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ANALYSIS OF TRABECULAR BONE AS A HIERARCHICAL MATERIAL

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Summary We study trabecular bone as a hierarchical material with a highly complex and random structure. First, we characterize trabecular bone's structure at several structural levels: nanoscale (apatite crystals and collagen fibril level), sub-microscale (single lamella level), microscale (single trabecular pocket or single trabecula level), and mesoscale (porous trabecular network level) using atomic, scanning, and transmission electron microscopy, and x-ray microtomography. Then, we model trabecular bone at each structural level as a linear elastic solid employing either classical elasticity or higher order elastic theory (Cosserat theory). Modeling techniques include analytical micromechanics theories and numerical simulations involving finite element, spring network, and beam network approaches. We compare our theoretical results at different scales with our experimental measurements.

INTRODUCTION

In mechanical terms bone is a composite material, consisting of several phases [1]. The main two components are apatite crystals of calcium phosphate that are embedded in collagen fibrils. The apatite crystals are in the shape of irregular platelets, 20-50 nm in length and width and 1-3 nm in thickness. In trabecular bone, these crystals are located either within or around collagen fibrils. The fibrils reinforced with crystals are aligned and arranged in lamellar sheets (3-7 μm in thickness). These are stacked at different orientations and wind around to form randomly oriented cylindrical (or platelike) struts called trabeculae, which are about 0.1 mm in diameter and 1 mm long. This gives trabecular bone a porous appearance. Thus, the trabecular bone has a complex hierarchical structure in which different geometrical features occur on several length scales. These scales are classified as follows: a) nanoscale (single fibril and crystals), b) sub-microscale (single lamella level), c) microscale (trabecular pockets, single trabecula level), and d) mesoscale (random network of struts or plates). The next structural level, macroscale, represents the whole bone, which includes both trabecular (porous) and cortical (solid) bone types. These structural levels are illustrated in Fig. 1.

CHARACTERIZATION AND TESTING OF TRABECULAR BONE TISSUE

We study trabecular bone structure using bone samples from both normal and osteoporotic bones. Osteoporotic samples are selected based on a) known T score at sample site of at least –2.0 (a measure of bone density) or b) calcium ash weight < 0.75 g/cm^3. We also obtain measurements of bone density using DEXA (dual excitation absorptiometry). We study trabecular bone's structure at different structural levels using scanning electron microscopy (SEM), transmission electron microscopy (TEM), and atomic force microscopy (AFM). Photographs, representing a wide range of magnifications (10-50,000), are analyzed by image analysis techniques using Micromorph software. The data collected includes the area fraction and orientation of fibrils at the sub-microscale level, and the porosity, pore size distribution, connectivity of microstructure, and thickness of trabeculae at the microscale level. We also store digitized images of bone structures at each scale for use in numerical simulations. SEM, TEM, and AFM investigations provide us with two-dimensional data. Alternately, we use the x-ray microtomography (Micro-CT), a nondestructive technique, which gives three-dimensional details of bone structure.

Measurements of mechanical properties are done using MTS machine and nanoindentation apparatus. We use the MTS testing machine to determine the constitutive response of bone at mesoscale (5-10 mm samples). We employ the nanoindentation apparatus (Hysitron) to determine bone's mechanical properties at lower scales. Nanoindentation method involves pressing of a tip of nanoindentor into a specially prepared surface. The output is a load-deformation curve from which elastic modulus and hardness can be obtained.

MODELING OF TRABECULAR BONE AS A HIERARCHICAL MATERIAL

Following the characterization and testing of bone, the theoretical mechanics analysis of trabecular bone is conducted. Inputs reflecting structural features at different scales are rectified with our experimental observations obtained by methods described in the previous section. In the analysis, for simplicity, we model bone as a linear elastic solid material and we do not include solid-fluid interactions. We determine material responses, at each structural level defined in the Introduction, either analytically (using micromechanics theories) or numerically (using finite element, spring network, or beam network approaches). Bone's structure is analyzed in an "ascending" order by starting with the nanoscale level.

At the nanoscale level apatite crystals are represented as platelike or ellipsoidal inclusions, which are embedded in collagen fibrils (matrix). First we use micromechanics effective medium theories, for simplicity, and then conduct numerical simulations to account for a discrete (molecular) structure of bone at that structural level. At the next higher up scale (sub-microscale) a single lamella consisting of aligned collagen fibrils reinforced with apatite crystals is modeled using a finite element based beam network approach. At the microscale level, involving a lamellar structure of a single trabecula, we use laminate theory from mechanics of composite materials. The challenges at that scale include curved, crescent like geometry of trabecular pockets which are made of several lamellar layers stacked at different orientations. Also, at that scale bone remodeling takes place and each trabecula undergoes a different stage of bone resorption and formation (remodeling). At the mesoscale level we have a random network of struts or plates in trabecular bone. The trabecular structure poses modeling challenges because of its complex and irregular geometry. Initially, we model this structure by assuming periodicity in the pores' arrangement and we represent this material as a micropolar one. This higher order theory allows us to account for microstructural features such as pore geometry via characteristic lengths. Then, we conduct large scale finite element computations accounting for random structural features of trabecular network. At this scale the inputs on geometry are taken from a Micro-CT digitized data of actual bone samples. Computational results are compared with experimental ones obtained by testing of digitized bone samples. Throughout this work we have studied the structure and properties of trabecular bone and we have focused on the differences between normal and osteoporotic bone at each scale of observation.

RESULTS

We found no distinct structural differences at the nanoscale, sub-microscale and microscale levels between normal and osteoporotic bone using AFM, SEM, and TEM, but observed a depleted trabecular network at the mesoscale level in trabecular bone. The latter observation is in agreement with the data reported in literature, while the information on bone's structure at lower levels is new [2].

In the numerical simulations we took properties of collagen fibrils as E = 1.5 GPa where E was elastic modulus, and ν = 0.3 where ν was a Poisson's ratio. Several models/approaches were used for each structural scale. Our computational prediction at mesoscale level (by bringing inputs from lower levels) was E = 600 MPa for a given osteoporotic trabecular bone sample (cylinder 5 mm in diameter and 5 mm high) while the experimental measurement gave E = 400 MPa.

CONCLUSIONS

We characterized, tested, and modelled trabecular bone as a hierarchical material. Good agreement was found between theoretical and experimental predictions, considering the complexity of bone's hierarchical structure and the idealizations used in modelling of each structural level. The approach taken presents a framework for studying other biological materials with complex hierarchical structures.

References

[1] Park, J.B. and Lakes, R.S., *Biomaterials: An Introduction*, 2nd ed., Plenum Press, NY, 1992.
[2] Rubin, M.A., I. Jasiuk, J. Taylor, J. Rubin, T. Ganey, R.P. Apkarian, *Bone* **33**:270-282, 2003

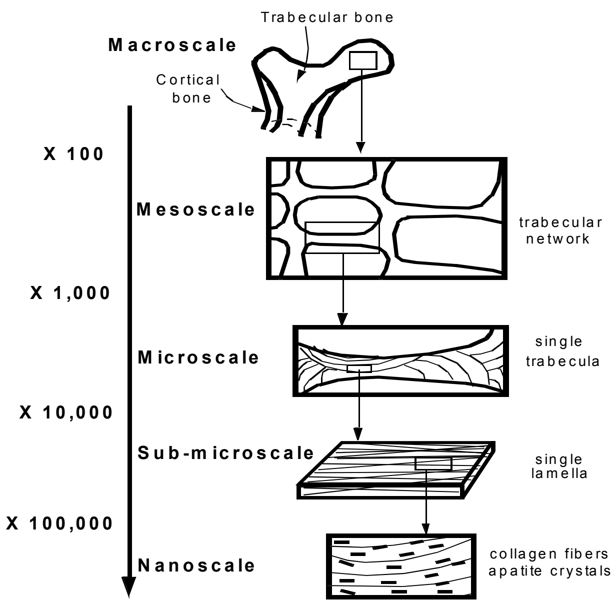

Fig.1 Schematic of a hierarchical structure of trabecular bone.