INFLUENCE OF LYOPHILIZATION, STERILIZATION AND REHYDRATION ON THE MECHANICAL PROPERTIES OF BOVINE CORTICAL BONE

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Abstract. *Metalic alloys are the most frequently used biomaterials for bone reconstruction or fixation. However, metalic implants presents a great number of inconvenients such as biologic rejection, bone loss at implant bondary proximity and displacements at bone-implant interface due stiffness incompatibility. In this context, bovine cortical bone appears as an alternative biomaterial for implant manufacturing because allows uniform stress and strain distribution at bone-implant system, and can be substituted by new bone. These caracteristics make possible host bone to recover its original structure once the regeneration process has finished. In this study, the mechanical properties of bovine cortical bone were determined through destructive mechanical tension and bending tests, and the fractures were analysed in stereoscope. The results were used to evaluate the influence that treatments employed on biomaterial exerts upon its mechanial properties. This kind of technical information is needed for the design and analysis of orthodontic and orthopedic bone implants.*

Keywords*: biomaterials, bovine cortical bone, mechanical properties*

1. Introduction

The biomaterials currently employed in implants for orthodontic and orthopedics applications are metal alloys. However, the use of these implants has associated complications like foreign body reactions, bone loss in implant neighbourhood (Figure 1) and implant loosening at bone-implant interface due material stiffness difference. For this reason, bovine cortical bone could be viewed as an alternative biomaterial for implant fabrication: it acts as structural element during healing and after it is later reabsorbed and substituted by new bone. The purpose of this study is to evaluate the mechanical properties of bovine cortical bone through mechanical tests. As the processing and sterilization techniques used to prepare these biomaterials may significantly affect their properties (Boyce et al., 1999), the effects of lyophilization, sterilization by irradiation gamma and rehydration upon the mechanical properties of bovine cortical bone were evaluated.

2. Materials and methods

Gênius Group (Biomaterials Baumer Company) supplied bovine cortical bone from femur and tibia of Nelore cows. The pieces had been previously submitted to chemical processes in order to clean and remove all fat and impurities in the bone. All the samples were manufactured according to machine parameters defined by Mora (2000). The ratio L / w < 20 was respected according to the specifications proposed by Spatz et al. (1996) , where L and w are the sample length and width, respectively.

Machined samples had been separate in three groups: G1 control group (submitted only to biochemical process before manufacture), G2 group (submitted to biochemical process before manufacturing and to biochemical, lyophilization and sterilization processes after it), and G3 group (submitted to the same treatment of group G2 plus rehydration).

The lyophilization (freeze-drying) process involved the removal of water from the frozen tissue, after which the tissues were vacuum-packed and stored at room temperature. The lyophilized samples, were sterilized by gamma irradiation of 20 kGy. Gamma irradiation is a common process of terminal sterilization. The rehydration was carried out through immersion in physiological serum three hours before the mechanical tests.

The tensile test samples were instrumented with two strain gauges bonded in longitudinal (load direction) and transversal direction. The measurement of these strain quantities during the tension tests allows the Poisson ratio determination.

The mechanical tests (four-point bending and tensile) were carried out with an EMIC DL 3000 machine, at a deformation speed of 1 mm/min. This velocity is in accordance with the limits proposed by Evans: of 0,889 - 1,143

mm/min (Evans, 1973). All samples were tested at room temperature, between 20 - 25ºC. In bending test, a pre-load of 5 N was applied to the sample to avoid rigid body movements.

The samples for tensile test have a useful dimension of 34 mm of length, 4 mm of width and 4 mm of thickness. The samples for bending test have a useful dimension of 75 mm of length, 7 mm of width and 4 mm of thickness.

The values of tensile strength, longitudinal and transversal strain, Young modulus and Poisson ratio were determined by the tensile test, while the value of bending strength come from the bending test. All values were submitted to statistics analysis to verify for significant differences of the collected data. The software Analyze i-t for Microsoft Excel was employed.

After the mechanical tests, sections where the fracture occurs were analyzed with an Olympus stereoscopic, which allowed identification of the fracture characteristics, and their comparison among the different samples.

(a) (b)

Figure 1. a) osseointegration obtained after implantation of cortical bone screw on a rabbit femur b) bone loss around the metalic screw. After Bento, 2003.

3. Results

The mean value of the mechanical properties obtained by the mechanical tests can be seen in Figure 2, while deviation between groups are shown in Table 1.

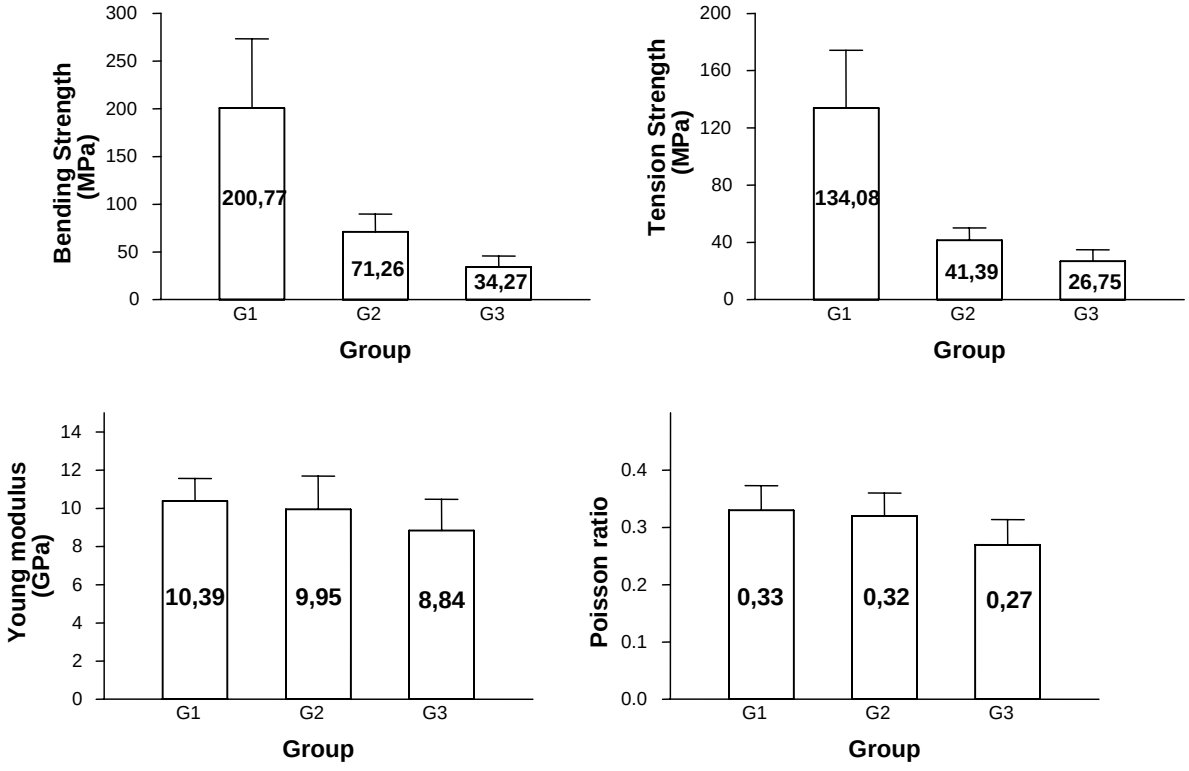


Figure 2. Mean values and standard deviation of mechanical properties obtained for three groups.

Table 1. Percentile difference between mean values obtained for the mechanical properties.

	Poisson ratio	Young modulus	Tension strength	Bending strength
G1 - G2 =	3,03%	4,22%	69,13%	64,51%
G1 - G3 =	18,18%	14,91%	80,05%	82,93%
G2 - G3 =	15,62%	11,15%	35,37%	51,91%

(1) : measured at 25°C

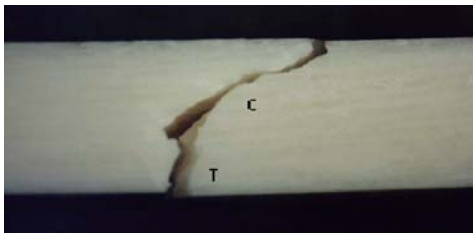
Comparing control group G1 (biochemical process) with group G2 (biochemical process before and after machining / liophilization and gamma irradiation after machining), almost negligible reductions of 3.03% for the Poisson ratio and 4.22% for the Young modulus are noted. These results suggests that it does not occur significant influences upon elastic properties of cortical bone when this material is submitted to a new biochemical process followed by liophilization and gamma irradiation. On the other hand, the occurrence of statistically significant reductions of 69.13% on material's strength limit, and of 64.51% on the bending strength limit, suggest that the process sequence can jeopardize the material's mechanical strength.

Comparing the control group G1 (biochemical process) with the group G3 (biochemical process before and after usinagem / liophilization, gamma irradiation and rehidration after usinagem), it can be noted significant reductions of 18.18% for the Poisson ratio, 14.91% for the Young modulus, 80.05% for the tension strength limit and 82.93% for the bending strength limit.

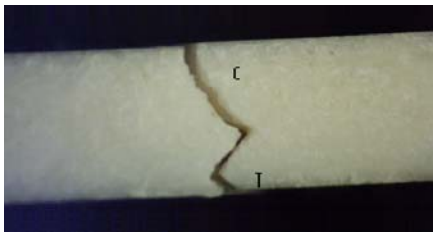
Comparing the control group G2 with group G3, reductions of 15.62% for the Poisson ratio, 11.15% for the Young modulus, 35.37% for the tension strength limit and 51.91% for the bending strength limit are verified. These results suggest that both, mechanical properties and strength, are jeopardized when biochemical processed bovine cortical bone is submitted to a new biochemical process followed by liophilization and gamma irradiation. This effect can be observed independently of the hidratation condition, but it appears more intensively when bovine cortical bone is rehidrated after biochemical processing, liophilization and gamma irradiation.

The characteristics of the fracture resulting from the mechanical tests are showed in Figure 3. The specimens of the three groups submitted to axial test present similar patterns (Fig. 3a); and the fracture is perpendicular to the load direction. The specimens submitted to bending loads, present a fracture approximately plane at the tractioned region of the transversal section and oblique at the compressed part of it (groups G1 and G2, Figs. 3b and 3c). In these cases, the formation of little particles of material was accomplished during breaking. For G3 group, the specimens tested through bending loading present a distinct fracture mode (Fig. 3d): breaking without loosening of material's little particles. This occurrence demonstrates that bovine cortical bone became more ductile when submitted to treatments of G3 group, although its mechanical strength is jeopardized.

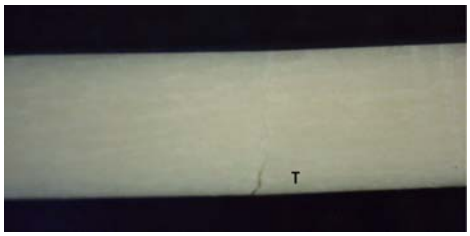
(a)



(b)



(c)



(d)

Figure 3. a) Bovine cortical bone of G1 group after tension (25X of enlargement). b) Bovine cortical bone of G1 group after bending (15X of enlargement). c) Bovine cortical bone of G2 group after bending (15X of enlargement). d) Bovine cortical bone of G3 group after bending (15X of enlargement).

4. Conclusions

According to Mora (2000), bone's mineral phase have greater influence on bone's elastic modulus. So, the small variation of elastic properties (Young modulus and Poisson ratio) observed by the comparison between groups G1 and G2 groups suggests that bovine cortical bone mineral phase is not influenced by the combination of biochemical process, liophilization and gamma irradiation. On the other hand, the reduction on mechanical strength observed in the specimens of the G2 group related to those of the group G1 suggests that the biochemical process, liophilization and gamma irradiation (separated or in combination) could lead to a great degradation on bone's colagenous matrix without affecting the mineral phase. Cowin (2001) points out that, when gamma irradiation travel 2mm or minus to cross over the specimen, the potential needed to degrade the tissue isn't produced because the Compton electrons get out of the tissue before maximum ionization potential activation. As the specimens used in this work has a length, width and thickness greater that 2mm, likely the gamma irradiation ionization carrying out alterations on the bovine cortical bone's molecular structure. In fact, it has been pointed out that gamma irradiation weaken the cortical bone, fundamentally with respect to torsion and bending strength (Hamer et al.,1996; Currey et al., 1997, Godette et al., 1996).

The statistically significant reduction observed on elastic properties and mechanical strength of G3 specimens With respect to G1 and G2 groups suggests that combination between biochemical process, liophilization, gamma irradiation and rehidratation is the cause of alterations on bone's mineral phase and colagenous matrix. This allows to suppose that the rehidration is the treatment responsible by mineral phase alteration, as long as the comparation between G2 and G1 groups demonstrates that biochemical process, liophilization and gamma irradiation likely do not prompt any influence upon this phase. It must be noted, however, that colagenous matrix may suffer influence from rehidration if this treatment is combined with biochemical process, liophilization and gamma irradiation.

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