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# Potential of autogenous or fresh-frozen allogeneic bone block grafts for bone remodelling: a histological, histometrical, and immunohistochemical analysis in rabbits

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**Abstract**

Our aim was to compare the wound healing of autogenous bone grafts with that of fresh-frozen allogeneic block bone in rabbits. We used 25 animals. One was killed before the experiment to provide the allogeneic bone, and the remainder were killed at four time points (n = 6 in each group). On histometrical analysis there was a significant difference between the two groups only at 45 days and between 15 and 45 days in the intergroup analysis. However, there was significantly more revascularisation ($p < 0.05$), resorption ($p < 0.05$), and bony replacement ($p < 0.05$) in the autogenous group in the immunohistochemical analysis. In later periods, the autogenous bone was replaced by newly-formed bone in all samples, whereas it was always possible to find regions of devitalised bone in the fresh-frozen allogeneic bone grafts. Autogenous grafts were completely replaced whereas, in the fresh- frozen allogeneic grafts, we found acellular tissue that had been incorporated into the receptor bed interface during the later evaluation times.


*Keywords:* Bone Bank; Implants; Bone Graft; Bone Remodelling; Tissue Scaffold

## Introduction

The advantages of using tissue banks for reconstruction of alveolar bone include reductions in anaesthetic and operating times, as well as in postoperative morbidity. No intervention is required in a donor area, and the surgeon is able to reconstruct large areas using only local anaesthesia.[1,2]

We know of no standardisation or consensus of information about the techniques used to treat bony tissue until it is frozen.[3,4] Other issues are the effect of freezing on bone repair and the behaviour of tissue over time; these cause surgeons to wonder about its applicability in implant dentistry.[5–8]

This study is justified by the need for further details about the dynamics of freezing allogeneic bone, because most previous reports have studied only the use of frozen allogeneic bone.[9,10]

### *Aim of the study*

Our aim was to evaluate the process of healing of autogenous bone grafts and fresh-frozen allogeneic block bone repairs in rabbit mandibles using histological, histometrical, and immunohistochemical analyses. The findings will later be incorporated into rehabilitation with dental implants.

Our null hypothesis was that the allogeneic block bone would be incorporated into the receptor bed in a similar way to that of the autografted block bone.

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## Material and methods

We studied 25 white, adult male (New Zealand) albino rabbits that were six months old and weighed 3 to 4 kg from the central vivarium of Araçatuba Dental School – UNESP. This research was approved by the Ethics Committee for Animal Experimentation (83/05).

One of the animals was killed at the beginning of the study to obtain allogeneic bone; its right metaphyseal tibia was removed, packed, and sent to the tissue bank in accordance with instructions. At the tissue bank the bone went through a pre-established decontamination process, after which it was packed and frozen at −70 °C.

The remaining animals were fasted preoperatively for eight hours before being weighed and sedated with ketamine 50 mg/kg and xylazine hydrochloride 5 mg/kg intramuscularly. The regions of the mandibular angle and right tibia were then shaved and disinfected bilaterally. Each animal was simultaneously used in two groups - the left mandible was the autogenous graft group, and the right mandible the frozen allogeneic bone group.

### *Autogenous graft group*

After local anaesthesia with 2% mepivacaine hydrochloride and 1:100 000 epinephrine, the internal superior face of the right tibia was exposed by incision and separation until bony tissue was exposed close to the tibiofemoral joint. A cortical block of bone tissue was removed with a trephine burr 8 mm in diameter mounted in a straight hand piece and powered by an electric motor. This was kept immersed in 0.9% physiological saline until it was fixed in the receptor bed.

A skin incision was then made in the region of the left mandibular angle. Bone was exposed and separated, and the receptor bed prepared with slight decortication using a spherical diamond burr N° 6. The autogenous bone block was then fixed in the receptor bed with a 1.5 mm × 8 mm titanium screw. The soft tissue was closed in layers with a 5/0 nylon suture.

### *Frozen allogeneic bone group*

An 8 mm trephine burr was used to remove a disc of the frozen tibia, which was kept in sterile physiological saline (0.9%) until it was fixed to the receptor bed in the right mandibular angle, using the same technique as described in the autogenous group.

During the postoperative period the animals received 0.2 ml of veterinary pentabiotic (Fort Dodge, Campinas, SP, Brazil) intramuscularly, one dose during the immediate postoperative period and the other five days later, together with three doses of sodium dipyrone (Novalgina – São Paulo, Brazil) 1 mg/kg/day. Animals were killed in groups of six 15, 45, 120, and 180 days postoperatively.

### *Laboratory processing*

The mandibles were dissected, and a bone sample including the entire graft was sent to the laboratory where the bone was fixed and decalcified, the screws were removed, and samples were embedded in paraffin. The serial cuts were obtained by placing the graft in its longitudinal direction in relation to the screw to see the graft in its receptor bed.

Slides were stained with haematoxylin and eosin for qualitative histological and histometrical evaluation by light microscopy.

*Histometrical analysis*: photomicrographs were imported to the ImageJ analysis program (Processing Software and Image Analysis, Ontario, ON, Canada) and the "freehands" option was selected to measure the amount of new bone ($\mu m^2$) that had formed at the graft/bed interface for all groups and periods.

*Immunohistochemical analysis:* for immunohistochemical processing, osteocalcin protein (OC, goat antioc, Santa Cruz Biotechnology®, USA), tartrate-resistant acid phosphatase lysosomal enzyme (TRAP, goat antitrap, Santa Cruz Biotechnology®), vascular endothelial growth factor (VEGF, goat antivegf, Santa Cruz Biotechnology®), and platelet endothelial cell adhesion molecule (PECAM-1, goat antipecam– Santa Cruz Biotechnology®) were used as primary antibodies. The biotinylated secondary antibody was donkey antigoat (Jackson Immunoresearch Laboratories, West Grove, USA). The reaction signal was amplified with the streptavidin-biotin system (DAKO Kit, DAKO Co, USA), and the reaction exposed using diaminobenzidine (DAB, DAKO Co) as the chromogen. Immunohistochemical controls were used to evaluate the effectiveness and specificity of the reactions.

The pattern of expression of each protein was analysed for each group after periods of 15, 45, and 120 days. The first period was considered as the initial analysis, the second as the intermediate analysis, and the third as the late analysis, in an attempt to involve the entire extent of the blocks. The expression of proteins was evaluated using the following scores: absent (score 0), positive (score 1), superpositive (score 2), and hyperpositive (score 3).[11]

### *Statistical analysis*

A normality test (Shapiro-Wilk) was applied to the data obtained from the histometrical analysis, which showed a normal distribution (Sigmaplot 12.3 software; Systat Software Inc, San Jose, CA, USA). We then used a two-way analysis of variance (ANOVA) and Tukey post-hoc test to compare the grafts used (autogenous and allogeneic) and the periods analysed (15, 45, 120, and 180 days) ($p < 0.05$).

For immunohistochemical analysis, we applied a normality test (Shapiro-Wilk), which showed normally distributed results for all proteins. A two-way ANOVA and Tukey post-hoc test were used for data that showed significant differences