

EFFECTS OF ELECTRON BEAM IRRADIATION ON BOVINE PERICARDIUM TISSUE

Roberta Polak¹, Andrea C. D. Rodas², Yasko Kodama³, Olga Z. Higa², Luci D. B. Machado³ and Ronaldo N. M. Pitombo¹

¹ Departamento de Tecnologia Bioquímico-Farmacêutica – Faculdade de Ciências Farmacêuticas - USP
Universidade de São Paulo
Av. Professor Lineu Prestes, 580
05508-900 São Paulo, SP
robertapl@gmail.com
pitombo@usp.br

² Instituto de Pesquisas Energéticas e Nucleares, IPEN – CNEN / SP – Centro de Biotecnologia
Av. Professor Lineu Prestes 2242
05508-900 São Paulo, SP
andrea.ipen@gmail.com
ozhiga@ipen.br

³ Instituto de Pesquisas Energéticas e Nucleares, IPEN - CNEN/SP – Centro de Tecnologia das Radiações
Av. Professor Lineu Prestes 2242
05508-900 São Paulo, SP
ykodama@ipen.br

ABSTRACT

In this work, electron beam irradiation was studied as a way for bovine pericardium (BP) tissue crosslinking. BP samples were irradiated in an electron beam accelerator at different doses (12.5 and 25 kGy), at three different dose ratios (4.67, 9.34 kGy/s), in the presence and absence of oxygen. Irradiated samples were analyzed by Differential Scanning Calorimetry (DSC), Thermogravimetry (TGA), Scanning Electron Microscopy (SEM) and swelling degree. DSC analysis showed a decrease in shrinkage temperature. However, for all irradiated samples, the energy required in the process was higher than the non irradiated BP. The TGA analysis showed that the thermal behavior, both the control and the irradiated samples, was characterized by three stages concerned in the loss of mass. The BP structure was characterized by swelling degree and SEM. The structure of the BP tissue suffered alteration, becoming looser, or more compact. By swelling degree, when the BP was irradiated in the presence of oxygen, the swelling degree value was higher than non irradiated BP, in the other hand the swelling degree value of BP irradiated in oxygen absence were lower than the non irradiated BP. Those results indicate that the BP irradiated in absence of oxygen could predominantly crosslinks. The BP degradation when it was irradiated in presence of oxygen was confirmed by SEM.

1. INTRODUCTION

Biological tissue grafts have been used since the 60s as an alternative to mechanical cardiac prostheses [1]. From 1974, bovine pericardium (BP) has become one of the most used materials for bioprosthesis preparation. BP, which is composed mainly of collagen type I [2],

has been used since then as material for the manufacture of bioprostheses and heart valves [3].

To improve the material resistance, several chemical and physical crosslinking processes have been developed. Nowadays the most consolidated method for bovine pericardium bioprosthesis is its reticulation by glutaraldehyde (GA), although GA reticulation can induce calcification *in vivo* leading to valve failure and the need of prosthesis changes [4]. In this work, electron beam irradiation was studied as a way for the of the BP tissue crosslinking and explores new opportunities in biomaterial treatments introducing the utilization of ionizing radiation.

2. MATERIAL AND METHODS

2.1. Bovine Pericardium Preparation

Bovine pericardium were collected at slaughterhouse, cleaned, washed, and stored in glycerol for preservation. Before use, BP was washed with saline solution (NaCl 0.9%). Samples of BP were lyophilized with annealing treatment as described by Leirner [5].

2.2. Ionizing Radiation

Irradiation was performed in an electron beam accelerator at CTR-IPEN São Paulo, Brazil. Samples of BP were packed separately in polyethylene (HDPE) wrapping material. The samples were irradiated in two different doses (12.5 and 25 kGy) and two different dose ratios (4.67, 9.34 kGy/s) with a 0.550 MeV radiation energy.

2.3. Differential Scanning Calorimetry (DSC)

DSC analyses were performed in a DSC50 Shimadzu to determine the shrinkage temperature of the irradiated samples. Samples were submitted to a temperature program starting at room temperature (RT), heating rate of 10°C/min up to 100°C, in dynamic N₂ atmosphere (50 mL/min).

2.5 Thermogravimetric Analysis (TGA)

The loss of mass was determined using a linear heating program (conventional TG). Samples were subjected to a controlled temperature program starting the experiment at RT up to 800°C, heating rate of 10°C/min in compressed dry air atmosphere (50 mL/min) in a TGA-50 Shimadzu.

2.6. Swelling Degree

The samples were previously heighted and immersed in excess of water. The water uptake was measured by the weight increment during the time. The swelling degree was determined by the relationship:

$$S\% = \frac{wt - wi}{wi} \times 100 \quad (1)$$

Where: S% is the degree of swelling in percentage, *wt* is the final mass in grams and *wi* is the initial mass in grams [6].

2.4. Scanning Electron Microscopy (SEM)

The BP samples were attached on the SEM support, and coated with gold for 5 seconds. BP micrographs were analyzed and captured at a Jeol - JSM 7401-F, using a magnification of 500 times.

3. RESULTS AND DISCUSSION

DSC analysis showed that the shrinkage temperature was shifted up to 17°C for less, but the energy involved in the event increased for all samples (Table 1). Tattini *et al* observed an endothermic peak at 57°C that is related to the shrinkage temperature of untreated bovine pericardium tissue. For the GA crosslinked bovine pericardium a higher temperature (60°C) was related to the thermal event, but the enthalpy energy involved in process was higher for BP treated with GA [7]. However, from these data is not possible to establish a relationship between the effects caused to the samples and the doses and dose rates applied. This can be caused by factors such as heterogeneity of tissue, with different thicknesses and due to the imbalance in the dose of radiation absorbed by the samples, one of the disadvantages of irradiation by electron beam.

Table 1. Shrinkage temperature of BP irradiated samples

Dose Rate (kGy/s)	Dose (kGy)	Shrinkage Temperature ^a (°C)	Enthalpy Energy ^b (J/g)	Peak for the First Derivative ^b (J/g)
0	0	69.56	4.85	1.87
4.67	12.5	57.12	6.28	3.77
4.67	25	55.20	14.28	2.31
9.34	12.5	54.81	5.40	2.92
9.34	25	53.43	10.00	3.36

a. Peak temperature; b. Endothermic event.

According to TGA curves, the thermal behavior of both the control (0 kGy) and irradiated samples is characterized by three stages of loss of mass (Table 2). The first, which occurs in the range 25 to 200°C, is related to the loss of water from the material (13%). The second stage occurs in the range of temperature from 200 to 450°C and is related to the degradation of the structure of collagen (41%). The third phase, which occurs in the temperature from 450

to 650°C, is related to the combustion of residual organic compounds (39. %). However, the use of this technique did not allow the observation of changes caused in BP tissue.

Looking at the results obtained by swelling tests (Fig. 1), it was possible to observe that even the presence or the absence of oxygen during the irradiation of lyophilized BP the structure of the tissue was altered. When it has lower degree of crosslink, the swelling degree is higher than the non irradiated tissue. This phenomenon was observed in the BP irradiated in the presence of oxygen. The decreament of the swelling degree value suggests more interactions between BP collagen fibers, characterizing crosslinking of the tissue. This phenomenon was observed in the BP irradiated in the absence of oxygen.

Table 2. Thermogravimetric behavior of BP irradiated samples

BP Sample		Loss of water (%)	Collagen degradation (%)	Combustion of residual organic (%)
Dose (kGy)	Dose rate (kGy/s)			
0 kGy		13.6	41.3	40.2
12.5	4.67	13.5	40.5	39.2
25	4.67	11.8	42.0	37.1
12.5	9.34	12.1	40.8	40.6
25	9.34	13.0	40.7	38.8

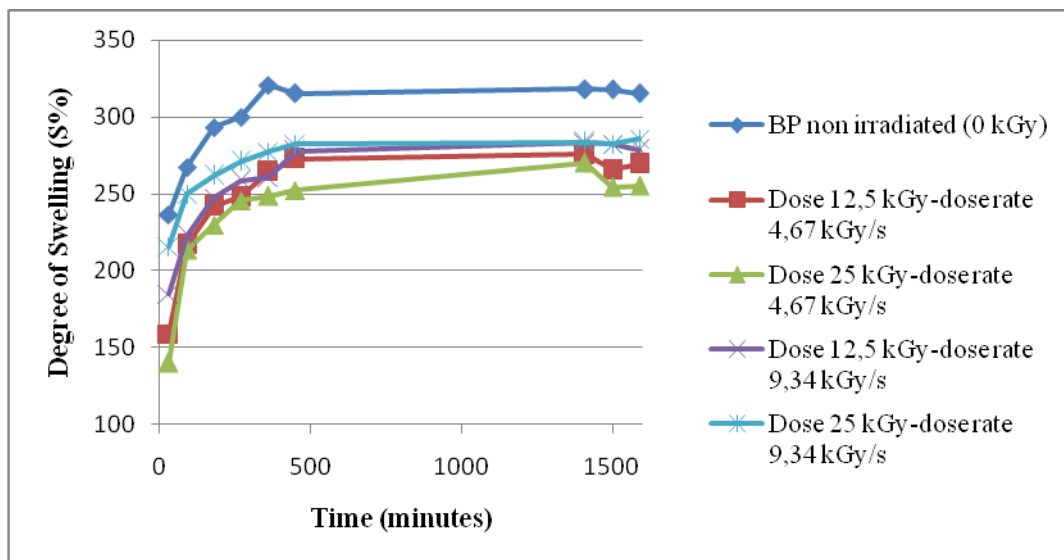


Figure 1. Bovine Pericardium swelling degree.

According to the micrographs it was possible to observe that the irradiation changed all samples. When samples are irradiated in the presence of oxygen (Fig. 2C and 2D) it can be observed that, superficially, some of the collagen fibers in BP tissue seem to be broken and then rolled discomposedly (indicated with arrows). When samples are irradiated in the

absence of oxygen (Fig. 2B) the tissue seems compacted, suggesting the crosslinking of collagen fibers, confirming the swelling results.

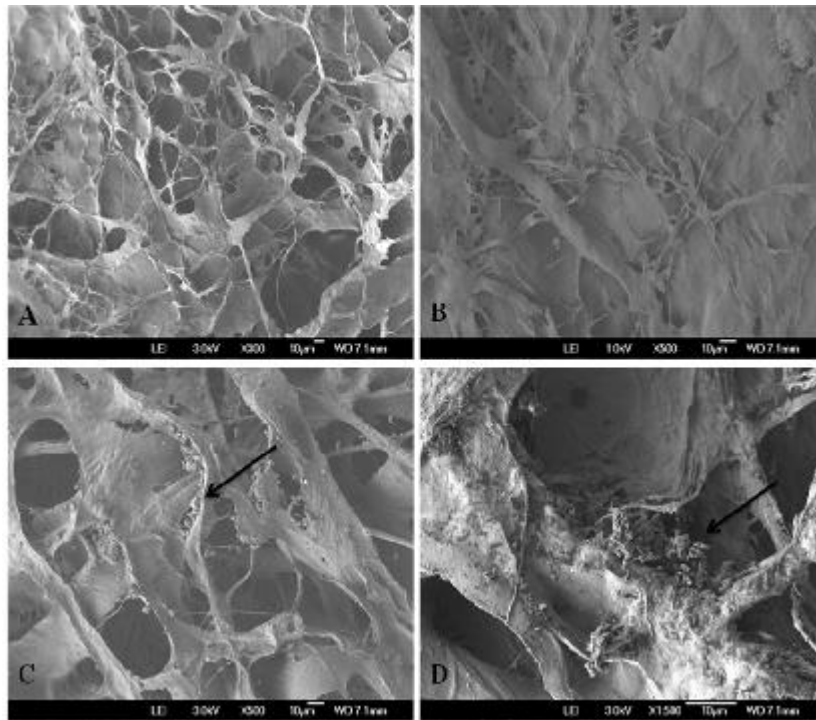


Figure 2. SEM images from BP: A- non-irradiated (500x); B- irradiated in the absence of O₂ (500x); C- irradiated in the presence of O₂ (500x); D- irradiated in the presence of O₂ (1500x). All irradiated samples refer to 25 kGy (4.67 kGy/s).

4. CONCLUSIONS

In this preliminary study to verify the effects of the irradiation on lyophilized BP, it can be observed that irradiation of tissue in the presence of oxygen favors chain scission of collagen molecules. However in the absence of oxygen, using the same parameters of irradiation, it was possible to note that the tissue undergoes preferentially to crosslinking. In order to verify the mechanical properties of the irradiated samples, tensile tests will be performed

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