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# Influence of bone marrow characteristic and trabecular bone morphology on bone remodelling process with FSI approach

**AAR Rabiatul[1], Devi Rianti[2*], SJ Fatihhi[3], Amir Putra Md Saad[4], Zulfadzli Zakaria[1], Anita Yuliati[3], MN Harun[2,5], MRA Kadir[5,6], Andreas Öchsner[7], Tunku Kamarul[8], Khalid M Saqr[9]**
GQ1 **and Ardiyansyah Syahrom[1,2*]**

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GQ2 **Abstract**
GQ4 While doing daily physiological activities, the trabecular bone will experience a certain amount of deformation which leads
GQ5 to the bone marrow movement. The movement can affect the bone remodelling process and the properties of the bone itself. The bone marrow plays a role as a hydraulic stiffening of the trabecular structure. However, previous studies analysed on trabecular bone and bone marrow separately, which is not considered as the actual condition. Thus, it is crucial to consider combine analyses of the bone marrow with the trabecular structure simultaneous. The aim of this study is to investigate the effect of bone marrow on the mechanical environment and the structure of trabecular bone during normal walking loading. Hence, this study used the Fluid-Structure Interaction (FSI) approach as a finite element method to discover the effect of bone marrow to the trabecular structure and vice versa. The findings show the shear stress value along normal walking phase was found in a range of 0.01–0.27 Pa which is sufficient to regulated cell response minimally. This study provides insight into understanding the related mechanobiological responds towards supply of nutrients onto bone cells.

GQ4 = All Artistic Work Originally by our work

**Keywords**
Fluid Structure Interaction, Trabecular Bone, Bone Marrow, Shear Stress, Stiffness, Bone Remodelling

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## Introduction

Physiological loading induced trabecular bone deformation that leads to the bone marrow movement within the porous structure which contribute to stimulating the osteogenic response to the bone cells.[1] The forces from the physiological loading cause both small strain and shear stress which known as a key to initiate the bone remodelling process.[2–5] Currently, researchers tried to discover the actual value necessary to stimulate the bone cells for the bone remodelling process. To this date, experimental and simulation study have been performing in order to capture the value of these biomechanical stimuli that encourage the remodelling process.[6–8] Bone marrow is a prime component in trabecular bone, in which it accommodates bone predecessors' cells for bone remodelling. Thus, it is important to consider it presence to better represent the actual conditions of trabecular bone. Therefore, knowledge of biomechanical environment that occur within the trabecular bone during daily physiological activities is necessary to comprehend on how the bone marrow can affect the bone remodelling.

Physiological loading includes daily activities such as house chores, daily walking, and sports activity helps in

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maintaining the bone health by transferring force to the bone structure. These can be seen on immobilization and bedrest individual that had reduce in their bone mass.[9,10] These relationship of loading and bone formation had been support with the Wolff's law since 1892.[11] The trabecular bone experience compression and tension in the microstructure due to the loading causing the micro strain which one of the mechanical stimuli. Previous study reported that the physiological activities initiate in range of 0.001–0.003 mm/mm on the trabecular structure.[12,13] However, minimal physiological activity cause 1000με which cause bone resorption process higher compare to the bone resorption.[14] Amazingly the bone can heal itself when there is external loads act upon the cells which can help in cells excite by signalling to the bones to start building themselves up. Then again, the loads from human daily life will also initiate the movement of bone marrow within the structure which cause the shear stress that act as response to the remodelling process.[15]

The osteogenic response include osteoprogenitor cells secrete autocrine factors, for example prostaglandins $E_2$ ($PGE_2$) and nitric oxide (NO), which can regulate the remodelling activity.[16] In addition, the proliferation rates have been found increasing when the bone marrow stromal cells were exposed to the fluid flow, which means higher number of cells participate in bone formation.[17] The mesenchymal stem cells (MSCs) has been actively investigate in experiment and simulation due to its ability to differentiate into other cells such as osteoblast (bone cells), chondrocytes (cartilage cells) and adipocytes (fat cells).[18–20] The shear stress known as one of the parameters required for the MSCs in the bone marrow to differentiate and assists the remodelling activity.[20,21] The range of shear stress need for the cells to response mention by previous study is about 0.02 to 1.0 Pa.[2,22–25] Undoubtedly, bone remodelling process also requires adequate nutrient transport through the bone cells. These were also with help of bone marrow which function to transport the nutrient and remove waste. However, knowledge on how shear stress value contributing in MSCs to differentiate to different cells are still shallow.

In the present work the movement of the bone marrow regulate osteogenic responds which relate to the trabecular bone deformation due to the physiological activity. Thus far, the is no study using physiological gait loading as boundary to examine the effect of interaction on mechanical stimulus and trabecular bone. Therefore, the aim of this study is to investigate the effect of bone marrow mechanical environment and trabecular bone structure during normal walking loading. A fluid structure interaction (FSI) approach was applied to determine the deformation of trabecular bone with corresponding of marrow shear stress in bone remodelling activity.

## Materials and methods

### Sample preparation

The fresh bovine femur bones were harvested from the local slaughterhouse and kept frozen at −18°C to 26°C. Specimens of trabecular bone were taken by using a Bosch circular saw with copious water irrigation. The femur bone was then divided and cut into a section of medial condyle, femoral neck and femoral ball with the vertical orientation due to the maximum extension of knee joint occurred. The trabecular bone was then again cut into a cubic shape (10mm × 10mm × 17mm) in length by using a precision cutting tool (Allied Techcut, USA). The precision cutter consists of diamond-resin bonded wafering blade with a minimum speed of 150–250 rpm with continuously water irrigation to prevent heat-related damages. Then, the specimens were placed in small airtight plastic bag with the purpose of reducing the thermal cycling and stored in the freezer with a temperature below −26°C. After that, the specimens will go through next procedure using the ultrasonic cleaner (Crest ultrasonic, model P11000SR, USA) additional with a chemical detergent (Pumicizedcitrius, Gent-l-kleen,USA) to cleaned from marrow. The specimens were then submerged for about 10–15 min at a temperature below 46°C. In order to remove the loose particles and excessive marrow, the specimens were then air-jetted and vacuumed suction. This procedure was repeated until all excessive marrow is removed (Fatihhi SJ et al. 2015). A custom jig was used to align the specimen for improved vertical oriented. Afterwards, the specimens sealed in an airtight bag placed in a −20° C freezer and frozen overnight while the adhesive completely cured. Only then, the samples are scan by using the μ-CT scanner (SkyScan 1172, Bruker MicroCT, Belgium).

### Model development

The two-dimensional image data sets from the μ-CT scan were stacked in sequence by Mimics software (MIMICS 12, Materialise, Belgium) and converted into rectangular shape to construct the trabecular model. The thickness of each images slice is 15 μm. The stacked image datasets were calculated into three-dimensional trabecular model through image segmentation by the Mimics software. Subsequently, the image datasets were thresholded to select the region of interest for three-dimensional constructed model. In addition, by using an adapted marching cubes algorithm, the triangular surface meshes were generated for the trabecular model. Then again, the result of triangular surface mesh was very fine, which needed to follow with step of removing noise, redundant parts and irregularities shape to construct accurate three-dimensional models. There were 10 models generated and tested in this study.

As for finite element analysis simulation, small sized sub volume region of interest was selected from the fine mesh trabecular bone constructed models (Figure 1) due to the limitation of computer capability to complete the simulation study. These models were then converted into finite element mesh for the simulation. In addition, the trabecular bone model surface meshes with jagged or bad sector are also repaired before importing the

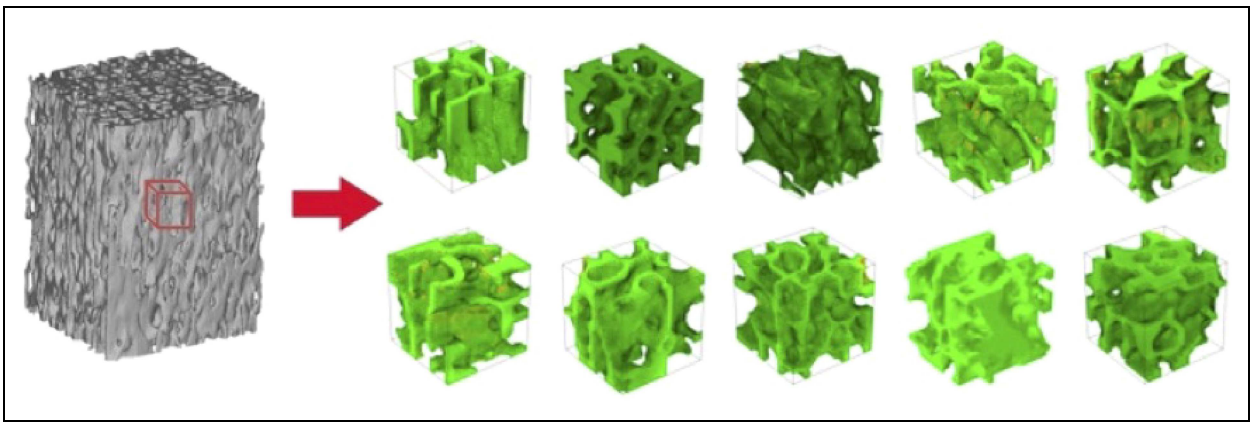

**Figure 1.** Development of three-dimensional model into sub volume model.

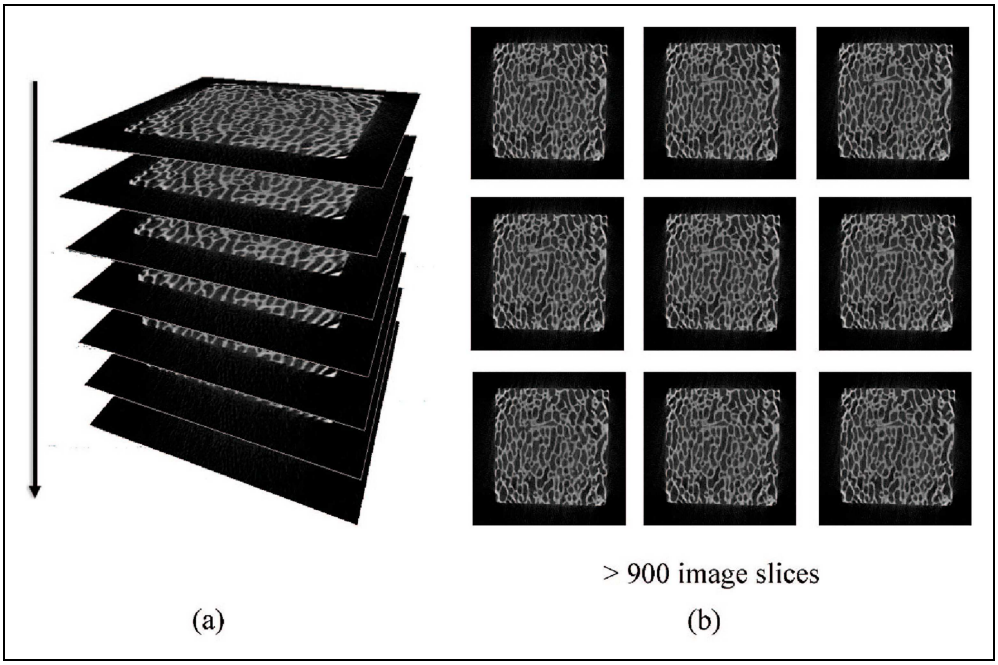


**Figure 2.** Images obtain from μ-CT scan with (a) images stacked in sequence according to sample orientation (b) raw scanned images file.

model. For the FSI study purpose, the outer wall of the models needed to convert into a flat surface. Thus, for the model preparation, the smaller size sub-models were then merged with cube surface mesh in the Mimics software. After that, the uneven surface of trabecular sub volume models was removed according to the sub volume model shape. Then, the space between the sub model and the cube were stitch together by creating a triangular mesh in between the space. These steps were applied to all six surfaces for the sub trabecular model. Finally, the surface mesh is exported to an STL format file.

*Morphology study.* From the μ-CT scan images, the morphological study was conducted. One of a trabecular bone sample contains approximately 900 image slices (Figure 2 (b)). The morphological indices were measured using ImageJ (ImageJ, National Institute of Health, USA). All these slices were import and stacked (Figure 2 (a)) by using BoneJ plugins in ImageJ software to obtain the trabecular morphological data. The parameters measured included BV/TV, Tb.Th, Tb.Sp, Tb.N, DA, MIL, etc. All data present in Table 1.

## Computational simulation

Two-way fluid-structure analysis were conducted using COMSOL Multiphysics software with purpose of investigate the fluid behaviour of bone marrow under gait loading conditions. A gait loading which representing normal walking were applied through the cap faces feature which considered as a rigid body. The gait loading (Figure 3) applied in multi-axis according to normal walking phase.[26] The normal walking phase of

**Table 1.** Morphological indices of trabecular bone sample.

| Parameter | Minimum | Maximum | Mean | SD |
|---|---|---|---|---|
| BV/TV | 0.318 | 0.477 | 0.379 | 0.057 |
| Tb.Th (mm) | 0.128 | 0.559 | 0.207 | 0.057 |
| Tb.Sp (mm) | 0.253 | 1.022 | 0.441 | 0.137 |
| BS/BV | 11.313 | 15.857 | 13.677 | 1.719 |
| DA | 0.38 | 0.684 | 0.611 | 0.146 |
| Conn.D ($mm^{-3}$) | 19.625 | 59.875 | 37.975 | 14.179 |
| SMI | 0.875 | 1.918 | 1.416 | 0.316 |
| Porosity (%) | 62 | 76 | 70 | 5 |
| Bone Surface Area ($mm^2$) | 28.802 | 37.518 | 32.447 | 3.134 |

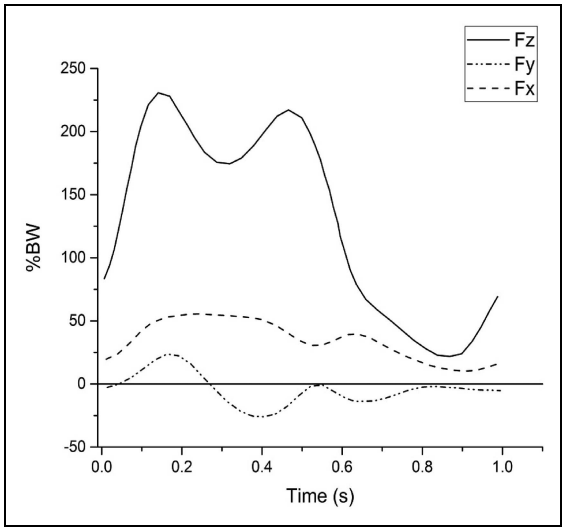


**Figure 3.** Gait loading of normal walking based on body weight percentage (bergmann G. *et al.*, 2001).

gait cycle was divided into 40 discrete points for the simulation. The cap faces feature is vital in the FSI study due to restriction coupling between the trabecular model and marrow model within the FEA. In addition, the prescribed displacement was applied to the cap, where the domain was restricted in the X and Y directions (Figure 4). As for the fluid boundary, the plane of the bottom boundary of fluid was applied as symmetry in order to ensure that the marrow volume remains within the domain when there is load applied through the structure. Moreover, in order to prevent normal velocity to the respective boundary, the marrow flow was model as symmetric in their normal directions. The convergence analysis in this study conclude that 400 thousand tetrahedral elements and shape function was used tessellation method is Delaunay on average were needed for accurate result computation (see Figure 5). In addition, the FSI interface uses an arbitrary Lagrangian-Eulerian (ALE) method, which allows moving boundaries without the need for the mesh movement to follow the material. This ALE method combined the fluid flow formulated using a Eulerian description and a spatial frame with solid mechanics formulated using a Lagrangian description and a material frame. The analysis was performed with the criterion of the von Mises stress criterion less than 5%.

The time-dependent solution is obtained for every gait cycle. Details on the force parameters implemented in this study was demonstrated in.[27] The solid trabecular structures were modelled as a linear elastic material.[28] An elastic modulus ($E$) of 1000 MPa[29] and Poison's ratio of 0.3 was attributed to the trabecular bone solid structure.[30] Additionally, the viscosity of fluid marrow was assigned 0.4 Pa.s and modelled as incompressible according to Bryant et al.[31] Newtonian fluid with density of 1060 kg/$m^3$.[32] The surface between the trabecular structure and marrow fluid is assigned as no-slip boundary.

In the present work, the bone marrow was modelled as an incompressible liquid. The incompressible Navier-Stokes equation was considered as the governing equation, in which;

$$\nabla \cdot u_{fluid} = 0 \tag{1}$$

On the other hand, the momentum equation was as follows;

$$\rho \frac{\partial u_{fluid}}{\partial t} + \rho(u_{fluid} \cdot \nabla)u_{fluid} = \nabla \cdot [-pI + \mu(\nabla u_{fluid} + (\nabla u_{fluid})^T)] + F \tag{2}$$

where the external force acting on the fluid was denoted by **F**, and gravity was neglected. Meanwhile, equation for solid at local equilibrium is given by;

$$\rho \frac{\partial^2 u_{solid}}{\partial t^2} - \nabla \cdot \sigma = F_v \tag{3}$$

where $\sigma$ and $F_v$ are the Cauchy stress tensor and body force, respectively. Deformed structure was demonstrated by $u_{solid}$, whereas the Piola-Kirchhoff stress, S was used to calculate the Cauchy stress using the following equation;

$$\sigma = J^{-1}\ \mathrm{FSF^T}. \tag{4}$$

Using the gradient of displacement vector $u_{solid}$, the deformation gradient, **F** can be expressed as;

$$F = (I + \nabla u_{solid}), \tag{5}$$

In which the identity matrix was denoted by **I**, and the Jacobian of the deformation is defined as;

$$J = \det(F). \tag{6}$$

Fluid domain was solved based on Eulerian formulation, while solid domain was solved based on Langrangian

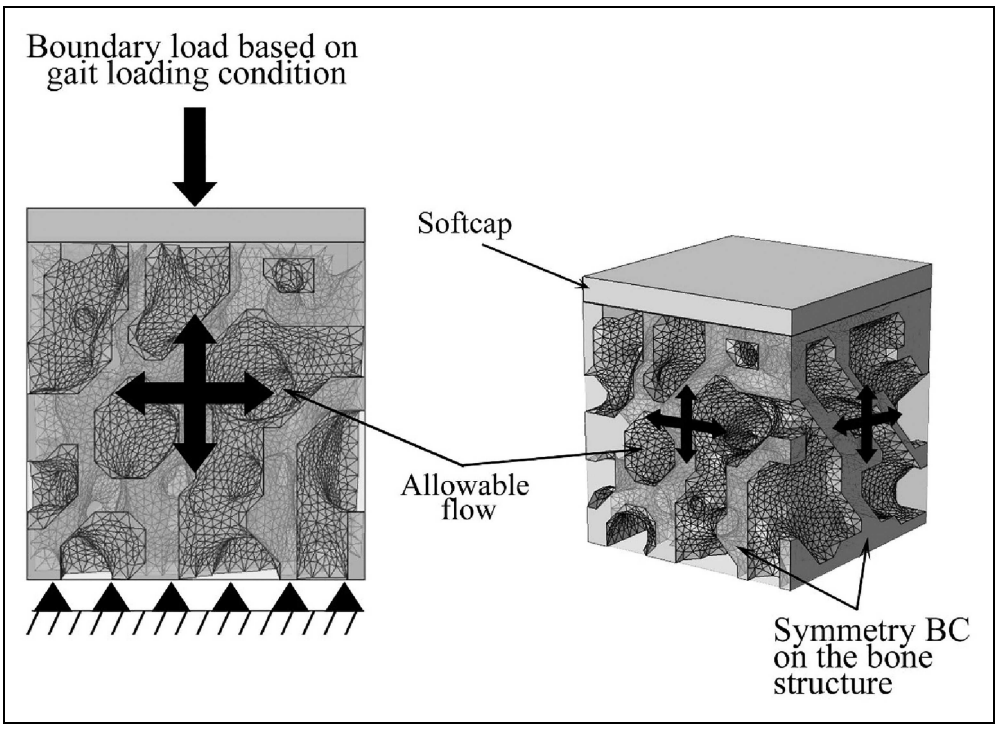


**Figure 4.** Boundary conditions (BC) of trabecular bone and bone marrow models.

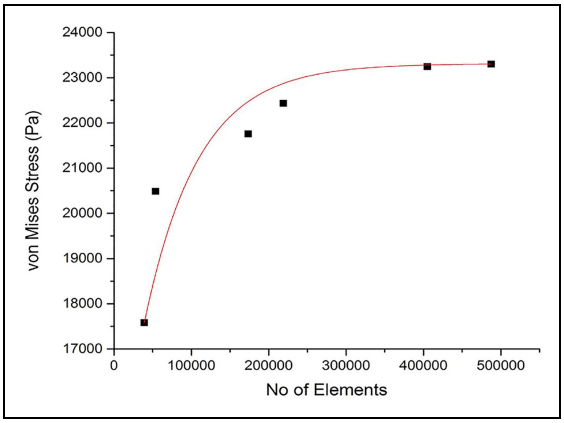


**Figure 5.** Convergence study for the trabecular structure model.

formulations. In coupling fluid-solid system, the arbitrary Lagrangian-Eularian method can be implemented with total force on the fluid-solid boundary was given as;

$$f_r = n \cdot [-pI + \mu(\nabla u_{fluid} + (\nabla u_{fluid})^T)], \tag{7}$$

With **n** is the normal acting outward at the boundary, the force at the structure's boundary is given by;

$$F_r = \boldsymbol{\sigma} \cdot \boldsymbol{n}. \tag{8}$$

In Spatial and material coordinate system, these forces can be coupled thru a force transformation using the arbitrary Eulerian-Lagrangian method as follows:

$$F_r = f_r \cdot \frac{dv}{dV} \ . \tag{10}$$

Mesh element scale factors $dv$ and $dV$ are the fluid and material frames, respectively. Further, the relationship of structural velocity of the moving wall with the fluid velocity is demonstrated as follows:

$$u_{fluid} = u_w, \tag{11}$$

Thus, the rate of change of the solid displacement is defined by the structural velocity.

$$u_w = \frac{\partial u_{solid}}{\partial t} \tag{12}$$

### Statistical analysis

All morphology indices are presented in mean and standard deviation (Table 1). The Pearson's correlation and linear regression analysis were performed to explore the interrelationship between the morphological indices and the mechanical properties of the trabecular bone sample. The multiple linear regression was perform using IBM SPSS Statistics 23 (IBM Corp, USA). For all comparison, the level of significant for p-value was <0.05.

## Results

The average von Mises stress distribution during normal walking with cycle duration was plotted as shown in Figure 6. The peak pressure reached as high as $11.35 \times 10^5$ Pa. Then again, the minimum stress for trabecular bone is $10.75 \times 10^4$ Pa at period of 0.86s. As can be seen, the behaviour of von Mises stress during gait cycle is similar to the force in the vertical direction.

From the computational FSI simulation, the von Mises stress distribution within the trabecular bone model along gait normal walking gait loading cycle at different time frame as illustrated in Figure 7. Comparing Figure 7(a) and (b), more area covered with high stresses at time 0.14 s. These results match with the graph of the von Mises stress over time.

The pressure and shear stress distribution during gait loading cycle are presented in Figure 8. This figure shows how the structure of trabecular bone affects the fluid characteristic during the gait loading cycle. It can be observed from the Figure 8 that the pattern of pressure distribution was similar to the von Mises stress results. The pressure was range from 380 to 4070 Pa during the normal walking cycle. Moreover, as discussed earlier, the trabecular structure experience shear stress due to bone marrow movement. With an average of 0.09 Pa, the shear stress was in the range of 0.01 to 0.27 Pa.

Based on 2D images of the trabecular model cross section, the pressure on top section was lower than below section when the structure at the highest compression deformation Figure 9(a). However, at period 0.86 s, the pressure on top section becomes higher than the lower section. Figure 10 shows velocity profile of marrow during gait loading cycle at different time frame. As can be seen, at period 0.14s, the velocity was higher than at period 0.86 s. The velocity was range of 0.09 μm/s to 81.2 μm/s at period of 0.14 s and 0.001 μm/s to 2.6 μm/s at period 0.86 s.

Multiple regression analysis for morphological parameters is tabulated in Table 2 and Table 3 with Pearson correlation and *p*-value for solid and fluid characteristic. Bone volume fraction and SMI shows good correlation

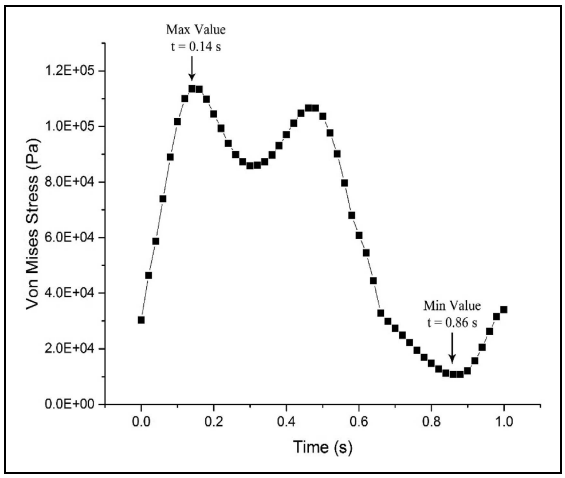


**Figure 6.** Von Mises stress distribution on the trabecular bone during normal walking.

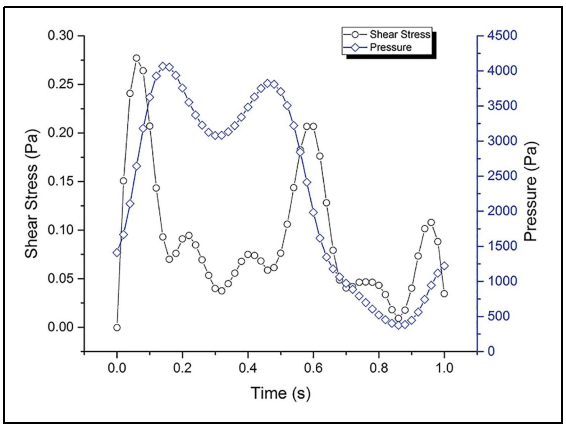


**Figure 8.** Maximum shear stress and pressure distribution on the trabecular bone along with normal walking loading.

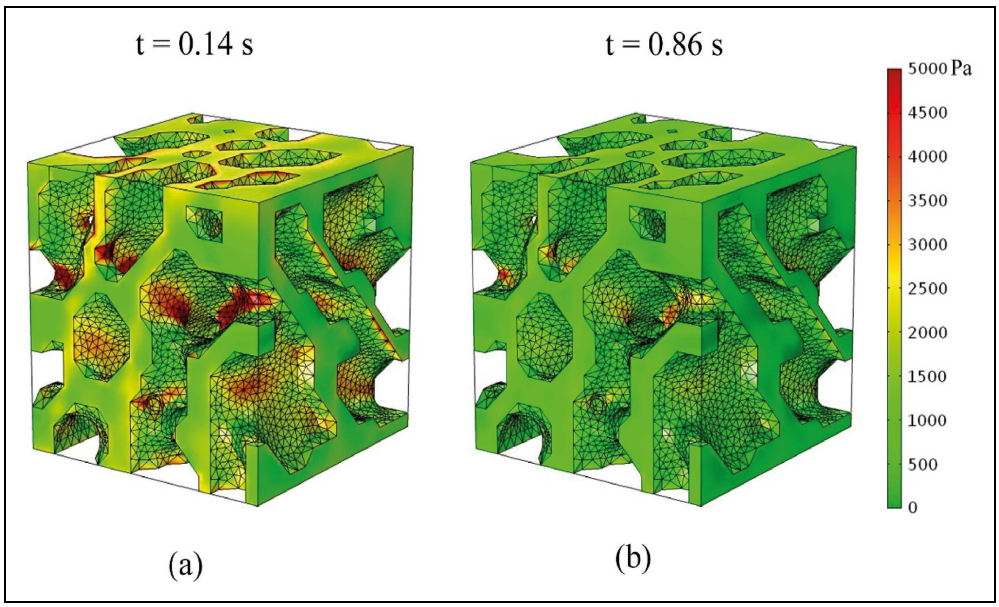


**Figure 7.** Comparison of von Mises stress on the trabecular bone at different time frame (a) t = 0.14 s and (b) t = 0.86 s.

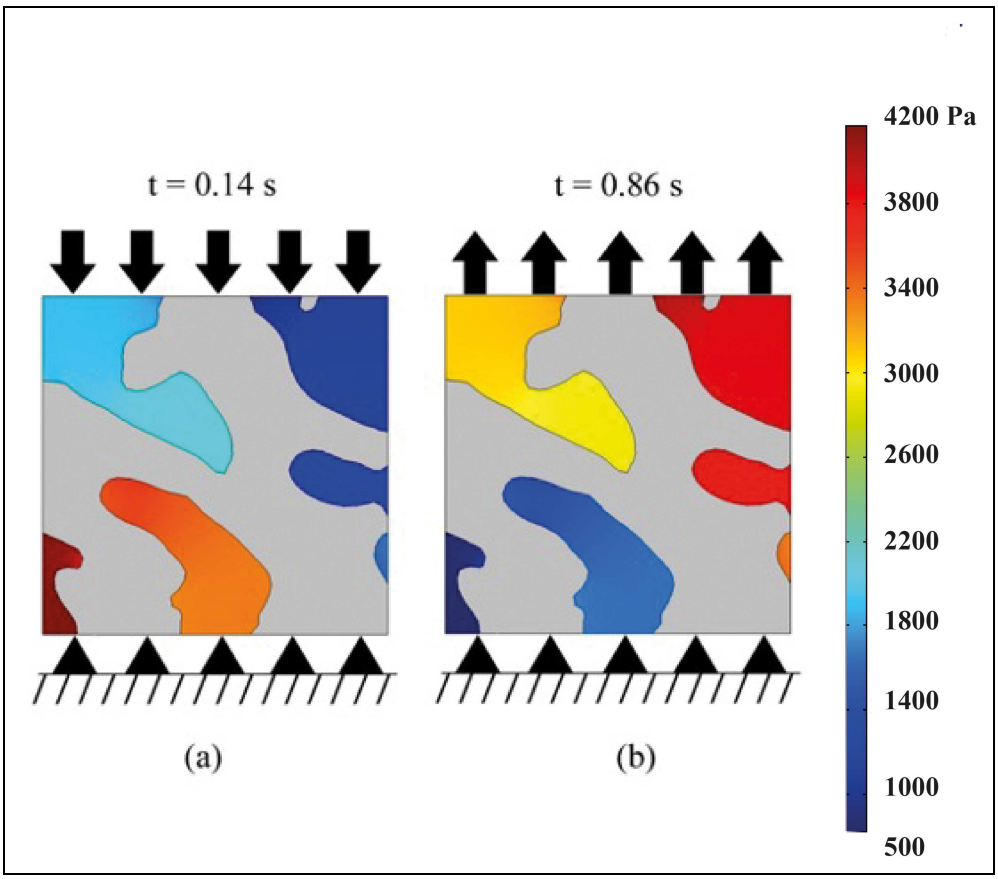


**Figure 9.** Comparison of pressure distribution on the trabecular bone cross section at different time frame (a) t = 0.14 **s** and (b) t = 0.86 s.

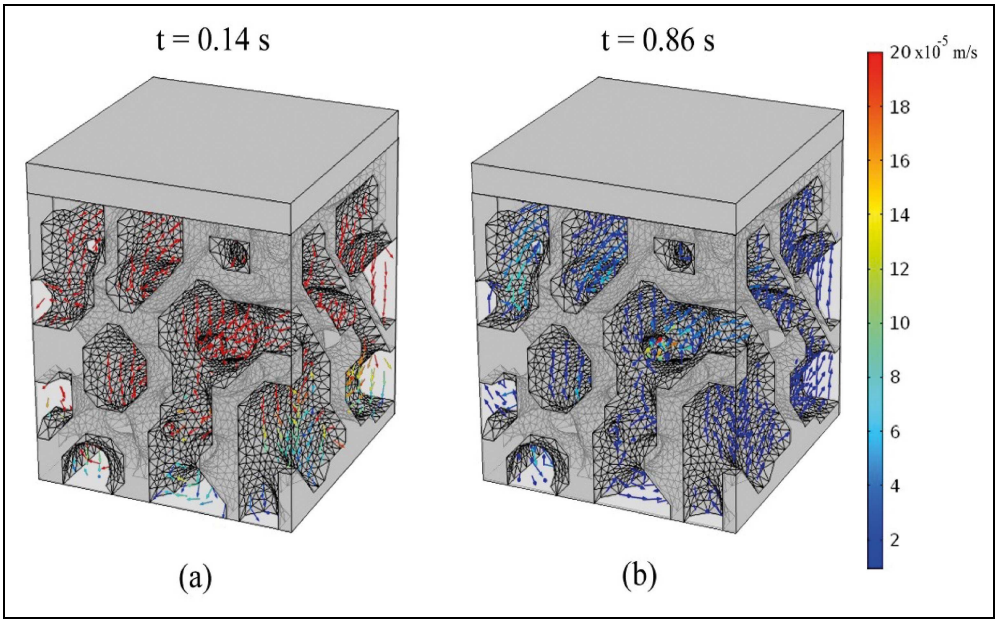


**Figure 10.** Velocity profile on the trabecular bone at different time frame (a) t = 0.14 s and (b) t = 0.86 s during normal gait loading.

with principal strain and von Mises stress. However, the SMI value only significant with principal strain and bone volume fraction solitary shows significant value with von Mises stress. As for marrow characteristic, velocity and pressure were significant to SMI, while shear stress is insignificant with all morphological parameters.

The volume fraction plays a major role in trabecular bone mechanical properties. The distribution of von

Mises stress on different model with different bone volume fraction is shown in Figure 11. As can be seen, low bone volume fraction model result in higher von Mises stress compare with a model which has high volume fraction.

The permeability and trabecular stiffness relationship to bone volume fraction and SMI are plotted in Figure 12. The graph showed a strong and negative correlation between the permeability and bone volume fraction ($r = -0.862$), whereas the correlations are strong and positive between permeability and SMI ($r = 0.835$). Meanwhile, in Figure 13 shows both bone volume fraction and SMI have a strong correlation with the trabecular stiffness ($r = 0.832$ and $r = -0.796$; respectively). However, the trabecular bone stiffness correlation was inversely compared to the permeability.

## Discussion

The trabecular bone structure is known as a porous structure which contributes to maximum strength while giving the bone less weight. Understanding the bone marrow flow and trabecular bone structure mechanism can provide insight into bone remodelling process and bone strength. It is vital to identify which architectural features that affect the trabecular strength. Thus, in this study, finite element analysis with FSI approach was used to identify the architecture contribution with the presence of bone marrow when there is physical loading involved.

During physiological activities, the bone will have a deformation due to the mechanical load.[3] The compressive and tensile stress is generated in the trabecular structure which causes the bone marrow within the structure to drift from region of compression to tension. Due to the complex structure of trabecular bone with small rods, plated and pores, there will be shear stress generated on the wall structure. Bone marrow function in activating the bone cells to start the bone remodelling process. Thus, this study analysed shear stress, pressure, and permeability to identify the fluid characteristic through physical activity. Moreover, the trabecular bone architecture and volume fraction play an important role in its mechanical properties. In addition, this study analysed the bone marrow permeability and trabecular stiffness with correlation to the trabecular morphology. Applied physiological gait loading in this present study to assure more reliable and safer prediction of bone marrow behaviour and trabecular stiffness.

In the analysis, it can be seen that the maximum peak von Mises stress range for all ten models are 91 kN/m$^2$ to 114 kN/m$^2$. The maximum value was identified in period 0.14s, which is due to the fact that higher contact force occurs at that period of time (Figure 7). Assessing the von Mises stress in the trabecular model was necessary with purpose of providing new insight in prediction of trabecular structure failure and evaluating the fracture risk. The permeability in this study was also in agreement with results from the literature.

The aim of this study is to identify the bone marrow movement behaviour within the trabecular structure sufficient for bone cell growth based on physiological activity. Normal walking loading was chosen since it is reported as the most frequent physiological activity.[26] The results show that during normal walking loading, the maximum shear stress occurs is all models are in range 0.05 Pa to 0.27 Pa. Furthermore, Li et al.,[17] Castillo and Jacobs[20] present that the shear stress was needed for the cells to differentiate and proliferate. Moreover, the previous

**Table 2.** Morphological parameters of trabecular bone sample with Pearson correlation and p-value in relation with mechanical behaviour.

| | Principle Strain | | Von Mises Stress | |
|---|---|---|---|---|
| Morphological Parameters | Pearson Correlation | *p*-value | Pearson Correlation | *p*-value |
| BV/TV | −0.830 | 0.123 | −0.798 | 0.006* |
| BS/TV | 0.449 | 0.452 | 0.28 | 0.132 |
| SMI | 0.850 | 0.002* | 0.715 | 0.689 |
| Conn. D | −0.032 | 0.916 | −0.303 | 0.16 |
| Tb.Th | −0.426 | 0.566 | −0.344 | 0.206 |
| Tb.Sp | 0.705 | 0.403 | 0.769 | 0.405 |
| DA | 0.410 | 0.966 | 0.559 | 0.218 |

*Significant *p*-value < 0.05.

**Table 3.** Morphological parameters of trabecular bone sample with Pearson correlation and p-value in relation with fluid characteristics.

| | Velocity | | Pressure | | Shear Stress | |
|---|---|---|---|---|---|---|
| Morphology Parameters | Pearson Correlation | *p*-value | Pearson Correlation | *p*-value | Pearson Correlation | *p*-value |
| BV/TV | −0.661 | 0.969 | −0.672 | 0.168 | 0.001 | 0.499 |
| BS/TV | 0.388 | 0.449 | 0.53 | 0.585 | 0.411 | 0.119 |
| SMI | 0.710 | 0.022* | 0.825 | 0.003* | 0.029 | 0.469 |
| Conn. D | −0.049 | 0.316 | 0.288 | 0.815 | 0.184 | 0.306 |
| Tb.Th | −0.296 | 0.395 | −0.346 | 0.209 | −0.424 | 0.111 |
| Tb.Sp | 0.607 | 0.735 | 0.594 | 0.754 | −0.255 | 0.239 |
| DA | 0.324 | 0.468 | 0.074 | 0.721 | 0.079 | 0.414 |

*Significant *p*-value < 0.05.

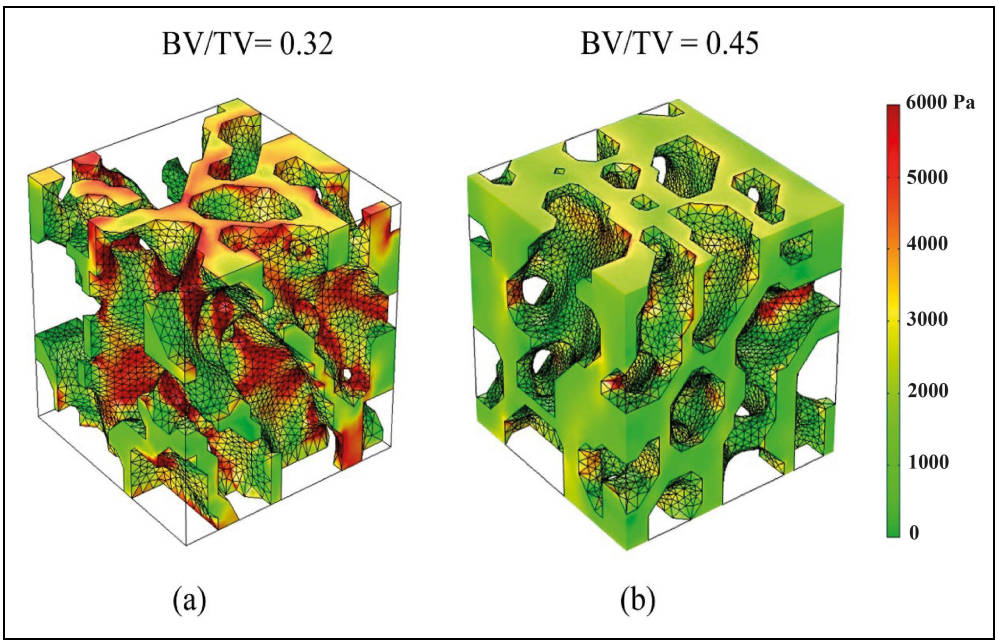


**Figure 11.** Comparison of von Mises stress on the trabecular bone with different bone fraction (a) BV/TV = 0.32 and (b) BV/TV = 0.45.

experimental study showed that shear stress in the range of 0.1 Pa to 1 Pa needed to stimulate bone cells in vitro. Thus, a much lower range of shear stress is suggested to simulate the stress on bone cells during normal walking. However, other previously study mention that 0.23 Pa of shear stress is sufficient for regulated bone cells to respond and synthesise bone matrix protein for bone remodelling process.[33] Additionally, there is separate study by Nauman et al.[34] stated that the bone cells stimulation is unaffected by difference shear stress levels.

Walking 10000 step per day has been proposed as daily activity for healthy adults. This recommendation was studied, and it is found that 10000 step per day can improve individual's health and sustainability. For example, previous studies stated higher step count per day could lower the prevalence of depression.[35] In addition, it is observed the Body Mass Index (BMI) of the group with higher step count per day has shown significantly lower compared with another group.[36] Nevertheless, there is no studies indicate on bone health based on daily step count. However, based on the results of this study, it is suggested that higher step count will lead to more shear stress. Thus, can help in improving the bone remodelling process and bone strength.

Moreover, while the trabecular bone deforms according to the physiological load, there would be pressure difference within the structure (Figure 8). Previous study stated bone formation increase with pressure. Welch et al.[37] in their study found that bone marrow pressure increased about 2000 Pa resulted in bone remodelling. Another study on the new bone formation of mouse tibiae, stated in dynamic compression induced a similar range of pressure value.[38] This study results for pressure distribution in normal walking gait loading for all models was in the range of 180 Pa to 4000 Pa. Addition to this study result analysis, it is found that higher bone volume fraction will lead to lower pressure value (Table 3). However, the pressure difference also depends on a variety of factors, including the bone marrow rheology, bone strain and permeability.[39,40]

In addition, the results show that the pressure gradient along the walking gait loading was different at period 0.14s and 0.86 s as in Figure 9. This is where the bone marrow function as hydraulic stiffening effect. Hydraulic stiffening effect refers to the reduction of bone stress during dynamic loading effect by the presence of fluid within the structure.[41] While at period 0.14s is when the maximum compression occurs, the pressure of bone marrow was high at the bottom. This pressure might support a certain amount of applied load which caused the apparent stiffness to the trabecular structure. Similarly, at period of 0.86s, where tensile occur, the pressure was high at the upper region giving the structure extra load barrier which prevents the structure to have high deformation.

This study used BoneJ plugin in the ImageJ software to analyse the morphological data of the trabecular model. In the correlation study, only bone volume fraction (BV/TV) and SMI shows a significant value with the simulation results. Thus, this study correlated the permeability and bone stiffness with these both morphological parameters. From the results, permeability and stiffness show good correlation with the BV/TV. Permeability in trabecular bone was vital since it demonstrated the biological based features of trabecular bone. Still, the simulation results were consistent to those found in the previous literature.[42] Additionally, the trabecular bone mechanical

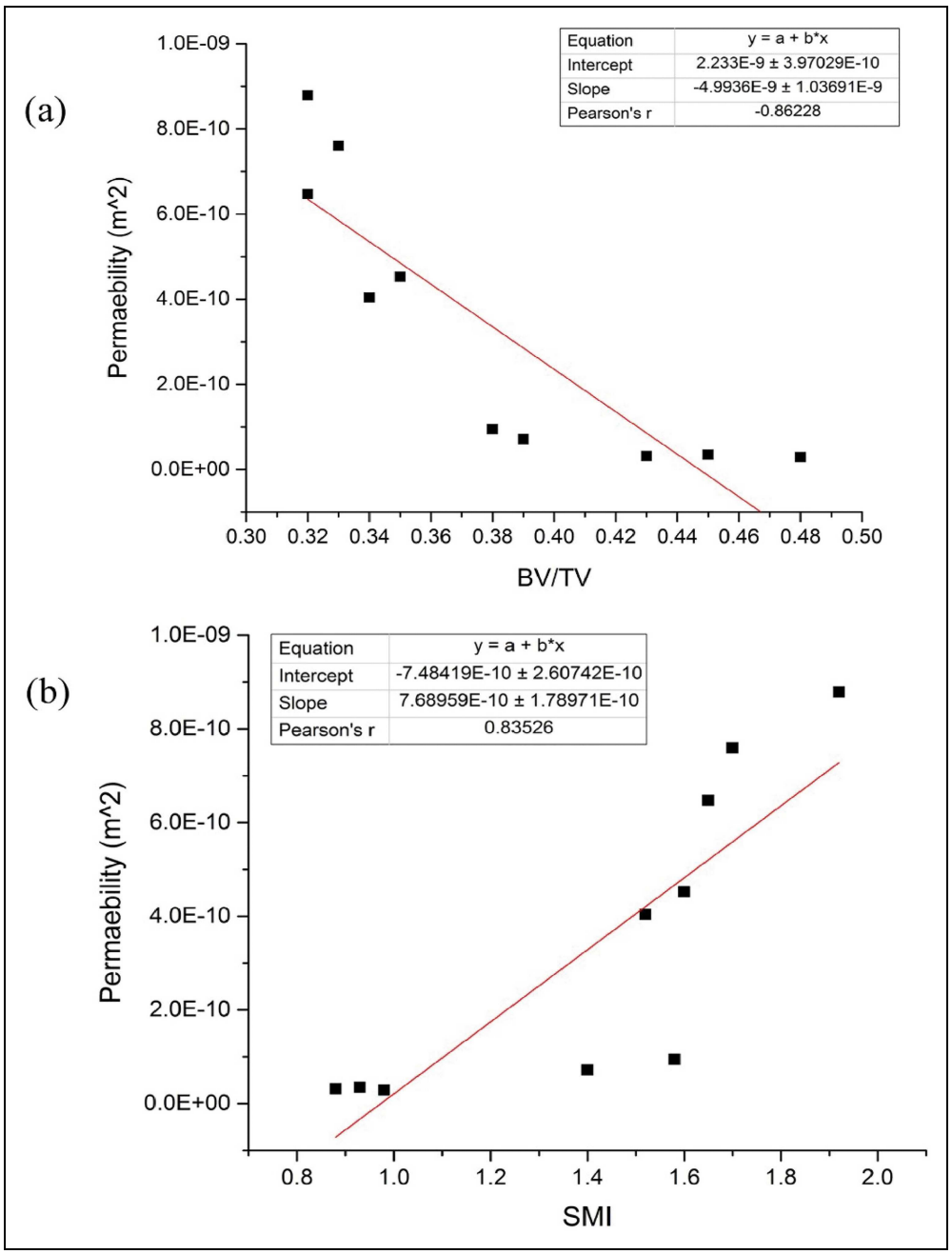


**Figure 12.** Linear relationship between (a) BV/TV and (b) SMI with permeability.

quality was depending on its stiffness, since the stiffness have a strong correlation with the strength.[43]

Hypothetically bone with lower bone volume fraction means porous structure (osteoporotic bone). Thus, as mention by Goldstein et al.[44] and Syahrom et al.,[45] the trabecular bone integrity and bone marrow permeability can be disarrange based on the porosity value. In addition, the results of this study suggests that enhancement of bone remodelling process can be achieved by optimization of BV/TV and permeability value. However, from the results analysis the reduction of BV/TV can cause higher stress on the trabecular structure (Figure 11) and the loss of trabecular structure stiffening effect (Figure 13). Therefore, important to find the optimum bone volume fraction which has a good stiffening value and yet able to deliver sufficient nutrient to bone cells.

Other than bone volume fraction, the SMI also shows high correlation with the stiffness. The SMI is a measurement which determines a porous structure is made of rod or plate-like structure. The value starts from 0 for ideal plate structures to 3 for ideal rod structure.[46] Theoretically, the plate-like structure could barricade more fluid flow compared to the rod-like structure. In contrast with the permeability, the stiffness is negatively correlated with the SMI. Moreover, previous study already stated that trabecular plates have more dominant role in mechanical integrity of trabecular bone structure.[47,48] Also, osteoporotic trabecular bone has been found to

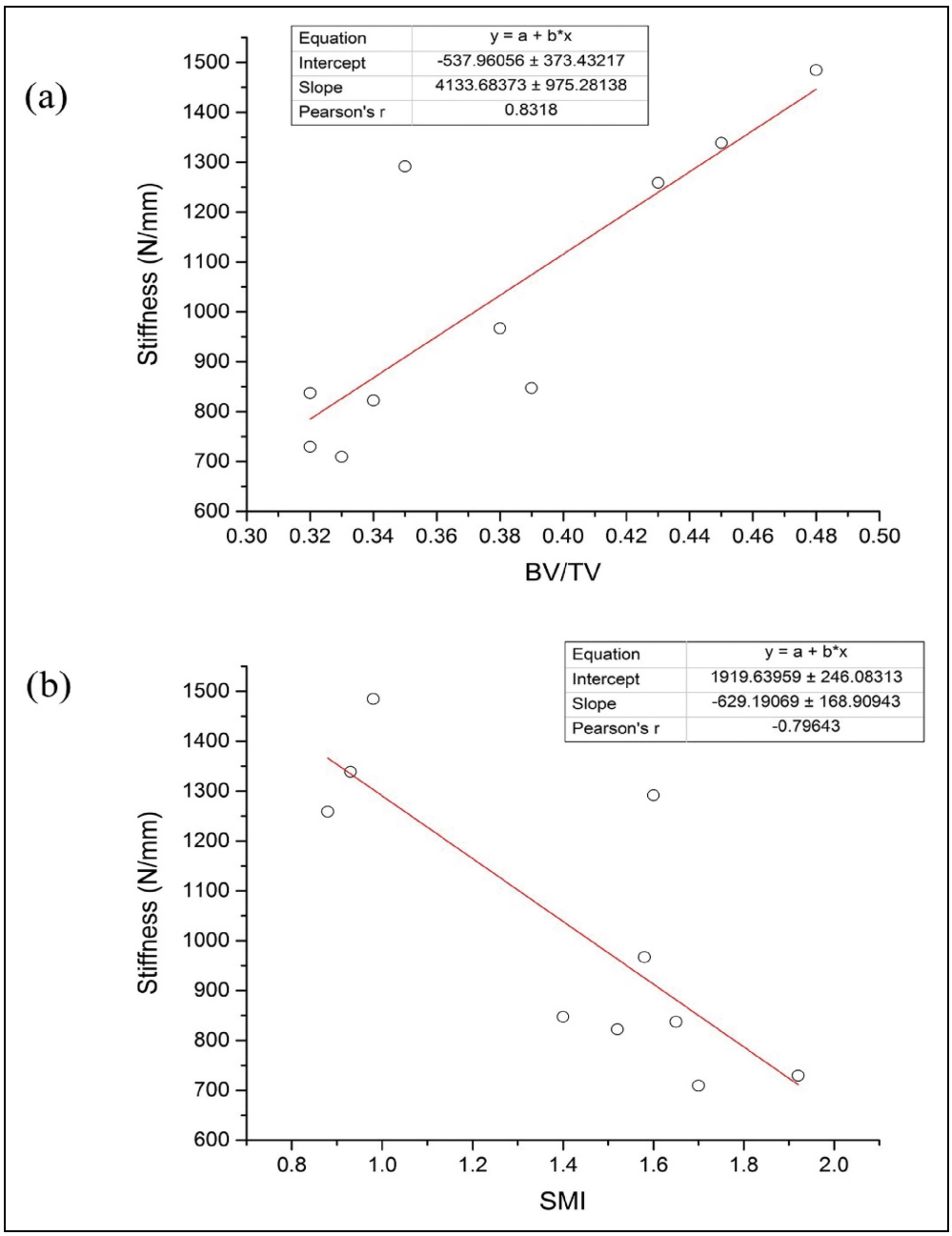


**Figure 13.** Linear relationship between (a) BV/TV and (b) SMI with stiffness.

have an apparent transition of microarchitecture, which is from plate like to rod-like structure.[47] Stein et al.[49] in their study found that the bone stiffness related with trabecular connectivity, trabeculae orientation and trabecular plates. Thus, in correspondence to previous studies, our results suggest that plate-like trabecular structure (lower SMI value) can contribute to higher trabecular stiffness.

There are a few limitations in the interpretation of results that should be considered. This study has employed trabecular samples from anatomic site of bovine femoral bone. As such, the microarchitecture parameters may differ from that of the human bone or other anatomic sites. However, there are a few works done that demonstrate agreement between the architecture as well as mechanical properties of bovine trabecular bone and that of a healthy human.[50,51] Furthermore, the impact of marrow phase on the trabecular structure can be further investigated in terms of its properties such as the variation of constituents and viscosity.

In summary, this study was investigated on the correlation of the morphology parameters onto the mechanical properties of trabecular bone with presence of bone marrow. The bone volume fraction and SMI were identified as the one that has a higher correlation with the trabecular permeability and stiffness compared with others morphological parameters. Moreover, the bone marrow behaviour through the physiological activity was identified in this study. This study provides insight into understanding how human daily physiological activities contribute to the bone remodelling process and nutrient transport with

the bone environment. However, more knowledge in this area was crucial to studying the bone adaption to the bone replacement and in estimating fracture risk.

## Conclusion

The overall aim of this study was to assess the importance on the interaction between the bone marrow and trabecular bone structure during mechanical loading by using the FSI approach. Trabecular bone is known as a highly porous structure with a significant volume of bone marrow, a compressive or tensile force on the trabecular bone will result in bone marrow movement with respect to the trabecular bone structure. It is believed that the fluid flow will cause the shear stress to the trabecular structure. The interaction between the fluid and trabecular bone will occur, and this incident might have several effects on the trabecular structure. Moreover, bone remodelling process was occurring due to the shear stress on the bone cell which triggers the process. In addition to shear stress, based on previous study the hydraulic stiffening effect occurs due to the presence of bone marrow within the trabecular bone structure. Therefore, this study proposed the used of FSI approach to model the trabecular bone behaviour and marrow flow characteristic.

The physiological activities in daily human life play a major role than calcium intake in the bone development process.[52] It contributes to mechanical stimuli in bone marrow and trabecular bone strain. Normal walking is one of human major daily activity is chosen in this study as a boundary condition in analysing the trabecular bone behaviour. The bone marrow behaviour was recorded during the normal walking cyclic loading. While the trabecular bone deforms according to the physiological load, the bone marrow within will encounter mechanical stimulation in mechanobiological response.[53,54] The shear stress value along normal walking gait loading was found in a range of 0.05 to 0.27 Pa which is sufficient to regulate cell response minimally.[33] However, due to ageing factor, bone resorption rate will become higher. Thus, higher shear stress was needed in order to have rapid bone remodelling process to encounter the bone resorption rate. Furthermore, this study also provides insight into understanding the related mechanobiological of bone cells and disease in deterioration of nutrient supplied to the bone.

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### References

1. Saad APM and Syahrom A. Study of dynamic degradation behaviour of porous magnesium under physiological environment of human cancellous bone. *Corros Sci* 2018; 131: 45–56.
2. Yourek G, McCormick SM, Mao JJ, et al. Shear stress induces osteogenic differentiation of human mesenchymal stem cells. *Regen Med* 2010; 5: 713–724.
3. Coughlin TR and Niebur GL. Fluid shear stress in trabecular bone marrow due to low-magnitude high-frequency vibration. *J Biomech* 2012; 45: 2222–2229.
4. Vaughan T, Voisin M, Niebur G, et al. Multiscale modeling of trabecular bone marrow: understanding the micromechanical environment of mesenchymal stem cells during osteoporosis. *J Biomech Eng* 2015; 137. page: 1-10 AQ2
5. You L, Cowin SC, Schaffler MB, et al. A model for strain amplification in the actin cytoskeleton of osteocytes due to fluid drag on pericellular matrix. *J Biomech* 2001; 34: 1375–1386.
6. Adachi T, Kameo Y and Hojo M. Trabecular bone remodelling simulation considering osteocytic response to fluid-induced shear stress. Philosophical transactions of the royal society A: mathematical. *Phys Eng Sci* 2010; 368: 2669–2682.
7. Wittkowske C, Reilly GC, Lacroix D, et al. In vitro bone cell models: impact of fluid shear stress on bone formation. *Front Bioeng Biotechnol* 2016; 4: 87.
8. Birmingham E, Niebur G, McNamara L, et al. An experimental and computational investigation of bone formation in mechanically loaded trabecular bone explants. *Ann Biomed Eng* 2016; 44: 1191–1203.
9. Shen V, Liang X, Birchman R, et al. Short term immobilization-induced cancellous bone loss is limited to regions undergoing high turnover and/or modeling in mature rats. *Bone* 1997; 21: 71–78.
10. Rodriguez N, Herndon D and Klein G. Evidence against a role of immobilization in the bone loss following burns. *Bone* 2011; 2: S190.
11. Frost HM. Wolff's Law and bone's structural adaptations to mechanical usage: an overview for clinicians. *Angle Orthod* 1994; 64: 175–188.
12. Frost HM. Bone "mass" and the "mechanostat": a proposal. *Anat Rec* 1987; 219: 1–9.
13. Burr DB, Milgrom C, Fyhrie D, et al. In vivo measurement of human tibial strains during vigorous activity. *Bone* 1996; 18: 405–410.
14. Fritton SP, McLeod KJ and Rubin CT. Quantifying the strain history of bone: spatial uniformity and self-similarity of low-magnitude strains. *J Biomech* 2000; 33: 317–325.
15. Gurkan UA and Akkus O. The mechanical environment of bone marrow: a review. *Ann Biomed Eng* 2008; 36: 1978–1991.

16. McAllister T and Frangos J. Steady and transient fluid shear stress stimulate NO release in osteoblasts through distinct biochemical pathways. *J Bone Miner Res* 1999; 14: 930–936.
17. Li YJ, Batra NN, You L, et al. Oscillatory fluid flow affects human marrow stromal cell proliferation and differentiation. *J Orthop Res* 2004; 22: 1283–1289.
18. Knight MN and Hankenson KD. Mesenchymal stem cells in bone regeneration. *Adv Wound Care* 2013; 2: 306–316.
19. Shao J, Zhang W and Yang T. Using mesenchymal stem cells as a therapy for bone regeneration and repairing. *Biol Res* 2015; 48: 62.
20. Castillo AB and Jacobs CR. Mesenchymal stem cell mechanobiology. *Curr Osteoporos Rep* 2010; 8: 98–104.
21. Klein–Nulend J, Van Der Plas A, Semeins CM, et al. Sensitivity of osteocytes to biomechanical stress in vitro. *FASEB J* 1995; 9: 441–445.
22. Case N, Sen B, Thomas J, et al. Steady and oscillatory fluid flows produce a similar osteogenic phenotype. *Calcif Tissue Int* 2011; 88: 189–197.
23. Bakker AD, Joldersma M, Klein-Nulend J, et al. Interactive effects of PTH and mechanical stress on nitric oxide and PGE2 production by primary mouse osteoblastic cells. *Am J Phys-Endocrinol Metab* 2003; 285: E608–EE13.
24. Arnsdorf EJ, Tummala P, Kwon RY, et al. Mechanically induced osteogenic differentiation–the role of RhoA, ROCKII and cytoskeletal dynamics. *J Cell Sci* 2009; 122: 546–553.
25. Yeatts AB and Fisher JP. Bone tissue engineering bioreactors: dynamic culture and the influence of shear stress. *Bone* 2011; 48: 171–181.
26. Bergmann G, Deuretzbacher G, Heller M, et al. Hip contact forces and gait patterns from routine activities. *J Biomech* 2001; 34: 859–871.
27. Fatihhi S, Harun M, Kadir MRA, et al. Uniaxial and multiaxial fatigue life prediction of the trabecular bone based on physiological loading: a comparative study. *Ann Biomed Eng* 2015; 43: 2487–2502.
28. Homminga J, Mccreadie BR, Weinans H, et al. The dependence of the elastic properties of osteoporotic cancellous bone on volume fraction and fabric. *J Biomech* 2003; 36: 1461–1467.
29. Sylvester AD and Kramer PA. Young's modulus and load complexity: modeling their effects on proximal femur strain. *Anat Rec* 2018; 301: 1189–1202.
30. Bayraktar HH, Gupta A, Kwon RY, et al. The modified super-ellipsoid yield criterion for human trabecular bone. *J Biomech Eng* 2004; 126: 677–684.
31. Bryant J, David T, Gaskell P, et al. Rheology of bovine bone marrow. Proceedings of the institution of mechanical engineers. *Part H: J Eng Med* 1989; 203: 71–75.
32. Haskill JS, McNeill TA and Moore MAS. Density distribution analysis of In vivo and In vitro colony forming cells in bone marrow. *J Cell Physiol* 1970; 75: 167–179.
33. Kreke MR, Sharp LA, Woo Lee Y, et al. Effect of intermittent shear stress on mechanotransductive signaling and osteoblastic differentiation of bone marrow stromal cells. *Tissue Eng, Part A* 2008; 14: 529–537.
34. Nauman E, Satcher R, Keaveny T, et al. Osteoblasts respond to pulsatile fluid flow with short-term increases in PGE2 but no change in mineralization. *J Appl Physiol* 2001; 90: 1849–1854.
35. McKercher CM, Schmidt MD, Sanderson KA, et al. Physical activity and depression in young adults. *Am J Prev Med* 2009; 36: 161–164.
36. Krumm EM, Dessieux OL, Andrews P, et al. The relationship between daily steps and body composition in postmenopausal women. *J Womens Health (Larchmt)* 2006; 15: 202–210.
37. Welch R, Johnston 2nd C, Waldron M, et al. Bone changes associated with intraosseous hypertension in the caprine tibia. *JBJS* 1993; 75: 53–60.
38. Hu M, Cheng J and Qin Y-X. Dynamic hydraulic flow stimulation on mitigation of trabecular bone loss in a rat functional disuse model. *Bone* 2012; 51: 819–825.
39. Teo J, Si-Hoe K, Keh J, et al. Correlation of cancellous bone microarchitectural parameters from microCT to CT number and bone mechanical properties. *Mater Sci Eng C* 2007; 27: 333–339.
40. Baroud G, Falk R, Crookshank M, et al. Experimental and theoretical investigation of directional permeability of human vertebral cancellous bone for cement infiltration. *J Biomech* 2004; 37: 189–196.
41. Liebschner MA and Keller TS. Hydraulic strengthening affects the stiffness and strength of cortical bone. *Ann Biomed Eng* 2005; 33: 26–38.
42. Kohles SS and Roberts JB. Linear poroelastic cancellous bone anisotropy: trabecular solid elastic and fluid transport properties. *J Biomech Eng* 2002; 124: 521–526.
43. ~~Lopez KW, Goldstein SA, Ciarelli MJ, et al. The relationship between the structural and orthogonal compressive properties of trabecular bone. 1994.~~ AQ3
44. Goldstein AS, Juarez TM, Helmke CD, et al. Effect of convection on osteoblastic cell growth and function in biodegradable polymer foam scaffolds. *Biomaterials* 2001; 22: 1279–1288.
45. Syahrom A, Kadir MRA, Abdullah J, et al. Permeability studies of artificial and natural cancellous bone structures. *Med Eng Phys* 2013; 35: 792–799.
46. Hildebrand T and Rüegsegger P. Quantification of bone microarchitecture with the structure model index. *Comput Methods Biomech Bio Med Eng* 1997; 1: 15–23.
47. Wang J, Zhou B, Liu XS, et al. Trabecular plates and rods determine elastic modulus and yield strength of human trabecular bone. *Bone* 2015; 72: 71–80.
48. Wang J, Zhou B, Parkinson I, et al. Trabecular plate loss and deteriorating elastic modulus of femoral trabecular bone in intertrochanteric hip fractures. *Bone Res* 2013; 1: 346–354.
49. Stein EM, Kepley A, Walker M, et al. Skeletal structure in postmenopausal women with osteopenia and fractures is characterized by abnormal trabecular plates and cortical thinning. *J Bone Miner Res* 2014; 29: 1101–1109.
50. Keaveny TM, Wachtel EF, Ford CM, et al. Differences between the tensile and compressive strengths of bovine tibial trabecular bone depend on modulus. *J Biomech* 1994; 27: 1137–1146.
51. Morgan EF, Yeh OC, Chang WC, et al. Nonlinear behavior of trabecular bone at small strains. *J Biomech Eng* 2001; 123: 1–9.
52. Anderson JJ. The important role of physical activity in skeletal development: how exercise may counter low calcium intake. *Am J Clin Nutr* 2000; 71: 1384–1386.
53. Metzger TA, Schwaner SA, LaNeve AJ, et al. Pressure and shear stress in trabecular bone marrow during whole bone loading. *J Biomech* 2015; 48: 3035–3043.
54. Birmingham E, Grogan J, Niebur G, et al. Computational modelling of the mechanics of trabecular bone and marrow using fluid structure interaction techniques. *Ann Biomed Eng* 2013; 41: 814–826.

# Influence of Bone Marrow Characteristic and Trabecular Bone Morphology on Bone Remodelling Process with FSI Approach

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| Keywords: | Fluid Structure Interaction, Trabecular Bone, Bone Marrow, Shear Stress, Stiffness, Bone Remodelling |
| Abstract: | While doing daily physiological activities, the trabecular bone will experience a certain amount of deformation which leads to the bone marrow movement. The movement can affect the bone remodelling process and the properties of the bone itself. The bone marrow plays a role as a hydraulic stiffening of the trabecular structure. However, previous studies analysed on trabecular bone and bone marrow separately, which is not considered as the actual condition. Thus, it is crucial to consider combine analyses of the bone marrow with the trabecular structure simultaneous. The aim of this study is to investigate the effect of bone marrow on the mechanical environment and the structure of trabecular bone during normal walking loading. Hence, this study used the Fluid-Structure Interaction (FSI) approach as a finite element method to discover the effect of bone marrow to the trabecular structure and vice versa. The findings show the shear stress value along normal walking phase was found in a range of 0.01-0.27 Pa which is sufficient to regulated cell response minimally. This study provides |

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| | insight into understanding the related mechanobiological responds towards supply of nutrients onto bone cells. |
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# Influence of Bone Marrow Characteristic and Trabecular Bone Morphology on Bone Remodelling Process with FSI Approach

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**Abstract**

While doing daily physiological activities, the trabecular bone will experience a certain amount of deformation which leads to the bone marrow movement. The movement can affect the bone remodelling process and the properties of the bone itself. The bone marrow plays a role as a hydraulic stiffening of the trabecular structure. However, previous studies analysed on trabecular bone and bone marrow separately, which is not considered as the actual condition. Thus, it is crucial to consider combine analyses of the bone marrow with the trabecular structure simultaneous. The aim of this study is to investigate the effect of bone marrow on the mechanical environment and the structure of trabecular bone during normal walking loading. Hence, this study used the Fluid-Structure Interaction (FSI) approach as a finite element method to discover the effect of bone marrow to the trabecular structure and vice versa. The findings show the shear stress value along normal walking phase was found in a range of 0.01-0.27 Pa which is sufficient to regulated cell response minimally. This study provides insight into understanding the related mechanobiological responds towards supply of nutrients onto bone cells.

Keyword: Fluid Structure Interaction, Trabecular Bone, Bone Marrow, Shear Stress, Stiffness, Bone Remodelling

## 1.0 INTRODUCTION

Physiological loading induced trabecular bone deformation that leads to the bone marrow movement within the porous structure which contribute to stimulating the osteogenic response to the bone cells (1). The forces from the physiological loading cause both small strain and shear stress which known as a key to initiate the bone remodelling process (2-5). Currently, researchers tried to discover the actual value necessary to stimulate the bone cells for the bone remodelling process. To this date, experimental and simulation study have been performing in order to capture the value of these biomechanical stimuli that encourage the remodelling process (6-8). Bone marrow is a prime component in trabecular bone, in which it accommodates bone predecessors' cells for bone remodelling. Thus, it is important to consider it presence to better represent the actual conditions of trabecular bone. Therefore, knowledge of biomechanical environment that occur within the trabecular bone during daily physiological activities is necessary to comprehend on how the bone marrow can affect the bone remodelling.

Physiological loading includes daily activities such as house chores, daily walking, and sports activity helps in maintaining the bone health by transferring force to the bone structure. These can be seen on immobilization and bedrest individual that had reduce in their bone mass (9, 10). These relationship of loading and bone formation had been support with the Wolff's law since 1892 (11). The

trabecular bone experience compression and tension in the microstructure due to the loading causing the micro strain which one of the mechanical stimuli. Previous study reported that the physiological activities initiate in range of 0.001-0.003 mm/mm on the trabecular structure (12, 13). However, minimal physiological activity cause 1000με which cause bone resorption process higher compare to the bone resorption (14). Amazingly the bone can heal itself when there is external loads act upon the cells which can help in cells excite by signalling to the bones to start building themselves up. Then again, the loads from human daily life will also initiate the movement of bone marrow within the structure which cause the shear stress that act as response to the remodelling process (15).

The osteogenic response include osteoprogenitor cells secrete autocrine factors, for example prostaglandins $E_2$ ($PGE_2$) and nitric oxide (NO), which can regulate the remodelling activity (16). In addition, the proliferation rates have been found increasing when the bone marrow stromal cells were exposed to the fluid flow, which means higher number of cells participate in bone formation (17). The mesenchymal stem cells (MSCs) has been actively investigate in experiment and simulation due to its ability to differentiate into other cells such as osteoblast (bone cells), chondrocytes (cartilage cells) and adipocytes (fat cells) (18-20). The shear stress known as one of the parameters required for the MSCs in the bone marrow to differentiate and assists the remodelling activity (20, 21). The range of shear stress need for the cells to response mention by previous study is about 0.02 to 1.0 Pa (2, 22-25). Undoubtedly, bone remodelling process also requires adequate nutrient transport through the bone cells. These were also with help of bone marrow which function to transport the nutrient and remove waste. However, knowledge on how shear stress value contributing in MSCs to differentiate to different cells are still shallow.

In the present work the movement of the bone marrow regulate osteogenic responds which relate to the trabecular bone deformation due to the physiological activity. Thus far, the is no study using physiological gait loading as boundary to examine the effect of interaction on mechanical stimulus and trabecular bone. Therefore, the aim of this study is to investigate the effect of bone marrow mechanical environment and trabecular bone structure during normal walking loading. A fluid structure interaction (FSI) approach was applied to determine the deformation of trabecular bone with corresponding of marrow shear stress in bone remodelling activity.

## 2.0 MATERIALS AND METHODS

### 2.1 SAMPLE PREPARATION

The fresh bovine femur bones were harvested from the local slaughterhouse and kept frozen at -18°C to 26°C. Specimens of trabecular bone were taken by using a Bosch circular saw with copious water irrigation. The femur bone was then divided and cut into a section of medial condyle, femoral neck and femoral ball with the vertical orientation due to the maximum extension of knee joint occurred. The trabecular bone was then again cut into a cubic shape (10mm x 10mm x 17mm) in length by using a

precision cutting tool (Allied Techcut, USA). The precision cutter consists of diamond-resin bonded wafering blade with a minimum speed of 150-250 rpm with continuously water irrigation to prevent heat-related damages. Then, the specimens were placed in small airtight plastic bag with the purpose of reducing the thermal cycling and stored in the freezer with a temperature below -26°C. After that, the specimens will go through next procedure using the ultrasonic cleaner (Crest ultrasonic, model P11000SR, USA) additional with a chemical detergent (Pumicizedcitrius, Gent-l-kleen,USA) to cleaned from marrow. The specimens were then submerged for about 10-15 minutes at a temperature below 46°C. In order to remove the loose particles and excessive marrow, the specimens were then air-jetted and vacuumed suction. This procedure was repeated until all excessive marrow is removed (Fatihhi SJ et al., 2015). A custom jig was used to align the specimen for improved vertical oriented. Afterwards, the specimens sealed in an airtight bag placed in a -20°C freezer and frozen overnight while the adhesive completely cured. Only then, the samples are scan by using the μ-CT scanner (SkyScan 1172, Bruker MicroCT, Belgium).

## 2.2 MODEL DEVELOPMENT

The two-dimensional image data sets from the μ-CT scan were stacked in sequence by Mimics software (MIMICS 12, Materialise, Belgium) and converted into rectangular shape to construct the trabecular model. The thickness of each images slice is 15 μm. The stacked image datasets were calculated into three-dimensional trabecular model through image segmentation by the Mimics software. Subsequently, the image datasets were thresholded to select the region of interest for three-dimensional constructed model. In addition, by using an adapted marching cubes algorithm, the triangular surface meshes were generated for the trabecular model. Then again, the result of triangular surface mesh was very fine, which needed to follow with step of removing noise, redundant parts and irregularities shape to construct accurate three-dimensional models. There were 10 models generated and tested in this study.

As for finite element analysis simulation, small sized sub volume region of interest was selected from the fine mesh trabecular bone constructed models (Figure 1) due to the limitation of computer capability to complete the simulation study. These models were then converted into finite element mesh for the simulation. In addition, the trabecular bone model surface meshes with jagged or bad sector are also repaired before importing the model. For the FSI study purpose, the outer wall of the models needed to convert into a flat surface. Thus, for the model preparation, the smaller size sub-models were then merged with cube surface mesh in the Mimics software. After that, the uneven surface of trabecular sub volume models was removed according to the sub volume model shape. Then, the space between the sub model and the cube were stitch together by creating a triangular mesh in between the space. These steps were applied to all six surfaces for the sub trabecular model. Finally, the surface mesh is exported to an STL format file.

**Figure 1:** Development of three-dimensional model into sub volume model.

## 2.3 MORPHOLOGY STUDY

From the μ-CT scan images, the morphological study was conducted. One of a trabecular bone sample contains approximately 900 image slices (Figure 2 (b)). The morphological indices were measured using ImageJ (ImageJ, National Institute of Health, USA). All these slices were import and stacked (Figure 2 (a)) by using BoneJ plugins in ImageJ software to obtain the trabecular morphological data. The parameters measured included BV/TV, Tb.Th, Tb.Sp, Tb.N, DA, MIL, etc. All data present in Table 1.

**Figure 2:** Images obtain from μ-CT scan with (a) Images stacked in sequence according to sample orientation (b) raw scanned images file

**Table 1**: Morphological indices of trabecular bone sample

## 2.4 COMPUTATIONAL SIMULATION

Two-way fluid-structure analysis were conducted using COMSOL Multiphysics software with purpose of investigate the fluid behaviour of bone marrow under gait loading conditions. A gait loading which representing normal walking were applied through the cap faces feature which considered as a rigid body. The gait loading (Figure 3) applied in multi-axis according to normal walking phase (26). The normal walking phase of gait cycle was divided into 40 discrete points for the simulation. The cap faces feature is vital in the FSI study due to restriction coupling between the trabecular model and marrow model within the FEA. In addition, the prescribed displacement was applied to the cap, where the domain was restricted in the X and Y directions (Figure 4). As for the fluid boundary, the plane of the bottom boundary of fluid was applied as symmetry in order to ensure that the marrow volume remains within the domain when there is load applied through the structure. Moreover, in order to prevent normal velocity to the respective boundary, the marrow flow was model as symmetric in their normal directions. The convergence analysis in this study conclude that 400 thousand tetrahedral elements and shape function was used tessellation method is Delaunay on average were needed for accurate result computation (see Figure 5). In addition, the FSI interface uses an arbitrary Lagrangian-Eulerian (ALE) method, which allows moving boundaries without the need for the mesh movement to follow the material. This ALE method combined the fluid flow formulated using a Eulerian description and a spatial frame with solid mechanics formulated using a Lagrangian description and a material frame. The analysis was performed with the criterion of the von Mises stress criterion less than 5%.

**Figure 3:** Gait loading of normal walking based on body weight percentage (Bergmann G. et al., 2001).

The time-dependent solution is obtained for every gait cycle. Details on the force parameters implemented in this study was demonstrated in (27). The solid trabecular structures were modelled as a linear elastic material (28). An elastic modulus (*E*) of 1000 MPa (29) and Poison's ratio of 0.3 was attributed to the trabecular bone solid structure (30). Additionally, the viscosity of fluid marrow was assigned 0.4 Pa.s and modelled as incompressible according to Bryant et al. (31), Newtonian fluid with density of 1060 kg/m$^3$ (32). The surface between the trabecular structure and marrow fluid is assigned as no-slip boundary.

**Figure 4** Boundary Conditions (BC) of trabecular bone and bone marrow models

**Figure 5** Convergence study for the trabecular structure model

In the present work, the bone marrow was modelled as an incompressible liquid. The incompressible Navier-Stokes equation was considered as the governing equation, in which;

$$\nabla \cdot \mathbf{u}_{fluid} = 0 \tag{1}$$

On the other hand, the momentum equation was as follows;

$$\rho \frac{\partial \mathbf{u}_{fluid}}{\partial t} + \rho(\mathbf{u}_{fluid} \cdot \nabla)\mathbf{u}_{fluid} = \nabla \cdot \left[ -p\mathbf{I} + \mu\left(\nabla \mathbf{u}_{fluid} + (\nabla \mathbf{u}_{fluid})^T\right)\right] + \mathbf{F} \tag{2}$$

where the external force acting on the fluid was denoted by **F**, and gravity was neglected. Meanwhile, equation for solid at local equilibrium is given by;

$$\rho \frac{\partial^2 \mathbf{u}_{solid}}{\partial t^2} - \nabla \cdot \boldsymbol{\sigma} = \mathbf{F}_v \tag{3}$$

where $\boldsymbol{\sigma}$ and $\mathbf{F}_v$ are the Cauchy stress tensor and body force, respectively. Deformed structure was demonstrated by $\mathbf{u}_{solid}$, whereas the Piola-Kirchhoff stress, S was used to calculate the Cauchy stress using the following equation;

$$\sigma = J^{-1}\,\mathrm{FSF^T}. \tag{4}$$

Using the gradient of displacement vector $\mathbf{u}_{solid}$, the deformation gradient, **F** can be expressed as;

$$\mathbf{F} = (\mathbf{I} + \nabla \mathbf{u}_{solid}), \tag{5}$$

In which the identity matrix was denoted by **I**, and the Jacobian of the deformation is defined as;

$$J = \det(\mathbf{F}). \tag{6}$$

Fluid domain was solved based on Eulerian formulation, while solid domain was solved based on Langrangian formulations. In coupling fluid-solid system, the arbitrary Lagrangian-Eularian method can be implemented with total force on the fluid-solid boundary was given as;

$$f_r = \mathbf{n} \cdot \left[ -p\mathbf{I} + \mu\left(\nabla \mathbf{u}_{fluid} + (\nabla \mathbf{u}_{fluid})^T\right)\right], \tag{7}$$

With **n** is the normal acting outward at the boundary, the force at the structure's boundary is given by;

$$\mathbf{F}_r = \boldsymbol{\sigma} \cdot \boldsymbol{n}. \tag{8}$$

In Spatial and material coordinate system, these forces can be coupled thru a force transformation using the arbitrary Eulerian-Lagrangian method as follows:

$$\mathbf{F}_r = f_r \cdot \frac{dv}{dV}. \tag{10}$$

Mesh element scale factors $dv$ and $dV$ are the fluid and material frames, respectively. Further, the relationship of structural velocity of the moving wall with the fluid velocity is demonstrated as follows:

$$\mathbf{u}_{fluid} = \mathbf{u}_w, \tag{11}$$

Thus, the rate of change of the solid displacement is defined by the structural velocity.

$$\mathbf{u}_w = \frac{\partial \mathbf{u}_{solid}}{\partial t} \tag{12}$$

## 2.5 STATISTICAL ANALYSIS

All morphology indices are presented in mean and standard deviation (Table 1). The Pearson's correlation and linear regression analysis were performed to explore the interrelationship between the morphological indices and the mechanical properties of the trabecular bone sample. The multiple linear regression was perform using IBM SPSS Statistics 23 (IBM Corp, USA). For all comparison, the level of significant for p-value was <0.05.

## 3.0 RESULTS

The average von Mises stress distribution during normal walking with cycle duration was plotted as shown in Figure 6. The peak pressure reached as high as 11.35 x $10^5$ Pa. Then again, the minimum stress for trabecular bone is 10.75 x $10^4$ Pa at period of 0.86s. As can be seen, the behaviour of von Mises stress during gait cycle is similar to the force in the vertical direction. From the computational

FSI simulation, the von Mises stress distribution within the trabecular bone model along gait normal walking gait loading cycle at different time frame as illustrated in Figure 7. Comparing Figure 7(a) and (b), more area covered with high stresses at time 0.14 s. These results match with the graph of the von Mises stress over time.

**Figure 6:** von Mises stress distribution on the trabecular bone during normal walking

**Figure 7:** Comparison of von Mises stress on the trabecular bone at different time frame (a) t = 0.14 s and (b) t = 0.86 s

The pressure and shear stress distribution during gait loading cycle are presented in Figure 8. This figure shows how the structure of trabecular bone affects the fluid characteristic during the gait loading cycle. It can be observed from the Figure 8 that the pattern of pressure distribution was similar to the von Mises stress results. The pressure was range from 380 to 4070 Pa during the normal walking cycle. Moreover, as discussed earlier, the trabecular structure experience shear stress due to bone marrow movement. With an average of 0.09 Pa, the shear stress was in the range of 0.01 to 0.27 Pa.

Based on 2D images of the trabecular model cross section, the pressure on top section was lower than below section when the structure at the highest compression deformation Figure 9(a). However, at period 0.86 s, the pressure on top section becomes higher than the lower section. Figure 10 shows velocity profile of marrow during gait loading cycle at different time frame. As can be seen, at period 0.14s, the velocity was higher than at period 0.86 s. The velocity was range of 0.09 μm/s to 81.2 μm/s at period of 0.14 s and 0.001 μm/s to 2.6 μm/s at period 0.86 s.

**Figure 8:** Maximum shear stress and pressure distribution on the trabecular bone along with normal walking loading

**Figure 9:** Comparison of pressure distribution on the trabecular bone cross section at different time frame (a) t = 0.14 s and (b) t = 0.86 s

**Figure 10:** Velocity profile on the trabecular bone at different time frame (a) t = 0.14 s and (b) t = 0.86 s during normal gait loading.

Multiple regression analysis for morphological parameters is tabulated in Table 2 and Table 3 with Pearson correlation and *p*-value for solid and fluid characteristic. Bone volume fraction and SMI shows good correlation with principal strain and von Mises stress. However, the SMI value only significant with principal strain and bone volume fraction solitary shows significant value with von Mises stress.

As for marrow characteristic, velocity and pressure were significant to SMI, while shear stress is insignificant with all morphological parameters.

**Table 2:** Morphological parameters of trabecular bone sample with Pearson correlation and *p*-value in relation with mechanical behaviour.

**Table 3:** Morphological parameters of trabecular bone sample with Pearson correlation and *p*-value in relation with fluid characteristics.

The volume fraction plays a major role in trabecular bone mechanical properties. The distribution of von Mises stress on different model with different bone volume fraction is shown in Figure 11. As can be seen, low bone volume fraction model result in higher von Mises stress compare with a model which has high volume fraction.

**Figure 11:** Comparison of von Mises stress on the trabecular bone with different bone fraction (a) BV/TV = 0.32 and (b) BV/TV = 0.45

**Figure 12:** Linear relationship between (a) BV/TV and (b) SMI with permeability

The permeability and trabecular stiffness relationship to bone volume fraction and SMI are plotted in Figure 12. The graph showed a strong and negative correlation between the permeability and bone volume fraction (r = -0.862), whereas the correlations are strong and positive between permeability and SMI (r = 0.835). Meanwhile, in Figure 13 shows both bone volume fraction and SMI have a strong correlation with the trabecular stiffness (r = 0.832 and r = -0.796; respectively). However, the trabecular bone stiffness correlation was inversely compared to the permeability.

**Figure 13:** Linear relationship between (a) BV/TV and (b) SMI with stiffness.

## 4.0 DISCUSSION

The trabecular bone structure is known as a porous structure which contributes to maximum strength while giving the bone less weight. Understanding the bone marrow flow and trabecular bone structure mechanism can provide insight into bone remodelling process and bone strength. It is vital to identify which architectural features that affect the trabecular strength. Thus, in this study, finite element analysis with FSI approach was used to identify the architecture contribution with the presence of bone marrow when there is physical loading involved.

During physiological activities, the bone will have a deformation due to the mechanical load (3). The compressive and tensile stress is generated in the trabecular structure which causes the bone marrow within the structure to drift from region of compression to tension. Due to the complex structure of trabecular bone with small rods, plated and pores, there will be shear stress generated on the wall structure. Bone marrow function in activating the bone cells to start the bone remodelling process. Thus, this study analysed shear stress, pressure, and permeability to identify the fluid characteristic through physical activity. Moreover, the trabecular bone architecture and volume fraction play an important role in its mechanical properties. In addition, this study analysed the bone marrow permeability and trabecular stiffness with correlation to the trabecular morphology. Applied physiological gait loading in this present study to assure more reliable and safer prediction of bone marrow behaviour and trabecular stiffness.

In the analysis, it can be seen that the maximum peak von Mises stress range for all ten models are 91 kN/m$^2$ to 114 kN/m$^2$. The maximum value was identified in period 0.14s, which is due to the fact that higher contact force occurs at that period of time (Figure 7). Assessing the von Mises stress in the trabecular model was necessary with purpose of providing new insight in prediction of trabecular structure failure and evaluating the fracture risk. The permeability in this study was also in agreement with results from the literature.

The aim of this study is to identify the bone marrow movement behaviour within the trabecular structure sufficient for bone cell growth based on physiological activity. Normal walking loading was chosen since it is reported as the most frequent physiological activity (26). The results show that during normal walking loading, the maximum shear stress occurs is all models are in range 0.05 Pa to 0.27 Pa. Furthermore, Li et al. (17), Castillo and Jacobs (20) present that the shear stress was needed for the cells to differentiate and proliferate. Moreover, the previous experimental study showed that shear stress in the range of 0.1 Pa to 1 Pa needed to stimulate bone cells in vitro. Thus, a much lower range of shear stress is suggested to simulate the stress on bone cells during normal walking. However, other previously study mention that 0.23 Pa of shear stress is sufficient for regulated bone cells to respond and synthesise bone matrix protein for bone remodelling process (33). Additionally, there is separate study by Nauman et al. (34) stated that the bone cells stimulation is unaffected by difference shear stress levels.

Walking 10000 step per day has been proposed as daily activity for healthy adults. This recommendation was studied, and it is found that 10000 step per day can improve individual's health and sustainability. For example, previous studies stated higher step count per day could lower the prevalence of depression (35). In addition, it is observed the Body Mass Index (BMI) of the group with higher step count per day has shown significantly lower compared with another group (36).

Nevertheless, there is no studies indicate on bone health based on daily step count. However, based on the results of this study, it is suggested that higher step count will lead to more shear stress. Thus, can help in improving the bone remodelling process and bone strength.

Moreover, while the trabecular bone deforms according to the physiological load, there would be pressure difference within the structure (Figure 8). Previous study stated bone formation increase with pressure. Welch et al. (37) in their study found that bone marrow pressure increased about 2000 Pa resulted in bone remodelling. Another study on the new bone formation of mouse tibiae, stated in dynamic compression induced a similar range of pressure value (38). This study results for pressure distribution in normal walking gait loading for all models was in the range of 180 Pa to 4000 Pa. Addition to this study result analysis, it is found that higher bone volume fraction will lead to lower pressure value (Table 3). However, the pressure difference also depends on a variety of factors, including the bone marrow rheology, bone strain and permeability (39, 40).

In addition, the results show that the pressure gradient along the walking gait loading was different at period 0.14s and 0.86 s as in Figure 9. This is where the bone marrow function as hydraulic stiffening effect. Hydraulic stiffening effect refers to the reduction of bone stress during dynamic loading effect by the presence of fluid within the structure (41). While at period 0.14s is when the maximum compression occurs, the pressure of bone marrow was high at the bottom. This pressure might support a certain amount of applied load which caused the apparent stiffness to the trabecular structure. Similarly, at period of 0.86s, where tensile occur, the pressure was high at the upper region giving the structure extra load barrier which prevents the structure to have high deformation.

This study used BoneJ plugin in the ImageJ software to analyse the morphological data of the trabecular model. In the correlation study, only bone volume fraction (BV/TV) and SMI shows a significant value with the simulation results. Thus, this study correlated the permeability and bone stiffness with these both morphological parameters. From the results, permeability and stiffness show good correlation with the BV/TV. Permeability in trabecular bone was vital since it demonstrated the biological based features of trabecular bone. Still, the simulation results were consistent to those found in the previous literature (42). Additionally, the trabecular bone mechanical quality was depending on its stiffness, since the stiffness have a strong correlation with the strength (43).

Hypothetically bone with lower bone volume fraction means porous structure (osteoporotic bone). Thus, as mention by Goldstein et al. (44) and Syahrom et al. (45), the trabecular bone integrity and bone marrow permeability can be disarrange based on the porosity value. In addition, the results of this study suggests that enhancement of bone remodelling process can be achieved by optimization of BV/TV and permeability value. However, from the results analysis the reduction of BV/TV can cause higher stress

on the trabecular structure (Figure 11) and the loss of trabecular structure stiffening effect (Figure 13). Therefore, important to find the optimum bone volume fraction which has a good stiffening value and yet able to deliver sufficient nutrient to bone cells.

Other than bone volume fraction, the SMI also shows high correlation with the stiffness. The SMI is a measurement which determines a porous structure is made of rod or plate-like structure. The value starts from 0 for ideal plate structures to 3 for ideal rod structure (46). Theoretically, the plate-like structure could barricade more fluid flow compared to the rod-like structure. In contrast with the permeability, the stiffness is negatively correlated with the SMI. Moreover, previous study already stated that trabecular plates have more dominant role in mechanical integrity of trabecular bone structure (47, 48). Also, osteoporotic trabecular bone has been found to have an apparent transition of microarchitecture, which is from plate like to rod-like structure (47). Stein et al. (49) in their study found that the bone stiffness related with trabecular connectivity, trabeculae orientation and trabecular plates. Thus, in correspondence to previous studies, our results suggest that plate-like trabecular structure (lower SMI value) can contribute to higher trabecular stiffness.

There are a few limitations in the interpretation of results that should be considered. This study has employed trabecular samples from anatomic site of bovine femoral bone. As such, the microarchitecture parameters may differ from that of the human bone or other anatomic sites. However, there are a few works done that demonstrate agreement between the architecture as well as mechanical properties of bovine trabecular bone and that of a healthy human (50, 51). Furthermore, the impact of marrow phase on the trabecular structure can be further investigated in terms of its properties such as the variation of constituents and viscosity.

In summary, this study was investigated on the correlation of the morphology parameters onto the mechanical properties of trabecular bone with presence of bone marrow. The bone volume fraction and SMI were identified as the one that has a higher correlation with the trabecular permeability and stiffness compared with others morphological parameters. Moreover, the bone marrow behaviour through the physiological activity was identified in this study. This study provides insight into understanding how human daily physiological activities contribute to the bone remodelling process and nutrient transport with the bone environment. However, more knowledge in this area was crucial to studying the bone adaption to the bone replacement and in estimating fracture risk.

## 5.0 CONCLUSION

The overall aim of this study was to assess the importance on the interaction between the bone marrow and trabecular bone structure during mechanical loading by using the FSI approach. Trabecular bone

is known as a highly porous structure with a significant volume of bone marrow, a compressive or tensile force on the trabecular bone will result in bone marrow movement with respect to the trabecular bone structure. It is believed that the fluid flow will cause the shear stress to the trabecular structure. The interaction between the fluid and trabecular bone will occur, and this incident might have several effects on the trabecular structure. Moreover, bone remodelling process was occurring due to the shear stress on the bone cell which triggers the process. In addition to shear stress, based on previous study the hydraulic stiffening effect occurs due to the presence of bone marrow within the trabecular bone structure. Therefore, this study proposed the used of FSI approach to model the trabecular bone behaviour and marrow flow characteristic.

The physiological activities in daily human life play a major role than calcium intake in the bone development process (52). It contributes to mechanical stimuli in bone marrow and trabecular bone strain. Normal walking is one of human major daily activity is chosen in this study as a boundary condition in analysing the trabecular bone behaviour. The bone marrow behaviour was recorded during the normal walking cyclic loading. While the trabecular bone deforms according to the physiological load, the bone marrow within will encounter mechanical stimulation in mechanobiological response (53, 54). The shear stress value along normal walking gait loading was found in a range of 0.05 to 0.27 Pa which is sufficient to regulate cell response minimally (33). However, due to ageing factor, bone resorption rate will become higher. Thus, higher shear stress was needed in order to have rapid bone remodelling process to encounter the bone resorption rate. Furthermore, this study also provides insight into understanding the related mechanobiological of bone cells and disease in deterioration of nutrient supplied to the bone.

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## 6.0 References

1. Saad APM, Syahrom A. Study of dynamic degradation behaviour of porous magnesium under physiological environment of human cancellous bone. Corrosion Science. 2018;131:45-56.
2. Yourek G, McCormick SM, Mao JJ, Reilly GC. Shear stress induces osteogenic differentiation of human mesenchymal stem cells. Regenerative medicine. 2010;5(5):713-24.

3. Coughlin TR, Niebur GL. Fluid shear stress in trabecular bone marrow due to low-magnitude high-frequency vibration. Journal of biomechanics. 2012;45(13):2222-9.
4. Vaughan T, Voisin M, Niebur G, McNamara L. Multiscale modeling of trabecular bone marrow: understanding the micromechanical environment of mesenchymal stem cells during osteoporosis. Journal of Biomechanical Engineering. 2015;137(1).
5. You L, Cowin SC, Schaffler MB, Weinbaum S. A model for strain amplification in the actin cytoskeleton of osteocytes due to fluid drag on pericellular matrix. Journal of biomechanics. 2001;34(11):1375-86.
6. Adachi T, Kameo Y, Hojo M. Trabecular bone remodelling simulation considering osteocytic response to fluid-induced shear stress. Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences. 2010;368(1920):2669-82.
7. Wittkowske C, Reilly GC, Lacroix D, Perrault CM. In vitro bone cell models: impact of fluid shear stress on bone formation. Frontiers in Bioengineering and Biotechnology. 2016;4:87.
8. Birmingham E, Niebur G, McNamara L, McHugh P. An experimental and computational investigation of bone formation in mechanically loaded trabecular bone explants. Annals of biomedical engineering. 2016;44(4):1191-203.
9. Shen V, Liang X, Birchman R, Wu D, Healy D, Lindsay R, et al. Short term immobilization-induced cancellous bone loss is limited to regions undergoing high turnover and/or modeling in mature rats. Bone. 1997;21(1):71-8.
10. Rodriguez N, Herndon D, Klein G. Evidence against a role of immobilization in the bone loss following burns. Bone. 2011;2(48):S190.
11. Frost HM. Wolff's Law and bone's structural adaptations to mechanical usage: an overview for clinicians. The Angle Orthodontist. 1994;64(3):175-88.
12. Frost HM. Bone “mass” and the “mechanostat”: a proposal. The anatomical record. 1987;219(1):1-9.
13. Burr DB, Milgrom C, Fyhrie D, Forwood M, Nyska M, Finestone A, et al. In vivo measurement of human tibial strains during vigorous activity. Bone. 1996;18(5):405-10.
14. Fritton SP, McLeod KJ, Rubin CT. Quantifying the strain history of bone: spatial uniformity and self-similarity of low-magnitude strains. Journal of biomechanics. 2000;33(3):317-25.
15. Gurkan UA, Akkus O. The mechanical environment of bone marrow: a review. Annals of biomedical engineering. 2008;36(12):1978-91.
16. McAllister T, Frangos J. Steady and transient fluid shear stress stimulate NO release in osteoblasts through distinct biochemical pathways. Journal of Bone and Mineral Research. 1999;14(6):930-6.
17. Li YJ, Batra NN, You L, Meier SC, Coe IA, Yellowley CE, et al. Oscillatory fluid flow affects human marrow stromal cell proliferation and differentiation. Journal of Orthopaedic Research. 2004;22(6):1283-9.
18. Knight MN, Hankenson KD. Mesenchymal stem cells in bone regeneration. Advances in wound care. 2013;2(6):306-16.
19. Shao J, Zhang W, Yang T. Using mesenchymal stem cells as a therapy for bone regeneration and repairing. Biological research. 2015;48(1):62.
20. Castillo AB, Jacobs CR. Mesenchymal stem cell mechanobiology. Current osteoporosis reports. 2010;8(2):98-104.
21. Klein-Nulend J, Van Der Plas A, Semeins CM, Ajubi NE, Erangos JA, Nijweide PJ, et al. Sensitivity of osteocytes to biomechanical stress in vitro. The FASEB Journal. 1995;9(5):441-5.

22. Case N, Sen B, Thomas J, Styner M, Xie Z, Jacobs C, et al. Steady and oscillatory fluid flows produce a similar osteogenic phenotype. Calcified tissue international. 2011;88(3):189-97.
23. Bakker AD, Joldersma M, Klein-Nulend J, Burger EH. Interactive effects of PTH and mechanical stress on nitric oxide and PGE2 production by primary mouse osteoblastic cells. American journal of physiology-endocrinology and metabolism. 2003;285(3):E608-E13.
24. Arnsdorf EJ, Tummala P, Kwon RY, Jacobs CR. Mechanically induced osteogenic differentiation–the role of RhoA, ROCKII and cytoskeletal dynamics. Journal of cell science. 2009;122(4):546-53.
25. Yeatts AB, Fisher JP. Bone tissue engineering bioreactors: dynamic culture and the influence of shear stress. Bone. 2011;48(2):171-81.
26. Bergmann G, Deuretzbacher G, Heller M, Graichen F, Rohlmann A, Strauss J, et al. Hip contact forces and gait patterns from routine activities. Journal of biomechanics. 2001;34(7):859-71.
27. Fatihhi S, Harun M, Kadir MRA, Abdullah J, Kamarul T, Öchsner A, et al. Uniaxial and multiaxial fatigue life prediction of the trabecular bone based on physiological loading: a comparative study. Annals of biomedical engineering. 2015;43(10):2487-502.
28. Homminga J, Mccreadie BR, Weinans H, Huiskes R. The dependence of the elastic properties of osteoporotic cancellous bone on volume fraction and fabric. Journal of biomechanics. 2003;36(10):1461-7.
29. Sylvester AD, Kramer PA. Young's modulus and load complexity: Modeling their effects on proximal femur strain. The Anatomical Record. 2018;301(7):1189-202.
30. Bayraktar HH, Gupta A, Kwon RY, Papadopoulos P, Keaveny TM. The modified super-ellipsoid yield criterion for human trabecular bone. J Biomech Eng. 2004;126(6):677-84.
31. Bryant J, David T, Gaskell P, King S, Lond G. Rheology of bovine bone marrow. Proceedings of the Institution of Mechanical Engineers, Part H: Journal of Engineering in Medicine. 1989;203(2):71-5.
32. Haskill JS, McNeill TA, Moore MAS. Density distribution analysis of In vivo and In vitro colony forming cells in bone marrow. Journal of Cellular Physiology. 1970;75(2):167-79.
33. Kreke MR, Sharp LA, Woo Lee Y, Goldstein AS. Effect of intermittent shear stress on mechanotransductive signaling and osteoblastic differentiation of bone marrow stromal cells. Tissue Engineering Part A. 2008;14(4):529-37.
34. Nauman E, Satcher R, Keaveny T, Halloran B, Bikle D. Osteoblasts respond to pulsatile fluid flow with short-term increases in PGE2 but no change in mineralization. Journal of Applied Physiology. 2001;90(5):1849-54.
35. McKercher CM, Schmidt MD, Sanderson KA, Patton GC, Dwyer T, Venn AJ. Physical activity and depression in young adults. American journal of preventive medicine. 2009;36(2):161-4.
36. Krumm EM, Dessieux OL, Andrews P, Thompson DL. The relationship between daily steps and body composition in postmenopausal women. Journal of women's health. 2006;15(2):202-10.
37. Welch R, Johnston 2nd C, Waldron M, Poteet B. Bone changes associated with intraosseous hypertension in the caprine tibia. JBJS. 1993;75(1):53-60.
38. Hu M, Cheng J, Qin Y-X. Dynamic hydraulic flow stimulation on mitigation of trabecular bone loss in a rat functional disuse model. Bone. 2012;51(4):819-25.
39. Teo J, Si-Hoe K, Keh J, Teoh S. Correlation of cancellous bone microarchitectural parameters from microCT to CT number and bone mechanical properties. Materials Science and Engineering: C. 2007;27(2):333-9.

40. Baroud G, Falk R, Crookshank M, Sponagel S, Steffen T. Experimental and theoretical investigation of directional permeability of human vertebral cancellous bone for cement infiltration. Journal of biomechanics. 2004;37(2):189-96.
41. Liebschner MA, Keller TS. Hydraulic strengthening affects the stiffness and strength of cortical bone. Annals of Biomedical Engineering. 2005;33(1):26-38.
42. Kohles SS, Roberts JB. Linear poroelastic cancellous bone anisotropy: trabecular solid elastic and fluid transport properties. J Biomech Eng. 2002;124(5):521-6.
43. Lopez KW, Goldstein SA, Ciarelli MJ, Kuhn JL, Brown M, Feldkamp L. The relationship between the structural and orthogonal compressive properties of trabecular bone. 1994.
44. Goldstein AS, Juarez TM, Helmke CD, Gustin MC, Mikos AG. Effect of convection on osteoblastic cell growth and function in biodegradable polymer foam scaffolds. Biomaterials. 2001;22(11):1279-88.
45. Syahrom A, Kadir MRA, Abdullah J, Öchsner A. Permeability studies of artificial and natural cancellous bone structures. Medical engineering & physics. 2013;35(6):792-9.
46. Hildebrand T, Rüegsegger P. Quantification of bone microarchitecture with the structure model index. Computer Methods in Biomechanics and Bio Medical Engineering. 1997;1(1):15-23.
47. Wang J, Zhou B, Liu XS, Fields AJ, Sanyal A, Shi X, et al. Trabecular plates and rods determine elastic modulus and yield strength of human trabecular bone. Bone. 2015;72:71-80.
48. Wang J, Zhou B, Parkinson I, Thomas CDL, Clement JG, Fazzalari N, et al. Trabecular plate loss and deteriorating elastic modulus of femoral trabecular bone in intertrochanteric hip fractures. Bone research. 2013;1:346-54.
49. Stein EM, Kepley A, Walker M, Nickolas TL, Nishiyama K, Zhou B, et al. Skeletal structure in postmenopausal women with osteopenia and fractures is characterized by abnormal trabecular plates and cortical thinning. Journal of Bone and Mineral Research. 2014;29(5):1101-9.
50. Keaveny TM, Wachtel EF, Ford CM, Hayes WC. Differences between the tensile and compressive strengths of bovine tibial trabecular bone depend on modulus. Journal of biomechanics. 1994;27(9):1137-46.
51. Morgan EF, Yeh OC, Chang WC, Keaveny TM. Nonlinear behavior of trabecular bone at small strains. J Biomech Eng. 2001;123(1):1-9.
52. Anderson JJ. The important role of physical activity in skeletal development: how exercise may counter low calcium intake. The American journal of clinical nutrition. 2000;71(6):1384-6.
53. Metzger TA, Schwaner SA, LaNeve AJ, Kreipke TC, Niebur GL. Pressure and shear stress in trabecular bone marrow during whole bone loading. Journal of biomechanics. 2015;48(12):3035-43.
54. Birmingham E, Grogan J, Niebur G, McNamara L, McHugh P. Computational modelling of the mechanics of trabecular bone and marrow using fluid structure interaction techniques. Annals of biomedical engineering. 2013;41(4):814-26.

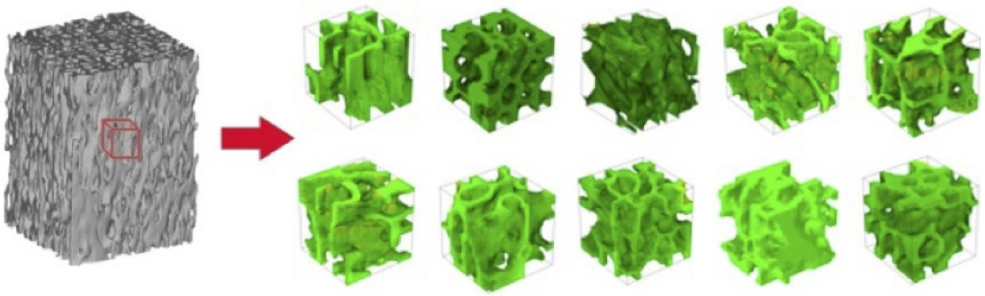

Figure 1: Development of three-dimensional model into sub volume model.

309x95mm (300 x 300 DPI)

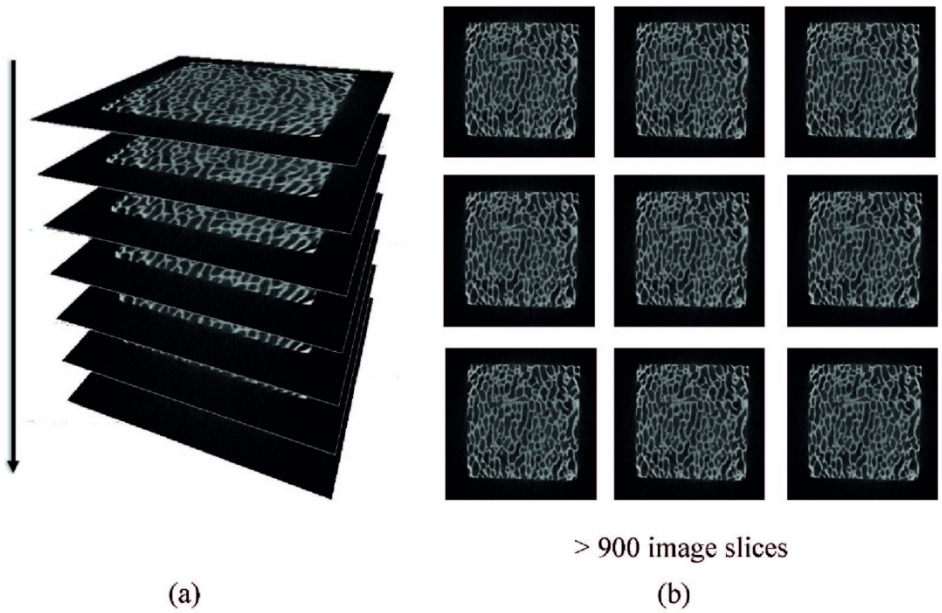


Figure 2: Images obtain from μ-CT scan with (a) Images stacked in sequence according to sample orientation (b) raw scanned images file

145x102mm (300 x 300 DPI)

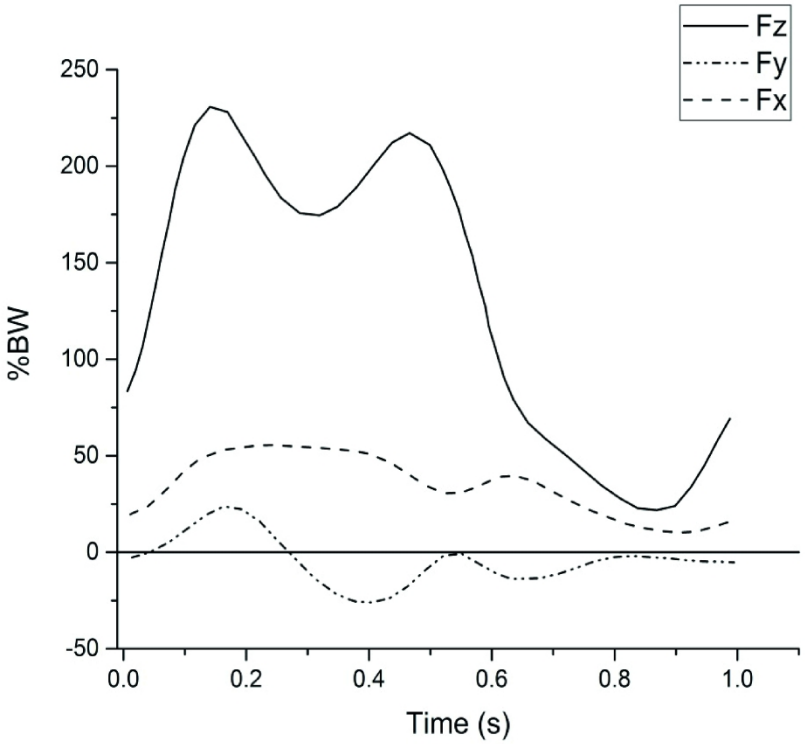


Figure 3: Gait loading of normal walking based on body weight percentage (Bergmann G. et al., 2001).

249x201mm (300 x 300 DPI)

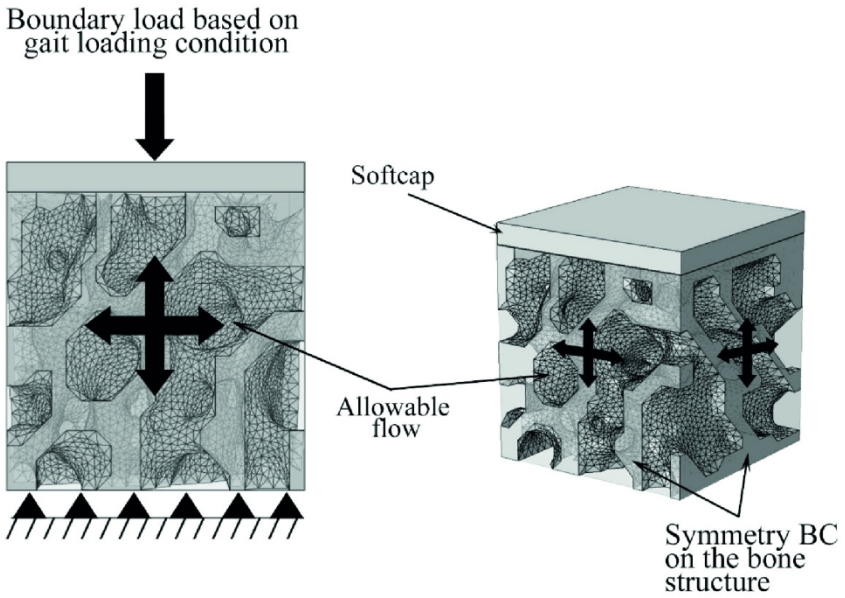


Figure 4 Boundary Conditions (BC) of trabecular bone and bone marrow models

159x98mm (300 x 300 DPI)

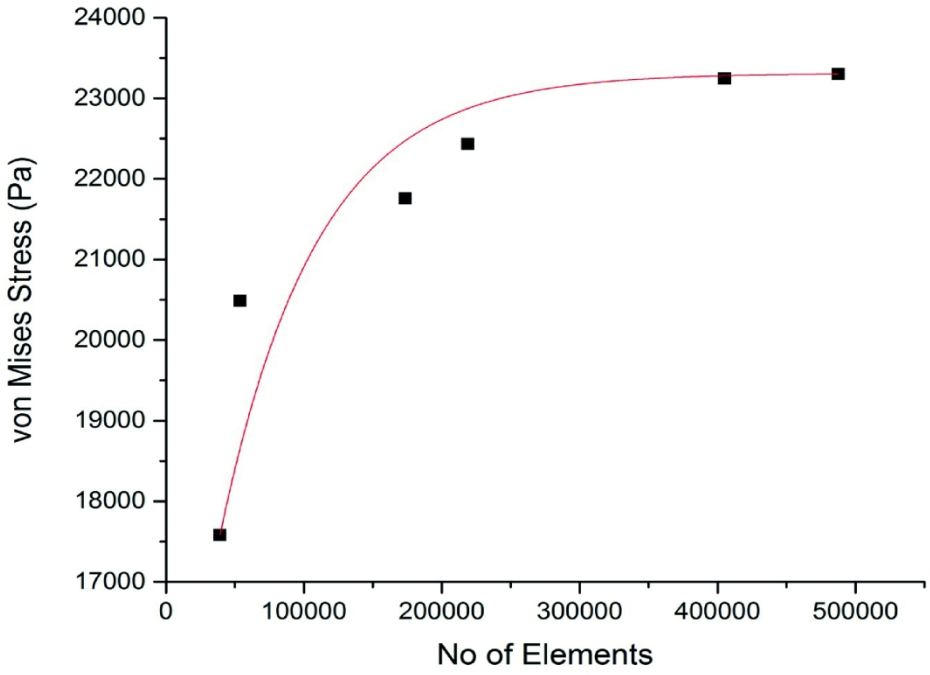


Figure 5 Convergence study for the trabecular structure model

210x147mm (300 x 300 DPI)

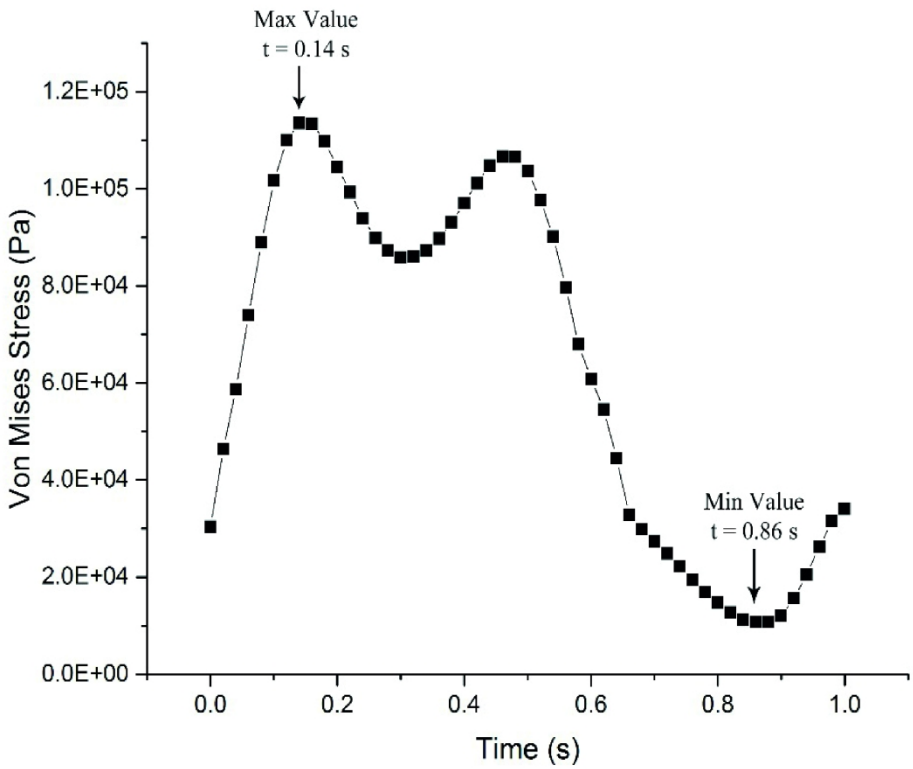


Figure 6: von Mises stress distribution on the trabecular bone during normal walking

210x169mm (300 x 300 DPI)

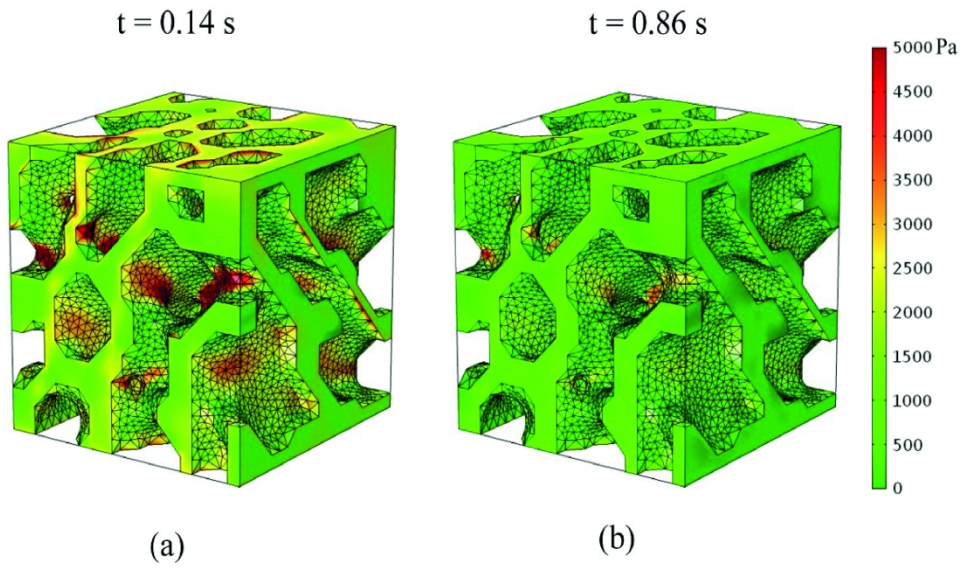


Figure 7: Comparison of von Mises stress on the trabecular bone at different time frame (a) t = 0.14 s and (b) t = 0.86 s

210x134mm (300 x 300 DPI)

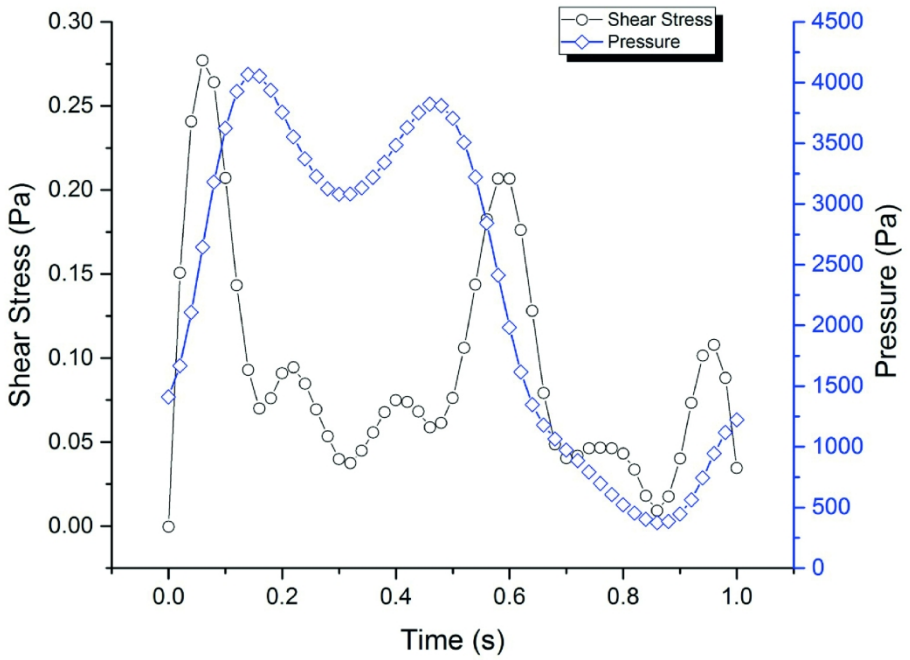


Figure 8: Maximum shear stress and pressure distribution on the trabecular bone along with normal walking loading

210x159mm (300 x 300 DPI)

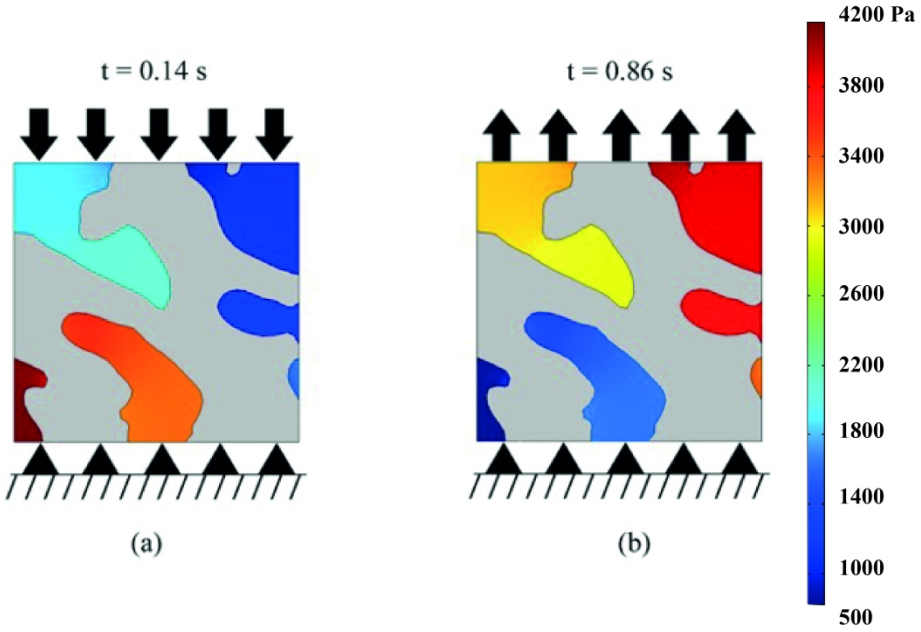


Figure 9: Comparison of pressure distribution on the trabecular bone cross section at different time frame (a) t = 0.14 s and (b) t = 0.86 s

308x220mm (300 x 300 DPI)

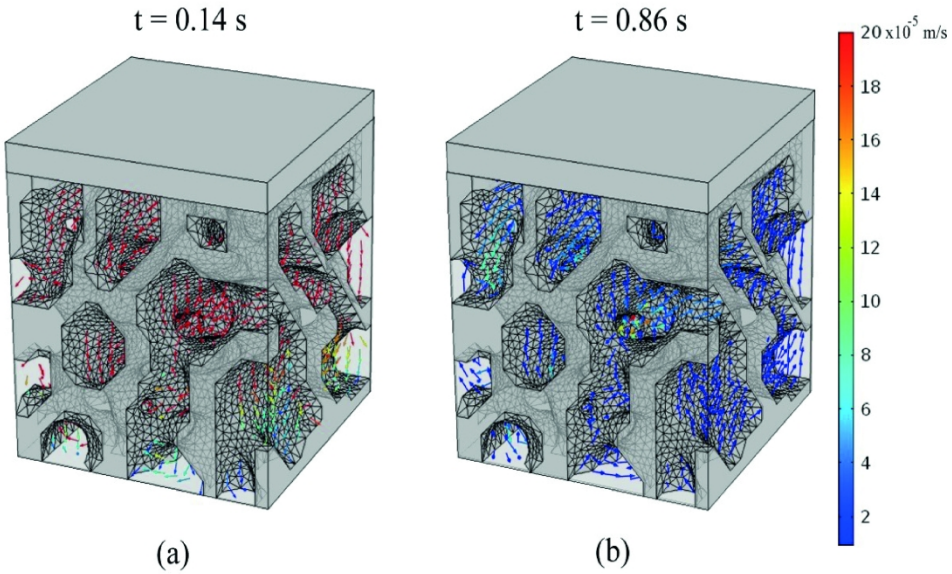


Figure 10: Velocity profile on the trabecular bone at different time frame (a) t = 0.14 s and (b) t = 0.86 s during normal gait loading.

292x184mm (300 x 300 DPI)

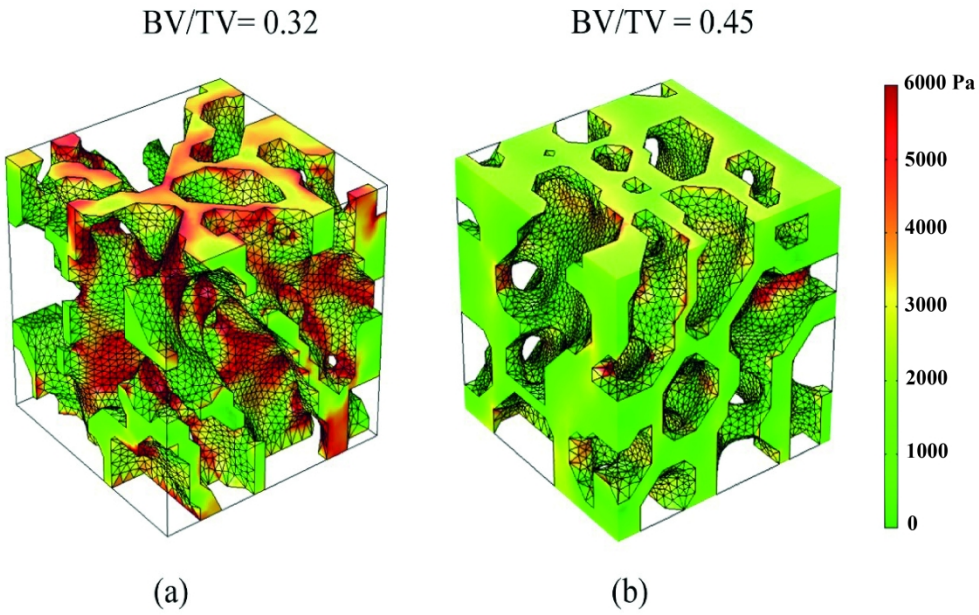


Figure 11: Comparison of von Mises stress on the trabecular bone with different bone fraction (a) BV/TV = 0.32 and (b) BV/TV = 0.45

298x212mm (300 x 300 DPI)

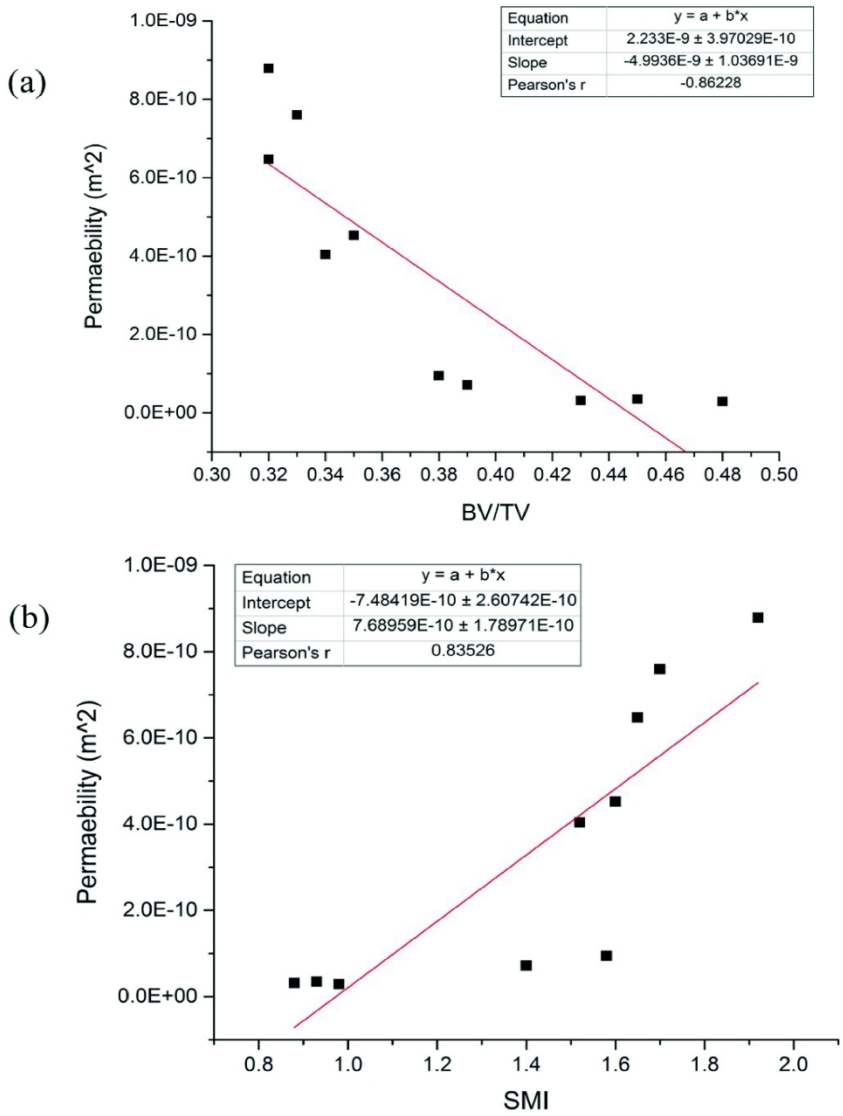


Figure 12: Linear relationship between (a) BV/TV and (b) SMI with permeability

209x241mm (300 x 300 DPI)

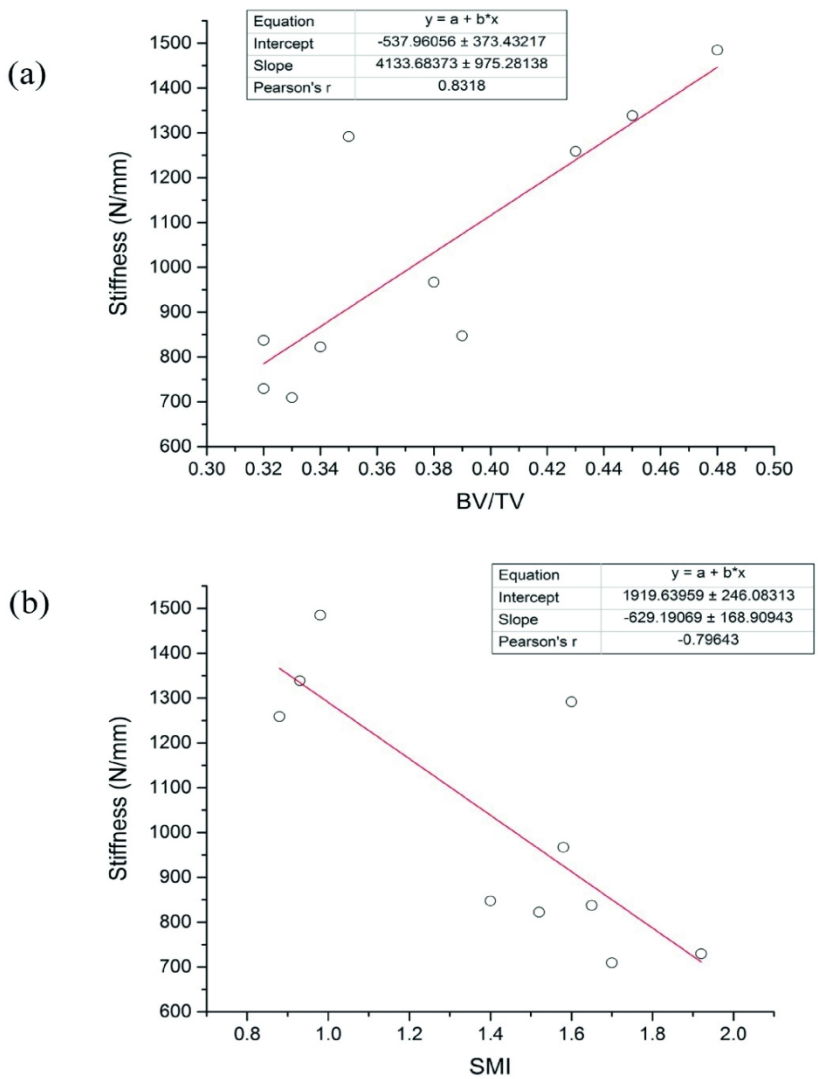


Figure 13: Linear relationship between (a) BV/TV and (b) SMI with stiffness.

209x238mm (300 x 300 DPI)

**Table 1**: Morphological indices of trabecular bone sample

| PARAMETER | MINIMUM | MAXIMUM | MEAN | SD |
|---|---|---|---|---|
| BV/TV | 0.318 | 0.477 | 0.379 | 0.057 |
| Tb.Th (mm) | 0.128 | 0.559 | 0.207 | 0.057 |
| Tb.Sp (mm) | 0.253 | 1.022 | 0.441 | 0.137 |
| BS/BV | 11.313 | 15.857 | 13.677 | 1.719 |
| DA | 0.38 | 0.684 | 0.611 | 0.146 |
| Conn.D ($mm^{-3}$) | 19.625 | 59.875 | 37.975 | 14.179 |
| SMI | 0.875 | 1.918 | 1.416 | 0.316 |
| Porosity (%) | 62 | 76 | 70 | 5 |
| Bone Surface Area ($mm^2$) | 28.802 | 37.518 | 32.447 | 3.134 |

**Table 2:** Morphological parameters of trabecular bone sample with Pearson correlation and *p*-value in relation with mechanical behaviour.

| Morphological Parameters | Principle Strain | | Von Mises Stress | |
|---|---|---|---|---|
| | Pearson Correlation | *p*-value | Pearson Correlation | *p*-value |
| BV/TV | -0.830 | 0.123 | -0.798 | 0.006* |
| BS/TV | 0.449 | 0.452 | 0.28 | 0.132 |
| SMI | 0.850 | 0.002* | 0.715 | 0.689 |
| Conn. D | -0.032 | 0.916 | -0.303 | 0.16 |
| Tb.Th | -0.426 | 0.566 | -0.344 | 0.206 |
| Tb.Sp | 0.705 | 0.403 | 0.769 | 0.405 |
| DA | 0.410 | 0.966 | 0.559 | 0.218 |

*Significant *p*-value < 0.05

**Table 3:** Morphological parameters of trabecular bone sample with Pearson correlation and *p*-value in relation with fluid characteristics.

| Morphology Parameters | Velocity | | Pressure | | Shear Stress | |
|---|---|---|---|---|---|---|
| | Pearson Correlation | *p*-value | Pearson Correlation | *p*-value | Pearson Correlation | *p*-value |
| BV/TV | -0.661 | 0.969 | -0.672 | 0.168 | 0.001 | 0.499 |
| BS/TV | 0.388 | 0.449 | 0.53 | 0.585 | 0.411 | 0.119 |
| SMI | 0.710 | 0.022* | 0.825 | 0.003* | 0.029 | 0.469 |
| Conn. D | -0.049 | 0.316 | 0.288 | 0.815 | 0.184 | 0.306 |
| Tb.Th | -0.296 | 0.395 | -0.346 | 0.209 | -0.424 | 0.111 |
| Tb.Sp | 0.607 | 0.735 | 0.594 | 0.754 | -0.255 | 0.239 |
| DA | 0.324 | 0.468 | 0.074 | 0.721 | 0.079 | 0.414 |

*Significant *p*-value < 0.05

Reviewer(s)' Comments to Author:

Reviewer: 1

Comments to the Author
The paper presents an interesting analysis on the effect of bone marrow on the mechanical environment and the structure of trabecular bone during normal walking loading.
However, there are comments and questions that need to be addressed.

1. Abstract – “FSI approach”, define the meaning of FSI.
   ➔ Definition added.

2. Line 72 – Units of micro-strain (με) are somehow different from what is used on mechanical engineering. Could it be replaced by mm/mm?
   ➔ Replaced accordingly.

3. Line 83 – Define MSCs
   ➔ Definition added.

4. Line 116 – the protocol for storage and cleaning was previously defined? If so, give a reference.
   ➔ Reference added.

5. Table 1 –If possible, also add Tb.N (trabecular number) and FD (fractal dimension)
   ➔ We decided not to include Tb.N and FD on the table as both indices do not directly related to the focus of this study.

6. Line 177 – Figure Caption – Was this figure obtained under the present work or does it belong to another author?
   ➔ Yes, it is belonged to another author. Citation added.

7. Line 175 – The authors should add in the text that the convergence study was performed with the criterion of the von Mises stress variation less than X %. Usual values are 2 to 5%. What was the value here?
   ➔ The variation was less than 5% of the von Mises stress criterion. Explanation added on line 177 – “The analysis was performed with the criterion of the von Mises stress criterion less than 5%.”

8. Line 196, page 11, provide a number of each equation.
   ➔ Numbers added to all equations.

9. Line 215 “……With n is the normal acting outward at the boundary,…” needs an English revision
   ➔ Sentence was revised accordingly – “With the normal, n is acting outward from surface boundary,…”

10. Line 250 – add in the caption that the time chosen corresponds to the highest and to the lowest von Mises stress.
    ➔ Added accordingly.

11. Line 271 – Could the authors add a scale of colors at Figure 9?
    ➔ Added accordingly.

12. Line 306 – The number of the figure is missing, but it should be 12.
    ➔ Added accordingly.

13. Line 308, also the number of the figure is missing.
    ➔ Added accordingly.

14. Line 400 and 401 – Figures number is missing
    → Added accordingly.

Reviewer: 2

Comments to the Author
The language used needs reviewing by a native English reader.
➔ Done, the manuscript was send for proofread.

The temperatures used are confusing, for example “frozen at -18ºC to 26ºC”, or “below 46°C”
→ revised accordingly.

There were 10 models generated and tested in this study. Can the models be shown ?
➔ Added accordingly. Please refer figure 1

Density is wrong: “Newtonian fluid with density of 1.06 kg/m^3”
→ Thank you very much, you are correct we wrong give a unit supposly (g/cm^3). We are update become 1060 Kg/m^3, Please refer line 193

S is not the Piola-Kirchhoff stress
→ Revised into second Piola-Kirchhoff stress.

What was the load applied to the model ?
➔ Load applied based on gait loading condition (Please refer figure 3)

In relation to the Fluid boundary conditions, marrow cannot enter or exit your model, is this correct ?
➔ The fluid boundary condition was used allowable flow, then the fluid region can enter and or exit the model.

What software was used for the FSI simulations ?

→ COMSOL Multiphysics.

No details are given for the Eulerian mesh
→ Please refer line 175-182 and convergence study figure 5.

What about accelerations and inertial effects? walking is a dynamical event, and it is common sense that this would have influence on the results.
→ In this study researcher not consider acceleration and inertial effect. However gait loading was used as load in boundary condition. we have agree acceleration and inertial effect may have influence on the result and we will consider for next study. Thank you for your suggestion.

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