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Elastic moduli of untreated, demineralized and deproteinized cortical bone: Validation of a theoretical model of bone as an interpenetrating composite material

E. Hamed [a], E. Novitskaya [b], J. Li [a], P.-Y. Chen [b,c], I. Jasiuk [a,*], J. 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 and Materials Science and Engineering Program, 9500 Gilman Dr., La Jolla, CA 92093, USA
[c] National Tsing Hua University, Department of Materials Science and Engineering, 101 Sec. 2 Kuang-Fu Rd., Hsinchu 30013, Taiwan, ROC

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ABSTRACT

A theoretical experimentally based multi-scale model of the elastic response of cortical bone is presented. It portrays the hierarchical structure of bone as a composite with interpenetrating biopolymers (collagen and non-collagenous proteins) and minerals (hydroxyapatite), together with void spaces (porosity). The model involves a bottom-up approach and employs micromechanics and classical lamination theories of composite materials. Experiments on cortical bone samples from bovine femur include completely demineralized and deproteinized bones as well as untreated bone samples. Porosity and microstructure are characterized using optical and scanning electron microscopy, and micro-computed tomography. Compression testing is used to measure longitudinal and transverse elastic moduli of all three bone types. The characterization of structure and properties of these three bone states provides a deeper understanding of the contributions of the individual components of bone to its elastic response and allows fine tuning of modeling assumptions. Very good agreement is found between theoretical modeling and compression testing results, confirming the validity of the interpretation of bone as an interpenetrating composite material.

1. Introduction

Bone tissue is a natural composite material consisting of an organic phase (90% type-I collagen and 10% non-collagenous proteins (NCP)), an inorganic phase (hydroxyapatite-like minerals) and water. On a volumetric basis, mammalian skeletal bone is made up of 32–44% organics, 33–43% minerals and 15–25% water [1]. These constituents assemble into a complex hierarchical structure, which gives bone its excellent mechanical properties [2–4]. In this paper, the hierarchical structure of cortical bone is described in terms of four structural levels (Fig. 1), spanning from nanoscale to mesoscale levels.

Nanoscale (Level I), which ranges from a few to several hundred nanometers, represents a mineralized collagen fibril level. A mineralized fibril has a composite structure made of organic and inorganic phases and water. Type I collagen, which is the major constituent of the organic phase, consists of triple helical tropocollagen molecules which are ~300 nm long [5,6] and ~1.5 nm in diameter [6,7]. These molecules assemble into a staggered arrangement with a periodicity of 67 nm [8,9], which includes gap and overlap regions. The inorganic phase consists of non-stoichiometric hydroxyapatite crystals ($Ca_{10}(PO_4)_6(OH)_2$), with 4–6% of the phosphate groups replaced by carbonate groups. The mineral crystals are in the form of platelets 40–60 nm long, 20–30 nm wide and 2–4 nm thick [10–14]. The remaining phase is water, which plays an important role in bio-mineralization. These constituents are combined into mineralized collagen fibrils (~100–200 nm in diameter [1,15]), which are the primary building blocks of bone. It is generally believed that crystals initially form within the gap regions of the collagen fibrils, further proceed into the overlap regions, and subsequently grow into the extrafibrillar space [16,17]. Consequently, minerals are found both within and outside the collagen fibrils, but the exact amount in each location is still a matter of contention [18–22]. Recent studies [23–25] on completely deproteinized and completely demineralized bones show that both the minerals and protein form continuous phases.

Sub-microscale (Level II), which spans from one to tens of microns, represents a single lamella level. A lamella, with thickness 3–7 μm [10], is made of preferentially oriented mineralized collagen fibrils. At this length scale, the elliptical cavities called lacunae (typically 5–10 μm wide and 15–25 μm long [26,27]) can be observed. Connecting the lacuna are small channels (~100–500 nm in diameter [28]), called canaliculi.

Microscale (Level III), ranging from tens to hundreds of microns, denotes lamellar structures, which are made of lamellae stacked together at different orientations, i.e., the fibrils in each lamella are oriented at a different angle with respect to the adjacent one

* Corresponding author. Tel.: +1 217 333 9259; fax: +1 217 244 6534.
E-mail address: ijasiuk@illinois.edu (I. Jasiuk).

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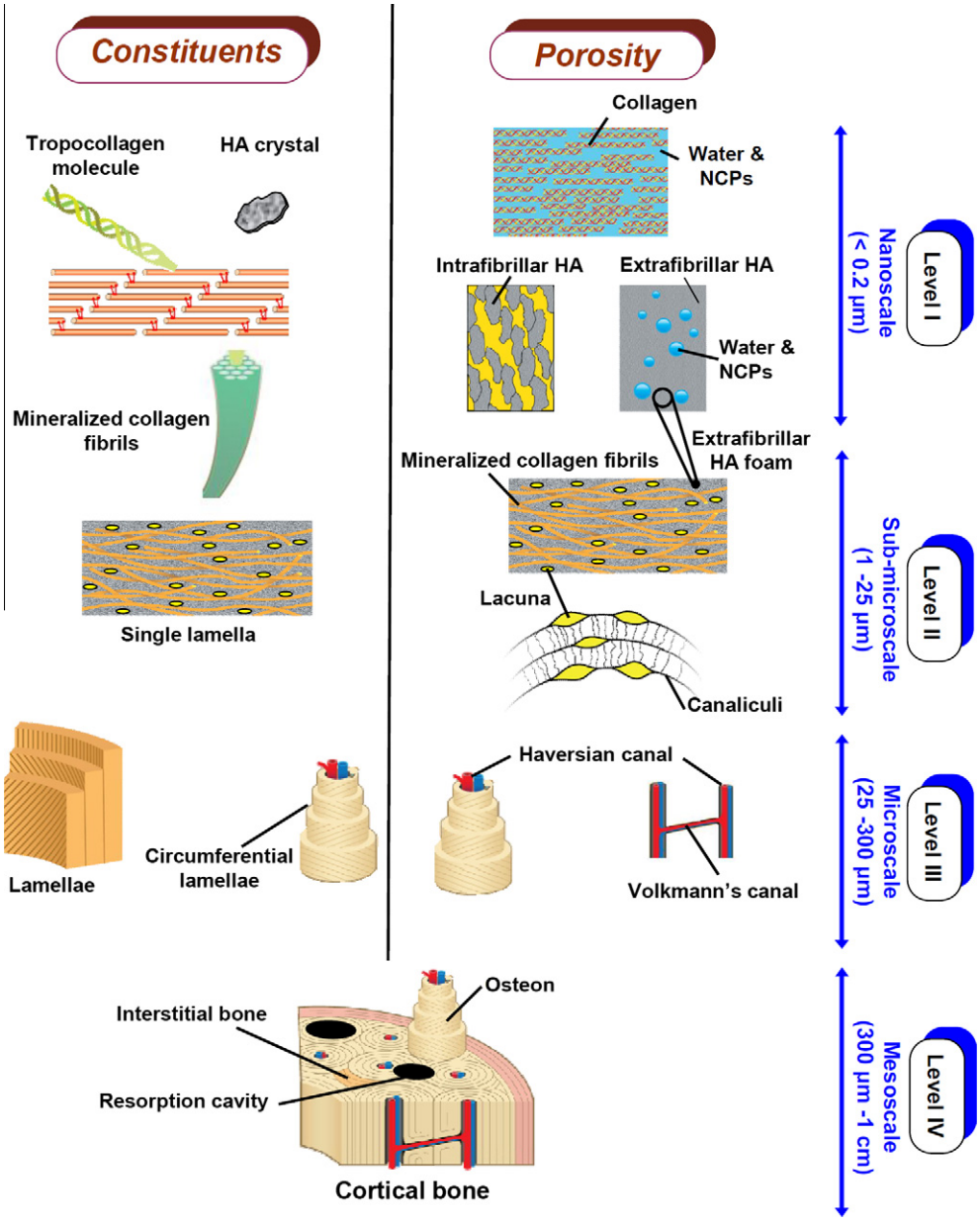

Fig. 1. Hierarchical structure of cortical bone constituents and associated porosity. HA = hydroxyapatite, NCP = non-collagenous proteins.

	Constituents	Porosity
I	At the nanoscale, tropocollagen molecules (~300 × 1.5 × 1.5 nm) and hydroxyapatite minerals (50 × 25 × 3 nm) combine to form the basic unit of all bone—the mineralized collagen fibril (~100 nm in diameter).	Gaps within and between the collagen molecules (~40–100 nm) configure the first level of porosity, where minerals, water, and non-collagenous proteins are deposited.
II	Lamellae (3–7 μm thick) are formed from preferentially oriented mineralized collagen fibrils.	Embedded in the lamellae are lacuna spaces (~25 × 10 × 5 μm) where bone cells reside, connected by small channels (canaliculi).
III	The lamellae form osteons with a central vascular channel (Haversian system, 100–200 μm diameter), the primary feature of cortical bone. The orientation of the mineralized collagen fibrils is different between the adjacent lamellae, which form a twisted plywood structure.	Porosity at this level includes the Haversian canals (~50 μm in diameter), which are oriented longitudinally, and Volkmann's canals (~50 μm in diameter), which are oriented in transverse direction.
IV	Osteonal and interstitial bones with resorption cavities form the cortical bone.	Resorption cavities (~300 μm in diameter), sites of bone remodeling, are the main porosity at this scale.

[29,30]. In cortical bone, several layers of the lamellae, arranged in concentric rings around the vascular channels, form osteons (Haversian system), while interstitial lamellae, which are remnants of old osteons, fill spaces between osteons.

Mesoscale (Level IV), which spans several hundred microns to several millimeters or more, depending on species, represents the cortical bone level. The cortical bone consists of osteons embedded in interstitial lamellae with some resorption cavities.

In order to understand the structure and mechanical properties of bone and its protein and mineral constituents, bone can be demineralized or deproteinized by aging in HCl or NaOCl solutions, respectively. Previous studies on the structure of cancellous and