Pilot Characterization of Human Bones by Spectroscopic Techniques

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Introduction

There are well known pathologies, such as osteoporosis, that affect the bone structure by decreasing the bone mineral density (BMD), and thus increasing the risks of suffering fractures. Mexico, like many other countries, is facing an epidemiological transition due to an increasing number of older people [1]. Since osteoporosis is associated with ageing, it is expected to become more common within the Mexican population [2]. In 2009, the World Health Organization (WHO) reported a global incidence of million cases of fractures due to osteoporosis, the most common fractures being of the total hip, femoral head, forearm, and vertebral body [3].

The current gold standard to diagnose osteoporosis is the Dual-Energy x-ray absorptiometry (DXA) [3,4], which measures the BMD. This diagnostic test has the advantage of being able to analyze the mineral bone structure in distinct stages of the pathology and is easy to apply. However, this DXA exposes the patient briefly to ionizing radiation and it focuses only on the structural dominion and morphology of the bone and thus it does not consider chemical composition, which is vital to bone quality [5,6].

Raman spectroscopy has been well established as a complementary technique to analyze osteoporosis, since it offers insight on the chemical composition [7]. This tool is based on the electromagnetic radiation interaction with the sample´s molecules, resulting in a spectrum with characteristic peaks corresponding to the vibrational modes of the molecular composition [8]. Hence, structural characteristics of the bone tissue that affect the mechanical properties of the bone can be studied with Raman spectroscopy. A study on the molecular information of femoral head, obtained with a DXR Raman Microscope, Thermofisher, is presented and is related to the various crystalline changes of the minerals found the femoral head [9].

Materials and Methods

Sample Preparation

Patients signed an informed consent form to authorize the use of the employment of the biological material. Six patients underwent arthroplasty at the UMAE Hospital de Especialidades #1 Centro Médico Nacional del Bajío, after suffering a proximal femoral fracture. An informed consent form was signed by all patients to employ the biological material. Three femoral head samples originated from male patients, whereas the remaining originated from female patients.

Samples were selected in 5mm slices using scalpel blade #10 (RIBBEL), consistent with what is described by Ciarelli et al [10], in order that the bone volume was sectioned in three parts: a) cortical section b) cancellous section, and c) compact section [11] (Figure 1).

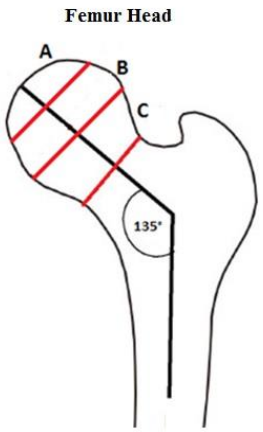


Figure 1. *Femoral Head Sections A) Cortical Section, B) Cancellous Section, C) Compact Section*

Raman Spectroscopy

A DRX Raman Microscope with a 785 nm laser centered in line was used to examine the chemical composition of the femoral head samples[12]. These were focused with a 10X./0.25 BD objective and were measured in a range of 100-3000 cm-1. To assess the composition homogeneity, an area of 2 × 2 µm2 was determined for Raman mapping. Subsequently, the data was acquired with Omnic software. The femoral head samples presented the integrated intensities of three characteristic bands: phosphate (approximately 903-991 cm-1), collagen (1445-145 cm-1), and amide I (1660-1720 cm1).

Data Analysis

Data from the femoral head spectra were analyzed by PCA (Principal Component Analysis), using the algebraic-statistical technique in two separate analyses. Exact tests were performed to evaluate significant differences between the proportions calculated from the tissues in every region. Throughout the statistical analysis, PCA and the Kruskal-Wallis test (non- parametric) were used with $P<0.01$.

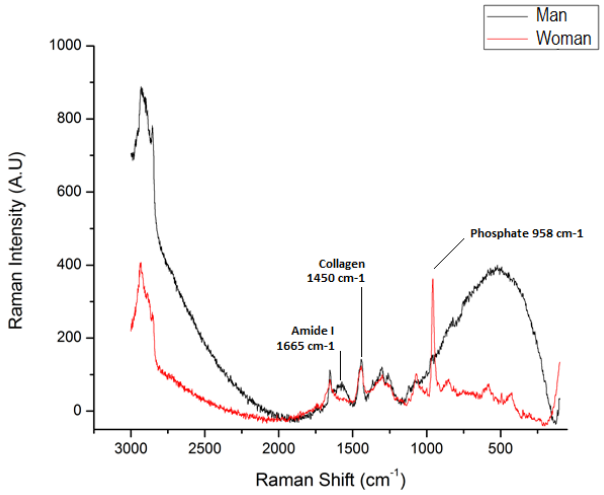


Figura 2. *Representative Raman spectra of ……. The black line and upper stroke is from a human male, whereas the red and lower stroke is from a human female.*

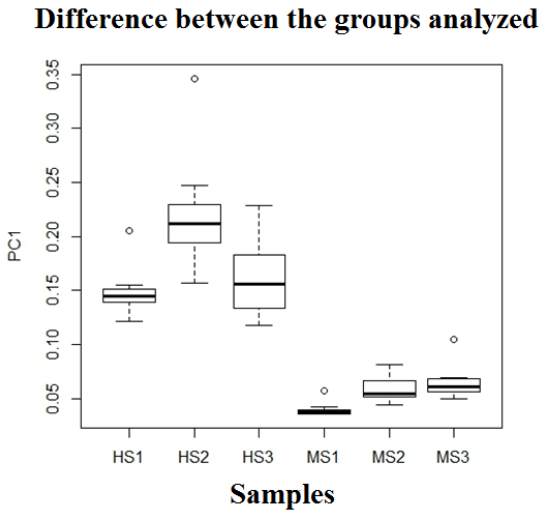


Figure 3*. Box diagram that shows differences between the groups of female and male patient, as well as a comparison of the bone sections.*

Discussion

The purpose of this study was to characterization of femoral heads by Raman spectroscopy to verify the physicochemical composition of the bones. The results demonstrate a clear difference of mineralized elements that could be interesting, from a clinical point of view, in a larger sample with chronic pathologies and osteoporosis. Also, a significant decrease in the bone´s mineral ratio can later be associated with the mineral ratio of the same patient´s hair.

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