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BONY TRABECULAR MICRO-STRUCTURE AND POROSITY WITH AGE IN YOUNG RABBIT'S FEMUR: IN-VIVO STUDY

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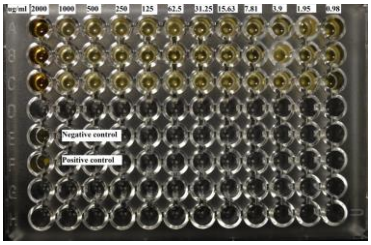
Graphical abstract



Abstract

The established of micro computed tomography (micro-CT) analysis software make it compatible to use to determine abnormality of bone morphology. This study investigated 90 new rabbits for bony trabecular microstructure and porosity after their dams were exposed to ultrasound at the second stage of pregnancy (duration - 90 minutes; frequency - 7.09 MHz; spatial peak temporal average intensity (SPTA) - 49.4 W/cm2; power - 56 W; thermal index (TI) - 0.2; mechanical index (MI) - 1.0). A femur of five groups of litters (n = 18 litters per group): 1, 2, 3, 4 and 5 month-old was excised and scanned using SkyscanTM 1176. For total porosity, a significant difference was shown in at two month-old group ($p = 0.017$). A significant difference was also shown in bony trabecular thickness for three month-old group ($p < 0.05$). For trabecular separation, there was a significant difference at four month-old group ($p = 0.040$). This study suggested that there might be some significant differences in bony trabecular structure and total porosity with ages. This may be due to heat created by ultrasound exposure, which can apply effect to bone morphology. To determine whether the findings are applicable to human, clinical trials should be carried out in the future.

Keywords: Micro-CT, bone morphology, bony trabecular structure, total porosity

1.0 INTRODUCTION

In the research of animal models of skeletal disease, imaging modalities such as computed tomography (CT) and magnetic resonance imaging (MRI) have been increasingly utilised [1]. They arise as possible methods for determining both bone density and structure [2]. In veterinary practice, radiological examination of animal musculoskeletal systems has also been used, commonly for the assessment of musculoskeletal function and the diagnosis musculoskeletal pathologies. Meanwhile, rabbits are the most commonly used subject for orthopaedic research. To detect any abnormalities on the bone rabbit femurs are often used [1].

Current studies worldwide have been focusing on measuring trabecular bone structure noninvasively. The high-resolution micro-computed tomography (micro-CT) system has now become an important tool for biological research. The micro-CT system facilitates a non-invasive study to screen anatomical changes in small animals [3]. Numerous parameters measured by micro-CT analysis software are being used to scrutinize anomalies of bone morphology. In this study, only selected parameters were analysed. These parameters were percentage of bone volume, total porosity, trabecular thickness distribution range (Tb.Th) and trabecular separation distribution.

2.0 LITERATURE REVIEW

2.1 Ultrasound Effect

In the late 1950s, ultrasound was used for diagnostic imaging, and many researches were carried out on the effect of ultrasound exposure on foetal development [4], [5], [6]. For this study, the basic B-mode diagnostic ultrasound system was applied, because the method is widely used for clinical purposes. The frequencies of the ultrasound system range between 2 and 12 megahertz (MHz). However, it is not possible to completely eliminate the possibility of adverse effects of ultrasound [7] [8], especially in undeveloped tissues, and it cannot be regarded as completely innocuous [9]. If the study results show no significant biological effect, it does not conclude that the effects are not present. A recent study [10] concluded that if ultrasound scans did affect foetal grow, it is more likely to have an effect on bone growth. Even thoughthere is lack of credible and substantiated evidence of the adverse effects on foetus, little information was obtained on the teratogenic effects for the safe utilization of diagnostic ultrasound [7].

2.2 In-Vivo Studies

Based on available research materials, the main areas of concern are congenital malformation, reproductive loss, neoplasm incidence, and genetic damage. A lot of in-vivo studies have been carried out using drosophila, rats, mice, chicken, frogs, guinea pigs, rabbits, sheep, pigs and monkeys [11]. However, for research purposes, as their physiology that is quite similar to that of humans [12], rabbits are most commonly used for in-vivo experimental studies. This is due to the possibility of drawing a sensible correlation of the result to humans [12]. Rabbit is mild-tempered, tame, and easy to handle and keep. At the same time, rabbit has a high reproductive potential [12], [13]. Rabbit was chosen because of the local trend that regards rabbit as a domestic pet; therefore, there is easy access to information on rabbits, their food supply, and health care requirements. Effects on various types of blood cells, bacteria, and single-cell organisms have also been studied. Since experimental conditions varied due to a number of reasons, there is great difficulty in relating their results in a consistent pattern.

2.3 Micro-Computed Tomography

Since 1989, micro-computed tomography (μCT) has become the gold standard for assessing the 3D architecture of trabecular bone. With little or no preparation of the sample μCT can provide non-destructive quantitative results. Since the application of μCT to trabecular bone has taken place, compact cortical bone study has become more limited. It is because the cortical bone of many vertebrates has a complex and dynamic microstructure that continually remodels throughout life [3].

μCT technique has been used in the study in calvarial bone defect models. The main advantages of using μCT is we can detect any bone rejuvenation that would be the non-appearance of extensive sample preparation as would be required during histological analysis. Previous study shown that μCT forms the main analytical tool in their experiment; its usage was furthered by the approach to track down spatial bone regeneration patterns within the defect [3].

3.0 METHODOLOGY

This in-vivo experimental study utilized six of 5 month-old New Zealand white rabbits (Oryctolagus Cuniculus), specially breed for research purposes [14] [15]. This study obtained approval from the community of Animal Research Ethics (CARE) UiTM in June 2012. Rabbit selection was made based on its appropriateness to the research plan. The rabbits were kept in a temperature-controlled room in separate cages with the ratio of 1:3 (male to female). The cages were placed in the Animal Handling Research Laboratory of Universiti Teknologi MARA (UiTM), Puncak Alam. Mating was done in male rabbits' cages. Once completed, female rabbits were returned to their cages where they would be monitored until it was time for full-term delivery. The first day of conception was determined after ovulation, which occurred 12 hours after mating.

Philips HD3 ultrasound machine from Koninklike Philips N.V, Netherlands 2D was used for this study. It operates on B mode pulsed-ultrasound. All ultrasound parameters such as focal distance, transducer frequency, mechanical index (MI), and thermal index (TI) were kept constant. The focal distance was set to 4.5 cm. A linear array transducer was used. L 5-9 is able to transmit frequency between 5 MHz and 9 MHz. The TI and MI displayed by the output display throughout the experiment demonstrated the value of 0.2 and 1.0, respectively. This in-vivo experimental study involved three groups of pregnant rabbits; T for the treated group, S for the sham group, and O for the control group. A 90-minute, real ultrasound exposure was given during the second trimester of pregnancy to the rabbits from the T group, while the rabbits from the S group received only simulated ultrasound exposure. No ultrasound exposure was given to the O group. Following this, all pregnant rabbits were returned to their individual cages while waiting for normal delivery which normally takes 31 to 33 days [16] [17].

All 90 young rabbits were treated equally and labelled accordingly. 6 kittens of each group control, sham and treated within (n - 18) the age of 1, 2, 3, 4 and 5 month were sacrificed after being euthanized using pentobarbital sodium, dolethal 250 mL from Ethical Agents LTD.NZ, 1mL/kg body weight by

intravenous injection or 0.75 mL/kg intra-cardiac injection [16]. Next, the femurs were excised. The femurs were also scanned with high-resolution microtomography system SkyscanTM 1176 at a source voltage of 100 kV, at a source current of 100 µA, and using an aluminium filter of 0.5 mm. Each sample was rotated until 180 degrees with a rotation step of 0.9 degrees and a frame averaging of 4. The pixel size was 9.5 µm. The reconstructions were performed using NRecon software (version 1.6.2.0) and the resulted JPEG images had 4000 × 4000 pixels with a pixel size of 6.5 µm. No correction was applied except for specific misalignment for each acquisition and a moderate ring artefact reduction. The image datasets in 8-bit JPEG format (approximately 1200 images) were analysed and binarized by CTAn software (version 1.10.1.3 -Skyscan, Belgium). In order to compare with the control and the sham groups, the data were analysed using SPSS version 21 to look for correlations of trabecular bone structure with young rabbit's age and porosity. Figure 1 shows the Rabbit Femur Scanning Skyscan 1176TM Micro CT Imaging Unit while Figure 2 represents the Scanning and Reconstruction using CTAn software (version 1.10.1.3 -Skyscan, Belgium).

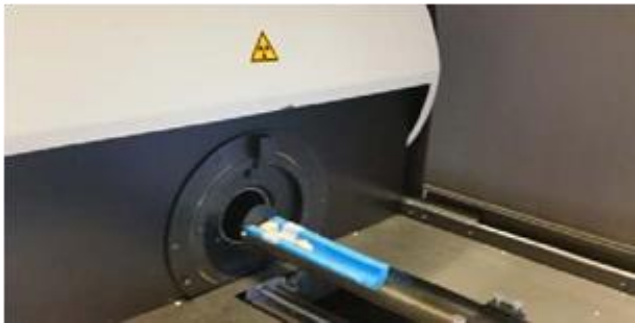


Figure 1 Rabbit Femur Scanning Skyscan 1176TM Micro CT Imaging Unit

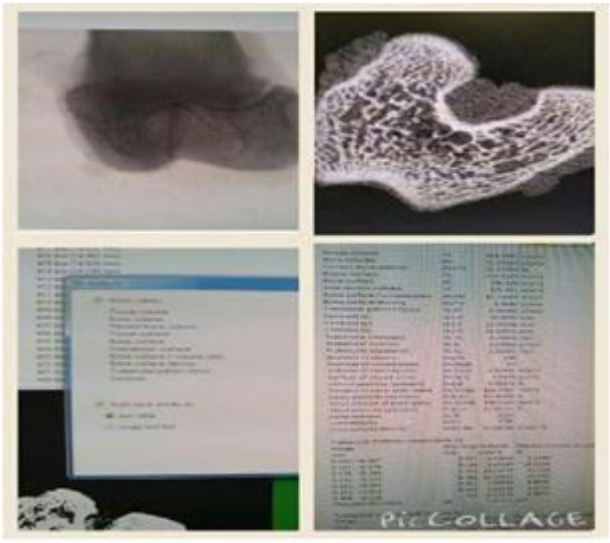


Figure 2 Scanning and Reconstruction using CTAn software (version 1.10.1.3 -Skyscan, Belgium)

The Non Parametric Test (Kruskal-Wallis) for the total porosity, trabecular thickness and trabecular separation have shown significant differences at 2, 3 and 4 month-old with p-values less than 0.05 (P < 0.05), Table 1. The results indicated that there was significant different in the total porosity at 2 month-old of young rabbits (p - 0.017). A significant difference was also shown in bony trabecular thickness for 3 month-old group (p < 0.05). For trabecular separation, there was a significant difference at 4 month-old group (p - 0.040). The correlation test results showed negative association between the total porosity, trabecular thickness and trabecular separation with ages p values less than 0.05 (P < 0.05), Table 2.

Table 1 Non Parametric Test (Kruskal -Wallis) for the total porosity, trabecular thickness and trabecular separation

Young Rabbit's Age/Parameters	Total Porosity (%)	Trabecular Thickness (mm)	Trabecular Separation (mm)
		P-value	
1 month	0.144	0.157	0.154
2 month	0.017*	0.390	0.260
3 month	0.125	< 0.05 *	0.117
4 month	0.143	0.320	0.040*
5 month	0.104	0.076	0.075

*p<0.05 shows a significant difference in mean values between the control and exposed.

Table 2 Correlation test for the total porosity, trabecular thickness and trabecular separation

Young Rabbit's Age/Parameters	Total Porosity (%)	Trabecular Thickness (mm)	Trabecular Separation (mm)
		P-value coefficient	
1 month	0.570 (-0.144)	0.533 (-0.157)	0.542 (0.154)
2 month	0.947 (0.017)	0.108 (-0.391)	0.286 (0.266)
3 month	0.620 (0.125)	1.000 (0.001)	0.645 (-0.117)
4 month	0.571 (0.143)	0.195 (0.320)	0.862 (-0.040)
5 month	0.682 (0.104)	0.075 (0.076)	0.0766 (0.075)

*P<0.05 shows a significant association in mean values between the control and exposed

4.0 DISCUSSION

This study suggested that there might be some significant differences in bony trabecular structure and total porosity with ages. The results may indicate that micro-CT may be useful for quantifying the difference in trabecular structure. The poor correlation in most of the parameters is predictable. This is because trabecular bone changes may be associated with cortical bone changes, but may not follow with age [2]. The poor correlation in this study could be due to the possibility of bone becoming osteoporotic with the increasing age. M.G Mullender *et al*. explained that the bone formation and wall thickness mean decreased in osteoporotic patients compared to normal patients [17]. A.J Bailey *et al*. stated [18] that a change in the material quality of the bone may reduce the bone collagen. It likely will indicate a relation in bone mineralization during aging. Meanwhile, P. Lip *et al*. showed that wall thickness mean in trabecular bone decreased with age [19].

The finding of this study may suggest that due to some external factors (for example, prenatal ultrasound effect), trabecular bone changes will decrease. J.L Kuhn *et al*. [20] recommended that single trabecular structures should be tested accordingly in order to notice subtle alterations in the material quality of cancellous bone. In previous laboratory studies have affirmed that there are moderate to strong correlations between trabecular bone architecture and biomechanical properties of trabecular bone. Nevertheless, trabecular bone microarchitecture characters are strongly correlated with trabecular bone mass. Previous clinical studies showing changes in trabecular microarchitecture with iliac bone biopsies in subjects with fragility fractures compared to age-matched controls with no fractures [21].

Nonetheless, this study has its own limitations. The first limitation was the small number of rabbits used in this study due to budget constraints. Secondly, this study assumed that all subjects have a "normal life" even though it is not always true. Even if the researcher uses the same room temperature, air quality, palette, and drinking water, as well as treatment for parasite and ringworm infections, some rabbits may react differently throughout the course of the study [15].

5.0 CONCLUSION

This fundamental in-vivo experimental study suggests that there might be some significant of trabecular bone structure and porosity with age. Although there are many modalities such as MRI in the current technologies, micro-CT with its own capabilities may have the potential in calculating several parameters of the bone, especially in animal studies. By providing high-resolution images, the reciprocal role of micro-CT mostly benefits the imaging process in micro-CT imaging. The ability of micro-CT standard configuration method will help in future research [3]. Clinical trials should be carried out in the future to determine whether the same findings are applicable to human.

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