Development of a biomechanical model of deer antler cancellous bone based on X-ray microtomographic images

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Aims
Finite element (FE) models accurately compute the mechanical response of bone and bone-like materials when the models include their detailed microstructure. The aim of this paper is to develop and validate a biomechanical model for deer antler cancellous bone tissue based on X-ray microtomographic images.

In order to simulate the mechanical behavior under compressive load using a finite element model, images obtained by X-ray microtomography were exported into \textit{Metafor}, which is a non-linear finite element software initially developed at University of Liège for metal forming processes. This software has recently found biomedical applications \cite{1,2}. The ultimate goal is to compare model predictions with the mechanical behavior observed experimentally using the \textit{Skyscan material testing stage} under compression mode.

The creation of the biomechanical model mesh from segmented \textit{µCT} images, its integration into the software \textit{Metafor} and the simulation of a compression test are described in this paper.

Material and methods
The material that has been tested is cancellous tissue of a deer (\textit{Cervus Elaphus}) antler, prepared at the Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Liège \cite{3,4}. Cervid antlers are constituted of bone tissue covered with velvet in the early stage of growth. This bone tissue is composed of a central core of cancellous bone surrounded by a thick outer layer of compact bone (Figure 1). The core cancellous bone, which is analyzed in the present study, presents a cellular structure. A cylindrical deer antler specimen of an apparent volume density of 18.7\% with following dimensions was used: 7.8 mm in diameter and 11.96 mm in height. It was collected before antler casting, during the active growth phase when the antler is still covered by velvet. The sample, made of primary bone tissue, was machined from the core of the antler main beam \cite{3}.
Images of the sample microstructure were acquired using an X-ray microtomography imaging system: the *Skyscan 1172 high-resolution micro CT* (Skyscan, Belgium) [5]. The cone beam source operated at 40 kV and 250 mA. The detector was a 2D, 1048 pixels x 4000 pixels, 16-bit X-ray camera. The distance source-object-camera was adjusted to produce images with a pixel size of 8.64 µm. The rotation step was fixed at 0.4° during the 360° scan. Frame averaging was set at 4 measurements per projection. From the 3D reconstructed images we were able to access data on the sample microstructure. The following structural parameters were determined using the *CTAn* (v.1.10) software (Skyscan, Belgium) [6]: tissue volume (TV), bone volume (BV), bone volume fraction (BV/TV = relative density ρ), percentage of closed and open porosity (Po). These data were used to link the intrinsic properties of the solid matrix with apparent properties of the cellular-type sample. In this work, these structural parameters were used to evaluate the trabeculae Young's modulus of the deer antler, starting from the measurement of the apparent Young’s modulus.

The finite element model used to describe the mechanical behavior of deer antler cancellous bone tissue was developed by the Aerospace and Mechanical Engineering Department from the University of Liège [7]. First, the images obtained by microtomography were segmented and filtered to obtain noise-free images preserving the pores continuity and the trabeculae geometry. This was done using *ImageJ* [8], a free software for image processing and image analysis written by the National Institute of Health (NIH). The following steps were applied to all the 2D reconstructed images: importation of original images in *ImageJ*, segmentation of imported images by thresholding (threshold determined manually = 8), binarization of segmented images, and finally use of the *Minimum Filter* [9] twice to remove artifacts.

The processed images were then introduced in the *Geniso* [10,11] software in order to generate a surface mesh of the solid structure. Three-dimensional images (Figure 2(c)) are provided in the form of successive cuts as shown in Figure 2(b). Initially we considered an anisotropic frame where the slice thickness (86.4 µm) was significantly larger than the cross section pixel size (8.64 x 8.64 µm²). The mesh was then improved using the same resolution in all directions (cubic voxels).

A tetrahedral volume mesh of the structure was finally created in the *TetGen* [12,13] software before being introduced in the finite element software *Metafor* [14] to simulate the deer antler mechanical behavior. The mechanical behavior of cancellous bone tissue under compressive load was assumed to follow Hooke’s law, which was thus used as constitutive law. Provided that the finite element model is built from the sample detailed microstructure, this law is able to capture the structural behavior of bone-like materials [15].
The methodology was validated by comparison of simulated results against experimental data obtained on deer antler samples tested under compression. The experimental compression tests of the cylindrical samples were performed using the Skyscan Material testing stage (Skyscan, Belgium) [16,17], i.e. with a compression cell put inside the microtomograph. The compression tests were performed using a continuous compression mode at constant displacement speed. The data were recorded according to the “load (N) vs. displacement (mm)” mode. Samples were compressed until the maximum stress of the cell (210 N) was reached which corresponds, in this work, to an apparent engineering strain ranging from 0% to 4.1%.

Results and discussion
The objective was to compare the results of finite element calculations performed with the software Metafor (simulation of a compression test on the “meshed” sample) to numerical values found in literature and to experimental results obtained with the compression cell (compression test performed on the “real” sample). To simulate the deer antler mechanical behavior using a finite element model, a volume mesh of the sample and a constitutive law describing its behavior were required.

The constitutive law used to simulate the deer antler mechanical behavior under compressive load is chosen to be Hooke’s law. The mechanical behavior is described by the two material parameters associated to a linear elastic material: the Young’s modulus $E$ and the Poisson’s ratio $\nu$ of trabeculae. Two quantities were compared: the apparent Young’s modulus of the whole sample (measured in the compression cell and computed from FE simulations) and the Young’s modulus of the bone trabeculae in the sample (found in literature and calculated from experimental data).

The successive steps leading to the volume mesh of the deer antler sample are illustrated in Figure 2, starting from images acquisition to volume mesh generation.

Figure 2: Realization of a biomechanical model for deer antler cancellous bone tissue
As explained in the material and methods part, meshes with two refining levels were created. The first surface and volume meshes were performed on a small cubic specimen (201 x 201 pixels, 21 slices) selected inside the cylindrical deer antler sample. The slice thickness (86.4 µm) was ten times larger than the cross section pixel size, so the voxel size was: 8.64 x 8.64 x 86.4 µm³. The final mesh consisted of 32918 nodes and 65590 triangles. The second surface and volume meshes were performed on approximately the same cubic specimen (245 x 245 pixels, 245 slices). In this case, the mesh was significantly improved using the same resolution in all directions (cubic voxel: 8.64 x 8.64 x 8.64 µm³). The final mesh consisted of 66013 nodes and 232859 tetrahedra. Respective characteristics of the two meshes are summarized in Table 1.

Table 1: Dimensions of the first and the second meshes

<table>
<thead>
<tr>
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<th>Mesh 1</th>
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<th>Mesh 2</th>
</tr>
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</tr>
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Evaluation of trabeculae Young’s modulus

Trabeculae Young’s modulus found in literature

According to the literature, the values of Young’s modulus of deer antlers trabeculae range between 5 GPa and 7 GPa, depending on the species [18,19], with most common values of 7.1 GPa et 7.4 GPa [20]. Young’s modulus of trabecular bone, whose the structure and the nature are quite similar to those of deer antler, ranges between 1 GPa and 10 GPa [21,22]. These values may be compared to experimental values measured with the compression cell.

Trabeculae Young’s modulus calculated from experimental data

Values of the trabeculae Young’s modulus may be computed from experimental values of the apparent Young’s modulus, provided that the sample microstructural parameters, obtained from microtomographic images, are taken into account. Indeed, correlations can be found in the literature (Figure 3), allowing to obtain the trabeculae Young’s modulus knowing the apparent Young’s modulus, taking into account the relative density of the sample and the relative percentages of closed and open pores [23,24].

So the application of the correlation presented on Figure 3 required to know the experimental apparent Young’s modulus, information on the topological structure of the sample porosity (closed or open) and its relative density.

The apparent Young’s modulus, i.e. related to the entire sample, can be determined from “stress vs. strain” experimental curve obtained using the compression cell, as shown in Figure 4. The presence of an initial plateau is probably due to an imperfect contact between the compression cell and the upper sample surface. Regarding the two “negative jump” of the stress during loading (“stepped” curve), they illustrate the “relaxation phenomenon” of the deer antler during two stops in the compression test, which correspond to two intermediate periods of scan. Depending on the portion of the experimental curve which is considered to compute the apparent Young’s modulus, values equal to 61.21 MPa (orange curve) or to 37.93 MPa (green curve) were obtained.
Figure 3: Correlation between the ratio of apparent Young's modulus and trabeculae Young’s modulus $E/E_s$, relative density $\rho$ and topology of the material (closed or open cell) [85]

Information on the topological structure of the sample porosity (closed or open) and its relative density were also needed to obtain the trabeculae Young’s modulus. These structural parameters may be directly computed from 3D microtomographic images using the CTAn software. For the tested sample, the percentage of closed porosity is very low (1.6%) and the “open cell” correlation was thus used: $E/E_s = \rho^2$. Regarding to the relative density $\rho$ used in the correlation, this one is equivalent to the “Percent Bone Volume” (BV/TV) and for the tested sample, this value was equal to $\rho = 0.0887$ (8.87%).

The experimental values of the apparent Young’s modulus (37.93 MPa or 61.21 MPa) and the sample relative density (8.87%) were then used to calculate the trabeculae Young’s modulus $E_s$, considering an “open cell” pore topology. The “experimental” values of trabeculae Young’s modulus are equal to: $E_s = 4.8$ GPa if one considers $E = 37.93$ MPa and $E_s = 7.8$ GPa if one considers $E = 61.21$ MPa.
These “experimental” values of trabeculae Young’s modulus (4.8 GPa and 7.8 GPa), are in very good agreement with values reported in the literature, ranging between 5 GPa and 7 GPa (see above).

**Finite element simulation of the mechanical behavior**

Several calculations were performed with the FE software *Metafor*, considering different values for Young’s modulus and Poisson’s ratio of bone trabeculae. Since the bone trabeculae Young’s modulus values reported in literature ranged between 1 and 10 GPa, these two extreme values were tested. Simulations were also performed considering the minimum (4.8 GPa) and the maximum (7.8 GPa) values of trabeculae Young’s modulus obtained from the experiments, using specific correlations. Regarding the Poisson’s ratio of bone trabeculae, the theoretical value 0.3 was tested.

The simulations of compression tests in *Metafor* were successively performed considering three different loading systems (boundary conditions) to achieve a total strain of 4.1%: displacement imposed on the upper surface of the sample via a plan (case 1, Figure 5(a)) or imposed on the upper (case 2, Figure 5(b)) or lower surface (case 3, Figure 5(c)) of the sample via a certain percentage of nodes belonging to the structure itself (2%). These three modes of loading were tested on the first two meshes. In all cases, the cubic sample was assumed initially at rest. All the values of the apparent Young’s modulus computed from FE simulations of the compression test are reported in Table 2.

![Figure 5](image)

**Figure 5**: Displacement imposed on the upper surface of the sample via a plan (a) or on the upper (b) or lower (c) surface of the sample via a certain percentage of nodes belonging to the structure itself (2%). Compression settings: mesh 2, \( E_{\text{trabeculae}} = 7.8 \) GPa, \( \nu_{\text{trabeculae}} = 0.3 \)

Results presented in Table 2 show that values of the “equivalent *Metafor*” apparent Young’s modulus calculated in the first loading mode (case 1) are slightly lower than in other loading modes (cases 2 or 3). This can be explained by the fact that the plan “does not adhere” completely to the sample’s surface. There are thus fewer contact points than when a displacement is imposed via a set of nodes to the rest of the sample.

We can also notice that values of the apparent Young’s modulus calculated in case 3 (433 MPa and 116 MPa) are slightly lower than the one calculated in case 2 (453 MPa and 120 MPa). This can be explained by the slight non-homogeneity of the deer antler sample. Indeed, the stress imposed by 2% of the nodes belonging to its upper (b) or lower (c) surface will not lead to the same displacement of the sample since the initial contact surface \( S \) (in \( \sigma = F/S \)) is not identical from one case to another.
Table 2: “Equivalent Metafor” apparent Young’s modulus [MPa] calculated according to different compression settings

<table>
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<th>Mesh</th>
<th>Loading mode</th>
<th>$E_{\text{trabecula}}$</th>
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<th>$E_{\text{Metafor}}$</th>
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Comparison and validity of the FE simulation

If results reported in Table 2 are compared to experimental data (37.93 MPa or 61.21 MPa), it appears that the values of the apparent Young’s modulus computed from FE calculations are all higher than the experimental ones. For the first mesh, the “equivalent Metafor” apparent Young’s modulus (453 MPa or 281 MPa) are seven times higher than the experimental ones, while the second mesh leads to “equivalent Metafor” apparent Young’s modulus only two (116 MPa) or three (101 MPa) times higher than the experimental ones. Generating a mesh from a 3D segmented image with the same resolution in all directions thus made it possible to calculate an apparent Young’s modulus closer to the experimental one.

These overestimated values could either be due to erroneous experimental values or to a poor quality of the mesh in Metafor. But they are most probably due to the fact that the mesh used in finite element calculations represents only a very small portion of the whole deer antler sample. Indeed, volumes of mesh 1 (5.49 mm$^3 = 0.25\%$) and mesh 2 (9.49 mm$^3 = 0.45\%$) both represent less than 1% of the total volume of the initial cylindrical sample (2121 mm$^3$). Since the size of the subsample meshed does not allow representing the behavior of the whole sample, the orders of magnitude obtained by simulation are not aberrant. One solution would be to mesh a larger sample, reaching the size of the so-called “representative elementary volume” or a large number of subsamples to be able to simulate an “average” mechanical behavior of the sample. This approach would allow us to understand even better the relationship between mechanical properties and the microstructure of cellular materials.

Conclusion

A methodology for microtomographic image processing allowing generating a good quality surface mesh of deer antler cancellous bone tissue was proposed. The volume mesh created from this surface mesh was used to simulate the mechanical behavior under compression of deer antler samples using a finite element method. In the FE model an elastic behavior of trabeculae was considered. Compared to the experimental ones, simulated values of the apparent Young’s modulus are not aberrant according to the fact that less than 1% of the total volume of the initial sample was meshed. Regarding the trabeculae Young’s modulus, values obtained
from the literature or determined from the experimental apparent Young’s modulus, taking into account the relative density of the sample and the relative percentages of closed and open pores were compared during the simulations. Values computed from the FE simulations are in very good agreement with ones reported in the literature. The proposed approach is thus validated as it allows modeling the mechanical properties of a sample under compressive load and to calculate Young’s modulus value of its trabeculae by combining experimental and structural data.

References:


