OSTEOPOROSIS DIAGNOSTIC THROUGH 3D µ-CT AND SR-µXRF

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ABSTRACT
Bone microarchitecture and its mechanical properties have been considered as an important determinant of several diseases such as osteoporosis. This disease is characterized by the weakness of the bones, which compromises all structure.

The microstructure evaluation of biological specimens can be accomplished with X-rays. The 3DX-ray microcomputed tomography (3DµCT) and the X-ray microfluorescence by synchrotron radiation (SRµXRF) are imaging techniques in microns order of space resolution (~10 µm). Those techniques can be decisive to understand the bone structures in order to improve the knowledge about the bone quality.

The 3DµCT and SRµXRF results reveal an increase of the porous diameter on osteoporotic bones, representing high bone porosity with a decrease in trabecular dimensions.

KEY WORDS
Bone, x-ray, synchrotron, osteoporosis, trace elements.

1. Introduction
Bone quality is related to the bone strength. The strength of bones depends on its structure (size and shape) and material properties (minerals and micro damages) which are affected by bone remodeling and modeling [1]. The common parameter used to express such concept is bone mineral density (BMD), which can be defined as bone density per unit surface area and can be measured mostly by dual x-ray absorptiometry (DXA). Over the years, however, BMD proved to be less than ideal tool for diagnosing postmenopausal osteoporosis [2] and [3].

In recent years, the X-ray microcomputed tomography (3DµCT) has become an useful method to access the internal microarchitecture of many kind of materials including bones [4,5,6,7]. This technique becomes a kind of static bone histomorphometry but with a great advantage that is its nondestructive nature and the analyses of all the internal structures in minutes directly in three dimensions (3D). The 3DµCT gives qualitatively and quantitatively data of all the microstructure, cortical and trabecular bones, based on its geometry without any information about the minerals concentration present on microstructure. The usual parameters are BV/TV (bone volume to total volume), BS/BV (bone surface to bone volume) ratios respectively, trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), trabecular number (Tb.N) and anisotropy (preferential degree of structural orientation). The procedure of calculation of the 2D anisotropy is based on the counting technique named Mean Intercept Length (MIL) [8]. The qualitative is based on the image quality and the quantitative part is fundamant on static histomorphometric data [9,10,11]. The x-ray microfluorescence by synchrotron radiation (SR-µXRF) technique is a powerful method used to obtain this information and the mapping of trace element distribution.

Synchrotron X-ray sources have been used for high resolution micro imaging based on the distribution of the chemical elements. SRµXRF is a powerful method that involves minimal (or none) sample preparation, causing any damage and providing a trace element detection of all kind of samples (homogeneous or not). Furthermore, the SRµXRF procedure is able to detect several elements at the same time with low detection limit and excellent spatial resolution. The intensity of the x-ray fluorescent radiation is proportional to the elemental concentrations take into account the matrix effects [12]. The use of capillary optics reduces the beam size (~10 µm) improving even more the space resolution. This technique is now becoming used as a helpful tool to improve the knowledge of bones [13,14,15].

The bone structures consists essentially of a protein (collagen) and hydroxyapatite (99% of Ca_{10}(PO_4)_6(OH)_2).
Inside the cortical bone three are neurovascular channels known as Harversian canals, which transport nutrients. Trace elements are found in both mineral and organic phases, although their roles in normal bone function and in bone pathology are not fully established. The abnormal accumulation or deficiency of trace elements may theoretically impair the formation of bone and contribute to osteoporosis [16,17].

Minerals can be found in bone as a normal component or incorporated by foods and environment. Minerals such as zinc (Zn) and strontium (Sr) are examples of a normal component and incorporations of bone, respectively.

Zinc can influence bone mineralization either directly, as a divalent cation acting on nucleation and mineral growth, or indirectly, as a cofactor of enzymes like alkaline phosphatase or other metalloenzymes involved in the process [18]. It seems that Zn plays a role in normal growth and bone mineralization during puberty onset [19], and the transient mobilization and restoration of skeletal Zn mainly occurs in trabecular bone but does not involve major changes in bone mass, macro mineral content, or bone tissue turnover [20].

Strontium has a great affinity for bone and can be incorporated by surface exchange or ionic substitution. [21] Once absorbed, Sr is distributed throughout the body but preferentially deposited in bone and teeth [22]. This mineral can be given as strontium ranelate. When this occurs in experimental animals, it increases bone Ca and reduces fracture rate in osteoporotic patients. So it could be hypothesized that one feature of osteoporosis may be a certain degree of Sr deficiency, but data on bone Sr in normals are scare [23].

In this context, the knowledge of major and trace elements is very important to elucidate some important questions that remain unknown or even not closed in bone samples such as osteoporosis.

The present paper was mainly dedicated to investigate bone microstructure in an experimental protocol of osteoporosis induced by two x-ray images techniques (3DµCT and SRµXRF). Therefore, this research hopes to increase some aspects of the bone quality knowledge.

2. Materials and Methods

The system that was used to do the 3DµCT was a microfocus Fein Focus, FXS 160.50 model that has an electromagnetic device which makes possible several degrees of freedom in relation to the movement and positioning of the test body (controlled by a microcomputer), as well as the adjustment of the magnification factor of the captured image. This tube operates with 160 kV of a maximum high voltage and 1 mA of a maximum current. The system generates a conic beam and an image intensifier of 9° of diameter and it was used to convert the X rays photons in light photons, which can be captured by a “ccd” video camera (that permits real time visualization). With the objective to filter the spectrum of X rays produced to reduce the incidence of photons of very low energy on the test body, a sheet of aluminum of 0.5 mm was used promptly after the exit of the X ray tube. Figure 1 shows the basic principle of the procedure.

![Figure 1 – Basic principle of 3DµCT.](image)

The equipment was calibrated to operate at 40 kV voltage and 0.1 mA current, which gives the best contrast between the bone and non-bone materials. The magnification factor used was equal to 11.8 and 12 µm of resolution.

The SRµXRF was carried out at National Synchrotron Laboratory (LNLS), in Brazil and the x-ray source came from the D09 dipole at XRF beam line. It was used a white beam with an energy range from 4 to 23 keV. The X-ray spectra were recorded by a GeHp detector with resolution of 140 eV at 5.9 keV and were analyzed by an AXIL (Analysis of X-ray Spectra by Iterative Least-Squares Fitting) program.

A micro-stage controlled by a computer was used to move the sample in the horizontal and vertical directions using 45°/45° geometry and a 20µm of capillary optics. The mapping of all elements was made with the scan in an area equal to 1.2 mm² (in both vertical and horizontal directions) with 30 µm and 9 seconds for each step, which gives an acquisition time approximately equal to 4 hours, for each sample. The accuracy was checked by a standard reference material, NBS 1577a bovine liver. Figure 2 shows photography of the set-up.
Bone specimens were obtained from 15 Wistar female rats (4 months-old; 150–200 g) from femoral head. The animals were separated into two groups: control and OVX. The animals were ovariectomized when they were 2 months-old and were killed, in accordance with an approved protocol submitted to the animal care and use committee of our institution, when they were 4 months-old, e.g., 2 months-old after OVX. After the sacrifice, the femurs were disarticulated and the tissue was manually cleaned to remove soft tissue and the femoral heads were cut with a diamond cutter in $(100 \pm 10) \, \mu\text{m}$ of thickness. Figure 3 shows an example of one sample used.

3. Results and Discursions

All the results show the capability of the techniques used in this research. They can be used qualitatively as well as quantitatively. The possibility of collecting selective images at the micrometer resolution is very attractive to look at very small objects as well as to map heterogeneous zones. The x-ray and synchrotron radiations based imaging techniques have to be explored in biomedical investigations to identify and characterized the microstructure of bone samples. They could be also helpful to characterize different bones pathologies like osteoporosis.

Figure 4 shows a 2D reconstruction of one slice. In this figure it is possible to note the appearance of ring artefacts, which is very common on the CT process. This kind of artefacts occurs because of the non-linearity of the photo elements on the detector in response to the same luminous excitation. The fact that those linearity appears in the same locate make the projections (2D or 3D) present a common gain profile.

The gain profile generates vertical grids and they are responsible for the appearance of the artefacts on the reconstructed process. So, a way to eliminate this kind of artefacts is removing the vertical grids. This procedure was done in this study.

The other way to remove the artefacts is to correct the profile directly on the detector before the acquisition process. This method is not always available because of the amount of the photo elements is so big that an analogical gain correction is prohibitive.

Figure 5 (1,4) shows an example of one 2D reconstructed slice for each sample and its segmented images (2,5) to do the quantification process, which is a very important step [4]. Figure 5 also illustrates the 3D reconstructions (only the region of interest) of a normal and osteoporotic bones (3,6). It can be noticed the difference between the microstructures, which corroborates the knowledge of the increase in trabecular dimensions. This fact can be reinforced by the quantification data in table 1. The bone volume ratio (BV/TV) decreases in osteoporotic samples creating a false interpretation in the BS/BV value. With osteoporosis the trabeculae becomes thinner (TbTh) with less bone volume (BV) and the separation (TbSp) among then increase. This fact contributes in the appearance of some fractures disconnecting the trabeculae.
Figure 5 – Representation of one slice in the reconstruction process (1,4) and its segmentation (2,5) to the quantification and the 3D visualizations (3,4). The figures represent the region of interest that was used to do the quantifications of normal bone (a) and osteoporotic (b).

Table 1 - 3DµCT quantification results.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Bone type</th>
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<tbody>
<tr>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>BV/TV (%)</td>
<td>59.1 ± 4.0</td>
</tr>
<tr>
<td>BS/BV (mm²/mm³)</td>
<td>53.2 ± 8.9</td>
</tr>
<tr>
<td>TbTh (mm)</td>
<td>0.038 ± 0.004</td>
</tr>
<tr>
<td>TbN (mm⁻¹)</td>
<td>15.8 ± 3.2</td>
</tr>
<tr>
<td>TbSp (mm)</td>
<td>0.027 ± 0.005</td>
</tr>
<tr>
<td>DA</td>
<td>1.04</td>
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</tbody>
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Concerning the anisotropy parameter, the interpretation of the generate ellipse is given by the ratio between the largest and the smallest axis (perpendiculars to each other) that indicate the anisotropy degree (DA): the semi-axes of the ellipse indicating the preferential directions of the structure, the adjustment of the ellipse and the angle. Table 1 shows that this parameter increase in osteoporotic bone sample, which seem that the bone structure is more anisotropic in osteoporosis due to preferential damage of trabeculae in some directions.

The interpretation of the absolute concentration of the chemical elements in the SRµXRF images is a little complex because it is not possible to do just a mean of the data. These images have to be analyzed and take into account each structure because they can be influenced by the relative amounts found in the organic and inorganic components [16].

Figures 6-9 show the SRµXRF images for Ca, P, Zn and Sr for normal and osteoporotic bones in concentration scale. Each gray value on the image corresponds to the concentration, e.g., the darkest level represents the highest concentration and the lightest level the lowest.

For P and Ca, which are the main elements in the bone, were found a highest concentration in normal bones, occurring the opposite behavior in some other parts. The trabecular diameter seems to increase while the cortical thickness decreases. The distribution of these elements is homogeneous, but they were succeeded to demarcate the region between the cortical and the trabeculae, which demonstrate the lack of these minerals in this part of the bone. It was possible to visualize the Harversians canals in the cortical region and once more the deficiency of P and Ca.
The concentration of Zn and Sr were lowest than Ca and P, which were expected because of the bone composition. Figure 8 demonstrates that zinc is preferentially located at trabecular region in highest concentrations, and only in the normal bone it was not possible to separate clearly the cortical and trabecular parts. Strontium concentration was higher than zinc and was distributed throughout trabeculae and cortical bone demonstrating no preferential region.

Figure 7 - The SRµXRF images: normal (up) and osteoporotic (down) bones for calcium element.

Figure 8 - The SRµXRF images: normal (up) and osteoporotic (down) bones for zinc element.

Figure 9- The SRµXRF images: normal (up) and osteoporotic (down) bones for strontium element.

4. Conclusion

The x-ray imaging techniques used in this work (3DµCT and SRµXRF) were able to characterized qualitatively and quantitatively osteoporotic bone samples. The results revealed a decrease in bone volume in osteoporotic samples making the bones weakness increasing its porosity. It was demonstrated the elemental distributions in tabeculae and cortical zones in microns order was very helpful the understand of the chemical elements functions in such structures.

In summary, it was demonstrated that the bone quality can and must be evaluate not only by the architecture of the
bones but also to take into account the concentration and the distribution of the minerals.

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References