Age-related change in the damage morphology of human cortical bone and its role in bone fragility

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Abstract

Application of cyclic loading results in the formation of distinct strain-dependent microdamage morphologies. It is still unknown; however, how the morphology of microdamage affects age-related increase in bone fragility. In this study, four-point bending fatigue tests were conducted on aging human bone (age 26 to 89) in conjunction with histological evaluation of the resultant tensile (diffuse damage) and compressive (linear microcracks) damage to identify the damage morphologies associated with an increase in age-related bone fragility. The results demonstrate that young donors (38 ± 9 years) had a longer fatigue life (P < 0.05) and formed more diffuse damage than the older donors (82 ± 5 years) (P < 0.05). In contrast, old donors had a shorter fatigue life and formed more linear microcracks than the younger donors (P < 0.05). Linear microcracks were longer in older than in younger donors (P < 0.05) and were associated with weak lamellar interfaces. Areas of diffuse damage were, however, larger in younger than in older donors (P < 0.05), and these showed no relationship with the lamellar arrangement of bone. These findings show, for the first time, that the propensity of bone to form a particular damage morphology is subject to change with age and that the propensity of young donors to form diffuse damage over interlamellae linear microcracks plays a critical role in the ability of bone to dissipate energy and resist a catastrophic fracture. Age-related changes in damage morphology may therefore be an important contributor to the increased bone fragility in the elderly.

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Introduction

Application of cyclic loading on cortical bone results in the formation of microdamage. In vivo, microdamage accumulation depends on the imbalance between the rate at which the remodeling process repairs fatigue damage and the rate at which microdamage is initiated [6,15,16]. In vivo, microdamage occurs in the form of linear microcracks [18,24,37] and diffuse damage [11,30]. Damage morphology (linear microcracks or diffuse damage) is a combined function of local strain and tissue properties [3,5,9,22]. Linear microcracks form under compressive loading [3,9,22] in the interstitial bone tissue and stop at osteonal boundaries [3]. In contrast, diffuse damage forms under tensile loading [3,9,22] as submicroscopic cracks in the interstitial bone [3].

Burr et al. [5] demonstrated that modulus loss has a linear relationship with diffuse damage and a quadratic relationship with linear microcracks. They also found that tensile damage accumulates more easily but crack growth is greater in compression [5]. Burr et al.’s results are consistent with the computational models of Martin et al. [17] and Griffin et al. [12] who showed that tensile damage is self-limiting but compressive damage is not. Furthermore, in a recent study, we showed that cortical bone compartmentalizes the damage morphologies in different regions and the sequence of damage production in different phases of cyclic loading to dissipate energy and resist a catastrophic fracture [9]. We hypothesize that the predisposition
of bone to form linear microcracks and not diffuse damage may be a significant contributor to bone quality and age-related increase in bone fragility.

Age-related changes at the microstructural level, especially at the lamellar interface (i.e. the interlamellar boundary), may alter the mode and magnitude of microdamage formation. Previous studies have shown that the lamellar interface is a site of shear crack formation [13] and contributes significantly to the fracture process under tensile creep [7]. Furthermore, it is well known that in fibrous composite materials the internal interfaces play a major role in damage development during fatigue loading [21]. However, the role of the lamellar interface and its relationship with damage morphology in bone has not been investigated.

Thus, the aim of this study is to investigate the role of linear microcracks and diffuse damage production in the age-related increase in bone fragility and to study the effects of the lamellar interface on damage morphology.

Materials and methods

Thirty-two rectangular parallelepiped beams (4 × 4 × 48 mm) were wet-machined on a CNC milling machine (Micromill 2000, Denford, Medina, OH 44256) from the anterior and posterior quadrants of sixteen human cadaveric tibiae (age 26 to 89). These specimens were separated into two groups (Young < 50 years, N = 16, mean age = 38 ± 9 years; and Old > 75 years, N = 16, mean age = 82 ± 5 years) and subjected to fatigue loading in a four-point bending configuration on a servo-hydraulic testing machine (Bionix Model 858, MTS, Eden Prairie, MN 55344).

All specimens subjected to bending fatigue were loaded in an anatomical configuration to induce compression on the endosteal side and tension on the periosteal side. Because the initial bending modulus was expected to vary between individual specimens, the testing protocol included cycling (20 cycles) of each specimen to a low load (100 N) in order to determine the modulus for each specimen. Specimen modulus was used to calculate the normalized load required to produce an initial strain of 500 μstrain at the mid-span using the following equation:

\[ F = \frac{2Ebh^3e}{3l} \]

where ‘E’ is the specimen modulus, ‘ε’ is strain (ε = 500 μstrain), ‘b’ is specimen width, ‘h’ is the specimen height and ‘l’ is the inner support length (l = 20 mm).

Each specimen was fatigue loaded inside an environmental chamber (37°C, Physiological Saline) under load control at 2 Hz up to its normalized load. During each test, the maximum cyclic load and displacement were measured to determine the change in specimen modulus as a function of loading cycle. The test was stopped at a point corresponding to 60% modulus loss. Boyce et al. [3] have demonstrated that loading of human cortical bone specimens in four-point bending load configuration to an initial strain of 5000 μstrain and 60% modulus loss successfully captures all three characteristic phases of fatigue-induced modulus loss without catastrophic failure.

Following fatigue loading, six randomly selected specimens in each group were selected for histological analysis of damage. Due to the symmetrical loading configuration in the four-point bending setup, only half the beam was assigned for histological assessment of damage (Fig. 1). The preparation of sections included en bloc staining in 1% basic fuchsin. Following staining, specimens were embedded in polymethyl methacrylate (PMMA). Serial longitudinal sections (100 μm thickness) along the endosteal (compressive) and periosteal (tensile) surfaces were obtained.

Diffuse damage and linear microcracks were observed on the longitudinal sections obtained from the tensile and compressive sides of the mid-span and unloaded (control) regions under 125× using a bright-field microscopy (Eclipse E600, Nikon, Melville, NY 11747). In basic fuchsin-stained longitudinal sections, linear microcracks appear as a sharply defined line, and diffuse damage appears as an area of pooled staining [3,9,30]. Selective areas of diffuse damage were examined under a laser confocal microscope (Zeiss, Thornwood, NY 10594; Excitation = 543 nm; Emission = 560 nm) to verify the presence of submicroscopic cracks (Fig. 2).

To investigate the role of the lamellar interface in the age-related increase in bone fragility, linear microcracks and diffuse damage were qualitatively assessed in the mid-span region using the following classification (Fig. 3): LM1 = linear microcrack forming at the lamellar interface, LM2 = linear microcrack intersects with the lamellar interface, DD1 = diffuse damage area is limited to one lamella, DD2 = diffuse damage area is spread over more than one lamella. No distinction was made between the lamellar interface and the cement line. Distinction between the lamellar interface and the cement line is often difficult on longitudinal sections, and this was the case here.

For statistical analyses, the differences in fatigue life (θ of cycles to failure or 60% E loss) and microdamage data (linear microcrack density (θ/mm²), diffuse damage density (mm²/mm²), crack length (μm), and diffuse damage area (μm²)) between the young and old groups were examined using a t test following a normality check. For those variables failing the test, a nonparametric Mann–Whitney rank sum test was used. The effects of the lamellar interface on damage morphology (LM1, LM2; DD1, DD2) were compared using a paired t test. Regression analysis was used to examine the relationship between linear microcracks and diffuse damage density. SigmaStat 2.0 (SYSTAT Software Inc, Chicago, IL 60606) was used for all the statistical analyses.

Results

The fatigue life of cortical bone was significantly different between the young and old groups. Young donors had four-fold longer fatigue life (P < 0.05, Fig. 4), and they exhibited very different patterns of damage formation than the bones from the older donors. Histological evaluation of the resultant damage showed that old donors formed 115% more linear microcracks on the compressive side than young donors (P < 0.05, Fig. 5a). In contrast, young donors had 240% more diffuse damage on the tensile side than old donors (P < 0.05, Fig. 5b). Furthermore, a significant negative correlation between linear microcracks and diffuse damage was found (r² = 0.56, P < 0.05).

In addition to the difference in the development of microdamage between the young and old groups, a post hoc analysis revealed that old donors formed longer (116%) linear microcracks than young donors (P < 0.05, Fig. 6a). In contrast, young donors showed a 287% increase in the diffuse damage area compared to old donors (P < 0.05, Fig. 6b). Linear microcracks and diffuse damage showed different associations with the lamellar interface. More linear microcracks developed along the lamellar interface (LM1) than intersecting with the lamellar interface (LM2) for both the
young and old donors (Fig. 3; LM1 = 77.7% ± 24.5%, LM2 = 22.3% ± 24.5%; P < 0.05). In contrast, diffuse damage formed over more than one lamella (DD2) rather than being limited to one lamella (DD1) for both the young and old donors (Fig. 3; DD1 = 0%, DD2 = 100%; P < 0.01).

Discussion

The differences in the fatigue behavior between young and old donors found in this investigation are consistent with Zioupos et al. [35] who showed that specimens from young
Fig. 6. Following bending fatigue, old donors form longer linear microcracks than young donors (a). In contrast, young donors form larger diffuse damage patches than old donors (b). *Indicates $P < 0.05$.

donors have a longer fatigue life than old donors following tensile loading. Damage development following fatigue loading is similar to Wank et al. [34] who found that younger rats had a longer fatigue life associated with the formation of diffuse damage. In contrast, old rats displayed a shorter fatigue life and formed a linear macrocrack with little collateral damage. Characterization of age-related changes in damage development following bending fatigue has not been reported previously. To our knowledge, this is the first study to report the effects of damage morphology on the fatigue behavior of human cortical bone and to identify the relationship between damage morphology and the lamellar interface.

For the same magnitude of global stiffness loss (60%), following the tertiary phase of bending fatigue, young and old donors showed markedly different energy dissipation mechanisms. Old donors dissipated energy by forming more linear microcracks on the compressive side than young donors (Fig. 5a). In contrast, young donors displayed more diffuse damage on the tensile side than old donors (Fig. 5b). In a recent study, we have shown that the formation of diffuse damage occurs at an early stage in the fatigue life, while the formation of linear microcracks occurs towards the end of the fatigue life [9]. The ability of cortical bone to compartmentalize damage morphology in different stages of cyclic loading, therefore, plays a critical role in dissipating energy and resisting a catastrophic fracture [9]. Results of this study show that the bones obtained from the older donors show a reduced tendency to form diffuse damage that dissipates energy, takes time to form and is self-limiting [5,9,17]. More significantly, older donor bones demonstrated a higher propensity of forming linear microcracks that are associated with the terminal phase of bone fracture [9] as well as rapid crack propagation and catastrophic fracture [5].

The relationship between the different damage morphologies and the lamellar interface was also distinct. 77.7% of the linear microcracks followed the lamellar interface; however, none of the diffuse damage areas was limited by lamellar interfaces. Thus, bone microstructure, especially the lamellar interfaces, plays a significant role in the development of linear microcracks but not diffuse damage. The intimate relationship between the lamellae and linear microcracks also implies that the orientation of the lamellae may affect the age-related toughness loss in compact bone. For example, the known increase in osteon density with aging [10,14,16] will inherently increase the number of the lamellae that are oriented at different angles to the applied load. The resultant increase in the number of the inclined lamellae in older donors will consequently result in the formation of more and longer linear microcracks in older than in young donors because a crack oriented at an angle to the applied load has more energy to propagate than a crack oriented parallel to the load [2,28].

Previous studies have shown that the arrest of microcrack growth during fatigue loading plays a major role in cortical bone’s resistance to fracture [1,19,27]. Several mechanisms may cause deceleration and arrest of fatigue crack growth. For example, the formation of a damage wake zone behind the crack tip [1,29,31] or the contact between crack faces following the reduction in crack tip opening displacement [1,28] will result in a reduction in the energy available for crack growth [1,26,29,31]. In addition, microstructural features could arrest microcrack growth [1,3,4,23,26,27] by blunting the crack tip or deflecting crack growth [4,20,26,36]. Therefore, the differences in crack length between the young and old donors, found in this study, indicate different or altered mechanisms of crack arrest and fatigue resistance. Specifically, older donor bones demonstrated a reduced tendency to form diffuse damage on the tensile side but formed linear microcracks on the compressive side that were longer than those in the bones of younger donors (Fig. 6a).

A submicroscopic crack in a diffuse damage patch will be shielded by the presence of other submicroscopic cracks (Fig. 2). Consequently, the submicroscopic cracks will not have enough energy to propagate. More submicroscopic cracks will therefore form and in a larger diffuse damage area as was found here (Fig. 6b). The above observation is consistent with Burr et al. [5] who found that tensile damage accumulates more easily but crack growth is greater in compression.

The age-related variations investigated in this study are limited to the damage mechanisms that affect fatigue life, and no attempt has been made to identify the ultrastructural differences that may cause such change. For example, it is known that aging bone is characterized by an increase in the fraction of highly mineralized bone [25] and modification of
collagen by denaturation [33] or non-enzymatic glycation [8,32]. All the above factors may explain the damage mechanism found here by reducing the diffuse-damage-induced ductility and increasing the linear-microcracks-induced brittle fracture. Further work is currently in progress to address this hypothesis.

In conclusion, the results of this investigation demonstrate that the propensity of bone to fail depends on the microdamage morphology. Following bending fatigue loading, young donors had a longer fatigue life associated with the presence of diffuse damage. In contrast, old donors had a shorter fatigue life concomitant with the formation of linear microcracks. The formation of linear microcracks but not diffuse damage was associated with the weak lamellar interface.

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References