

Tensile Resistance of Mineralized and Demineralized Rat Bones in Different Regions (Calvarial and Femur)

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The aim of this study was to evaluate the tensile resistance of mineralized and demineralized bones. Twelve mice were used. Specimens were collected and divided into groups 1 and 2, mineralized and demineralized calvarial bone, and groups 3 and 4, mineralized and demineralized femoral bone. There was not a statistically significant difference (analysis of variance) between the regions; however, when comparing the demineralized and mineralized groups, a statistically significant difference (Student test) for the mineralized group was noticed.

Key Words: bone, biomaterial, tensile strength

INTRODUCTION

When there is tooth loss due to caries, periodontal disease, trauma, trepanation, or endodontal 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).

Because of this difficulty, the literature has demonstrated that the use of biomaterials to fill up the sockets after tooth extraction may contribute to the maintenance of the alveolar ridge in both height and thickness¹⁻³; 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, and indications vary according to their mechanism of action and origin. These biomaterials have been submitted to several laboratory evaluations (animal and human studies) before being introduced into the market. The calvaria of rats is an experimental model used 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, and quality and quantity of newly formed bone tissue among others.

Defects in calvarias of rats may be classified into 2 types: critical (greater than 6-mm diameter) or noncritical (smaller than 6-mm diameter).⁴⁻¹² Creation of a critical-size defect is indicated when the biomaterial might have the ability of induction and/or conduction to bone formation in cases without natural closure of this area (ie, the biomaterial would be able to induce and/or conduct bone formation beyond the physiological repair capacity of the organism). In this type of study, animals are killed at different periods to allow histological and radiographic follow-up of bone repair, thus allowing quantification of the period of repair of the defect.

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In the case of noncritical 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 (eg, 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 their 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 study¹⁵ 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 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 and modulus of elasticity of mineralized and demineralized bone in calvarias and femurs of rats by microtensile testing and to compare the difference between different testing bone regions. The hypothesis of this study is that there are statistically significant differences between the mineralized and demineralized bone in calvarias and femurs of rats.

MATERIALS AND METHODS

This study was approved by the Committee of Animals of Bauru Dental School–USP with process number 22/2004.

The study was conducted on 24 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 5 boxes (4 boxes contained 5 animals and 1 box with only 4 animals) lined with wood shavings, which were regularly replaced; after 5 months (adult age), the animals were killed by anesthetic overdose.

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 and femurs of the animals were removed with a bone saw from the Anatomy Department of Bauru Dental School.

The specimens were divided according to their type and localization: group 1 (n = 12), mineralized calvarial bone; group 2 (n = 12), demineralized calvarial bone; group 3 (n = 12), mineralized femoral bone; and group 4 (n = 12), demineralized femoral bone.

Mineralized group

After the collection of calvarias and femurs (calvaria and femur figure), 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 to 12 mm in length, 3 to 4 mm in width, and 1 mm thickness (Figure 1), always observing the parietal region. After this procedure, specimens were stored in deionized water at 37°C until use 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 the aid of cyanoacrylate adhesive¹⁶ 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 (cm²), which was obtained by measurement of width × thickness of the central area of the specimen and multiplied by a universal

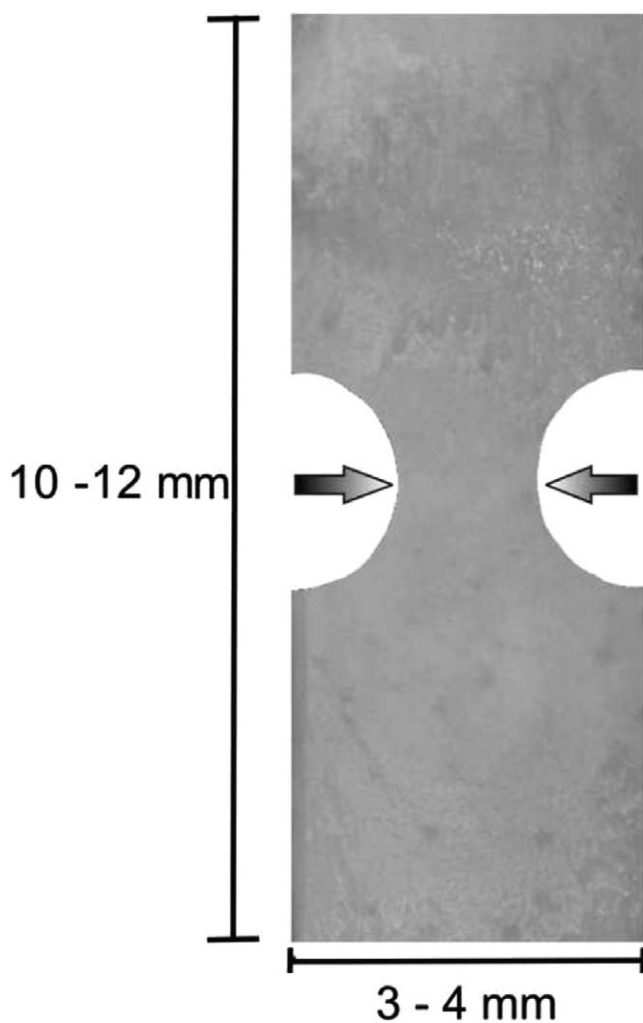


FIGURE 1. Scheme of specimen's microtensile group.

constant (0.0981) to provide a maximum tensile value in mega Pascal (MPa).

Demineralized group

The specimens were sectioned as described above; however, these specimens were submitted to demineralization. Before demineralization, the edges of specimens were protected from the demineralizing agent with nail varnish because the specimens were affixed to the testing machine by their edges with the aid of clamps. After protection of edges, the specimens were immersed in 0.5 M ethylenediamine tetraacetic acid for an undetermined period. Complete demineralization was checked by radiographic examination, which allowed observation of presence or absence of mineral remnants.

The microtensile tests were conducted on the same testing machine as described above; however,

TABLE		
Average values of microtensile resistance (MPa \pm SD)*		
	Calvarial Bone	Femoral Bone
Mineralized	11.445 \pm 3.316 ^a	12.668 \pm 2.937 ^a
Demineralized	1.931 \pm 0.417 ^b	1.868 \pm 0.496 ^b

*Means identified by different symbols are significantly different ($P < .001$).

the specimens were kept immersed during both microtensile testing due to the need of hydration of specimens during the test. In the present study, specimens were kept immersed in distilled water.

After the microtensile test, the average and standard deviations were calculated for each group by descriptive analysis followed by the Student *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 2-way analysis of variance to investigate the possible significant differences between groups.

RESULTS

The Table shows the means and standard deviations of the groups: group 1 (mineralized calvarial bone) was 11.445 \pm 3.316 MPa, group 2 (demineralized calvarial bone) was 1.931 \pm 0.417, group 3 (mineralized femoral bone) was 12.668 \pm 2.937, and group 4 (demineralized femoral bone) was 1.868 \pm 0.496.

Two-way analysis of variance showed no significant differences when comparing the different regions in the same type of bone (femoral mineralized \times calvarial mineralized and femoral demineralized \times calvarial demineralized; Figure 2). By comparing the mineralized and demineralized bones, a statistically significant difference ($P < .001$) was noticed, being greater in the mineralized bone in both areas (femur and calvaria; Table).

DISCUSSION

The results of this study confirm the hypotheses that there are statistically significant differences between the mineralized and demineralized bone in calvarias and femurs of rats.

This study employed the experimental model of calvarias of rats for mechanical testing based on the

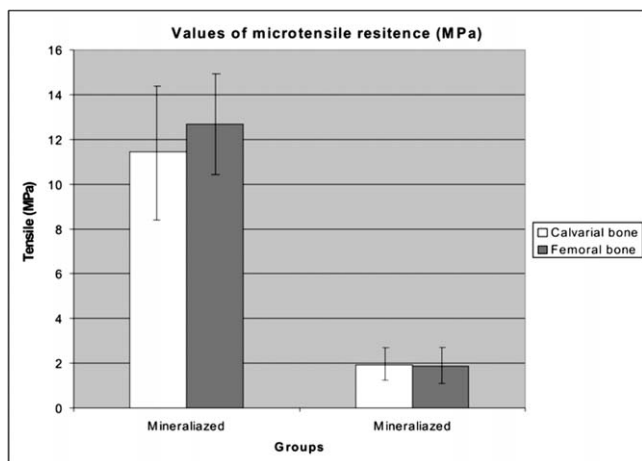


FIGURE 2. Graphic representation of mean values of tensile bond strength in MPa for each subgroup. Bars inside the columns represent the standard deviation.

literature. The proven scientific validity of this model with regard to the biological aspect, including a wide range of biomaterials, such as bone morphogenic proteins,^{11,17,18} polymers,^{9,10} xenografts,¹⁹ membranes,²⁰ allografts,²⁰ and platelet rich plasma²¹ further validated its use. The femur bone has been used in many works in the field of orthopedics to conduct many mechanical resistance tests. However, in this study, the calvaria and femur were analyzed by microtensile testing, which allowed achievement of numerical values of microtensile strength.

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 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 because of ethical concerns.

After removal of the calvarias and femurs, the specimens were divided into mineralized and demineralized groups and submitted to microtensile testing. The microtensile strength values observed were 12.9814 ± 3.49217 MPa for the mineralized group and 1.8547 ± 0.3682 MPa for the demineralized group. A study analyzing the cortical

bone and vertebrae of humans reported that strength values depended on the direction and type of load applied.²⁶

Analysis of the microtensile strength results observed for the mineralized group (12.9814 ± 3.49217 MPa) revealed similar values as those observed for the cortical bone of femur submitted to longitudinal testing. Despite the use of small-sized specimens ($10 \times 3 \times 1$ mm), the present results are similar to previous reports in the literature.²⁷

With regard to the demineralized group, it is known that it is composed only of collagen fibers, thus leading to analysis of values of cancellous bone, which contains a large amount of collagen and few mineral structures. Despite these similarities, their structures are different and impair further comparisons. This analysis is plausible because no studies have yet been conducted to compare results of mineralized bone submitted to tensile testing.

Comparison of outcomes in the calvarial bone (11.445 ± 3.316 MPa, mineralized; 1.931 ± 0.417 MPa, demineralized) and femoral bone (12.668 ± 2.937 MPa, mineralized; 1.868 ± 0.496 MPa, demineralized) with those in the literature revealed a difference among values due to several factors, including the type of experimental model employed; most studies in this field (medical/orthopedic) are conducted on corpses^{13,28} or bovine bone because these studies usually aim to investigate the resistance to fatigue of long bones and repaired areas after fracture. The present study aimed to evaluate and establish a pattern of value of resistance on the most representative experimental model in dental studies, especially in the field of grafting for implantology.

Other factors that might influence the differences in outcomes²⁶ might be the different locations of areas investigated (femur, tibial head, and tibial body), type of bone (cancellous or cortical), structural type (osteons or lamellae), and type of test applied for achievement of resistance values.

This study did not aim to achieve similar values for comparison with the literature but rather maximum and minimum microtensile strength to allow comparison with these patterns in future studies evaluating the repair of calvarias or femurs of rats with biomaterials, and it also demonstrated the power of including mechanical investigations in addition to biological tests. In the short term, these

results might be helpful in implantology; however, further studies are required to compare the values to the present study, which are presented as comparative values for these situations.

CONCLUSION

According to the results of microtensile test obtained and analyzed in this study, the following can be concluded:

- There were no significant differences when comparing the different regions in the same type of bone (femoral mineralized × calvarial mineralized and femoral demineralized × calvarial demineralized)
- There were statically significant differences ($P < .001$) between mineralized and demineralized bones, being greater in the mineralized bone in both areas (femur and calvaria).

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