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Microscopic assessment of bone toughness using scratch tests

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ABSTRACT

Bone is a composite material with five distinct structural levels: collagen molecules, mineralized collagen fibrils, lamellae, osteon and whole bone. However, most fracture testing methods have been limited to the macroscopic scale and there is a need for advanced characterization methods to assess toughness at the osteon level and below. The goal of this investigation is to present a novel framework to measure the fracture properties of bone at the microscopic scale using scratch testing. A rigorous experimental protocol is articulated and applied to examine cortical bone specimens from porcine femurs. The observed fracture behavior is very complex: we observe a strong anisotropy of the response with toughening mechanisms and a competition between plastic flow and brittle fracture. The challenge consists then in applying nonlinear fracture mechanics methods such as the *J*-integral or the energetic Size Effect Law to quantify the fracture toughness in a rigorous fashion. Our result suggests that mixed-mode fracture is instrumental in determining the fracture resistance. There is also a pronounced coupling between fracture and elasticity. Our methodology opens the door to fracture assessment at multiple structural levels, microscopic and potentially nanometer length scale, due to the scalability of scratch tests.

1. Introduction

Bone has evolved to a fascinating structure, being lightweight and yet tough. Nonetheless, accidents, injuries or diseases make bone fracture a common phenomenon with an estimated average of 2 fracture events per individual during their lifetime. Unfortunately, bone fracture is also among the 20 most expensive medical conditions, leading to a heavy socio-economical burden(Office of the Surgeon General (US), 2004). Like most biological tissues, bone has a complex hierarchical design. It is mostly made up of collagen, hydroxyapatite and water molecules arranged precisely into 5 distinct length scales - nanoscale (mineralized collagen fibril), sub microscale (single lamella), microscale (lamellar structure), mesoscale (osteons) and macroscale (whole bone). Fracture occurrences in bone are related to the quality of bone, which in turn is influenced by several biological factors, the mechanical behavior and the micro-structure. Hence, understanding the toughness and fracture properties of bone will help in developing better orthopedic treatments.

Despite many investigations, the fracture response of bone is not fully understood. On the one hand, previous approaches have been limited by the use of single-size specimens in single mode (mode I or mode II) fracture. The challenge here consists in capturing the inelastic behavior that manifests at several length scales under complex loadings. To-date most fracture assessment methods for compact bone rely on pure mode I testing and very few mixed-mode investigations (Norman et al., 1996; Zimmermann et al., 2009, 2010) have been reported. Early studies focused on linear elastic fracture mechanics (Norman et al., 1995; Phelps et al., 2000), based on traditional fracture tests such as the compact tension test (Behiri and Bonfield, 1984, (1989; Bonfield and Datta, 1976; Norman et al., 1995; Wright and Hayes, 1977) or the three point bending test on single-edge notched specimens (Lucksanasombool et al., 2001; Robertson et al., 1978; Yan et al., 2006). However, bone exhibits a rising *R*-curve behavior (Malik et al., 2003; Nalla et al., 2004a, 2005a; Vashishth, 2004) due to multiple toughening mechanisms (Yeni et al., 1997) such as microcracking (Vashishth et al., 2000), diffuse damage (Diab and Vashishth, 2007; Parsamian and Norman, 2001), fiber bridging (Nalla et al., 2004b), crack deflection (Koester et al., 2008) or osteon pull-out (Cooke et al., 1973). As a result, the fracture energy or critical energy release rate is a better metric than the critical stress intensity factor because it also accounts for the non-linearity of the

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behavior or the presence of plastic flow during the fracture of bone (Yan et al., 2007). Moreover, recent works (Yang et al., 2006a,b) have recommended the use of a multi-parameter fracture criteria such as cohesive fracture to fully capture the complex behavior. Yet the coehsive fracture model was calibrated by testing specimens of the same size. In contrast, a recent study (Kim et al., 2013) showed that due to significant size effects, the fracture parameters must be assessed by testing specimens of different sizes. The reason is the non-uniqueness of the work-of-fracture method or cohesive fracture method for tests on same-size specimens. Thus, new methods are needed that can incorporate nonlinear fracture mechanics, size effects and mixed-mode fracture.

On the other hand, previous studies have evaluated the fracture parameters of the macrostructure instead of probing the behavior at the level of the microstructure (haversian system, osteon or single trabeculae), sub-microstructure (single lamella) or the nanostructure (fibrillar collagen). Because bone is a multi-scale material, it is important to capture the local behavior at smaller length scales to gain a better understanding of crack initiation, propagation as well as toughening mechanisms. A couple of methods have been proposed—indentation fracture and nanoscratch tests–yet a rigorous framework is still lacking. Although indentation fracture was introduced (Mullins et al., 2007) to access the fracture toughness of bone as a function of crack length, those tests tend to be unreliable and highly subjective due to the need to measure the crack length, which highly depends on the observer's skill (Quinn and Bradt, 2007). In another study, nanoscratch tests were employed in an attempt to assess fracture toughness at the ultra-structural level (Islam et al., 2012). However, owing to the use of a plastic limit model, the resulting metric, scratch work consumed per unit volume of the scratch groove, is very akin to a strength measurement. Thus, new methods must be developed to evaluate the fracture characteristics at the microscopic level.

Herein, we implement a novel approach, micro-scratch testing, to quantify the fracture toughness of bone at the mesoscale (osteon level) and micro-scale (concentric lamella). We use a recently introduced experimental and analytical framework (Akono et al., 2012, 2011; Akono and Ulm, 2011, 2012) based on non-linear fracture mechanics. The technique used here allows for fracture characterization in both the longitudinal and transverse directions at the micro scale. In what follows, we first present the experimental procedure followed by a brief review of the scratch test model and a detailed discussion of results.

2. Materials and methods

2.1. Specimen preparation procedure

Fresh bones from 22–26 weeks old porcine animals were harvested 24 h after slaughter from the Department of Animal Sciences at the University of Illinois, Urbana-Champaign. The donor pigs had an average diet of corn and weighed about 275 lbs. To keep the bone fresh, femurs were stored at −20 °C before use. Cortical bone specimens were thawed and then sampled using first a table-top band saw and then a diamond precision saw (Isomet 5000®, Buehler, LakeBluff, IL). Two set of specimens were prepared to observe the fracture behavior in two different orientations. On the one hand, longitudinal-transverse (LT) specimens were used to study fracture perpendicular to the long axis of bone and they were cut into about 5-mm thick sections as shown in Fig. 1. On the other hand, short longitudinal (SL) specimens, approximately 15-mm long and 10-mm wide, were used to study fracture parallel to the long axis of bone. After sectioning, these smaller pieces were defattened, defleshed and then cleansed in a solution of 1.5% Alconox + 5% bleach. For further ease of handling during polishing and testing, each individual section was embedded in polymethylmethacrylate, allowed to cure for 8–9 hours, cut into 5-mm thick discs using the linear precision diamond saw and mounted onto aluminum discs using cyanoacrylate adhesive. To assure a smooth and flat surface finish, a rigorous grinding and polishing operation was carried out. Samples were ground using consecutively a 400, 600, 800 and 1200 grit size of alumina oxide abrasive pads. Wet coarse and fine polishing was ensued using consecutively 3, 1 and 0.25 μm diamond suspension solutions along with TexMet P® (Buehler, LakeBluff, IL) polishing cloths. Finally, a 0.05 μm Microcloth (Buehler, LakeBluff, IL) pad was used for ultra fine polishing. The specimens were wrapped in a gauge soaked in Hank's Balanced Salt Solution to keep them wet and then stored at 4 °C until tested.

2.2. Scratch testing

A scratch test is performed by pulling a hard stylus across the surface of a softer material, under a linearly increasing vertical force. The method can be traced back to the mineralogist Friedrich Mohs in 1820 and is currently extensively used in many materials science applications, including strength assessment of rocks (Bard and Ulm, 2010), damage of polymers (Wredenberg and Larsson, 2009), and adhesion and cohesion properties of thin films and coatings (C1624-05, 2015; Randall et al., 2001). We employed an Anton Paar Micro Scratch Tester (MST) (Anton Paar, Switzerland), with a maximum loading capacity of 30 N, and used a Rockwell C diamond scratch probe. The Rockwell C is a conical probe with a half-apex angle of $\theta = 60^\circ$ and a spherical tip of radius $R = 200$ μm: in particular, the transition from hemisphere to cone happens for $d/R = 0.13$, d being the penetration depth. During the test, the scratch forces (both vertical and horizontal) and the penetration depth are continuously measured using high-accuracy linear variable differential transformer sensors. In addition, fracture-induced acoustic waves are recorded by the embedded acoustic sensors.

3. Microscopic scratch testing of cortical bone

Fig. 2 illustrates the micro-scratch test procedure on cortical bone for both the longitudinal-transverse and short-longitudinal directions. LT and SL specimens characterize the fracture toughness perpendicular and parallel to the long axis of bone respectively. Due to the small scale of our experiments and the natural variability of bone, numerous complications needed to be addressed. First, bone exhibits a high local variability in osteon density, shape and size. Since, the toughening mechanisms and hence the fracture toughness of bone are greatly influenced by surface features, this variability demands meticulous efforts in finding appropriate spots to create scratches. Second, the specimens must always be kept wet in order to mimic *in-situ* conditions, requiring a constant relative humidity during experimentation. Last but not least, a major problem, especially for LT specimens, is the surface area available to create scratches of a desired length. Porcine femur bones are thin-walled allowing only 2-mm long scratches in LT specimens. For the same reason, in SL orientation, samples are thin and need careful polishing to ensure a reasonable specimen thickness.

3.1. Experimental protocol

In order to obtain accurate measurements, it is important to use a scratch probe that is clean and damage-free. Before conducting scratch tests, the tip of the stylus is observed under an optical microscope to make sure it is pristine. The sample is then mounted on the testing platform and a desired area to create scratches is found by monitoring the surface through optical microscope. Scratches are carried out at a loading rate of 60 N/min on both LT and SL specimens as per the parameters shown in Table 1.