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Technical Note

Random field assessment of nanoscopic inhomogeneity of bone

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ABSTRACT

Bone quality is significantly correlated with the inhomogeneous distribution of material and ultrastructural properties (e.g., modulus and mineralization) of the tissue. Current techniques for quantifying inhomogeneity consist of descriptive statistics such as mean, standard deviation and coefficient of variation. However, these parameters do not describe the spatial variations of bone properties. The objective of this study was to develop a novel statistical method to characterize and quantitatively describe the spatial variation of bone properties at ultrastructural levels. To do so, a random field defined by an exponential covariance function was used to represent the spatial uncertainty of elastic modulus by delineating the correlation of the modulus at different locations in bone lamellae. The correlation length, a characteristic parameter of the covariance function, was employed to estimate the fluctuation of the elastic modulus in the random field. Using this approach, two distribution maps of the elastic modulus within bone lamellae were generated using simulation and compared with those obtained experimentally by a combination of atomic force microscopy and nanoindentation techniques. The simulation-generated maps of elastic modulus were in close agreement with the experimental ones, thus validating the random field approach in defining the inhomogeneity of elastic modulus in lamellae of bone. Indeed, generation of such random fields will facilitate multi-scale modeling of bone in more pragmatic details.

Introduction

Material properties of bone are inhomogeneous in nature at multiple length scales. At the macroscopic level, material properties of bone are significantly different at various anatomic locations [1,2]. Microscopically, marked variations of elastic modulus in bone lamellae have been observed in osteons by nanoindentation testing [3,4]. In addition, inhomogeneity of mineralization in bone was detected by quantitative backscattering imaging and described by bone mineral density distribution [5,6]. At the nanoscopic scale, variations in the elastic modulus of individual collagen fibrils have also been reported in the literature [7]. It has been well documented that bone inhomogeneity may give rise to the increased bone fragility in terms of energy dissipation at nanoscopic scales [7] and the toughness of bulk bone specimens [6]. Thus, quantification of inhomogeneity becomes necessary in both analytical and experimental research of bone.

Current techniques to quantify the inhomogeneous properties of bone include basic and descriptive statistics such as mean (μ), standard deviation (σ) and coefficient of variation (COV). However, these parameters only give an overall estimate of variations, but not the spatial distribution of tissue properties in bone. The recent advance of nanotechnology has made it possible to experimentally map the distribution of elastic modulus within a single lamella [7]. How to quantitatively analyze and describe the spatial distributions of these properties still remains unsolved. To address this issue, a novel statistical method based on the random field theory was proposed and validated in this study to quantitatively characterize spatial variations of tissue properties of bone at nanoscopic scales.

Materials and methods

Maps of nanoindentation modulus

Nanoscopic inhomogeneity of bovine cortical bone samples was investigated in this study by assessing the spatial distribution of the elastic modulus of lamellae using atomic force microscopy (AFM)-based nanoindentation techniques [7]. In brief, a square region of 2 µm × 2 µm in a lamella was selected randomly within an osteon (Fig. 1). The region of interest was divided in rectangular grids by 100 nm. Nanoindentation measurements in the grids were carried out at a peak indentation force of 5 µN, with the indentation depth being around 50 nm.

Two maps of nanoindentation modulus were obtained on the cross-section perpendicular to the longitudinal axis of two bovine

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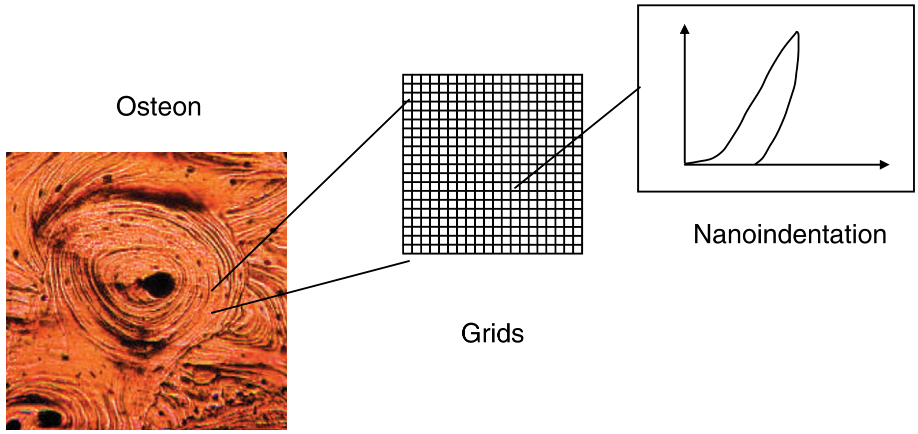

Fig. 1. Schematic of nanoindentation measurements of modulus in bone.

cortical bone samples and used to develop the statistical model of inhomogeneity in this study. The elastic modulus was calculated from the unloading curve of nanoindentation testing (Fig. 1) using the Oliver–Pharr method [8].

Assessment of inhomogeneity using variograms

In this study, the spatial variation was evaluated using a so-called variogram, which could be expressed in two parameters: semi-variance and lag [9,10]. The semi-variance (γ) was defined as the half of the expected squared difference between any paired data values $\{z(\mathbf{x}), z(\mathbf{x}+\mathbf{h})\}$:

$$\gamma(\mathbf{h}) = \frac{1}{2}E\left[\{z(\mathbf{x})-z(\mathbf{x}+\mathbf{h})\}^2\right] \tag{1}$$

where $\mathbf{z}$ is a random function of the indentation modulus of bone that varies continuously in space; $\mathbf{x}$ denotes the spatial coordinates of locations; and $\mathbf{h}$, also known as lag, is a vector representing the Euclidean distance and direction between any two data locations. In this paper, the terms, lag and separation distance, are interchangeable.

The experimental variogram for the nanoindentation modulus maps was computed as an average of semi-variance values at different locations that have the same value of lag:

$$\hat{\gamma}(\mathbf{h}) = \frac{1}{2m(\mathbf{h})}\sum_{i=1}^{m(\mathrm{h})}\{z(\mathbf{x}_i)-z(\mathbf{x}_i+\mathbf{h})\}^2\} \tag{2}$$

where $m(\mathbf{h})$ is the number of data pairs $\{z(\mathbf{x}_i), z(\mathbf{x}_i+\mathbf{h})\}$ for observations separated by $\mathbf{h}$.

Correlation length

To fit the variogram obtained from the experimental data, a mathematical model must satisfy the following conditions: an intercept on the ordinate, a monotonically increasing section, and conditional negative semi-definite [9]. Among the simple functions (*i.e.*, exponential, Gaussian, and spherical models) that meet these conditions, the exponential model was selected in this study because it fitted well with the experimentally obtained maps of nanoindentation modulus and had the highest R-squared value.

$$\begin{aligned}\gamma(\mathbf{h}) &= c_0 + c\left(1-e^{-\mathbf{h}/L}\right)\\ \gamma(\mathbf{0}) &= 0\end{aligned} \tag{3}$$

where $\gamma(\mathbf{h})$ is the semi-variance as a function of lag ($\mathbf{h}$), L is referred to as the correlation length, c_0 is the nugget variance. In theory, c_0 should be naught. Due to the discrete data acquisition during the experiment, however, it may not be zero. c is the difference between the nugget variance and the converging value of $\gamma(\mathbf{h})$ when $\mathbf{h}$ approaches infinity, which is actually the value of variance of the map.

The correlation length of experimental modulus maps can be mathematically determined by fitting the exponential model to the observed variogram using the least square estimation or the maximum likelihood estimate [9]. In this study, the ordinary least square estimation was implemented in MATLAB (MathWorks, Natick, MA) with the function “lsqcurvefit.” In brief, the parameters in the exponential model (L, c_0, and c) can be estimated by minimizing the residual sum of squares for semi-variance

$$R(\tau;\hat{\gamma}_k) = \sum_{j=1}^{k}\left\{\hat{\gamma}\left(\mathbf{h}_j\right)-\gamma\left(\mathbf{h}_j,\tau\right)\right\}^2 \tag{4}$$

where τ represents the vector of parameter estimates [L, c_0, c]; $\hat{\gamma}_k$represents the vector of semi-variance estimates, each estimate being denoted by $\hat{\gamma}(\mathbf{h}_j)$; k is the size of the semi-variance vector; The quantity $\gamma(\mathbf{h}_{\mathrm{j}},\tau)$ is the jth semi-variance expected from the fitted model and depends on the parameter estimates.

The correlation length is an important parameter to characterize spatial variations of a random field. Relatively, a large correlation length ($L=500$ nm, Fig. 2a) implies a smooth variation, whereas a small correlation length ($L=100$ nm, Fig. 2b) corresponds to rapid changes in the property over the space domain. The mean, standard deviation, c_0 and c were the same between these two maps and only the correlation length was different.

Random fields

A random field is a statistical manifestation that represents the continuous random distribution of properties across the space. For example, each observed map of nanoindentation modulus actually corresponds to one realization of the random field. In fact, random fields are represented by random variables that are correlated with each other through the covariance function. To avoid intensive computation induced by the large number of independent random variables, the covariance matrix from the exponential model was decomposed in this study through an eigenvalue analysis known as the Karhunen–Loeve (KL) transformation with a cutoff value of 90% [11]. By doing so, only a limited number of random variables would be needed for realization of a random field. Furthermore, if a model is determined for the random field, other realizations could be readily generated using the covariance function [12].