Abstract 89: Orthopaedic Research Society; Vol. 36. 2011

What Can Antler Microstructure Teach Us About Human Bone Toughening?

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INTRODUCTION:

Antlers are highly unusual because they regenerate yearly and become extraordinarily tough without undergoing significant osteonal remodeling. Consequently, antler tissue is under scrutiny in order to determine the relative importance of its specific microstructural and nanostructural toughening mechanisms (1-4). The clinical impetus for this research is clear — the degradation of bone toughness is a major factor in the compromised bone quality (tissue mechanical properties) in aging humans (5). This is also associated with senescence of remodeling dynamics and osteonal collagen/lamellar organization. Novel ways to enhance toughness before the onset of skeletal fragility may be gleaned from understanding the toughening mechanisms in antler. This study examined antler for microstructural toughening mechanisms in addition to the hypermineralized seams ("regions") as observed by Launey et al. (2010) and Skedros et al. (1995). In this earlier study highly heterogeneous collagen fiber orientation (CFO) was also observed in circularly polarized light (CPL) images, but quantitative data were not reported. These observations contrast with suggestions that microstructural characteristics have subsidiary importance when compared to nanostructural toughening mechanisms (3); this is because primary osteons and CFO in antler are considered to be typically longitudinal (3,4). We hypothesize that antler has several microstructural toughening mechanisms that, when recognized, will prompt additional studies aimed at discerning their relative importance in ensuring its extraordinary toughness.

METHODS:

Antlers were obtained from 11 adult two-point (each side) Rocky Mountain mule deer in late October in Utah, USA. Two 10mm thick segments were cut transversely from each antler starting at four centimeters distal to the main bifurcation. Bulk mineral content was determined by ashing two cortical fragments from the proximal segment. Two specimens obtained from the distal segment were embedded in polymethyl methacrylate and were glued together to form a single aggregate of specimens. Two 1.0mm sections were obtained from the aggregate; one was used for backscattered electron (BSE) imaging, the other for CPL analysis. Each surface was milled to a high-luster finish to achieve 100±5µm overall thickness. Two 50x grayscale CPL images were obtained from each specimen and a weighted mean gray level (WMGL) was obtained of each image and osteon: darker gray levels represent relatively more longitudinal CFO and brighter gray levels represent relatively more oblique-to-transverse CFO (7). CFO variations are considered as differences in WMGL and CFO heterogeneity variations are expressed as the mean of the full-width at ½ maximum (FWHM) of the two peaks of the gray level (GL) profiles of the antler images and FWHM of the main one or two peaks of the GL profiles from human and non-human bones from our on-going and previous studies (7,8). Images from the antlers were also analyzed in order to determine the: (1) number, area, and circularity of individual osteons; where 1.0 = perfect circle, (2) aspect ratio of each osteon structure (= max/min chord lengths), (3) angle of the max chord with the medial-lateral axis (0-180 degrees), and (4) distances between interfaces at osteon peripheries.

RESULTS:

The CPL and BSE images showed extensive primary osteon structures with apparent tortuous orientations. There are ~20 primary osteons/mm^2, and each image had <3 secondary osteons. The circularity and aspect-ratio data support the high heterogeneity of osteon orientations; many of which also had evidence of multiple and/or obliquely coursing vascular canals (see Table and antler CPL image). The range of osteon areas also revealed substantial differences in their sizes and/or orientations. The broad range of chord angles suggests quasi random osteon orientations. The BSE images showed the extensive presence of "bright lines" at the osteon interfaces, which we refer to as "hyper-mineralized seams" (data and images not shown). These seams course through the lower mineralized bone (mean ash fraction is 59%; human bone is typically 65-68%). Gray-level histogram profiles from antler CPL images showed multiple peaks, reflecting a high degree of CFO heterogeneity (see Figures). These FWHM measurements typically exceed the range of the human and non-human bones that we have studied previously. CFO heterogeneity can, in part, be explained by the positive correlation between CFO/WMGL and the aspect ratio of the osteons ($r = 0.46$; $p < 0.01$) (i.e., more oblique-to-transverse CFO correlates with elongated osteons).

Deer Antler Osteon Data Means and Ranges

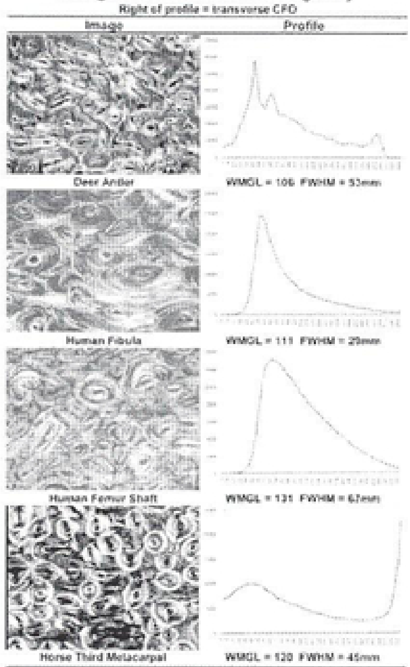
	O.A. (mm^2)	Circularity	Aspect Ratio	Chord Angle	IF Distance (mm)
Mean	0.036	0.75	1.8	110	0.146
Range	0.007-0.139	0.30 - 0.94	1.0 - 4.2	1 - 177	0.032-0.586

DISCUSSION:

These data support the hypothesis that there are several potentially important microstructural toughening mechanisms in deer antler, including highly heterogeneous: (1) osteon size, shape, and orientation, and (2) CFO, which correlates with vascular orientation. The interfaces formed by the osteons have a thin seam of material that appears to be analogous to hypermineralized cement lines of human secondary osteons, which are important *microstructural* toughening mechanisms (9). They are 'analogous', not 'homologous', because in antler they are rarely formed by the osteon remodeling process. The interfacial (IF) distances are of a mean magnitude (~150µm) that could have evaded detection as being mechanically important in recent studies where microstructural mechanisms are de-emphasized — this view is based on data obtained from small-angle x-ray diffraction patterns obtained from 200µm thick sections loaded in longitudinal tension (3). An overview of the CPL images from various bones that we have studied showed that CFO in antler is among the most heterogeneous. This heterogeneity is at the microstructural level of the hierarchical organization of bone (10) and may be one of several important microstructural toughening mechanisms. Although CFO heterogeneity has not yet been recognized as potentially important in this context, it may prove to strongly correlate with toughness that ensures adequacy of a bone's tissue mechanical properties, and might be an important factor in age-related reduction of bone quality in the human skeleton.

Collagen Fiber Orientation Heterogeneity

Right of profile = transverse CFO



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