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# A Comparative Study of Anorganic Xenogenic Bone and Autogenous Bone Implants for Bone Regeneration in Rabbits

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The aim of this study was to investigate the possible use and ultimate fate of anorganic xenogenic bone for the restoration of defects in the maxillae and mandibles of adult rabbits. Furthermore, anorganic xenogenic bone was compared with implanted autogenous bone particles with regard to the response of the surrounding connective tissue and possible resorption of these implants by multinucleated cells. Results showed that after 12 weeks, the implanted autogenous bone was actively resorbed by multinucleated cells, and new bone was formed in close apposition to the particles. In contrast, implanted anorganic xenogenic bone was degraded to a much lesser extent, and new bone was seen adjacent to the anorganic bone particles without signs of resorption. Further long-term studies are needed to determine whether anorganic xenogenic bone may be regarded as a resorbable material and whether any side effects occur as a result of this material's tendency to linger on in the recipient bed.

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**Key words:** anorganic bovine bone, Bio-Oss, implant, rabbit

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There is considerable interest in developing substitute materials for autogenous bone grafts. Autografts require additional surgical procedures, which sometimes result in morbidity at the donor site. Furthermore, the harvesting procedure itself may considerably limit the benefits of the primary operation. Also, mineralized autogenous bone grafts often decrease in volume postoperatively because of resorption,<sup>1</sup> and this might have an impact on the outcome of the procedure. Use of autolyzed, antigen-extracted, allogenic bone as a substitute for autogenous bone transplants was described in 1975<sup>2</sup> by Urist et al, but was thereafter avoided for many years because of fear of disease transmission. These grafts are reported to

be quite resistant to resorption, although in specific cases, advanced reduction in volume of the grafts has occurred.<sup>3</sup>

To overcome the limitations of autografts and allografts, several different bone substitutes have been developed. Anorganic xenogenic bone mineral (Bio-Oss, Geistlich-Pharma, Wolhusen, Switzerland) is such a material, and the objective of the present study was to investigate the host tissue reactions to it and the biodegradability of this material in bone defects in the maxillae and mandibles of adult rabbits. Comparison was also made with autogenous bone particles regarding the response of the surrounding connective tissue and possible resorption of the material by multinucleated giant cells.

The ultimate bone substitute should eventually be resorbed and encourage new bone formation, thus permanently replenishing lost bone. Bio-Oss is a reportedly resorbable, anorganic bone mineral produced from bovine bone.<sup>4</sup> The material has so far been tested in different animal models<sup>4-8</sup> and a few studies have reported the use of Bio-Oss in patients.<sup>9-11</sup> The results from these studies are contradictory as to whether this material may be

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**Table 1** Experimental Data

	Group 1	Group 2	Group 3	Group 4
Total no. of animals	6	6	6	4
No. of animals excluded as the result of death	0	2	1	0
Experimental material used	auto	bio	auto + bio	contr
Experimental site	max + mand	max + mand	max + mand	max + mand

Auto = autologous bone; bio = Bio-Oss; contr = untreated control group; max = maxilla; mand = mandible.

regarded as a resorbable and effective bone substitute; therefore, further research is considered necessary.

### Materials and Methods

Twenty-two adult male and female New Zealand white rabbits, weighing between 300 and 460 grams, were kept in standard laboratory conditions of light-dark schedule and relative humidity. Stock diet and tap water were provided ad libitum. Preoperatively, the animals were anesthetized with an intramuscular injection of fluanisone and fentanyl (10 mg/mL and 0.2 mg/mL, respectively; 0.6 mL/kg body weight) and a submucosal injection of lidocaine with adrenaline (20 mg/mL; adrenaline 12.5 µg/mL). A U-shaped incision was made posterior to the incisors in the maxilla and mandible. A mucosal flap was elevated and the underlying bone was exposed using a mucoperiosteal elevator. Bone defects were produced in the midline using a 3-mm trephine bur mounted on a low-speed dental handpiece. During bone drilling, the surgical field was continuously irrigated with sterile saline to reduce thermal damage. The bone plugs were gently removed, rinsed with sterile saline to wash off any soft tissue debris, and crushed several times with pliers to a fine particulate for later use.

Equal amounts of Bio-Oss (particle size 0.25 to 1.00 mm) and autogenous bone particles were used to randomly fill the bony defects in such a way that 4 experimental groups were formed (Table 1): group 1, defects filled with autogenous bone; group 2, defects filled with Bio-Oss; group 3, defects filled with autogenous bone and Bio-Oss; and group 4, untreated control defects. Following implantation, the mucoperiosteal flaps were placed in their original position using 4.0 polyglactin 910 sutures. Three rabbits died during surgery; the remaining 19 rabbits were checked several times during the first day and weighed once daily throughout the first postsurgical week. The experimental data are summarized in Table 1.

Twelve weeks postoperatively, the rabbits were sacrificed using an overdose of pentobarbital (100 mg/mL), and the implantation areas were dissected free and fixed in 4% neutral buffered formalin. The specimens were radiographed for orientation of possible hard tissue formation, demineralized in 17% formic acid, dehydrated, and embedded in paraffin wax. Serial sections 7 µm thick were cut transversely in the midpart of the lesion, stained with hematoxylin and eosin, and analyzed using light microscopy. The experiment was approved by the local animal ethics committee for the southern areas of Stockholm, Sweden.

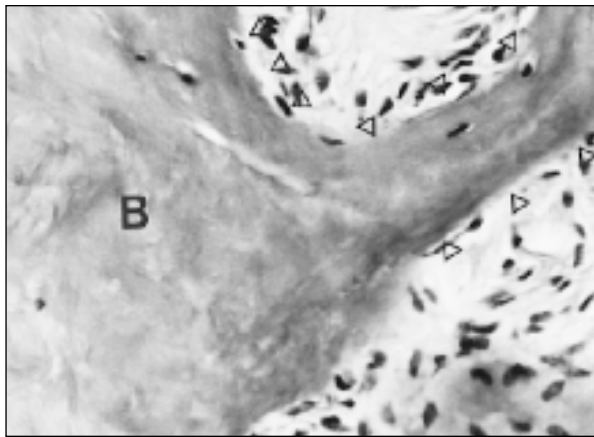
### Results

All 19 remaining animals gained weight during the 12-week observation period. There was no evidence of postsurgical infection or extrusion of implant material. Furthermore, there were no observable differences in the tissue response to the different implant materials in the maxilla compared with the mandible.

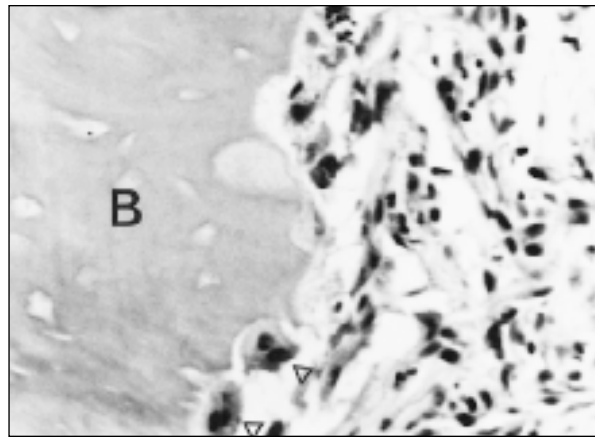
Bone defects that were rinsed with sterile saline only and left empty were filled with fibrous tissue, but bone ingrowth was evident from the edges of the defects. However, in no animal was complete bony continuity seen throughout the defect.

Defects filled with autogenous bone particles showed formation of new bone in close apposition to the implanted bone, which was easily recognized (Fig 1). Howship's lacunae were frequently seen in the particles harboring large, multinucleated osteoclasts (Fig 2), and occasionally a few areas with scattered inflammatory cells were seen. Thus, the autogenous bone graft was undergoing active resorption. The surrounding vascular and cellular rich connective tissue stroma did not show signs of acute or chronic inflammation.

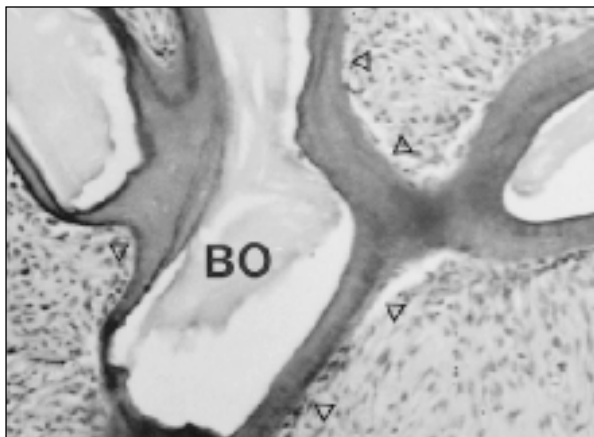
In defects filled with autogenous bone particles and Bio-Oss, the interstitial space between the grafted particles was occupied by a very dense connective tissue stroma, and osteoclasts were noted



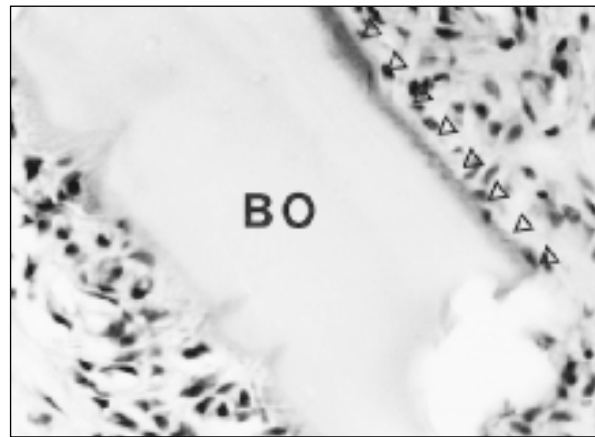
**Fig 1** Defects filled with autogenous bone particles (B) show new bone formation (*arrowheads*) in close apposition to the particles, which were easily recognized (hematoxylin and eosin; original magnification  $\times 200$ ).



**Fig 2** Howship's lacunae are evident in the implanted autogenous bone particles (B), and multinucleated cells are seen resorbing the bone (*arrowheads*) (hematoxylin and eosin; original magnification  $\times 200$ ).



**Fig 3** Several Bio-Oss particles (BO) are evident, and adjacent to the implant, new bone is laid down (*arrowheads*) (hematoxylin and eosin; original magnification  $\times 160$ ).



**Fig 4** A seemingly unaffected Bio-Oss particle (BO) is surrounded by highly cellular connective tissue stroma. A thin, basophilic, acellular tissue is seen outlining the implant on one side (*arrowheads*) (hematoxylin and eosin; original magnification  $\times 320$ ).

**Table 2** Summary of Histologic Evaluation

Histology	Group 1	Group 2	Group 3	Group 4
Experimental material	auto	bio	auto + bio	control
Bone formation	+	+	+	-
Resorption of material	+	-	+	-
Multinucleated cells	+	-	+	-
Inflammation	-	-	-	-
Dense connective tissue stroma	-	+	+	-
Vascular and cell-rich stroma	+	-	-	-

Auto = autologous bone; bio = Bio-Oss; + = present; - = not present; \* = resorption of bone implant only, and multinucleated cells were seen only adjacent to autologous bone.

in resorption lacunae in the implanted autogenous bone particles. Newly formed bone was seen in direct apposition to the bone particles undergoing active resorption. New bone was formed in close proximity to Bio-Oss particles, seemingly without resorption of this material (Fig 3). A strongly basophilic, thin acellular tissue was sometimes noted outlining the particles (Fig 4). When only Bio-Oss particles were implanted, a highly cellular, dense connective tissue stroma surrounded the particles, with no evidence of resorption of the material. Acute or chronic inflammatory cells could not be detected. Thin bone trabeculae were evident in direct contact with some of the Bio-Oss particles. The results are summarized in Table 2.

### Discussion

The most popular implant procedure today involves the use of autogenous bone. This technique is based on the concept of creeping substitution. This means that the autogenous bone graft serves to provide immediate mass and stability and also acts as a scaffold that is gradually resorbed and vascularized as new bone is synthesized.<sup>12</sup> With time, the graft, particularly those in the craniofacial region, appear to melt away to an undetectable degree. This problem may result from graft resorption exceeding bone ingrowth. Further operative procedures are then often required.

The bone inductive process is a multistep cascade; it comprises chemotaxis and attachment of stem cells to a demineralized matrix, followed by the proliferation of progenitor cells and the formation of cartilage, bone, and subsequently hematopoietic marrow. It has become clear that a class of regulatory proteins governs the cellular and molecular biology of osteogenesis.<sup>13-16</sup> One such protein is bone morphogenetic protein (BMP), which can be found in mineralized tissues such as bone and is released during resorption.<sup>17-19</sup> The release of BMP into the surrounding matrix induces new bone formation, and the simultaneous occurrence of bone resorption and bone apposition has been termed the "coupling phenomenon."<sup>20,21</sup> Various bone substitutes have been developed because of the shortage of autogenous bone, the sometimes complicated harvesting procedure, and the reported unpredictable reduction in volume of mineralized bone grafts. One of the bone substitutes now in routine use, Bio-Oss, is an anorganic hydroxyapatite from bovine bone. The material is regarded as resorbable and osteoconductive, although some studies refute this.<sup>9,22,23</sup>

The purpose of this investigation was to compare the effects of particulate Bio-Oss and autogenous mineralized bone particles in an experimental model of active osteogenesis under comparable healing conditions in the anterior maxillary and mandibular region in rabbits. Furthermore, signs of resorption and the ultimate fate of the implants were studied. Implantation of autogenous mineralized bone resulted in resorption of the bone particles, and osteoclasts were a frequent finding in Howship's lacunae. Furthermore, ongoing formation of new bone was seen in close proximity to these bone particles, thus the coupling phenomenon was evident. The connective tissue stroma surrounding the bone particles was vascular and cell-rich, with no signs of inflammation. Frequent osteoblast-like cells were seen lining the newly formed bone. In cases where only Bio-Oss was implanted, the connective tissue stroma was conspicuously dense around the particles. Multinucleated giant cells were seldom seen in the vicinity of this material. However, the outline of the Bio-Oss particles was often irregular, and presumably the material was degraded rather than resorbed. These observations corroborate the findings of Schlichewei et al<sup>24</sup> and Jensen et al.<sup>25</sup> The implantation of both autogenous bone and Bio-Oss resulted in resorption of the bone particles and apposition of new bone, whereas newly formed bone was seen adjacent to Bio-Oss without prior resorption of the Bio-Oss. However, the current observation time (12 weeks) may not be sufficient for Bio-Oss to completely disappear, but the question still arises whether this material indeed is resorbable, as other studies have indicated.<sup>26</sup> The results obtained in the present study support findings in a recently published study in humans in which Bio-Oss was used to augment atrophic alveolar ridges, and residual material was evident in the recipient bed for as long as 44 months after the initial operation.<sup>9</sup>

### Conclusion

It is concluded that implantation of anorganic xenogenic bone, Bio-Oss, in preformed bone defects in rabbits results in bone regeneration. However, resorption of the implanted material was not evident. Further long-term studies are needed to determine whether Bio-Oss can be regarded as a resorbable material, and whether any side effects can be observed attesting to the material's tendency to linger on in the recipient bed.

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