>
<tr>
<td>Control and L-Osteon</td>
<td>0.011</td>
<td>0.007*</td>
<td>0.064</td>
</tr>
<tr>
<td>Control and S-Osteon</td>
<td>0.013*</td>
<td>0.009*</td>
<td>0.072</td>
</tr>
</tbody>
</table>
Discussion:

Schmitz and Hollinger defined a critical-size defect as the smallest osseous defect in a particular bone that would not heal naturally during the lifetime of the animal. This has been redefined as a defect that shows bone regeneration of less than 10% during the mentioned period. The absolute value of a critical-size defect depends on the breed, age, and phylogenetic order of the animal.

There is still considerable debate over the definition of critical-size defects for bone bioengineering. The three-dimensional (3D) nature and the discontinuity of the defect are important parameters that need to be assessed before a defect can be considered critical in size. Defect repair in rabbit mandibles has been attempted before by Ren et al. They showed that a defect of $5 \times 12 \ mm^2$ in the mandible was of critical size and did not show any sign of bone union.

The rabbit is recognized as an appropriate model of study for bone bioengineering in the craniofacial region; as it allows the creation of a large mandibular osteoperiosteal discontinuity, critical-size bone defects can be made to simulate the clinical setting without jeopardizing animal wellbeing. The rabbit model is also a mammalian model that is biologically similar to humans. Depending on the animal model used, the length of the follow-up before sacrifice varied from 5 weeks to 8 months in minipigs, from 6 weeks to 12 weeks in rats, and from 3 weeks to 8 weeks in rabbits.

In addition to the chemical composition and physiological conditions of bone graft materials, features such as crystallinity, crystal and particle size, porosity, and surface roughness affect biological performance and determine the nature and extent of scaffold biodegradation. In this study, histological and histomorphometric properties of regenerated bone in defects in the rabbit model were assessed following the application of two commercially available xenografts (Bio-Oss and Osteon). More specifically, inflammation, foreign body reaction, newly formed bone, and remaining biomaterial were analyzed.

The least amount of inflammation was observed in the control group with S-Osteon, Bio-Oss, and L-Osteon showing increased amounts of inflammation in an ascending order. Artzi et al investigated the influence of Bio-Oss grafted particles on the histopathological pattern of the intra-socket regenerated bone. They also histomorphometrically evaluated the healed porous bovine bone mineral (PBBM) grafted extraction sockets and found some lymphocytes present. Piatelli et al used Bio-Oss in sinus augmentation procedures and found small capillaries, mesenchymal cells, and osteoblasts inside some Haversian canals in the specimens harvested at 6 months with no inflammation present. Bae et al clinically evaluated the use of Osteon as a sinus bone grafting material. They concluded that bone healing is not greatly affected if the perforation site is reconstructed using appropriate techniques.

Conclusion

The results of this study showed that Bio-Oss and Osteon appear to be highly biocompatible and osteoconductive and can successfully be used as a bone substitute in augmentation procedures.

Ethical approval

The study was conducted with the approval of the Ethics Committee of the Faculty of Dentistry of Islamic Azad University of Medical Sciences, Tehran, Iran.

Conflict of interest

The authors have no financial or other conflicting interest to declare concerning the content of this article.
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