Reconstruction of the anterior wall of the frontal sinus using bovine bone matrix. Histological study

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Abstract
The purpose of this study was to evaluate the repair process in the reconstruction of the anterior wall of the frontal sinus of monkeys with bovine bone matrix. Four adult Cebus apella monkeys underwent an ostectomy of the anterior wall of the frontal sinus. The frontal sinus mucosa and the nasofrontal duct were not manipulated. Reconstruction occurred with implants of bovine bone matrix laminae measuring 2.0 x 2.5 cm and 0.4 mm thick, stabilized under pressure in the lateral wall of the frontal sinus. The monkeys were sacrificed over a period of 150 days and routine laboratory procedures were followed for hematoxylin-eosin staining and histologic evaluation of the specimens. Neofomed bone tissue was observed in contact with the frontal sinus mucosa and the bovine bone matrix. The frontal sinus mucosa remained whole without fibrous tissue or cystic formations. There was no occurrence of cellularization as well as revascularization of the bovine bone matrix, though it has permitted bone conduction on this surface. It was possible to conclude that the demineralized bovine bone matrix was biotolerable, being incorporated into the bone without the presence of inflammatory cells with characteristics of inertness and antigenicity and behaved as an osteoconductive material.

Key words
Reconstruction; Frontal Sinus; Bone Matrix

Introduction
The main objective of the treatment of frontal sinus fractures is to avoid intracranial infectious complications10, as well as to re-establish the morpho-functional characteristics of the region. Obliteration of the frontal sinus is used to treat these fractures with involvement of the nasofrontal duct and the posterior wall of the frontal sinus, anterior wall communication, chronic infections and other non-malignant conditions. 20

The incidence of frontal sinus fracture varies from 2% to...
15% of all the facial fractures. Fractures of the cranium are frequently associated with other facial fractures. The most adequate treatment for frontal sinus fractures is still controversial. However, the objectives of the treatment are well established: isolation of the intracranial content, prevention of infections and sequelae, restoration of the functional integrity of the structures involved, frontal contour and esthetic restoration.

Frontal sinus obliteration can be performed with autogenous fat, muscular flap, autogenous bone and spontaneous osteogenesis. The ideal method for obliteration has been discussed and has stimulated clinical and experimental studies about the comparison of these referential methods. However, there are no long term clinical and experimental follow-up reports comparing different types of treatment. Among the materials used for obliteration are homogeneous and heterogeneous bone, bone morphogenetic proteins, platelet rich plasma, hydroxyapatite, and others. These materials have brought great advances in the substitution and maintenance of bone tissue.

In reconstructive craniomaxillofacial surgery, autogenous bone continues to be the most widely used graft material due to its osteogenic, osteoinductive and osteoconductive biological properties, which greatly enhance the formation of new formed bone tissue. However, in this case, two surgical sites are required and the source of bone is limited. Therefore, several different categories of materials have been developed and are under test for bone-substitution, such as bioactive glass, calcium sulphate, ceramics (i.e., hydroxyapatite), and polymers.

Demineralized bone matrices are used in periodontal clinical procedures, reconstructions, pre-prosthetic surgeries, and surgery and traumatology. The bone morphogenetic proteins (BMP) existent in the demineralized bone matrices are responsible for cellular events that occur in the bone tissue, leading to its regeneration.

The process of obtaining bone matrix by means of demineralization cycles does not assure the conservation of protein content and does not make it possible for pathogenic microorganisms to survive. The advantages of bovine bone include the minimal risk of disease transmission when adequately processed, and low cost. These characteristics have favored the use of demineralized bovine cortical bone as an osteoconductive and osteoinductive biomaterial for the treatment of perennial bone defects, especially in dental surgeries.

Considering the biological properties of bovine bone matrix, the purpose of this study was to investigate the repair process in the reconstruction of the anterior wall of the frontal sinus monkeys with bovine bone matrix.

Material and Methods
Four adult male monkeys (Cebus apella) in good health from the Tufted Capuchin Monkey Procreation Center at the School of Dentistry of Araçatuba, São Paulo State University (UNESP, Brazil) were used in this study. The research protocol was approved by the institutional Animal Care and Research Use Committee. All guidelines regarding the care of animal research subjects were strictly followed.

The age was determined according to the Schultz criteria. After being food deprived for 10 hours, the animals received intramuscular injections of ketamine hydrochloride (Dopalen®; Agribrands do Brasil LTDA, Paulínea, SP, Brazil; 15 mg/kg body weight) and benzodiazepine (Diazepan®; Sigma Pharma, São Bernardo do Campo, SP, Brazil; 0.1 mL/kg body weight) to promote sedation and muscular relaxation, respectively, and were anesthetized with an intraperitoneal injection of sodium thiopental (Thionembutal®; Abbott Laboratórios do Brasil LTDA, São Paulo, SP, Brazil; 30 mg/kg body weight). During the pre- and post-operative periods, the animals were fed with a diet based on bananas, rations, corn, fruits and water.

Bovine bone was obtained from high quality Cold Storage Depots with federal inspection by the Ministry of Agriculture – S.I.F. (Federal Inspection Service, São Paulo, SP, Brazil). Immediately after the animal was sacrificed, a portion of the mandibular ramus was removed and stored in a cold sodium chloride solution at 0.9% and at temperature ranging between 0 and 4°C for a period of 60 minutes. Next, for decalcification, the bone tissue was immersed in a decalcified hydrochloric acid solution (HCL) at 0.4 mol with temperature ranging between 6 and 8°C, and remained for a period of 10 days with daily change of solution. After this period, the tissue was cut and so, rectangular laminae measuring approximately 2.0 x 2.5 cm and 0.4 mm thick were obtained, stored in glycerin at 98% and kept in a refrigerator at a temperature ranging around 8 degrees centigrade for a period of 20 days.

Ninety minutes before the implant surgical procedure, the bone matrices were removed from the 98% glycerin conservation medium, irrigated and stored in 0.9% cold saline irrigation until implantation.

Trichotomy on the frontal area and anti-sepsis with 10% PVP-I (polyvinyl pyrrolidone iodine (Riodeine, Rioquímica Ltda. São José do Rio Preto, SP, Brasil) topical and degerming solutions were performed. To prevent excessive bleeding, 1 mL of prilocaine with felipressin was infiltrated along the region to be incised. The incision used was the gull wing on
Maillefer S/A, Ballaigues, Switzerland) under external and intense irrigation with physiologic saline solution, the anterior wall of the frontal sinus was ostectomized. The mucosa was removed with curettes.

After, the anterior wall of the frontal sinus was reconstructed with implants of bovine bone matrix laminae, fixed under pressure on the lateral walls of the frontal sinus thus maintaining and rebuilding the anatomic contour of the frontal region (Figure 2).

Suture of the internal plane was performed with a number 4.0 polyglactin 910 wire and of the superficial plane with number 4.0 nylon wires. All surgical procedures were performed by the same operator. During the course of the experiment, the animals received 0.4 mL of sodium cephalothin (Antibióticos do Brasil LTDA, Cosmópolis, SP, Brazil), 0.2 mL of sodium diclofenac (Laboratório Delta, Carazinho, RS, Brazil) and 0.2 mL of dipyrone (NeoQuimica, Anápolis, GO, Brazil) intramuscularly. A penicillin V antibiotic (Pen Ve Oral 250 mg; Eurofarma, São Paulo, SP, Brazil; 3 drops/day) was administered for 7 days.

One hundred and fifty days post-operative the animals were anesthetized with sodium pentobarbital (30 mg/kg, i.p.) and perfused transcardially. The anatomic pieces containing the frontal sinus were removed and fixed in neutral buffered 10% formalin, decalcified in a formic acid/sodium citrate solution and embedded in paraffin, in order to allow cuts in the anterior-posterior direction of the frontal sinus. The blocks were sectioned semi-serially and 6-µm-thick longitudinal sections were obtained, stained with hematoxylin and eosin and examined under light microscopy by a skilled observer blinded to the treatment groups.

The histological analysis was performed using an optical microscope (Axiolab – Zeiss, Germany) coupled to a computerized image capture system, to analyze the incidence and intensity of the inflammatory processes present in the neoformed tissues (bone and conjunctive), quality of the conjunctive tissue and of the neoformed bone tissue next to the bovine bone matrix, the occurrence of pathology in the frontal sinus mucosa, and the union of the bovine bone matrix with frontal bone tissue.

**Results**

Clinically, the animals presented no post-operative complications. Histological analysis revealed that the frontal sinus mucosa remained intact without fibrous tissue or cystic formation and without bone union between the experimental cavity wall and bovine bone matrix, which remain separated by the conjunctive tissue without the occurrence of inflammatory cells of a chronic or severe nature (Figure 3).
interaction with the conjunctive fibers, similar to a neoformed periosteum for its protection (Figure 7).

Discussion
In reconstructions of facial bone defects there is a constant concern not only with replacing of lost or injured tissue, but also with re-establishing of morphophysiological functions of the injured area. Therefore, autogenous grafts or alloplastic materials, capable of supporting muscular efforts, are implanted in the injured bone area and they lead to bone formation.27

Deminerlized bovine bone matrix is a great source of grafting material and its almost neutral pH helps the bone repair process during the first week. Moreover, the chemical composition, structure and porosity of deminerlized bone are almost identical to properties of human bone, thus
preventing microfractures between the grafted material and the surgical site. 14

The results obtained in this study with the implantation of bovine bone matrix allow affirmation that this material did not interfere in the local process of bone neoformation. Besides, this material worked as a scaffold allowing bone tissue growth and adhesion of dermal conjunctive tissue to its bed. Furthermore, no local inflammatory process occurred. Queiroz et al. 19 considered that the use of demineralized bovine bone matrix worked as a barrier to inflammatory cell migration, and it formed a structure for bone neoformation. The results observed in our study were similar to that, since the presence of bone matrix prevents the migration of inflammatory cells and also favored bone neoformation.

When the behavior of the bovine bone matrix used in this study was compared with that of other studies and bioinert materials, such as hydroxyapatite, 13 it was verified that it did not allow chronic or severe inflammatory processes to occur, and almost always behaved as an inert compound. 13 This behavior also was similar to the histological condition presented in this study. In studies related to obliteration of the infected frontal sinus, 7 hydroxyapatite also behaved in a safe and efficient manner with minimum morbidity and excellent post-operative contour.

A retrospective study that assessed the results of bioactive glass and of hydroxyapatite in head and neck surgeries concluded that bioactive glass does not provide microbial growth and presents osteoconductive properties. 1 These materials are stable, reliable, present good functional and esthetic results and are well tolerated by the body.

Stal et al. 22 concluded that the behavior of bovine bone matrix and Lactosorb membrane are similar. Both behave as osteoconductive materials. Lactosorb membrane is constituted of 82% polyactic acid and 18% polyglycolic acid and due to its constituents it is totally reabsorbed after 12 weeks, it is biotolerable and it is not associated with inflammatory reactions. This membrane can act as a physical barrier allowing guided bone regeneration.

A study conducted by Hochuli-Vieira et al. 18 in monkeys, assessed the results of spontaneous of the frontal sinus in comparison with obliteration using human heterogeneous bone. In group I, the frontal sinus was obliterated with heterogeneous bone and in group II the frontal sinus was treated to obtain spontaneous osteogenesis. When compared with the specimens in group I, the group II presented better organization of Harvers’ system and a quantity of osteocytes very similar to the quantity of pre-existent bone, characterizing greater bone maturity. The authors concluded that both techniques are efficient for obliterating the frontal sinus.

In a similar study conducted by Altman et al., 2 the behavior and histological condition of the bovine bone matrix were similar to behavior of this study. The authors affirm that the bovine bone matrix allows complete obliteration of the frontal sinus. In this study, the bovine bone matrix also behaved as an osteoconductive material and maintained the volume of the frontal sinus.

When compared with other synthetic materials, bovine bone matrix presents innumerable advantages. 2 For instance, this material does not require heat to be handled, as opposed to methacrylate, which has the disadvantage of hardening due to the exothermic reaction causing cell destruction and foreign body reaction. Moreover, the bone matrix was easy to handle, particularly when compared with hydroxyapatite, which is a material that is difficult to handle in the absence of complete surgical hemostasis.

Decortication of the internal wall of the frontal sinus is reported in the literature with the objective of removing all the sinus mucosa and bone septs inside the sinus, 26 stimulating the formation of a blood clot, facilitating bone repair and providing an adequate receptor bed for grafts and implants. 4 In the proposed study, decortication was not performed in the internal walls of the frontal sinus and no cystic formations were found inside it. This can be explained because the surgical trauma was located only in the anterior wall of the frontal sinus and the other anatomic areas were preserved.

In this study, after 150 post-operative days, it was possible to consider that bovine bone matrix graft was well tolerated; it allowed bone growth and presented conjunctive tissue without severe or chronic inflammatory reaction around it, showing osteoconductive potential. The use of bovine bone matrix for reconstruction of the anterior wall of the frontal sinus can eliminate the need of a donor bed, and consequently, the patient’s morbidity.

With regard to the morphophysiological functions of the frontal sinus, complete anatomic-functional re-establishment of the injured structures was observed.

Conclusion

Based on the results of the present study, it may be concluded that: The demineralized bovine bone matrix was biotolerable, behaved as an osteoconductive material and was incorporated by the bone without the presence of inflammatory cells characteristics of ineriticity and antigenicity.
References