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BIOMECHANICAL EFFECTS ON LONG-TERM FROZEN HUMAN COSTAL CARTILAGE

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ABSTRACT

Currently, the progresses on treatment of musculoskeletal diseases with the evolving of artificial implants and the success of tissue transplantation between genetically different individuals have conducted to an increase in radiosterilization. Regarding to tissue transplantation, it is essential to have sterile tissue and many tissue banks use radiosterilization as an effective method to sterilize these tissues. However, high doses of ionizing radiation and the preservation method may induce structural modifications in the tissues, as degradation of structural scaffold, decreasing its mechanical properties. Particularly, cartilage have been preserved in high concentrations of glycerol or deep-frozen at -70 ºC for storage after radiosterilization. Therefore, it is important to study the modifications induced in cartilage by preservation methods and by radiosterilization to determine the appropriated parameters for high quality of human allografts.
Costal cartilages were obtained from cadaveric donors and were frozen at -20 ºC for 2 years long in order to compare with previous studies for fresh, deep-frozen and glycerolized cartilages. The mechanical tests were carried out in a universal testing machine until sample failure. According our results, there is no significant statistical difference between stress at break of fresh, long-term – 20 ºC frozen cartilages and deep-frozen cartilage. This early result suggests, regarding to tensile property, that long-term – 20 ºC frozen cartilages corresponds to glycerolized costal cartilages irradiated with 25 kGy or deep-frozen cartilages irradiated with 25 and 50 kGy. Thus, this long-term frozen cartilages may be used for tissue banks, but more studies about effects of ionizing radiation are necessary.

1. INTRODUCTION

One of the most important concerning about use of ionizing radiation as a sterilization method is its effects on grafts, mainly when high doses are used. Despite of its effects over the tissues are relatively well known, the association of radiosterilization and long-term storage of grafts may promote further modification on tissue structure, decreasing the safety on its use for transplantation. Indeed, several studies have been done concerning cartilage preservation methods [1,2] and the main aim of these studies is maintenance of cell viability [3,4,5]. On other hand, for tissue banks, the need for a method that preserve cell viability is not a concerning, once radiosterilization promote cell death in whole tissue [6]. Thus, tissue

banks need a range of different preservation methods that assure high quality grafts with respect to biomechanical properties, for example.

Human costal cartilage has been used for thoracic reconstruction of chest wall in children affect by Pectus Excavatum [7] and Poland's syndrome [8]. Also, cartilage grafts have been used in bronchial repair [9] and in reconstructive surgeries of nose, external ear and penis [10,11]. Collagen type II is the main component of hyaline cartilage and its organization into the tissue depends of the depth. Cartilage is divided in four distinct layers (fig. 01) and its composition and properties vary according these layers. In the superficial layer, collagen fibrils are parallel arranged to the surface, and water content reach almost 85% of total weight. In transitional layer the collagen fibrils are more randomly arranged and proteoglycan content is greater than into superficial layer. A perpendicular orientation of collagen fibrils is find into radial layer and this layer is responsible for attach the cartilage to subchondral bone. Calcified layer is the deepest layer of cartilage and promote structural and mechanical integration [12].

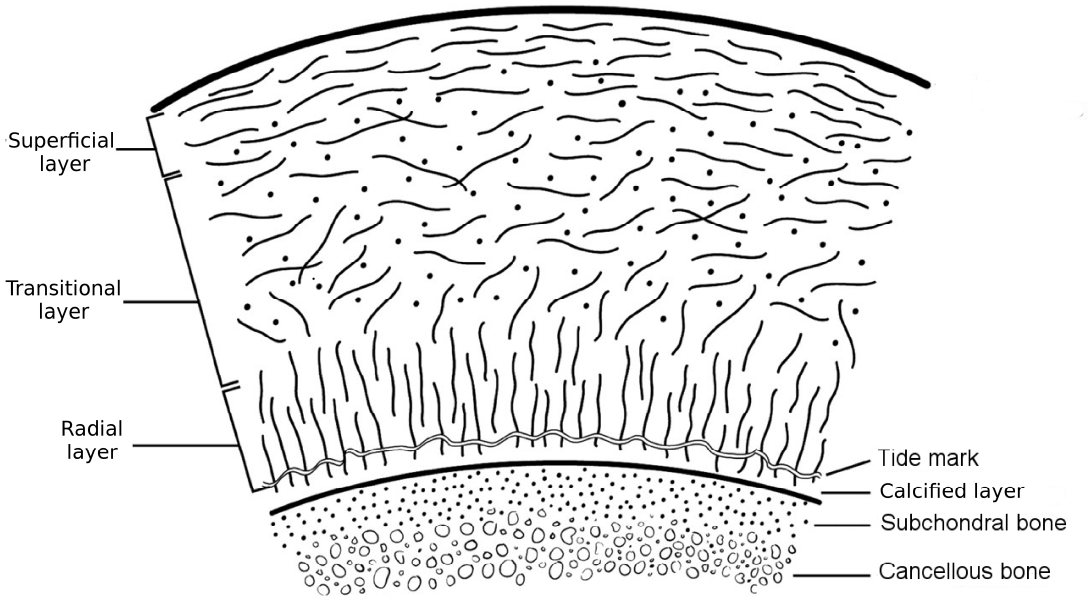

Figure 01. Collagen network organization in cartilage. (Adapted from: Alford and Cole [13])

According Herson and Mathor [14], preservation in high concentrations of glycerol (>98%) and deep-frozen at -70 ºC are the most common forms of tissue preservation in tissue banks. However, some studies have used cartilages frozen at -20 ºC [7] and no effects over biomechanical properties were observed. However, at the best of our knowledge, no work concerning long-term preservation at -20 ºC of cartilage grafts was published. Thus, it's very important to tissue banks know the biomechanical consequences of long-term preservation at -20 °C before use ionizing radiation to sterilize cartilage grafts.

In this work, we present our results of biomechanical tests for long-term preservation at -20 ºC cartilages, providing new options for tissue banks to long-term storage cartilage grafts.

2. MATERIALS AND METHODS

Human costal cartilages were obtained from eight cadaveric donors, both sexes, with age ranging from 18 to 45 years old. After remove whole adjacent tissue, each cartilage was divided in 5 fragments which were conditioned in appropriated plastic bags used for tissue banks. Fresh cartilages were kept in saline solution (0.9% NaCl) for 6 hours before mechanical test. The other 4 fragments of each donor were preserved in three different ways: frozen at -20 ºC for 2 years long and 2 were preserved according current protocol [14] - deep-frozen at -70 ºC for three months and preserved in high concentrations of glycerol for three months. Moreover, one fragment was preserved in glycerol with saline solution (1:1) for 14 days and, after this period, the fragments were kept in high concentration glycerol.

To avoid variation on the size of the samples owing to defrosting and rehydration, deep-frozen and frozen samples were thawed at room temperature and glycerolized samples were rehydrated in saline solution (0.9% NaCl) before the cut of the specimens. The specimens were obtained from superficial layer of samples, once in this region the collagen fibrils are arranged parallel to the cartilage surface and it is responsible for tension property of the this tissue [15]. Samples were trimmed in dumbbell format according fig. 02. Biomechanical tests of tension were carried out in a universal testing machine (Instron ®, CA, USA) set to move at 5 mm/min until sample failure with a load module of 1 kN. The tests were programed previously in the BlueHill® (Instron, USA) software and stress at break (MPa) was recorded automatically in a computer.

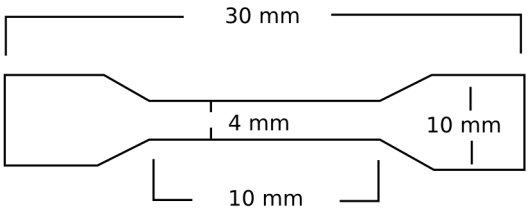

Figure 02. Specimen dimensions for mechanical tests obtained from superficial layer of cartilage.

Results are expressed as mean ± uncertainty and are compared with results obtained for fresh cartilages and for frozen at -20 ºC costal cartilages obtained for Feng et al. [7]. One-way ANOVA was used to look for significant differences among experimental groups.

3. RESULTS

Results of stress at break of fresh, deep-frozen, frozen and glycerolized cartilages are showed in figure 03, comparing them with the results obtained by Feng et al. [7].

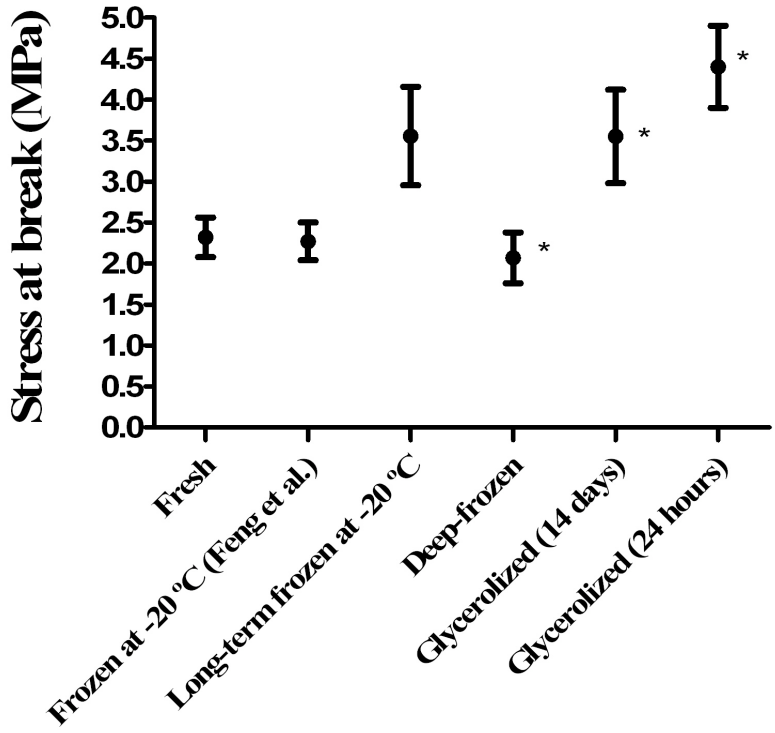

Figure 03. Stress at break of different methods of preservation. The value for frozen cartilages was obtained from Feng et al. [7]. The symbol "*" was used indicate significant statistical differences between groups ($p<0.05$).

4. DISCUSSION

According our results (fig. 03), long-term frozen cartilages have a stress at break equals to 3.55 ± 0.6 MPa while deep-frozen, glycerolized cartilages for 14 days and glycerolized cartilages for 24 hours presented 2.07 ± 0.31, 3.55 ± 0.57 and 4.4 ± 0.5 MPa, respectively. There is no significant statistical difference between long-term frozen cartilages and fresh, deep-frozen or glycerolized cartilages, but there is a significant difference between deep-frozen and glycerolized cartilages (24 hours and 14 days). Comparing this result with our fresh cartilages and for that found by Feng et al. [7] (fig. 03), we can note an increase in stress at break of about 70% for long-term frozen cartilages. Moreover, log-term frozen and glycerolization increase the stress at break when compared with fresh cartilages.

When we compare the results obtained in this work with previous work that use ionizing radiation at different doses (data not published), we can note that long-term frozen cartilages corresponds to glycerolized costal cartilages irradiated with 25 kGy or deep-frozen cartilages irradiated with 25 and 50 kGy, which demonstrate that preservation has more influence in biomechanical properties than ionizing radiation by itself. Also, the modifications caused in tissue structure in both frozen at -20 ºC storage for long time periods and in glycerolized conduced to an increase in stress at break.

Salai et al. [16] demonstrated that long-term deep-frozen bones do not present mechanical differences for fresh bones and no culture was contaminated over long-term storage. Our work suggests that long-term preservation at -20 °C does not cause decreasing in mechanical property of tension in cartilages.

5. CONCLUSION

Long-term frozen cartilages can be used as a safety preservation method once keeps the biomechanical properties of cartilage grafts but trends to increase the tension property as occurs with glycerolized cartilages.

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