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IMAGING OF IRRADIATED HUMAN COSTAL CARTILAGE BIREFRINGENCE BY PS-OCT

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ABSTRACT

Sterilization by ionizing radiation is a technique used for tissue banks around the world to avoid transmission of infectious diseases by human allografts. However, high doses of ionizing radiation may cause undesirable changes in tissue structure, decreasing its mechanical properties, for example.
Optical Coherence Tomography (OCT) is a non destructive, non ionizing and real time method to investigate biological tissues without promote any change in tissue structure. Polarization Sensitive Optical Coherence Tomography (PS-OCT) is an OCT technique that combines polarimetry with low coherence reflectometry to provide depth resolved measurements from birefringent structures as collagen.
Costal cartilages from 15 cadaveric donors were preserved in high concentration glycerol and each individual sample was divided in 6 fragments. One of them was kept as a control group and the others were irradiated with gamma radiation from a Co-60 source with doses of 15, 25, 50, 75 and 100 kGy. OCT and PS-OCT images of the same region of the samples were obtained from a device OCS 1300 SS (Thorlabs, USA) with a coupling polarization module PSOCT 1300 (Thorlabs, USA).
According with our results, birefringence may be visualized in all test groups as well in the control group, suggesting that sterilization by ionizing radiation does not affect the collagen structure significantly to cause total loss of birefringence, even if high doses as 75 and 100 kGy are used. The next step of our work is to develop a new method to quantify the birefringence using the optical properties of the tissue.

1. INTRODUCTION

The technical support of International Atomic Energy Agency (IAEA) for tissue banks around the world was started in 1983 and until now hundreds of thousands of grafts was produced using ionizing radiation as a safety method for sterilization [1].

Although radiosterilization does not promote increase in both pressure and temperature, high doses of ionizing radiation may cause undesirable changes on tissues, decreasing its mechanical properties, e.g., making it inappropriate for use [2]. Indeed, one of the main concerning of tissue banks is relative to tissue modifications promoted by radiosterilization and its impact on receptors.

Radiosterilized cartilage have been used for reconstructive surgeries of nose, external ear and penis [3,4], in bronchial repair [5] and for thoracic reconstruction of chest wall of children affected by Poland's syndrome [6] and *Pectus Excavatum* [7]. In cartilaginous tissue, collagen type II is the major component of extracellular matrix of cartilage, accounting for almost 80% of dry weight and does not form fibers, only fibrils [8] that are arranged parallel in the superficial layer [9], providing the birefrigency property to cartilage and allowing the use of some techniques to identify this property, as confocal microscopy and PS-OCT, for example [10].

Optical Coherence Tomography (OCT) is a relative new technique that provide high resolution images. Its successful in scientific fields and in medicine is due to be a contactless and non-destructive technique and to generate images in real time without any sample preparation, avoiding modifications caused by tissue dehydration as occurs in optical and electronic microscopy, for example. OCT measure the backscattering intensity of infrared laser (Fig. 01). Once backscattering intensity cannot be measured directly due to the high speed of the light, the OCT use a technique known as low coherence interferometry. Basically, in an OCT system the broadband light is coupled into a fiber-optic Michelson interferometer and is divided in two light beams by a beam splitter. One beam is directed to a reference mirror that is precisely controlled by a computer and another light beam is directed to the sample. Light backscattered by the sample and the light reflected by the mirror are recombined at the beam splitter generating an interference pattern which is collected by the detector [11].

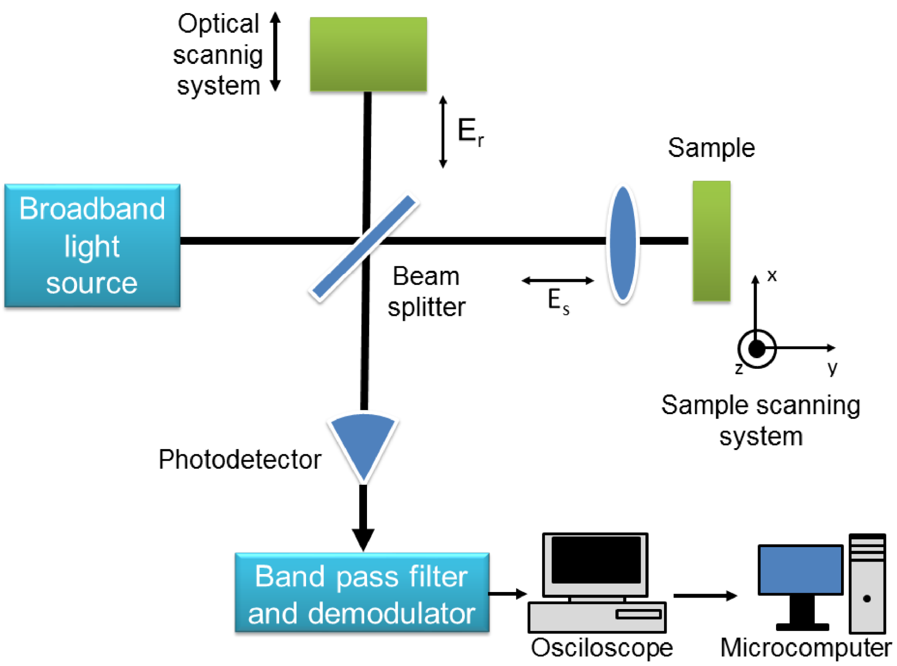

Figure 01. Main components of the OCT setup

Polarization Sensitive Optical Coherence Tomography (PS-OCT) is a variation of OCT imaging technique used for determining phase retardation in biological birefringent tissues as cartilage and have been used to investigate the topological variations of collagen fibrils of articular cartilages [12], to evaluate the characteristics of degenerative joint disease [13] and the influence of laser-assisted cartilage reshaping [14].

Light is represented by an electric and magnetic transversal fields oscillation. For a wave light propagating in z axis and the electric field propagating exclusively in x axis, the electric field can be written as:

$$\mathbf{E_x}\,(z, t) = E_{0x}\cos(kz - \omega t)\mathbf{i} \qquad (01)$$

If we consider a similar wave, with its electric field oscillating exclusively in y axis, with the same frequency but not necessarily with the same phase, it can be written as:

$$\mathbf{E_y}\,(z, t) = E_{0y}\cos(kz - \omega t + \varepsilon)\mathbf{j} \qquad (02)$$

If we assume ε=0 or a integer multiple of 2π, then the sum of two electric fields is represented by:

$$\mathbf{E}(z, t) = \mathbf{E_x}\,(z, t) + \mathbf{E_y}\,(z, t) = E_{0x}\cos(kz - \omega t)\mathbf{i} + E_{0y}\cos(kz - \omega t + \varepsilon)\mathbf{j} = (E_{0x}\mathbf{i} + E_{0y}\mathbf{j})\cos(kz - \omega t) \qquad (03)$$

Therefore, the electric field is constant in orientation with angle relative to x axis. By simple geometry, the angle can be represented by;

$$\frac{sen\ \phi}{\cos\phi} = \tan\phi = \frac{(E_{0y})}{(E_{0x})} \qquad (04)$$

If $E_{0x} = E_{0y}$, we have a linearly polarized light as result, which position in relation to x axis is constant and equal to 45 degrees.

On other hand, if we assume that ε equal to a multiple of $-\pi$, where $E_{0x} = E_{0y}$, since the second cosine equal to -1, **E**(z,t), is given by

$$\mathbf{E}(z, t) = (E_{0x}\mathbf{i} - E_{0y}\mathbf{j})\cos(kz - \omega t) \qquad (05)$$

that again result in a linearly polarized light with a different axis from that eq. 03, and its angle is given by

$$\tan\phi = -\frac{E_{0y}}{E_{0x}} \qquad (06)$$

and, again, we have a linearly polarized light. Therefore, light is linearly polarized when the relation of phase between two light waves is between zero and an integer of ±nπ [11].

Although light does not penetrate deepest enough on human body to make possible generate high-resolution images without an invasive technique, new methods minimally invasive have been developed [15] providing important data concerning to real time tissue state, what may help surgeons in take the best decision during surgeries.

Thus, in this paper we discuss the use of PS-OCT as a new method to evaluate collagen network structure before and after radiosterilization, providing new evidences for use of ionizing radiation as a secure sterilization method for cartilage grafts.

2. MATERIALS AND METHODS

2.1 Material processing and irradiation

Before the tests were carried out, this study was approved by Ethical Committee of Faculdade de Saúde Pública of Universidade de São Paulo. Thirty costal cartilages were obtained from fifteen cadaveric donors (aged from 18 to 45 years), both sex. Right costal cartilages were deep-frozed at -70 °C [3,16] and left costal cartilages were preserved in high concentration of glycerol (>85%) [17]. After removing whole adjacent tissue with a scalpel, the deep-frozen and glycerolized samples were divided in six groups with fifteen samples in each one. One group was kept as control group and another five were irradiated by a ^{60}Co source with doses of 15, 25, 50, 75 and 100 kGy. During irradiation, the control group was kept in the same environmental conditions of the experimental group. To avoid variation of temperature, deep-frozen costal cartilages were irradiated with dry-ice and glycerolized cartilages were irradiated with refrigerant blocks. As dose control were used Red Perspex polymeric dosimeters with a range of 50 kGy. Samples irradiated with 75 and 100 kGy had exchanged its dosimeters by a novel one after dose of 50 kGy.

Before testing, glycerolized cartilages were rehydrated in saline solution (0.9% NaCl) and deep-frozen cartilages were thawed at room temperature.

2.2 PS-OCT

To look for visual disruptions on internal collagen structures of irradiated cartilages, PS-OCT images were obtained from PSOCT 1300 coupled to OCS1300SS OCT system (Thorlabs ® - USA) (10 mW, λ=1325 nm). To perform the test, the longitudinal axis of the samples were aligned perpendicularly with the laser beam. Once phase retardation occurs when birefringence structures form 45º with the laser beam [11] , the samples were fixed in its sagittal axis and rotated in its transversal plan, until the phase retardation can be viewed.

For each sample were recorded five OCT and PS-OCT images of distinct tissue regions, totaling seventy-five images from each dose.

3. RESULTS

In the fig. 02 we can see the phase retardation according to rotational angle. Note that phase retardation rises when θ = 45º.

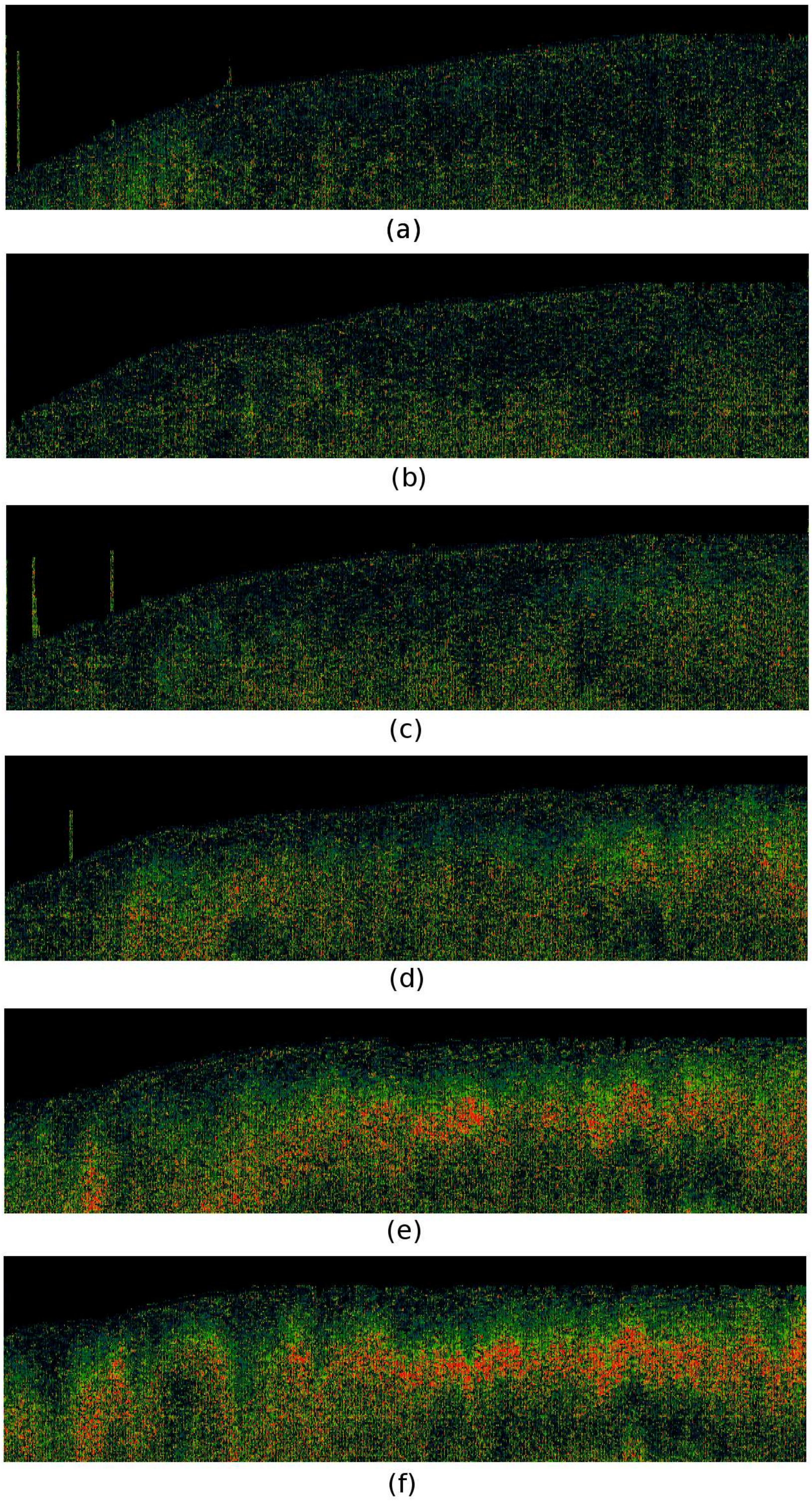

Figure 02. Phase retardation according rotational sample angle. (a) 0 degree; (b) 10 degrees; (c) 20 degrees; (d) 30 degrees; (e) 40 degrees; (f) 45 degrees;

Figures 03 and 04 represents OCT and PS-OCT images of the same region of the cartilage for deep-frozen cartilages and glycerolized cartilages, respectively.

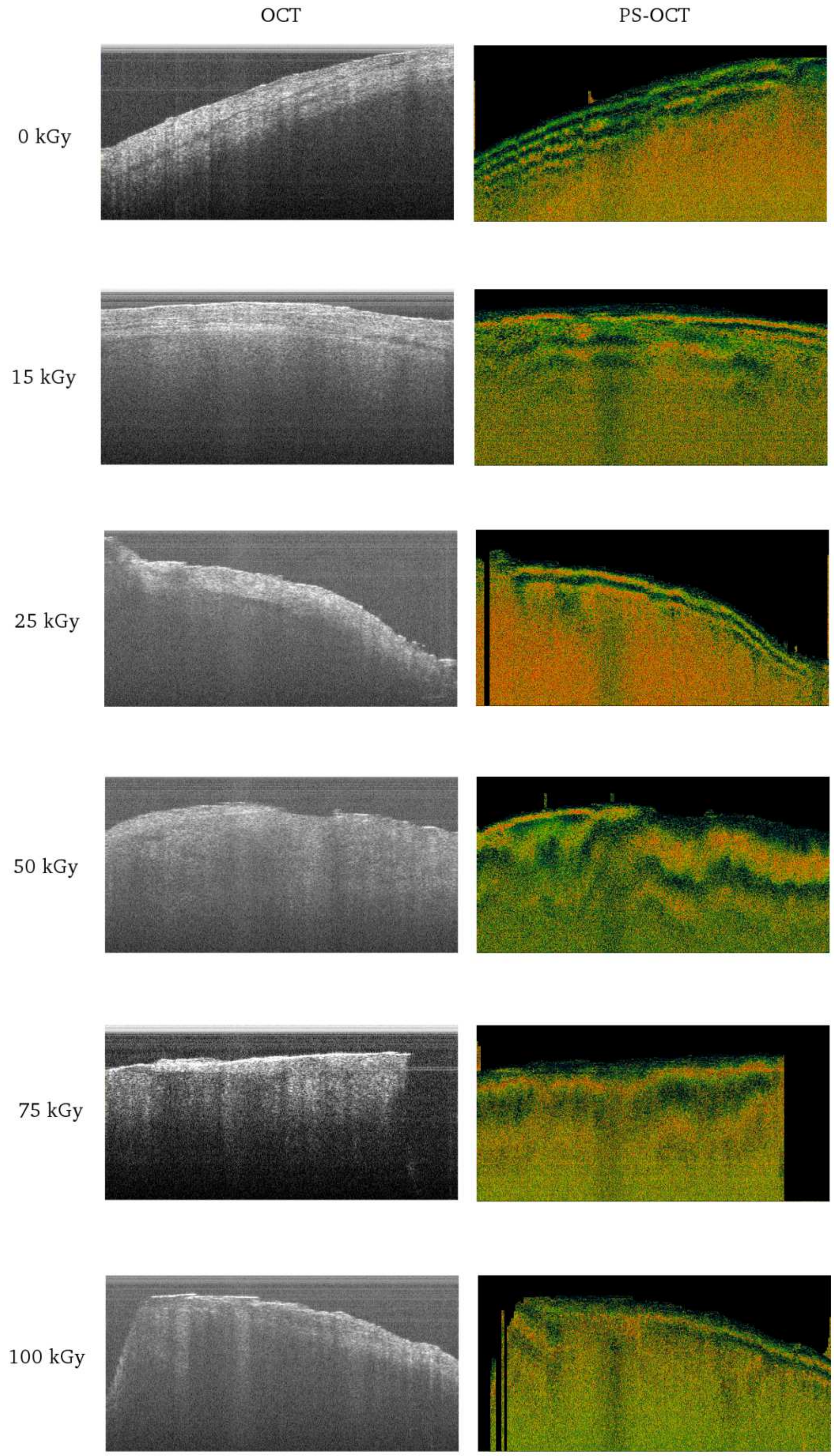

Figure 03. OCT and PS-OCT images from deep-frozen human costal cartilage.

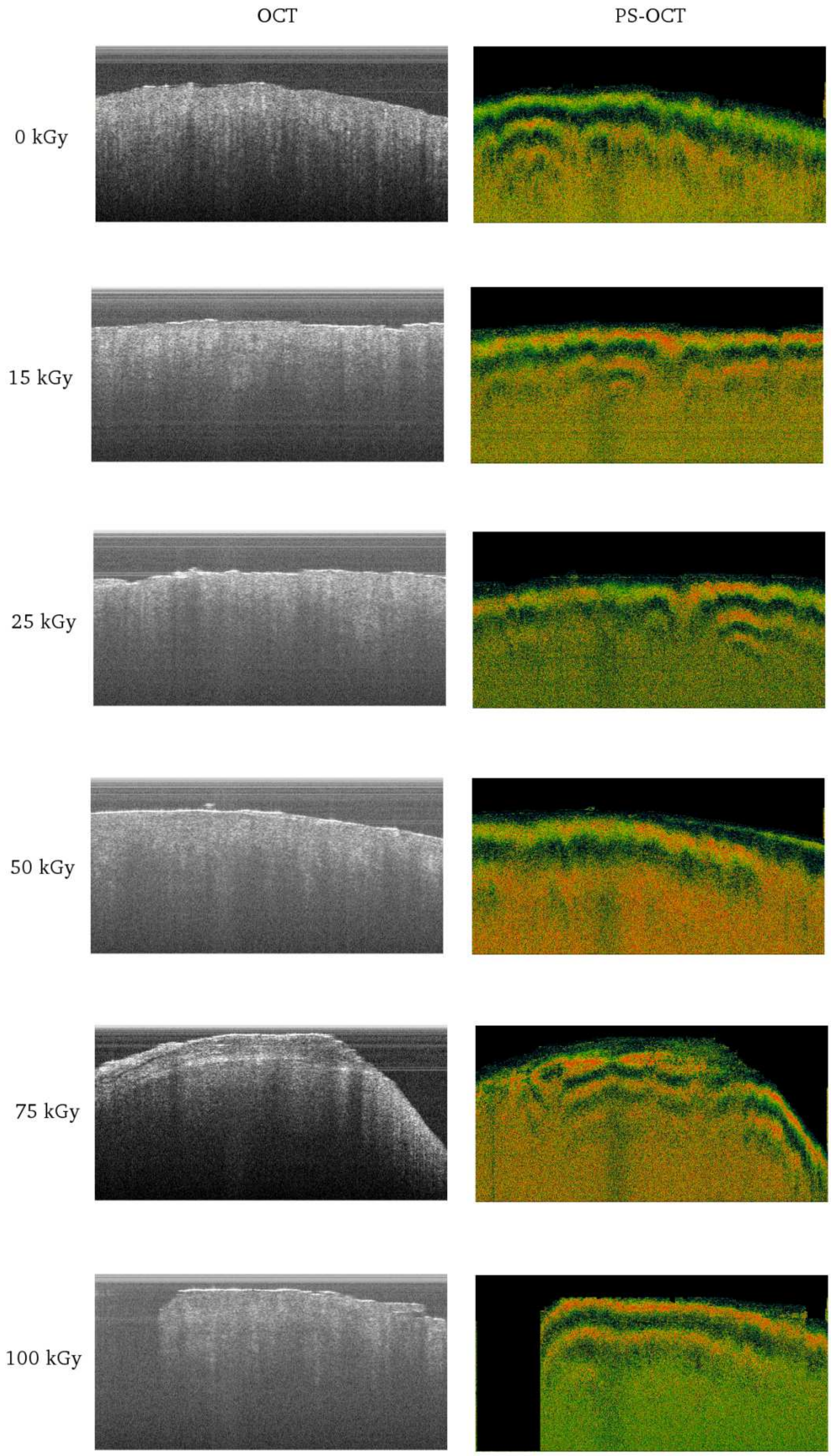

Figure 04. OCT and PS-OCT images from glycerolized human costal cartilage

4. DISCUSSION

Despite of PS-OCT have been used in studies of articular degenerative diseases [11,12,18], at the best of our knowledge, there is no work concerning PS-OCT and structural modifications induced by ionizing radiation in cartilages allografts for tissue banks.

As we can see in fig. 02, phase retardation only arise when the longitudinal orientation of collagen fibrils form 45º with the laser beam, corroborating with the theory and demonstrating that the type II collagen fibrils present in the costal cartilage are arranged longitudinally in the superficial zone of the tissue.

According to our results (fig. 03 and fig. 04), preservation method and irradiation, even if in very high doses (75 and 100 kGy), does not promote whole disorganization of the structure of the cartilage, once retardation phase is present in all doses for both deep-frozen and glycerolized samples. This is an important finding because in degenerative articular diseases as osteoarthritis, the phase retardation is not observed in affected areas, indicating a loss of the collagen structural organization, which lead to a decrease in biomechanical properties of the tissue [13]. Moreover, our results also demonstrate a normal variation on polarization sensitivity at different regions in the same sample and between different samples, as indicated previously by Xie et al. [12].

However, as demonstrated by Youn et al. [18], when PS-OCT image are analyzed quantitatively by plotting phase retardation versus depth, even a simple dehydration by using glycerol can change the phase retardation. Thus, when tissue banks around the world use glycerol as a preservation method, they must be in mind that this kind of tissue preservation modifies the internal structure of the water rich tissues as cartilages.

5. CONCLUSIONS

PS-OCT imaging is a high-resolution, real time and non-destructive technique that is capable to identify structural tissue modifications in human cartilage allografts storaged in tissue banks without any change in tissue structure caused by other imaging conventional methods as microscopy, for example.

Our results suggest that tissue preservation by deep-frozen or glycerolization followed by ionizing radiation sterilization does not promote whole disruptions in type II collagen network as occurs in degenerative diseases as osteoarthritis.

However, qualitative analysis is not enough to identify subtle modifications on internal structure of type II collagen network and, therefore, quantitative analysis as phase retardation versus depth is needed to better understand the consequences of radiosterilization.

The next step of this work is to compare phase retardation versus depth in human costal cartilages before and after sterilization by ionizing radiation to ensure for tissue banks high quality tissues for transplantation.

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