



Osteopontin deficiency increases bone fragility but preserves bone mass

Philipp J. Thurner^{a,b}, Carol G. Chen^a, Sophi Ionova-Martin^{c,d}, Luling Sun^{c,d}, Adam Harman^e, Alexandra Porter^e, Joel W. Ager III^c, Robert O. Ritchie^{c,d}, Tamara Alliston^{a,f,g,h,*}

^a Department of Orthopaedic Surgery, University of California San Francisco, CA, USA

^b School of Engineering Sciences, University of Southampton, UK

^c Materials Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA, USA

^d Department of Materials Science and Engineering, University of California, Berkeley, CA, USA

^e Department of Materials, Imperial College London, London, UK

^f Department of Bioengineering and Therapeutic Sciences, University of California San Francisco, CA, USA

^g Department of Otolaryngology, University of California San Francisco, CA, USA

^h Eli and Edythe Broad Center for Regeneration Medicine and Stem Cell Research, University of California San Francisco, CA, USA

ARTICLE INFO

Article history:

Received 10 November 2009

Revised 8 February 2010

Accepted 9 February 2010

Available online 18 February 2010

Edited by: D. Burr

Keywords:

Osteopontin

Rodent

Fracture toughness

Bone matrix properties

Mineralization

ABSTRACT

The ability of bone to resist catastrophic failure is critically dependent upon the material properties of bone matrix, a composite of hydroxyapatite, collagen type I, and noncollagenous proteins. These properties include elastic modulus, hardness, and fracture toughness. Like other aspects of bone quality, matrix material properties are biologically-defined and can be disrupted in skeletal disease. While mineral and collagen have been investigated in greater detail, the contribution of noncollagenous proteins such as osteopontin to bone matrix material properties remains unclear. Several roles have been ascribed to osteopontin in bone, many of which have the potential to impact material properties. To elucidate the role of osteopontin in bone quality, we evaluated the structure, composition, and material properties of bone from osteopontin-deficient mice and wild-type littermates at several length scales. Most importantly, the results show that osteopontin deficiency causes a 30% decrease in fracture toughness, suggesting an important role for OPN in preventing crack propagation. This significant decline in fracture toughness is independent of changes in whole bone mass, structure, or matrix porosity. Using nanoindentation and quantitative backscattered electron imaging to evaluate osteopontin-deficient bone matrix at the micrometer level, we observed a significant reduction in elastic modulus and increased variability in calcium concentration. Matrix heterogeneity was also apparent at the ultrastructural level. In conclusion, we find that osteopontin is essential for the fracture toughness of bone, and reduced toughness in osteopontin-deficient bone may be related to the increased matrix heterogeneity observed at the micro-scale. By exploring the effects of osteopontin deficiency on bone matrix material properties, composition and organization, this study suggests that reduced fracture toughness is one mechanism by which loss of noncollagenous proteins contribute to bone fragility.

© 2010 Elsevier Inc. All rights reserved.

Introduction

Currently, bone mineral density (BMD) is the most common diagnostic used to assess fracture risk [1,2], yet less than half of non-vertebral fractures can be explained by BMD alone [3]. The limitations of using solely BMD for the prediction of fracture risk can even be shown *in vitro*. The mechanical competence of healthy human bone is

compromised when exposed to chemical treatments that affect the organic matrix but not BMD [4–7]. These findings are not surprising given that the inorganic mineral is only one of several constituents that make up the complex hierarchical composite that is bone. To better reflect these complexities, the scientific and medical communities have adopted the term “bone quality” to summarize the aspects of bone that contribute to fracture risk but are not encompassed by BMD measurements [8]. Bone quality, the subject of growing interest and research efforts, comprises a number of parameters such as the microarchitecture of trabecular bone, prevalence of microcracks, bone geometry and, importantly, bone matrix material properties.

Bone matrix material properties, including elastic modulus, hardness, and fracture toughness, reflect the ability of bone to resist deformation and catastrophic failure. Even without changes in bone mass, average mineralization or bone shape, the alteration of bone matrix material properties can dramatically impact the mechanical

* Corresponding author. Department of Orthopaedic Surgery, University of California San Francisco, 533 Parnassus, UC Hall 452, San Francisco, CA 94143-0514, USA. Fax: +1 415 476 1128.

E-mail addresses: p.thurner@soton.ac.uk (P.J. Thurner), carol.chen@ucsf.edu (C.G. Chen), sophi@berkeley.edu (S. Ionova-Martin), llsun@berkeley.edu (L. Sun), adam.harman05@imperial.ac.uk (A. Harman), a.porter@imperial.ac.uk (A. Porter), JWAger@lbl.gov (J.W. Ager), roritchie@lbl.gov (R.O. Ritchie), tamara.alliston@ucsf.edu (T. Alliston).

competence of bone. For example, it is known clinically that defects in bone matrix material properties due to increased collagen cross-linking likely contribute to bone fragility in diabetes [9,10], whereas collagen point mutations contribute to bone fragility in osteogenesis imperfecta [11]. Even in healthy tissue, bone matrix material properties are biologically regulated [12,13] and anatomically distinct [14]. The unique material properties of a specific bone are present across multiple species, suggesting that their regulation is functionally advantageous and evolutionarily conserved. However, the mechanisms that specify the material properties of bone matrix remain largely unknown.

Bone matrix is a composite of osteoblast-derived collagen and noncollagenous proteins that undergoes mineralization. Both the mineral and organic components of bone matrix contribute to its characteristic hardness and toughness, and defects in the composition or organization of either can cause bone fragility. While the roles of mineral and of collagen type I in bone quality have been investigated in some detail [15–20], we know little about the influence of the noncollagenous proteins on bone matrix material properties. Noncollagenous proteins such as osteocalcin, osteopontin (OPN) and others comprise a relatively small percentage of the bone matrix volume or weight, but they may contribute to bone matrix quality in a number of ways. Noncollagenous proteins control hydroxyapatite crystal nucleation, growth, shape and size as well as facilitate attachment between the major organic (collagen) and inorganic (hydroxyapatite) phases [21–26]. In addition to their effects on mineral, it has more recently been suggested that the intrinsic properties of certain noncollagenous proteins could also be important for the material properties of bone matrix [27]. Networks of highly phosphorylated proteins such as OPN exhibit a molecular self-healing character allowing them to repeatedly dissipate large amounts of energy when loaded in tension [6]. Such protein networks were also found to have an energy storage mechanism and, importantly, exhibit large cohesion and toughness [28]. Hence, the role of noncollagenous proteins, particularly OPN, as determinants of bone matrix material properties and fracture resistance warrants further study.

Although OPN deficiency has previously been shown to impair the macro-mechanical properties of whole bones, the mechanism for this impairment remains unclear. Duvall et al. [29] found a decreased maximum load and torque, as well as work to failure in OPN-deficient mice, but these tests do not explore the underlying changes in bone matrix material properties and ultrastructure. Therefore, the origin of the effect of OPN on bone quality is unclear, which is in part due to the multiple roles attributed to it. OPN facilitates osteoclast attachment and guides mineral deposition by influencing crystal shape and size [23,25,30]. OPN, as well as other noncollagenous proteins, are enriched in cement lines, lamellar interfaces, and interfibrillar spaces of mineralized collagen fibrils [31–34]. High-resolution imagery shows that bone ultimately fails through delamination of mineralized collagen fibrils [6,35–37]. Therefore, perturbation of either the mineral or organic components at these interfaces due to genotype, such as OPN deficiency, or disease, such as osteoporosis, could dramatically impact fracture resistance. Therefore, we hypothesized that OPN, because of its effects on bone mineral and its localization in interfibrillar spaces, is critical for the material properties of bone matrix.

In order to investigate this hypothesis, bones of male OPN-deficient (OPN^{−/−}) mice and wild-type littermates (WT) were evaluated to determine the role of OPN in bone structure, composition, and mechanical and material behavior. Our most important result was a significant decrease in fracture toughness due to OPN deficiency. Fracture toughness is influenced by a multitude of factors including uncracked-ligament bridging, microcracking, crack deflection, and porosity [38–40]. As these factors arise at multiple length scales, our analysis extended from the whole bone level down to the composition and organization of individual matrix constituents.

Materials and methods

Mice

Osteopontin-deficient mice (OPN^{−/−}) on a C57BL/6 background were purchased from the Jackson Laboratory [41]. Protocols were performed as approved by the Institutional Animal Care and Use Committee. Male mice were sacrificed at 8 weeks of age. Harvested hind limbs and spines were cleaned of soft tissue. Left tibiae were stored in Hanks' balanced salt solution (HBSS) containing 0.05% NaN₃ at 4 °C for a maximum of 5 days, whereas all other bones were wrapped in HBSS soaked gauze and stored in sealed plastic pouches at −80 °C.

Fracture toughness

Dissected femora from 9 mice of each genotype were tested in bending to measure the fracture toughness. For such measurements, ASTM standards [42] require that fracture is initiated from a sharp precrack. Generally, this is achieved by fatigue precracking, but this is not really feasible for small mouse bones. Because of this, machined notches have often been used, but more often than not, these are not sharp enough to obtain an accurate toughness measurement. Accordingly, we use a micro-notching technique here, where machined notches are sharpened by “polishing” with a razor blade using 1 μm diamond polishing solution to cut the bone midshaft through the posterior wall of the femur. The resulting micro-notches were maintained at ~1/3 of the bone diameter in length with a reproducible notch root radius of ~10 μm [43]. In this study, femora were tested in 37 °C HBSS in a three-point bending configuration with a custom-made rig for the ELF 3200 mechanical testing machine (ELF3200, EnduraTEC, Minnetonka, MN), in general accordance with ASTM Standard E-399 [42] and E-1820 [44] and as discussed in previously developed methods for small animal bone testing [43]. Testing was conducted in displacement control at a cross-head displacement rate of 0.001 mm/s. Half-crack angle at point of instability was determined by scanning electron microscopy. Fracture toughness, K_{IC} , was calculated using a stress-intensity solution for a circumferential through-wall flaw in cylinders [45]. This methodology and its motivations are discussed in more detail by Ritchie et al. [43].

Areal bone mineral density (aBMD) measurement

aBMD of dissected spines ($N=9$ WT, 11 OPN^{−/−}) and femurs ($N=6$ WT, 7 OPN^{−/−}) was measured using a PIXImus mouse densitometer (GE Lunar II, Faxitron Corp., Wheeling, IL). Importantly, it should be noted that this areal BMD measurement is a combined measure of tissue mineral content as well as the structural organization of bone.

Micro-computed tomography

Dissected femora from 6 mice of each genotype were subjected to micro-computed tomography to determine cortical thickness. Femora were scanned fully hydrated in a benchtop CT 160Xi tomography system (XTEK, Tring, UK) using an acceleration voltage of 75 kV and a current of 60 mA. 1920 projections were recorded for each bone. Bone was segmented from reconstructed volumes using an adaptive threshold algorithm [46], from which the region of interest (ROI) containing only the midshaft of each bone was chosen for quantitative analysis. Segmentation and processing of ROIs was done using algorithms programmed in IDL (ITT, Boulder, CO). As each slice was roughly perpendicular to the long axis of the bone, a 2D component labeling algorithm allowed detection of the main bone compartment as well as the medullary cavity and outside void space. A thickness map of the cortical bone was obtained by filling the outside void space and performing a 3D distance transformation. The individual

Download English Version:

<https://daneshyari.com/en/article/2781247>

Download Persian Version:

<https://daneshyari.com/article/2781247>

[Daneshyari.com](https://daneshyari.com)