# EVALUATION OF TENSILE STRENGTH RESISTANCE OF DIFFERENT BIOMATERIALS IN CALVARIAL RATS


## ABSTRACT

**AIM:** The aim of this study was to determine the effect of different grafting materials on bone tensile strength after 6-month wound healing. **MATERIAL AND METHODS:** Non-critical size defects (3-mm diameter) were created in calvarium of 30 three-month-old Wistar rats. Animals were divided into 3 groups (n=10) treated with different grafting materials: GenOx® (Group 1A); GenMix® (Group 1B); no treatment (blood clot, Group 3). Six months after the surgery, rats were sacrificed; bone specimens were harvested and submitted to tensile strength test using a universal testing machine. The bone fracture surface morphology was evaluated using scanning electron microscopy (SEM) at 200X magnification. Data were compared by One-Way ANOVA at 5% significance. **RESULTS:** No significant difference was found among the groups although tensile strength decreased in the following order: Group 3 (9.56±3.74MPa), Group 1B (8.58±3.60MPa), Group 1A (7.70±2.41MPa). All tested materials showed similar effects on bone tensile strength, no matter the source (xenogenic or blood clot). **CONCLUSION:** After six months of bone healing, the type of grafting material is irrelevant to the final outcome and bone tensile strength.



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## KEYWORDS

Implant. Bone. Biocompatible materials.



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| **Received** 03 Oct 2014 **Received in revised form** 07 Oct 2014 **Accepted** 11 Oct 2014

## INTRODUCTION

When there is tooth loss due to caries, periodontal disease, trauma, trepanation, endodontic lesions among other reasons, a physiological process of bone resorption in height or thickness is triggered in the area, which is called alveolar ridge resorption[1-3]. This bone remodeling affects the functioning of any prosthesis supported on the residual alveolar ridge and impairs prosthetic rehabilitation of patients either with complete dentures, removable partial dentures or implant-supported dentures (affixed or removable).

Due to this difficulty, the literature has demonstrated that the utilization of biomaterials to fill up the sockets after tooth extraction may contribute to the maintenance of the alveolar ridge in both height and thickness[1-3]; the advances in medical and dental technology have led to an increase in the development of biomaterials within the context of bone resorption caused by tooth extraction.

Several biomaterials are commercially available, which indications vary according to their mechanism of action and origin. These biomaterials are submitted to several laboratory evaluations, animal and human studies before being introduced into the market. The calvarial of rats is an experimental model to evaluate the repair of grafted areas; this model comprises creation of defects in calvarias and filling them with different biomaterials for evaluation of their biocompatibility, repair time, cell type, quality and quantity of newly formed bone tissue among others.

Defects in calvarias of rats may be classified into two types: critical (greater than 6-mm diameter) or non-critical (smaller than 6-mm diameter) [4-12]. In case of non-critical defects in calvarias of rats, the aim is to evaluate the quality and quantity of tissue formed by the biomaterial, either by its osteoinductive, osteoconductive or osteogenic property. Because this experimental model allows certainty of closure of the created defect, only the cell type, bone quantity and quality are evaluated.

There are other important factors in the evaluation of these biomaterials, such as their mechanical properties (resistance, modulus of elasticity, tenacity, plasticity, etc.). Many published reports address the evaluation of bone tissue in the field of orthopedics, especially in long bones (e.g., human or bovine femurs or tibias); these studies usually employ tensile or compressive tests on long bones, nanoindentation, ultrasonic measurements and microtensile testing [13-14].

Despite its importance, in dentistry (and especially in Implantology) newly formed tissues are not often evaluated for their resistance; after tooth loss, endosseous implants may be predictably placed in the area

with utilization of biomaterials to maintain the bone tissue of the socket. One study[15] has performed histological evaluation of this area both in animals and humans. However, the mechanical properties of grafted areas have not been evaluated in either in animals or humans.

Considering the lack of investigation of bone resistance, the present study aimed to combine two methodologies widely used in the literature (evaluation of bone repair in calvarias of rats and investigation of resistance by microtensile testing) to evaluate the resistance of interface newly bone and bovine graft. The hypothesis of this study is that not there are statistically significant differences between bovine grafts in calvarias of rats.

## MATERIAL AND METHODS

This study was approved by the Committee of animals of Bauru Dental School – USP with process number 023/2007.

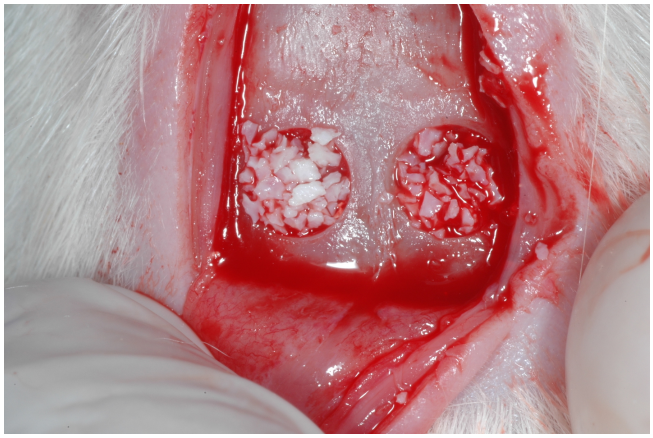
The study was conducted on 30 adult male Wistar rats (Rattus norvegicus), weighing 250 to 300 g, supplied by the central animal laboratory of Bauru Dental School. The animals received normal diet "ad libitum" throughout the study period, including rat chow and water. At birth, the animals were randomly grouped into five boxes (four boxes contained five animals and one box had only four animals) lined with wood shavings, which were regularly replaced.

After 3 months (adult age), the animals were submitted by surgery procedures and insert the biomaterial.

The anesthesia was used 0.4 mL of solution (0.2 mL of ketamine hydrochloride and 0.2 mL of xilazina hydrochloride) intraperitoneally. After anesthesia, in head region, realized the trichotomy and antisepsis with povidone iodine.

In the two parietals were created the non-critical defect with trefina (3 mm diameters). The defects were filled with biomaterials according groups (Figure 1). Before, the biomaterials were irrigated with saline into dappen pot. After fill with biomaterial, the periosteum was sutured. Group A: Anorganic bovine bone microgranulate (GenOx®, Baumer S.A., Mogi Mirim, SP, Brasil, Registro Ministério da Saúde: 103.455.00001); Group B: Mix bovine bone microgranulate (GenMix®, Baumer S.A., Mogi Mirim, SP, Brasil, Registro Ministério da Saúde: 103.455.00080); Group C: Blood Clot .

Figure 2. The defects were filled with biomaterials according groups.



After 6 months, animals were killed according to the protocol of the central animal laboratory of Bauru Dental School – USP. All animals in the study groups were killed by anesthetic overdose and muscle relaxant applied directly to the animal's heart.

The amount of drug used in this procedure was established in a pilot study on 4 animals, which revealed that 0.8 mL of solution (0.4 mL of ketamine hydrochloride and 0.4 mL of xilazina hydrochloride) could be used when injected directly into the animal's heart.

The entire calvarias of the animals were removed with a bone saw from the Anatomy Department of Bauru Dental School.

After the collection of calvarias, the specimens were dissected and sectioned with stainless steel discs mounted on a low-speed handpiece under constant cooling for achievement of specimens measuring 10 - 12 mm in length, 3 - 4 mm in width and 1-mm-thickn, always observing the parietal region. After this procedure, specimens were stored in deionized water at 37°C until utilization in the test.

Microtensile testing was performed in a universal testing machine, which allows the specimens to affix to the machine. Specimens were fixated with aid of cyanoacrylate adhesive[16] and submitted to microtensile testing at a crosshead speed of 1 mm/min. After testing, the machine revealed the maximum tensile value generated until occurrence of fracture/rupture of the specimen; the value was projected into kilograms (Kg) and divided by the cross-sectional area ($cm^2$), which was obtained by measurement of width x thickness of the central area of the specimen and multiplied by a universal constant (0.0981) to provide a maximum tensile value in MegaPascal (MPa).

After the microtensile test, the average and standard deviations were calculated for each group by descriptive analysis followed by the Student's t test for comparison of microtensile values between the mineralized and demineralized groups and between the calvarial and femoral bone.

Data were statistically analyzed by one-way analysis of variance to investigate the possible significant differences between groups.

## RESULTS

Table 1 shows the means and standard deviations of groups: Group A (Gen-Ox) was 7,709±2,416 MPa; Group B (Gen-Mix) was 8,587±3,602; Group C (Clot) was 9,563±3,740.

One-way analysis of variance showed no significant differences when comparing the different bone grafts.

## DISCUSSION

The results of this study confirm the hypotheses that not there are statistically

significant differences between the different bovine grafts in calvarias in rats.

This study employed the experimental model of calvarias of rats for mechanical testing based on the literature. The proven scientific validity of this model with regard to the biological aspect, including a wide range of biomaterials, such as BMPs[11, 17-18], polymers[9-10], xenografts[19], membranes[20], allografts[20] and PRP[21] further validated its use.

The microtensile test may be employed for evaluation of mechanical properties of substrates such as enamel[22], dentin[23], dental materials[24] and mechanical properties of bone[25, 26]. It should be mentioned that the microtensile test is a method, rather than a purpose; it may be adapted to the needs of different study hypotheses, knowing that these adaptations do not impair the fundamental mechanical principles of the test. The microtensile test allows several possibilities and has advantages (e.g., working with animals with reduced bone structure without the need for a large number of animals); this test in animals is especially important because evaluation in humans would not be feasible due to ethical concerns.

Table1. Means and standard derivation (MPa).

| **Groups** | **Means** | **SD** |
|---|---|---|
| GenOx | 7,709 | 2,416 |
| GenMix | 8,587 | 3,602 |
| Clot | 9,563 | 3,74 |

After the microtensile test observed for Clot Group (9,56 ± 3,74MPa) > GenMix (8,58 ± 3,60MPa) > GenOx (7,7±2,41MPa).

Ratier et al. (2001)[27] observed that using treated in calcium phosphate cement, it was possible to introduce at least 6% of active ingredient whilst still allowing the reaction to proceed to completion i.e. the formation of hydroxyapatite with good mechanical properties. And in this study, the tensile strength was 2,0 MPa, less than our result in all groups. The result can be better due in tensile area has collagen fibers, increasing the resistance in interface, because the fibers behave to elastic scaffold.

The collagen fibers could be useful to improve the resistance, but in large quantify the fibers can disturb in repair process, because the fibers have a quickly proliferation and preventing that bone tissue develop in this region [20, 28-34].

The best method to prevent that there is no cell proliferation of fibroblasts and collagen fibers give rise would be to use a membrane that isolate the grafted region of the fabric covering, which is responsible for cell migration of fibroblasts (Guided Tissue Regeneration - GTR)[31, 33-34]. This fact was

confirmed by analysis at the time of death of the animals himself, as was visible to the rest of the dressing interlaced fibers.

When comparing the results among the groups, it is observed that the clot (control group) was very similar to the other results, it reinforces what is histologically in several works in alveolar repair using only clot [31,35] demonstrating that the alveolar repair is carried naturally in the presence of clots and the presence of a biomaterial for alveolar only enables the maintenance of cellular longer time due to delay absorption of debris biomaterials grafted.

## CONCLUSION

According to the results of microtensile test obtained and analyzed in this study, it can be concluded that: (1) confirmed the null hypothesis proposed in this work which groups had no statistically significant difference between them (P <0.5). The control group/clot (9.56 ± 3.74 MPa) showed better results between groups, followed by GenMix (8.58 ± 3.60 MPa) and GenOx (7.70 ± 2m41MPa); (2) it was observed that the tensile strength was directly influenced by the large amount of collagen fibers in the region, requiring a comparative study with the use of mechanical barriers (membranes - guided bone regeneration). However, in assessing the groups presented, it is noted that the material was irrelevant to the final result of the tensile strength, showing a good quality of materials used, both when comparing products as their source (xenogeneic or clot).

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