Nanomechanics of antler bone

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Abstract

Antler bone is an example of a biologically produced composite that contains a high volume fraction of nanofibrous mineralized collagen fibrils (MCFs). As antler bone is a material with high toughness, the failure of antler bone is a considerable energy absorbing process and involves pullout of MCFs from the surrounding bone material. In this work the failure of antler bone is evaluated by pullout of individual MCFs from the bone material using in situ nanomechanical atomic force microscopy (AFM) testing combined with scanning electron microscopy (SEM) imaging in order to directly quantify the interfacial shear strength between MCFs. The interfacial shear strength is observed to vary with pullout rate from around 0.4 to 0.8 MPa. Critically, the interfacial strength increases as the pullout rate decreases, indicating potential for interfacial bond reformation at the nanoscale.

1 Introduction

Antler bone is acknowledged as possessing extraordinary mechanical properties where both high strength and fracture toughness is achieved due to the unique architecture of an organized organic–inorganic composite system [1]. In recent years, significant effort has been directed toward the study of mechanical properties especially the considerable fracture toughness of bone. Specifically, bone is hierarchically structured and the origin of its excellent mechanical properties is contentious with the structural organization over different length scales requiring consideration. However, mineralized collagen fibrils (MCFs) can be defined as a building block of bone and is believed to be crucial in defining the mechanical and fracture behavior of bone in recent studies [2]. While the structure of bone varies over various length scales, MCFs are the distinct unit at the nanoscale with typical diameters of 100 nm that are used to construct the larger length scale structures. Figure 1 highlights the importance of MCFs in the composition of antler bone with a fracture surface clearly displaying a high volume fraction of MCFs protruding from the surface. The small spaces between MCFs are defined as non-collagenous proteins (NCPs) and typically ensure MCF spacing of the order of 1-2nm [3]. The antler bone in Figure 1 can therefore be considered as a high fiber volume fraction reinforced polymer composite. Importantly, the NCP region between the collagen fibrils is expected to be critical in defining the toughness of whole bone but is often poorly understood.

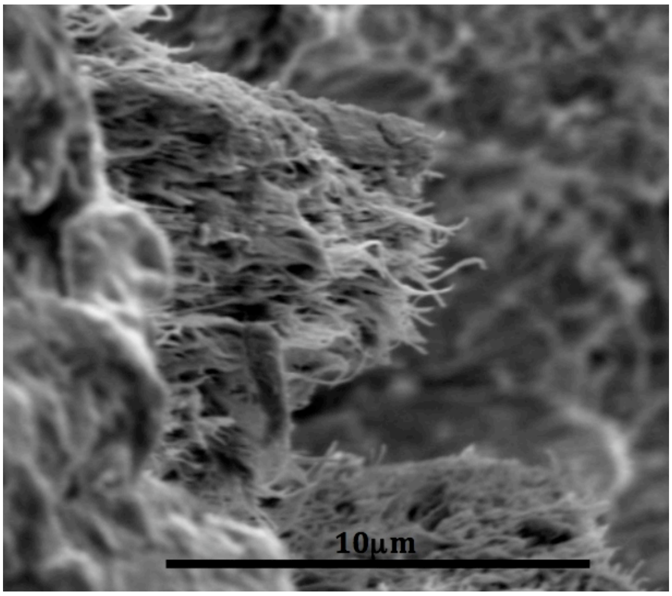

Figure 1. Scanning electron micrograph of the fracture surface of antler bone. The high volume fraction of mineralized fibrils exposed at the fracture surface is clearly observable.

Recent work has indicated deficiency of specific proteins in NCPs cause loss of bone strength, while the transfer of stresses between collagen fibrils is expected to be critically dependent on the mechanical behavior of the NCP region [4, 5]. Indeed, classical mechanical analysis of composites incorporating fibers bound together by a polymer matrix highlights the importance of the fiber-matrix interfacial adhesion on stress transfer. Composite theory has been extensively exploited in nanocomposite interfacial mechanics, such as efficient stress transfer at carbon nanotube-polymer interfaces [6], but is rarely utilized in understanding the effect of nanoscale interfaces between the collagen fibrils in bone. The mechanical characteristics of the NCP interface region between collagen fibrils in bone therefore remains elusive despite its importance in bone and other fibrous material toughness.

Direct evaluation of synthetic nanoscale interfaces has been achieved using advanced atomic force microscopy (AFM) techniques, utilized to manipulate and remove nanofibers partially embedded within a polymeric matrix material [6]. These direct nanofiber pullout measurements give quantitative information on the mechanical behavior of the interface between the nanofiber and matrix, allowing the evaluation of both strong and weak nanocomposite interfacial mechanics. This paper therefore exploits the direct mechanical testing ability of the AFM combined with the imaging capabilities of scanning electron microscopy (SEM) to evaluate the interfacial properties at collagen fibril-NCP interfaces in bone using a pullout configuration.

2 Materials and testing methods

Individual mineralized collagen fibrils were pulled out from rehydrated bone sample using in situ AFM-SEM. The details of this experimental setup are described in previous publications [2]. Figure 2 shows the SEM image of an AFM system where the pyramidal AFM tip is attached to an individual MCF protruding from the antler bone fracture surface. This attachment is achieved by first moving the apex of the AFM tip into an epoxy glue (Poxipol, Arg.) and then translating the tip position towards the free end of the MCF until contact between the glue and fibril is achieved. Curing of the glue within the SEM occurs over a time frame of the order to 10 minutes.

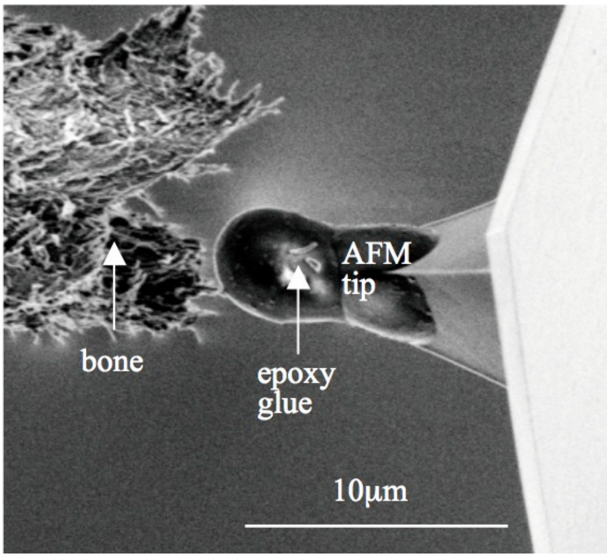

Figure 2. Scanning electron micrograph of the pullout experimental setup. Note the individual MCF is between the epoxy glue and the bone surface.

The AFM tip can be translated away from the bone surface until, at a critical force, the MCF is pulled away from the fracture surface. We note that pullout is observed from the SEM imaging when the length of the pulled out MCF is larger than the initial length of the MCF between the AFM tip and bone surface in Figure 2. Many attempted MCF pullouts cause fracture of the MCF and have been evaluated previously [2]. The AFM system recorded a linear increase of applied force during the translation of the AFM tip away from the bone fracture surface in pullout experiments. This linear force increase indicates that the fibril was pulled out as opposed to fracture of the fibril length, which would cause a drop in the recorded force, at a position within the bone followed by pullout.

The mechanical properties of the NCP interphase region around the MCFs is calculated by recording the maximum force $F_{\max}$ applied to the MCF by the AFM system. The strength of the interface between the MCF and surrounding NCP is characterized by the interfacial shear strength (τ_i) and is calculated from the maximum force applied to the exposed MCF to cause pullout from the surrounding NCP using:

$$\tau_i = \frac{F_{\max}}{\pi D l_e} \qquad (1)$$

Where D is the fibril diameter and l_e is the length of MCF embedded within the bone. Both of these parameters are measured from the SEM imaging and gave values of $D = 115.6 \pm 33$ nm and $l_e = 674 \pm 223$ nm. Equation (1) assumes the stress generated at the MCF-NCP interface during pullout is uniformly distributed along the fibril embedded length. The strain in the free length of the MCF during pullout testing was less than 1 %, indicating negligible fibril strain contributions to the measured mechanical behavior in this work. The MCF-NCP interfacial shear strength was measured for a series of pullout tests carried out at various pullout velocities. The results of these tests are shown in Figure 3.

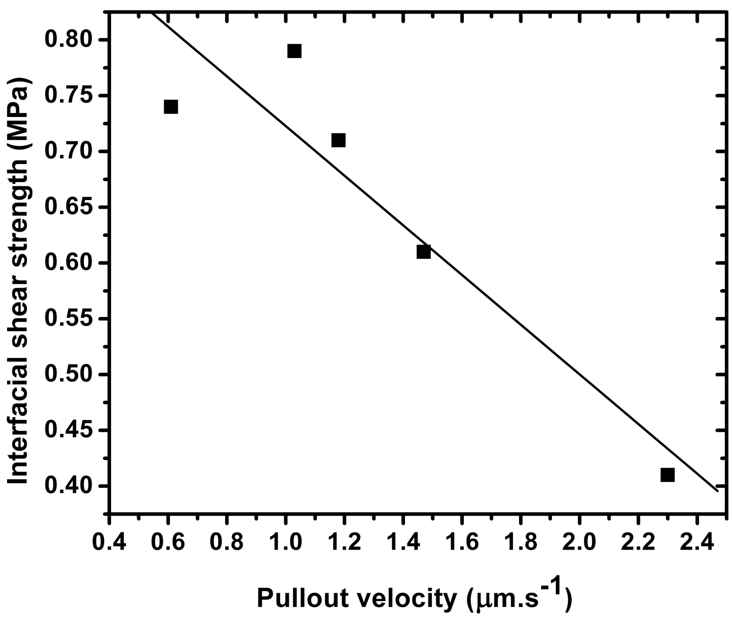

Figure 3. Plot of the velocity when pulling out individual MCFs from antler bone against the resultant interfacial shear strength calculated from equation (1).

The results in Figure 3 indicate that the MCF-NCP interfacial shear strength τ_i is lower than values recorded from pullout of engineering fibers in conventional fiber reinforced polymer composites. However, engineering composites are often optimized for effective stress transfer between the reinforcing fibers and require relatively high τ_i values up to approximately 50 MPa [7]. The MCFs in this work therefore exhibit low interfacial shear strength, which is conducive for toughness as cracks propagating through bone will be deflected at the weak interfaces between the MCFs and surrounding NCP as shown in Figure 1. However, the vast area available at these nanoscale interfaces in antler bone may provide a considerable energy absorbing process.

A clear increase in the interfacial shear strength is observed in Figure 3 when the pullout test velocity decreases. These results suggest that potential interfacial reforming occurs during pullout testing. Thus, low pullout velocities provide sufficient time for bonds to reform at the MCF-NCP interface, resulting in a relatively high interfacial shear strength, whereas larger pullout velocities restrict the ability of the MCF-NCP interface to reform during pullout and results in a relatively low interfacial shear strength. This work therefore indicates that the toughness of antler bone incorporates two distinct energy absorbing mechanisms through i) failure of a large interfacial area, per unit volume of bone material, between MCFs and binding NCPs ii) bond reforming at MCF-NCP interfaces that increase the energy required to fail the interface, especially at lower loading rates. This last mechanism is perhaps of particular interest during repetitive loading, particular during impact loading of antler, as interfaces may potentially completely reform to enhance the structural integrity of the antler bone nanocomposite over its lifetime.

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