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PERSPECTIVES

Mechanisms of Bone Deformation and Fracture

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Abstract

The fracture of bone involves deformation and failure at multiple levels, ranging from the nanoscale mineral platelet/tropocollagen molecule interface, through micron-scale lamellae, to crack deflection at mesoscopic osteons. In order to understand the key structural mechanisms contributing both to high toughness in normal bone tissue and its deterioration in osteoporosis, quantitative analysis at length scales ranging from the nanometer scale up to the μm scale must be performed and reconciled. This *Perspective* reviews key research results from the last few years that have begun to clarify the multiple hierarchical fracture-toughening mechanisms in both healthy and diseased bone. Specifically, at the nanoscale, the existence and importance of a non-collagenous protein phase has been recognized, as well as its possible role in interfibrillar shearing and separation, enhanced fracture strength of the mineral phase due to their small size (~3-5 nm), and the occurrence of highly heterogeneous deformation at scales below 1 μm. At the same time, it has been found that the maximal toughness of bone is achieved due to mechanisms such as ligament/crack bridging and cement line deflection that operate at the higher-length scale of the whole tissue. This has important implications for osteoporosis, where tissue-level structural changes can reduce fracture toughness. This review concludes that a quantitative link between the now-measured deformation and damage initiation at the fibrillar nanoscale level, and the operative crack-deflection and other mechanisms at higher levels, is both necessary and accessible with current high-resolution structural techniques. *IBMS BoneKEy*. 2010 June;7(6):218-228.

Introduction

The structure of bone can be considered at multiple hierarchical levels, from the ordered fibrillar nanostructure up to the macroscopic shapes and lengths of whole bones. While this is well-known, the relative mechanical importance of each length scale in giving bone its high stiffness and toughness is not well-recognized. Considering the molecular level elements it is made of (mainly type I collagen fibres and nanocrystalline apatite), bone achieves a near optimal compromise by maintaining both high stiffness as well as high toughness (1). It is of great importance to understand quantitatively how all elements in the hierarchy – fibrils, fibril arrays, osteons and the whole organ – contribute to these mechanical properties. Equally, the effects of osteoporosis and drug treatments against such disorders need to be evaluated not just at the level of the tissue or whole bone but also in regard to whether the deformation mechanisms of the altered tissue matrix-material are mechanically efficient in preventing fracture (2;3).

Briefly, bone can be considered a structure with up to seven different hierarchical levels (2). At the nanoscale, these are type I collagen molecules, elongated platelets of carbonated apatite and a small fraction (< 5 % by weight) of non-collagenous proteins like osteopontin and osteocalcin. These form a mineralized fibrillar composite, which assembles into arrays with an orientation depending on the developmental stage (woven early bone versus lamellar ordered bone) and mechanical function (parallel orientation in tensile loaded tissues like tendon versus perpendicular orientation in tissues like dentin). In bone specifically, these fibril array patterns form primary and secondary osteon structures in compact bone and elongated trabeculae in spongy bone. Both are, however, made from the

same lamellar building block that is typically 5 μm in diameter (4).

There is evidence that the reduction of mechanical properties of bone in aging and disease is due to a combination of a reduction in bone mass, a change in bone architecture as well as changes in bone quality at the nanoscale (5). While the first two factors are well-known, less is known about the alterations in the collagen/mineral nanocomposite. Changes in the collagen triple helical structure and organization, for example through the *COL1A1* gene (6) or the *Mov13* mutation (7), have been shown to correlate well with fracture incidence and poorer mechanical properties. In an avian osteoporosis model (8), post-translational modification of collagen including alterations in cross-linking were observed. Both in this example and in a study of the femoral heads of osteoporotic women (9), alterations in hydroxylation of the lysine residues in collagen were observed. Bisphosphonates have been shown to alter trabecular bone crosslinking in beagle dog vertebrae (10).

When bone is subjected to physiological loading conditions leading to fracture, each structural level listed above will undergo a deformation (and eventually failure). To prevent this, we need to know quantitatively the deformation and fracture mechanisms at each length scale. At the gross organ level down to the scale of about 100 microns, such measurements can be done with routine mechanical test equipment. However, it becomes progressively more difficult technically to measure mechanics at the microscale and below. In the last few years, however, advances in specialized instrumentation related to micro- and nanoscale imaging have enabled significant progress in answering this question. These advances, and the insights they have delivered into the micro- and nanomechanics underlying bone deformation and fracture, are the focus of this *Perspective*.

Nanoscale Deformation and Fracture Mechanisms

The first direct investigations at the nanoscale focused on the mechanical role of the extrafibrillar matrix proteins (11;12) as a glue binding fibrils together. Using scanning probe microscopy to measure the forces holding freshly cleaved bone surfaces together, Hansma and coworkers found a force-displacement curve shaped like a sawtooth, with multiple small drops and increases in force as the strain was increased (11). Electron microscopic images revealed a number of small, bead-like aggregates on fracture surfaces of bone, which were immunohistochemically revealed to be highly phosphorylated proteins (13). The maximum force observed in the force-displacement curves was strongly dependent on local divalent ion concentrations like Ca^{+2} (11;13;14). Based on these results, Hansma and coworkers proposed a model where multiple weak ionic bonds in the extrafibrillar matrix proteins break during bone fracture, leaving the fibrils essentially intact. The large energy dissipation needed to absorb impact loading of bone is accounted for by the large number of bonds that can break in the extrafibrillar matrix. With these results, the authors shifted the focus of the field away from solely focusing on collagen and mineral content as determinative of bone fracture resistance. However, direct measurement of the strains and stresses in the fibrillar composite was still lacking.

At the nanoscale, bone has been considered a composite of collagen fibrils interlinked and reinforced with mineral (15), or alternatively as a porous mineral network reinforced with collagen (16). However, considerable regularity exists at this scale and can be exploited to learn more about the fibrillar strains under load. Due to the axially regular arrangement of collagen molecules, the fibrils in bone can be considered crystalline (ordered) with a periodically repeating electron density of 65-67 nm. As a result, these fibrils generate regular Bragg peaks in a small-angle X-ray scattering (SAXS) experiment. Under external force, the deformation and engineering strain at the fibrillar level can be directly measured by the percentage shift in the peak position. Similarly, the hexagonal cubic structure of the mineral phase is experimentally observed to align with the collagen fibrils. Specifically, the c-axis

(0002) of the mineral platelets is parallel to the fibril orientation (17), and as a result the Bragg peak shifts in a conventional X-ray diffraction experiment can measure directly the mineral phase strain. The drawback until recently was the long time (of the order of hours) required to get a statistically significant X-ray diffraction or scattering signal from a laboratory-based X-ray apparatus, which precluded real-time imaging of deformation at the nanoscale. With the advent of very intense X-ray beams at third-generation synchrotron sources, rapid measurements (of the order of seconds) have now become possible, although care must be taken to avoid altering the mechanical properties of bone by high radiation doses.

Using this technique on the well-ordered fibrolamellar bone from bovine periosteum as a model system, it was found (18;19) that bone fibrils deform in tension via a shearing mechanism (Fig. 1). Approximately half the external deformation occurs in the extrafibrillar matrix, which consists of mineral particles and noncollagenous proteins. When deformed past the onset of nonlinear stress-strain behavior (damage threshold stress) fibrils showed no further elongation, implying slippage. These results were consistent with the noncollagenous protein model proposed previously. In human secondary osteonal bone, we expect that the shearing deformation would be combined with lamellar and osteonal deformation mechanisms. The picture appears different in compression, with high prestresses and strains (20) measured on canine bone.

The shearing mechanism proposed above (18;19) was extended to a multi-level deformation picture of bone, of which a part is shown in Fig. 1. X-ray diffraction (measuring mineral platelet strain) and SAXS (measuring fibrillar strain) were combined on the same specimen to obtain the ratios of macroscopic, fibril and mineral platelet strain as a function of stress (21). The results showed that the hierarchical structure of bone results in a hierarchical pattern of strains being passed down from the macro- to the nano-level. Quantitatively this means that for every 1% of strain applied macroscopically on bone, the mineral particle sees only 0.16%. There was also a significant negative correlation between the hydration state of the organic matrix of bone and the fraction of strain in the mineral phase, implying that mineral platelets would be more likely to break and be overloaded if the bone was dry than if it was wet. While dry bone is not a relevant physiological state, the result is important in that mechanical changes in the organic matrix, as in disorders like osteogenesis imperfecta, could cause directly an overloading and consequent failure of the mineral phase. Altogether, these findings showed a natural biological stress-shielding design, where the brittle mineral phase was loaded sufficiently less to avoid sudden catastrophic fracture.

At the same time, lab-based X-ray imaging of quasi-static deformation during load showed residual strain accumulated at the nanoscale around naturally occurring hole regions like the foramen of bovine femurs and metacarpals (22). These results indicate that the functional loading history of the tissue can lead to variable pre-strains at different tissue locations. Secondly, the mineral particle strain depended strongly on the orientation between the fiber direction and the direction of loading (23), as is expected for a fiber composite where the axial strength of the fibers is lower than the interfacial adhesion force. These results are relevant because in realistic fracture situations extending across bone matrix with differently-oriented fibrils, the mechanically anisotropic response of the tissue needs to be considered (24).

Macroscopic tests on large tissue samples can also provide insight into the nanoscale mechanics of fracture of bone. Thermal activation analysis is a technique originally developed for studying plasticity in metals (25). It provides a measure of the atomic-scale energy barriers and bond breakage volumes in which plastic deformation takes place, through measuring an activation enthalpy and volume, respectively. Our group applied this technique to bone (26), to find that the activation volume of ~ 1 nm^3 was much smaller than in metals, and the activation enthalpy of ~ 1 eV was

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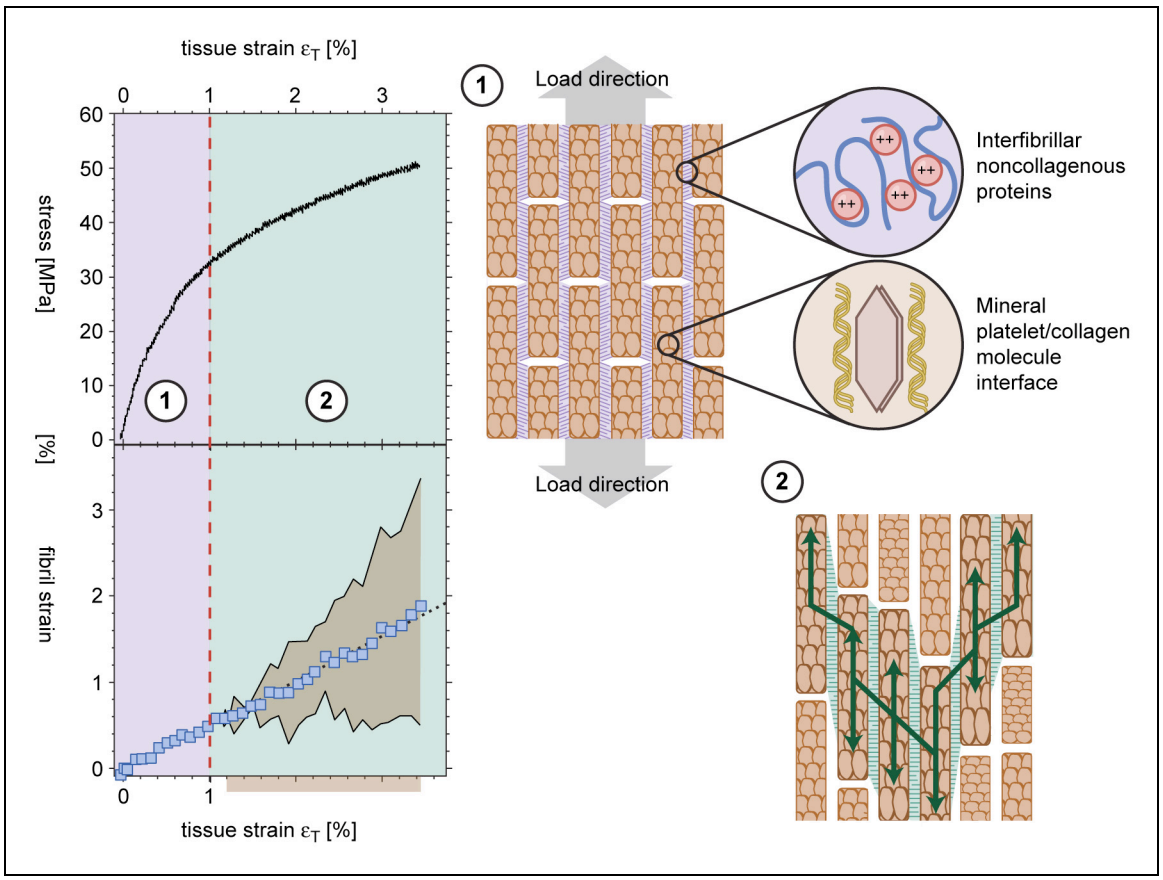

Fig. 1. *Left*: Stress/strain curve for antler cortical bone in tension (27) and fibrillar strain as a function of applied tissue strain. The fibril strain is directly proportional and ½ of the total tissue strain. As the experiment was carried out at a constant tissue strain rate, this implies a linear increase of fibril strain with time. This implies an interfibrillar shearing, as shown in the *upper right* schematic of the bone nanocomposite under load. However, in the zone of inelastic deformation (②), a wide range of fibril strains is observed (shaded area in the *bottom left* graph), which corresponds to interfibrillar decoupling. The interfibrillar decoupling is shown on the schematic on the *bottom right*, with some fibrils extended and carrying the load, while others have decoupled from the interfibrillar matrix and relax. Arrows show the load transfer pathway through the damaged mineralized collagen composite.

characteristic of the strength of ionic bonds. These conclusions led us to propose that ionic bonds breaking in the extrafibrillar matrix were the rate-limiting step in bone plasticity, which is very similar to the model put forward by Hansma and coworkers (11;13;14).

The quantitative deformation fibrillar level mechanics in the region of plastic deformation remain controversial, however. In this region, large bands of high strain appear across the length of the bone, typically a few hundreds of microns in width (28). These correspond to the whitening zones observed in compression (29) and tension (30). As a consequence, X-ray measurements become unreliable unless performed with a micron-scale beam, as there is no guarantee that the X-ray is illuminating the damaged zones in preference to the undamaged intermediate regions. To overcome this, it is beneficial to investigate bone-like tissues that have lower degrees of heterogeneous micro-scale deformation in the inelastic zone. Antler, the annually regenerated bone tissue found in male deer, is an ideal example. It has slightly lower mineralization compared to bone (59% versus 65 wt %) but much higher toughness. It also exhibits a homogeneous pattern in micro-scale deformation, and is thus better suited to *in-situ* X-ray methods.

In-situ X-ray imaging of fibrillar deformation in antler tissue revealed that the toughness

arose from a fibrillation (splitting of fibrils) phenomenon (27;29). The elastic behavior was exactly the same as that of bone, with half the tissue strain occurring in the extrafibrillar matrix. However, in the zone of inelastic deformation, a different behavior was observed, with the fibrils exhibiting varying degrees of strain at the same time. We interpreted this to mean that cracks were developing at the nanoscale between fibrils, altering the load transfer pathway. As a result, some fibrils were decoupled from the rest of the tissue and relaxed back to zero strain, while others were disproportionately strained to bear the remaining load. A schematic describing this mechanism is shown in Fig. 1. Because the mechanism involves individual collagen fibrils separating from each other, we described this as a nanoscale toughening phenomenon in contrast to the microscale toughening mechanisms discussed in the next section.

Tai and co-workers (31;32) have used small-scale nanoindentation measurements (at scales of 100 nm or less) to measure localized elastic/plastic deformation in wet bone. By combining the results with finite element modeling, they obtained evidence for highly heterogeneous deformation in compressive loading. Whether this heterogeneity is the same as the heterogeneous interfibrillar decoupling proposed above (27) for antler tissue is not clear. In contrast to the interfibrillar sacrificial bond mechanism (which is an intra-organic molecule mechanism), this group proposes the main energy-dissipating mechanism to be friction between the nanograins of mineral or between mineral and collagen fibrils.

Considering these different nanoscale mechanisms, some of which give only mechanical, some structural, and some structural/mechanical information, it becomes apparent that a missing link at the nanoscale is structural. We need a better understanding of where the mineral nanoplatelets are located with respect to the collagen phase, and whether this differs between tissue types, for example between antler and mature bone. Our synchrotron data used as a starting point the model of Landis *et al*. (33). In this model, the mineral platelets are regularly arranged inside the collagen fibril. In contrast the models of Hellmich and Ulm consider bone to be primarily an open nanoporous mineral network crosslinked with the ductile collagen phase (16;34). Electron microscopic measurements have not been conclusive one way or another (35). Chemical decollagenization of the bone network is well-known to leave an open connected mineral network, but this may differ depending on the stage of bone mineralization. Resolving this is difficult because the volume fractions of the two phases are roughly equal, making it hard to sustain the conventional fiber-composite convention of a “low” volume fraction fiber (mineral) in a “high” volume fraction matrix (collagen). An equally important gap is the lack of knowledge of the deformation processes within the mineralized collagen fibril. While little direct experimental evidence exists, modeling work has shown that nonenzymatic crosslinking strongly influences the mechanical response of bone and reduced toughness, by inhibiting collagen sliding (36).

Microscale Deformation and Fracture Resistance

It has been known for a long time that when bone is deformed to the point where it yields and breaks, damage and cracks appear at the scale of several microns and larger (37-40). The focus of this section is to describe recent (in the last decade) advances in our knowledge of the structural mechanisms that underlie these events. Bone differs from a brittle material in that when a crack is formed at the microscale, it becomes progressively more difficult to make it longer and longer, eventually leading to fracture. Quantitative fracture mechanics analysis uses the concept of a stress-intensity factor K to determine the stress field in the vicinity of a sharp crack. The fracture toughness K_C in a brittle material remains constant regardless of how long the crack extends, but in bone a different behavior is observed. As shown first by Vashishth *et al*. (41) and later in extensive studies by Robert Ritchie’s group (42-46), the fracture toughness (crack growth resistance K_R) increased with crack

length, implying that the entire bone becomes tougher as the length of the crack increases.

The first mechanism proposed to explain this toughening behavior was microcracking, or the formation of diffuse damage and cracks in the matrix of bone, ahead and around the crack tip (41;47;48). Microcrack growth and coalescence was held responsible for the absorption of energy away from the crack tip. More recently, extensive studies by Ritchie and coworkers (42;45;46;49) have led to two new mechanisms that take into account the lamellar and osteonal microstructural motifs. The first mechanism of ligament bridging occurs when bundles of collagen fibrils span the crack behind the sharp end of the crack tip. They effectively hold or pull the faces of the crack together as the tip extends, making it more difficult to extend the crack. The second mechanism of crack deflection occurs mainly in transverse fracture of bone (42), when a crack is diverted about 90° due to a transversely oriented interface such as a cement line or lamella in its path. Indeed, the energy required to propagate a crack parallel to the lamellar boundary is more than a factor of 100 less than the energy required to drive the crack through it (50). Because a crack encounters more of these microstructural motifs as it extends, the toughness of bone progressively increases, reaching maximal values at near macroscopic dimensions of ~ 500 μm and above. This is a clear example of how the hierarchical structure itself acts as a toughening mechanism. The reduction in fracture resistance due to aging was found to correlate well with the variation of the fracture toughness (51). Specifically, the increase of crack growth resistance with crack extension went down when comparing bones from patients in the age-range of 30-40 years to those from patients about 85-99 years of age (51).

The mechanical relevance of the microstructural motifs like interlamellar boundaries and cement lines is thus very clear. Early classic work by Lakes and Saha (52) had recognized this, by observing cement line creep and motion under loading. Recent studies by Ebacher *et al*. (53;54) have shown that even without considering crack growth explicitly, under compression the circular laminate structure of the osteons can lead to strain patterns at 45° to the main axis of loading. High resolution electron microscopic images of the fracture patterns thus generated showed that inside a single lamella, a range of incipient microcracks formed. These microcracks had a curved pattern, being diverted to a more parallel orientation with the lamellar boundary. This result supports the role of the intra-lamellar architecture, specifically the rotated plywood structure (4), in controlling the way cracks develop and propagate in bone.

A second aspect that has attracted recent attention is the interplay between these microdamage mechanisms and the stimulation of osteoblast and osteoclast activity (55-57). The basic finding is that the remodeling units of bone are oriented toward the presence of damage and respond to it (58), with the response dependent on size, shape and orientation of the microcracks. *In vivo* microcracks have a sheet-like morphology, visualized using laser scanning confocal microscopy (59). The biophysical mechanism proposed is the breakage of cellular processes that extend across crack faces (60). Recently computer models have incorporated the interaction of bone multicellular units with microcracks. Equilibrium states with continuous addition and removal of cracks can be obtained (61).

Perspective and Outlook

Both at the micro- and at the nanoscale, recent advances have added both considerably more information as well as new concepts regarding the deformation and toughening mechanisms in bone. The concept of a hierarchical shearing mode at the nanoscale (18;19;21), interfibrillar sacrificial bonds (11;13;14;62), and ionic interactions at the nanoscale (11;14;26;63), as well as the role of microstructural mechanisms like microcracking, ligament bridging and crack deflection (44-46;51;64;65) at the microscale, are significant examples. However, from a clinical perspective, we do not yet know how these mechanisms can be used to correlate alterations in bone quality and quantity to

observed fracture risk. For this purpose, combination of techniques at the micro- and nanoscale will be vital. In particular, we can see that the link between the nanoscale mechanisms and the eventual growth and propagation of a crack at the microscale remains incomplete. Bridging this gap will be a significant achievement. Our group is currently attempting to do so by combining nanoscale synchrotron techniques with microscale strain mapping methods.

A very recent demonstration of how to link bone quality changes to alterations in fracture risk is provided by an *in vivo* microindentation test to assess bone mechanical quality (66) via an ingenious subcutaneous insertion. The indentation distance, which is a measure of the combined plastic and elastic deformation induced by a localized load, was found to exhibit a strong negative correlation with the work to fracture measured by R-curve tests of the type described above. This opens up the possibility of directly correlating fibrillar separation and noncollagenous protein networks, induced via the indentation, with macroscopically relevant fracture mechanisms.

In conclusion, fracture and deformation in bone proceed by a hierarchical transfer of load from the macro- to the nanoscale, where interfacial matrix properties (either interfibrillar, interlamellar or between osteons and interstitial bone) play a crucial role. Energy absorption occurs due to interfacial delamination and sliding at multiple scales, and direct evidence for interfibrillar decoupling exists. These results point to a concerted effort to bridge the gap between nanoscale deformation processes and the critical microlevel toughening mechanisms, especially as they may be altered in metabolic bone diseases like osteoporosis.

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References

1. Ashby MF, Gibson LJ, Wegst U, Olive R. The mechanical properties of natural materials, I: material property charts. *Proc R Soc A*. 1995;450(1938):123-40.

2. Weiner S, Wagner HD. The material bone: Structure-mechanical function relations. *Annu Rev Mat Sci*. 1998 Aug;28:271-98.

3. Rho JY, Kuhn-Spearing L, Zioupos P. Mechanical properties and the hierarchical structure of bone. *Med Eng Phys*. 1998 Mar;20(2):92-102.

4. Weiner S, Traub W, Wagner HD. Lamellar bone: structure-function relations. *J Struct Biol*. 1999 Jun 30;126(3):241-55.

5. Fratzl P, Gupta HS, Paschalis EP, Roschger P. Structure and mechanical quality of the collagen-mineral nano-composite in bone. *J Mater Chem*. 2004;14(14):2115-23.

6. Langdahl BL, Ralston SH, Grant SF, Eriksen EF. An Sp1 binding site polymorphism in the COLIA1 gene predicts osteoporotic fractures in both men and women. *J Bone Miner Res*. 1998 Sep;13(9):1384-9.

7. Jepsen KJ, Goldstein SA, Kuhn JL, Schaffler MB, Bonadio J. Type-I collagen mutation compromises the post-yield behavior of Mov13 long bone. *J Orthop Res*. 1996 May;14(3):493-9.

8. Knott L, Whitehead CC, Fleming RH, Bailey AJ. Biochemical changes in the collagenous matrix of osteoporotic avian bone. *Biochem J*. 1995 Sep 15;310(Pt 3):1045-51.

9. Köwitz J, Knippel M, Schuhr T, Mach J. Alteration in the extent of collagen I hydroxylation, isolated from femoral heads of women with a femoral neck fracture caused by osteoporosis. *Calcif Tissue Int*. 1997 Jun;60(6):501-5.

10. Allen MR, Gineyts E, Leeming DJ, Burr DB, Delmas PD. Bisphosphonates alter trabecular bone collagen cross-linking and isomerization in beagle dog

vertebra. *Osteoporos Int*. 2008 Mar;19(3):329-37.

11. Fantner GE, Hassenkam T, Kindt JH, Weaver JC, Birkedal H, Pechenik L, Cutroni JA, Cidade GA, Stucky GD, Morse DE, Hansma PK. Sacrificial bonds and hidden length dissipate energy as mineralized fibrils separate during bone fracture. *Nat Mater*. 2005 Aug;4(8):612-6.

12. Thompson JB, Kindt JH, Drake B, Hansma HG, Morse DE, Hansma PK. Bone indentation recovery time correlates with bond reforming time. *Nature*. 2001 Dec 13;414(6865):773-6.

13. Fantner GE, Rabinovych O, Schitter G, Thurner P, Kindt JH, Finch MM, Weaver JC, Golde LS, Morse DE, Lipman EA, Rangelow IW, Hansma PK. Hierarchical interconnections in the nano-composite material bone: Fibrillar cross-links resist fracture on several length scales. *Compos Sci Technol*. 2006 Jul;66(9):1205-11.

14. Fantner GE, Oroudjev E, Schitter G, Golde LS, Thurner P, Finch MM, Turner P, Gutsmann T, Morse DE, Hansma H, Hansma PK. Sacrificial bonds and hidden length: unraveling molecular mesostructures in tough materials. *Biophys J*. 2006 Feb 15;90(4):1411-8.

15. Jäger I, Fratzl P. Mineralized collagen fibrils: a mechanical model with a staggered arrangement of mineral particles. *Biophys J*. 2000 Oct;79(4):1737-46.

16. Hellmich Ch, Ulm FJ. Are mineralized tissues open crystal foams reinforced by crosslinked collagen? Some energy arguments. *J Biomech*. 2002 Sep;35(9):1199-1212.

17. Wagermaier W, Gupta HS, Gourrier A, Paris O, Roschger P, Burghammer M, Riekel C, Fratzl P. Scanning texture analysis of lamellar bone using microbeam synchrotron X-ray radiation. *J Appl Cryst*. 2007 Feb;40(1):115-20.

18. Gupta HS, Wagermaier W, Zickler GA, Hartmann J, Funari SS, Roschger P, Wagner HD, Fratzl P. Fibrillar level fracture in bone beyond the yield point. *Int J Fract*. 2006 Jun;139(3-4):425-36.

19. Gupta HS, Wagermaier W, Zickler GA, Raz-Ben Aroush D, Funari SS, Roschger P, Wagner HD, Fratzl P. Nanoscale deformation mechanisms in bone. *Nano Lett*. 2005 Oct;5(10):2108-11.

20. Almer JD, Stock SR. Internal strains and stresses measured in cortical bone via high-energy X-ray diffraction. *J Struct Biol*. 2005 Oct;152(1):14-27.

21. Gupta HS, Seto J, Wagermaier W, Zaslansky P, Boesecke P, Fratzl P. Cooperative deformation of mineral and collagen in bone at the nanoscale. *Proc Natl Acad Sci U S A*. 2006 Nov 21;103(47):17741-6.

22. Giri B, Tadano S, Fujisaki K, Todoh M. Understanding site-specific residual strain and architecture in bovine cortical bone. *J Biomech*. 2008 Nov 14;41(15):3107-15.

23. Giri B, Tadano S, Fujisaki K, Sasaki N. Deformation of mineral crystals in cortical bone depending on structural anisotropy. *Bone*. 2009 Jun;44(6):1111-20.

24. Seto J, Gupta HS, Zaslansky P, Wagner HD, Fratzl P. Tough lessons from bone: extreme mechanical anisotropy at the mesoscale. *Adv Funct Mater*. 2008 Jul 9;18(13):1905-11.

25. Kocks UF, Argon AS, Ashby MF. *Thermodynamics and Kinetics of Slip. Progress in Materials Science*. Vol. 19. Oxford, UK: Pergamon Press; 1975:1-281.

26. Gupta HS, Fratzl P, Kerschnitzki M, Benecke G, Wagermaier W, Kirchner HO. Evidence for an elementary process in bone plasticity with an activation enthalpy of 1 eV. *J R Soc Interface*. 2007 Apr 22;4(13):277-82.

27. Krauss S, Fratzl P, Seto J, Currey JD, Estevez JA, Funari SS, Gupta HS. Inhomogeneous fibril stretching in antler starts after macroscopic yielding: indication for a nanoscale toughening mechanism. *Bone*. 2009 Jun;44(6):1105-10.

28. Benecke G, Kerschnitzki M, Fratzl P, Gupta HS. Digital image correlation shows localized deformation bands in inelastic loading of fibrolamellar bone. *J Mater Res*. 2009 Feb;24(2):421-29.

29. Thurner PJ, Erickson B, Schriock Z, Langan J, Scott J, Zhao M, Weaver JC, Fantner GE, Turner P, Kindt JH, Schitter G, Morse DE, Hansma PK. High-speed photography of the development of microdamage in trabecular bone during compression. *J Mater Res*. 2006 May;21(5):1093-1100.

30. Zioupos P, Currey JD, Sedman AJ. An examination of the micromechanics of failure of bone and antler by acoustic emission tests and Laser Scanning Confocal Microscopy. *Med Eng Phys*. 1994 May;16(3):203-12.

31. Tai K, Dao M, Suresh S, Palazoglu A, Ortiz C. Nanoscale heterogeneity promotes energy dissipation in bone. *Nat Mater*. 2007 Jun;6(6):454-62.

32. Tai K, Ulm FJ, Ortiz C. Nanogranular origins of the strength of bone. *Nano Lett*. 2006 Nov;6(11):2520-5.

33. Landis WJ. The strength of a calcified tissue depends in part on the molecular structure and organization of its constituent mineral crystals in their organic matrix. *Bone*. 1995 May;16(5):533-44.

34. Hellmich C, Ulm FJ. Micromechanical model for ultrastructural stiffness of mineralized tissues. *J Engrg Mech*. 2002 Aug;128(8):898-908.

35. Rubin MA, Jasiuk I, Taylor J, Rubin J, Ganey T, Apkarian RP. TEM analysis of the nanostructure of normal and osteoporotic human trabecular bone. *Bone*. 2003 Sep;33(3):270-82.

36. Siegmund T, Allen MR, Burr DB. Failure of mineralized collagen fibrils: modeling the role of collagen cross-linking. *J Biomech*. 2008;41(7):1427-35.

37. Zioupos P, Wang XT, Currey JD. Experimental and theoretical quantification of the development of damage in fatigue tests of bone and antler. *J Biomech*. 1996 Aug;29(8):989-1002.

38. Zioupos P, Currey JD. The extent of microcracking and the morphology of microcracks in damaged bone. *J Mater Sci*. 1994 Jan;29(4):978-86.

39. Schaffler MB, Choi K, Milgrom C. Aging and matrix microdamage accumulation in human compact bone. *Bone*. 1995 Dec;17(6):521-25.

40. Carter DR, Caler WE. A cumulative damage model for bone fracture. *J Orthop Res*. 1985;3(1):84-90.

41. Vashishth D, *et al*. Toughening mechanisms in cortical bone. In 42nd Annual Meeting of the ORS. 1996.

42. Koester KJ, Ager JW 3rd, Ritchie RO. The true toughness of human cortical bone measured with realistically short cracks. *Nat Mater*. 2008 Aug;7(8):672-7.

43. Nalla RK, Stölken JS, Kinney JH, Ritchie RO. Fracture in human cortical bone: local fracture criteria and toughening mechanisms. *J Biomech*. 2005 Jul;38(7):1517-25.

44. Ritchie RO, Kinney JH, Kruzic JJ, Nalla RK. A fracture mechanics and mechanistic approach to the failure of cortical bone. *Fatigue Fract Eng Mater Struct*. 2005 Apr;28(4):345-71.

45. Nalla RK, Kruzic JJ, Ritchie RO. On the origin of the toughness of mineralized tissue: microcracking or crack bridging? *Bone*. 2004 May;34(5):790-8.

46. Nalla RK, Kinney JH, Ritchie RO. Mechanistic fracture criteria for the failure of human cortical bone. *Nat Mater*. 2003 Mar;2(3):164-8.

47. Zioupos P. On microcracks, microcracking, in-vivo, in-vitro, in-situ and other issues. *J Biomech*. 1999 Feb;32(2):209-11.

48. Zioupos P, Wang XT, Currey JD. The accumulation of fatigue microdamage in human cortical bone of two different ages in vitro. *Clin Biomech (Bristol, Avon)*. 1996 Oct;11(7):365-75.

49. Nalla RK, Kruzic JJ, Kinney JH, Ritchie RO. Mechanistic aspects of fracture and R-curve behavior in human cortical bone. *Biomaterials*. 2005 Jan;26(2):217-31.

50. Peterlik H, Roschger P, Klaushofer K, Fratzl P. From brittle to ductile fracture of bone. *Nat Mater*. 2006 Jan;5(1):52-5.

51. Nalla RK, Kruzic JJ, Kinney JH, Ritchie RO. Effect of aging on the toughness of human cortical bone: evaluation by R-curves. *Bone*. 2004 Dec;35(6):1240-6.

52. Lakes R, Saha S. Cement line motion in bone. *Science*. 1979 May 4;204(4392):501-3.

53. Ebacher V, Wang R. A unique microcracking process associated with the inelastic deformation of Haversian bone. *Adv Funct Mater*. 2009 Jan 9;19(1):57-66.

54. Ebacher V, Tang C, McKay H, Oxland TR, Guy P, Wang R. Strain redistribution and cracking behavior of human bone during bending. *Bone*. 2007 May;40(5):1265-75.

55. Taylor D, Hazenberg JG, Lee TC. Living with cracks: damage and repair in human bone. *Nat Mater*. 2007 Apr;6(4):263-8.

56. Taylor D. Fracture and repair of bone: a multiscale problem. *J Mater Sci*. 2007 Nov;42(21):8911-18.

57. Verborgt O, Gibson GJ, Schaffler MB. Loss of osteocyte integrity in association with microdamage and bone remodeling after fatigue in vivo. *J Bone Miner Res*. 2000 Jan;15(1):60-7.

58. Lee TC, Staines A, Taylor D. Bone adaptation to load: microdamage,as a stimulus for bone remodelling. *J Anat*. 2002 Dec;201(6):437-46.

59. O'Brien FJ, Taylor D, Dickson GR, Lee TC. Visualisation of three-dimensional microcracks in compact bone. *J Anat*. 2000 Oct;197 Pt 3:413-20.

60. Hazenberg JG, Freeley M, Foran E, Lee TC, Taylor D. Microdamage: a cell transducing mechanism based on ruptured osteocyte processes. *J Biomech*. 2006;39(11):2096-103.

61. Hazelwood SJ, Bruce Martin R, Rashid MM, Rodrigo JJ. A mechanistic model for internal bone remodeling exhibits different dynamic responses in disuse and overload. *J Biomech*. 2001 Mar;34(3):299-308.

62. Fantner GE, Adams J, Turner P, Thurner PJ, Fisher LW, Hansma PK. Nanoscale ion mediated networks in bone: osteopontin can repeatedly dissipate large amounts of energy. *Nano Lett*. 2007 Aug;7(8):2491-8.

63. Kirchner H. Ductility and brittleness of bone. *Int J Fract*. 2006 Jun;139(3-4):509-16.

64. Cook RB, Zioupos P. The fracture toughness of cancellous bone. *J Biomech*. 2009 Sep 18;42(13):2054-60.

65. Zioupos P, Hansen U, Currey JD. Microcracking damage and the fracture process in relation to strain rate in human cortical bone tensile failure. *J Biomech*. 2008 Oct 20;41(14):2932-9.

66. Diez-Perez A, Güerri R, Nogues X, Cáceres E, Peña MJ, Mellibovsky L, Randall C, Bridges D, Weaver JC, Proctor A, Brimer D, Koester KJ, Ritchie RO, Hansma PK. Microindentation for in

vivo measurement of bone tissue mechanical properties in humans. *J Bone Miner Res*. 2010 Feb 23. [Epub ahead of print]