Morphology-based prediction of elastic properties of trabecular bone samples

FRANCESCA COSMI*

Department of Mechanical Engineering, University of Trieste, Trieste, Italy.

Morphological characteristics of the trabecular structure, identified by micro-tomography, can be quantified by volume fraction and second-order fabric tensors. These parameters have been proved to be related to bone structural properties but the formulations so far developed between volume fraction, fabric and elastic properties are bone specific and the coefficients found for one bone are not directly applicable to other bones. In this work, a general relationship was determined that links volume fraction and Mean Inter cept Length (MIL) to the trabecular structure stiffness as computed by means of numerical models on which compression tests are simulated. Preliminary results obtained for three pig and two rat bone structures show that, for the pooled data set, the model could predict approximately 99% of the variation of the numerically computed elastic moduli.

Key words: cancellous bone, elastic properties, Mean Intercept Length, Cell Method, apparent Young’s modulus

1. Introduction

The behaviour of materials with a complex structure strongly depends on the spatial arrangement of their components. In particular, it is well known that the mechanical behaviour of cancellous bone depends not only on its mineral content but also on the trabecular architecture, which appears to be the main factor responsible for trabecular bone anisot- ropy [1].

Once the trabecular structure has been identified, i.e. by micro-tomography, it is possible to quantify its morphological characteristics such as bone volume fraction, the degree of anisotropy and the second- order fabric tensors [2]–[6]. These parameters have been proved to be related to structural properties such as the trabecular structure stiffness obtained by converting the same images into numerical models on which compression tests are simulated using the Finite Element Method [7]–[9] or alternative methods such as the Cell Method [10], [11].

COWIN [12] developed a framework within which the fourth-order tensors that describe the elastic properties of the material can be related to the second-order fabric tensors that describe bone anisotropy. This relationship has been quantified for several bones using FEM models derived from high-resolution 3D reconstructions of trabecular bone [13]–[16]. Alternative formulations have also been developed and a review can be found in [17]. Unfortunately, the relationships so far developed between volume fraction, fabric and elastic properties are bone specific, and the coefficients found for one bone are not directly applicable to other bones [18].

The aim of this work was to investigate whether a different approach could lead to a general relationship that allows us to predict the differences in elastic properties of trabecular bone structures from morphological anisotropy parameters such as the Mean Intercept Length (MIL).
2. Materials

In order to give maximum generality to this study, samples from different species and anatomic locations were examined: three bone samples extracted from the same pig shoulder in differently loaded sites and two tibial proximal epiphyses explanted from different rats were investigated. During the experiments, animals were handled in accordance with the National guidelines for care and use of laboratory animals.

2.1. Pig samples

The pig bone sample $P1$ was extracted from an in vivo weakly loaded region, while samples $P2$ and $P3$ came from in vivo weight bearing regions of the same pig shoulder. According to the well-known Wolff’s law, different loading conditions give rise to different spatial organizations of the trabeculae within the same bone, so that different structural properties were expected in the samples. The explants were treated with formaldehyde and hypochlorite in order to extract the mineral matrix from the biological tissue and to reduce images distortion. The samples were subjected to micro-CT with synchrotron radiation at SYRMEP beamline (ELETTRA, Trieste, Italy). A subset of these volumes ($200^3$ voxel, $2.8 \times 2.8 \times 2.8$ mm$^3$) was used in the subsequent analyses. The same reconstructed volumes had been used by the author in her previous researches and are described in detail in [10]. The different architectures, due to the different loading conditions, are clearly visible in the 3D reconstructed images of the samples, as shown in figure 1.

2.2. Rat samples

The rats bone explants $R1$ and $R2$ consisted of tibial proximal epiphyses obtained immediately after death from different rats, washed with phosphate buffer, fixed for 7 days with 10% buffered formaldehyde solution and let to dry. Within each bone sample, a cubic portion ($200^3$ voxel, $12.8 \times 1.8 \times 1.8$ mm$^3$) of anatomically and structurally homologous trabecular structure was examined (figure 2). Although these samples have not been previously discussed, details on the procedure followed to ensure this condition are the same as those described in [11].

3. Methods

3.1. Mean Intercept Length

Mean Intercept Length (MIL), evaluated from 3D micro-CT reconstructions, is one of the methods that can be applied to identify trabeculae orientation. MIL is
computed by sending a line through a 2-phase volume and dividing its length by the number of times it crosses one of the phases. By changing the orientation of the test line, a 3D graphic representation of the MIL values along the different directions, called a rose diagram, can be obtained. As an example, the rose diagram – obtained using Quant3D software [19] – of the examined volume of sample R1 is shown in figure 3. The analysis only took a few seconds. The morphological characterization depicted by the rose diagram can be approximated by an ellipsoid or, in an equivalent mode, by a second-order fabric tensor, whose eigenvalues represent the principal axes of the ellipsoid. For the examined volume of sample R1, the effect of this approximation is shown in figure 4.

3.2. Cell Method model

The Cell Method is a numerical method in which the equilibrium equations are directly obtained in a discrete form [20]. Its results are comparable with those obtained with FEM commercial codes and in some cases even better from the accuracy/computational requirements points of view. Without going into the details of the applications developed, which can be found in [21]–[26] and at http://www.dic.units.it/perspage/discretephysics, it can be said the Cell Method is particularly advantageous when discontinuities are present, because discretization and heterogeneities may have similar characteristic lengths.

The model developed for the analysis of trabecular structures, described in detail in [10] and [11], was used to simulate compression tests along the cubic portions axes. The same 812905 tetrahedra cells mesh was used for all the 200³ voxels cubic volumes examined. Material properties in each cell were assigned by scaling the linear elastic base material properties according to the presence/absence of bone phase in the vertexes of tetrahedra. An elastic modulus $E_{base} = 1000$ MPa and Poisson’s coefficient $v_{base} = 0.3$ were assumed as base material properties. Output of the model was the apparent elastic moduli along the three coordinate axes depicted in figures 1 and 2 for the pig and rat bone structures, respectively. Computing time was approximately 2.5 hours/axis.
4. Predictive relationship

The morphological parameters, i.e. bone volume fraction \( V_f \) and MIL values \( H_x, H_y, H_z \) along the coordinate axes, are summarized in table 1. Following the common procedure, the MIL values in table 1 have been normalized so that for each sample \( H_x + H_y + H_z = 1 \).

<table>
<thead>
<tr>
<th>Samples</th>
<th>( H_x )</th>
<th>( H_y )</th>
<th>( H_z )</th>
<th>( V_f )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( P1 )</td>
<td>0.34</td>
<td>0.40</td>
<td>0.26</td>
<td>0.27</td>
</tr>
<tr>
<td>( P2 )</td>
<td>0.33</td>
<td>0.29</td>
<td>0.38</td>
<td>0.42</td>
</tr>
<tr>
<td>( P3 )</td>
<td>0.29</td>
<td>0.34</td>
<td>0.37</td>
<td>0.42</td>
</tr>
<tr>
<td>( R1 )</td>
<td>0.26</td>
<td>0.28</td>
<td>0.46</td>
<td>0.14</td>
</tr>
<tr>
<td>( R2 )</td>
<td>0.28</td>
<td>0.27</td>
<td>0.45</td>
<td>0.14</td>
</tr>
</tbody>
</table>

The apparent elastic moduli of the trabecular structures along the coordinate axes, \( E_{\text{sim}} \), obtained from the simulations, are summarized in table 2. The values reported for the pig specimens are different from those reported in [10] because different elastic properties were assumed for the base material. In fact, the aim of this work was to relate information obtained with MIL to that obtained with numerical models of cancellous bone structures, not of particular pieces of bone. For this reason, the same base elastic properties were used in all the simulations.

<table>
<thead>
<tr>
<th>Samples</th>
<th>( E_x )</th>
<th>( E_y )</th>
<th>( E_z )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( P1 )</td>
<td>116</td>
<td>139</td>
<td>91</td>
</tr>
<tr>
<td>( P2 )</td>
<td>237</td>
<td>209</td>
<td>260</td>
</tr>
<tr>
<td>( P3 )</td>
<td>206</td>
<td>237</td>
<td>257</td>
</tr>
<tr>
<td>( R1 )</td>
<td>21</td>
<td>19</td>
<td>50</td>
</tr>
<tr>
<td>( R2 )</td>
<td>20</td>
<td>18</td>
<td>49</td>
</tr>
</tbody>
</table>

As already stated in the introduction, the general polynomial relationship between elastic properties, volume fraction and fabric tensor was established by COWIN [12] and requires the determination of a number of constants that are not of general value, so that the parameters obtained for one particular bone structure cannot in principle be adopted for another one. Accordingly, normalized Mean Intercept Length values show a poor correlation to the stiffness components computed using the Cell Method model, as shown in figure 5 for the pooled data set. However, it can be observed that these relationships directly link normalized quantities, the MIL eigenvalues, to the stiffness components, which are not normalized.

![Fig. 5. Computed elastic moduli (MPa) vs. MIL normalized values for pooled data set](image)

In this work, a simpler but general relation is proposed, arising from basic considerations.

<table>
<thead>
<tr>
<th>Samples</th>
<th>( E_x )</th>
<th>( E_y )</th>
<th>( E_z )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( P1 )</td>
<td>0.33</td>
<td>0.40</td>
<td>0.26</td>
</tr>
<tr>
<td>( P2 )</td>
<td>0.34</td>
<td>0.30</td>
<td>0.37</td>
</tr>
<tr>
<td>( P3 )</td>
<td>0.29</td>
<td>0.34</td>
<td>0.37</td>
</tr>
<tr>
<td>( R1 )</td>
<td>0.23</td>
<td>0.21</td>
<td>0.56</td>
</tr>
<tr>
<td>( R2 )</td>
<td>0.23</td>
<td>0.21</td>
<td>0.56</td>
</tr>
</tbody>
</table>

Table 3 reports the values of apparent elastic moduli, normalized so that for each sample \( E_x + E_y + E_z = 1 \). In figure 6, the normalized computed elastic moduli \( E_i \) vs. the MIL normalized values \( H_i \) along the same directions for the pig and rat bone structures, respectively, are compared. In the rat structures, for which the direction of the z-axis is the predominant load direction, a transversely isotropic behaviour appears and is well pronounced.

The predictive value of normalized Mean Intercept Length for the normalized elastic moduli computed from the Cell Method model is very high for both species, with a correlation coefficient \( R^2 = 0.9895 \) for the pig bone structures and \( R^2 = 0.9852 \) for the rat bone structures. Even more interestingly, the correlation coefficient becomes \( R^2 = 0.9332 \) for the pooled data set if the normalized values of the stiffness \( E_i \) are used instead of the apparent elastic moduli \( E_{\text{sim}} \) (figure 7).
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However, it must be noted that these normalized values are only able to reflect anisotropy properties, that is a preferred orientation of trabeculae coming from morphological or structural computations, and do not reproduce the actual changes in stiffness among the trabecular structures, which instead clearly emerge in the apparent elastic moduli obtained from simulations.

According to [3], it is expected that the apparent elastic modulus depends on the squared volume fraction value so that the simplest formulation that predicts the apparent elastic properties $E_{i\_pred}$ along a Cartesian frame from the normalized values of MIL along the same directions $H_i$ will be given by

$$E_{i\_pred} = KH_i Vf^2,$$

where the value of $K$ can be found by simply imposing that $E_{i\_pred} = E_{\_base}$ when the entire cubic volume is occupied by the base material, so that the volume fraction becomes $Vf = 1$, $H_i = 1/3$ because of isotropy and, substituting,

$$K = 3E_{\_base}.$$  
(1)

Having assumed $E_{\_base} = 1000$ MPa, we obtain $K = 3000$ MPa.

5. Results

The predicted elastic moduli computed by (1), $E_{x\_pred}$, $E_{y\_pred}$, $E_{z\_pred}$ along the coordinate axes, are summarized in table 4.

<table>
<thead>
<tr>
<th>Samples</th>
<th>$E_{x_pred}$</th>
<th>$E_{y_pred}$</th>
<th>$E_{z_pred}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>74</td>
<td>87</td>
<td>57</td>
</tr>
<tr>
<td>P2</td>
<td>176</td>
<td>153</td>
<td>201</td>
</tr>
<tr>
<td>P3</td>
<td>155</td>
<td>180</td>
<td>193</td>
</tr>
<tr>
<td>R1</td>
<td>15</td>
<td>16</td>
<td>27</td>
</tr>
<tr>
<td>R2</td>
<td>17</td>
<td>16</td>
<td>26</td>
</tr>
</tbody>
</table>

In figure 8, the predicted elastic moduli $E_{i\_pred}$ along the coordinate axes vs. the computed elastic moduli $E_i$ along the same directions for the pig and rat bone structures, respectively, are compared. The already noted transversely isotropic behaviour of the rat structures is well reproduced by the elastic moduli predicted by (1).

The predictive value of elastic moduli computed with (1) for the elastic moduli obtained by the Cell Method model for the pig bone structures is higher ($R^2 = 0.9957$) than the one for the rat bone structure ($R^2 = 0.9765$). Despite the extreme simplicity of equation (1), the correlation coefficient is very high for both species and, as shown in figure 9, for the pooled data set ($R^2 = 0.9916$). However, it should be pointed out that these values should be regarded only as indicative, as they have been obtained based on a limited number of samples.
6. Conclusions

The MIL computation on a commercial notebook (Intel Core 2 Duo T5600 1.83 GHZ processor with a 2Gb RAM) requires a few minutes, while numerical methods need several hours for the solution of trabecular bone models. A general relationship that allows the elastic properties of trabecular bone structures to be derived from a morphological anisotropy parameter such as the Mean Intercept Length (MIL) was determined and tested. Preliminary results obtained for three pig and two rat bone structures show that, for the pooled data set, approximately 99% of the variance of the numerically computed apparent elastic moduli can be accounted for by the changes predicted by the formulation proposed. Further research is now needed in order to confirm these findings by a larger number of data and is currently under way.

Acknowledgements

The author wishes to thank Diego Dreossi from Sincrotrone Trieste for his valuable contribution to image acquisition and analyses activities. The author is thankful to Professor Franco Vittur (the University of Trieste) and Professor Giovanna Mazzoleni (the University of Brescia) who provided the samples examined. Patent: PCT WO03/082118 2002, “Method to identify the mechanical properties of a material” deposited by the University of Trieste.

References

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