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Histocompositional organization and toughening mechanisms in antler

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ABSTRACT

Mechanical testing studies by Krauss et al. (2009) and Gupta et al. (2013) suggest that the extraordinary toughness of antler bone is primarily achieved by intrinsic/nanostructural mechanisms instead of extrinsic/microstructural mechanisms. However, this conclusion is based on data from extremely small specimens from one antler loaded only in tension, which impedes discernment of the relative importance of intrinsic vs. extrinsic mechanisms. In the present study we conducted analyses into the microstructural features of antler for details of potential additional microscale toughening characteristics, as suggested by recent mechanical testing studies of *bulk* specimens. The data are also considered in view of the above-mentioned studies concluding that extrinsic/microstructural toughening mechanisms are less important than nanoscale/intrinsic toughening mechanisms in antler. Mule deer antlers were evaluated using: (1) backscattered electron imaging for micro-mineralization, (2) circularly polarized light for osteonal interfacial complexity and collagen fiber orientation (CFO) heterogeneity, and (3) X-ray 3D micro-computed tomography for osteon/vessel orientation, density, and size. Results showed: (1) hyper-mineralized seams of approximately 3–4 microns thickness within relatively hypermineralized "zones" that course circuitously along osteonal interfaces, (2) highly heterogeneous CFO, including increased oblique-to-transverse CFO near/adjacent to osteon peripheries, and (3) osteons are often highly elongated in 2D. 3D reconstructions show that a considerable percentage of the vascular canals course obliquely with respect to the antler long axis. While results show multiple possible extrinsic-level histological characteristics in antler bone, it remains to be determined if microstructural characteristics become subsidiary to nanostructural characteristics in enhancing toughness during the majority of post-yield behavior of antler bone when loaded in a biologically relevant fashion.

1. Introduction

Antler is considered bone because it is comprised of hydroxyapatite, collagen, non-collagenous proteins, water, and predominantly primary osteons (Bloebaum et al., 1997; Chapman, 1975; Currey, 2002; Gomez et al., 2013; Kierdorf et al., 2000; Krauss et al., 2011; Landete-Castillejos et al., 2007a; Launey et al., 2010b). The typical paucity of secondary osteons (Haversian systems) in antler (Gomez et al., 2013; Krauss et al., 2011) reflects the fact that nearly all species cast off these structures annually. They re-grow at a very fast rate with the majority of the growth being completed in just 3–4 months (Banks et al., 1968; Chapman, 1975), hence leaving insufficient time for the coupled osteoclastic/osteoblastic remodeling process that forms traditional secondary osteons (Gomez et al., 2013; Krauss et al., 2011). Because antler breakage can significantly reduce reproductive success (Clutton-Brock, 1982), natural selection favors resilient/tough tissue that can withstand higher impact loads, bending moments, and torsion seen frequently in the male-to-male combat of the rutting (mating) season. Elk antler has been shown to have the highest strain to failure of all bones studied, with an ultimate tensile strain of ~12%, which is six times higher than the ultimate tensile strain of human cortical bone (~2%) (Currey, 2002). In this perspective it is notable that in compression tests of bulk specimens of North American elk antler, Kulin et al. (2011) reported that their specimens often did not break even at 25% strain.

The seminal work of Currey (1979, 1984, 1990) suggested that the extraordinary toughness of antler is strongly influenced by its relatively low mineral content, which reduces its material stiffness and is somehow coupled with mechanisms that enhance its

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capacity to develop microdamage without catastrophic failure (i.e. fracture). Subsequent studies revealed microcracking behavior associated with microstructural features of the tissue but detailed analysis of antler microstructure was not pursued in these studies (Vashishth, 2004; Vashishth et al., 2003; Zioupos and Currey, 1994; Zioupos et al., 1994).

In a recent set of elegant studies, Krauss et al. (2009) and Gupta et al. (2013) reported experimental data suggesting a very unusual toughening mechanism present in antler that has not been identified as being prominent in human or bovine bone. Based on changes in small-angle X-ray diffraction patterns (SAXD) that occurred while progressively loading (in tension) matchstick-like specimens (16 mm × 400 μm × 200 μm) from antlers of Iberian elk (red deer) (*Cervus elaphus hispanicus*)[1], they drew fundamentally important conclusions: (1) this mechanism is at the collagen fibrillar (nanoscale) level of the antler material, and (2) it not only begins during pre-yield loading but dominates during post-yield loading. Therefore, they emphasized that this *nanoscale* toughening mechanism is unusual primarily because it is more important and effective than *microscale* mechanisms that dominate in human and bovine bone. This is a significant distinction because, while suggested toughening mechanisms in bone occur across the spectrum from nanostructure to microstructure, the most important of these have typically been considered to be at the *microstructural* level, especially during post-yield deformation as shown in the more highly mineralized "typical" bones that have been studied (Barth et al., 2010; Ciarelli et al., 2009; Dong et al., 2011; Koester et al., 2008; Launey et al., 2010b; Skedros et al., 2005; Zimmermann et al., 2009; Zioupos and Currey, 1994). Gupta, Krauss and co-workers' emphasis on the dominance and time course (i.e. starting in the pre-yield/post-yield transition) of nanostructural toughening mechanisms is a revolutionary idea in bone biomechanics and, therefore, has important implications for understanding intricacies of the structural biology and mechanical behavior of other natural mineralized composite materials in the contexts of normal, aged, and disease states.

By contrast, mechanical tests conducted by Launey et al. (2010b) and Kulin et al. (2011) provide clear evidence that *microstructural* toughening mechanisms are at work — and prominently so — in North American elk antler. For example, Launey et al. (2010b) utilized real-time (in-situ) videography during environmental SEM imaging to view microcrack propagation during bending tests in specimens with much greater tissue volume (specimen size: 12 mm × 3 mm × 2.0–2.5 mm) than those of Krauss et al. (2009) and Gupta et al. (2013). Analysis of these bulk specimens revealed at the peripheries of the primary osteons the presence of "hypermineralized regions" (below we call these "zones", and there is a hypermineralized "seam", or "cement line", contained within the "zone") that help ramify and disperse developing microcracks, thus helping to avoid fracture by absorbing energy and reducing stress concentrations. Nevertheless they also argued that *intrinsic* toughening mechanisms dominate during plastic deformation. This conclusion in microcrack propagation tests in bending contrasts with findings of Kulin et al. (2011) who found that, in their bulk specimens of North American elk antler (6 mm × 4 mm × 4 mm) tested in compression, the primary osteons and their interfaces are the dominant loci for the extrinsic mechanisms that enhance both pre- and post-yield toughness. Notably, when compared to the prior studies of antler mechanical properties the experiments of Kulin et al. (2011) more closely mirror natural conditions because they tested their specimens using strain rates that more likely resemble those produced by combat loads.

Despite these insights the four landmark studies that have reported data supporting and/or stimulating discussions of these important ideas in bone biomechanics and structural biology are based on experiments conducted on only one antler in each study ($n = 3$) (Gupta et al., 2013; Krauss et al., 2009; Kulin et al., 2011; Launey et al., 2010b). Nevertheless, in these studies of antler biomechanics an important duality has emerged in devising comprehensive mechanical tests of this tissue — nanostructural and microstructural toughening characteristics and mechanisms must be studied to determine the extent that both are present and their relative importance. This distinction is based on important toughening mechanisms in bone that are, respectively, either "intrinsic" (ahead of a crack tip and <1 μm in scale and largely material independent) or "extrinsic" (behind a crack tip and >1 μm in scale and largely material dependent) (see Appendix 1). The importance of microcracking extrinsically is that it results in both microcrack bridging and deflection, which are the most potent toughening mechanisms shown in bone (at least in non-antler bone) and have been shown to typically involve osteonal structures and/or other microstructural interfaces (Dong et al., 2011; Hoo et al., 2011; Koester et al., 2008; Launey et al., 2010a; Ritchie et al., 2005).

As noted above, and in contrast to these studies of antler specimens in bending and tension (Gupta et al., 2013; Krauss et al., 2009; Launey et al., 2010b), Kulin et al. (2011) found a more important role for osteon-level characteristics in toughening, as shown by their compression tests at high strains (up to 25%) and multiple strain rates (10^{-3}, 10, 10^{3}). Although scanning electron microscopy of fracture paths showed that, as expected, failure of antler occurs at multiple scales of its hierarchical organization, from macrostructure to nanostructure, the involvement of microstructural characteristics seemed preeminent in the data of Kulin and co-workers. For example, splitting and sliding between osteons, with localized osteon buckling was observed in transversely loaded specimens. By contrast, splitting and tearing of osteons was observed in longitudinally tested specimens. They concluded that large plastic deformations are:

> . . . attributed to the reduced mineral content [of antler] relative to long bones that allows the osteons to deform more easily, as well as (and perhaps more importantly) the irregular shape of the osteons. The irregular osteon shape leads to mechanical "interlocking" between osteons, and increases the load necessary for slipping between osteons to occur. This is supported by microstructural observations indicating failure tends to propagate between osteons, but is occasionally forced through osteons due this interlocking. (pg. 1038).

In addition, they found that during bulk deformation the tissue exhibited shearing along osteonal boundaries at approximately a 45-degree angles, resembling microstructural/extrinsic-based failure mechanisms in secondary osteonal bone in human long bones (Ebacher et al., 2007). In fact, previous studies have shown that shear deformation in oblique orientations (cross-hatched microcracks) is the major mechanism of the post-yield deformation of human cortical bone in compression (Ebacher et al., 2007; Leng et al., 2009). Because these findings show the relative importance of extrinsic mechanisms in compression tests of antler, they contrast with the emphasis on intrinsic failure mechanisms gleaned from the tension tests of Krauss et al. (2009) and Gupta et al. (2013). Placing heavy emphasis on one of these mechanisms

[1] North American elk are *Cervus elaphus canadensis* and are less commonly referred to as wapiti. In Europe there are many subspecies of *Cervus elaphus* and these are considered "red deer". In North America the moniker "red deer" is not used to describe elk, hence in this study we use "elk", not "red deer", to describe all subspecies of *Cervus elaphus*. For the purpose of this study we refer to red deer as "elk" in order to remain consistent with common usage of "elk" in North America and because this usage: (1) is also consistent with recent studies of North American elk antlers (Kulin et al., 2011; Launey et al., 2010b) that are heavily referenced in the present investigation, and (2) helps to avoid additional confusion that can result from the fact that the species referred to as "elk" in Europe are called "moose" in North America (http://en.wikipedia.org/wiki/Deer) (Nowak, 1999).