Effect of environment on mechanical properties of micron sized beams of bone fabricated using FIB

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Failure of bone at the sub-lamellar level using *in situ* AFM-SEM investigations

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ABSTRACT

In this paper we examine the mechanical properties of individual lamellae from bone material using novel atomic force microscopy (AFM)-scanning electron microscopy (SEM) techniques. Individual lamellar beams were selected from bone using focussed ion beam (FIB) microscopy and mechanically deformed with the AFM while observing failure modes using SEM. Both the elastic and fracture behavior of the bone lamellae were determined using these techniques.

INTRODUCTION

Bone is a fibrous biological nanocomposite material, which is optimized to avoid catastrophic failure [1, 2]. The fracture behavior of bone is expected to be controlled by the various structural features present across the many existing hierarchical length scales [3]. However, micron sized lamellae in bone present the simplest composite unit in bone consisting of mineralized collagen fibrils within a protein matrix, with some work suggesting that this length scale dominates the fracture of whole bone [2]. The fracture process of bone can be described by considering the range of energy absorbing mechanisms such as the plasticity of the collagen phase, crack deflection along bone cement lines, diffuse micro-cracking and the bridging of cracks by ductile phases [4]. Peterlik et al. examined osteonal lamellar bone by controlled crack extension experiments of test specimens with millimeter dimensions and concluded that the micron sized lamellae dominated the fracture of whole bone [2]. The determination of the elastic properties of bone lamellae has been investigated using nanoindentation [5-7] and provides little information on fracture behavior. This paper therefore extends the understanding of bone mechanics by evaluating the deformation behavior of bone to failure at the micron length scales that correspond to the lamellar to sub-lamellar level of bone.

EXPERIMENTS

Femurs of 8-month-old sprague dawley rats were used as a source of bone material. Sub-lamellar bone micro-cantilever beams from these rat femurs were isolated using a dual beam system. The dual beam system is composed of a scanning electron microscope (SEM) and focused ion beam (FIB) working simultaneously. The FIB allows bone material to be milled out in order to pattern fine cantilever beams of bone for subsequent mechanical testing [8]. Micro cantilevers of bone with dimensions 10 x 2 x 2 m were produced using FIB as described in previous work [8]. These dimensions ensured that the shear stress contribution to the bending stress was minimal (<~3.5%) [8].

Mechanical testing was performed using a custom-built atomic force microscope (AFM) (Attocube GmbH, Germany) incorporated within the vacuum chamber of an SEM. The setup allows in situ bending testing of micro-cantilever beams [9, 10] while maintaining the hydrated state of mineralized tissue [8]. Six unnotched bone micro-cantilever beams were tested in cantilever bending to failure.

RESULTS

The elastic modulus and the work-of-fracture for bone cantilever bending to failure tests were calculated from a variation of the equation described by Liu et al. [11]. The nominal work-of-fracture is the area under the force-deflection curve, divided by twice the cross-sectional area of the specimen [11, 12]. The cross-sectional area measurements were taken from top and front SEM images of the each of the micro-cantilever beams.

The elastic modulus and the work to fracture of each of the testing methods were calculated from the force deflection curves using the formulas below.

$$E = \frac{12l^3}{3bh^3} \cdot \frac{f}{\delta} \qquad \text{Equation (1)}$$

$$W = \frac{2A}{bh} \qquad \text{Equation (2)}$$

Where *l, b* and *h* are the length from the base of the sample to testing contact point, breadth and height of the rat bone beam respectively and f/δ is the slope of the force-displacement curve. W is the work-of-fracture and A is the area under the force-displacement curve.

Figure 1 shows the force-displacement curves for six unnotched beams tested to failure. Figure 2 shows SEM screenshots of in situ testing of rat femur bone micro-cantilever beam in bending to failure. There are slight variations in the amount of force that was needed to fracture each beam and can be attributed to changes in collagen orientation within bone as shown previously [2]. Table I details the different Young's modulus and work-of-fracture values calculated for each beam. Since there are no other bone features such as lamellar boundaries and cement lines that could affect the fracture properties of these bone samples, the differences in mechanical properties are expected to be directly correlated to collagen fibril orientation.

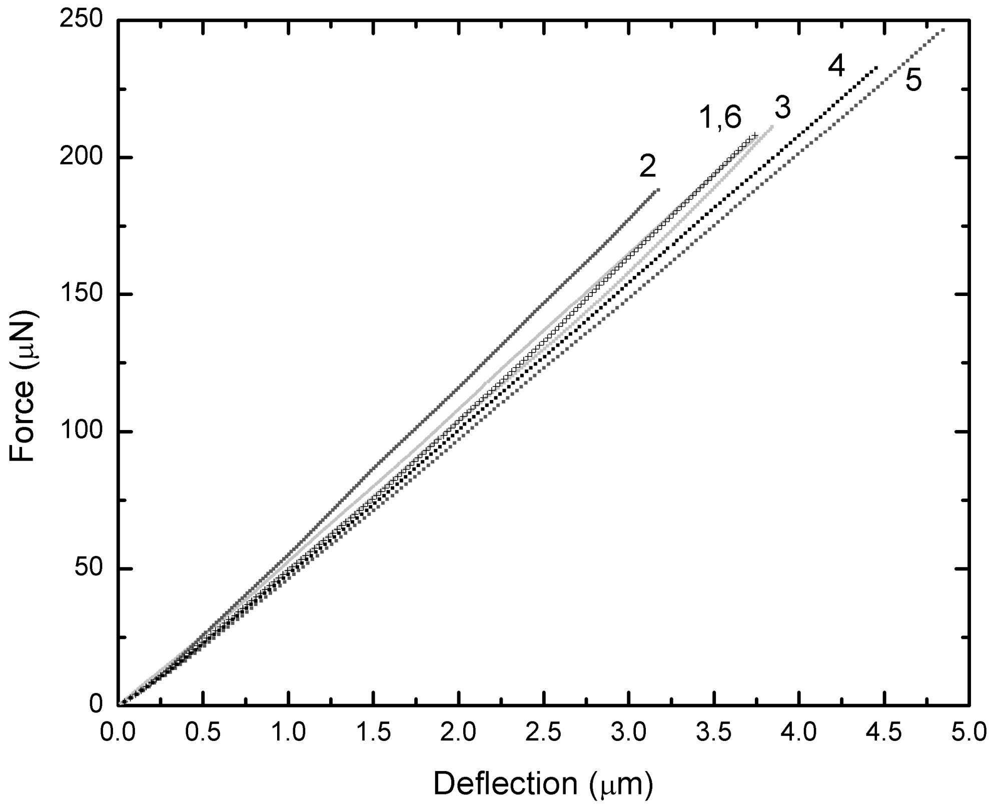

Figure 1. AFM force-deflection curves of the 6 sub-lamellar micro-cantilever beams tested to fracture in bending in this paper. The labeling corresponds to the test number and correlates with the data presented in Table I.

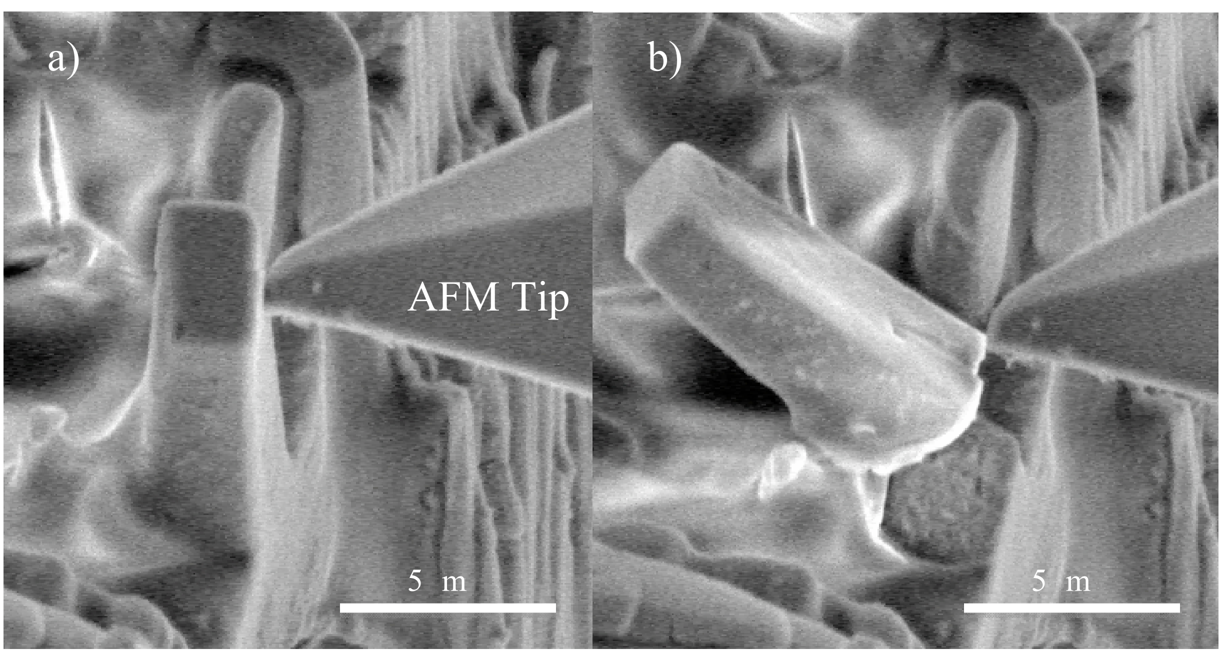

Figure 2. SEM images of in situ cantilever beam testing in bending a) micro-cantilever beam in bending b) failed micro-cantilever beam. There is a 25 tilt to the SEM beam.

Table I. Work-of-fracture and Young's modulus values of rat femur micro-cantilever bone beams tested in bending to failure. Work-of-fracture values are arranged from lowest to highest.

Test No.	Work-of-fracture (Jm^{-2})	Young's Modulus (GPa)
2	99.31	8.1
1	108.80	3.8
6	124.71	6.03
3	141.70	9.4
4	146.61	4.8
5	162.25	3.7

DISCUSSION

Table I shows both the work-of-fracture and the Young's modulus for 6 micro-cantilever beams mechanically tested in bending. Generally, except in the case of test number 1 and 3, the Young's modulus is observed to decrease as the work-of-fracture increases. Work-of-fracture is impossible to compare to previous literature experimental results as it is critically dependent on specimen size and geometry [12]. However, the Young's modulus measured through these experiments are comparable to previous work [6, 11]. The relationship between Young's modulus and work-of-fracture in the bone micro-cantilever beams can be described by consideration of the potential collagen fibril orientation within the beams. Collagen fibrils oriented predominantly along the principal long axis of the beam will provide the highest Young's modulus due to the fibrils lying in the direction of the applied load. Conversely, collagen fibrils oriented away from this principal axis will provide less effective resistance to deformation, resulting in a lower Young's modulus, but failure will be able to occur predominantly at interfaces between the collagen fibrils. We therefore expect that the interfacial failure between the collagen fibrils is extensive during fracture of the micro-cantilever beams when the fibrils are not aligned in the direction of the bending and provide an enhanced work-of-fracture.

CONCLUSIONS

Sub-lamellar bone micro-cantilever beams were created using FIB methods and tested in-situ via a custom built AFM within an SEM. Unnotched bone cantilever beams were tested in cantilever bending. The work-of-fracture and Young's modulus was calculated for each of the beams tested with an inverse correlation between the Young's modulus and work-of-fracture observed.

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