Quantification and Improvement of Stiffness Measurement Techniques of Trabecular Bone Using Porcine Mandibular Condyles

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This thesis titled
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ABSTRACT

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Quantification and Improvement of Stiffness Measurement Techniques of Trabecular Bone Using Porcine Mandibular Condyles

Director of Thesis: John R. Cotton

This study improves the agreement between two stiffness measurement techniques of trabecular bone harvested from porcine mandibular condyles. The previous method of Zaylor (2013) measured stiffness with digital finite element and experimental compression tests using 2 mm bone cubes. The current study improves the agreement between methods by examining the effect of sample size using 3 mm and 4 mm cubes, the effect of strain range used in compression tests, and investigating the geometric accuracy of the digital finite element models.

It was found that larger specimens improved the stiffness agreement in medial-lateral, and superior-inferior directions of the condyles, ($R^2 > 49$). The agreement was not affected by the strain limits of 0.4-0.5%, 0.5-0.6%, and 0.4-0.6%. The average digital cube lengths were 2% smaller than the physical lengths. The improved agreement justifies digital modeling of trabecular bone to measure the stiffness of mandibular condyles.
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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>3</td>
</tr>
<tr>
<td>Acknowledgments</td>
<td>4</td>
</tr>
<tr>
<td>List of Tables</td>
<td>7</td>
</tr>
<tr>
<td>List of Figures</td>
<td>8</td>
</tr>
<tr>
<td>Chapter 1 - Introduction</td>
<td>10</td>
</tr>
<tr>
<td>1.1 Trabecular Bone</td>
<td>11</td>
</tr>
<tr>
<td>1.2 Mechanical Properties of Trabecular Bone</td>
<td>12</td>
</tr>
<tr>
<td>1.3 Microcomputer Tomography</td>
<td>13</td>
</tr>
<tr>
<td>1.4 Finite Element Modeling of Trabecular Bone</td>
<td>14</td>
</tr>
<tr>
<td>1.5 Uniaxial Non-destructive Compression Testing</td>
<td>15</td>
</tr>
<tr>
<td>1.6 Pig as a Model for Human TMJ Study</td>
<td>15</td>
</tr>
<tr>
<td>1.7 Background of the Work</td>
<td>16</td>
</tr>
<tr>
<td>1.8 Thesis Objectives</td>
<td>19</td>
</tr>
<tr>
<td>1.8.1 Objective 1:</td>
<td>19</td>
</tr>
<tr>
<td>1.8.2 Objective 2:</td>
<td>19</td>
</tr>
<tr>
<td>1.8.3 Objective 3:</td>
<td>20</td>
</tr>
<tr>
<td>Chapter 2 - Literature Review</td>
<td>21</td>
</tr>
<tr>
<td>2.1 Use of FE Modeling in the Study of Trabecular Bone</td>
<td>21</td>
</tr>
<tr>
<td>2.2 Compression Testing of Trabecular Bone</td>
<td>22</td>
</tr>
<tr>
<td>2.3 Combined FE Modeling and Compression Testing</td>
<td>24</td>
</tr>
<tr>
<td>2.4 Summary</td>
<td>26</td>
</tr>
<tr>
<td>Chapter 3 - Measurement of Stiffness Using FE Modeling and Compression Testing</td>
<td>28</td>
</tr>
<tr>
<td>3.1 Specimen Preparation and Cutting Procedure</td>
<td>28</td>
</tr>
<tr>
<td>3.2 Micro Computed Tomography Scanning</td>
<td>32</td>
</tr>
<tr>
<td>3.3 Image Segmentation and Smoothing Operation</td>
<td>33</td>
</tr>
<tr>
<td>3.4 Tetrahedral Mesh Preparation of Surface Mesh</td>
<td>37</td>
</tr>
<tr>
<td>3.5 FE Modeling Process</td>
<td>38</td>
</tr>
<tr>
<td>3.6 Digital Length Measurement</td>
<td>40</td>
</tr>
<tr>
<td>Section</td>
<td>Page</td>
</tr>
<tr>
<td>------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>3.7 Compression Testing Procedure</td>
<td>41</td>
</tr>
<tr>
<td>3.8 Physical and Digital Measurements of Mass</td>
<td>45</td>
</tr>
<tr>
<td>Chapter 4 - Results</td>
<td>48</td>
</tr>
<tr>
<td>4.1 Comparison of the Experimental Stiffness With Respect to Strain Ranges</td>
<td>48</td>
</tr>
<tr>
<td>4.2 Comparison of the Average Apparent Modulus With Respect to Direction</td>
<td>49</td>
</tr>
<tr>
<td>4.3 Agreement between the FE and Experimental Apparent Modulus</td>
<td>51</td>
</tr>
<tr>
<td>4.4 Comparison of the Apparent Densities of 2, 3, and 4 mm Cubes</td>
<td>53</td>
</tr>
<tr>
<td>4.5 Comparison of the Digital and Physical Mass</td>
<td>54</td>
</tr>
<tr>
<td>4.6 Comparison of the Digital and Physical Geometry of the Cubes</td>
<td>55</td>
</tr>
<tr>
<td>Chapter 5 - Discussion and Conclusion</td>
<td>58</td>
</tr>
<tr>
<td>5.1 Conclusion</td>
<td>64</td>
</tr>
<tr>
<td>Bibliography</td>
<td>68</td>
</tr>
<tr>
<td>Appendix A – Mesh Convergence Study</td>
<td>71</td>
</tr>
</tbody>
</table>
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 1: Estimated coefficients in regression analysis</td>
<td>59</td>
</tr>
<tr>
<td>Table 2: Comparison of the average stiffness with respect to loading directions</td>
<td>60</td>
</tr>
<tr>
<td>Table 3: Average mesh density and element size for 3 mm and 4 mm cubes</td>
<td>74</td>
</tr>
<tr>
<td>Table 4: Modulus extrapolation from the edge length of tetrahedral elements</td>
<td>75</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-1</td>
<td>The human TMJ, temporal bone, and mandible</td>
<td>10</td>
</tr>
<tr>
<td>1-2</td>
<td>Scanning electron micrographs of the trabecular bone structure</td>
<td>12</td>
</tr>
<tr>
<td>1-3</td>
<td>Porcine mandibular condyle (circled)</td>
<td>16</td>
</tr>
<tr>
<td>1-4</td>
<td>Porcine mandibular condyle with anatomical directions and a digital cube of side 2 mm</td>
<td>17</td>
</tr>
<tr>
<td>3-1</td>
<td>A mandibular condyle with anatomical planes</td>
<td>29</td>
</tr>
<tr>
<td>3-2</td>
<td>Sequence of cutting operations</td>
<td>30</td>
</tr>
<tr>
<td>3-3</td>
<td>A specimen holder containing four 3 mm cubes and top view of four tubes placed in tube holders</td>
<td>33</td>
</tr>
<tr>
<td>3-4</td>
<td>A 2D µCT image of 4 mm trabecular bone cube structure</td>
<td>34</td>
</tr>
<tr>
<td>3-5</td>
<td>Screenshot of magic wand tool in Avizo</td>
<td>35</td>
</tr>
<tr>
<td>3-6</td>
<td>Image segmentation procedure</td>
<td>36</td>
</tr>
<tr>
<td>3-7</td>
<td>A tetrahedral mesh of trabecular bone sample</td>
<td>37</td>
</tr>
<tr>
<td>3-8</td>
<td>The boundary conditions of a typical FE model of a trabecular sample</td>
<td>38</td>
</tr>
<tr>
<td>3-9</td>
<td>Four readings of the digital length measurement from the FE model of 3 mm trabecular bone</td>
<td>41</td>
</tr>
<tr>
<td>3-10</td>
<td>The MTS Q-10 load frame with a 500 N load cell, LVDT, and a bone sample between the top and bottom platen</td>
<td>42</td>
</tr>
<tr>
<td>3-11</td>
<td>The stress strain curve showing the 15 cycles for a particular compression test</td>
<td>44</td>
</tr>
</tbody>
</table>
Figure 3-12: Sequential workflow of the experimental mass calculation.......................46

Figure 4-1: Uniaxial compression test histograms showing the variation in the average apparent modulus in ML, AP, and SI direction with respect to strain range .................48

Figure 4-2: The average FE apparent modulus of 3 mm and 4 mm cubes ....................50

Figure 4-3: The average experimental apparent modulus of 3 mm and 4 mm cubes .......51

Figure 4-4: Correlation between predicted FE and experimental apparent modulus of 3 mm cubes .................................................................................................52

Figure 4-5: Correlation between predicted FE and experimental apparent modulus of 4 mm cubes .................................................................................................53

Figure 4-6: Average apparent density measured from the air dried mass. .................54

Figure 4-7: The relationship between the digital mass and the experimental mass of bone cubes .................................................................................................................55

Figure 4-8: The relationship between the digital and physical lengths of 3 mm cubes ..56

Figure 4-9: The relationship between the digital and physical lengths of 4 mm cubes. 57

Figure A-1: Relationship between the number of triangular faces and FE modulus......73
CHAPTER 1 - INTRODUCTION

The temporomandibular joint (TMJ) is a joint between the temporal bone of the skull and the mandible. The mandibular condyle is the part of the mandible that articulates with the disc which articulates the skull (Figure 1-1).

Figure 1-1: The human TMJ, temporal bone, and mandible.

TMJ disorders involve pain, limited movement of the mandible or misalignment of the biting or chewing motion (Buescher, 2007). TMJ disorders affect than 10 million Americans (NIDCR.nih.gov). One of the TMJ disorders is osteoarthritis (Liu and Steinkeler, 2013). Osteoarthritis results in a degeneration of the bone and cartilage; which produces pain and can damage the joint with reduced or permanent loss of motion (Felson, 2013). Diagnostic tests for osteoarthritis, of the TMJ include imaging techniques such as CT scans, X-rays, or magnetic resonance imaging (MRI) (Liu and Steinkeler, 2013). TMJ disorder diagnosis has showed significant changes in the bone tissues,
deformation of the condyle and changes in the position and morphology of the articular
disc with MRI imaging technique (Liu and Steinkeler, 2013). These progressive changes
in the shapes of the condyles can provide evidence of osteoarthritis (Liu and Steinkeler,
2013).

The most commonly used noninvasive treatment for TMJ disorders is the use of
intra-oral splints (Buescher, 2007). One type of commonly used splint functions to
improve the alignment of the upper and lower teeth. A second type of splint opens the
jaw in an attempt to relieve muscle strain and prevent teeth clenching (Buescher, 2007).
The purpose of these types of splints is to minimize the pain by changing the location of
the load on the condyle and prevent the wear of the TMJ and articular disc (Liu and
Steinkeler, 2013). However, research is mixed regarding the effectiveness of these
devices (Buescher, 2007; Liu and Steinkeler, 2013). It is clear that further investigation is
necessary to evaluate the effectiveness of the splint therapy for the TMJ dysfunction.

1.1 Trabecular Bone

Trabecular bone is a foam-like structure which is defined by Gibson (2005) as a
“three dimensional cellular solid”. Trabecular bone is located at the expanded heads of
long bones, in the core of shell-like bones, and inside vertebral bodies (Gibson, 2005).
The function of trabecular bone is to transmit loads in long bones while inside vertebral
bodies to resists compressive loads (van Rietbergen and Huiskes, 2001). In order to
support the compressive loads acting on the bone, the trabecular bone structure orients
itself in the direction of the load (Goulet et al., 1994). Trabecular bone structure varies
from a low-density rod-shaped to a high-density plate-shaped structure, as seen in Figure
1-2 (Gibson, 2005). Nevertheless, the detailed relationship between the trabecular bone structure and its loading condition is unknown (Boyd et al., 2002; Goulet et al., 1994).

![Figure 1-2: Scanned electron micrographs of trabecular bone structure, retrieved from Gibson (1985). (a) Low-density rod shaped structure of femoral head specimen, (b) High-density plate shaped structure of femoral head specimen, (c) Femoral condyle specimen with intermediate density.]

The structural properties of trabecular bone include trabecular thickness, trabecular number, density, and bone volume fraction (BVF). The trabecular bone structure varies between different anatomical locations in the same body and between different bodies (Augat et al., 1998).

### 1.2 Mechanical Properties of Trabecular Bone

Trabecular bone is highly anisotropic accounting for the fact that the stiffness of trabecular bone varies in different loading directions (Brozovsky and Pankaj, 2007). Longitudinal loading in a vertically oriented structure results in higher stiffness, compared to transverse loading (Gibson, 2005). The tissue modulus of trabecular bone,
$E_t$, is the elastic modulus of the individual trabecula (Keaveny et al., 2001). The structure of trabecular bone is highly porous and its stiffness is measured from the samples harvested on a millimeter scale. If there was no porosity in the structure, the elastic modulus of the sample would be equal to the tissue modulus. However, because of porosity, the elastic moduli of all the individual trabecula together with the trabecular structure contribute together, and the stiffness is referred to as apparent modulus. As a result, the apparent modulus is always lower than the tissue modulus (Keaveny et al., 2001).

Apparent density is a strong estimator of the stiffness of trabecular bone (Rice et al., 1988). It is defined as the bone mass divided by the total volume of the bone specimen. Nonlinear regression analyses are commonly used to determine the parameters of the empirical relationship between the apparent modulus and the apparent density of trabecular bone. Linde (1994) reviewed various studies for the modulus-density relationship and stated that the majority of the studies have obtained power coefficients between 1 and 2; however a few studies have also showed cubic relationships. Similar to apparent density, BVF is an important morphological parameter used to determine an empirical relationship with the apparent modulus of trabecular bone (Kim et al., 2007; Ding et al., 1999; Hara et al., 2002). BVF is defined as the volume of bone tissue divided by the total volume of trabecular bone specimen.

1.3 Microcomputer Tomography

Micro computed Tomography (μCT) can be used to measure the 3D geometry of trabecular bone microstructure (Rüegsegger et al., 1996). The working principle of the
μCT scan is that when the 3D object is placed in between the X-ray source and the detector system, the X-rays passes through the object and reduce its intensity. The intensity is reduced due to the absorption, reflection and deflection derived from the density of the object (Rüegsegger et al., 1996). This is called X-ray attenuation; the attenuation at every position in the field of view is determined by a special algorithm and correlated to a scale called a Hounsfield Unit (HU). Ruegsegger et al. (1996) showed that the 3D architecture of trabecular bone can be represented with μCT to examine the mechanical properties without damaging trabecular bone.

1.4 Finite Element Modeling of Trabecular Bone

Finite element (FE) analysis derived from μCT images of trabecular bone is a digital technique that analyzes trabecular bone for the estimation of mechanical properties. FE models of trabecular bone require inputs of geometry, boundary conditions, material properties, and loads. When μCT images are segmented to represent the bone structure, the segmented images can be converted into a three-dimensional FE mesh. Once the mesh is created, and the material properties, boundary conditions, and loads defined, the FE analysis of the trabecular bone structure can estimate displacements, stresses, and strains in the trabecular bone. Numerous studies such as those by Kabel et al. (1999), Kim et al. (2007), and van Rietbergen et al. (1995) have determined the mechanical properties of trabecular bone using μCT based FE modeling. These studies compared estimated FE properties with experimental compression tests using linear regression analysis and validated the accuracy of the FE modeling.
1.5 Uniaxial Non-destructive Compression Testing

The mechanical properties of trabecular bone can be measured by using a nondestructive compression test method (Linde et al., 1988). The method is called nondestructive because the mechanical properties are estimated below the failure strain of trabecular bone (Linde, 1994). The advantage of using this type of testing is that it allows the estimation of the modulus of a cube specimen in all three directions. The principle of the test is to apply strain on the trabecular bone specimens and to measure the load. The slope of the linear elastic region of the stress-strain curve, is defined as the apparent Young’s modulus of the specimen (Linde et al., 1988). Several studies including Linde et al. (1988), Linde et al. (1992), and Goulet et al. (1994) have determined the stiffness of trabecular bone from experimental compression testing.

1.6 Pig as a Model for Human TMJ Study

Pigs are used as a model for the humans in studies of the TMJ because pigs are omnivorous, have bunodont dentition, the articular surface sizes are similar and tissue histology is alike (Bermejo et al., 1993). Figure 1-3 shows a porcine mandibular condyle.
The trabecular bone samples in this thesis will be harvested along the medial lateral axis of the porcine mandibular condyle.

1.7 Background of the Work

Zaylor (2013) investigated the effects of occlusal splinting on the stiffness and BVF of the subchondral trabecular bone in the porcine mandibular condyle. Zaylor (2013) used µCT based FE models to estimate the digital stiffness and BVF of the trabecular bone cubes. Digital bone cubes with 2 mm side lengths were obtained from the micro CT scanned images of the porcine mandibular condyles (Figure 1-4).
Zaylor (2013) compared the stiffness and BVF values of cubes from splinted animals with cubes from unsplinted animals. Zaylor (2013) found that splinting altered the stiffness and reduced the BVF of the trabecular bone in the mandibular condyle. All this work was done on images of dried condyles from a previous study by Sindelar et al. (2000, 2002). As these were dried, physical testing would not have reflected in the vivo condition.

In order to validate the FE modeling method used, Zaylor (2013) performed non-destructive compression tests on a new set of bone cubes to measure the experimental stiffness. For the compression tests, stiffness was calculated from the slope of the stress strain curve in the strain range of 0.5 - 0.6 %. Stiffness was measured in three orthogonal directions which represented the anterior-posterior, (AP), medial-lateral, (ML), and superior-inferior, (SI), directions of the condyle. Linear regression between the two
measures was performed to quantify the accuracy of the FE method to predict experimental stiffness. A coefficient of determination (R²) of 0.31 and 0.33 in the AP and ML directions respectively was achieved, but no relation was found in the SI direction.

The current study hypothesizes that by increasing the side length of cube samples to 3 mm and 4 mm from 2 mm, we will improve the correlation between the digital FE and experimental compression tests measures in all three directions. The 2 mm cubes used by Zaylor (2013) may have been too small to treat trabecular bone as a homogeneous material. Harrigan et al. (1988) suggested that in order to treat trabecular bone as a continuum, the size of the specimens should represent more than 5 intertrabecular widths. Bone cubes of side 3 mm and 4 mm will have higher number of intertrabecular widths. In addition, it is assumed that the accuracy of the stiffness measurement was reduced because the small 2 mm cubes were affected by the structural artifact phenomenon described by Keaveny et al. (1997), who proposed that when the bone sample is harvested from the surrounding structure, the sample loses surrounding connective tissues along its length and width. Consequently, substantial loss in the apparent modulus of the trabecular bone occurs. Linde et al. (1992) also showed that structural artifact phenomenon reduces as the length and cross-sectional area of the specimen increases. It is assumed that 3 mm and 4 mm cubes will improve the agreement of the modulus estimation because these cubes will have considerably higher surface area and volume compared to the 2 mm cubes.
1.8 Thesis Objectives

The aim of this study is to improve the agreement of digital FE and experimental stiffness measurement techniques of trabecular bone from porcine mandibular condyles. To achieve that, bone specimens will be harvested from the TMJ of new porcine mandibular condyles. To measure the digital stiffness of trabecular bone, I will build linear FE models from μCT images of 3 mm and 4 mm cube specimens. I will also perform experimental uniaxial nondestructive compression tests on all cubes to measure the experimental stiffness. Stiffness will be measured in three directions, which will represent the AP, ML and SI direction of the mandibular condyle.

1.8.1 Objective 1:

To test if the accuracy of stiffness measurements obtained from both the methods improved, I will perform regression analysis between both the measures of the stiffness. If the coefficient of determination ($R^2$) between the two measures improves, then an improvement in the accuracy of measures is demonstrated. I will also investigate the effects of specimen size on the agreement of the digital and physical measurement techniques. To do that, I will compare the coefficient of determination of the regression analysis between the digital FE and experimental measures of the apparent modulus of 3 mm and 4 mm cubes with 2 mm cubes used in the study by Zaylor (2013) in the ML and AP directions.

1.8.2 Objective 2:

To test the accuracy of the image segmentation, I will estimate the mass of the digital models and compare to physical mass of cubes. I will measure the mass of 3 mm
and 4 mm cubes experimentally. I will compare this to the digital mass calculated from scanned images. Linear regression between the digital mass with the experimental mass will measure the accuracy of the image segmentation procedure used to develop digital FE models.

1.8.3 Objective 3:

To analyze whether the stain limit effects the agreement between FE and the experimental stiffness, I will measure the stiffness of 3 mm and 4 mm cubes from the compression testing in the strain limit of 0.4-0.5%, 0.5-0.6%, and 0.4-0.6%. The stiffness estimated in three different ranges will be compared with one-way ANOVA analysis for the significant differences. The experimental stiffness will also be correlated to the FE stiffness using linear regression to estimate the accuracy of the FE model.
CHAPTER 2 - LITERATURE REVIEW

2.1 Use of FE Modeling in the Study of Trabecular Bone

Finite element analysis (FEA) is an important tool in engineering analysis as it can be used to estimate the displacement, stress, or strain of a body when it is subjected to an external load. The external load may be a combination of one or different types of loads. Unlike analytic techniques, FEA adapts well to highly complex shapes. This characteristic of FEA makes it suitable for the analysis of trabecular bone, which is extremely complex in structure.

Van Rietbergen et al. (1995) showed that μCT based FE models can be used to measure the mechanical properties of trabecular bone. In this study, FE models were used to predict trabecular bone tissue modulus, apparent modulus, and anisotropy. The apparent modulus from FE models was calculated first, by using an idealized uniform isotropic tissue modulus of 1 GPa for the elements of the FE models. Later, the actual bone tissue modulus for each sample was calculated by taking the ratio of the experimental apparent modulus to that of simulation and multiplying the ratio by the simulated tissue modulus of 1 GPa. Outcomes of this study suggested that the trabecular bone tissue modulus varies in the range of 2.23 to 10.1 GPa for the proximal human tibia.

Hara et al. (2002) showed that the accuracy of FE models depends on the accuracy of the segmentation procedure of the μCT images. Segmentation is an image processing operation, which separates the bone from the marrow. By defining a threshold value in the threshold segmentation procedure, all the voxels greater than the selected threshold value represent bone whereas the voxels smaller than or equal to the threshold
value represents marrow. Hara et al. (2002) suggested that if higher threshold values are selected, these produce an artificially weak trabecular structure due to loss of connectivity in the structure, whereas lower values produce thickening of trabecular plates and rods which results in an artificially strong trabecular structure. Hara et al. (2002) revealed that with an alteration of 0.5% in threshold value in observer-based thresholding, the architectural parameters and mechanical parameters of the specimens under study changed. The authors found that the specimens with BVF below 0.15 showed changes in apparent modulus and BVF of 9% and 5% respectively. On the other hand the specimens with BVF above 0.2 showed small variation of 3% and 2% respectively (Hara et al., 2002).

2.2 Compression Testing of Trabecular Bone

Compression testing is the traditional method used to estimate the mechanical properties such as stiffness, strength, and anisotropy of the trabecular bone. In the nondestructive test, a strain controlled cyclic load is applied. Cyclic loading involves loading and unloading of trabecular bone at low strain rates to definite strain range. A slow rate of loading, such as 0.01s$^{-1}$, is recommended to minimize the viscoelastic material effect of trabecular bone (Linde and Hvid, 1987). The application of a preload is necessary to ensure surface contact of the top platen with the specimen (Linde et al., 1988). The authors proposed that the strain value at the preload can be defined as the zero strain and the strain range of 0.4 to 0.6% can be used as upper limit in cyclic loading to estimate the stiffness.
Linde et al. (1988) showed that trabecular bones’ stiffness and strength can be estimated by a non-destructive compression test method. Linde et al. (1988) used 121 cylindrical trabecular bone samples of diameter and length 7.5 mm, which were harvested from human knees. The test procedure used by Linde et al. (1988) included cyclic loading of the specimens at a strain rate of 0.01 s\(^{-1}\) with a frequency of 0.2 Hz. The specimens underwent loading cycles where load was applied until 0.6 % strain was achieved, after which specimens were unloaded back to zero strain. The modulus was calculated as a slope of the linear portion of the stress-strain curve at a strain of 0.6 %. Linde et al. (1988) suggested that 10 preloading cycles are necessary for trabecular bone before measuring the modulus in order to achieve a viscoelastic steady state. In cyclic loading, the modulus increases with successive cycles and after 10 cycles the variation in the modulus is reduced significantly (Linde et al., 1988).

According to Linde et al. (1988), the test can be stopped when the increase in the modulus value from the consecutive loading cycles is less than 5%. Moreover, the average value of the modulus of these three consecutive loading cycles was defined as the stiffness of the specimen. The authors compared the stiffness measured by this nondestructive method with the stiffness estimated by a destructive method and found a excellent correlation (r = 0.99).

Linde et al. (1992) investigated the effect of specimen geometry and size on the modulus estimated by a uniaxial nondestructive compression test on human knee bone specimens. The authors compared a total of 41 cubes with side lengths of 5.8 mm to 41 cylinders of diameter and lengths of 6.5 mm to investigate the effect of geometry and
cylinders of diameter 5.5, 6.5, and 7.5 mm with length to diameter ratio of 1 to
investigate the effect of size. Results indicated that the effect of specimen geometry,
cubes verses cylinders, showed no significant difference in the stiffness values. Whereas,
the stiffness significantly (p < 0.0001) increased with the larger specimens. The average
apparent modulus of the 7.5 mm cylinders was 58% higher than the 5.5 mm cylinders and
30% higher than 6.5 mm cylinders.

2.3 Combined FE Modeling and Compression Testing

In this section, I review those studies in which the mechanical and structural
properties of trabecular bone are measured by using both compression testing and FE
models. The reason for measuring the mechanical properties using two different measures
is to validate the FE models of trabecular bone by comparing the model properties with
the experimental tests.

Kabel et al. (1999) used FE models to show that the apparent modulus of
trabecular bone can be predicted using a uniform isotropic bone tissue modulus. 29
trabecular bone cubes of side 10 mm were harvested from the vertebra of a sperm whale.
The apparent modulus and anisotropy were calculated by both FE models and uniaxial
compression tests. In compression testing, using a maximum strain of 0.3% at a rate of
0.001s⁻¹ the apparent modulus was measured. The authors found a strong correlation of
determination for apparent modulus (R² = 0.92) and the anisotropy ratio (R² = 0.95). The
same study found a mean value of 5.6 ± 0.2 GPa for the uniform isotropic tissue
modulus. Considering the results of regression analysis, the authors concluded that the
elastic modulus of an anisotropic trabecular bone structure can be estimated accurately using isotropic homogeneous tissue modulus in FE models.

Another study by Kim et al. (2007) inspected the consequences of thresholding procedures on the accuracy of µCT based FE models for the prediction of apparent modulus. Three different thresholding procedures: adaptive, global, and BVF compensated global thresholding were used by the researchers to produce seventeen µCT-based FE models of cylindrical bovine tibiae, with diameter 6 mm and length 10 mm. The thresholding approach used by Kim et al. (2007) followed three steps. First, in the adaptive threshold method, graylevel values of for each row of voxel in three directions of the 3D image were obtained from a regional histogram. Based on this, the values of local thresholds were defined for each voxel. Second, for the global thresholding method, each specimen was assigned a distinctive threshold value from the histogram distribution. Third, in BVF compensated global thresholding method, the global threshold value was selected so that it gave the same BVF as the one obtained from a physical measure of apparent density (Kim et al., 2007).

The apparent elastic modulus was calculated from FE models and uniaxial compressions tests for all the specimens. For the compression test, the author carried out a nondestructive compression up to 0.8% of strain at a strain rate of a 0.0005 s⁻¹. The authors defined modulus as the slope of a linear portion of 0.0-0.1% strain of the stress-strain curve. The study found that the apparent moduli range from 300 MPa to 4500 MPa for the bovine tibiae when using both FE models and compression testing methods. The authors found a strong agreement (R² > 0.85) between the FE and the experimental
estimation of apparent modulus. The results of Kim et al. (2007) also showed that measurements of BVF and apparent modulus from μCT based FE models using the three different thresholding procedures resulted in no significant variation (p > 0.05).

2.4 Summary

Considering the studies mentioned in the literature review section, the mechanical properties of trabecular bone can be measured alone by either digital FE modeling or experimental compression testing. Compression testing is a traditional method to measure the mechanical properties and it is considered as a reference standard for the FE method. An advantage of the compression test is that it can measure the compressive strength and stiffness without knowing the trabecular bone tissue modulus. However, some disadvantages are that the process is time consuming and the specimens cannot be tested more than once in destructive testing. Advantages of the FE method over the experimental method is that, the digital FE method is cost and time saving. It allows testing of the digital specimen an infinite number of times with different load conditions. It can also help to understand the relation between mechanical properties and structural anisotropy. The major disadvantage of the FE method is that the accuracy of the results depends on the accuracy of the inputs of trabecular bone geometry, materials properties and boundary conditions.

Some studies mentioned above have used both FE modeling and experimental techniques to measure the stiffness and anisotropy ratio. The comparison of the FE properties with the experimental can validate the accuracy of the FE models. By using methods of the above studies, I will build μCT based FE models assuming a
homogeneous isotropic bone tissue modulus similar to Kabel et al. (1999). To study the effect of the specimen size, I will measure apparent modulus of trabecular bone cubes with sides of 3 mm and 4 mm with both FE and nondestructive compression tests. I will use 10 preconditioning cycles in the nondestructive compression tests as described by Linde et al. (1988) to overcome the viscoelastic material effects of trabecular bone. To understand the accuracy of the segmentation procedure used in the FE models, I will compare the physical mass of the specimens with the digital mass obtained from the segmentation procedure. The mass comparison will consider the effect of the thresholding procedure as described by Hara et al. (2002) and Kim et al. (2007). Lastly, to compare with the FE modulus with experimental modulus, I will measure the experimental stiffness as a slope of stress-strain curve over strain ranges of 0.4-0.5%, 0.5-0.6%, and 0.4-0.6% similar to Linde et al. (1988).
CHAPTER 3 - MEASUREMENT OF STIFFNESS USING FE MODELING AND COMPRESSION TESTING

In the present study, μCT based FE models and compression testing are used to validate the FE apparent modulus measure of the trabecular bone. The current study uses a cubic geometry of trabecular bone specimens as this allows stiffness measures in three directions of the specimens. The three directions of the cube correspond to the AP, ML and SI directions of the articular surface of the mandibular condyle.

3.1 Specimen Preparation and Cutting Procedure

In the current study, samples are harvested from the porcine mandibular condyles of eight pig heads collected from a local slaughterhouse. The cutting procedure of the cube specimens used in this study is identical to the cutting procedure used by Zaylor (2013). First, mandibles from the pig heads were dissected out from the skull packed in saline, and refrigerated at -20˚ C. The first step in the cutting procedure involved the removal of the condyles from the dissected mandibles. Two cuts were taken to remove each condyle from the mandible; the first cut was taken parallel to the horizontal plane whereas the second cut was taken with a frontal cut at the mandibular notch as shown in Figure 3-1. After removing the condyles from the mandible, the condyles were kept moist in 0.9% saline solution in airtight plastic bags and stored immediately in a -20˚ C freezer.
The condyles stored in the freezer were thawed to cut cubic samples. Linde and Sørensen (1993) found that the apparent modulus of trabecular bone specimens does not vary significantly by the repetitive storage in freezer and thawing. First, cube samples with 3 mm sides were cut from 11 condyles, from the remaining 5 condyles the 4 mm cube samples were cut. The cutting procedure was carried out at room temperature. The medial-lateral (ML) axis was defined on the specimen by recognizing and highlighting the medial and lateral poles on the condyle. After that, the condyle was placed on the specimen fixture with the ML axis normal to the set screws of the fixture and the set screws were tightened to hold the condyle flat into the specimen fixture. A LECO VC-50 saw and a diamond blade were used to section the condyle into cubes. Distilled water was used during the cutting to avoid damage and heating due to dry cutting of the samples. The micrometer provided on the saw was used to measure the distance between two neighboring cuts on the condyle.
By positioning the specimen fixture as shown in Figure 3-2 (a), two cuts separated by a total distance of the cube side length plus the blade kerf (0.35564 mm) parallel to the ML axis were made as shown in Figure 3-2 (b).

![Figure 3-2](image)

Figure 3-2: Sequence of cutting operations. 3-2 (a) shows the condyle and blade position for the cuts on anterior and posterior faces. 3-2 (b) shows two horizontal cuts parallel to ML axis. 3-2 (c) shows vertical cuts on the ML axis.

After cutting the AP faces to cut ML faces, the specimen fixture was rotated by 90° about the SI axis. Approximately 7-8 successive cuts of cube width plus kerf were made perpendicular to the ML axis to cut the ML faces as shown in Figure 3-2 (c).

Finally, two cuts in the plane perpendicular to the SI axis were necessary to free the cubes from the condyle. For these cuts, the specimen fixture was rotated 90°. The samples in the study were harvested from the ML axis therefore, the first cut was made (1.5 + 0.1778 mm) above the medial- lateral axis for the 3 mm cubes, and for the 4 mm cubes same cut was made (2 + 0.1778 mm) above the ML axis. The superior faces of the cube samples along the ML axis were visible after this cut. In order to identify the superior inferior (SI) direction, the superior faces of samples were marked with a green
marker. Similarly, the medial face of cubes was marked with red marker for identification (Zaylor, 2013). The last cut was taken below the medial lateral axis similar to the first cut as shown in Figure 3-2 (d).

Figure 3-2 (d) shows the inclination of the specimen fixture with respect to the blade for the last cut.

The final cut was made slowly to cut each individual sample. The saw blade was stopped after cutting each cube free and the anterior face of the harvested cube was marked with a blue marker. Each cube sample with marked faces was placed inside a labeled test tube filled with saline solution. Before cutting the next cube, the medial face of the next cube was marked red (Zaylor, 2013). A micrometer with accuracy of $10^{-4}$ mm was used to measure the dimensions of the samples. All test tubes were filled with saline solution and the sample was stored in a -20°C freezer. In the present study, a total of forty-six 3 mm cubes, and sixteen 4 mm cubes were harvested from eight pigs.
3.2 Micro computed Tomography Scanning

The specimens under study were shipped to the University of Toledo, OH, for the scanning. An insulated container filled with two reusable gel ice packs along with the specimens were used for overnight shipping. The scanning was done at room temperature using SCANCO μCT 35. The samples were kept refrigerated except when in the scanner. From thaw to refreeze the time needed to prepare, ship, scan, and return the samples took 15 days.

During the μCT scanning, to avoid movement of the samples in the test tubes, specifically designed rectangular specimen holders made from plexiglass acrylic sheets were used. The holders had rectangular slots in which specimens were firmly positioned by wrapping a thin band of cellophane tape around the cube. The specimens positioned in a holder can be seen in Figure 3-3 (a). The holder was then inserted into a test tube filled with 0.9% saline solution, to keep the bone samples hydrated during the scanning. The tube was sealed at the end using silicone gel to prevent and reduce leakage of saline, and to prevent the movement of the holder in the test tube during scanning. The scanning was done with four scans, each containing four tubes per scan. The top view of the arrangement of the tubes in the holders for a scan set is shown in Figure 3-3 (b). The orientation and position of all samples in the slots of the specimen holder as well as the tube labels were recorded to identify the scanned location of each cube sample.
3.3 Image Segmentation and Smoothing Operation

A two dimensional slice of the μCT image is shown below in Figure 3-4, it represents trabecular bone tissues (white portion), marrow (black portion) within a sample, and a specimen holder made of plexiglass acrylic sheets (black portion outside the sample).
Zaylor (2013) explained the image segmentation described below. I have briefly restated the methods for the general knowledge of the readers. Scanned μCT images of each specimen were imported into Avizo 7.1 (Visualization Sciences Group, Merignac, France) to perform image segmentation, smoothing, and tetrahedral mesh generation.

Image segmentation was the first step in creating the FE mesh from the μCT images. The segmentation procedure labels the bone voxels from the marrow in the scanned images. This segmentation uses a magic wand tool in Avizo. A specific magic wand threshold value obtained from the histograph in the segmentation editor was defined. Voxels with grey values larger than the defined threshold value represent bone tissue and the trabecular structure is identified from marrow. The histograph of a typical scanned sample and the magic wand tool are shown in Figure 3-5. The upper limit of the grey value was recorded by selecting the right end of the histograph. The lower limit was 5/8th of upper limit value (Zaylor, 2013).
Figure 3-5: Screenshot of magic wand tool in Avizo, where the horizontal axis is HU and the vertical axis is number of voxels for the histograph. The lower limits of grey values, 8705 are $5/8$th of upper limit 13928, are seen in the left and right boxes of the magic wand tool.

After segmentation, a complete 3D volume was smoothed twice which made a bone surface smooth in appearance by removing jagged edges and artifacts. Figure 3-6 shows the sequence of the processes followed in the image segmentation.
Figure 3-6: The image segmentation procedure 3-6(a) shows a scanned 2D slice of 4 mm cube. 3-6(b) the brighter pixels are bone tissues, which were selected by assigning $5/8^{th}$ of the maximum HU value of that specimen. 3-6(c) shows a segmented slice before the smoothing operation, jagged edges at the boundaries of bone can be seen in the segmented image. 3-6(d) Image obtained after smoothing operation shows smooth and consistent bone region boundaries.

The surface models from the smoothed images were created in *Generate Surface* module in Avizo. This module creates the surface model with a very large number on the order of $10^7$ of triangles.
3.4 Tetrahedral Mesh Preparation of Surface Mesh

The surface model was simplified to reduce the number of triangles of the generated surface because the large number of triangles increases the complexity to create the tetrahedral mesh. For 3 mm and 4 mm cubes, I could create a mesh with a maximum of 350000 and 400000 triangles respectively. The minimum and maximum edge length of the triangle was requested as 10 µm and 100 µm with a maximum aspect ratio of 15, similar to the study by Zaylor (2013). The 3D geometry defined by the triangulated surfaces was processed into a tetrahedral mesh with the Generate Tetra Grid module. The tetrahedral mesh created in Avizo is shown in Figure 3-7. The solid mesh represented the architecture of trabecular bone. The mesh was then imported into the finite element package Marc 2012 (MSC Software, Santa Ana, CA, USA).

Figure 3-7: A tetrahedral mesh of trabecular bone sample.
3.5 FE Modeling Process

The three dimensional solid tetrahedral mesh was assigned material properties for all the elements representing trabecular bone microstructure. Two homogeneous isotropic tissue material properties were defined: Young’s modulus and Poisson’s ratio. Van Eijden (2004) reviewed the literature to find the variation in the average tissue modulus of trabecular bone and suggested a range of 3.5 GPa to 18.7 GPa. For the current study, a tissue modulus of 6.25 GPa was selected and applied to all the elements similar to the previous study by Zaylor (2013). Poisson’s ratio of 0.3 was assigned for all the elements in FE model (Kabel et al., 1999; Zaylor, 2013).

After the material properties were assigned, the FE model was given boundary conditions shown in Figure 3-8 below. The boundary conditions were applied to replicate the experimental uniaxial unconfined compression testing.

Figure 3-8: The boundary conditions of a typical FE model of a trabecular sample (left and right), nodes fixed in the X and Z direction on the X and Z edges of the cube (right).
As a result, all nodes at the bottom surface of the model were constrained not to move in the direction of the load. In addition to that, the nodes on two orthogonal edges of the same bottom surface were constrained to not move laterally, which removed the rigid body motion in the FE simulation but allowed lateral strains similar to experimental compression tests.

After defining the boundary conditions, the load condition was defined. For that purpose, the nodes at the top surface of each FE model were linked in the load direction’s degree of freedom to a load node. This method applies a uniform load on all the nodes at top surface and moves them together as would the top platen. Finally a load of 20 N was applied at the load node, although as the FE analysis are linear, the magnitude will divide out during the analysis and not change modulus results.

The model was simulated in a linear elastic analysis in the AP, ML and SI directions identical to the simulations performed by Zaylor (2013). After performing simulations, the apparent modulus, \( E_{app} \), was calculated as defined by equation 3.1.

\[
E_{app} = \frac{PH}{Ah}
\]  

(3.1)

Where,

\( P \) is the load of 20 N, applied at the load node.

\( H \) is the specimen height (mm) in the loading direction, measured FE model.

\( A \) is cross sectional area (mm\(^2\)) of the face in the loading direction, calculated as the product of the orthogonal lengths measured from FE model.

\( h \) is displacement (mm) of the linked node at the top surface of the sample, recorded from the FE simulation.
The apparent modulus was calculated in the AP, ML, and SI direction for each sample. A total of thirty four of the 3 mm cubes, and sixteen of the 4 mm cubes were simulated, each in AP, ML, and SI directions. A total of 12 samples of 3 mm cubes were rejected from the study due to low connectivity of structure.

3.6 Digital Length Measurement

Geometry is an important input parameter in FE analysis. The accuracy of FE analysis depends on the accuracy with which inputs are defined. In the present study, the input geometry is obtained after processing the scanned images in Avizo. To investigate the accuracy of the methods used to create a digital cube, the dimensions of the digital cubes were compared with the dimensions of the physical cubes.

The digital lengths of the cubes were measured from the FE models. The lengths were calculated by measuring the nodal distance between two opposite faces of the cube in the respective AP, ML and SI directions. Figure 3.9 shows a typical FE model of 3 mm trabecular bone cube. The digital length was recorded as the maximum distance between the two nodes on the opposite faces of the cube. Four readings were taken by selecting different nodes at different heights, approximately one at the top, one at the bottom, and two readings roughly at equal interval from top and bottom readings as shown in Figure 3-9. The average of all the four readings was defined as the digital length in the particular direction. The physical lengths of all samples were measured using a micrometer. Only one reading was taken for physical length measurement unlike four readings for digital length measurement. The difference between the digital and physical length was defined as an error.
Figure 3-9: Four readings of the digital length measurement from the FE model of 3 mm trabecular bone.

3.7 Compression Testing Procedure

The apparent moduli of all the specimens were calculated using a nondestructive compression test to validate the FE apparent moduli. The specimens were taken out of the freezer one hour before the compression tests and kept in saline solution. Uniaxial compression testing was carried out at room temperature. Mineral oil was applied on the top and bottom faces of the platen before each test to reduce the effect of the friction (Linde et al., 1994). The setup of the compression test is as shown in the Figure 3-10.
Figure 3-10: (a) The MTS Q-10 load frame with a 500 N load cell, LVDT, and a bone sample between the top and bottom platen.

Figure 3-10 (b) a closed view of the sample, LVDT, and top and bottom platen.
A sample was taken out of the test tube and placed on the bottom platen of the MTS Q-10 load frame. A sample was aligned at the center on the bottom platen, to avoid eccentric loading and the bending on the sample. Once centered in the platens, a drop of saline was injected with a syringe to keep the sample wet. The samples were small enough that the drop encompassed the samples. Samples were kept moist to avoid drying of bone tissue. Before starting the test, the height and cross-sectional area of each specimen in the direction of the load, measured with micrometer, was input to the MTS Testworks 4 computer program (Zaylor, 2013).

The compression testing protocol described below, is identical to the one used by Zaylor (2013). The test included a 500 N load cell to measure the force, and a linear variable displacement transducer (LVDT) to measure the displacement between the top and bottom platen. The test was conducted at a constant velocity of 0.01 mm/s with data recorded at 100Hz throughout. In the beginning of the test, a preload of 2 N was applied on the test specimen. The preload was necessary to ensure contact between the top platen and surface of the specimen. The position of the top platen at preload was recorded as the preload height and the strain was reset to 0. The strain was applied up to 0.6%, when the strain reached 0.6% the strain was removed. The cycle was programmed to stop when the load value returned to the preload value of 2 N. This concluded the first cycle. For the second cycle, the strain at the end of the first cycle was redefined as zero strain. A total of 15 strain controlled loading and unloading cycles were applied to measure the apparent modulus (Zaylor, 2013).
The apparent stress was calculated as the ratio of the load divided by the cross sectional area of the specimen in the loading direction. The computer program simultaneously plotted a graph of the apparent stress against apparent strain for each cycle during the testing. A stress-strain curve of a complete test for one of the samples is shown in Figure 3.11.

Figure 3-11: The stress-strain curve shows the 15 cycles for a particular compression test. The strain exceeded 0.6 % strain due to inertia of the crosshead.
As a standard from Zaylor (2013), the MTS Testworks 4 computer program calculated the apparent modulus of each cycle as the slope of the stress-strain curve between the strains of 0.5-0.6% while loading. The average apparent modulus of the last three cycles was reported as the modulus of that sample (Zaylor, 2013).

In addition to the 0.5-0.6% strain range, I calculated the stiffness of each sample in the strain range of 0.4 to 0.5% and 0.4 to 0.6%. The objective of this was to analyze the variation in the stiffness and if the range effected the agreement between FE and the experimental stiffness in these strain ranges.

To calculate the stiffness in strain ranges of 0.4 to 0.5% and 0.4 to 0.6%, I imported the experimental data into Matlab, Dr. John Cotton (Russ College of Engineering and Technology, Ohio University) wrote the code. In the code, the linear regression was performed for all the data points between the defined strain limits for the last three cycles. The average of the last three cycles was reported as stiffness. To check the accuracy of the code, the stiffness was calculated for each sample in the strain range of 0.5-0.6%. The results were compared with the stiffness values estimated from the computer program. The comparison showed no difference between the results, which confirmed the accuracy of the Matlab code.

3.8 Physical and Digital Measurements of Mass

The experimental mass was measured to test if the segmented digital models agree with the physical models. To achieve this, the physical mass of the sample was measured (see method below) and compared with a digital mass calculated from the scanned images. In creating the FE models, the scanned images were segmented and the
digital mass was calculated as follows. The volume of the bone tissue was recorded from the material statistics menu in the label field editor of Avizo. The mass of the sample was calculated by multiplying the bone volume, $BV$, of the sample to the bone tissue density, $\rho_t$.

$$\text{Mass} = BV \times \rho_t \quad (3.2)$$

The bone tissue density, $\rho_t = 1.9 \text{ g/cm}^3$ suggested by Linde (1994) is used for all the samples.

I assumed the same value of tissue density for all the samples. The calculated mass can be scaled to the lower or higher values by changing the tissue density values, but it will not affect the correlations of the linear regression analysis between the digital and physical mass.

To measure the experimental mass of bone content in the specimen, I attempted to remove the marrow from the specimen as shown by Linde et al. (1992) and Kabel et al. (1999) described here. The subsequent steps can be seen in Figure 3.12.

Figure 3-12: Sequential workflow of the experimental mass calculation.
First, an air jet was used to remove superficial marrow. The masses of the samples were measured by weighing them on a digital scale (AB104-S Mettler Toledo, Made in Switzerland) with an accuracy of 0.001g. After using the air jet, the specimens were soaked in ethanol for 48 hours and stirred in the solution after interval of roughly 10-12 hours. After 48 hours, air jet was used one more time to remove residual fat and liquid present in the specimens. Finally, the specimens were dried at room temperature for 24 hours. The masses were measured again and were recorded as defatted mass.
CHAPTER 4 - RESULTS

4.1 Comparison of the Experimental Stiffness With Respect to Strain Ranges

The experimental apparent modulus was calculated in the strain range of 0.4-0.5%, 0.5-0.6%, and 0.4-0.6%. The average apparent modulus in each strain range can be seen in Figure 4-1. The mean values of the experimental modulus in these strain ranges were not significantly different (One way ANOVA analysis; p > 0.05). Therefore, the strain range of 0.5-0.6% is used for all the statistical comparisons between the experimental and FE values of apparent modulus. All samples showed a typical stress-strain curve shown in figure 3-12 in the methods section. One 4 mm sample exceeded the 500 N limit of the load cell in the SI direction and was eliminated from the analysis.

Figure 4-1: Uniaxial compression test histograms showing the variation in the average apparent modulus in ML, AP, and SI direction with respect to strain range of 0.4-0.5%, 0.5-0.6%, and 0.4-0.6%.
4.2 Comparison of the Average Apparent Modulus With Respect to Direction

The FE average apparent modulus of 3 mm and 4 mm cubes calculated in the ML, AP, and SI loading directions with a tissue modulus of 6250 MPa are shown in Figure 4-2. ANOVA analysis was used for the average apparent modulus measured in ML, AP, and SI directions of the cube to determine if the modulus is significantly different in different loading directions. Minitab 17 (State college, PA, USA) was used for the general linear model of ANOVA. In the analysis, the response was stiffness and the factors were animals, condyles, samples, and direction. Respective samples were nested within the condyles. The average apparent modulus measured in all three directions for 3 mm cubes were significantly different (p < 0.05). There was a significant effect of all the factors for the variation in the stiffness (p < 0.05). In order to find the nature of variances between the modulus measured in the loading directions, a post hoc Tukey test was performed. The Tukey test showed that the average FE apparent moduli measured in the ML, AP, and SI directions of 3 mm cubes were significantly different (p < 0.05). For 4 mm cubes, the average FE apparent modulus measured in the SI direction was significantly different than the ML and AP directions (p < 0.05).
The average experimental apparent modulus of 3 mm and 4 mm cubes calculated in the ML, AP, and SI loading directions are shown in Figure 4-3. ANOVA analysis followed by a Tukey test showed that the average experimental apparent modulus measured in the SI direction of 3 mm cubes is significantly different ($p < 0.05$). However, no significant difference ($p = 0.63; n=16$) was found between the average experimental apparent modulus of 4 mm cubes with respect to loading direction.
Figure 4-3: The average experimental apparent modulus of 3 mm and 4 mm cubes measured in the ML, AP, and SI directions (*p < 0.05, n = 34 and 16 for 3 mm and 4 mm cubes respectively).

4.3 Agreement between the FE and Experimental Apparent Modulus

In the linear FE analysis, all models were assigned a tissue modulus of 6250 MPa. The values of the apparent modulus are proportional to the tissue modulus. The ratio of the apparent modulus to the tissue modulus ($E_{app} / E_t$) is a constant value in the linear FE analysis. I have used this ratio as a relative modulus, $E_{rel}$, to represent the FE apparent modulus irrespective of the tissue modulus.

A linear regression analysis between the relative apparent modulus calculated from the FE models and experimental apparent modulus of 3 mm cubes is shown in Figure 4-4. A coefficient of determination, $R^2$, of 0.85, 0.27, and 0.49 are obtained in the ML, AP, and SI directions, respectively.
Figure 4-4: Correlation between predicted FE and experimental apparent modulus of 3 mm cubes in ML, AP, and SI loading directions. The slope can be interpreted by inverting it to determine $E_t$.

A linear regression analysis between the FE and experimental apparent modulus of 4 mm cubes is shown in Figure 4-5. Coefficients of determination, $R^2$, of 0.65, 0.15, and 0.55 are obtained in the ML, AP, and SI direction, respectively. The intercept of AP direction is significantly different from 0, ($p < 0.05$).
Figure 4-5: Correlation between predicted FE and experimental apparent modulus of 4 mm cubes in ML, AP, and SI loading directions. The slope can be interpreted by inverting it to determine $E_t$.

4.4 Comparison of the Apparent Densities of 2, 3, and 4 mm Cubes

In the present study, the apparent modulus of 3 and 4 mm cubes is estimated with both FE and experimental methods. The correlation between the two measures of apparent modulus is compared with the correlation obtained with 2 mm cubes used in the previous study by Zaylor (2013). In order to understand if the 3 and 4 mm cubes used in the current study are significantly different than 2 mm cubes, the apparent densities measured from the air dried mass of all the cubes were compared. The average apparent densities of 2, 3, and 4 mm cubes are significantly different (One way ANOVA, $p < 0.05$) as shown in Figure 4-6.
In order to find the nature of the variances between the means, post hoc Tukey test was performed between the average values of the apparent densities of 2, 3, and 4 mm cubes. I found no significant difference between the average densities of 3 mm and 4 mm cubes (p > 0.05), however the average density of 2 mm cubes is significantly higher (p < 0.05) than the average densities of both the 3 mm and 4 mm cubes. This tells us our cancellous bone cubes are less dense than those 2 mm cubes tested by Zaylor (2013).

4.5 Comparison of the Digital and Physical Mass

The digital mass of cubes is calculated from scanned images by assuming a bone tissue density of 1.9 g/cm³. A linear regression between the experimental and digital mass
showed a coefficient of determination of \( R^2 = 0.85 \) and \( R^2 = 0.69 \) for 3 mm and 4 mm cubes respectively. The plot of the experimental mass against digital mass is shown in Figure 4-7.

![Figure 4-7: The relationship between the digital mass and the experimental mass of bone cubes.](image)

**4.6 Comparison of the Digital and Physical Geometry of the Cubes**

The scanned images of the cubes were segmented first to label the bone tissue, and then processed to generate FE mesh of the bone cube. This digital cube geometry was simulated in the FE analysis to measure the apparent modulus. In order to investigate the accuracy of the digital geometry of cubes with respect to the physical geometry of cubes, dimensions of the digital and physical cubes are compared. Linear regression between the
digitally measured and micrometer measured dimensions showed a strong correlation of 
$R^2 = 0.80$, 0.81, and 0.78 in the ML, AP, and SI directions, respectively, which is shown
in Figure 4-8.

![Figure 4-8: The relationship between the digital lengths of 3 mm cubes, measured from
the FE models and the physical lengths, measured with micrometer in ML, AP and SI
direction.](image)

Similarly, for 4 mm cubes, regression analysis showed a correlation of $R^2 = 0.95$, 0.67, and 0.50 in the ML, AP, and SI direction respectively which is shown in Figure 4-9.
Figure 4-9: The relationship between the digital lengths of 4 mm cubes, measured from FE models and the physical lengths, measured with micrometer in ML, AP and SI direction.

I found no change in the correlation of the regression analysis, even after removing the samples which showed difference up to 10% in length measurements.
CHAPTER 5 - DISCUSSION AND CONCLUSION

The purpose of the study is to quantify and improve the accuracy of digital FE modeling and experimental uniaxial nondestructive compression stiffness measurement techniques of trabecular bone from the porcine mandibular condyle. To do this, I calculated the stiffness of trabecular bone samples in the ML, AP, and SI direction of the mandibular condyle. Samples under study are 34 cubes of side length 3 mm and 16 cubes of side length 4 mm. Stiffness was estimated from FE models and uniaxial nondestructive compression tests.

The purpose of using a larger sample size of 3 mm and 4 mm cubes as compared to 2 mm cubes was to investigate the effect of the sample size on the agreement of the stiffness prediction. The agreement is computed by performing linear regression analysis between the stiffness measures obtained by FE models and experimental tests. I found that there is improvement in the values of coefficient of determination in the ML and SI direction using larger 3 mm cubes and 4 mm cubes as compared to the 2 mm cubes used in the previous study by Zaylor (2013). The values of coefficient of determination for 2, 3, and 4 mm cubes for the stiffness are showed in Table 1 below.
In the previous study by Zaylor (2013) the stiffness was estimated from the strain range of 0.5-0.6%. In order to check the variation in the stiffness estimated from different strain ranges, I measured the stiffness in the strain range of 0.4-0.5%, 0.5-0.6%, and 0.4-0.6%. The stiffness’s were compared by one way ANOVA analysis. There was no significant difference found in the stiffness between these strain ranges, (p > 0.05). Even though the average apparent modulus in all strain ranges was not significantly different than each other, average experimental modulus in the strain range of 0.5 - 0.6% was 5.6%, 6.8% and 6.5% higher as compared to the strain range of 0.4 - 0.5% in AP, ML, and SI directions respectively. However, none of these strain ranges could predict a better FE modulus.

The average stiffness of all the cubes in the current study follows the findings of Teng and Herring (1996). Teng and Herring (1996) performed experimental testing on rectangular specimens to study the anatomic and directional dependence of the stiffness of the porcine mandibular condyles. The average height of specimen in the test direction was 8.92 mm and the average cross sectional area was 6.09 mm x 4.98 mm. Table 2

<table>
<thead>
<tr>
<th>Sample Length</th>
<th>Sample size</th>
<th>Loading direction (R²) values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>AP</td>
</tr>
<tr>
<td>2 mm*</td>
<td>46</td>
<td>0.32</td>
</tr>
<tr>
<td>3 mm</td>
<td>34</td>
<td>0.27</td>
</tr>
<tr>
<td>4 mm</td>
<td>16</td>
<td>0.15</td>
</tr>
</tbody>
</table>

(∗) - From Zaylor (2013); NS, not significant.
below shows the values of the average stiffness obtained in the current study and those by Teng and Herring (1996).

Table 2: Comparison of the average stiffness with respect to loading directions

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<thead>
<tr>
<th>Loading direction</th>
<th>(Teng and Herring, 1996)</th>
<th>3 mm Cubes FE</th>
<th>3 mm Cubes Experimental</th>
<th>4 mm Cubes FE</th>
<th>4 mm Cubes Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-L</td>
<td>1310 ± 980</td>
<td>229 ± 192</td>
<td>109 ± 77</td>
<td>220 ± 144</td>
<td>223 ± 96</td>
</tr>
<tr>
<td>A-P</td>
<td>1760 ± 610</td>
<td>309 ± 254</td>
<td>128 ± 75</td>
<td>306 ± 170</td>
<td>254 ± 136</td>
</tr>
<tr>
<td>S-I</td>
<td>3030 ± 840</td>
<td>485 ± 348</td>
<td>224 ± 154</td>
<td>458 ± 294</td>
<td>314 ± 137</td>
</tr>
</tbody>
</table>

Teng and Herring (1996) found that the average stiffness for all the specimens were maximum in the SI direction, minimum in the ML directions and intermediate in AP direction (SI>AP>ML). The stiffness measured by them is considerably high compared to the specimens used in the current study. It should be noted that the authors’ specimens are considerably larger in size as compared to the specimens used in my study. In addition, anatomic locations and age of the pigs are also different. I have also found the same variation of the average stiffness in the respective loading directions for 3 and 4 mm cubes.

I also found that with an increase in the specimen size from 3 mm to 4 mm the average values of the experimental stiffness was significantly increased (p < 0.1). The average experimental stiffness of the 4 mm specimens is 103%, 98%, and 40% higher in the ML, AP, and SI directions respectively. These results are in accordance with the findings of Linde et al. (1992), who investigated the effects of trabecular bone sample
size on the estimation of experimental stiffness. Linde et al. (1992) found an increase in the experimental stiffness of the specimen with increase in the cross sectional area and length of the specimen. The authors used trabecular bone specimens harvested from human knees; cylinders with diameter 5.5 mm, 6.5 mm, and 7.5 mm and length to diameter ratio of 1. The stiffness was measured in the SI direction with nondestructive testing at a strain of 0.4 %. The average stiffness was 192 MPa, 255 MPa, and 318 MPa for the cylinders of diameter 5.5, 6.5, and 7.5 mm respectively (Linde et al., 1992). This reveals that the average stiffness of 7.5 mm cylinders was 66% and 25% higher than the 6.5 mm and 5.5 mm cylinders respectively.

The authors explained that during experimental testing, friction between the loading surface of the specimen and the test platens initiates a varying stress distribution. This stress grows towards the center of the specimen conically whose depth depends on the cross sectional area and the length of the specimen (Linde et al., 1992). During testing, larger loads are required to achieve the same longitudinal strain on the specimen as compared to a steady stress field, which results in the increase in the stiffness of the specimen (Linde et al., 1992). To reduce this effect of the friction, I applied mineral oil on the test platens before testing each cube, but in real conditions, friction can be minimized but cannot be eliminated.

Furthermore, Linde et al. (1992) explained when the bone specimens are harvested from the surrounding structure, structural damage occurs around the edges of the sample. This structure around the edges is not supported as it normally would before cutting the specimen. As a result, it leads to underestimation of the apparent modulus.
The effect of structural artifacts decreases as the specimen length and area increases (Linde et al., 1992). Although the structural end effect error is present in both 3 and 4 mm cubes, with the larger cross sectional area and length of the 4 mm cubes, the effect of error is reduced. These theories explain the reason of higher experimental apparent modulus of the 4 mm specimens seen in the current study.

A direct comparison of the FE apparent modulus between 2 mm, 3 mm, and 4 mm cubes will not be fair due to under meshing of the cubes. There is a difference of 64.85% in the average mesh density and 34.04% in the average edge length of tetrahedron between the meshes of 3 and 4 mm cubes. The average length of tetrahedra for 2 mm, 3 mm, and 4 mm cubes are 30 µm, 39 µm, and 55 µm, respectively. The effect of the larger edge length of tetrahedra results in a higher stiffness. From the mesh convergence study, I estimate that FE models of 3 mm cubes are 7% stiffer as compared to FE models of 2 mm cubes and FE models of 4 mm cubes are 13% stiffer as compared to FE models 3 mm cubes. The detailed explanation of the mesh convergence study is provided in appendix A.

The average values of the experimental and the FE apparent modulus of 3 and 4 mm cubes were found to be smaller than the apparent experimental and FE modulus of 2 mm cubes used by Zaylor (2013). The stiffness of trabecular bone has been reported to be well predicted by its apparent density according to $E = A\rho^\beta$. Where, $\beta$ is an empirically determined value from 2 to 3 (Rice et al., 1988). In order to test if the samples reflected inherent differences in material properties, the apparent density of 2 mm cubes was compared to 3 mm and 4 mm cubes. The apparent densities of all samples calculated
from the air dried mass were compared by using ANOVA analysis. I found the average apparent density of 2 mm cubes was significantly higher, 19% and 17%, as compared to 3 mm and 4 mm cubes respectively. These higher values of apparent density in the 2 mm cubes could cause a higher stiffness as compared to 3 mm and 4 mm cubes. A quick examination with a $\beta = 3$, shows the 19% difference in the apparent density values from 3 mm to 2 mm cubes could explain, $1.19^3 = 1.69$ or a 69%, increase in the apparent modulus values. However, the average increase in the modulus from 3 mm to 2 mm was 300%. Similarly, between 2 mm and 4 mm cubes, the apparent density could explain about 60% increase for the average modulus increase of 117%.

Another important source of error in the FE process may be segmentation. Image segmentation is a very important step in the development of the µCT based FE models. It is necessary to identify and segment the trabecular bone geometry from the surrounding marrow present in the scanned grey images to create FE meshes for the FE analysis. Based on the threshold used in the segmentation procedure, the bone structure is distinguished from the marrow. The calculated digital mass predicted the experimental mass with a correlation of 85% and 69% for 3 and 4 mm cubes. I believe for the 4 mm cubes, the lack of correlation is mostly due to the overestimation of the experimental mass. After defatting, microscopic inspection showed some matrix inside the trabecular space. I think the defatting procedure described by Linde et al. (1992) and Kabel et al. (1999) was more effective for 3 mm cubes, than 4 mm cubes, and the defatting procedure needs to be improved. The further directions which may turn out to be effective are allowing more time for specimens to remain immersed in ethanol, or using a shaker or
ultrasonic bath to stir the specimens constantly during the defatting. In addition, I think the bone tissue density of 1.9 g/cm³ may be a bit high for both 3 and 4 mm cubes.

To test geometric accuracy, I also investigated the image segmentation procedure further by comparing the digital cube geometry with respect to physical geometry of the cube. I measured the lengths of the digital cubes in all three directions from its FE model and compared with the original lengths measured with micrometer. I found few FE cubes have length variation up to 10% in one of the direction. The average FE lengths are roughly 2% smaller than the micrometer measured lengths. There is, however no correlation between the error in the length and the modulus agreement. The linear regression analysis between the digital and physical lengths showed (R² = 0.80, 0.81, and 0.78) values in ML, AP, and SI direction for 3 mm cubes and (R² = 0.95, 0.65, and 0.50) for 4 mm cubes respectively. Theoretically, a 2% loss in length results into overestimation of the apparent modulus; as the cross sectional area, A, and the specimen height, H, used in the calculations of apparent modulus in equation 3.1 are underestimated. This overestimates the apparent stress by 4% and strain by 2%, which increases the modulus by 2%.

5.1 Conclusion

The aim of this study was to quantify and hopefully improve the accuracy of digital FE and physical stiffness measurement techniques of trabecular bone from the porcine mandibular condyle. To measure the digital stiffness of trabecular bone, I built linear FE models from μCT images of 3 mm and 4 mm cube specimens assuming a homogeneous isotropic tissue modulus of 6.25 GPa. I also performed experimental
uniaxial nondestructive compression tests on all cubes to measure the physical stiffness in the strain range of 0.4-0.5%, 0.5-0.6%, and 0.4-0.6%. Stiffness was measured in three directions of the cubes, which represented the ML, AP, and SI direction of the mandibular condyle.

I investigated the effects of specimen size on the accuracy of the digital and physical stiffness measurements. I performed regression analysis between the measures of stiffness to test if the agreement between the stiffness measurements obtained from both the methods improved with larger specimens. The results show that FE models predict 85%, 27%, and 49% of the experimental stiffness with 3 mm cubes and 65%, 15%, and 55% with 4 mm cubes in ML, AP, and SI direction respectively. The coefficient of determination obtained in the present study between both measures of the stiffness is improved in ML and SI direction as compared to the previous study of 2 mm cubes by Zaylor (2013). With 2 mm cubes, FE models predicted 31% and 33%, of experimental stiffness in the AP and ML direction and no relation was obtained in SI direction. This study succeeded to achieve the correlation in the SI direction which was not achieved with 2 mm cubes. The realistic tissue moduli of trabecular bone were seen in the range of 2890 MPa to 6100 MPa.

I have used a homogeneous tissue modulus in the FE models to measure the FE apparent modulus. Therefore the FE models only measured the influence of the architecture on apparent modulus. However, the experimental apparent modulus is influenced by both the architecture and the heterogeneous tissue modulus. I think this might be a reason for poor agreement in the AP direction using large cubes. The results
obtained with larger cubes are improved in ML and SI direction, but the relationship between FE and experimental prediction of modulus is still poor.

To test if a realistic mass of the segmented digital models agrees with the physical models, I measured the mass of 3 mm and 4 mm cubes experimentally and compared the mass calculated from the digital files. A strong agreement of the digital mass with the experimental mass is obtained. The experimental mass is predicted with accuracy of 85% and 69% with 3 mm and 4 mm cubes respectively. For 4 mm cubes, the defatting procedure was likely not as effective as the 3 mm cubes as some matrix was observed in the samples under microscopic inspection for both.

I also found there are some errors associated with the geometric modeling procedure. The FE models have roughly an average of 2% smaller lengths as compared to the physical lengths. The loss of geometry in the FE analysis is not desired. FE analysis generally assumes that the physical geometry of the structure is accurately presented digitally. In the future, the geometric modeling procedure can be improved by preventing the loss of trabecular bone structure in segmentation process. This can be done by processing images with Gaussian smoothing to remove the noise and for better edge detection. In addition, the geometry of trabecular bone developed by the $5/8^{th}$ of maximum threshold method can be compared with the geometry developed by different segmentation methods, such as OTSU’s method or the histogram method. With an increase in the accuracy of the image segmentation procedure, accuracy of the FE models to predict apparent modulus should increase.
I also examined the experimental stiffness of 3 mm and 4 mm cubes measured over the strains of 0.4-0.5%, 0.5-0.6%, and 0.4-0.6%. The reason to choose these three stiffness strain ranges was to check if the arbitrarily chosen range of 0.5-0.6% used to calculate the stiffness of 2 mm cubes was significantly different from other strain ranges. Results show that there is no significant difference in the stiffness values estimated from these three strain ranges (p > 0.05). These results help to gain confidence in the uniaxial nondestructive compression test method used with 2, 3, and 4 mm specimens.

The improved correlation between the stiffness measurement methods justifies digital modeling of TMJ condyle to measure the stiffness.


APPENDIX A – MESH CONVERGENCE STUDY

Introduction:

The surface generator in Avizo assigned triangular faces to the segmented label field to create a triangular surface. A triangular surface was processed using Generate tetrahedral mesh module to create a tetrahedral mesh. The number of the tetrahedral elements generated in the mesh was depended on the number of triangular faces assigned to the surface. The accuracy of the FE solution is higher with a higher number of tetrahedral elements. However, the computational and user time to create the mesh and to perform FE simulation increases exponentially with respect to the number of elements used in the mesh. The objective of the mesh convergence study is twofold. First, to select an optimum number of triangular faces based on the total time required to create a tetrahedral mesh and perform FE simulation. Second is to investigate the effect of tetrahedral elements size on the accuracy of FE solution.

Methods:

I performed a mesh convergence study for 3 mm cubes using three samples with low, medium, and high BVF values. The samples were 703 RM, 203 LM, and 202 LM with BVF of 0.2, 0.33, and 0.45 respectively. For each sample five tetrahedral meshes were created by requesting 100k, 200k, 300k, 350k, and 400k triangular faces. From the generated tetrahedral meshes, the total number of nodes, elements, and the mesh volume were recorded. For each mesh, tetrahedral edge length and mesh density were calculated as

\[ \text{Mesh density} = \frac{\text{Number of elements}}{\text{Mesh volume}} \]  

(3.2)
By inverting mesh density, the average tetrahedral volume, $V$, was calculated. Then the edge length, $a$, was calculated as

$$ V = \frac{a^3}{6\sqrt{2}} \quad (3.3) $$

Results and Discussion:

The time required to create a mesh and simulate a cube increased with the increase in the requested triangular faces. To create a mesh with 350k faces and perform simulation for one cube, the user and computational time was approximately 4 hours. The major portion of the time was consumed in the creation of the mesh. With 400k faces, the initial time mesh quality was poor and it took approximately 6 hours for one cube to repair and then simulate the mesh. Up to 90% of the time was consumed in Avizo. The problems associated with the mesh generated from 400k faces were repetitive crashing of the Avizo software, elements with intersection and wrong orientation, higher aspect ratios, and holes in the mesh. It was necessary to resolve all the problems to improve the quality and successfully generate the mesh.

The results of the mesh convergence study are shown in figure A-1 below. It can be seen as the edge length decreased, the apparent modulus decreased.

The average tetrahedral edge lengths for three samples in the pilot study for the meshes with 100k, 200k, 300k, 350k, and 400k triangular faces are 67 µm, 52 µm, 43 µm, 40 µm and 38 µm respectively. There is a difference of 2 µm in the average tetrahedral edge lengths of meshes generated with 350k and 400k triangular faces. The results reveal that stiffness values are overestimated with these edge lengths due to under
meshing. To calculate the overestimation of the modulus, the linear regression equations between the stiffness and the edge length, shown in figure A-1 can be used.

Figure A-1: The relationship between modulus and edge length.
In my study, I have used a total of 34 cubes of 3 mm and 16 cubes of 4 mm side. I requested 350k faces for 3 mm cubes and 400k triangular faces for 4 mm cubes to create tetrahedral meshes. Table 3 below shows the average values of the mesh density and edge length obtained for the cubes.

<table>
<thead>
<tr>
<th>Size</th>
<th>Sample size</th>
<th>Average Mesh density</th>
<th>Average edge length</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 mm</td>
<td>34</td>
<td>151,000/mm³</td>
<td>39 µm</td>
</tr>
<tr>
<td>4 mm</td>
<td>16</td>
<td>53,000/mm³</td>
<td>55 µm</td>
</tr>
</tbody>
</table>

There is a difference of 64.85% in the average mesh densities and 34.04% in the average edge length of tetrahedra between the meshes of 3 and 4 mm cubes. In order to directly compare the modulus of 3 mm and 4 mm cubes, the edge length of tetrahedral elements should be the same. To calculate the overestimation of the modulus, I used the linear regression equations obtained in the pilot study. From these equations, the percent change in modulus with a change of 10 µm edge length was calculated. Then the percent change in a modulus for the average edge length difference between Zaylor’s 2 mm, and my 3 mm and 4 mm cubes were calculated. The average length of tetrahedra for 2 mm, 3 mm, and 4 mm cubes are 30 µm, 39 µm, and 55 µm respectively. I estimate that the 3 mm cubes are 7% stiffer as compared to the 2 mm cubes and the 4 mm cubes are 13% stiffer as compared to 3 mm cubes. Table 4 below shows the pilot study calculations.
Table 4: Modulus extrapolation for the edge length of tetrahedral element

<table>
<thead>
<tr>
<th>Sample</th>
<th>Slope</th>
<th>Intercept</th>
<th>Modulus at edge length = 30 (MPa)</th>
<th>ΔE for Δ edge length of 10 μm</th>
<th>% ΔE for Δ edge length of 10 μm</th>
<th>% ΔE from 4 mm to 3 mm cubes</th>
<th>% ΔE from 3 mm to 2 mm cubes</th>
</tr>
</thead>
<tbody>
<tr>
<td>SI</td>
<td>2</td>
<td>4696</td>
<td>911</td>
<td>1032</td>
<td>47</td>
<td>5</td>
<td>7</td>
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<tr>
<td>3</td>
<td>7575</td>
<td>553</td>
<td>780</td>
<td>76</td>
<td>10</td>
<td>16</td>
<td>9</td>
</tr>
<tr>
<td>ML</td>
<td>2</td>
<td>4117</td>
<td>574</td>
<td>697</td>
<td>41</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td>5167</td>
<td>190</td>
<td>345</td>
<td>52</td>
<td>15</td>
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<td>13</td>
</tr>
<tr>
<td>AP</td>
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<td>36</td>
<td>7</td>
<td>11</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Average</td>
<td>8</td>
<td>13</td>
</tr>
</tbody>
</table>

Conclusion:

The outcome of the study is that for 3 mm cubes and 4 mm cubes I could use maximum of 350k and 400k triangular faces to generate the tetrahedral meshes respectively. It is not possible to have converged meshes with the available resources because of the software limitations. In order to have converged meshes, other software packages should be considered to generate the meshes with a larger number of elements. The pilot study showed that the 2 mm, 3 mm, and 4 mm cubes have different average edge lengths of the tetrahedral elements. The meshes used in the current study are under meshed and the resulting stiffness is overestimated. Even though the stiffness is overestimated, I have an estimation of the error resulting due to numerical under meshing.