Analysis of bone architecture in 3D microtomographies

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ABSTRACT

The 3D computer microtomography (3Dµ-CT) in real time, is an example of the progress of the inspection technique for X rays, in what tells respect the space resolution of the tomography system, that is in microns order (10⁻⁶m). One of the great 3Dµ-CT applications in the medical area meets characterization of bone trabecular seeking the osteoporosis diagnosis. The study of those trabecular structures is very important when it is spoken in that pathology, that is a disease of the skeleton characterized by low bony mass and deterioration of the architecture of the fabric with consequent increase of the fragility to the fracture. This work shows the preliminary results about the use of three-dimensional computer microtomography (3Dµ-CT) to characterize bone tissue through bi-dimensional and three-dimensional histomorphometric quantification that are based on stereological concepts.

1. INTRODUCTION

There are many applications in relationship to X Ray Computer Tomography and, between them we can detached the study of the internal structures, such as cancellous bone. The biggest interest by this kind of structures is liked to the kind of analysis that through the X rays microtomography can be done: 2D and 3D evaluations on all trabecular architecture without destroy the samples, in others words, it is a potential non invasive technique [1,2]. Among the many parameters that can be quantified sand out: BV/TV (bone volume/tissue volume), BS/BV (ration between bone surface to bone volume), TbN (trabecular number), TbTh (the thickness of the trabecular structure) and TbSp (trabecular separation) [3]. This kind of study it has be done since 1989 by Feldkemp[4], with several pathologies, and continuous all long the years in human and animals bone, such rat and mice. This class of analysis is particularly important when it is spoken in osteoporosis. This disease is defined as a systemic skeletal problem characterized by low bone mass and microarchitectural deterioration of bone tissue, resulting in reduced bone strength and increasing risk of fracture [5]. In Brazil, this category of examination starts to be investigation by Alves et al [6] and I. Lima et al [7] with human samples. In this paper it can be seen a continuous study in this subject but now with femurs head on Wistar rat.
2. MATERIALS AND METHODS

The equipment used to do the microtomographies in real time is an X ray microfocus, model is FSX-160-50 and it is in the Nuclear Instrumentation Laboratory at the Federal University of Rio de Janeiro (UFRJ). The system is composed of basically an X ray microfocus source; a detection system that consists in an image intensifier with a CCD camera and a mechanical arm to translated and rotated the sample. The electronic sign, sent by CCD to the monitor, is transformed in digital for an interface. That sign is processed in real time by a microcomputer and presented in a video monitor, being stored under the file form. With the digital sign, the process of the reconstructed begins and this process is based in Feldkamp algorithms [8], which uses the cone-beam reconstruction method to account for the conical geometry of the X rays source. After the reconstruction, that can last of hours to days, depending on the used resolution, the analysis of the obtained images is had.

3. RESULTS

In this paper the images were done in six femurs of Wistar rat samples that were given by the Nutrition Institute at the UFRJ. After the retreat of the bone of each animal, without sample preparation or decalcification, the scan were done in the femurs head of the samples and parameters to the tomography were: 40 kV to the High Voltage, 0.1 mA for the current, 600 projections, 32 pictures and 11.8 magnification factor. The step of each sample rotation was 2° over 360° and the pixel size was 0.143 mm. Later than the images were captured and reconstructed, the regions of interest (ROI) were study. In this stage VOI is determined and corresponds to the maximum possible volume of trabecular bone in the femurs head. This was standardized so that for all specimens the same volume was manually chosen. The cortical was not included in that region, as we can see in the figure 1.

After the definition of the ROI, bone should be separated from the non-bone to proceeds the quantifications. Figure 2 shows the binary and the segmented processes respectively that are needed to do the quantifications of the parameters [9]. In those processes it is necessary determinates the threshold value.

Figure 1. (A) Photo of an example of one sample, (B) the determination of the ROI
The threshold value is an important factor that influences directly in the proposed parameters, as we can see in the figure 3 and 4.

Figure 3. Different threshold values in a bidimensional image
The threshold value was determined based on the procedure used for human bone [10,11]: the same data was analyzed several times by changing only threshold value. Then, BV/TV (that is the main parameter) values were plotted against this threshold value and the partial derivatives calculated ($\frac{\partial[BV/TV]}{\partial[threshold\ value]}$). When this partial derivative was equal to zero (or approximately equal to), changes in the threshold did not induce changes in the bone volume and thus corresponded to the correct value, as we can see on the figure 5.

Figure 4 – Some different threshold values in three-dimensional details images

Figure 5 – Graphic that represents the variations of the BV/TV parameter against some threshold values.
Once choose the threshold value for all the images, the quantifications are had in an area equal to 1.52mm$^2$ (only for the trabecular structures), presented in table 1.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Female</th>
<th>Male</th>
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<tbody>
<tr>
<td>BV/TV (%)</td>
<td>88.3 ± 1.4</td>
<td>88.0 ± 1.2</td>
</tr>
<tr>
<td>BS/BV (mm$^2$/mm$^3$)</td>
<td>81.86 ± 0.54</td>
<td>80.20 ± 2.08</td>
</tr>
<tr>
<td>TbTh (mm)</td>
<td>0.024 ± 0.0006</td>
<td>0.025 ± 0.001</td>
</tr>
<tr>
<td>TbN(mm$^{-1}$)</td>
<td>36.15 ± 0.54</td>
<td>35.30 ± 0.75</td>
</tr>
<tr>
<td>TbSp(mm)</td>
<td>0.003 ± 0.0006</td>
<td>0.003 ± 0.001</td>
</tr>
</tbody>
</table>

The values are Mean ± Standard deviation

The figure 6 shows the 3d visualization of one sample, and it is important to understanding the morphology.

Figure 6 – (A, B) 3D visualization of one sample, (C) the region that was quantified.

4. CONCLUSIONS

The results shown in this paper tells that the 3D-μCT is a non-destructive potential technique able to characterization the internal regions of bone samples (specifically femurs head). The quality of the three-dimensional reconstruction depends directly on the quality of the X rays, of the number of the radiographies and of the precision of the calculations in flotation point of the computer used for the reconstruction. The appearance of some artifacts (as we can see on figure 3, original image) in the reconstruction is function of the number of the radiographies projected. Thus, increasing this number, the occurrence of artifacts is minimized. But as it is not possible to increase that parameter indefinitely, the reconstruction will always present such structures

It was found any significant difference between female and male parameters to $\alpha=0.05$ with a “t” test. The 3D visualization assists in the agreement of the samples morphology a time that in it we have a notion of the internal connection and how the trabecular are linked.
ACKNOWLEDGMENTS

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REFERENCES