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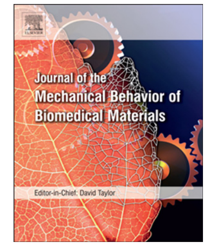

Structural orientation dependent sub-lamellar bone mechanics

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ABSTRACT

The lamellar unit is a critical component in defining the overall mechanical properties of bone. In this paper, micro-beams of bone with dimensions comparable to the lamellar unit were fabricated using focused ion beam (FIB) microscopy and mechanically tested in bending to failure using atomic force microscopy (AFM). A variation in the mechanical properties, including elastic modulus, strength and work to fracture of the micro-beams was observed and related to the collagen fibril orientation inferred from back-scattered scanning electron microscopy (SEM) imaging. Established mechanical models were further applied to describe the relationship between collagen fibril orientation and mechanical behaviour of the lamellar unit. Our results highlight the ability to measure mechanical properties of discrete bone volumes directly and correlate with structural orientation of collagen fibrils.

1. Introduction

Bone is a natural composite material and possesses a structural complexity across a range of length scales. This structural complexity is commonly based on the organizations of fibrils within lamellar units that allow bone to maintain biological function while performing a number of mechanical roles (Fratzl and Weinkamer, 2007; Wainwright et al., 1982). Thus, the mechanical properties of the lamellar unit are critical in defining overall bone mechanics. Determining mechanical properties of lamellae is compromised by the micron length scale of the unit as well as the anisotropy of the constituents including the collagen predominantly in the form of fibrillar framework, hydroxyapatite platelets, non-collagenous protein and water (Gupta et al., 2006). Approaches to understand mechanics of the lamellar unit therefore consider a three phase composite material with plate-like hydroxyapatite minerals reinforcing collagen fibrils bound together in a relatively small volume fraction of non-collagenous proteins (Akiva et al., 1998). These hydroxyapatite minerals are platelet-shaped and embedded within and around the collagen fibrils, with the principal axis of the mineral oriented in the same direction as the long axis of the collagen fibrils (Fratzl et al., 2004; Landis et al., 1996; Wagermaier et al., 2007; Wagner and Weiner, 1992). Thus, the organization of the collagen fibrils within the lamellar unit defines the mineral orientation within this same unit. The orientation of collagen fibrils and, thus, the mineral phase in the lamellar unit has been the subject of considerable research due to the resultant influence on bone mechanics. Layered

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arrays of aligned mineralized collagen fibrils have been previously proposed to organize into five subunit layers within the lamellar unit (Weiner et al., 1997). The orientation of the sub layers has been shown to conform to a rotated plywood-like structure and can be generally grouped into two subunits (Boyd and Nigg, 2007; Gupta et al., 2006; Weiner et al., 1999); the 'thick' subunit where the collagen fibrils run parallel or at 30° to the long axis of bone, thus contributing significantly to the elastic modulus (Ascenzi et al., 1982), and the 'thin' subunit for fibrillar arrays oriented at 60°, 90° and 120° to the long axis. More recent work indicated considerably more disorder when examining bone volumes using 3D imaging due to fibril dispersions, suggesting subunit layers are better described using three sub-lamellar structures (Reznikov et al., 2013).

The overall mechanical properties of bone have been shown to depend on both the volume fraction of constituents, most notably mineral phase, and the organization of these constituents represented by the lamellar unit. Previous works have indicated the importance of the mineral phase in defining overall bone mechanical behaviour by direct investigations on mineral volume fraction in a variety of different bone samples (Currey, 2006). A general increase in the elastic modulus of bone was correlated with an increase in the mineral volume fraction but a number of exceptions were noted where the mineral volume fraction alone does not determine the mechanical properties of bone. Currey suggested a mineral organizational factor that further defined the effectiveness of the reinforcement in bone, which has also been considered by Rho et al. (1998) and Sasaki et al. (1991). The plywood collagen organization within the lamellar unit has also been shown to act as a crack blunter to enhance toughening mechanisms at this submicrostructural level (Gupta et al., 2006; Peterlik et al., 2006). The mechanical properties of bone material are therefore not determined solely by mineral volume fraction but by both the mineral content and the mineral platelet orientation defined by the collagen fibril orientation (Sasaki et al., 1991). This mineralized fibril orientation will therefore give rise to the overall mechanical properties in bone material.

The influence of constituent organization on bone mechanical behaviour was first conclusively highlighted in studies on individual osteons. Polarized light microscopy was previously used to identify collagen fibril orientation and related to the mechanical properties of individual osteons in tension (Ascenzi et al., 1982), bending (Ascenzi and Bonucci, 1990) and compression (Ascenzi and Bonucci, 1968). Increases in the elastic modulus of individual osteons were found to occur when the majority of the collagen fibrils were oriented in the loading direction and supported theories that established the existence of lamellar orientations in bone material (Ascenzi et al., 1965; Giraud-Guille, 1988). Further works have more specifically highlighted the relationship between overall bone mechanics and collagen fibril orientation, including increased tensile strength (Martin and Ishida, 1989) and higher elastic modulus (Ramasamy and Akkus, 2007; Riggs et al., 1993) when collagen fibrils are predominantly oriented along the longitudinal, as opposed to the transverse, loading direction. The importance of the collagen fibril orientation in determining overall bone mechanical properties led to mechanical testing at smaller submicrostructural length scales. In particular, direct mechanical testing of bone at sub-millimetre length scales has been previously achieved in order to determine the effect of lamellar orientation on the elastic properties of baboon tibiae (Liu et al., 1999). The results of the work by Liu et al. (1999) indicated clear anisotropic behaviour at different spatial positions along a bone fracture surface, and correlated lamellae orientation from mechanical behaviour and observed fracture surfaces. Further improvements to measure the mechanical properties of bone at the submicrostructural level have been attained through nanoindentation, which allows localized testing to be performed on individual constituents such as individual lamellae (Gupta et al., 2006; Lewis and Nyman, 2008; Xu et al., 2003). However, structural heterogeneities in bone coupled with the complex stress analysis formed from indentation of bone surfaces make direct understanding of bone component mechanics particularly fraught (Gupta et al., 2006; Isaksson et al., 2010; Rodriguez-Florez et al., 2013; Xu et al., 2003). A comprehensive review of nanoindentaton in mineralized tissue particularly emphasizes issues of indentation-sample contact area, critical in determining mechanical properties of samples, as an unexplored area of study (Lewis and Nyman, 2008). A more recent study on nanoindentation as a technique to test the mechanical properties of bone has revealed the strong effect of the hydration state, tip geometry and the assumptions of the analysis methods on the results (Rodriguez-Florez et al., 2013) that provides discrepancies in the mechanical properties of bone measured via nanoindentation. Further determination of mechanical properties of constituents has been more recently available with the advent of more sophisticated and higher force resolution techniques, which are able to elucidate constituent mechanics directly. Such components have been investigated by a series of experiments such as nanoindentation (Tai et al., 2007), tensile testing of individual mineralized collagen fibrils (Hang and Barber, 2011) and collagen fibril pullout to determine the mechanical properties of non-collageneous protein regions (Hang et al. 2014).

Variations in the mechanical properties of bone when testing at different orientations to the bone's long axis are considered to be mainly due to the alignment of the collagen fibrils and mineral plates relative to the loading axis, and highlight the influence of the lamellar unit on overall bone mechanical behaviour (Fratzl et al., 2004; Martin and Ishida, 1989; Ramasamy and Akkus, 2007; Riggs et al., 1993). Techniques suitable for elucidating mechanical anisotropy defined by lamellae include nanoindentation (Gupta et al., 2006; Franzoso and Zysset, 2009), scanning acoustic microscopy (Granke et al., 2013) and micropillar compression (Schwiedrzik et al., 2014), with this latter work presenting one of the few data sets that examine failure beyond elastic limits. Computational validation has additionally stressed the importance of anisotropy on overall mechanical properties of bone (Geers et al., 2010; Ghanbari and Naghdabadi, 2009). Understanding the mechanical properties of the lamellar unit and the effects of orientation within the lamellae therefore provides a link between constituent and overall bone mechanical performance. While mechanical properties of constituents are instructive in defining overall bone behaviour, testing of bone at larger length scales approaching a few microns perhaps best represents the synergy between the constituents in bone but ignores the higher order structural effects such as osteonal canals or the curvature of whole bone.

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