Materials Science and Engineering C 54 (2015) 207–216

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Experimentally-based multiscale model of the elastic moduli of bovine trabecular bone and its constituents

Elham Hamed [a,1], Ekaterina Novitskaya [b,*], Jun Li [a,2], Iwona Jasiuk [a], Joanna McKittrick [b]

[a] University of Illinois at Urbana-Champaign, Department of Mechanical Science and Engineering, 1206 West Green Street, Urbana, IL 61801, USA
[b] University of California, San Diego, Department of Mechanical and Aerospace Engineering, Materials Science and Engineering Program, 9500 Gilman Dr., La Jolla, CA 92093, USA

ARTICLE INFO

Article history:
Received 21 October 2014
Received in revised form 14 January 2015
Accepted 24 February 2015
Available online 11 March 2015

Keywords:
Trabecular bone
Elastic moduli
Multiscale modeling
Demineralization
Deproteinization
Micro-computed tomography

ABSTRACT

The elastic moduli of trabecular bone were modeled using an analytical multiscale approach. Trabecular bone was represented as a porous nanocomposite material with a hierarchical structure spanning from the collagen–mineral level to the trabecular architecture level. In parallel, compression testing was done on bovine femoral trabecular bone samples in two anatomical directions, parallel to the femoral neck axis and perpendicular to it, and the measured elastic moduli were compared with the corresponding theoretical results. To gain insights on the interaction of collagen and minerals at the nanoscale, bone samples were deproteinized or demineralized. After such processing, the treated samples remained as self-standing structures and were tested in compression. Micro-computed tomography was used to characterize the hierarchical structure of these three bone types and to quantify the amount of bone porosity. The obtained experimental data served as inputs to the multiscale model and guided us to represent bone as an interpenetrating composite material. Good agreement was found between the theory and experiments for the elastic moduli of the untreated, deproteinized, and demineralized trabecular bone.

1. Introduction

Bone is a hierarchically structured biological material composed of an organic phase, inorganic phase, and water. The organic phase (32–44 vol.%) contains a type-I collagen and a small amount of non-collagenous proteins (NCPs). Hydroxyapatite minerals form the inorganic phase (33–43 vol.%) [1]. Water occupies about 15–25 vol.% of the bone material. There are two bone types: cortical (compact, dense) and trabecular (cancellous, porous). The cortical bone is a dense tissue (porosity <10 vol.%), which forms a protective shield around the more porous (porosity ~70–90 vol.%) trabecular bone. Different levels of hierarchy of trabecular bone, including nanoscale, sub-microscale, microscale, and mesoscale, were discussed in detail in our previous study [2].

Mechanical properties of trabecular bone have been extensively studied using various theoretical and experimental approaches. At the nanoscale, the mineralized collagen fibril was modeled either as a matrix-inclusion composite material consisting of collagen and hydroxyapatite crystals [3–6] or as an interpenetrating composite built up by the two phases [2,5,7]. The collagen–mineral interactions were also investigated using computational methods, including a finite element method (FEM) [8–12] and molecular dynamics simulations [13–15]. Modeling of bone at the nanoscale is reviewed in [12]. At the sub-microscale, a single lamella was modeled analytically [2,7,16,17] or computationally [12,18] as a network of mineralized collagen fibrils including lacunar cavities. Elastic properties of a single lamella were also measured by a nanoindentation technique [19–24]. At the microscale, various experimental techniques including a microtensile test [25–27], bending test [28,29], and ultrasound [24,26,30] were used to estimate the elastic properties of a single trabecula. Some studies measured the Young's modulus of trabecular bone experimentally and used that data to back-calculate the elastic constants of trabecular bone tissue [31,32]. At the mesoscale, the majority of analytical studies modeled trabecular bone as a cellular foam and expressed its Young's modulus by power law relations in terms of bone density [33–38]. Other researchers took into consideration the density as well as the fabric tensor [39–41], which characterizes the structural anisotropy of bone. Since the trabecular bone architecture, including thickness, number, separation distance, and connectivity of trabeculae, affects bone's mechanical response, high-resolution imaging techniques such as a micro-computed tomography (μ-CT) have been widely used to characterize the architecture of trabecular bone. Accordingly, many researchers used μ-CT based FEM to model the mechanical behavior of trabecular bone [21,42–47]. Advances in theoretical calculations and experimental measurements of the elastic moduli of trabecular bone were recently summarized in

* Corresponding author.
E-mail address: eevdokim@ucsd.edu (E. Novitskaya).
[1] Now at Northwestern University, Civil and Environmental Engineering Department, 2145 Sheridan Road, Evanston, IL 60208, USA.
[2] Now at California Institute of Technology, Division of Engineering and Applied Science, 1200 E California Blvd, MC 301-46, Pasadena, CA 91125, USA.

http://dx.doi.org/10.1016/j.msec.2015.02.044
0928-4931/

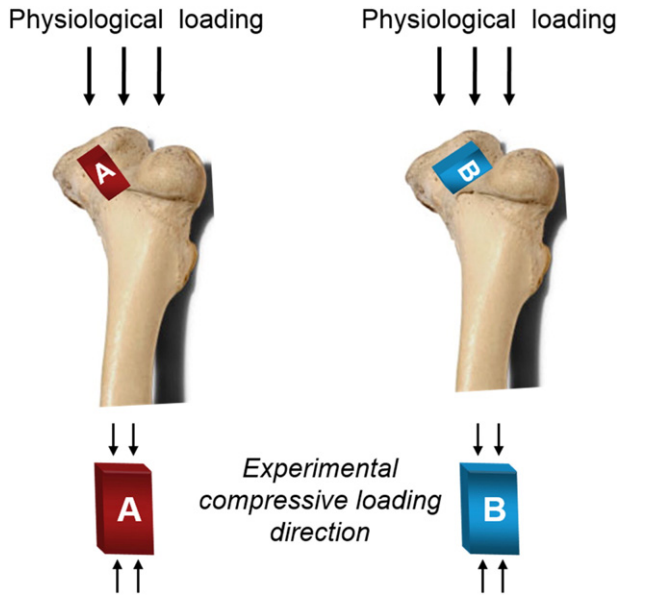

Fig. 1. Schematic illustration of physiological and experimental loadings on a bovine femur head, and the sample orientation for two directions: A and B. The samples are not shown to scale. Image adapted from [81].

[48]. It was illustrated that the complex hierarchical structure of trabecular bone plays a crucial role in the mechanical response and makes the modeling of elastic properties of bone very challenging.

Several recent studies [49–51] demonstrated that the two main constituents of bone (collagen and hydroxyapatite) can be separated by aging bone in hydrochloric acid (demineralization) or sodium hypochlorite (deproteinization) solutions, and their mechanical properties can be examined separately. These studies proposed that bone tissue could be considered as an interpenetrating composite of collagen and minerals.

Despite advances in characterization of bone's mechanical properties, a comprehensive multiscale model that incorporates experimental observations of trabecular bone as an interpenetrating composite material and elucidates the structure–property relations in bone is still absent. As a step toward developing such a model, here, we introduce a multiscale modeling approach, which spans from the nanoscale to the mesoscale, to predict the elastic moduli of trabecular bone, representing the minerals and collagen as interpenetrating phases. The developed model is employed to investigate the elastic behaviors of the untreated (UT), demineralized (DM), and deproteinized (DP) bovine trabecular bone. This is a follow up study to a similar analysis performed on bovine cortical bone, where we demonstrated that the predicted elastic moduli, using a multiscale model of bone as an interpenetrating composite of minerals and collagen, compared well with those obtained experimentally [49]. We further developed ideas generated in a previous study, where preliminary results presented were based on an isotropic model of bone at the mesoscale [52]. In particular, the model outlined here retains the features of our previous study at the nano and submicro scales [49], while different modeling approaches are employed at the micro and meso scales, due to inherent differences in the structure of cortical and trabecular bones at the two latter scales. The obtained modeling results are compared to the experimentally measured elastic moduli of trabecular bone samples tested in compression. Volume fraction, shape, and distribution of porosity at different levels of hierarchy estimated by μ-CT are the inputs to the model. Experimentally-based theoretical models of these three types of bone (UT, DM, and DP) can provide additional insights into the structure and structure–property relations of trabecular bone and allow to fine tune modeling approaches.

2. Materials and methods

2.1. Sample preparation

One trabecular bovine femoral bone from an approximately 18-month-old animal was obtained from a local butcher. Bovine bone was chosen for the current study since it is readily available and is commonly used as a model material for the investigation of bone mechanical properties [53–56]. The bone marrow was carefully removed with water using a water pick. Altogether fifty-six samples (6 mm × 6 mm × 8 mm) were prepared for compression testing. Sample sizes were chosen in accordance with [57], who suggested to use an aspect ratio between 1 and 1.5 for compression tests on trabecular bone samples. The samples were first roughly cut by a handsaw and then precisely with a diamond blade under a constant water irrigation with the surfaces as parallel as possible. Samples were cut in two directions. The direction oriented along the femur neck axis was called as the A-direction, while the direction normal to the A-direction was labeled as the B-direction (Fig. 1). Seventeen UT samples in the A-direction and eleven UT samples in the B-direction were prepared. Samples were stored in closed zip lock bags filled with Hank's balanced saline solution in refrigerator (T = 4 °C) for 1–2 days until chemical procedure and mechanical testing.

Five DM samples in the A-direction and seven DM samples in the B-direction were prepared following the procedures given in [51]. In parallel, four DP samples in the A-direction and four DP samples in the B-directions were prepared following the procedures given in [51]. To avoid additional uncertainties associated with samples from different animals, only one bovine femur bone was used for the current study. Therefore, there was a limitation to the number of samples that could be prepared in the A- and B-directions. The DM and DP processes brought additional challenges to the sample preparation (since DP and DM samples are extremely fragile). Therefore, several samples were excluded from the investigation due to their failure during sample preparation. As a result, there is a difference in the number of samples in the A- and B-directions. All samples were stored in closed zip lock bags filled with Hank's balanced saline solution in refrigerator (T = 4 °C) until mechanical testing.

2.2. Micro-computed tomography characterization

All samples cut in the two directions (A and B) were scanned using Xradia MicroXCT-200 (−400 for DM, due to low X-ray absorption of protein) (Carl Zeiss X-ray Microscopy, Inc., Pleasanton, CA). For each sample the scan generated 729 radiographic images over a range of 182° with a 12 s exposure time for each image and no frame averaging. The raw images were then reconstructed using the Xradia TXMReconstructor software, where ring artifacts and beam hardening effects (BHE) were corrected. For the DP group (hydroxyapatite only), due to the high X-ray attenuation of minerals [58], the BHE were so pronounced that a 3.7-mm-Al beam-flattening filter was placed in the X-ray path during the scan. The reconstructed tomographic images consisted of 1024 slices (1024 × 1024 image pixels per slice) with a resolution of 10 μm and a field of view of roughly 1 cm^3 cube. The stacks of two-dimensional (2D) slices were post-processed in the software Amira 5.2 (FEI Visualization Sciences Group, Burlington, MA) for image visualizations and quantifications of 3D microstructures. No image filtering was used on images of UT samples, while for treated cases (DM and DP) a median image filter with kernel size 3 was applied for noise reduction.

Initially, the 2D tomographic slices were 16 bits gray scale images (0 ~ 65536 from black to white) and, then, they were segmented to black-white binary images for the separation of voids and bone tissues. A global threshold was selected around the middle point between the two peaks of black and white in gray level histograms [59] and verified by an external method of porosity measurements [60].