THE MECHANICAL STRENGTH OF A BIPHASIC POLYCAPROLACTONE BONE SCAFFOLD FOR MEDIAL OPEN WEDGE HIGH TIBIA OSTEOTOMY IMPLANTATION

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ABSTRACT

Mechanical strength is a requirement of the bone scaffold for osteoarthritis treatment by the medial open wedge high tibia osteotomy (MOWHTO). The mechanical compression of the scaffold which depends on material concentration and structure must be concerned in MOWHTO substitution due to help to prevent the delayed bone healing process from scaffold collapse. This study was divided into two sections: (i) the influence of varying concentrations between DBM-HA mixed PCL scaffold (20/80, 30/70, and 40/60 % wt./wt. DBM-HA/PCL) on compressive strength, and (ii) the compressive strength of the 0°-90° orientations DBM-HA/PCL scaffold (20/80 % wt./wt.) constructed with 300-500 µm pore dimension by the extrusion-based bioprinting method. The results exhibited that the concentrations of DBM-HA/PCL affected mechanical properties in the scaffold. The low DBM-HA concentrations scaffold showed high compressive strength. The 20/80 % wt./wt. DBM-HA/PCL represented the 23.25 MPa and 157.63 MPa of compressive stress and modulus respectively. The 0°-90° orientations scaffold with 20/80 % wt./wt. DBM-HA/PCL showed the 2.90-16.03 MPa and 70.92 MPa of compressive stress and modulus. In conclusion, the mechanical compression of 20/80 % wt./wt. DBM-HA/PCL scaffold fabricated with 300-500 µm pore size has a range within tibia cancellous bone and it is suitable for an alternative bone in the MOWHTO.

Keywords: bone scaffold, polycaprolactone, demineralized bone matrix, hydroxyapatite, mechanical strength, and open wedge high tibia osteotomy

1. INTRODUCTION

A bone defect with 10-14 mm is a critical problem following the correcting knee alignment for osteoarthritis with the medial open wedge high tibia osteotomy (MOWHTO) without bone graft surgery because there is a lack of bone formation and delayed bone union process [1-2]. Normally, the minimum critical size of bone defect is greater than or equal to 4 mm leading to failure of osteogenesis [3-4]. Alternatively, adding a bone graft can promote the bone healing process in 3-8 months after substitution [5-8]. On the other hand, infective viral transmission disease, autoimmune rejection, and donor site morbidity are risk factors for implantation with grafting. Furthermore, the allograft cannot induce osteoinduction, which promotes bone-cell differentiation and is critical in osteogenesis [9-10]. Figure 1 illustrates the MOWHTO.

Keywords: bone scaffold, polycaprolactone, demineralized bone matrix, hydroxyapatite, mechanical strength, and open wedge high tibia osteotomy

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Newly technique, a bone scaffold which is an alternative synthetic bone was developed to risk factor reducing, bone deflection preventing, and bone union improving. Therefore, the bone scaffold required biological and mechanical properties similar to the native bone. The osteogenesis scaffold should be constructed with biocompatible materials and appropriate architecture according to biological and mechanical properties. Earlier, the scaffold was fabricated with pure polycaprolactone (PCL) because of
its properties. The PCL is a synthetic biopolymer with a melting point of 60 degrees Celsius, which is higher than the melting point of human body temperature [11]. The PCL scaffold has been used in plastic surgery because of its biocompatible, biodegradable, non-toxicity, and slow degradation [12-14]. Conversely, the PCL limitation is low compressive strength and non-favorable cell attachment. To improve osteogenesis, hydroxyapatite (HA) is added to the polymeric scaffold which is called the hybrid scaffold. Afterward, the hybrid scaffold explored increasing cell adherence, cell proliferation, and compressive strength after implantation because the HA is an inorganic component in human bone providing biocompatible and bioactive properties [15-18].

In addition, the demineralized bone matrix (DBM) is a popular material for scaffolds due to promoting osteoinduction on the scaffold. The DBM is made from decalcification of cortical or spongy bone with acidosis until 2% calcium, collagen type I, and growth factor remains. The DBM approved and classified as a medical device by Food and Drug Administration (FDA) is an alternative allograft and biocompatible material for bone regeneration [19-21]. Previous studies demonstrated successful bone union in 4-6 months after the DBM bone reconstruction. Additionally, the bone scaffold combined with the DBM could promote cell proliferation through osteoinduction and osteoconduction [22-24]. Recently, the DBM mixed HA with a 1:3 ratio explored the high significance of osteogenesis after implantation [25]. Moreover, the appropriate HA concentration for osteogenesis scaffold ranges from 20-50 % by weight. According to the architecture, interconnected pores of 300-500 µm, 30-50 % porosity, and pattern with 0°-90° orientations are suitable for osteogenesis scaffolds [26-36]. A previous study found that the patterns with 0°-90°, 0°-60°-120°, and 0°-45°-90°-135° orientations showed insignificance in the cell proliferation. They suggested that bone geometry influenced mechanical strength more than cell growth [37].

The synthetic bone in MOWHTO should have the same mechanical strength as the target bone which is a proximal part of the tibial cancellous bone. There is compressive strength ranging from 6-10 MPa. Inadequate mechanical strength leads to critical gab size and instability which relates to the delayed bone union process [38]. The scaffold should be concerned with material concentrations and structure according to mechanical compression. Previous research showed that increasing the HA concentrations on PCL demonstrated increased compressive strength in the bone scaffold [39]. Likewise, the increasing HA concentrations explored decreased the mechanical strength [40-41]. In addition, a popularly architectural scaffold is 0°-90° orientation due to its high strength and easy manufacturing [37, 42-44]. However, the effect of DBM-HA combined with PCL concentrations on the compressive strength of bone scaffold substituted in MOWHTO remains inconclusive. Therefore, the study focuses on the compressive strength of bone scaffold with a 1:3 ratio of DBM-HA mixed PCL among 3 concentrations including 20/80, 30/70, and 40/60 % wt/wt. DBM-HA/PCL. The best concentration providing the highest compressive strength is then utilized to find the mechanical compression of osteogenesis scaffold with 300-500 µm pore size, 30-50 % porosity, and pattern with 0°-90° orientations for applying with the MOWHTO.

2. MATERIALS AND METHODS

In our study, the 3D bone scaffold mimicking had 4 processes: (i) image processing (scaffold design), (ii) materials preparation, (iii) printing, and (iv) mechanical testing. All procedures will be described below. The architectural scaffold is represented in Figure 2.

2.1 Scaffold design

Rectangular specimen preparation

SolidWorks generated the 6×6×12 mm (width×length×height) solid rectangle block for compression test in varied material proportions (Figure 3). The solid block was sliced with the Perfactory RP program before printing with the bio-plotter. The program generated the layer for 3D printing. The distance between layers is 0.64 mm following the strut dimension.

Cubic specimen preparation

SolidWorks was used to build the STL file of a cubic with dimensions of 10×10×10 mm for compressive testing in structural (0°-90°) orientations. After that, the Perfactory RP software cut the block into 16 layers, with 0.64 mm spacing between each layer before printing (Figure 4). A cubic specimen is depicted in Figure 3. The cubic scaffold had a 300-500 µm pore size and 49 % porosity. The porosity of the 0°-90° orientations scaffold was estimated from equation (1) [45].

\[
\text{Porosity} = \frac{\left(\frac{F_{\text{dia}} \times N_1 \times L_2}{2}\right) \pi \times L \times N_1 \times N_2}{\left(\frac{F_{\text{dia}} \times N_1 \times L_2}{2}\right)} \times 100 \tag{1}
\]
where \( F_{\text{dia}} \) is the diameter of the filament (mm). 
\( N_l \) is the number of layers. 
\( N_f \) is the number of filaments per layer. 
\( L \) is the length of the scaffold.

### 2.2 Material Preparation

Bio-inks for 3D bioprinting were combined with 3 components: (i) the 3 mm PCL pellet (EnvisionTEC PCL 45K RG, Germany, \( M_w = 90,000 \text{ g/mol} \)), (ii) HA powder (CN Lab Nutrition, China, 50 µm particles), and (iii) the 80-100 µm particles DBM powder (Bone & Tissue Bank, King Chulalongkorn Memorial Hospital, Faculty of Medicine Chulalongkorn University, Bangkok, Thailand). The DBM particles were synthesized from the radius and tibia in human bone following King Chulalongkorn Hospital protocols. The DBM and HA were combined with a constant 1:3 ratio. The experiment materials are separated into three distinct groups based on concentrations of 20/80, 30/70, and 40/60 % wt./wt. DBM-HA/PCL. The materials were prepared by melting DBM-HA and PCL at 80-100 °C with magnetic stirring until visibly homogeneous and cut into 2 mm granules in each group [25]. Table 1 describes the material concentrations in three groups.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>% wt./wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>DBM</td>
<td>HA</td>
</tr>
<tr>
<td>20/80</td>
<td>5</td>
</tr>
<tr>
<td>30/70</td>
<td>7.5</td>
</tr>
<tr>
<td>40/60</td>
<td>10</td>
</tr>
</tbody>
</table>

### 2.3 Scaffold fabrication

The bio-plotter (EnvisionTEC™, Bioplotter®, Germany) is used to fabricate the scaffold. The scaffold is printed with an 18G dispensing needle tip, a heated cartridge at 100°C, and a 29°C of the platform. The characteristic of scaffold and printing parameters are shown in Table 2. Figure 5 showed the 3D bio-plotter.

<table>
<thead>
<tr>
<th>Characteristic scaffold</th>
<th>Nonporous structure</th>
<th>Porous structure (0°-90° orientation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pore size (µm)</td>
<td>-</td>
<td>400-500</td>
</tr>
<tr>
<td>Porosity (%)</td>
<td>-</td>
<td>48%</td>
</tr>
<tr>
<td>Filament diameter (mm)</td>
<td>0.8</td>
<td>0.8</td>
</tr>
<tr>
<td>Dispensing velocity (mm/min)</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Pressure (psi)</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Temperature platform (°C)</td>
<td>25-30</td>
<td>25-30</td>
</tr>
<tr>
<td>Humidity (°C)</td>
<td>35</td>
<td>35</td>
</tr>
</tbody>
</table>

### 2.4 Compression Test

The compressive strength test was measured by the ComeTech™ Universal Testing Machine (UTM) which controlled a continuous compression speed at 1 mm/min and applied with 1 kN according to ASTM D695-15. The compressive testing is evaluated until the strain reached 50%. The compressive stress and strain are calculated by equations (2) and (3). The young’s modulus \( (E) \) is evaluated from the slope at the initial phase of the stress-strain curve and calculated by equation (4).

\[
\text{Stress (}\sigma\text{)} = \frac{F}{A} \quad (2)
\]

\[
\text{Strain (}\varepsilon\text{)} = \frac{\Delta L}{L} \quad (3)
\]

where \( F \) is the applied force (N). 
\( A \) is the cross-sectional area (m²). 
\( \Delta L \) is the change in length (mm). 
\( L \) is the original length (mm).
where $\sigma$ is the compressive stress (N/m$^2$), 
$\varepsilon$ is the strain (mm/mm).

$$\text{Young's modulus (E)} = \frac{\sigma}{\varepsilon}$$ (4)

Figure 5. The 3D bio-plotter

### STATISTICAL ANALYSIS

The SPSS® program (PASW Statistics for Windows, Version 18.0, Chicago) was used to test the statistical analysis. Shapiro-Wilk test was performed for the evaluation of the data distribution of compressive stress and a compressive modulus. Kruskal-Wallis test was performed to compare compressive stress among three groups and the Mann-Whitney U test for comparison between groups. One-way ANOVA test with Sheff’s Post hoc test was performed for comparison compressive modulus. Statistical significance was defined as a $p$-value below 0.05.

### 3. RESULT AND DISCUSSION

A solid rectangular scaffold was constructed with 18 layers of 0.8 mm strands dimension. The sample was composed of 6 pieces in each group ($n = 6$). The compressive strength of 20/80, 30/70, and 40/60 % wt./wt. DBM-HA/PCL concentrations are shown in Figure 6. The compressive modulus of 20/80, 30/70, and 40/60 % wt./wt. DBM-HA/PCL concentrations are represented by the stress-strain curve. Figure 7 shows the compressive modulus.

The Shapiro Wilk test indicates that the abnormal distributions were significant for 20/80 % wt./wt. DBM-HA/PCL scaffold ($W = 0.827, p < 0.001$), 30/70 % wt./wt. DBM-HA/PCL scaffold ($W = 0.866, p < 0.001$), and 40/60 % wt./wt. DBM-HA/PCL scaffold ($W = 0.807, p < 0.001$). On the non-parametric analysis, the compressive stress is reported with the median and interquartile ranges (Mdn, Q1-Q3). According to a Kruskal Wallis test, the variation in DBM-HA mixed PCL concentrations significantly affected the mechanical compressive stress on the bone scaffold (H (2) = 165.65, $p < 0.001$), with a mean rank of 179.50 for 20/80 % wt./wt. DBM-HA/PCL, 95 for 30/70 % wt./wt. DBM-HA/PCL, and 47 for 40/60 DBM-HA/PCL scaffolds. A Mann-Whitney test indicated that the significant statistic was demonstrated in all paired groups: (i) U (N20/80 % wt./wt. = 72, N30/70 % wt./wt. = 72) = 0.000, Z = -10.36, $p < 0.001$, (ii) U (N20/80 % wt./wt. = 72, N40/60 % wt./wt. = 71) = 0.000, Z = -10.32, $p < 0.001$, and (iii) U (N30/70 % wt./wt. = 72, N40/60 % wt./wt. = 71) = 786.00, Z = -7.15, $p < 0.001$. As a result, the compressive stress of DBM-HA/PCL concentrations with 20/80 % wt./wt. (23.25 MPa, 22.39-24.11 MPa) > 30/70 % wt./wt. (17.72 MPa, 15.76-18.76 MPa) > 40/60 % wt./wt. (14.64 MPa, 13.62-15.76 MPa). From the finding, the low DBM-HA concentrations in the PCL group show the high compressive stress and the 20/80 % wt./wt. providing the maximum stress.

The normal distribution of compressive modulus among the 3 groups was performed by the Shapiro Wilk test for 20/80 % wt./wt. DBM-HA/PCL scaffold ($W = 0.943, p = 0.683$), 30/70 % wt./wt. DBM-HA/PCL scaffold ($W = 0.878, p = 0.259$), and 40/60 % wt./wt. DBM-HA/PCL scaffold ($W = 0.912, p = 0.452$). The one-way ANOVA and Sheff’s Post hoc tests were used to compare the three groups. An analysis of variance showed that the effect of the DBM-HA concentrations in the PCL scaffold on the compressive modulus was a significant difference, $F (2,15) = 43.65, p < 0.001$. Afterward, Post hoc comparisons were conducted with the Scheffé test indicating that the mean modulus (M) for all groups was significantly different in the various DBM-HA concentrations and the highest modulus was shown in 20/80 % wt./wt. DBM-HA/PCL scaffold ($M = 157.63$ MPa, SD = 16.54), 40/60 % wt./wt. DBM-HA/PCL scaffold ($M = 118.80$ MPa, SD = 16.54), and 30/70 % wt./wt. DBM-HA/PCL scaffold ($M = 98.19$ MPa, SD = 8.70) respectively.

According to the results, the maximum compressive stress and modulus of the 20/80 % wt./wt. DBM-HA/PCL (23.25 MPa and 157.63 MPa) is consistent with previous research. Lu et al. [46] exhibited the ultimate compressive strength of 20/80 % wt./wt. HA/PCL was 25.8 ± 1.1 MPa. They found that incorporation with 20/80 % wt./wt. HA/PCL concentrations showed the most compressive young’s modulus and hardness of the HA/PCL scaffolds. Kim et al. [47] & Huang et al. [48] studied the biological and mechanical behaviors on the scaffolds with various HA concentrations (0%, 10%, 15%, and 20%). They discovered that the 20% by weight HA composition showed the highest compressive young’s modulus due to the higher HA concentration giving the high stiffness particle dispersion on polycaprolactone, which enhanced the scaffold’s strengthening. Additionally, Choi et al. [49] proposed that the increased inorganic particle in HA on PCL scaffolds significantly improved the mechanical and biological properties of the scaffolds by inducing cell attachment and rigidity. Moreover, the decreased compressive stress and modulus were demonstrated in 30/70 % wt./wt. DBM-HA/PCL (17.72 MPa and 118.80 MPa) and 40/60 % wt./wt. DBM-HA/PCL (14.64 MPa and 98.19 MPa) because of a decrease in PCL concentrations in both groups which resulted in poor mechanical compression.
In comparison to previous studies, several groups observed that a large amount of polycaprolactone (PCL) concentrations not only provided loading support reinforcement but also reduced brittleness [40–41, 50]. Furthermore, previous studies explored that over 40 percent weight HA concentrations showed the weakening compressive strength of the scaffolds. They gave the reason that the excessive HA concentrations resulted in poor mechanical strength due to the bonding interruption of PCL chains leading to very brittle [5, 51–52]. Our finding suggested that the 20/80 % wt/wt. DBM-HA/PCL providing the highest mechanical compression is the optimal concentration for utilizing bone scaffold construction [53].

![Figure 6](image1.png)

**Figure 6.** Average of compressive stress was measured at 3 of strain (mm/mm) and compared among difference concentrations of the DBM-HA/PCL (n = 6 of each group). The box graph represented the interquartile of compressive strength and statistical analysis (The Kruskal-Wallis test and a Mann-Whitney test: * p < 0.05).

![Figure 7](image2.png)

**Figure 7.** Average of compressive modulus was measured by sloped of stress-strain curves and compared among difference concentrations of the DBM-HA/PCL (n = 6 samples of each group).

<table>
<thead>
<tr>
<th>Strain (mm/mm)</th>
<th>0°-90° orientations scaffold (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Compressive stress (MPa)</td>
</tr>
<tr>
<td></td>
<td>Mdn</td>
</tr>
<tr>
<td>0.05</td>
<td>2.90</td>
</tr>
<tr>
<td>0.1</td>
<td>6.77</td>
</tr>
<tr>
<td>0.15</td>
<td>10.00</td>
</tr>
<tr>
<td>0.2</td>
<td>11.28</td>
</tr>
<tr>
<td>0.25</td>
<td>12.72</td>
</tr>
<tr>
<td>0.3</td>
<td>13.81</td>
</tr>
<tr>
<td>0.35</td>
<td>14.75</td>
</tr>
<tr>
<td>0.4</td>
<td>16.03</td>
</tr>
</tbody>
</table>

![Figure 8](image3.png)

**Figure 8.** (a) Compression testing with solid and 0°-90° orientations scaffold and (b) A 0°-90° orientations scaffold with 500 µm pore dimension fabricated by the 3D bio-plotter in top and front views.

Ideally, the MOWHTO synthetic bone must be concerned with biological and mechanical properties. Biological functions represent the bone cell growth within the scaffold. Thus, the MOWHTO DBM-HA/PCL scaffolds were constructed with a 300-500 µm pore size, 30-50% porosity, and a pattern with 0°-90° orientations, which the designs allow osteogenesis within the scaffold. In addition, the 20/80 % wt/wt. DBM-HA/PCL was utilized as material to fabricate the scaffold since it provided the highest compressive strength among the three groups. Figure 8 depicts the scaffold's architectural design. Typically, the scaffold should have mechanical properties like natural bone and the target region to avoid delayed bone union process from minimal critical size deflection. Several studies showed that the 1.5-2 mm bone gap size allowed for the normal bone healing process while the over 4 mm defect size caused a delayed bone union process [4, 54-55]. Thus, the compressive strength was evaluated as less than 0.4 mm/mm of the strain. According to the Shapiro-Wilk test, the compressive
stress of the MOWHTO scaffold showed an abnormal distribution (W = 0.950, p < 0.001), and it was represented in the median and interquartile ranges. The compressive modulus had a normal distribution (W = 0.967, p = 0.856) and was shown in the mean and standard division. The compressive strength and modulus of the MOWHTO scaffold are represented in Table 5.

In previous studies, the compressive stress and modulus in human cancellous bone have ranged from 4-12 MPa and 20-500 MPa [57-58]. Additionally, the compressive stress in the tibia trabecular bone ranged from 5.7-7.7 MPa [59]. Moreover, the stress distribution in bone after medial open wedge high tibia osteotomy ranged from 5-15 MPa depending on fixation types [60-61]. Besides, the ranges of average young’s modulus in tibia cancellous bone are 40.12-84.92 MPa [62]. According to the finding, the 0°-90° orientations scaffold with 20/80 % wt./wt. DBM-HA/PCL had mechanical compression within the mechanical compression ranging (2.90-16.03 MPa, 70.92 MPa of compressive stress and modulus). However, the osteogenesis scaffold should be considered compressive stress at 0.1-0.15 mm of strain because it results in instability and a narrowing vertical pore dimension after implantation if the scaffold has an excessive strain, and leads to a limitation of the bone union process and bone growth within the scaffold.

From the findings, the scaffold can be used for substitution in the MOWHTO with the tomofix plate because the plate provided the lowest compressive stress on the tibial cancellous bone [61]. Moreover, the scaffold with 20/80 % wt./wt. DBM-HA/PCL is necessary to improve mechanical compression because it cannot reach the maximum compressive stress of the tibial cancellous bone after the MOWHTO with other fixations.

To improve mechanical compression, a decrease in pore size and porosity may be a strategy for enhancing the modulus in the MOWHTO scaffolds. Several studies discovered that the increased porosity resulted in poor mechanical strength and decreased compressive modulus [44, 52, 63-64]. Moreover, poor compressive strength was shown in the scaffold with a large pore size [28, 37]. However, the study had numerous drawbacks. Firstly, our study focused on the mechanical compression of the scaffold among three groups of different DBM-HA concentrations in which the DBM-HA is less than the PCL percent by weight. There are undetermined mechanical compression findings of different PCL concentrations in which the DBM-HA is higher than the PCL percent by weight, maybe providing greater mechanical compression because of the influence of high stiff particle concentrations. Secondly, our examination provided the data on mechanical properties without biological properties including cell survival, osteoconduction, and osteoinduction which are important to the scaffold development in the tissue engineering field. Thirdly, the finding represented only the mechanical compression in the 0°-90° orientations which other patterns maybe show better strength. The mechanical simulation will be used in future research to provide the compressive stress of the MOWHTO scaffold.

To improve our studies, we will investigate the mechanical compression (concentrations ranging from 20/80, 50/50, and 80/20 % wt./wt. DBM-HA/PCL) and the biological properties (the cell surviving, osteoconduction, and osteoinduction) to determine the optimal concentration providing the highest compressive stress and biological properties.

4. CONCLUSION

This study provides information on the mechanical compression of the difference in DBM-HA/PCL concentrations for the bone tissue engineering field and scientists to improve alternative bone synthesis. According to our study, the various ratios of biphasic scaffolds (DBM-HA/PCL) were successfully fabricated with the bio-ploter. The over concentrations of inorganic particles caused scaffold weakening by PCL bonding interruption and easy brittle of high Ceramic compound. The 20/80 % wt./wt. of DBM-HA/PCL scaffold were suitable ratio and the highest mechanical properties (23.25 MPa and 157.63 MPa respectively). The compressive stress and modulus of the 0°-90° orientations scaffold with 20/80 % wt./wt. DBM-HA/PCL had the 2.90-16.03 MPa and 70.92 MPa respectively which is within the range of trabecular tibia bone in MOWHTO. Finally, the designed scaffold with 0°-90° orientations, 300-500 µm pore size, 49 porosities, and 20/80 % wt./wt. DBM-HA/PCL is accessible for alternative synthetic bone for MOWHTO implantation.

5. ACKNOWLEDGMENT

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REFERENCES


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