RESEARCH ARTICLE

Measurement of trabecular bone parameters with different bone thicknesses and voxel sizes in mice using micro-CT

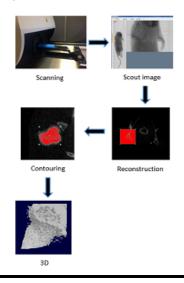Nurin Nadzlah Abu Bakar, Basri Saidi, Lyana Shahirah Mohamad Yamin[*]

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Graphical abstract

Scanning — Scout image — Contouring — Reconstruction — 3D

Abstract

Micro-CT is one of the best modalities in assessing bone morphology and microarchitecture in small animal models. Voxel size is directly related to the image resolution as it influences the bone morphology results. The purpose of this study was to assess the effects of different thicknesses of structures on the trabecular bone qualitative parameters. It was also to find out the most appropriate voxel size when scanning a certain or specific body part with different thicknesses. Five BALB-C breed mice carcasses were scanned using two different voxel sizes of 18 and 35 µm. The scanning acquisition times were recorded to be compared and the trabecular bone parameters measurements were taken. Both trabecular number and trabecular separation were increased in thicker structures meanwhile bone volume fraction and trabecular thickness values were inconsistent with the increment of the structure thickness. The bone volume fraction, trabecular thickness and trabecular separation were higher in larger voxel size and vice versa for trabecular number. The scanning acquisition time has no apparent correlation with the trabecular bone parameters. The thickness of the bone structure did affect trabecular number and trabecular separation significantly but less affecting bone volume fraction and trabecular thickness. All trabecular bone parameters were found affected by the size of scanning voxel size used. The usage of 35 µm voxel was more recommended than 18 µm to save time and give out less radiation dose to specimen unless the detailed features of the trabecular pattern was very important.

Keywords: Micro-CT, trabecular bone parameter, bone thickness, voxel size, mice

INTRODUCTION

Nowadays, micro-CT is widely known for assessing small animal's bone morphology because it can produce high resolution images as there is a big difference in signal contrast between bone and soft tissue (Holdsworth & Thornton, 2002; Li *et al.*, 2008). Numbers of bone morphometry parameters of trabecular bone can be assessed by micro-CT. The parameters that are usually being reported in studies are the bone volume fraction (BV/TV), bone surface to volume ratio (bone specific surface area [BS]/BV), the trabecular number (Tb.N), the trabecular thickness (Tb.Th) and the trabecular separation (Tb.Sp) (Bouxsein *et. al*, 2010).

Micro-CT has few steps in producing cross-sectional images including image acquisition, image processing, analysis of the image, and the yielding of the results. In the image acquisition process, image resolution and voxel size are included. Voxel is the basic element of the scan volume in 3D form that represents two dimensions within the slice and the slice thickness. The sizes of the voxels are directly related to the resolution of the image (Kim *et al.,* 2004).

Usage of smaller voxels can produce higher image resolutions. However, using smaller voxel size takes longer acquisition time and eventually produces more dose which are unnecessary in all examinations. It is said that the primary limitations of micro-CT

imaging are the associated radiation dose and relatively poor soft tissue contrast (Clark & Badea, 2014).

This study investigated the most optimum scanning voxel size to be used when scanning humerus and femur of mice in order to reduce the scanning time according to the voxel sizes used. The general objective for this study was to measure the trabecular bone parameters with different bone thicknesses and voxel sizes in mice using micro-CT.

The specific objectives for this study were to measure the trabecular bone parameters such as bone volume fraction (BV/TV), the trabecular number (Tb.N), the trabecular thickness (Tb.Th) and the trabecular separation (Tb.Sp) in the humerus and femur of mice, in order to assess the effects of different thicknesses of structures on the trabecular bone qualitative parameters, to investigate the effects of different voxel sizes on the trabecular bone qualitative parameters and to correlate between the trabecular bone parameters with scanning acquisition time.

EXPERIMENTAL

Study design

This research was done as an in-vivo experimental study. Five mice carcasses' humerus and femurs were scanned using 2

different isotropic nominal voxel sizes of 18 and 35 µm. Bruker's SKYSCAN 1176 in-vivo micro-CT scanner was used to carry out this experiment in the Research Laboratory of Medical Imaging Department Faculty of Health Sciences UiTM Puncak Alam. The post-processing of image reconstruction process was done in the Medical Imaging Department's research laboratory computer using the software NRecon Version 1.6.4.8 © Skyscan, 2011. In order to obtain the trabecular bone parameters, the software CT Analyser Version 1.16.4.1 © 2003-11 Skyscan, © 2012-16 Bruker microCT was used.

Study scope

The inclusion criteria for the specimens were 6-7 weeks BALB-C breed male mice carcasses. The exclusion criteria were mice with diseases especially bone disease such as osteoporosis or osteoarthritis. Both femur and humerus of the mice bones scanned were further divided into three parts of distal, midshaft and proximal, where head of humerus was considered proximal, the midshaft as the mid part and the epicondyles as distal humerus. Head of femur was regarded as the proximal part, midshaft as the mid part and the condyles as the distal femur. Hence, there were 6 structures to be compared which were the proximal humerus, mid humerus, distal humerus, proximal femur, mid femur and distal femur.

Mice was chosen instead of rat because the bone size of mice is smaller than rat. Smaller size specimens could give out more accurate results in micro-CT. Phantoms could not be used in this study as the results might be different than using real mice due to the different composition. For this study, real bone tissue composition was very crucial as the image of trabecular pattern was more complex in real tissue compared to in phantoms.

Study method

Firstly, the mice carcasses were scanned using the Bruker's SKYSCAN 1176 in-vivo micro-CT scanner. The carcass was put horizontally inside a polystyrene foam tube as shown in Fig.1 to avoid any possibilities of movement during the scan. The specimen was then put on the sample bed and aligned to the vertical axis of the scanner (Nurzawani, Isa, Dom, & Hashim, 2015).
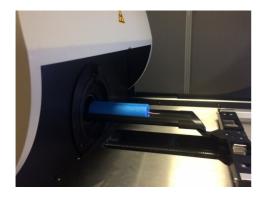

Fig. 1 The carcass was put inside a polystyrene foam and placed on the sample bed of the scanner.

For this whole process, the Control Software for SKYSCAN 1176 was used. Scout image was obtained first as shown in Fig. 2 to view the whole part of the carcass and to select the region of interest to be scanned. On the scout screen, the desired region was selected by drawing a measurement pink line to activate the scan dialog. The filename and data directory were named appropriately.

The scanning was done separately for humerus and femur. Both right and left humerus were scanned together, then followed by another scanning of both right and left femur of the same carcass. This was done by using the same voxel size. After this was done, the same procedure was repeated using a different voxel size. The scanning acquisition time was recorded. All of these steps were repeated for the remaining carcasses.

The exposure parameters settings were automatically set by the scanner according to the voxel size used. For voxel size 18µm, the

average exposure selected was 76kV and 230µA while for voxel size 35µm, the average exposure used was 70 kV and 226 µA. After the scanning was completed, NRecon software was executed to start by reconstructing the images into axial cross-sections. The bone dataset was loaded. Preview was done prior to the real reconstruction process. In order to avoid blurring or doubling of the image, alignment was done. All parameters adjustments were done manually by trial-and-error method to get the optimum image to be analysed (Nurzawani *et al.,* 2015).
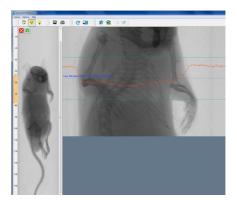

Fig. 2 The whole specimen was scanned to obtain the scout image before continuing with scanning only the desired part.

After reconstruction process, CT Analyser software was opened to get the measurement readings of trabecular bone parameters. The desired slice of each image dataset was chosen to undergo segmentation process qualitatively where the region of interest (ROI) was selected by the operator using naked eyes and experience. In this process, a method called contouring was done where a certain shape would be masked around all the bone area of interest. This was done to define the area in each slice to be included for segmentation (Bouxsein *et al.,* 2010). The contouring was done by hand drawing the area of interest inside the bone excluding the cortex as shown in an example in Fig. 3 below.
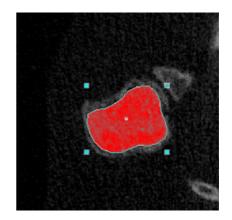

Fig. 3 The freehand drawing of contouring the trabecular bone part not including the cortical shell.

Next, thresholding was done manually and kept constant for all images with the range of 50 to 100 chosen. This step was done to extract the pixels from the images which represented the trabecular patterns. 3D analysis was then done to get all the bone parameters measurements. The acquisition time was also recorded to compare from one scanning to another. The results were then used to estimate the amount of dose of one scanning as the longer the acquisition time, the higher the radiation dose.

Statistical analysis

All results were analysed using the Statistical Package for Social Science (SPSS) version 20.0 for Windows. The means were calculated for all parameter measurements. One-way analysis of

variance (ANOVA) was used to test the differences in trabecular bone parameters between the six structures as well as to test differences between the two voxel sizes. Pearson correlation test was used to study the correlation between the trabecular bone parameters and scanning acquisition time. The correlation between the micro-CT scanning voxel size and scanning time was also investigated. A P value of < 0.05 was considered to be significant.

RESULTS AND DISCUSSION

Descriptive analysis

Fig. 4 below shows the 3D reconstructions of trabecular bone volume from the same distal femur analyzed from scans using two different voxel sizes of 18 and 35 µm. It could be visualized that the bone details using 18 µm voxel size showed more detailed trabecular bone patterns compared to the 35 µm.
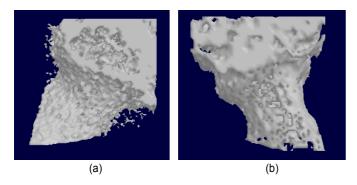

| (a) | (b) |

Fig. 4 The three-dimensional reconstructions of the trabecular bones of distal femur (a) using 18 µm voxel size (b) using 35 µm voxel size.

Table 1 below shows the data collected from the experiment carried out in this study.

Table 1 The results of trabecular bone parameters at different bone regions using different voxel sizes.

		Humerus		Femur	
VOXEL SIZE (µm)		**18**	**35**	**18**	**35**
DISTAL	BV/TV (ratio)	0.44425	0.61141	0.59991	0.87829
	Tb.N (1/mm)	1.69998	1.45031	2.00227	1.79637
	Tb.Th (mm)	0.06776	0.09461	0.10231	0.14158
	Tb.Sp (mm)	0.66261	0.78106	0.98127	L24864
MID	BV/TV (ratio)	0.24074	0.47261	0.37776	0.53486
	Tb.N (1/mm)	1.29286	1.02018	1.52475	1.131302
	Tb.Th (mm)	0.04513	0.07106	0.05979	0.08672
	Tb.Sp (mm)	0.44260	0.60894	0.53269	0.70964
PROXIMAL	BV/TV (ratio)	0.62484	0.82123	0.34139	0.54599
	Tb.N (1/mm)	1.98446	1.62294	1.51793	1.29288
	Tb.Th (mm)	0.07346	0.12089	0.05605	0.07626
	Tb.Sp (mm)	0.6769	0.92795	0A9987	0.64261
SCANNING TIME (min)		12.8	9.8	16.6	10.6

Table 1 shows the results of trabecular bone parameters at three different regions of the humerus and femur scanned at scanning voxel sizes of 18 and 35 µm. The results were expressed as mean of both left and right humerus and femur of five mice carcasses. The charts were divided according to their respective morphometric parameters and divided into two parts of humerus and femur.

Relationship between structure thickness and trabecular bone parameters

A one-way ANOVA was conducted to compare the effect of thickness of structure on the trabecular bone parameters. It was suggested that the structure thickness did not impose any significant effect on BV/TV and Tb.Th (p = 0.178 and p = 0.201, respectively). For Tb.Sp and Tb.N, the differences were significantly affected by the structure thickness (p < 0.05).

Not all trabecular bone parameters were affected by the thickness of the structure. For instance, the bone thickness did not affect BV/TV and Tb.Th due to the fact that the thickness of the structure did not guarantee the thickness of the trabeculae. The research done in 2007 was found that the correlation of BV/TV between sites was weak to moderate (Bae et al., 2010; Eckstein et al., 2007). Kennedy et al., (2009) discovered that the trabecular bone was varied from one site in the bone to another. Meanwhile the Tb.N was found to be directly related to structure thickness while Tb.Sp was found to be inversely proportional to Tb.N.

In answering the study objectives, the difference in structure thickness did affect the trabecular bone parameter measurements. As different regions of the bone were made up of different compositions, the number of trabeculae was also different. The amounts of external mechanical loadings and principal stress trajectories projected onto a certain part of the bone were affected the thickness of the structure as the bone adapted to them. This attribute led to significant difference in trabecular bone parameter measurements (Boyle & Kim, 2011)

Relationship between scanning voxel size and trabecular bone parameters

Another one-way ANOVA was carried out to study the effect of voxel sizes on the trabecular bone parameters measurements. It was found that the voxel size has significant effect on BV/TV and Tb.Th with p < 0.05 for both, respectively. On the other hand, it was suggested that Tb.N and Tb.Sp were not significantly related to voxel size with p = 0.143 and p = 0.167, respectively.

BV/TV, Tb.Th and Tb.Sp were found to increase along with increment in scanning voxel size but otherwise for Tb.N. Higher voxel size resulted in lower spatial resolution where there was higher risk of loss of trabeculae or fusion at nearby branches of trabeculae due to thickening. This resulted in fluctuation in Tb.N as well as occurrence of the separation of trabeculae. Consistent with the present results, Kim *et al*., (2004) found that the degradation of the trabecular bone parameters was due to separation of the trabeculae where it was highly suggested because of loss or fusion of the trabeculae as the voxel sizes increased. It was found that the BV/TV was increased as the voxel size was started to be larger within the same volume of interest (Kothari et al., 1998).

For BV/TV and Tb.Th, the scanning voxel size did affect these parameters significantly but not for Tb.N and Tb.Sp where they were not strongly dependent on the scanning voxel size. These results were highly supported by the studies done by Christiansen (2016) where the effects of micro-CT scanning voxel size and different segmentation methods on trabecular bone microstructure measurements were studied. It was found that the Tb.Th, connectivity density, and bone tissue mineral density were strongly depended on scanning voxel size, regardless of the segmentation method utilized. In contrast, Tb.N, Tb.Sp, and apparent bone mineral density were not strongly dependent on scan resolution for voxel sizes. As for voxel size of 30 µm, it might give out poor results of trabecular bone parameters.

Correlation between scan acquisition time and trabecular bone parameters

In addition, according to Pearson correlation test conducted to investigate the correlation between the scanning acquisition time and each of the trabecular bone parameters, there was not a significant, negative fair correlation between time and BV/TV with p = 0.108 and r = -0.488. It was also found that there were not significant, positive fair correlation between time and Tb.N with p = 0.184 and r = 0.412, negative fair correlation with Tb.Th with p= 0.179 and r = -0.416 and negative poor correlation with Tb.Sp with p = 0.371 and r = -0.284.

As scanning voxel size could determine the level of image quality, it would generally take longer time for the micro-CT to scan and resolve the image when smaller voxel size was chosen instead of a larger one. It was also found that it took longer time to scan femur than humerus. This was most likely due to the length of the scanning chosen by operator which was longer in femur than humerus. Thus, as longer scanning measurement was chosen, longer time was taken to scan the structure (Christiansen, 2016; Clark & Badea, 2014)

The mouse structure could also affect the results. Since the circumference of the mouse front limbs were narrower compared to the hind limbs area, it was understandable that the scanning of the whole 360^0 rotation would take longer for hind limbs. It could also be due to the computer system where sometimes there was delay that could take up to half a minute to resolve after just finishing one scanning and continuing with another (Baum et al., 2010)

It could be concluded that all the trabecular bone parameters did not have a significant correlation with scanning acquisition time. The correlation with BV/TV was negative and fair, as well as with Tb.Th. There was a positive fair correlation between scanning acquisition time and Tb.N but a negative poor correlation with Tb.Sp. A positive correlation was meant that as time was increased, the parameter measurement was also increased as well while a negative correlation was meant that as time was increased, the parameter value was decreased. There was no any good or strong correlation between time and any of the trabecular bone parameter measurement. This could be concluded that longer scanning time did not ensure a dramatic difference in the trabecular bone parameter readings.

From Table 1, it showed that the scanning voxel size was directly related to the scanning time where smaller voxel size could consume longer scanning time. For the whole humerus region, the difference of the average scanning acquisition time between the voxel size 18 and 35 µm was 3 minutes while it was 6 minutes for femur. This was proven that different voxel sizes would take up different scanning time consumption.

An indirect result of this study was that it also gave results about the radiation dose irradiated onto the specimens. It was important to note that in taking the exposure parameters into consideration when scanning with smaller voxel size, higher source current and voltage settings were automatically selected. This was directly meant that smaller voxel size did not only consume more time but it also exhibited higher radiation dose to the specimens.

CONCLUSION

From this study, it could be concluded that the structure of the thickness affected the trabecular bone parameters measurement. Generally, all the morphometry bone readings was favoured for thicker structure. This was because of the trabeculae proportion that was higher in amount for the thicker structure due to the larger amount of mechanical force was put onto it. The second conclusion that could be made from this study was the usage of 18 µm voxel size would consume slightly longer scanning time to acquire series of images compared to the usage of 35 µm. Depending on the results from the statistical analysis, the bone morphometry measurements

between the two voxel sizes were not very apparent, meaning that 18 µm voxel size did not produce a dramatically better image than 35 µm. Thus, 35 µm voxel size was more recommended to be used than 18 µm as it would save time and produce quite similar quality image unless minute details were very necessary.

ACKNOWLEDGEMENT

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