EVALUATION OF THE RESISTANCE AND MODULUS OF ELASTICITY OF BONE MINERALIZED AND DEMINERALIZED FOR THE TEST OF MICROTENSILE

ABSTRACT

Regeneration is made through bone grafts that be one scaffold for the tecidual repairing, increasing bone tissue in the resultant defects, filling the alveolous after extraction to preserve the height and thickness of the alveolar rigde. These procedures are carried through the use of bone grafts. With the development of the osseointegrated implants, it was seen necessity to keep a good quantities and quality bone. The aim is evaluate the resistance the traction and modulus of elasticity of the mineralized and demineralized bone of calvarial of rats to determine initial standards (maximum and minimum) for comparisons with possible biomaterials of fulfilling of osteoinduction and osteoconduction. The 24 animals divided in 2 groups had been used. Group modulus of elasticity being that we will use the two parietals of the animal, having made possible 24 specimens, subdivided in mineralized (n=12) and demineralized (n=12). Group microtensile, being that 12 hemi-calvarial had been used for the demineralized sub-group and the others 12 hemi-calvarial for the mineralized sub-group. After the death of the animals, the specimens will be collected and treated in accordance with the sub-group (mineralized and demineralized). The tests had been carried through in a machine of universal test. The resistance averages the tensile of the mineralized one was mineralized 129,814 ± 34.921 MPa and the demineralized one was 18,547 ± 3,682 MPa. For the modulus of elasticity, the values of 1377,792 ± 208.331 MPa for the mineralized group and 49,669 ± 11,204 MPa for the demineralized group, when comparing the resistance and modulus of elasticity the tensile between the demineralized and mineralized sub-group observe a estatistical significant difference for the mineralized group ($p<0,001$). Concluded that the microtensile test can be used to evaluate the mechanical properties in this biological experimental model.

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KEYWORDS

Dental Implants. Bone. Biocompatible Materials.

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| Received 01 Nov 2012 **Received in revised form** 08 Jan 2013 **Accepted** 01 Mar 2013

INTRODUCTION

When there is tooth loss due to caries, periodontal disease, trauma, trepanation, endodontic lesions and others, 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 these patients, either with complete dentures, removable partial dentures or implant-supported dentures (fixed or removable) [3-5].

Due to this difficulty, the literature has demonstrated that utilization of biomaterials to fill the sockets after tooth extraction may contribute to maintain the alveolar ridge in both height and thickness [2]; the advances in medical and dental technology led to an increase in the development of biomaterials within the context of bone resorption caused by tooth extraction.

Several biomaterials are commercially available, whose 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 in the market.

The calvaria of rat is an experimental model to evaluate the repair of grafted areas; this model comprises creation of defects in calvarias and filling with different biomaterials, for evaluation of their biocompatibility, repair time, cell type, quality and quantity of newly formed bone tissue, and others.

Defects in calvarias of rats may be classified into 2 types: critical (greater than 6-mm diameter) or non-critical (smaller than 6-mm diameter) [6-24]. Creation of a critical size defect is indicated when the biomaterial should have the ability of induction and/or conduction to bone formation in cases without natural closure of this area, i.e. 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. 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, since this experimental model allows certainty of closure of the created defect, and thus 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)[25]. 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; the studies usually employ tensile or compressive tests on long bones, nanoindentation, ultrasonic measurements and microtensile testing.

In Dentistry, newly formed tissues are not often evaluated as to their resistance, despite its importance, especially in Implantology; after tooth loss, endosseous implants may be predictably placed in the area with utilization of biomaterials to maintain the bone tissue of the socket. Many studies performed histological evaluation of this area, both in animal and human studies. The mechanical properties of grafted areas are not evaluated, either in animal or human studies.

Considering the lack of investigations on 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 of rats by microtensile testing, thus allowing the establishment of maximum and minimum patterns of resistance and modulus of elasticity for future comparison of areas repaired with different biomaterials in calvarias of rats.

MATERIAL AND METHODS

The study was conducted on 24 adult male Wistar rats (Rattus norvegicus), weighing 250 to 300g, 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. Since birth, the animals were randomly grouped in 5 boxes (4 boxes contained 5 animals and 1 box had 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 into the animal's heart. The amount of drug to be used in this procedure was established in a pilot study on 4 animals, which revealed that 0.8mL of solution (0.4mL of Dopalen® and 0.4mL of Anasedan®) might be used when injected directly into the animal's heart.

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

1. MINERALIZED GROUP

Specimen preparation for the microtensile test and microtensile test:

After 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 of length, 3 - 4 mm of width and 1 mm of thickness, always observing the parietal region. After this procedure, specimens were stored in deionized water at 37ºC until utilization in the test.

Microtensile testing was performed in a universal testing machine Virtodyne model V1000 (Liveco Inc., Burlington, VT), which allows fixation of specimens in the machine. Specimens were fixated with aid of cyanoacrylate adhesive [26] and submitted to microtensile testing at a crosshead speed of 1mm/min. After testing, the machine reveals the maximum tensile value generated until occurrence of fracture/rupture of the specimen; the value is projected into kilograms (Kg), divided by the cross-sectional area (cm^2), which is obtained by measurement of width x thickness of the central area of the specimen (Figure 4c, blue arrow) and multiplied by a universal constant (0.0981) to provide a maximum tensile value in MegaPascal (MPa).

Specimen preparation for the modulus of elasticity test and modulus of elasticity test:

After collection of calvarias, the specimens were dissected and trimmed with stainless steel discs mounted on a low-speed handpiece under constant cooling, for achievement of specimens measuring 10 - 12 mm of length, 3 - 4 mm of width and 1 mm of thickness. For the purposes of standardization, a section was performed on the central region of the specimen to reduce the area to be tested, reducing the load of the testing machine. After this procedure, the specimens were kept in deionized water at 37ºC until testing.

The modulus of elasticity test was performed on the same universal testing machine Virtodyne model V1000 (Liveco Inc., Burlington, VT), which allows fixation of specimens on the machine. Specimens were fixated with aid of cyanoacrylate adhesive [26] and submitted to tensile testing at a crosshead speed of 1.00 mm/min. The same test was applied for both microtensile and modulus of elasticity test, because the Vitrodyne machine calculates the displacement of the specimen until occurrence of fracture, as well as the maximum tensile value generated, thus allowing calculation of the modulus of elasticity.

2. DEMINERALIZED GROUP

Specimen preparation for the microtensile and modulus of elasticity tests:

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 (Colorama, L'Oréal), because the specimens were fixated to the testing machine by their edges with aid of clamps. After

protection of edges, the specimens were immersed in 0.5 M EDTA for an undetermined period. Complete demineralization was checked by radiographic examination, which allowed observation of presence or not of mineral remnants.

Microtensile and modulus of elasticity tests:

The microtensile and modulus of elasticity tests were conducted on the same testing machine as described above; however, the specimens were kept immersed during both microtensile and modulus of elasticity testing due to the need of hydration of specimens during the test. In the present study, specimens were kept immersed in distilled water.

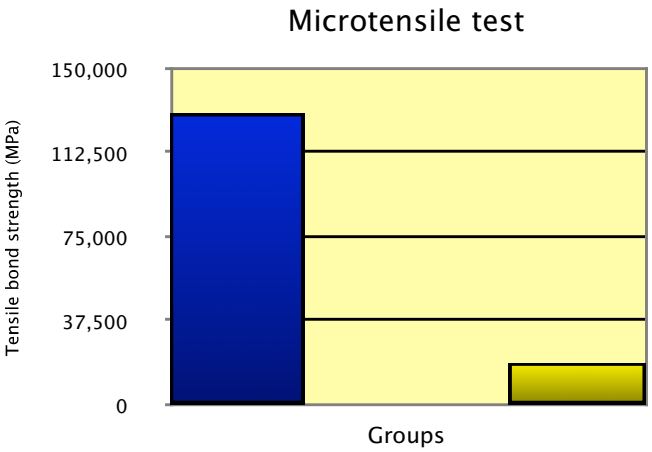
After the microtensile and modulus of elasticity tests, the means and standard deviations were calculated for each group, by descriptive analysis followed by the Student's t test for comparison of microtensile and modulus of elasticity values between the mineralized and demineralized groups. Statistical analysis was performed on the software SigmaStat 3.1 (Systat Software Inc., Richmond, California, USA).

RESULTS

1. MICROTENSILE TEST

The following mean values were found: 129.814 ± 34.9217MPa for the mineralized group and 18.547 ± 3.682MPa for the demineralized group. Data were submitted to the Student's t test on the statistical software SigmaStat 3.1, for comparison between subgroups (mineralized and demineralized), with statistically significant difference between them. Figure 1 allows better observation of outcomes, with graphic presentation of means and standard deviations of groups.

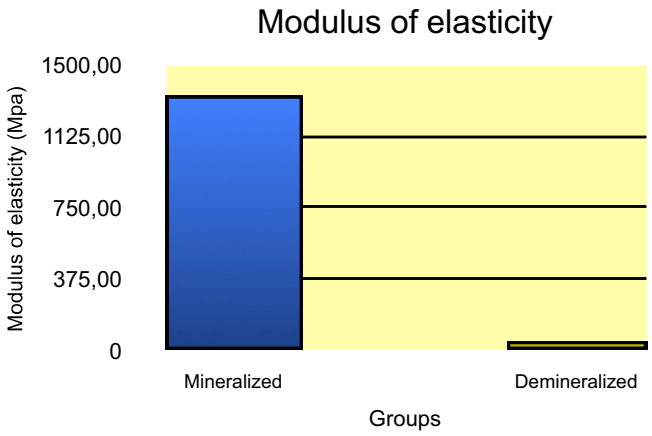
Figure 1 – Graphic representation of mean values of tensile bond strength, in MPa, for each subgroup. Bars inside the columns represent the standard deviation.



2. MODULUS OF ELASTICITY

The following mean values were found: 1377.792± 208.3316 MPa for the mineralized group and 49.66985 ± 11.2046 MPa for the demineralized group. Data were submitted to the Student's t test on the statistical software SigmaStat 3.1, for comparison between subgroups (mineralized and demineralized), with statistically significant difference between them. Figure 2 allows better observation of outcomes, with graphic presentation of means and standard deviations of groups.

Figure 2 – Graphic representation of mean values of modulus of elasticity, in MPa, for each group. Bars inside the columns represent the standard deviation.



DISCUSSION

This study employed the experimental model of calvarias of rats based on the literature review for mechanical testing, considering the proven scientific validity of this model with regard to the biological aspect, including a wide range of biomaterials, such as BMPs [11, 14, 19, 22, 27], polymers [10, 12, 14, 21, 22, 28, 29], xenografts [13, 21, 30, 31], membranes [9, 15, 30-32], allografts [6, 32], and PRP [17, 20, 33]. The calvaria was analyzed by microtensile testing, which allows achievement of numerical values of microtensile strength and modulus of elasticity.

The microtensile test may be employed for evaluation of mechanical properties of substrates such as enamel [34], dentin [35, 36], dental materials [37] and mechanical properties of bone [25, 38]. It should be mentioned that the microtensile test is a method, rather than a purpose; it may be adapted to the needs of investigation of different study hypotheses, provided 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 need of a large number of animals; this is especially important, since evaluation in humans would not be feasible due to ethical concerns.

After removal of calvarias, the specimens were divided into mineralized and demineralized subgroups and submitted to microtensile testing.

The microtensile strength values observed were 129.814 ± 34.9217 MPa for the mineralized group and 18.547 ± 3.682 MPa for the demineralized group. A study analyzing the cortical bone of femurs and vertebrae of humans reported the strength values found according to the direction and type of load applied.

Analysis of the microtensile strength results observed for the mineralized group (129.814 ± 34.9217MPa) revealed similar values as those observed for the cortical bone of femur submitted to longitudinal testing. Despite the utilization of small sized specimens (10x3x1 mm), the present results were similar to previous reports in the literature [39-42].

With regard to the demineralized group, it is known that it is only composed of collagen fibers, thus leading to analysis of values on 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, since no studies are available in the literature comparing results of mineralized bone submitted to tensile testing.

The tests most often employed to evaluate the modulus of elasticity of bone include microtensile [38], nanoindentation [43-47], compressive [48-50], acoustic microscopy [44], finite element/three-dimensional images [51-54] and tensile testing [50]. There is a large amount of tests and great variability of modulus of elasticity values, which may be explained by other variables present in studies, which might positively or negatively interfere with the outcomes.

The modulus of elasticity value found by microtensile testing in the present study was 1377.792 ± 208.3316 MPa for the mineralized group and 49.66985 ± 11.2046 MPa for the demineralized group.

Comparison of outcomes (1377.792 ± 208.3316 MPa – mineralized and 49.66985 ± 11.2046 MPa – demineralized) with those in the literature revealed a difference among values due to several factors, including the type of experimental model employed, since it is known that most studies in this field (medical/orthopedic) are conducted on corpses [43-51, 53, 54] or bovine bone [53], since 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 modulus of elasticity and 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 outcomes [43-47, 50, 51, 53-57] might be the different locations of areas investigated (femur, tibial head, tibial body), type of bone (cancellous or cortical), structural type (osteons or lamellae) and type of test applied for achievement of modulus of elasticity values.

The modulus of elasticity values found in the literature are usually determined by analysis of specimens obtained from the cancellous or cortical regions of bones. The present study was conducted on cortical bone from calvarias of rats and only comprised variations in the treatment of specimens after their achievement, different from the literature comparing cortical and cancellous regions of human or bovine long bones, impairing comparison with the present results.

CONCLUSION

This study did not aim to achieve similar values for comparison of the literature, but rather maximum and minimum microtensile strength and modulus of elasticity values, to allow comparison with these

patterns in future studies evaluating the repair in calvarias of rats with biomaterials, also 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 with the present study, which are presented as comparative values for these situations.

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