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Technical Note

Effect of age on mechanical properties of the collagen phase in different orientations of human cortical bone

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ABSTRACT

The collagen phase plays an important role in mechanical behaviors of cortical bone. However, aging effects on the mechanical behavior of the collagen phase is still poorly understood. In this study, micro-tensile tests were performed on demineralized human cortical bone samples from young, middle-aged, and elderly donors and aging effects on the mechanical properties of the collagen phase in different orientations (i.e. longitudinal and transverse directions of bone) were examined. The results of this study indicated that the elastic modulus and ultimate strength of the demineralized bone specimens decreased with aging in both the longitudinal and transverse orientations. However, the failure strain exhibited no significant changes in both orientations regardless of aging. These results suggest that the stiffness and strength of the collagen phase in bone are deteriorated with aging in both longitudinal and transverse directions. However, the aging effect is not reflected in the failure strain of the collagen phase in both longitudinal and transverse orientations, implying that the maximum sustainable deformation of the collagen phase is independent of aging and orientation.

Introduction

Bone is a composite material consisting of collagen fibrils, mineral phase, and water [1]. Changes in each of the constituents may inflict significant effects on the fragility of bone. Among them, the mineral phase with greater elastic modulus (~100 GPa) imparts rigidity to bone and makes bone behave anisotropically due to the anisotropic nature and uneven distribution of mineral crystals in different orientations [2]. In the past, the effect of the mineral phase in bone was mainly considered when studying the age-related degradation of bone mechanical properties, such as bone mass loss and the decreased bone mineral density [3,4]. However, recent studies have found that the collagen phase may also play a significant role in the mechanical behavior of bone [5–10].

It has been reported that aging has significant effects on mechanical properties of the collagen phase in cortical bone [9]. Experimental evidence shows that the collagen phase maintains a preferentially organized arrangement of collagen fibrils in healthy bones, but somehow altered in unhealthy tissues [11,12]. Also observed is that the ultimate strength of the collagen network in dentin varies with loading orientations [13]. Since human cortical bone undertakes different loading modes at distinct anatomic locations during daily activities, collagen fibrils at the locations may have different preferred orientations [14,15]. However, it is still not clear whether the aging effect on the mechanical behavior of the collagen phase is orientation-dependent.

In this study, the mechanical behavior (i.e. the elastic modulus, ultimate strength, and failure strain) of demineralized human cortical bone samples was measured in different orientations using a micro-tensile test. We hypothesized that aging effects on the mechanical behavior of the collagen phase are reflected in both longitudinal and transverse orientations of bone.

Materials and methods

Specimen preparation

Middle shafts of fresh-frozen cadaveric femurs from fifteen male donors were procured from the National Disease Research Interchange (NDRI, Philadelphia, PA). These samples were divided into three age groups ($N = 5$): young (20, 24, 25, 26 and 36 years old), middle aged (49, 51, 51, 52 and 55 years old), and elderly (72, 76, 76, 77 and 87 years old) groups. Briefly, bone slices with a thickness around 400 μm were sectioned longitudinally from the same anatomic region (anterior region) of each femur using a low speed diamond saw (Buehler Isomet 2000 Precision Saw, Buehler, Lake Bluff, IL) (Fig. 1). The slices

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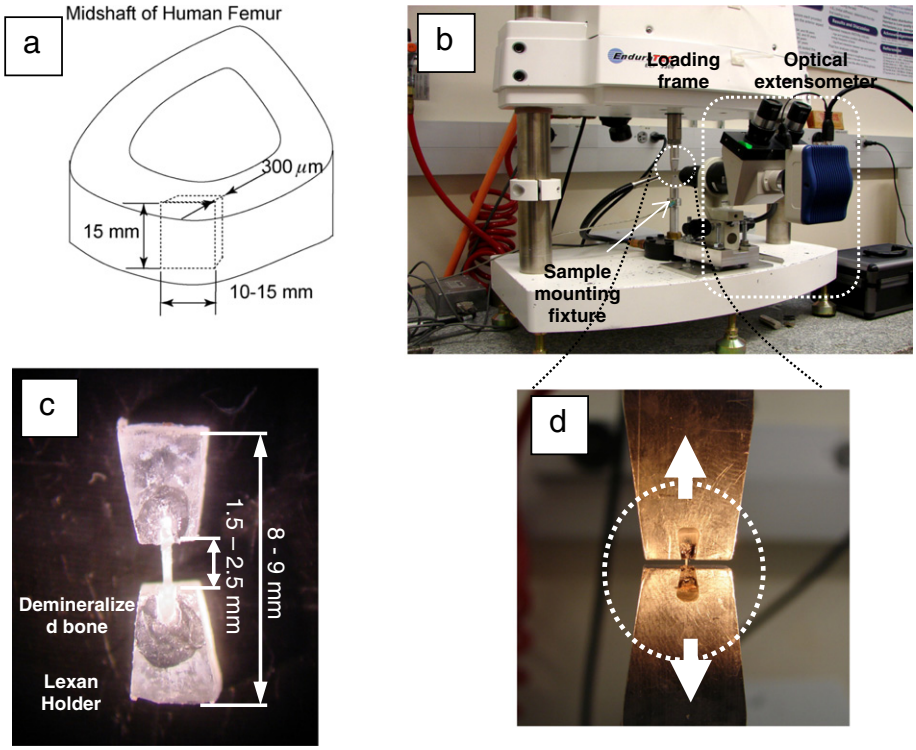

Fig. 1. Preparation of demineralized bone specimens (a) and the setup for the tensile test (b). The specimens were glued onto a pair of plastic (Lexan) holders (c) and mounted on the loading fixtures (d) in the mechanical test machine.

were then lapped down to 300 μm thick in sequential grits of grinding abrasives. Thereafter, all slices were demineralized by soaking them in a sodium citrate solution with formic acid for five (5) days with the solution changed once a day following the protocol reported elsewhere [16]. The completion of decalcification was verified by checking the concentration level of free calcium in the solution by neutralizing it with approximately 5 ml of 0.5 N NaOH and 1 ml of 5% ammonium oxalate solution. Upon completion of decalcification, the samples were washed six times in a sonic water bath with distilled water to remove all traces of acid and soaked in PBS overnight. For ease of cut, all demineralized bone slices were partially dried for 1 hour at room temperature in a vacuum cascader with Drieriter granules. Strips about 500 μm wide and 3.0 mm long were dissected using a surgical scalpel under microscope in both the transverse and longitudinal orientations of bone. The dissected (collagen network) strips were secured in a pair of plastic (Lexan) holders using a cyanoacrylate glue for ease of later tensile tests (Fig. 1). All the specimens were soaked in PBS for at least 1 hour to ensure full hydration prior to mechanical tests. The areas of cross-sections of the specimens were measured under microcopy in reference with a standard micro-graduated slide.

Mechanical testing

The tensile specimens with the plastic holders were attached to a loading fixture in a bench-top mechanical testing system (currently Bose ElectroForce@ 3330) (Fig. 1). All specimens were first preloaded to 4.0 grams in tension, wiped off the water on the surface, and then loaded to failure in tension at a constant loading rate of 0.01 mm/s recommended by the previous studies [17]. Since the collagen phase is viscoelastic in nature, the consistent loading rate can help avoid potential effects of viscoelasticity on the measurements. The image (1280 × 960 pixels) of the side view of the specimens during loading was recorded at a rate of one image per second using a digital microscopic photo system (a monocular microscope with a Motic 3000 CCD camera). Representative images of the specimen pre and post failure are shown in Fig. 2. Strain was calculated using a custom Matlab script that automatically captures the elongation of the distance (in pixels) between two reference spots along the gage length of the specimen on an image-by-image basis. Stress was calculated by dividing the applied force by the cross-sectional area of the specimens measured prior to load. Representative loading strain–stress curves for the transverse and longitudinal specimens are presented in Fig. 2. Elastic modulus was determined as the slope of the linear part of the strain–stress curve measured in a strain range of 0.05. The ultimate strength (σ_u) of each specimen was determined as the maximum stress and the failure strain (ε_f) as the corresponding maximum strain at the failure of the specimen.

Statistical analysis

Two-way analysis of variance (ANOVA) (JMP 5.1, SAS institute Inc., Cary, NC) was used to determine the effects of age and orientation on the ultimate strength and failure strain of the demineralized bone (collagen network) specimens. Simple *t*-tests were performed to detect the difference between the groups. The significance level for all tests was $p < 0.05$.

Results

The descriptive statistics of the mechanical properties of the mineralized bone samples in different age groups and loading orientations are shown in Table 1. Two-way ANOVA analyses indicated that both aging and orientation imposed significant effects on the elastic modulus and ultimate strength, but had little influences on the failure strain of the demineralized bone specimens (Table 2).

The multiple comparisons indicated that the elastic modulus (E) of the collagen network decreased with age in both longitudinal and transverse directions. The significant difference in E was observed