The Role of the Collagen Matrix in Skeletal Fragility

Deepak Vashishth, PhD

Corresponding author
Deepak Vashishth, PhD
Center of Biotechnology and Interdisciplinary Studies, Room 3137,
Rensselaer Polytechnic Institute, 110 8th Street, Troy, NY 12180, USA.
E-mail: vashid@rpi.edu

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The collagen network in bone provides resistance against fracture and may be susceptible to changes with aging and disease. This review identifies the changes in quality of collagen matrix as contributors to bone fragility. With aging and in diabetes, cross-links accumulate in bone collagen as a result of nonenzymatic glycation and consequently impair matrix properties, increasing bone fragility. Cell-culture and animal studies suggest that the accumulation of cross-links induced by nonenzymatic glycation may be related to a reduction in bone turnover resulting from the altered responses of osteoblasts and osteoclasts to advanced glycation end products.

Introduction

Age-related nontraumatic fractures are a major health problem in the United States and elsewhere, resulting in morbidity, mortality, and substantial economic costs [1]. Historically, only bone mass was considered to be a significant predictor of fracture risk, but the current consensus is that bone loss is necessary but not a sufficient condition to cause fracture [2]. It has been demonstrated that, for a given bone mass, an individual's risk of fracture increases with age [3], and mechanical variables directly related to fracture risk are either independent of bone mass or are not totally accounted for by bone mass [4]. Furthermore, the epidemiologic evidence shows a considerable overlap in bone density values between groups with and without fracture, suggesting that low bone quantity alone is insufficient to cause fragility fractures [5]. Thus, factors other than the loss of bone mass may be of crucial importance to the understanding of age-related skeletal fragility. These factors include changes in the quality of bone material [6] and increased frequency of falls related to reduced proprioceptive efficiency and impaired reflexes [7].

Bone Quality and the Extracellular Matrix of Bone

Bone quality can be altered by a number of factors, including the changes in its extracellular matrix caused by the variations in the composition, arrangement, and interaction of its organic (collagen and noncollagenous matrix proteins) and inorganic (hydroxyapatite) constituents [6]. Senescent human bone is characterized by an increasing heterogeneity of aspects at ultrastructural and microstructural levels. At the ultrastructural level, there is an age-related decrease in bone collagen content [8] and an increase in high-density mineralized bone [9]. Furthermore, both the chemical composition and the physiochemistry of mineral and collagen change with age [10,11•]. At the microstructural level, the average size of an osteon decreases with age, but the number of osteons, the size of haversian canals and resorption pores, and the amount of interstitial bone increase [12,13]. The microstructural arrangement of aging bone is therefore characterized by greater porosity and greater proportion of cement line interfaces. Additionally, bone accumulates microdamage with aging because its ability to target and repair microcracks is reduced [14,15]. It is noteworthy that all of the above changes except porosity reflect changes in the extracellular matrix quality and not the quantity of bone.

Some of the above determinants of bone quality have been implicated in enhanced skeletal fragility, but the role of others remains to be investigated. For example, although a number of studies have determined the influence of mineralization, osteon morphology, and porosity [12,16,17], the role and magnitude of age-related alterations in the quality of the organic matrix and their relationships with fracture risk remain poorly understood. One of the reasons for the lack of research into organic-level modifications and their relationships with the measures of bone fracture is the widely held belief that brittle bone results from increased mineralization. However, the lack of correlation between the measures of bone mineralization and bone fracture indices [17], as well as the growing body of evidence showing that collagen-level modifications can also introduce brittle bone behavior [6,18,19], has motivated researchers to explore the role of the collagen modifications in promoting skeletal fragility.

Collagen in Bone

Type I collagen is the principal structural protein of bone; it accounts for 90% of the organic matrix. Type I collagen consists of tropocollagen molecules that contain three polypeptide chains. Each chain is a left-hand helix characterized by the occurrence of a unique amino acid sequence involving glycine-proline-X or glycine-X-hydroxyproline, where X is another amino acid (eg, lysine, arginine). The unique amino acid sequence makes it possible for the three polypeptide chains to wrap around each other in a right-hand sense and form a triple helix in which glycine lies at the center. The other amino acids are present at the triple helix surface. The amino acids present on the triple helix surface and at the N- and C-telopeptide terminals participate via two different biochemical pathways to form covalent cross-links with their neighboring tropocollagen molecules and facilitate the aggregation of molecules in fibrils.

Cross-linking of Bone Collagen

The first pathway of cross-linking requires the action of an enzyme, lysyl oxidase, to produce lysyl and hydroxylysyl aldehydes by oxidative deamination of lysyl or hydroxylysyl residues in the nonhelical telopeptide regions of the collagen molecules. The aldehydes between adjoining collagen molecules subsequently condense and eventually convert to more mature, trivalent, intrafibrillar or interfibrillar cross-links, which are detectable as pyridinolines (HL-Pyr, L-Pyr) and pyrrolic cross-links [20].

In contrast, the second cross-linking pathway does not require the action of an enzyme and is accordingly categorized as nonenzymatic. Under this scheme, an aldehyde of the open-chain form of glucose reacts with the ε-amino group of lysine or hydroxylysine and the resultant aldimine (glucosyl-lysine) undergoes a rearrangement to form a Schiff base adduct and/or an Amadori product [21]. Both the Schiff base adduct and the Amadori product undergo further reactions with other amino groups to form advanced glycation end products (AGEs). To date, the intermolecular cross-links identified from AGEs include, among others, pentosidine [22], vesperlysine [23], and nonfluorescent component-1 (NFC-1) [24]. Pentosidine and vesperlysine, fluorophores consisting of an imidazo-pyridinium ring containing lysine and arginine side chains [22–25], are the commonly measured nonenzymatic cross-links. NFC-1 is a nonfluorescent compound composed of lysine and arginine moieties; its exact structure is currently unknown [24].

Collagen Cross-linking and Bone Fracture

Cross-links formed by both pathways are known to change and affect bone fragility in a number of diseases. For example, Knott et al. [26] demonstrate that the introduction of osteoporosis in an avian model is characterized by an increase in lysyl hydroxylation, which, in turn, results in decreased pyrrolic cross-link content and a consequent decrease in the mechanical strength of bone. Similar changes have been found recently in biopsy specimens from the iliac crest of patients with osteoporotic and multiple spontaneous fractures [27•]. Mutations in the collagen gene responsible for osteogenesis imperfecta have also been shown to result in decreased collagen content, altered cross-link profile, and decreased bone ductility [18]. Saito et al. [28] demonstrated that the introduction of diabetes in rats results in increased collagen-linked fluorescence (a measure of AGEs) and a corresponding decrease in bone strength.

Despite this evidence of the extent of alterations in collagenous proteins and their influence on bone fragility, the role of collagen modifications in age-related skeletal fragility remains unclear. To date, only a limited number of studies have investigated the effect of organic matrix modifications on age-related bone fragility [8], and all these studies have been limited to enzymatic cross-links. Furthermore, as enzymatic cross-links were not found to accumulate with age, no correlation could be made between the cross-link content and the mechanical characteristics of the organic network or of the whole bone. However, consistent with the changes in other collagenous tissues in the human body, evidence is now emerging that bone collagen is also susceptible to age-related accumulation of AGEs and that such modifications may play a significant role in age-related skeletal fragility [11•,29,30].

The accumulation of cross-links mediated by nonenzymatic glycation (NEG) in bone collagen in vitro is highly correlated with the stiffness of the organic matrix of bone [31], and the increased stiffness of the organic matrix has been shown to reduce measures of collagen deformation and microcracking [31,32]. Bone derives its resistance against fracture from collagen deformation [33] and from its ability to form microcracks during crack propagation [34]. Collagen deformation and microcracking are the primary mechanisms of toughening in bone [35••], and any alteration in toughening mechanisms will alter bone toughness. Thus, it is likely that in vivo accumulation of NEG cross-links in collagen [11•,29] may explain age-related loss of bone toughness based on a stiffer collagen network and loss of collagen-based and microcrack-based toughening mechanisms.

Until recently, approaches to measure the toughening magnitude were lacking in bone mechanics. Vashishth et al. [34] applied an experimental fracture mechanics approach, which not only allows for the characterization of the toughening behavior but also elucidates the fracture processes occurring in the postyield region. In this approach, crack growth resistance (K_R) is continuously monitored as a function of crack extension (Δa), and the slope of resistance curve (K_R vs $\sqrt{\Delta a}$) is used as a measure of bone toughness. In contrast to the initiation approach used previously, this new approach successfully differ-

entiates a tough bone like antler from bovine bone [36]. Unlike yield-based, initiation-based approaches, this new approach uses a postyield parameter (slope of resistance curve) to characterize toughness and hence can fully account for any energy-dissipating mechanisms during crack propagation, including (but not limited to) collagen deformation [33] and microcracking [34].

The new crack-propagation approach described above is also more appropriate to measure age-related changes in bone quality than previously described cyclic fatigue crack growth approaches [37]. Cyclic loading tests simulate fatigue fractures commonly seen in young athletes. Such tests typically involve slow crack propagation (maximum 1E-6 m/s [37]) and deceleration due to the opening and closing of the cracks. In contrast, the resistance curve–based approach utilizes quasistatic loading, which more closely approximates a fall, the most common cause of fractures in the elderly [7]. Quasistatic loading results in a rapid increase in the crack tip strains, causing a crack to initiate. This crack continues to accelerate (from 20E-6 m/s to 1200E-6 m/s) unless the material behavior slows the crack by forming additional microcracks in the vicinity of the crack tip [38]. Consequently, any alterations in bone quality related to age and disease, including the NEG-mediated cross-links that alter the microcrack-forming potential of bone [31], could be readily identified by measuring the slope of the resistance curve. A material with a steep resistance curve is less likely to experience unstable crack growth and catastrophic fractures. The applications of this technique demonstrate that the resistance-curve approach can indeed identify age-related changes in bone's fracture resistance [35••].

A fracture-mechanics approach to the toughness of cancellous bone, unlike cortical bone, has not been developed. Postyield and damage behavior of cancellous bone is a relatively new area. Failure energy (area under the stress/strain curve up to the ultimate point) and energy absorption (area under the force-deformation curve) have been used in the past to estimate cancellous bone toughness [39], but these measures are not specifically related to fracture. Keaveny et al. [40] developed postyield approaches that characterize the degradation of apparent mechanical properties in the postyield region, including percent-modulus and strength reductions as a measure of damage. The unloading and reloading of specimens in conjunction with the measurement of the loss of apparent mechanical properties and the characterization of microdamage have demonstrated that the damage behavior of cancellous bone is similar to that of cortical bone [40,41]. When loaded in the postyield region, both cortical and cancellous bones form microcracks at the tissue level and display a similar profile of modulus loss [40,41]. More significantly, by simulating the effects of trabecular microfracture and microdamage on apparent modulus reduction, Yeh and Keaveny [42] found that "extensive microdamage" was the primary reason for the loss of apparent mechanical properties in the postyield region, strikingly similar to what happens in cortical bone, where crack propagation and fracture involve extensive microcracking [34]. Thus, it seems likely that any modifications in the microcracking potential of cancellous bone, including the age-related and NEG-mediated stiffening of the organic matrix, will have considerable effects on its damage and fracture behavior.

When postyield strain energy and the rate of change of postyield modulus have been used to characterize cancellous bone toughness, the results have demonstrated that NEG alters both these variables in a manner consistent with increased bone fragility [11•,43,44•]. Again, these results are consistent with the current notions of loss of toughness in cortical bone and can be explained by the NEG-mediated stiffening of the organic matrix [11•].

Collagen Cross-linking and Bone Turnover

The evidence that AGEs accumulate in tissue with low turnover, including cartilage and tendons [24], has motivated a number of recent studies on the effect of AGEs on osteoblast and osteoclast behavior and on the relationship between tissue turnover and AGE accumulation. Consistent with the accumulation of AGEs in diabetic animals and a consequent decrease in bone healing [45], AGEs have been shown to impair osteoblast proliferation and differentiation [46] while increasing [47] or decreasing [48••] osteoclastic bone resorption.

An increase in osteoclastic bone resorption combined with decreased bone formation would indicate that AGEs will not accumulate in vivo. In contrast, reductions in both resorption and formation will result in reduced removal of AGEs and their consequent accumulation. The accumulation of AGEs with reduced bone turnover seen in bisphosphonate-treated animals [49], as well as in humans with aging [11•,29] and diabetes [28], seem to support this possibility, but further work is necessary to identify the mechanism by which AGEs accumulate in bone. Both the above scenarios would result in increased bone fragility, however. Increased osteoclastic bone resorption combined with decreased bone formation will cause osteopenia-induced bone fragility, whereas decreased osteoclastic bone resorption combined with decreased bone formation will cause bone fragility due to impaired bone matrix properties and reduced fracture resistance.

Conclusions

The literature reviewed here identifies changes in the quality of the collagen matrix that contribute to bone fragility. With aging or diabetes, NEG-induced cross-links accumulate in bone collagen and consequently impair matrix properties, causing increased bone fragility. Cell-culture and animal studies suggest that the accumulation of NEG-induced cross-links may be related to a reduc-

tion in bone turnover resulting from altered responses of osteoblasts and osteoclasts to AGEs.

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