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RESEARCH ARTICLE

Mechanical Behaviour of Cancellous Bone: Compression and Three-Point Bending Test

Farah Amira Mohd Ghazali[a], **Ardiyansyah Syahrom**[a,b,*]

[a] School of Mechanical Engineering, Faculty of Engineering, Universiti Teknologi Malaysia, Johor, Malaysia; [b] Medical Device Technology Center (MEDiTEC), Institute Human Centred Engineering (iHumEn), Universiti Teknologi Malaysia, Johor, Malaysia

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Abstract The mechanical properties of cancellous bone are fundamental in providing structural support and flexibility during physical activities. Characterization of cancellous bone properties and its mechanical behaviour were found crucial as information of the elastic and failure properties of the tissue may potentially be used to study the effects of drug treatments, aging and disease at the tissue level. This study aims to present the difference of mechanical properties of cancellous bone between compressive and three-point bending loads. Cancellous bone specimens from the femoral and tibial condyles of bovines were mechanically tested using three-point bending test and compression test and correlated with morphological parameters such as bone volume fraction and porosity. From the results, significant difference of mechanical properties was found between cancellous bone specimens tested with compressive and three-point bending load. From compressive load, the maximum stress reached 4.2 MPa, whereas in three-point bending, maximum flexural stress reached 17.0 MPa. Cancellous bone strength was found to be much higher when tested with three-point bending load, although correlations with morphological parameters such as bone volume fraction (BV/TV) and porosity were found lower compared to that in compressive load. In conclusion, there are no correlation between compression and three-point bending with morphology indices (BV/TV and porosity).

Keywords: Cancellous bone, biomechanics, compression, three-point bending..

***For correspondence:**
ardi@utm.my

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Introduction

Different mechanical loading may reflect the different daily physiological activities that are conducted by a person. A person undergoing high impact sports may experience different mechanical bone behaviour compared to a normal person walking. Cancellous bone, primarily found in the ends of long bones, are highly porous structure consisting of hard and soft tissue as these bones are fundamental in providing structural support and flexibility during physical activities. Microscopically, cancellous bone architecture is organized to optimise load transmission whereby mechanical properties of cancellous bone tissue depends on architecture (Ruiz, Schouwenaars, Ramírez, Jacobo, & Ortiz, 2010), cancellous bone properties, and volume fraction (Bayraktar et al., 2004; Osterhoff et al., 2016). Characterization of cancellous bone properties and its mechanical behaviour were found crucial as information of the elastic and failure properties of the tissue may potentially be used to study the effects of drug treatments, aging and disease at the tissue level. Meanwhile, investigating the strength of cancellous bone is vital as it can be associated with damage and bone fracture, which causes the bone remodeling, and failure of bone implants.

Experimental techniques used for determining the mechanical properties of bulk tissue specimens excised from cortical or cancellous regions of bone includes tensile, torsion, compression, and also three-point bending testing (Bayraktar et al., 2004; El Masri, de Sapin Brosses, Rhissassi, Skalli, & Mitton, 2012). In compression and three-point bending tests, the mechanical properties of cancellous bone developed are mainly elastic modulus, yield stress, ultimate stress (Burgers, Mason, Niebur, & Ploeg, 2008; Morgan, Unnikrisnan, & Hussein, 2018; Oftadeh, Perez-Viloria, Villa-Camacho, Vaziri, & Nazarian, 2015; Syahrom, Abdul Kadir, Abdullah, & Öchsner, 2011; Zhao et al., 2018), flexural strength and flexural modulus (Roberts et al., 2013; Silva, 2017). Today many studies have conducted compression and three-point bending test on cancellous bone, although, this study focuses on the differences in mechanical behaviour of the bone between the two tests. The severity or type of fracture in cancellous bone depends on different types of loading condition that represent types of daily activities. Therefore, this study determines the different mechanical behaviour of cancellous bone under different types of biomechanical testing; compression and three-point bending.

Materials and Methods

Specimen preparations

Thirty-eight cancellous bone specimens were extracted from the mediolateral femoral and tibial condyles of healthy bovine cadavers using a slow speed ±150 rpm diamond saw (Behringer GmbH, type SLB 230 DG HA, Kirchardt) under constant lubrication whereby twenty-four specimens were used for compression test, while the remaining was used for three-point bending test. Saline water was used as the lubricant to ensure the temperature did not exceed 46°C and therefore protecting the specimens from heat- related damage (Shin & Yoon, 2006). The cutting and drilling process were ceased at several stages to measure the cancellous bone specimen temperature and ensure temperature did not exceed 46°C. The hard cortex was removed and cancellous bone specimens were cored using diamond cutter (12mm outer diameter (10mm inner diameter) Bosch Diamond Tipped Tile Drill Bit) into cylindrical forms with a total length of 25 mm and a diameter of 10 mm for compression test. For the specimens in three-point bending test, the specimen was cut into 30 mm x 20 mm x 10 mm (L x W x Th). Each specimen was visually inspected and discarded for any visible signs of damage or disease. Specimens were immersed into a mixture of water and chemical detergent (Pumicized, Gent-1-kleen, USA) and placed inside an ultrasonic cleaner (Shim, Yang, Liu, & Lee, 2005) for a period of 3h with a temperature of 30^{o}C to remove remaining bone marrow. Bone marrow, fatty tissue and excess water were removed from the specimens with the aid of compressed air. Specimens were then individually wrapped with a cloth soaked in 0.15M Phosphate Buffered Saline (PBS) solution (Qrec Asia Sdn Bhd., Malaysia) and kept frozen at -20^{o}C in an airtight plastic bag until testing (Burgers et al., 2008; Kohles et al., 2001; Rapillard, Charlebois, & Zysset, 2006; Teo, Si-Hoe, Keh, & Teoh, 2007; van Lenthe, Stauber, & Müller, 2006). For the morphological study, all the specimens were used to determine the morphological parameters, whereby twenty-four were cylindrical shaped specimens for uniaxial compression testing, and the remaining fourteen specimens were for three-point bending test purposes. Distinction between these studies may be due to differing processes of extraction, machining, and preserving the bone specimens as well as different types of specimen regions extracted from the bone.

Testing of Cancellous bone specimens

Uniaxial Compression Test

Uniaxial compression test was conducted using an Instron universal testing machine (The FastTrack ™ 8801, Instron, Norwood, USA) with accuracy class 0.0025 and resolution to (± 0.000015 in), as shown in Figure 1 a and 1b. Load was measured through the Instron load cell with a maximum load capacity of 10 kN (Burgers, Mason, Niebur, & Ploeg, 2008). The relative displacement between the endcaps was measured using an extensometer (Instron 2620 Series Dynamic Extensometer with travel 5 mm/mm and gauge length 12.5 mm/mm). The test was conducted under displacement control (Kang, An, & Friedman, 1998) at a strain rate of 0.001/s (Shim, Yang, Liu, & Lee, 2005). The initial yield stress was determined from the 0.2% offset method (Rapillard, Charlebois, & Zysset, 2006) and the ultimate stress was the maximum stress value from the compression test. For the compression test, the test followed ISO 13314 and ASTM D1621.

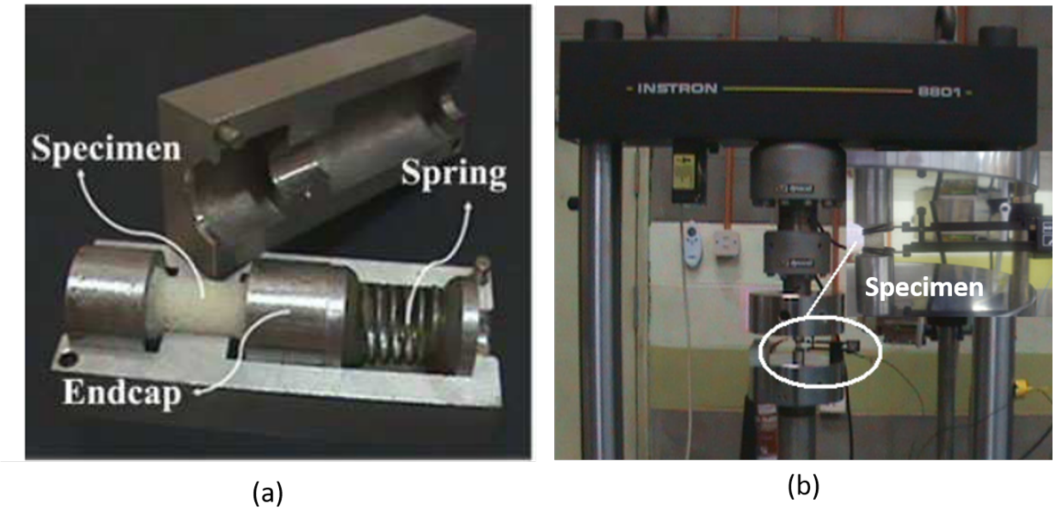


Fig. 1 Layout of specimen a) during alignment in jig device before compression test, and b) setup of specimen mounted on universal testing machine during compression test

Three-point bending Test

Frozen cancellous bone specimens were thawed in a PBS solution bath for 72 hours in 4^{o}C temperature, then gradually thawed in room temperature (25^{o}C) for 6 hours. Three-point bending loads were tested on fourteen cancellous bone specimens in room temperature (Cristofolini & Viceconti, 2000; Leppänen, Sievänen, Jokihaara, Pajamäki, & Järvinen, 2006; Sadeghi, Espino, & Shepherd, 2017). Each specimen was secured on a bending device attached to the universal testing machine (Instron 8874) with a setup load cell of 25 kN, loading rate of 0.01 mm/s (Nobakhti, Katsamenis, Zaarour, Limbert, & Thurner, 2017). To prevent any incidental movements of the specimen during the test, the specimens were first given initial load (5 N) between the attached impact rod and the bending apparatus and the distant between the support span were maintained (20 mm) as shown in Figure 2. All the specimens were placed in the same configuration in which the medial side is facing front view. The bending load started until failure. Flexural mechanical properties of the cancellous bone specimens were determined from the load-deformation curve. For this bending test, the standard operational procedure (SOP) was referred and followed from previous studies (Leppanen, Sievanen, Jokihaara, Pajamaki, & Jarvinen, 2006; Sadeghi, Espino, & Shepherd, 2017) that conducted three-point bending test. For the osteochondral specimen, the size is unable to fit standard, therefore other strategies were used to make sure it is close to the standard.

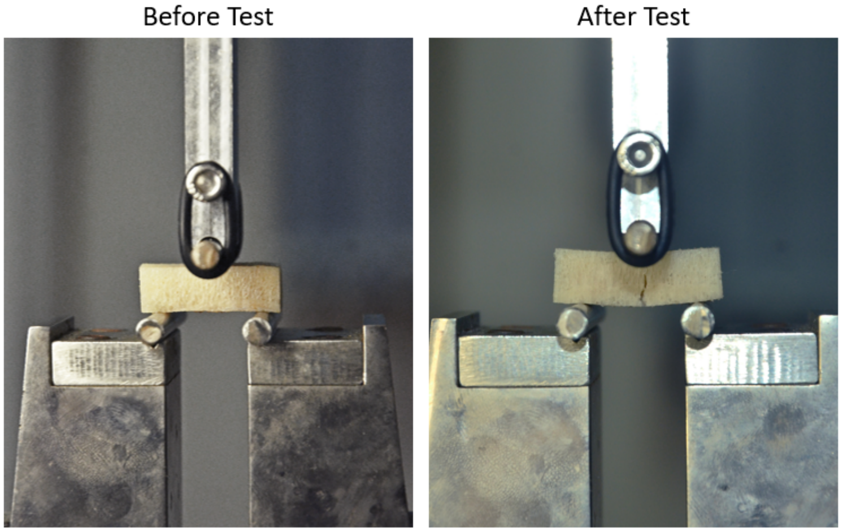


Fig. 2 The cancellous bone specimen before (left) and after test (right).

Flexural strength for the osteochondral specimen is calculated from the measured load, given as Equation 2, whereas flexural modulus is determined from Equation 3.

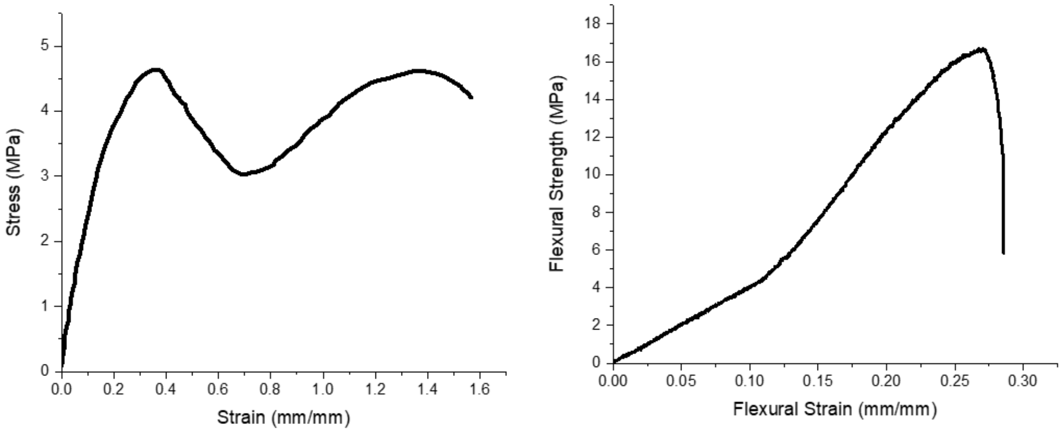
$$\sigma = \frac{3\,F\,L}{2bt^2} \tag{1}$$

$$E = \frac{FL^2}{4bt^3d} \tag{2}$$

where F is the maximum load (in newtons), $L$is the distance between the supports (in millimeters), b is the width of the specimen (in millimeters), t the thickness (in millimeters), d is the deflection (in millimeters) corresponding to load F.

Results

From the stress-strain response in Figure 4a and 4b, cancellous bone was found to fail at a lower stress rate (mean 5.75, stdev 3.54) compared to the cancellous bone specimen under three-point bending test (mean 19.04, stdev 7.01) .



(a) (b)

Fig. 3 a) Stress strain curve of cancellous bone specimen characteristics during compression test, and b) flexural strength- strain curve of cancellous bone specimen characteristics during three-point bending test.

The P value, mean and range for the parameters of the specimens taken from experiments are shown in Table 1. Porosity and bone volume fraction were separated into two categories whereby the first is used for uniaxial compression test (n= 24), and the second, three-point bending test (n=14) . Correlations were made between morphological parameters bone volume fraction and porosity and mechanical characteristics as shown in 4a-4h. As illustrated in Table 1, the mean ultimate stress and young's modulus was 5.75 MPa and 25.48 MPa respectively. While the mean for flexural strength and flexural modulus was 19.04 MPa and 11.84 MPa respectively.

Under compression test, the correlations between ultimate stress and bone volume fraction resulted in 19.4%, while in three-point bending test, 8.1 %. Meanwhile, correlations with bone volume fraction, ultimate stress was higher (R^2 19.4% , p 0.052) and lower with flexural strength (R^2 8.1%, p 0.324). The modulus properties of cancellous bone under compression showed a higher correlation with bone volume fraction (R-sq 37.0% p 0.047) while under three-point bending test, the correlations were much lower (R-sq 5.2% p 0.445). Relationships between ultimate stress and porosity was found high in cancellous bone specimens under compression test (R-sq 19.3% p 0.052), and were found low in cancellous bone specimens tested in three-point bending. Relationships between modulus and porosity were also found high in specimens tested under compression test (R-sq 20.1% p 0.047) and were found low (R-sq 5.0% p 0.4454) in specimens tested under three-point bending.

Table 1 P value, range, mean and SD values of cancellous bone specimens.

Parameters	P value	Min	Max	Mean	SD
Porosity(compressive)	0.229	2.83	77.35	39.13	22.44
BV / TV (compressive)	0.229	22.64	97.16	60.86	22.44
Porosity (three-point bending)	0.199	33.33	90.80	51.02	15.34
BV/ TV (three-point bending)	0.199	9.20	66.67	48.98	15.34
Ultimate Stress (MPa)	0.011	0.641	14.44	5.75	3.544
Young's Modulus (MPa)	0.044	4.51	77.35	25.48	18.34
Flexural Strength (MPa)	0.595	8.99	34.68	19.04	7.010
Flexural Modulus (MPa)	0.149	4.69	23.04	11.84	5.720

Discussions

This study limits to compression and three-point bending test using bovine cancellous bone. Bone is a complex organ which plays a major role in movement, protection, support, mineral storage, and formation of blood cells in the human body. Bones are divided into two types of different apparent density; cortical or compact bone (high density) and cancellous bone (low density) whereby both bones have important functions in the human body. While cortical bone is compact with small passages for fluid flow, cancellous bone is filled with holes (porous structure). The material and structural properties of bone subtly determine its behaviour under mechanical load, dictating its performance under stress and strain to transport mechanical stiffness and structural strength to the skeleton (Ammann & Rizzoli, 2003; Bouxsein & Karasik, 2006; Cucchiarini et al., 2016; Friedman, 2006; Russo, 2009). As cancellous bone structure was discovered to support 75% of mechanical loading of the human body, the study of its mechanical properties have drawn major attention in the etiology of osteoporosis as it is broadly postulated that this feature of bone "quality" contributes to fracture risk (Heaney, 1993; Osterhoff et al., 2016).

One of the morphological parameters which is also available to be obtained from micro-CT scan (Abu Bakar, Saidi, & Mohamad Yamin, 2019) in bones which was found to have a strong influence on the mechanical properties is bone volume fraction or BV/TV (Nazarian, Von Stechow, Zurakowski, Müller, & Snyder, 2008). Bone volume fraction is the volume of mineralise bone per unit volume of the sample (Doube et al., 2010) and was found to vary across anatomic cites (Hudelmaier et al., 2005; Nazarian et al., 2008; Nicholson et al., 1998; Portero-Muzy et al., 2007) which also differed between human and animal (Teo et al., 2007). In this study, specimens were extracted from the mediolateral femoral and tibial condyles of bovine hind legs. The findings show the percentage of bone volume fraction in specimens extracted from the mediolateral femoral condyles varied with specimens extracted from the tibial condyles. Correlations between bone volume fraction and mechanical properties also showed certain variation. In cancellous bone specimens tested under compression as shown in Figure 4a, and 4c, high correlations were found between bone volume fraction and ultimate stress, and modulus, while correlations with flexural strength and flexural modulus were lower in cancellous bone specimens tested under three-point bending as shown in Figure 4b and 4d. Generally, bone can resist to higher compression loads than tension loads (Lotz, Cheal, & Hayes, 1991). Due to the microarchitectural properties, cancellous bone exhibits anisotropic mechanical properties which are strongly influenced by its volume fraction. However, in this case, the results may have varied according to the specimen size and mechanical load applied. In future studies, a more detailed observation could be conducted using scanning electron microscope (SEM) to analyse the crack propagation in each specimen after the test. In compression test, specimens of uniform cylindrical sizes were statically compressed with constant load, while in three-point bending test, uniform rectangular sizes specimens were subjected to compressive stress on the top surface, and tensile stress on the bottom surface.

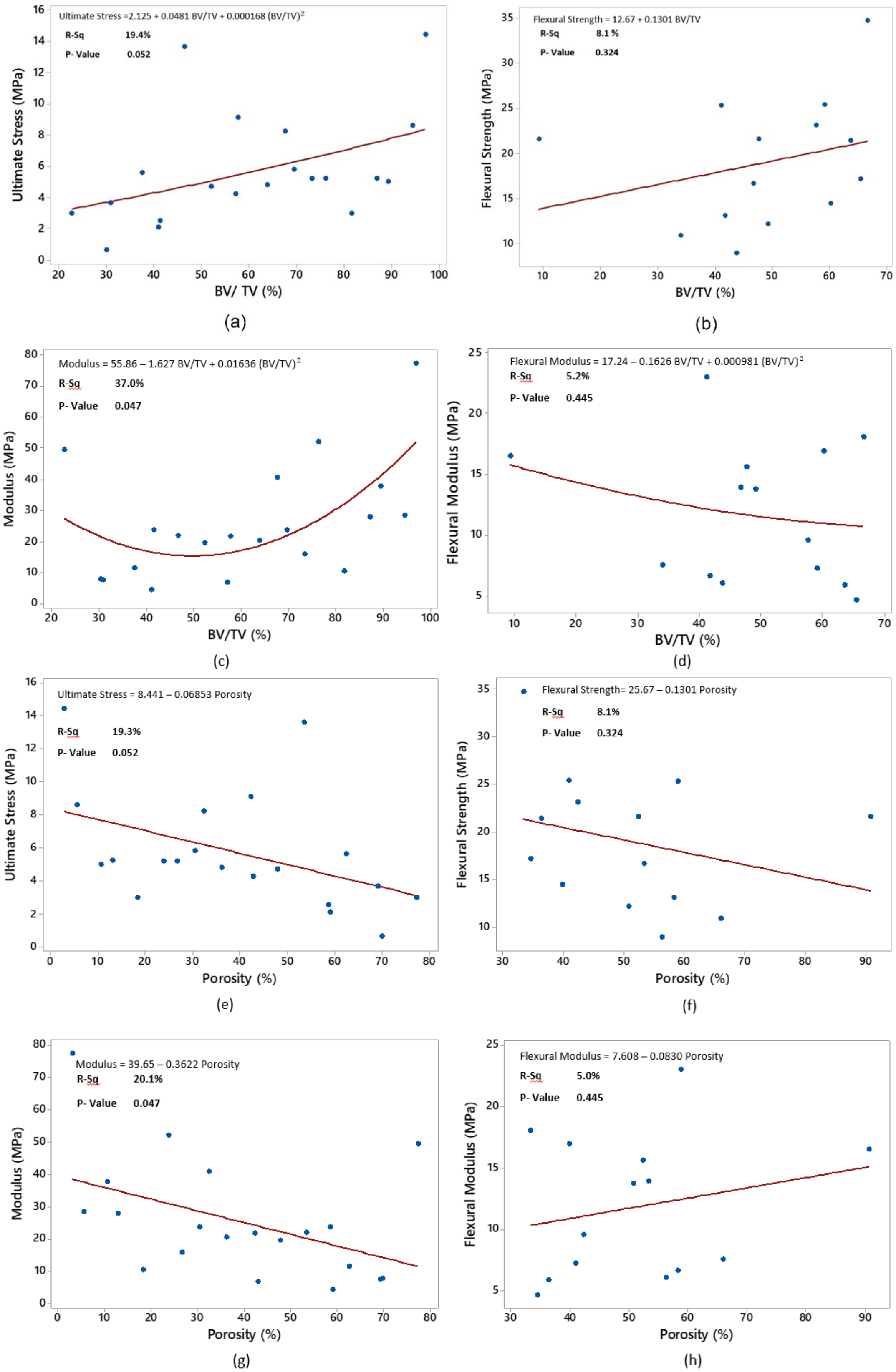


Fig. 4 Scatter plot a) Ultimate Stress vs BV/TV , b) flexural Strength vs BV/TV, c) modulus vs BV/TV d) flexural modulus vs BV/TV, e) ultimate stress vs porosity and f) flexural strength vs porosity g) modulus vs porosity and h) flexural modulus vs porosity.

As ageing or skeletal diseases such as osteoporosis and osteoarthritis has known to have direct effect on cortical and cancellous bone (Ding, Odgaard, Linde, & Hvid, 2002; Rincón-Kohli & Zysset, 2009; Stauber & Müller, 2006) whereby the trabeculae becomes thinner, porosity is one other morphological parameter that was discovered to influence mechanical strength and properties in cancellous bone structure. Porosity in cancellous bone play two primary roles which are to decrease the total weight whilst sustaining the locally required strength (Kohles et al., 2001) and to allow nutrient transmissions (Ochia & Ching, 2002). Based on the results, it was discovered that correlations between porosity and ultimate stress from compression test were higher compared to three-point bending test as shown in Figure 4e and 4f. Higher correlations were also found between porosity and modulus properties in compression test, compared to in three-point bending test as shown in Figure 4g and 4h. The similar results shown possibly indicates a difference in terms of correlations between morphological parameters bone volume fraction and porosity with the mechanical properties conducted from compression and three-point bending test.

Some previous studies have reported that the mechanical properties for instance, elastic modulus of cancellous bone in compression and tension remains the same (Bureau, Denault, Perrin, & Dickson, 2001) although others reported otherwise (Morgan et al., 2018). This may be related to the difference in the stress and strain rate applied onto the cancellous bone structure in which different stiffness and strength is exhibited in different directions (longitudinal or transverse). However, the transverse direction of cancellous bone specimen is found weaker than in longitudinal in both compressive and tensile behaviour. The difference in trabeculae orientation and porosity in femur and tibial condyles also contributes to varied mechanical strength of the bone (He et al., 2020; Shi, Sherry Liu, Wang, Edward Guo, & Niebur, 2010). This would therefore explain the difference in mechanical properties of cancellous bone under different mechanical loading. The priority of using three-point bending instead of four-point bending in this study was mainly due to the size of the cancellous bone specimens. Since the size of the specimens are only 30 mm long each, the most suitable and available type of bending was the three-point as it suited the specimen size. Moreover, the type of loading condition of three-point is different compared to four-point bending as there are no shear stress between the two loading in four -point bending. The four-point bending test is therefore particularly suitable for brittle materials that cannot withstand shear stresses very well. Whereas shear stress does occur in cancellous bone (Garrison, Gargac, & Niebur, 2011).

Conclusions

Studying the ultimate stress and elastic modulus behaviour of cancellous bone is crucial as it primarily functions in vertebral bodies and also transmits the load within joints to the cortical bone. Moreover, it is also interrelated with the strength and affects fracture risk of the bone structure (Ciarelli, Goldstein, Kuhn, Cody, & Brown, 1991). From the work performed, significant mechanical behaviour was found between cancellous bone under compression and three-point bending test. Mechanical behaviour of cancellous bone structure was discovered to be much higher in cancellous bone specimens when tested with three-point bending load although correlations with morphological parameters were lower compared to when tested in compressive loading. Not only the mechanical load is different, but the load intensity subjected onto the bone is varied.

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