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In situ observation of fracture behavior of canine cortical bone under bending

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ABSTRACT

Cortical bone provides many important body functions and maintains the rigidness and elasticity of bone. A common failure mode for bone structure is fracture under a bending force. In the current study, the fracture behavior of canine cortical bone under three-point bending was observed in situ using an atomic force microscope (AFM), a scanning electron microscope (SEM), and an optical microscope to examine the fracture process in detail. Nanoindentation was carried out to determine the elastic modulus and hardness of different building blocks of the canine cortical bone. The results have shown that the special structure of Haversian systems has significant effects on directing crack propagation. Although Haversian systems contain previously believed weak points, and microcracks initiate within Haversian systems, our findings have demonstrated that macro-cracks typically form around the boundaries of Haversian systems, i.e. the cement lines. Micro-cracks that developed inside Haversian systems have the functions of absorbing and dissipating energy and slow down on expanding when interstitial tissue cannot hold any more pressure, then plastic deformation and fracture occur.

1. Introduction

Bone as an organ plays an essential role in performing many important functions for the human body. These functions include supporting weight; framing body shapes; protecting inner soft tissues; assisting in movement; providing sites for muscle, tendon and ligament attachments; and operating as chemical and mineral reservoirs. Therefore, it is critical to understand how bone remodels itself by constantly degrading and reforming, to identify the plastic deformation threshold under different types of pressures, and to accurately monitor the crack initiation and fracture development process in order to generate an effective way to maintain a healthy bone structure, to avoid possible skeletal cleavages, and to be able to treat and heal bone damages more efficiently.

The basic unit of mature human cortical bone is the Haversian system, or secondary osteon. Haversian systems form when disordered, immature woven bone is resorbed and replaced by highly organized, mature lamellar bone during bone remodeling [27]. A fully grown Haversian system shapes as a round or oval cylinder of 200–300 μm diameter and is composed of a central Haversian canal of blood vessels, nerves, loose connective tissue, and up to 20 concentric lamellae, all running in the longitudinal direction of a bone [31,45]. Between adjacent Haversian systems appear the interstitial lamellae, which are remnants of earlier Haversian systems after remodeling. The boundary that separates Haversian systems from each other and neighboring interstitial lamellae is called the cement line. Small spaces, named lacunae, house osteocytes and bone cells and are arranged circumferentially inside Haversian systems, while canaliculi interconnect osteocytes as a web of small canals. This system is clearly very different from other forms of mammalian bones, such as primary and plexiform bones, which are incapable of remodeling. The presence of Haversian systems physically weakens bone because of a reduction in the actual bone amount, thus in calcium, in order to fill in weaker materials such as blood vessels [9,28]. This raises questions such as why Haversian systems exist and what mechanical strengths they can provide, especially under heavily pressured conditions.

Most mechanical properties of bone are provided by the cortical, or compact, portion of a skeleton. Calcium phosphate hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$, makes up 70% of human bone, and provides a rigid and hard texture, while the other 30% of bone is composed of water and organic substances such as type I collagen, which provides elasticity

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and dissipates fracture energy [14,45]. When a bone is highly mineralized, it can be hard but also brittle at the same time, losing the ability to deform [14]. Thus, it is important to understand the unique balance between toughness and elasticity of a natural bone and to study how bone as a whole reacts to extrinsic strengths with this specially combined property.

Compression, tension, and shear are the three major forces confronted in nature and can lead to bony failure. Fracture in bone is considered to be especially strain-controlled [43,52]. Often, long bones fail as a result of torsion or bending loads, or the two combined [7,11,16]. In case of trauma with direct, concentrated impact force from the outside environment, healthy long bone can often break in a transverse fashion, which can be resembled by a bending force at a lab setting. The rigid texture of cortical bone helps the body to stand against outside pressures such as bending, torsion, compression, and tension. However, once beyond its yield strength, plastic deformation occurs and newly developed micro-cracks that cannot be rapidly repaired can lead to macro fracture. In the current study, a micro-crack is defined as a crack that cannot be identified by the naked eye alone and is measured in μm or even millimeter, while a macro-crack is the major fracture line that breaks through a bone specimen and is detectable without the aid of any device. Recent research has provided limited information on the patterns of micro-crack and macro-crack formations in Haversian cortical bone under pressure in both lab animals and cadavers [11,12,27,28,35,36,40,41,49,50], and few studies have explained the exact developing process under gradually increased load [24]. It has been reported and summarized that in order to resist fracture, bone's toughness is derived through four mechanisms with structures in scales from submicrometers to hundreds of micrometers interacting with each other: 1) micro-cracks consume energy and affect the course of macro-crack development with deflection and twist at the weak boundaries of Haversian systems, deviating it from the direction of maximum tensile stress; 2) between a macro-crack and the surrounding micro-cracks exist regions that are bridged, i. e. unbroken, in order to oppose macro-crack propagation; 3) gaps formed by micro-cracks are bridged by collagen fibrils in order to resist micro-crack expansion; and 4) micro-cracks around a developing macro-crack are constantly repaired by the bone, and the micro-cracks dilate the area around the developing macro-crack to compress the macro-crack [25,42,53]. The emphasis of the micro-crack behavior has been focused in the vicinity of a macro-crack, but the relationship to Haversian systems and the benefit of this particular structure has yet been unclear.

Furthermore, most experiments have been done under a macro- or micro-scale [12], but a more subtle examination is still, to a large extent, absent. The mechanical properties of bone are determined by its composition along with not only macro- and micro- but also some nano-structures [24]. Previous studies have pointed out the importance of bone's nano-scale building blocks in bone's mechanical robustness and functions. Mineral crystal platelets from a typical 10–20 nm up to 200 nm in diameter embedded in a soft matrix of collagen fibrils of typically 80–100 nm in diameter help to dissolve energy from outside and to tolerate flaws [4,14,15,18,22,24,46,51]. Gupta et al. examined tissue, fibrillar, and mineral strain under tension and calculated a ratio of 12:5:2 [17]. Although they discussed the load transfer among nano-structures could protect the brittle mineral component from failing [17], the exact behavior of nano-scaled particles in the fracture process is still unclear. Therefore, further research is needed to use devices that allow detection of the behavior of extremely small structures inside the bone matrix.

The atomic force microscope (AFM) and scanning electron microscope (SEM) are devices that can identify features that might be missed by an optical microscope and are utilized in the current study. AFM specifically is known to examine structures on a nano-scale in research settings and provides the advantage of enabling observation on the fracturing process while step by step bending. It is valuable to detect micro-cracks. However, AFM's sensitivity decreases around a macro-crack as the crack deepens and enlarges. Therefore, an optical microscope and an SEM are also utilized to aid observation.

Past research has concentrated on interval or end results of fracture behavior but not followed through the process step by step under constant loading on detailed images, although mechanical quantification has been achieved step by step [23,28,30]. Therefore, observation on the behavior of micro-cracks that have eventually merged with macro-cracks could be lost. The current study focuses on subtle structural behavior changes on a micro-structure level during a step by step fracture process and the relation of these changes to Haversian systems. We hypothesize there is a difference in structural behavior between the areas inside and outside Haversian systems during the fracture process due to their unique mechanical properties. The objectives of the current study are to better understand the fracture development process of cortical bone; to examine the behavior of Haversian systems and their surrounding structures under a stressful environment, i.e. under a fracturing bending force; and to elucidate the functions and values of the Haversian systems in such a fracture process.

2. Materials and methods

2.1. Sample preparation

Canine cortical bones were used in this study due to their micro morphological comparability to human bones. Among non-human primates, canine cortical bones, especially the ones from small dogs, seem to be one of the most competent among the popular research animals [1,2,20]. For example, canine hind leg bones have similar weight bearing properties to the ones of humans and often contain abundant Haversian structures.

Two fresh femoral shafts with diameters of approximately 1.3 cm were removed from the two hind legs of a healthy one year old male beagle. The materials were obtained from the Department of Orthopaedic Lab at the Medical University of South Carolina. The bones were stored under −20 °C prior to testing. Specimens were made from a transverse section of canine hind leg bone (Fig. 1a) for both AFM

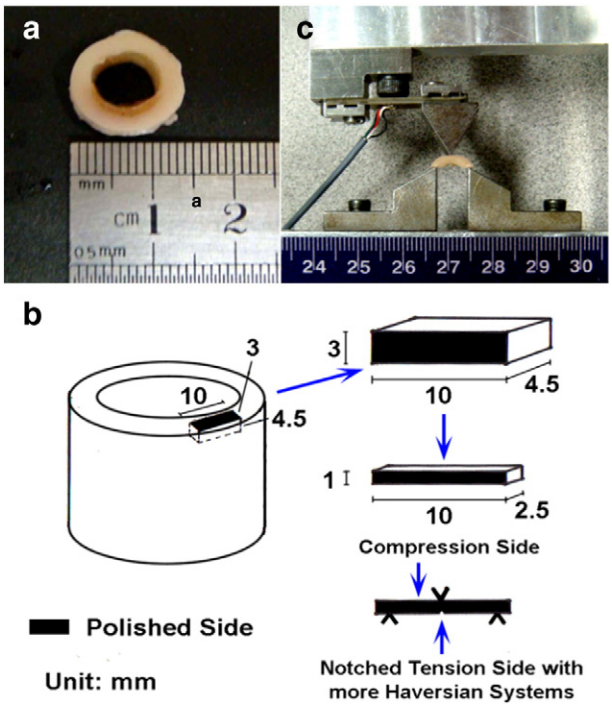

Fig. 1. (a) Transverse section of canine femoral bone; (b) bending test specimen dimensions; (c) the three-point bending tester holding a bone specimen.