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Editorial

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ABSTRACT

Animal models, in particular mice, offer the possibility of naturally achieving or genetically engineering a skeletal phenotype associated with disease and conducting destructive fracture tests on bone to determine the resulting change in bone's mechanical properties. Several recent developments, including nano- and micro-indentation testing, microtensile and microcompressive testing, and bending tests on notched whole bone specimens, offer the possibility to mechanically probe small animal bone and investigate the effects of aging, therapeutic treatments, disease, and genetic variation. In contrast to traditional strength tests on small animal bones, fracture mechanics tests display smaller variation and therefore offer the possibility of reducing sample sizes. This article provides an analysis of what such tests measure and proposes methods to reduce errors associated with testing smaller than ideal specimens.

Introduction

There has been considerable interest in the measurement of the mechanical properties of small animal bones. Animal models, in particular mice, offer the possibility of naturally achieving or genetically engineering a skeletal phenotype associated with disease and conducting destructive fracture tests on bone to determine the resulting change in bone's mechanical properties. Unfortunately the repertoire of mechanical tests, available for small animal bones, is limited due to inherent limitations imposed by bone size.

In recent years, however, there has been an explosion in the type of mechanical tests proposed for mouse bones. And because mouse bone has well defined organizational hierarchy, these tests scale the natural length scale from mineral and protein levels to whole bone tests. For example nano- and micro-indentation testing have been done on inbred or genetic knockout mouse bones in monotonic or cyclic mode to determine the local elastic and viscoelastic properties associated with bone mineral and protein modifications [1–4]. Next, at a scale comparable to lamellar level human bone specimens [5–7], Ramasamy and Akkus [8] have recently demonstrated successful machining and testing of microtensile and microcompressive mouse bone specimens (0.5×0.7 mm gage section; 0.15 mm thickness). Finally, at the whole bone level, three- and four-point bending and torsional tests to failure have been popular due to the inherent simplicity of such tests in determining the mechanical properties associated with changes in the structure and material due to exercise, variations among different inbred mouse strains, growth factor deficiency, accelerated senescence and ovariectomy [9–13]. Other less common testing methods for mouse bones include femoral neck tests to determine the effect of fluoride treatment on mineralization and whole bone fracture [14].

The inherent hierarchy of bone's extracellular matrix (ECM) has specific microstructural features and energy dissipation mechanisms at different length scales that allow the bone to effectively resist the different components of applied loading [15]. Tensile loading interacts with sublamellar structures including the mineralized collagen fibril matrix to produce time dependent diffuse damage containing submicron cracks <1 μm [15–17]. Compressive loading interacts with lamellae and produces primarily cycle dependent short linear microcracks of the order of tens of microns in length in a long bone cross-section [15–17]. Torsional and other forms of mixed mode loading interact with osteons and produce primarily time dependent microcracks that either get deflected by the osteonal structure or penetrate the osteon and are consequently of the order tens to hundred microns in length in a long bone cross-section [18–19]. Thus, in the selection of appropriate test/tests, a prior understanding of the length scales present in an animal bone and its expected modification by disease or treatment is often helpful.

In the study of engineering materials and structures, the theory of fracture mechanics and related experimentation has proven to be more effective than some of the strength-based testing methods described above. Reviewing the application of fracture mechanics to bone, three decades ago, Bonfield [20] was first to note that the presence of inherent flaws in bone, in the form unrepaired fatigue microcracks, caused significant variations in strength-based measures and that the initiation and controlled propagation of a fracture crack from a sharp pre-machined notch (representing a flaw) in bone specimen reduced variations in the measured fracture properties. Working with Bonfield, the author found that, in contrast to initiation, properties measured during crack propagation more comprehensively captured the fracture behavior of bone [21] and accurately distinguished tough from less tough bones [22]. Furthermore, Norman et al. [23] and Wang and Agrawal [24] extended the measurement of bone fracture from mode I (tensile) to mode II (in plane shear) and introduced smaller specimen designs. Zioupos and Currey [25] evaluated the effectiveness of various fracture mechanics based

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parameters and their comparison with traditional strength and work-to-fracture parameters.

Based on their recent work [26], in this issue of *Bone*, Ritchie et al. [38] have extended the application of the above concepts to small animal bones obtained from mice and rats. They present detailed techniques to characterize whole bone toughness by assuming femoral diaphysis to a pipe of uniform cylindrical shape and thickness. Through two- and six-point average of bone radius and thickness, they go on to reduce the error associated with the assumption. More importantly, compared to strength-based measures, they find reduced scatter in some of the fracture mechanics parameters. This is an important new development and the further use of the proposed techniques is likely to improve the estimation of fracture in small animal bones. However, there are a number of issues and limitations that one must consider before applying these methods to interpret the effects of therapeutic treatments, disease, genetic variations and knock-outs. Also, the methods proposed in the tutorial have not been extensively investigated and additional considerations and testing will be needed in order to standardize the mechanical testing procedures. These are discussed below in turn.

What is being measured?

Fracture toughness of bone, measured at initiation as critical stress intensity factor (K_c) or strain energy release rate (G_c) or at propagation as slope of crack growth resistance curve, is a material property only when certain conditions at the crack tip are met. For example, specimens below the recommended thickness yield plane stress conditions at the crack tip and result in higher K_c and G_c values that are geometry dependent and can only be compared among specimens with the similar dimensions. Norman et al. [27] demonstrated that measured values of K_c and G_c for 3 mm thick bovine compact tension specimens, giving plane stress conditions at the crack tip, were higher than 7 mm thick specimens, giving plane strain conditions at the crack tip. Because cortical thickness frequently varies in inbred mouse strain and with aging, treatment and disease, the measured difference from fracture tests on whole bone may reflect both the changes in geometry and material properties.

Which bone to test?

Both mouse and rat skeletons offer a choice of at least five different long bones for whole bone fracture tests including femur, humerus, third metatarsal, radius and the tibia. The methods proposed by Ritchie et al. [38] are valid for both thick and thin bones, however, the selection of an appropriate long bone for testing may be dictated by biological considerations including the turnover rate, site of interest (for example, site of fracture healing) and by mechanical considerations that reduce the errors related to deviations from theory and provide values close to the published material properties. Although neither material level fracture toughness values nor whole bone fracture toughness values of long bones from same mouse skeletons are currently available, a recommendation for an appropriate bone to test can be based on similar tests on unnotched specimens. In particular, similar to a three point bending test on unnotched whole bone, the fracture toughness test of a whole bone that is notched and subjected to crack initiation and propagation under tensile mode requires that bone has a straight morphology with a uniformly round and thick cross-section. Using the above criterion, and testing different long bones from skeletons of thirteen C57BL/6H (B6) and twelve C3H/HeJ (C3H) mice, Schriefer et al. [28] found that mouse radius produced accurate and most consistent results. When tested in the largest possible aspect ratio (see below), the ratio of high cortical thickness to periosteal radius in the mouse forearm radius increases the ratio of bending to shear contributions and minimizes the ring-type deformation associated with thin bones that lose circular shape and become oval under load [28]. Fig. 1 provides representative cross-sectional images of each of these bones from B6 and C3H and photographs of the testing set-up.

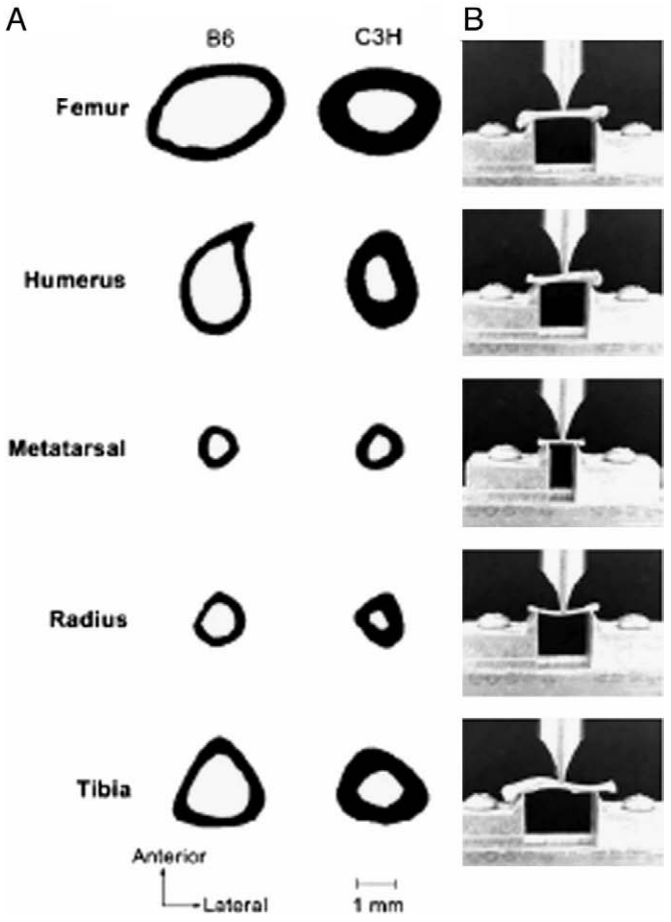

Fig. 1. (A) μCT images of different mouse long bones at the midshaft region where notching and failure occur. (B) Testing set-up showing largest possible span length for different mouse bones (reprinted from the Journal of Biomechanics Vol. 38. Schriefer J, Robling A, Warden S, Fournier A, Mason J and Turner C. A comparison of mechanical properties derived from multiple skeletal sites in mice. p.469 ©2005, with permission from Elsevier).

Additional testing considerations specific to mouse bones

Unlike machined human bone specimens where machining of a specialized notch and precracking are accomplished through automated machining of standard specimens held in fixtures [21,27], mouse bones are of the order of a few centimeters and do not lend themselves to similar procedures. Thus, ease of handing while notching and testing is an important factor to improve reproducibility of results in mouse bones. In general, the strength tests do not follow the practice of sawing off the ends of long bones and are able to achieve fracture through the use of a narrow and long upper loading fixture (see Fig. 1). The use of the whole mouse bone (Length 12–15 mm), instead of a 4.5 mm diaphyseal section recommended by Ritchie et al., [38] is likely to improve the ease of handling without affecting the results because the beam overhung outside the three loading points does not influence the test. More importantly, the use of whole mouse bone will allow an increase in span length to 10 mm [28] and increase the aspect ratio (Length/Diameter) of the femur to 6.0 mm from 2.74 reducing the error in toughness measurements due to relatively higher shear deformation.

Furthermore, the use of a microcomputed tomography (μCT) equipment is suggested as an alternative to digital calipers/SEM