Use of Bovine Bone Graft and Bone Membrane in Defects Surgically Created in the Cranial Vault of Rabbits. Histologic Comparative Analysis

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Purpose: *This study was proposed to analyze histologically the process of repairing bone defects created surgically in the cranial vaults of rabbits.* ***Materials and Methods:*** *Thirty adult male rabbits* (Oryctolagus cunilicus) *received, under general anesthesia, bilateral parietal osteotomies by means of a 6-mm-diameter trephine. The bony defects were divided into 4 groups. In group 1 the defect did not receive any treatment; in group 2 the defect was filled with lyophilized bovine bone (Biograft); in group 3 it was filled with bovine bone and covered with a bone matrix membrane (Bioplate); in group 4 it was covered with a bone matrix membrane. Animals were sacrificed in 3 equal groups at 15, 30, and 60 days. The specimens were subjected to routine laboratory procedures to evaluate the degree of bone repair.* ***Results:*** *After 60 days, new bone formation in group 2 was not satisfactory when compared to that of group 3. Large amounts of new bone formation in maturation were seen in group 3. In the defects covered with a membrane the results were similar to those of group 1 (ie, the cavity was filled with fibrous connective tissue). The implanted bone and membranes were totally resorbed.* ***Discussion and Conclusions:*** *The use of a membrane served as a barrier against the migration of cells from the adjacent tissue and the bone graft/membrane preserved the cavity space, resulting in an enhanced osteogenic effect.* Int J Oral Maxillofac Implants 2006;21:29–35

Key words: *bone grafting, bone regeneration, cranial vault reconstruction, resorbable membranes, xenografts*

Bone tissue has a high capacity for regeneration, and it can completely restore its original structure and function. However, in some situations, because of the defect size, the bone tissue does not completely regenerate by itself. To facilitate or promote bone restoration, various materials have been used for grafting in bone defects.[1]

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In surgery and oral maxillofacial trauma bone tissue is commonly used in preprosthetic surgeries for the treatment of congenital defects and dental or facial abnormalities to promote fracture union and to prevent collapse of bone segments in the iatrogenic defects.[2,3]

Bovine bone has been widely used as a graft material. Like other substitute bones, it has osteoconductive characteristics. This material has a morphologic structure and chemical composition comparable to human bone. It also has a wide internal surface and is similar in porosity to human bone.[4–6] According to Zitzmann and associates,[7] the techniques of guided bone regeneration using a protective membrane are frequently combined with the use of autogenous bone graft or bone substitutes. The graft material supports the membrane; it also controls blood coagulation and reduces its volume and contraction.[7–10]

Utilizing the principle of guided bone regeneration, a protected space is created with a protective membrane. The membrane prevents the penetration of soft tissue but allows the space to be invaded by cells with the capacity for bone formation. However, the pressure of the external soft tissue and the consequent collapse of the protective membrane are considered to be a main reason for failure of the regenerative process. It has also been suggested that the bone particles promote bone formation because of osteoconductive activity and by transference of stimulation factors provided by the source of the bone.[10]

Since lyophilized bovine bone and the bovine bone membrane have received attention in recent years as materials for bone cavity filling, the study of these materials is appealing. This study was proposed to analyze histologically the process of the repair of bone defects created surgically in the cranial vaults of rabbits and subsequently either filled with lyophilized bovine bone (Biograft; FOB-USP, Bauru, SP, Brazil) and covered with a membrane from a bovine bone source (Bioplate; FOB-USO) or left unfilled and/or uncovered.

MATERIALS AND METHODS

Thirty male adult *Oryctolagus cunilicus* rabbits weighing from 2 to 2.5 kg were used. The animals received general anesthesia through the intramuscular administration of ketamine chloride (10 mg/kg) and xylazine hydrochloride (5 mg/kg). The study was approved by the UNESP Ethical Committee for Animal Research of the Araraquara Dental School. All surgical procedures were done under strict aseptic protocol. After induction of anesthesia and trichotomy of the anterior parietal region, the rabbits were placed in a ventral position. A sagittal incision of approximately 20 mm was made on the interparietal suture, followed by the displacement and reflection of epidermal tissues, muscular tissue, and periosteum until the parietal bones were exposed. Using a 6-mm-diameter trephine (3i/Implant Innovations, West Palm Beach, FL) at a slow speed of rotation, bilateral parietal ostectomies were prepared in total thickness under constant irrigation with 0.9% physiologic serum.

The bone segments were carefully removed with a Freer detachment instrument (Schobell Industrial, Rio Claro, São Paulo, Brazil) and the integrity of the dura mater and encephalon were maintained.

The bone defects were divided into 4 equal groups of 15 and treated as follows:

- **Group 1:** Bone defect did not receive any treatment.
- **Group 2:** Bone defect was filled with lyophilized bovine bone (Biograft).
- **Group 3:** Bone defect was filled with lyophilized bovine bone and covered with a bone matrix membrane.
- **Group 4:** Defect was not filled but was covered by a bone matrix membrane.

The grafts were protected by repositioning the soft tissues. The tissue was closed with continuous sutures with mononylon 4-0 (Ethicon; Johnson & Johnson, Somerville, NJ). Immediately postoperatively, the animals received 100 mg of benzathine ampicillin (30 mg/kg) administered subcutaneously and 0.1 mL of sodium dipyrone administered intramuscularly every 6 hours in a total of 3 doses.

Euthanasia of the animals occurred after periods of 15, 30, and 60 days postsurgery. Ten animals were sacrificed per period. After sacrifice, to prepare the histologic specimens, the cranial vault was removed in such a way that the soft supraperiosteal tissues, as well as the dura mater and part of the encephalon, were removed. Block sections of the sites were fixed in 10% neutral buffered formalin, decalcified in a 50% formic acid solution and 20% sodium citrate solution, washed in running tap water, and dehydrated in a graded series of ethanols. The specimens were then embedded in paraffin and sectioned semiserially (6 µm thickness) sagitally. The sections were stained with hematoxylin-eosin (H&E) and Masson's trichrome (MT) stains and viewed under a light microscope, and photomicrographs of selected sections were made.

RESULTS

In general, the animals did not present any disturbances or postoperative problems. There were no open wounds, infections, or other complications.

15 Days

Group 1. The cavity was filled with blood. In all animals the borders of the cavity presented a lower quantity of neoformed bone tissue than those found in the other experimental groups, and the cavity was filled by fibrous connective tissue. The presence of inflammatory infiltrate, acute or chronic, was not seen (Fig 1a).

Group 2. The cavity was filled with lyophilized bovine bone. The borders of the cavity showed a little formation of bone tissue and remodeling. The bone tissue observed was intermixed with particles

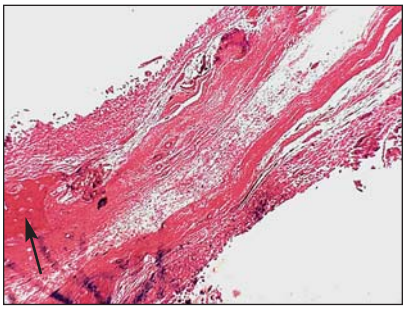


Fig 1a Group 1 at 15 days. Dense fibrous connective tissue filled the cavity. Note the border; the quantity of newly formed bony tissue *(arrow)* is small in comparison to the other groups (H&E; original magnification ×32).

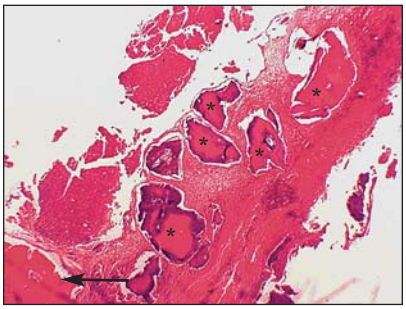


Fig 1b Group 2 at 15 days. Fibrous connective tissue intermixed with the lyophilized bone graft (*) filled the cavity. Signs of inflammation were observed around the implanted bone. Note the remodeling at the border of the cavity *(arrow)* (H&E; original magnification ×32).

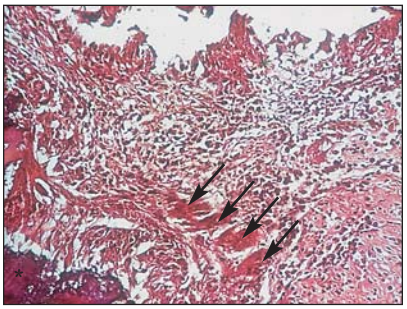


Fig 1c Group 2 at 15 days. Intense chronic inflammatory infiltrate was observed around the implanted lyophilized bone (*). Note the presence of giant cells *(arrow)* (H&E; original magnification ×100).

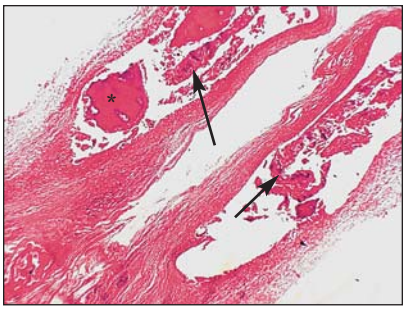


Fig 1d *(Left)* Group 3 at 15 days. Fibrous connective tissue was intermixed with the implanted lyophilized bone (*) and intense chronic inflammatory infiltrate. Note the membrane *(arrow)* surrounded by fibrous connective tissue (H&E; original magnification ×32).

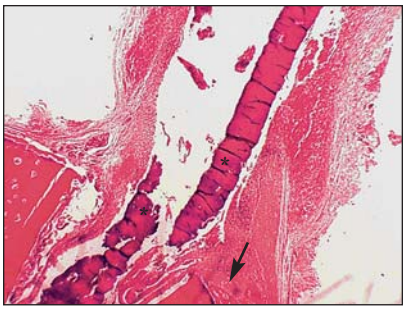


Fig 1e *(Right)* Group 4 at 15 days. Fibrous connective tissue surrounded the membrane (*). Chronic and moderate inflammatory infiltrate were found. Note the remodeling at the border of the cavity *(arrow)* (H&E; original magnification ×32).

of lyophilized bone in different stages of resorption by mononuclear cells and surrounded by fibrous connective tissue. Chronic inflammatory infiltrate was present (macrophages and giant cells were seen). The beginning of bone neoformation was seen next to the fragments of the bone that had been introduced (Figs 1b and 1c).

Group 3. Lyophilized bovine bone was observed at the borders of the cavity. The formation of bone tissue involving dense fibrous connective tissue was seen, intermixed with particles of lyophilized bovine bone and inflammatory infiltrate with a large amount of giant cells. The membrane was almost totally intact, surrounded by dense fibrous connective tissue and a moderate amount of inflammatory infiltrate characterized by the presence of a small quantity of giant cells (Fig 1d).

Group 4. The cavity was covered with the bovine bone matrix membrane. At the borders of the cavity, bone neoformation, remodeling, and spaces filled with fibrous connective tissue were observed. The membrane presented signs of resorption. It was surrounded by dense fibrous connective tissue and chronic and moderate inflammatory infiltrate with some giant cells (Fig 1e).

30 Days

Group 1. The borders of the cavity were remodeled with significant bone neoformation. Dense fibrous connective tissue filled the cavity (Fig 2a).

Group 2. The borders of the cavity were remodeled, and fragments of lyophilized bone were seen as part of a more intensive resorption process. Newly formed and associated bone tissue and chronic and intensive inflammatory infiltrate with a larger quantity of giant cells (Fig 2b) were seen.

Group 3. In all cases the borders of the cavity showed intensive bone neoformation. Within the cavity few fragments of the grafted bone had begun the resorption process. Neoformed and associated bone tissue were also observed. The presence of chronic and moderate inflammatory infiltrate was observed. The membrane was almost totally resorbed. The resorption of the membrane was associated with chronic inflammatory infiltrate (Figs 2c and 2d).

Group 4. Bone neoformation was observed at the borders of the cavity, which was filled with fibrous connective tissue with the presence of chronic inflammatory infiltrate. The membrane presented a higher degree of resorption than was observed at 15 days (Fig 2e).

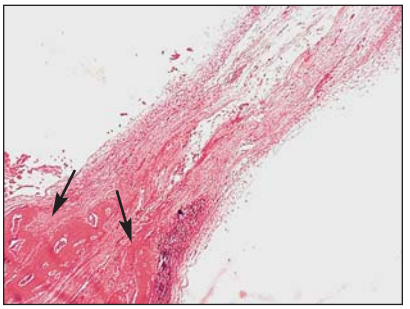


Fig 2a Group 1 at 30 days. Connective tissue filled the cavity. Note the remodeling at the border of the cavity *(arrow)* (H&E; original magnification ×32).

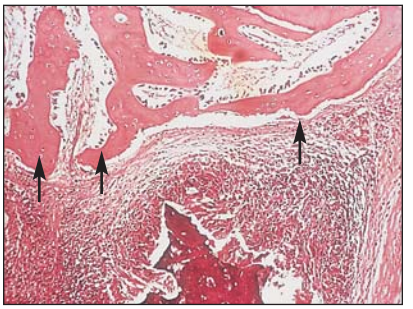


Fig 2b Group 2 at 30 days. Intensive chronic inflammatory infiltrate was present around the remains of the implanted lyophilized bone (*). Note remodeling *(arrow)* at the border of the cavity (H&E; original magnification ×400).

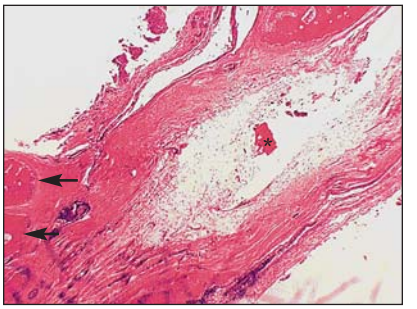
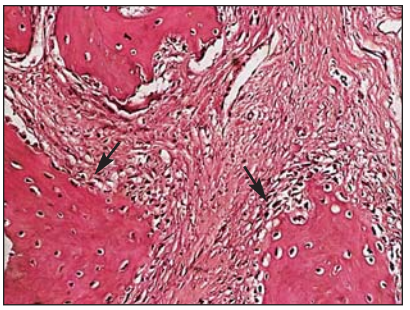


Fig 2c Group 3 at 30 days. Fragments of the bovine bone were in the process of being resorbed (*). Note the bone neoformation at the border of the cavity *(arrow)* and the almost complete resorption of the membrane *(arrow)* (H&E; original magnification ×100).



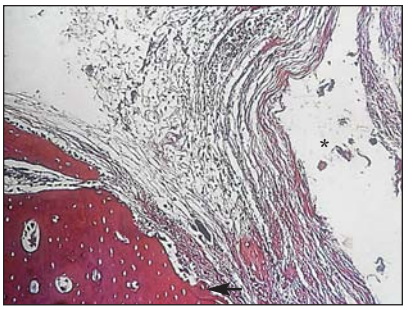


Fig 2d *(Left)* Group 3 at 30 days. Bone neoformation occurred within the cavity. Note the osteoblasts around the neoformed tissue *(arrow)* (H&E; original magnification ×100).

Fig 2e *(Right)* Group 4 at 30 days. Dense fibrous connective tissue was observed around the remains of the membrane (*) with chronic inflammatory infiltrate. Note the remodeling at the border of the cavity *(arrow)* (H&E; original magnification ×400).

60 Days

Group 1. The borders of the cavity were remodeled. Inside the cavity there was dense fibrous connective tissue. Some newly formed bone was observed adjacent to the borders of the cavity; however, there were no signs of cavity closure. No inflammatory reaction could be seen (Fig 3a).

Group 2. Intensive neoformation was observed at the borders of the cavity, and in the center of cavity there was a larger quantity of bone islands. However, these bone islands had a certain degree of immaturity and did not completely fill the lesion. The inflammatory infiltrate was chronic. No implanted bone could be seen (Fig 3b).

Group 3. The borders of the cavity showed significant neoformation and remodeling. The cavity was filled with newly formed bone tissue. The implanted bone and the membrane were totally resorbed. A chronic inflammatory infiltrate was seen in some areas of dense fibrous connective tissue (Figs 3c and 3d).

Group 4. The borders of the cavity were remodeled, and inside the cavity, immature newly formed bone was observed in a smaller quantities in comparison with groups 2 and 3. A significant quantity of fibrous connective tissue filled the cavity. The membrane was totally resorbed, and an inflammatory infiltrate was seen (Figs 3e and 3f).

DISCUSSION

In spite of the high potential of bone tissue for regeneration, some types of bone defects cannot be repaired because of local mechanical instability, defect size, or the existence of competitor tissues in the region. Various materials and techniques have been utilized in the treatment of such defects.[11]

One type of treatment is based on guided tissue regeneration (GTR), which utilizes a biologic membrane to cover the defect so as to prevent the proliferation and migration of adjacent competitor tissues from invading the defect area where it can partially or totally block neoformation in the area.[12,13]

One type of membrane is made of resorbable lyophilized bovine bone, which obviates a second surgery for its removal, thus reducing the general

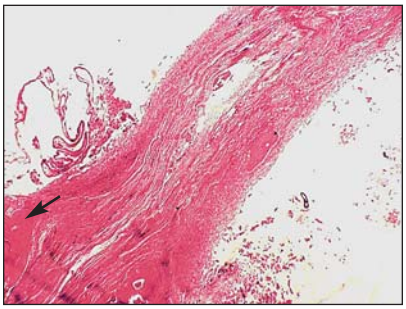


Fig 3a Group 1 at 60 days. Dense fibrous connective tissue filled the cavity. Some neoformation was observed at the borders of the cavity *(arrow)* (H&E; original magnification ×32).

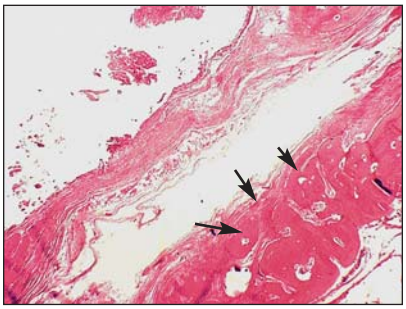


Fig 3b Group 2 at 60 days. Dense fibrous connective tissue filled the cavity. Bone islands *(arrow)* with a certain degree of immaturity were observed (H&E; original magnification ×32).

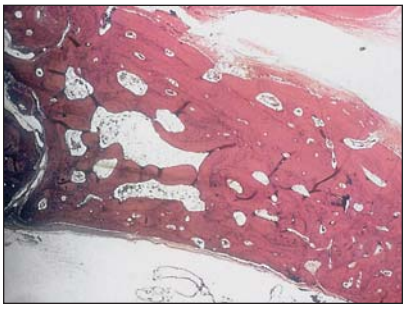


Fig 3c Group 3 at 60 days. Immature newly formed bone tissue filled the cavity (H&E; original magnification ×250).

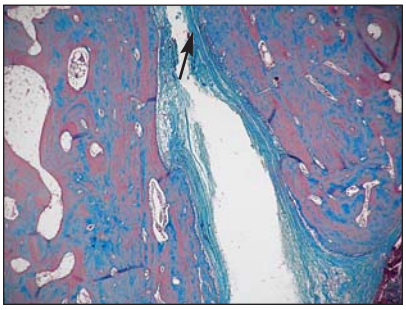


Fig 3d Group 3 at 60 days. Note the border of the cavity, where significant neoformation and remodeling were observed *(arrow)* (MT; original magnification ×32).

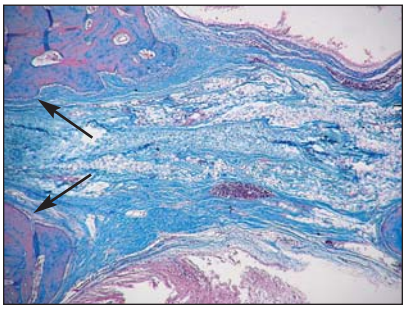


Fig 3e Group 4 at 60 days. Fibrous connective tissue filled the cavity. Note the remodeling at the border of the cavity *(arrow)* (MT; original magnification ×32).

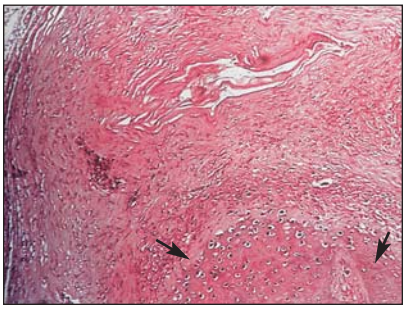


Fig 3f Group 4 at 60 days. Dense fibrous connective tissue filled the cavity, and newly formed bone was observed *(arrow)* (H&E; original magnification ×100).

morbidity of the treatment and providing better comfort to the patient. Many studies have been reported utilizing these membranes for various types of bone defects, with satisfactory results.[14–16]

In the present work, for animals in Group 2 at 60 days postsurgery, bone neoformation in the cavity was not satisfactory compared with the results obtained in group 3, but they differed significantly from the results in group 1 (the control group). In a study of male *albinus* rats, Mardas and coworkers[17] noted bone neoformation with more density at 120 days in a group treated with demineralized bone matrix than in control group capsules in the mandibular ramuses of rats. The difference found between groups 2 and 3 could indicate that the use of a membrane was favorable for the creation of a protected space for bone neoformation.[10]

At the end of 2 months, bone neoformation was seen throughout the cavity in group 3. In this case, the bovine bone graft may have acted as an osteoconductive material, as suggested by Stephan and colleagues[18] and Buser and associates,[4] providing backup to the bone formation, membrane stabilization, and structural support.[19] These results are also in accordance with those of Taga and coworkers,[20] who obtained 73.3% closure of the defect area by newly formed bone tissue when using a mixture of demineralized bovine bone matrix and ultrathick hydroxyapatite, recovered by lyophilized bovine bone cortical membrane. In this case, bovine bone matrix could be used not only as support for the membrane but also to facilitate and increase the speed of bone repair in the experimental defects while impairing the proliferation of other tissues inside the cavity.

Many authors[21–24] have demonstrated that the combination of bone graft with GTR has produced better results when compared to the use of graft material or a membrane alone. Possible explanations for the advantages observed with GTR include better space maintenance by the barrier to enable cell events and the facilitation of mineralized tissue formation because of the osteoconductive and/or osteoinductive properties that may be inherent in the implanted material.

Other studies have reported that there is no evidence of differences in bone neoformation between

defects treated with GTR with or without demineralized bone, verifying only an increase in the density of neoformed bone.[25–27] The reasons for these results are not completely understood. Surgical procedures, anatomic topography, and the dimensions and geometric conformation of defects can influence bone regeneration after implantation of the demineralized bone.[28]

In the defects recovered with membrane (group 4), the results observed were similar to those for the control group. These results can probably be related to local collapse of the membrane, since the particular membrane used in the present study was incapable of withstanding external pressure. As the coagulum that filled the lesion at the onset was resorbed early in the study, the membrane could have collapsed into the defect, reducing its space. These results have been previously observed by Aukhil and associates,[29] who concluded that the GTR method using only a reabsorbable membrane resulted in a limited quantity of bone formation. To prevent the collapse of the membrane into the defect and consequent obstruction of osteogenesis, GTR techniques can be used with an autogenous bone graft or bone substitutes.

Sculean and colleagues[30] showed that bovine xenograft has excellent osteoconductivity and can be integrated into bone tissue. However, in the current experiment, such treatment did not provide for the closure of the defect in any animal. Complete closure might have occurred if the experimental period had been longer, so that complete bone formation would have resulted.

The absorption of bovine bone and membrane occurred through the activities of mononuclear cells that invaded the membrane through its porosities with little inflammatory infiltrate associated with it, suggesting a low antigenic character.

Regarding the difference in the intensity of the inflammatory infiltrate observed around the membrane and implanted bone, it may be inferred that the use of a membrane works like a barrier against the migration of inflammatory cells from adjacent tissue and of exudates with many chemical mediators of inflammation.[20] Thus, it impairs the formation of intense inflammatory infiltrate and reduces the quantity of giant cells around the implanted material, in contrast with the specimens from group 2 at 30 days, where the inflammatory reaction was intensive, suggesting advanced resorption.

CONCLUSIONS

According to these results the use of a membrane covering the entire defect can act as a barrier against migration of the cells of adjacent connective tissue, creating a favorable microspace for angiogenesis and bone neoformation. However, not enough hard structure was demonstrated when the membrane was used without graft material. The combination of bovine bone graft and a membrane preserved the cavity space while avoiding membrane collapse, resulting in an increased osteogenic effect.

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