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THERMOGRAVIMETRY OF IRRADIATED HUMAN COSTAL CARTILAGE

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ABSTRACT

Costal cartilage has been sterilized with gamma radiation using ^{60}Co sources at two different doses, 25 kGy and 50 kGy, for storage in tissue banks. Samples of costal cartilage were deep-freezing as method of preservation. Thermogravimetry (Shimadzu TGA-50) was used to verify the water release of costal cartilage before and after irradiation. The TG tests were carried out at heating rate of 10°C/min from room temperature to 600°C under a flow rate of 50 mL/min of compressed air. Samples of costal cartilage were divided in 2 parts. One part of them was kept as reference material; the other part was irradiated. This procedure assures better homogeneity of the sample and reproducibility of the experimental results. The obtained data have shown that the TG curves have the same pattern, independently of the sample. Non-irradiated samples showed great variability of thermogravimetric curves among different donors and for the same donor. Further experimental work is being carried out on human cartilage preserved in glycerol in high concentration (> 98%) to compare with those deep-freezing.

1. INTRODUCTION

In the last years the use of costal cartilage allografts have grown, specially on reconstruction of nose and penis[1,2], on the repair of bronchius[3], on ocular surgeries[4] and for reconstruction of chest wall of children with Poland's syndrome[5], and others.

To avoid transmition of bacterial and fungical diseases, the allografts stored in tissue banks around the world have been sterilized with gamma radiation. According to same authors, the irradiation process may cause damage on the structure of the allografts, decreasing their biomechanical behavior[6]. In accordance with such studies, the main effect of ionizing radiation is the disruption of collagen fibers, promoting chemical degradation of the tissue[7,10]. On the other hand, other studies have demonstrated that the cartilage is a radioresistant tissue. The use of ionizing radiation for sterilization of grafts contaminated by virus has been not recommended because the high doses needed may cause several damages in the graft. Thus, tissues suspected of viral contamination are excluded on the donor screening. However, the

Central Tissue Bank of Warsaw has been used doses of 33 kGy (± 10%) to sterilize tissues, including bones[8].

The great amount of water in cartilaginous tissue allow the cartilage play a role of impact absorver[6], therefore, the comprehension concerning the behavior of water releasing of the cartilage is very important for tissue banks.

To optimize conservation and sterilization processes, the special necessity of each kind of tissue must to be taking in consideration. The most common forms of cartilage preservation by tissue banks include deep-freezing.

The literature about irradiated cartilage TGA (Thermogravimetric analysis) is poor. However, other methods have been used to analyze the water content in cartilage, like Karl Fischer method. Recently, Spahn *et al.*[9] used Karl Fischer titration to analyze the water content in intact and defective cartilage, but no data were found in the literature about irradiated cartilage.

To verify how water release occurs in costal cartilage before and after sterilization by ionizing radiation, we carried out a thermogravimetric tests samples non-irradiated as well as in samples irradiated with different gamma radiation doses (TGA).

2. MATERIAL AND METHODS

2.1. Sample Preparing and Irradiation

Samples of human costal cartilage were obtained from cadaveric donors between 20 and 45 years old (average 36,25), of both sex (50% men, 50% women), from "Serviço de Verificação de Óbitos (SVO), Faculdade de Medicina (FMUSP)". After harvest, the samples were cleaned and the perichondrium was removed. Costal cartilage was deep-freezing (-80 °C) and storage for 6 months until TGA tests.

To ensure better homogeneity of the samples and reproducibility of the experimental results, the samples were distributed randomly in three groups with three samples each ($n=3$), the first group was used as control (0 kGy). The others were divided in two fragments: one was irradiated and the other was kept as specific control of each irradiated sample. To verify how is the behavior of non-irradiated samples, this group also was divided in two fragments. The irradiation sample was carried out using ^{60}Co irradiator at 2 different doses (25 kGy and 50 kGy). During the irradiation process, the temperature of the samples was kept with dry ice.

2.2 Thermogravimetry of the Samples

The study of cartilage water release before and after irradiation process was carried out with thermogravimetric analyser Shimadzu TGA-50 connected a computer with specific software for analysis. Sample weight ranging from 4 to 10 mg were heated up to 600°C with heating rate of 10°C/min under a compressed air flow rate of 50 ml/min.

The thermogravimetric tests were started at room temperature using frozen samples, to avoid loss of water.

3. RESULTS AND DISCUSSION

According to the thermogravimetric curves (fig. 01), the water content is different between the tested samples. This trait is decurrent of several biologic features as age, sex and normal physiological condition of the donor. For example, oldest donors have a more calcified cartilage owing to decreasing synthesis of proteoglycans, therefore, decreasing the water content in tissue. Even in the same donor occurs a water content variation. It is at a function of heterogeneity of water distribution in the cartilage.

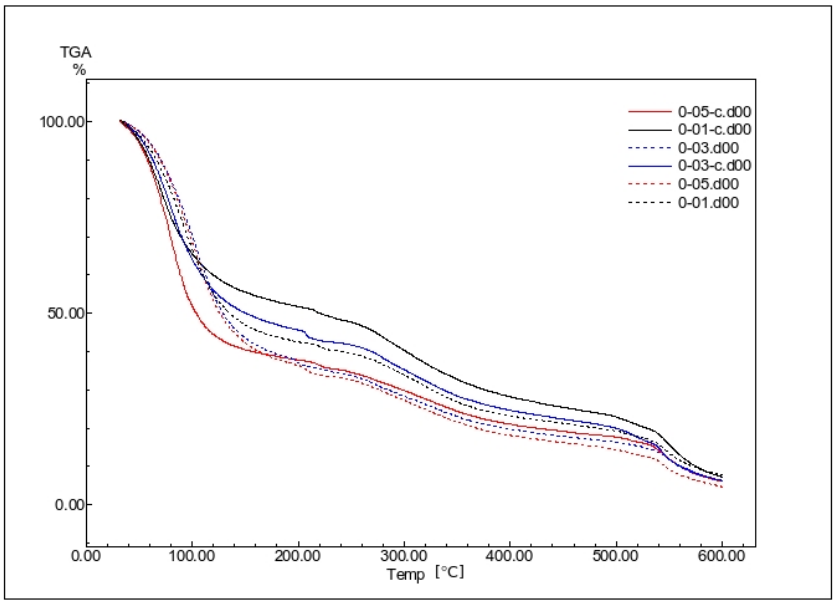

Figure 1: Thermogravimetric curves of non-irradiated samples from 3 different donors. Each donor is identified by a different color of the curve. Two fragments of the same donor were tested. The first number in the nomenclature of the sample represents the dose given, the second number represents the number of the donor and the "c" the copy of the sample of the same donor.

From TG curves it can be observed a great weight loss from the room temperature until 200 °C is at a function of sample dehydration. The other events of loss of mass may be related to thermal decomposition of the samples. A temperature variation is observed where the main event occurs. This variation is at a function of the variation in the initial weight of the test specimens. On the other hand, the solid residual content varies very little around 10% at 600 °C for all samples.

The variation of mass showed by the samples is at a function of the used methodology to test the samples still frozen. During the assays, the test bodies have been cut with the maximum of possible similarity.

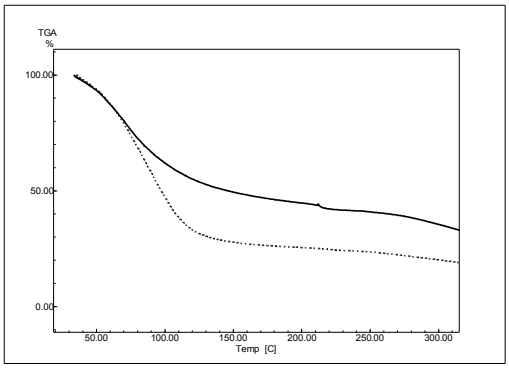

Figure 2: donor *a* irradiated with 25 kGy. The full line is the non-irradiated sample and the hatched line is the irradiated sample.

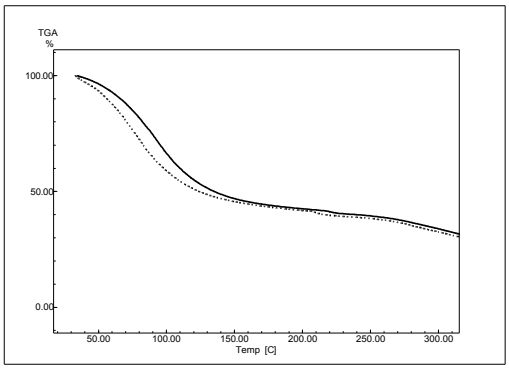

Figure 3: donor *b* irradiated with 25 kGy. The full line is the non-irradiated sample and the hatched line is the irradiated sample.

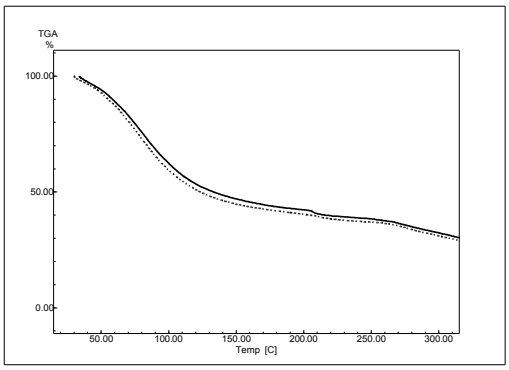

Figure 4: donor *c* irradiated with 25 kGy. The full line is the non-irradiated sample and the hatched line is the irradiated sample.

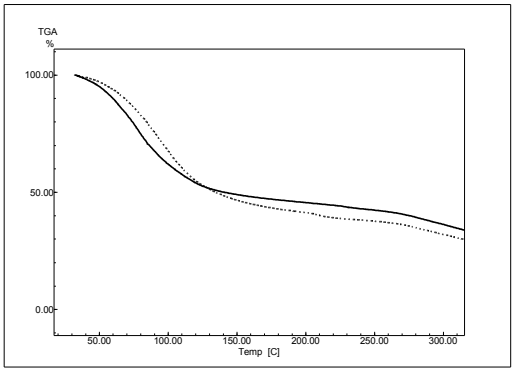

Figure 5: donor *d* irradiated with 50 kGy. The full line is the non-irradiated sample and the hatched line is the irradiated sample.

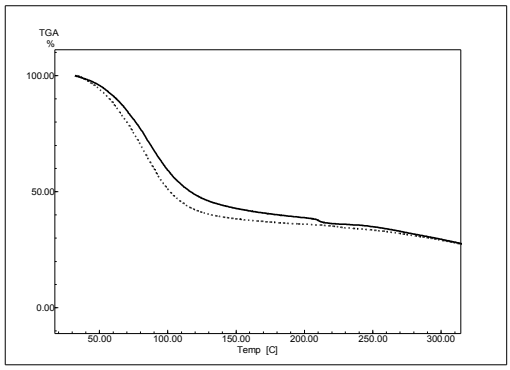

Figure 6: donor *e* irradiated with 50 kGy. The full line is the non-irradiated sample and the hatched line is the irradiated sample.

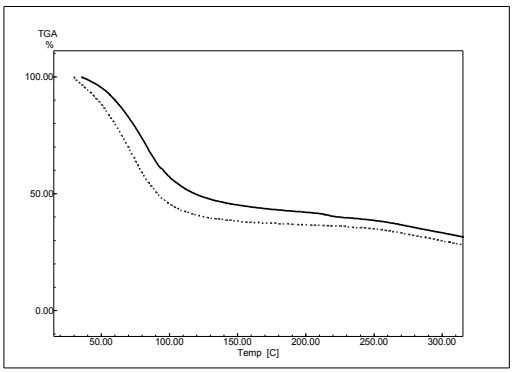

Figure 7: donor *f* irradiated with 50 kGy. The full line is the non-irradiated sample and the hatched line is the irradiated sample.

As the samples are not completely homogeneous, the thermogravimetric results obtained reveal little trustworthy (Fig. 02 to 07).

4. CONCLUSIONS

The thermogravimetry data from non-irradiated human costal cartilage show great variability of water content among different donors and even for different samples of the same donor.

The thermogravimetry did not allow the detection of significant difference between non-irradiated and irradiated samples behavior.

The obtained results have shown that new studies must be carried out with a greater number of samples for a better comprehension of the ionizing radiation effects on human costal cartilage.

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