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Research Paper

Insights into reference point indentation involving human cortical bone: Sensitivity to tissue anisotropy and mechanical behavior

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ABSTRACT

Reference point indentation (RPI) is a microindentation technique involving 20 cycles of loading in "force-control" that can directly assess a patient's bone tissue properties. Even though preliminary clinical studies indicate a capability for fracture discrimination, little is known about what mechanical behavior the various RPI properties characterize and how these properties relate to traditional mechanical properties of bone. To address this, the present study investigated the sensitivity of RPI properties to anatomical location and tissue organization as well as examined to what extent RPI measurements explain the intrinsic mechanical properties of human cortical bone. Multiple indents with a target force of 10 N were done in 2 orthogonal directions (longitudinal and transverse) per quadrant (anterior, medial, posterior, and lateral) of the femoral mid-shaft acquired from 26 donors (25–101 years old). Additional RPI measurements were acquired for 3 orthogonal directions (medial only). Independent of age, most RPI properties did not vary among these locations, but they did exhibit transverse isotropy such that resistance to indentation is greater in the longitudinal (axial) direction than in the transverse direction (radial or circumferential). Next, beam specimens (~2 mm × 5 mm × 40 mm) were extracted from the medial cortex of femoral mid-shafts, acquired from 34 donors (21–99 years old). After monotonically loading the specimens in three-point bending to failure, RPI properties were acquired from an adjacent region outside the span. Indent direction was orthogonal to the bending axis. A significant inverse relationship was found between resistance to

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indentation and the apparent-level mechanical properties. Indentation distance increase (IDI) and a linear combination of IDI and the loading slope, averaged over cycles 3 through 20, provided the best explanation of the variance in ultimate stress (r^2=0.25, p=0.003) and toughness (r^2=0.35, p=0.004), respectively. With a transverse isotropic behavior akin to tissue hardness and modulus as determined by micro- and nano-indentation and a significant association with toughness, RPI properties are likely influenced by both elastic and plastic behavior of bone tissue.

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1. Introduction

Standard bone densitometry measurements by dual energy x-ray absorptiometry (DXA) do not fully explain the increase in fracture risk with age (Johnell et al., 2005; Kanis et al., 2001) or diseases such as diabetes (Oei et al., 2013; Saito and Marumo, 2013; Vestergaard, 2007). While these measurements are sensitive to bone loss, they do not necessarily assess the various ways aging and diseases can lower fracture resistance as DXA is insensitive to certain tissue-level properties that contribute to bone's resistance to fracture (e.g., damage accumulation, mineral and collagen quality, collagen cross-linking pattern, and mineralization heterogeneity) (Friedman, 2006; Ruppel et al., 2008). A striking example is the association of long-term use of bisphosphonates – a drug that inhibits osteoclast activity – and atypical femoral fractures (Shane et al., 2014), even though bisphosphonates increase bone mineral density (Boivin et al., 2000). There are likely deleterious changes in the material properties of the bone tissue with suppression of bone resorption (Donnelly et al., 2012; Gourion-Arsiquaud et al., 2010) that make bone less resistant to fracture (Allen and Burr, 2007; Ettinger et al., 2013).

This calls for the development of diagnostic tools that would provide, not just a surrogate of fracture resistance, but also a direct measure of bone properties at the material level (i.e., independent of mass or structure). In this sense, promising technologies for in vivo fracture risk assessment include reference point indentation (RPI), a new microindentation method that can probe bone material properties of a patient's tibia on a length scale of 100 μm. Two clinical studies involving relatively small patient cohorts (N=35 to 70) (Diez-Perez et al., 2010; Guerri-Fernandez et al., 2013) have reported that a measure of indentation depth using RPI can discriminate fractured patients from age-matched, non-fracture controls. However, the mechanisms that sustain the association between the RPI parameters and overall fracture resistance are not well identified. Hence, despite encouraging preliminary clinical observations, further basic investigations are needed to identify the determinants of RPI parameters and clarify what mechanical behavior they assess. For example, it is unclear whether RPI provides a measure of hardness (i.e., the resistance to plastic deformation) or brittleness (i.e., a higher propensity to propagate cracks), or a combination thereof. Moreover, RPI can involve successive indentation cycles, and, as such, potentially provides a wealth of information other than indentation depth that remains underexploited.

Therefore, we aimed to assess to what extent RPI measurements explain the mechanical behavior of human cortical bone at the apparent level. We hypothesized that (i) similar to properties from nanoindentation (Fan et al., 2002; Franzoso and Zysset, 2009; Rho et al., 1999) and fracture mechanisms of micron-scale cracks (Akkus et al., 2000; Koester et al., 2008; Nalla et al., 2005; Ural and Vashishth, 2007), RPI measurements are sensitive to bone anisotropy; and (ii) RPI parameters are related to conventional mechanical parameters acquired at the millimeter length scale. Our hypotheses were evaluated via two independent ex vivo experiments. Such knowledge should provide insight into interpreting differences or changes in RPI properties and guide the application of RPI to bone in future studies.

2. Material and methods

2.1. *Reference point indentation (RPI)*

The tissue-level mechanical properties of excised human bone samples (see Section 2.2) were assessed using a BioDent™ instrument (Active Life Scientific, Inc., Santa Barbara, CA). The general principle of RPI has been described in detail by others (Aref et al., 2013; Diez-Perez et al., 2010; Guerri-Fernandez et al., 2013; Hansma et al., 2008; Rasoulian et al., 2013). Briefly, the instrument measures the displacement (relative to the bone surface) of a stainless steel test probe (375 μm diameter, 90° cono-spherical, 2.5 μm radius tip) that indents into the bone to a given load, dwells for a short period of time (typically <200 ms), and unloads to ~0 N. In our experiments, each load-controlled indentation consisted of 20 cycles at 2 Hz with a maximum force of 10 N per cycle. Throughout RPI testing, the samples were kept hydrated with phosphate buffered saline (PBS) at room temperature.

2.1.1. *Data processing*

At the time that indentation data was collected, the manufacturer software primarily provided averaged values for each parameter, instead of cycle-by-cycle analysis (Aref et al., 2013), with a set number of significant digits. In order to take full advantage of the raw load vs. displacement data as well as to control for round-off error and to adjust the significant digits of each properties, we used custom MATLAB® code (The MathWorks, Natick, MA) to determine a number of indentation resistance properties as follows.