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# On the mechanistic origins of toughness in bone

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**Abstract:** One of the most intriguing protein materials found in Nature is bone, a material composed out of assemblies of tropocollagen molecules and tiny hydroxyapatite mineral crystals, forming an extremely tough, yet lightweight, adaptive and multi-functional material. Bone has evolved to provide structural support to organisms, and therefore, its mechanical properties are of great physiological relevance. In this article, we review the structure and properties of bone, focusing on mechanical deformation and fracture behavior from the perspective of the multi-dimensional hierarchical nature of its structure. In fact, bone derives its resistance to fracture with a multitude of deformation and toughening mechanisms at many of these size-scales, ranging from the nanoscale structure of its protein molecules to its macroscopic physiological scale.

## 1. INTRODUCTION

By manipulating combinations of minerals and organic polymers into hierarchical structures spanning multiple length-scales, Nature has developed a wide range of hybrid materials with specific properties matched to function (1-4). Indeed, these biological systems represent an inexhaustible source of inspiration to materials scientists in offering potential solutions for the development of new generations of structural materials (5-7). Nature's key role here is in the complex hierarchical structuring of the materials (8-12) which, unlike engineering materials that are *fabricated* according to specific requirements, are *grown* using the principles of biologically controlled self-assembly (2). The act of "growing" rather than "fabricating" leads to the possibility of dynamic strategy, which allows for flexibility at all levels. The concept of multi-scale hierarchical structures, where the microstructure at each level is adapted to local needs (13), allows the adaptation and optimization of the material form and structure at each level of hierarchy to meet specific functions (11,12,14). Indeed, the complexity and symbiosis of structural biological materials has generated enormous interest of late, primarily because these composite biological systems exhibit mechanical properties that are invariably far superior to those of their individual constituents (15).

One of the most intriguing and complex materials found in nature is bone. Like any other biological systems, bone is a highly hierarchical composite material composed primarily of assemblies of collagenous protein molecules, water, and mineral

nanoparticles made of carbonated hydroxyapatite, forming an extremely tough, lightweight, adaptive and multi-functional material. Bone is often stereotyped as simply a protective and supportive framework for the body; though it does perform this function, it is actually a very dynamic organ that is constantly remodeling and changing shape to adapt to the daily forces placed upon it. Like all natural materials, its mechanical properties are determined by its structure (2,15,16), which in turn is motivated by its (primarily mechanical) function (17,18). The adaptation of compact bone to its mechanical environment includes both alteration of its shape and adaptation of its internal structure, *i.e.*, its material properties. Long bones, such as the femur or the tibia, provide stability against bending and buckling. Short bones, *e.g.*, vertebra, provide stability against compression, while plate-like bones such as the skull protect vital organs. The diversity of structures within this family reflects the fine-tuning or adaptation of the structure to its function.

In addition, a remarkable property of bone is its well-known capacity of self-repair (19). In bone, the combination of growth and remodeling (resorption and replacement of old material) occurs via specialized cells (osteoclasts) that are permanently removing material, while other cells (osteoblasts) are depositing new tissue. This continuous remodeling allows structural adaptation to changing external conditions, as well as the removal and replacement of damaged material (16,20,21). Unfortunately, excessive remodeling and other aging-related changes to the musculoskeletal system are known

to increase susceptibility to bone fracture (22). In the case of the elderly, such changes are critical as the consequent fractures can lead to significant mortality. While a number of extraosseous variables, such as loading regime, incidence of traumatic falls, prior fractures, etc., are involved, it is apparent that the bone tissue itself deteriorates with age (22-24). A primary factor here is *bone quality*, which is a loosely defined term used to describe some, but probably still not all, characteristics of the bone-matrix nano/microstructure that can influence mechanical properties such as stiffness (resistance to elastic deformation), strength (resistance to plastic deformation) and toughness (resistance to fracture).

Traditional thinking on the deterioration of bone with age has focused on *bone quantity* as a predictor of such fracture risk, where quantity is described by the bone mass or bone-mineral density (BMD), defined as the amount of bone mineral per unit cross-sectional area. For example, the elevation in bone remodeling activity, concurrent with menopause in aging women, can lead to osteoporosis, a condition of low bone mass associated with an increased risk of fracture. However, although bone mass can explain some of the fracture risk, there is now mounting evidence that low BMD alone is not the sole factor responsible for the aging-induced fracture risk; specifically, a landmark study by Hui *et al.* (22) revealed a roughly ten-fold increase in fracture risk with aging that was independent of BMD. This result and the concurrent realization that bone mass alone cannot explain the therapeutic benefits of anti-resorptive agents in

treating osteoporosis (24), has re-emphasized the necessity for understanding the factors that control bone quality. Indeed, there has been a renewed interest of late in the strength and fracture properties of bone, motivated by the notion that an appropriate assessment of bone quality could potentially be used to predict the risk of bone fracture.

As there is still only limited understanding of the physics-based mechanisms of bone fracture and how they relate to its hierarchical, multi-dimensional structure (25,26), one vital question that is paramount is the origin of bone's fracture resistance in relation to these structural length-scales. To understand the toughness of bone and discern the roles of the observed toughening mechanisms, it is pertinent to note that the fracture resistance is a multiple-scale process with each level of structural hierarchy adapted to provide optimal toughness. Traditionally, toughness has been thought of as the ability of a material to dissipate deformation energy without propagation of a crack. However, fracture is actually the result of a mutual competition of *intrinsic* damage mechanisms ahead of a crack tip that promote cracking and *extrinsic* shielding mechanisms mainly behind the tip that impede it (27,28). Intrinsic toughening mechanisms increase the microstructural resistance to crack initiation, as exemplified by the role of plasticity ahead of the crack tip in metals. Extrinsic toughening involves microstructural mechanisms that act primarily behind the crack tip to inhibit crack growth by effectively reducing the crack-driving force actually experienced at the crack tip, as illustrated by crack-tip shielding mechanisms such as crack bridging (29,30). A

discussion on how the fracture toughness can be quantitatively measured in light of these different classes of mechanisms is presented in the Appendix.

In this review, we describe the mechanistic roles that the multi-level structural constituents of bone play in affecting its resistance to fracture, in terms of both the initiation and subsequent propagation of cracks. Specifically, we show that the toughness of bone is a result of a suite of potent extrinsic (shielding) mechanisms, specifically crack deflection and bridging, acting primarily at the micro length-scales and above (typically at dimensions above  $\sim 1 \mu\text{m}$ ) (31), coupled with an additional role of intrinsic toughening due to the significant “plasticity” in the material generated at multiple length-scales (although primarily at dimensions below  $\sim 1 \mu\text{m}$ ). Whereas the extrinsic shielding mechanisms affect only the propagation of cracks (as assessed by the crack-resistance or R-curve), the “plasticity” mechanisms in bone affect both initiation and growth and include a number of inelastic, non-recoverable deformation mechanisms, such as local collagen fibrillar shearing and viscoplasticity at sub-micrometer scales and microcracking at somewhat coarser scales (32).

## **2. HIERARCHICAL STRUCTURE OF BONE**

The structure of bone can be viewed as cascaded arrangements of building blocks at defined length scales (Fig. 1) that form a hierarchical structure which controls its properties, *e.g.*, deformation and toughness (25,26,33). Bone tissue can be either

trabecular (spongy) or cortical (compact). Trabecular bone, also known as cancellous bone, fills the insides of many bones; it is spongy with struts of order 100-300  $\mu\text{m}$  in diameter. This review is primarily focused on cortical bone which is the dense bone that is found at the outer surface of all bones. The cortical bone shell can reach a thickness between several tenths of a millimeter (in vertebrae) to several millimeters (in the mid-shaft of long bones). Structurally, the basic building blocks of such bone are an organic matrix (90% type-I collagen, 10% amorphous ground substance) and a mineral phase (calcium phosphate based hydroxyapatite), although its composition and structure are not invariant; they vary with factors such as skeletal site, age, sex, physiological function and mechanical loading, making bone a very heterogeneous structure. Different bones grow at different rates, and the kind of primary bone laid down depends on the rate of accretion. The mineral content varies substantially from bone to bone, antlers having the lowest mineral content in the bone family (55-60 wt.%), and whale fins the highest (~90%) (16). The main determinant of mechanical properties is generally the amount of mineral in the tissue (16,18). Accordingly, different bones have different mechanical properties depending on the growth, structure and adaptation, all of which are interconnected to serve a specific function.

Typical structures found at a lower hierarchical level in cortical bone are shown in Fig. 2. Cortical bone is a particularly neatly arranged laminar bone. Blood vessels are more irregularly disposed or do not form a network, and the laminar arrangement gives



way to one in which the blood vessels anatomize in three dimensions, each surrounded by more or less concentric layers of lamellar bone; these structures are called primary osteons. They resemble secondary osteons, or Haversian systems, which provide the basis for the growth of new bone. The microstructures of two different types of compact bone, *e.g.*, primary bone in elk antler and secondary bone in adult human bone, are compared in Figs. 2b-c. Antlers are young bones that undergo limited remodeling and are predominantly composed of primary osteons (34) that contain vascular channels (15-25  $\mu\text{m}$  diameter) surrounded by concentric bone lamellae (Fig. 2c). Secondary osteon remodeling arises in response to mechanical stress. In human bones, the whole remodeling process takes about 2-4 months (16). Since antlers do not sustain mechanical loads during the growth process and are only used in sporadic combat for 1-2 months before shedding, secondary osteons do not develop under these conditions. The entire primary osteons are 100 to 200  $\mu\text{m}$  in diameter. In comparison, human bone is a secondary (replacement) bone that is the product of resorption of previously existing bone tissue and the deposition of new bone in its place. This process results in the formation of secondary osteons that have central vascular channels (Haversian canals) some 50 to 90  $\mu\text{m}$  in diameter (Fig. 2b); these are surrounded by a series of concentric lamellae containing osteocytes arranged in nominally circular fashion. The entire secondary osteons (or secondary Haversian systems) are about 200 to 300  $\mu\text{m}$  in diameter. In antler bone (Fig. 2c), a hyper-mineralized region surrounding

the primary osteons (34) is present whereas in the human bone (Fig. 2b), a thin mineralized region, the cement line (35), surrounds the secondary osteons. As prominent sites for microcracking, both microstructural features have strong implications for fracture behavior and bone toughness (36,37).

While a diversity of structural motifs exist between bone tissues at different microstructural length-scales (26), the most common in bone is the lamellar unit (38,39). As mentioned above, osteons (lamellar bone) have a lamellar structure with adjacent lamellae some 3-7  $\mu\text{m}$  thick (40,41). Each of these lamellae consists of a series of fibril layers oriented at different angles of the osteon axis (Fig. 1). Several studies (38,39) have suggested the existence of a “plywood” type structure, where the fibrils not only rotate with respect to the osteon axis, but also around their own axis across the width of the lamella. Indeed, novel measurement methods combining synchrotron x-ray texture measurements and scanning of thin sections of secondary osteons (42,43) indicate that each lamellae consists of a series of fibril layers oriented at different angles to the osteon axis with angles being always positive.

At the nano-scale level of hierarchy, each individual fiber is composed of an array of mineralized collagen fibrils. The mineralized collagen fibril, which is roughly 100 nm in diameter and ~5-10  $\mu\text{m}$  or more in length, is the basic building block of the bone matrix. The fibrils consist of an assembly of 300 nm long and 1.5 nm thick collagen molecules, which are deposited by the osteoblasts (bone forming cells) into the extracellular space

and then self-assemble into fibrils. The collagen fibrils are filled and coated by tiny mineral crystals of hydroxyapatite that assemble in the gap between collagen fibrils, and the fibrils become mineralized as the bone tissue grows and matures. These crystals are mainly flat and are mostly arranged parallel to each other and to the long axis of the collagen fibrils in the bone composite, in a regularly repeating, staggered arrangement (44,45). In bone tissue from several different mammalian and non-mammalian species, bone mineral hydroxyapatite crystals are very small with a thickness of 1.5-4.5 nm (33,46-51) (with implications for its fracture behavior, as discussed in ref. (52)<sup>1</sup>). At the lowest dimension level, the collagen fibrils are formed by staggered arrays of tropocollagen (TC) molecules, each built from three polypeptides arranged in a 300 nm long triple-helical “nanorope” geometry and stabilized by hydrogen bonding between residues (53-55). Every third residue in each of these molecules is a glycine (GLY) amino acid, and about one fourth of the tropocollagen molecule consists of proline and hydroxyproline (56,57).

Collagen is one of the most abundant proteins on earth, and is arguably the most important structural protein in biology. As we will discuss, the soft collagen gives bone its elasticity and the ability to dissipate energy under mechanical deformation. Notably,

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<sup>1</sup> With its high elastic modulus (~100 GPa), the hydroxyapatite (HA) crystals provide for the stiffness of bone. However, to offset its extreme brittleness (HA has a fracture toughness less than 1 MPa√m), the mineral crystals are nanoscale in size such that their (Griffith) fracture stress is very high, *i.e.*, at nanoscale dimensions, they are insensitive to flaws (52).

even minute structural changes in the collagen molecule's architecture can have severe consequences for the macro-scale tissue behavior, as is evident in genetic bone diseases.

### 3. INTRINSIC TOUGHENING (DEFORMATION) MECHANISMS

The mechanical properties of the constituents of bone largely control its strength and plasticity. However, these properties are strongly dependent on the scale of observation. Recent experimental and theoretical studies have provided new insight into the mechanisms underlying this behavior, most notably through the use of advanced instrumentation that permits the examination of bone properties at ever decreasing length scales, *e.g.*, transmission electron and x-ray microscopy, atomic force microscopy and Raman spectroscopy, as well as “bottom-up” multi-scale simulation modeling. The integration of results from these studies suggests that permanent deformation (“plasticity”) in bone is the result of concurrent multiple deformation mechanisms that are predominantly active at sub-micrometer ( $<\mu\text{m}$ ) levels (Fig. 3). However, even though individual mechanisms have been identified, there is still no integrated model of plastic deformation in bone. Plasticity at molecular scales is particularly challenging to identify. Whereas it has been shown that the breaking of H-bonds is a crucial mechanism of large and permanent deformation of protein molecules, fibrils and fibers, this mechanism is actually reversible when applied forces are removed (see Fig. 4 as an illustration of how H-bonds break as forces are applied to a collagen protein molecule). The force required to break these bonds is highly

dependent on the deformation rate (time-scales), which provides a mechanism to dissipate energy under rapid loading (58-60). Indeed, such breaking of H-bonds can be regarded as a viscoelastic effect. This suggests that plastic deformation in bone must also involve larger-scale mechanisms such as intermolecular sliding, which would not be reversible unless remodeling of bone occurs. In general, the salient deformation mechanisms in bone are associated with different structural levels (Fig. 3), as described below.

### **3.1. Hydrogen bond breakage in pure collagen: Molecular uncoiling**

The basic deformation mechanism of individual collagen molecules is molecular stretching and unwinding due first to entropic and then energetic mechanisms that involve breaking of H-bonds (Fig. 4). In collagen fibrils, molecular stretching competes with intermolecular sliding and breaking of weak and strong bonds between tropocollagen molecules. These sliding motions provide the basis for large plastic strains without catastrophic “brittle” failure. Severe mechanical tensile loading of collagen is significant under physiological conditions in various related tissues, representing one of its key performance features, as shown by its ability to stretch up to 50% tensile strain before breaking while reaching force levels of more than 10 nN (per molecule), or 10-20 GPa stress (obtained by normalization of the force by the cross-sectional area of the molecule) (61-65). Fig. 5 shows a tensile test of a tropocollagen molecule for deformation up to 40% strain. The plot also shows the progression of H-

bond breaking as the molecule unravels (65). H-bond breakage at 10 to 20% strain provides one of the major mechanisms that mediates the deformation of collagen fibrils; it is a reversible process and may thus provide a means to dissipate energy through large-deformation behavior of the soft collagen bone matrix. The specific structure of intermolecular cross-links thereby plays a crucial role in defining the particular deformation paths. Aged collagen tends to show a high cross-link density, whereas young collagen features few cross-links. The larger the cross-link density, the lower the material's ability to dissipate energy without failure; at large cross-link densities collagen fibrils tend to involve molecular fracture and breaking of cross-links (66) leading to increasingly brittle material behavior. This is certainly one factor associated with the increased fracture risk in older bone.

### **3.2. (Fibrillar) sliding of mineralized collagen fibrils**

The mineralized collagen fibrils are of particular significance for bone as they are its nanostructural building blocks. As the mineral phase has over an order of magnitude higher elastic modulus than the collagen, the presence of the hydroxyapatite phase is critical to the stiffness of bone. Indeed, experimental results show a continuous increase in Young's modulus with mineralization of the collagen fibrils, ranging up to a factor of three for high mineral contents (16). Molecular modeling and experimental x-ray analysis suggest that continuous glide between tropocollagen molecules and between hydroxyapatite particles and tropocollagen molecules, initiated by slip at the

hydroxyapatite-tropocollagen interface, enables a large regime of dissipative deformation once yielding begins, thus effectively increasing the resistance to fracture (66). The larger stresses in the fibrils, preserved due to mineralization, can be maintained after slip initiation due to additional resistance to slip at the interface between the tropocollagen molecules and hydroxyapatite particles; this leads to a several fold increase in energy dissipation.

Figs. 5(b,c) show a nano-mechanical analysis of bone deformation, along with structural mechanistic details of the associated deformation mechanisms. This result was obtained by a combination of molecular dynamics simulation and theoretical analysis, where it has been shown that the characteristic nanostructure of mineralized collagen fibrils is vital for its high strength and its ability to sustain large deformation. Fig. 5(a) shows stress-strain curves under tensile loading for a pure collagen fibril as compared to that of a mineralized collagen fibril, for tensile strains up to 50%; the resulting constitutive behavior is quite different, indicating that precipitation of hydroxyapatite crystals during bone formation significantly alters mechanical behavior. Specifically, the mineralized collagen fibrils display a higher strength and greater energy dissipation under deformation. Plastic deformation starts at ~5% tissue strain in a pure collagen fibril and at roughly 30% higher tensile strains (~6.7%) when mineralized. Further, the mineralized collagen fibrils show higher stiffness and significant softening at larger strains, with a characteristic saw tooth shaped stress-

strain curve due to repeated intermolecular slip. Images of the molecular geometry under increasing tensile load (Figs. 5(b-c)) imply a deformation mechanism of intermolecular slip which characterizes fibrillar yield (see red circle highlighting a local area of repeated molecular slip). Slip leads to the formation of regions with lower material density, as can be seen in the images.

In addition to providing a mechanism of “plastic” deformation, such molecular behavior of the protein and mineral phases under large-scale deformation of mineralized collagen fibrils also represents a fibrillar toughening mechanism that leads to an increase of energy dissipation as compared to fibrils without mineral phase (66,67). This is an intrinsic toughening mechanism (Fig. 3) which enhances the resistance to fracture by forming larger local yield regions around crack-like defects, a mechanism that protects the integrity of the entire structure by allowing for localized failure. As a consequence, mineralized collagen fibrils are able to tolerate microcracks on the order of several hundred micrometers size without causing any macroscopic failure of the tissue.

Specifically, the inclusion of the nanoscale mineral platelets on collagen fibrils not only increases their Young’s modulus, but also their yield and fracture strengths. In terms of numbers, the Young’s modulus, tensile yield strain and fracture strength for mineralized collagen fibrils are, respectively, 6.2 GPa, 6.7% and 0.6 GPa, as compared to corresponding values of 4.6 GPa, 5% and 0.3 GPa for pure collagen fibrils.



### **3.3. (Fibrillar) sliding of collagen fiber arrays**

The long (>5-10  $\mu\text{m}$ ) and thin (~100 nm) mineralized collagen fibrils are themselves twisted into collagen fibers, which are “glued” together by a thin layer (1-2 nm thick) of extra-fibrillar matrix (68,69). When the tissue is externally loaded in tension, the load is resolved into tensile deformation of the mineralized fibrils and shearing deformation in the extra-fibrillar matrix (70). While no precise data on its mechanical behavior or its composition are available, it is likely that the extra-fibrillar matrix is comprised of non-collageneous proteins, such as osteopontin, and proteoglycans, such as decorin. Indeed, single-molecule spectroscopy of fractured bone surfaces has confirmed that the extra-fibrillar matrix has properties similar to a glue-layer between the fibrils – specifically, it is relatively weak but ductile and deforms by the successive breaking of a series of “sacrificial bonds” (68,71). Specifically, the separation of individual fibrils and the larger fibers during deformation and fracture can be considered to be resisted by this macromolecular glue via a process of sacrificial bonds that break at a fraction (~0.1-0.5) of the force required break the backbone of the macromolecules (72). The matrix may also be partially calcified (73), which would increase its shear stiffness and reduce its deformability. These results point towards a deformation mechanism where the matrix/fiber interface is disrupted beyond the yield point, and the matrix moves past the fibers forming and reforming the matrix/fiber bonds. An alternative explanation

would be the disruption of bonds between the matrix and hydroxyapatite particles and a modification of the frictional stress between individual fibers (74).

### **3.4. Microcracking**

At several length-scales from the sub-micrometer to the tens of micrometers, the process of microcracking in bone provides the prevalent mechanism of microscale deformation (75-78). As described in the next section, such microcracking is not only a process of “plastic” deformation, thereby providing an intrinsic contribution to the toughness of bone, but it is also an essential phenomenon for the development of the most potent extrinsic toughening mechanisms, notably crack bridging and crack deflection, that predominate at larger length-scales (31). Microcracking may also play a crucial role in signaling the remodeling of the bone, which occurs in so-called basic multi-cellular units (BMUs), *i.e.*, combination of cells that are able to remove (osteoclasts) and form (osteoblasts) bone tissue (21).

## **4. EXTRINSIC TOUGHENING (SHIELDING) MECHANISMS**

The (primary) sub-micrometer deformation mechanisms described above contribute intrinsically to the fracture toughness of bone by forming “plastic zones” around crack-like defects, thereby protecting the integrity of the entire structure by allowing for localized failure through energy dissipation. However, at micro- to macro-scale dimensions, the toughness of cortical bone is associated with a very different kind of

toughening associated with crack-tip shielding (Fig. 3). Perhaps surprisingly, the main structural feature that appears to control such extrinsic toughening is quite large, namely the osteons, which are several hundred micrometers in size (79). Akin to many biological materials, these structural features provide a source of toughening which arises during crack growth, rather than crack initiation. To better understand this distinction, it is important to consider that many of the macroscopic fracture toughness properties of bone can be understood mechanistically in terms of the nature of the crack path. A central feature of this fracture behavior is that certain features in the microstructure, especially the interfaces of the osteons, provide microstructurally 'weak' or preferred paths for cracking. As these features have a specific alignment in bone, this provides the basis for the marked anisotropy of the fracture properties of bone (bone is easier to split and to break) (31,37,80-82) and for the fact that the toughness is actually lower in shear than in tension (80,83,84).

#### **4.1. Constrained microcracking**

In cortical bone, the path of least microstructural resistance is invariably along the cement lines (80,83,84), which are the hyper-mineralized interfaces between the bone matrix and secondary osteon structures (*e.g.*, in human bone), or along the similarly hyper-mineralized boundaries of the primary osteons (*e.g.*, in antler bone). These regions are therefore preferential sites for major microcracks to form, particularly as bone ages and the osteon density increases with remodeling. These microcracks

therefore have a typical spacing in the tens to hundreds of micrometers and are primarily aligned along the long axis of the bone, an orientation which directly results in the strong anisotropy of toughness in bone. Fracture of hydroxyapatite crystals surrounding collagen fibers or delamination at the crystal-fiber interfaces have been suggested as the cause of such microcracking damage (85).

As such microcrack formation predominates in the vicinity of a growing (macro) crack where the local stresses are highest, it had been thought that this process was responsible for the marked R-curve toughness behavior in bone (75-78). However, recent calculations (79,80,83,86,87) have clearly shown that although microcracking is important intrinsically for toughness as a deformation mechanism, its direct extrinsic contribution to the toughness is minimal<sup>2</sup>. However, the importance of the microcracking extrinsically is that it is the essential process which results in both crack bridging and crack deflection, which are the most potent toughening mechanisms in bone.

#### **4.2. Crack bridging and crack deflection/twist**

The occurrence of microcracking is the source of extrinsic toughening in bone that is primarily associated with such mechanisms as crack deflection/twist and crack bridging

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<sup>2</sup> This would arise from the creation of a zone of microcracking surrounding the crack where the stresses are highest. As microcrack formation leads to a dilation in this zone, which is constrained by surrounding regions that are less microcracked, the concept of "constrained microcracking" has the crack advancing into a zone of compression, thereby inducing extrinsic toughening and R-curve behavior. However, for realistic volume fractions of microcracks in bone, the contribution to the overall toughness from this mechanism has been estimated to be less than  $0.1 \text{ MPa}\sqrt{\text{m}}$  (79,80,83,86,87).

(31,37,80-82). In fracture, crack trajectories result from the competition between the direction of maximum mechanical driving force (defined by the maximum strain energy release rate,  $G_{\max}$ , or where the mode II (shear) stress intensity is zero,  $K_{II} = 0$ ) and the path of “weakest” microstructure resistance (84,88). Where these two paths conflict, generally high toughness results. In contrast to the longitudinal orientations where these preferred mechanical and microstructural crack paths are nominally in the same direction (Figs. 6f-j), these two requirements are incommensurate in the transverse (breaking) orientation, with the maximum driving force oriented parallel and directly ahead of the crack tip (which promotes coplanar cracking), and the “weakest” paths oriented perpendicular to the crack tip along the direction of the major microcracks (Figs. 6a-e). In human cortical bone, as the cement lines are oriented nominally along the longitudinal axis of the bone, preferred cracking paths will tend to be along this direction – this can lead to the significant (macroscopic) deflection of cracks which are attempting to propagate in the transverse direction (Figs. 6a,e). It is this phenomenon that makes the transverse orientation so much tougher and why bone is much more difficult to break than to split (80). Recent fracture-mechanics measurements show that after only 500  $\mu\text{m}$  of cracking, the fracture toughness, specifically the driving force for crack propagation, is more than five times higher in the transverse (breaking) direction than in the longitudinal (splitting) direction (Fig. 7) (80).

The major distinction in the toughness between these two orientations in bone can be directly attributed to the marked difference in crack paths (Fig. 6), which in turn results in different toughening mechanisms, primarily involving crack deflection/twist in the transverse direction and crack bridging in the longitudinal directions.

*Crack deflection/twist:* In the transverse orientation, the cement-line microcracks are roughly aligned perpendicular to the crack path, where they act as “delamination barriers”; this serves to blunt any growing cracks, cause marked deflections and twists in the crack path (Fig. 3), generate highly tortuous crack paths, extremely rough fracture surfaces, and correspondingly high toughness (Figs. 6a-e). The latter follows because of the reduced local stress field due to crack blunting and the need to reinitiate the crack following local arrest at such delaminations; in addition, such gross crack-path deviations away from the plane of maximum tensile stress greatly diminish the local stress intensity at the crack tip, thereby necessitating higher applied loads to continue cracking (Fig. 7) (80,83).

In addition to its important role in human cortical bone, the process of major crack deflections/twists at the osteons boundaries is also a potent source of toughening in the transverse orientation in other forms of bone, particularly in elk antler bone which is the toughest biological material on record. Three-dimensional images of crack propagation in antler reveal extensive (out-of-plane) crack twisting at angles of up to ~90 degrees (Figs. 6c, 8a,b) in addition to in-plane crack deflections (Figs. 8a,b). Linear-elastic

calculations using crack-deflection mechanics (89) show that for *in-plane* deviations of the crack path, the resulting fracture toughness can be increased by up to a factor of two compared to that for an undeflected crack; where the crack twists *out-of-plane*, this increase can be significantly higher (factor of six or more) (90).

*Crack bridging:* The crack path and consequent salient mechanisms of toughening in the longitudinal orientations are quite different. In human bone, the cement line microcracks are now aligned roughly parallel to the growing crack, and thus form ahead of, and parallel to, the main crack tip (termed the “mother and daughter” crack syndrome). The nature of the coalescence of such microcracks to the growing crack leads to the formation of uncracked regions along the crack length (Figs. 6h, 8c,d) which act to ‘bridge’ the crack and carry load that would otherwise be used to further crack propagation (79,80,83,86,87).<sup>3</sup> This mechanism of crack bridging, termed “uncracked-ligament bridging” also results in toughening but is a far less potent mechanism than that due to crack deflection/twist (31,80).

At smaller dimensions, there are other modes of crack bridging in bone. One prevalent form of bridging at micrometer to sub-micrometer length-scales is that due to fibrils which span many of the microcracks that form in bone (Fig. 3) (78). Although for a *single* propagating crack, fibril/fiber bridging does not generate much of a contribution

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<sup>3</sup> Crack bridging is a common mechanism of extrinsic toughening in many other classes of materials, including the generation of intact fibers spanning crack paths in many fiber-composite materials, the formation of interlocking grain bridges in monolithic ceramics and ductile-phase bridging in ceramic/metal and ceramic/polymer laminates and composites (29).

to the overall toughness ( $\sim 0.1 \text{ MPa}\sqrt{\text{m}}$ ) (80,83), it is important for its cumulative, integrated effect throughout the numerous microcracks that are generated in bone.

## 5. CASE STUDY: TOUGHENING IN ANTLER BONE

As noted above, one of the toughest materials in Nature is the cortical bone in elk antlers. We choose this here as an example of how bone is toughened at multiple length-scale by both intrinsic (plasticity) and extrinsic (shielding) mechanisms (91). Elk antler has the highest strain to failure of the entire bone family, with an ultimate tensile strain of  $\sim 12\%$  (Fig. 9), which is six times higher than the ultimate tensile strain of the human cortical bone ( $\sim 2\%$ ) (16). Evaluation of the R-curve behavior using nonlinear elastic fracture mechanics (see Appendix) clearly demonstrate that antler, like human bone, derives most of its resistance to fracture extrinsically during crack growth.  $J$  values reach exceptionally high values of  $\sim 60 \text{ kJ/m}^2$  over the first  $600 \mu\text{m}$  of crack extension (Fig. 10c,d), representing the highest toughness reported for any biological material reported to date (92). This is twice the toughness of human cortical bone (humerus) in the same orientation ( $J \sim 30 \text{ kJ/m}^2$ ) (80). In contrast, antlers tested in their longitudinal orientations (in-plane and anti-plane) are not as tough, but still reach (steady-state) fracture toughnesses of  $J_c \sim 4\text{-}5 \text{ kJ/m}^2$ , *i.e.*, more than an order of magnitude higher than in human cortical bone (to extend a crack  $\sim 500 \mu\text{m}$ ). Analogous to human cortical bone, the extrinsic component to this toughening is associated with extensive crack deflection and twisting in the transverse (breaking) orientation and



uncracked-ligament bridging in the longitudinal (splitting) orientations, both motivated by extensive microcracking along the hyper-mineralized interfaces of the primary osteons (91).

Traditionally, however, the high fracture toughness of antler has been attributed to the relatively high organic volume fraction and a higher potency of microcracking (77,93,94). A characteristic of its mechanical properties is its lower transverse strength and much lower stiffness compared to human bone, which is associated with its extensive plasticity (Fig. 9). This is the other major contribution to its toughness, but now intrinsically. The low yield strength in the longitudinal direction allows crack-tip plastic zones to form at lower stresses than in human bone (77,93), which contributes to the large inelastic deformation and thereby to its intrinsic toughness. Such contributions from nanoscale “plasticity” within the mineralized collagen fibrils are important, although little is known about these mechanisms in antler. Recently, *in situ* tensile testing on compact antler bone combined with small angle x-ray diffraction measurements (95) has revealed that while both antler and (bovine) bone show similar nanoscale fibril shearing (96) mechanisms during elastic deformation, during inelastic deformation, *i.e.*, after macroscopic yielding, they are different with inhomogeneous fibril stretching in antler leading to defects and consequent debonding between neighboring fibrils. The result is that strain localization in antler is suppressed at the microscale; moreover, this process is thought to cause the formation of nano- and

microcracks, which further contributes to its extensive inelastic deformation prior to failure.

The observed characteristic length-scales at each hierarchical level in bone is a result of structural adaptation towards maximizing target materials properties such as strength and toughness. The significance of this is that compared to human bone, for example, antler is tougher yet is not as strong, highlighting the vital distinction between strength and toughness in biological materials (97). In general, it is this combination between the intrinsic toughening contributions from (primarily sub-micrometer) “plasticity” mechanisms and the potent extrinsic toughening contributions from crack deflection/twist and crack bridging mechanisms (primarily at micrometer dimensions and above) that provides the basic description to how bone derives its resistance to fracture.

## **6. GENETIC DISEASES IN BONE**

Even though the mineral phase is crucial to create stiffness and toughness of bone tissue, the collagenous protein phase plays an elementary role in defining the physiological properties of the material. This is evident from the fact that structural alterations, or mutations in the collagen gene, can lead to severe diseases that are associated with a severe deterioration of the mechanical properties of bone. Notably, these mutations can be minute, and encompass only one out of thousands of amino

acids, yet, has catastrophic effects on the performance of collagenous tissues and bone in the physiological setting.

One of the most well known genetic diseases is *osteogenesis imperfecta*, or brittle bone disease, where tiny single point mutations (*i.e.* molecular defects at the level of single amino acids, or a few Angstrom in length-scale) in the collagen type I gene leads to catastrophic effects at larger scales (98). Experimental analysis of the mechanical properties showed that the stiffness and strength of *osteogenesis imperfecta* tissue shows mechanical weakness (99). Recent materiomics studies, carried out using a hierarchy of atomistic and molecular multi-scale simulation techniques, elucidated the molecular and mesoscale mechanism of the disease, and showed that the mutation leads to the formation of tiny cracks at the molecular scale, due to a softening of the tropocollagen molecule's stiffness and a significant reduction of the intermolecular adhesion, effectively resembling the formation of nano-cracks (100,101) (see, Fig. 11).

The presence of these nano-cracks results in a change of the stress distribution in the fibril; leading to locally large shear stresses that induce the propensity of material failure due to intermolecular shear. This example shows that under disease conditions, the intrinsic repair and toughening mechanisms of bone can fail to function properly and lead to a rapid breakdown of the tissue (4). Other collagen gene related diseases include some forms of dwarfism, Alport's syndrome, and Ehlers-Danlos syndrome (56,102-104).

## 7. DEGRADATION WITH AGING

Despite the development of such fracture resistance in human bone, the risk of bone fracture increases markedly with aging. As noted above, this has been traditionally related to a loss in bone mass (osteoporosis) but now it is realized that there is an additional degradation in the quality of bone with age. Fracture toughness measurements on 34 to 99 year old human cortical bone show some 40% reduction in the crack-initiation toughness and almost complete elimination of the crack-growth toughness with increasing age (Fig. 12) (86). The reasons for this are complex and not totally clear (79,86). With aging, at the nanoscale there are distinct changes in the collagen environment specifically with regard to an increased degree of cross-linking, at the sub-micrometer scale the nature and properties of individual collagen fibrils deteriorate, and at the scale of 1 to 100  $\mu\text{m}$  the microstructure of the bone level changes due to an increased density of osteons, the latter perhaps having the largest effect on the macroscale fracture toughness properties (79). With progressive aging, bone is remodeled, in human bone at the interior of the cortex (Haversian remodeling) which then stimulates further remodeling as the growing cement lines sever the tiny canals that connect interstitial bone cells (this leads to cell death) (16). The result of this is that the density of osteons increases with age (79). As the cement lines provide the prime sites for microcrack formation, the increased osteon density gives rise to a higher microcrack density; this can be associated with a diminished crack-initiation toughness,

but more importantly to smaller uncracked-ligament bridges spanning the crack, which in turn significantly degrades the crack-growth toughness. Such age-induced degradation in the potency of crack bridging in bone can has been directly measured, and is a major reason for the severe reduction in fracture resistance of bone with aging (31,79).

## **8. CLOSING REMARKS**

As bone is such a complex biological material, we still possess only a limited quantitative understanding of how it deforms and fractures, together with the salient plasticity and toughening mechanisms active at the various structural length- and time-scales. The advent of multi-scale observations, high-resolution structural imaging using transmission electron microscopy and computed x-ray micro-tomography, and vastly more powerful theoretical/computational tools, however, now enables the analysis of bone as a “material” with rigorous physical science techniques. These types of studies that seek to uncover, model and quantify the fundamental nano to macroscale mechanisms that govern the properties of bone are quite distinct from those that have been traditionally followed in the bone and medical communities. From the current work, we can conclude that origins of fracture resistance in bone can be traced to a suite of mechanisms acting at numerous length-scales, from nano to macro. These mechanisms can be classified as “plasticity” mechanisms which operate principally at sub-micrometer dimensions to promote intrinsic toughness, involving molecular

uncoiling of collagen molecules, fibrillar sliding of mineralized collagen fibrils and individual collagen fibers, and microcracking, and crack-tip shielding mechanisms which operate at length-scales of  $\sim 1$  to  $100 \mu\text{m}$  to promote extrinsic (crack growth) toughness via such mechanisms as crack deflection/twist and crack bridging. On the other hand, certain molecular level alteration of the structure of constituting collagen molecules due to genetic mutations can result in a catastrophic breakdown of bone tissue, indicating a lack of an ability of bone to deal with certain structural changes. Although not mainstream medical science, we believe that the results from such materials science based studies can positively impact the medical field by providing new and different insights into bone-related diseases, aging and the remodeling properties of bone, and as such help in the search for new cures and treatment options for a variety of bone diseases.

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#### **APPENDIX: Quantitative Assessment of Toughness in Bone**

As described above, significant efforts have been made over the past ten years or so to elucidate how bone is toughened. However, one important feature of this is how to quantitatively measure the macroscopic toughness of bone. Traditionally, this has been achieved by measurements of the *work of fracture*,  $W_t$ , which is obtained by dividing the area under the load-displacement curve measured during the toughness test by twice the nominal crack-surface area. This approach has been widely used in the past to quantify the toughness of cortical bone in unnotched (nominally “flaw-free”) specimens (16,17,105-110) but suffers from the fact that the results can be both specimen size- and geometry-dependent. Consequently, work of fracture measurements are generally not too useful for comparing toughness values determined in different studies that utilized different sample geometries, but may be used successfully to assess trends when the nominal sample size and geometry are held constant.

Akin to other structural materials, the fracture of bone is best characterized using fracture mechanics methods. In its simplest form, this involves linear-elastic fracture mechanics (LEFM) assessments of toughness, which have been widely used for bone. Here an assumption that bone is a linear-elastic material must be made, specifically that any inelastic (*e.g.*, yielding) behavior is limited to a small near-tip region such that the stress and displacement fields local to the tip of a pre-existing crack can be described by the stress-intensity factor,  $K$ , where  $K$  can be defined for mode I (tensile-opening loading), mode II (shear loading), or mode III (tearing or anti-shear loading) (97,111). There is a large body of results in the literature involving assessments of the fracture toughness of cortical bone using LEFM, which involved single-parameter characterization of the toughness using either the critical value of the mode I linear-elastic stress intensity,  $K_{Ic}$  (110,112-115), or the related strain-energy release rate,  $G_c$  (116,117).<sup>4</sup>

Whereas such a fracture mechanics approach represents a significant improvement over measurements of toughness involving parameters such as the work of fracture, single-value linear-elastic fracture parameters based on crack initiation, such as  $K_{Ic}$ ,

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<sup>4</sup> The fracture toughness,  $K_{Ic}$ , (where  $i = I, II$  or  $III$ ) is the critical value of the stress intensity  $K$  for unstable fracture in the presence of a pre-existing crack, *i.e.*, in mode I when  $K_I = Y\sigma_{app}(\pi a)^{1/2} = K_{Ic}$ , where  $\sigma_{app}$  is the applied stress,  $a$  is the crack length, and  $Y$  is a function (of order unity) of crack size and geometry. The alternative LEFM approach is to express the toughness as a critical value of the strain-energy release rate,  $G_c$ , defined as the change in potential energy per unit increase in crack area. The relationship between

these two parameters is given by:  $G_c = \frac{K_{Ic}^2}{E} + \frac{K_{IIc}^2}{E} + \frac{K_{IIIc}^2}{2\mu}$ , where  $E$  and  $\mu$  are, respectively, the Young's and shear modulus.



cannot capture, or even represent, the multiple length-scale toughening acting in cortical bone. As described in this article, although intrinsic (deformation) mechanisms primarily govern the crack-initiation toughness, the predominant toughening mechanisms in bone are extrinsic and hence operate largely in the wake of the crack to govern the crack-growth toughness. This results in some degree of stable cracking before unstable fracture, leading to characteristic resistance-curve (R-curve) behavior (76,77,86,94,118,119) where the fracture resistance actually increases with crack extension.<sup>5</sup>

Although such linear-elastic stress-intensity based R-curves do provide a means to characterize crack propagation, the underlying assumptions for such  $K_R$  calculations do not account for the energy associated with *plastic* deformation during bone fracture, which is associated with intrinsic toughening in bone. The LEFM approach is only valid where small-scale yielding conditions apply (111), *i.e.*, where the extent of local (crack-tip) inelasticity is small compared to the size of the bone or test sample. This places restrictions on how large a test specimen has to be for “valid” toughness measurements, specifically, that the in-plane specimen dimensions of crack size and the width of the remaining uncracked ligament must be at least an order of magnitude

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<sup>5</sup> The R-curve provides an assessment of the fracture toughness in the presence of subcritical crack growth. It involves measurements of the crack-driving force, *e.g.*,  $K$  or  $J$ , as a function of crack extension ( $\Delta a$ ). The value of the driving force at  $\Delta a \rightarrow 0$  provides a measure of the crack-initiation toughness, whereas the slope (or maximum value) of the R-curve can be used to characterize the crack-growth toughness.

larger than the plastic-zone size (“small-scale yielding”); additionally, for geometry- and thickness-independent toughness values, the out-of-plane thickness dimension must be equally larger than the plastic zone (“plane strain” conditions). For example, in antler bone a LEFM  $K_{Ic}$  value of  $\sim 10 \text{ MPa}\sqrt{\text{m}}$  (120) would require test specimen dimensions (in terms of crack size, ligament depth and thickness) in excess of 50 mm for a valid linear-elastic  $K_{Ic}$  based on current ASTM validity criteria (121). Since the thickness of the cortical shell in antler bone is typically only  $\sim 5 \text{ mm}$ , LEFM  $K_{Ic}$  measurements are simply not an appropriate means to measure its toughness, as the distribution in local stresses and displacements near a crack tip (*i.e.*, near a the fracture origin) would not be represented by the  $K$ -field (122) such that the resulting  $K$ -based toughness values would be highly questionable. This may not be the situation for all bones, but in the majority of cases the bone size in comparison to the extent of local “plasticity” will be insufficient for LEFM toughness measurements to be reliable (and geometry- and size-independent).

Accordingly, to assess the toughness of bone material with its large post-yield deformation and subcritical cracking behavior, an R-curve approach based on nonlinear elastic fracture mechanics approach is essential to provide a more realistic description of the crack-tip stress and displacement fields and furthermore is able to additionally capture the contribution to the toughness from the energy consumed in “plastic” deformation prior to and during fracture (80,123). The best approach here

appears to be the use of the  $J$ -integral as the crack-driving force, where  $J$  is the nonlinear strain-energy release rate, *i.e.*, the rate of change in potential energy for a unit increase in crack area in a nonlinear elastic solid (the nonlinear elastic equivalent of  $G$ ). In analogous fashion to  $K$  in linear-elastic materials,  $J$  characterizes the stress and displacement fields at a crack tip in a nonlinear-elastic solid, and as such can be used to define the onset of fracture there. Descriptions on the use of  $J$ -integral measurements for bone can be found in refs. (80,81,91,123). Indeed,  $J$ -integral measurements have now been used to quantify the toughness of several biological materials, including bone (80,81,91,123), dentin (124) and nacre (125).

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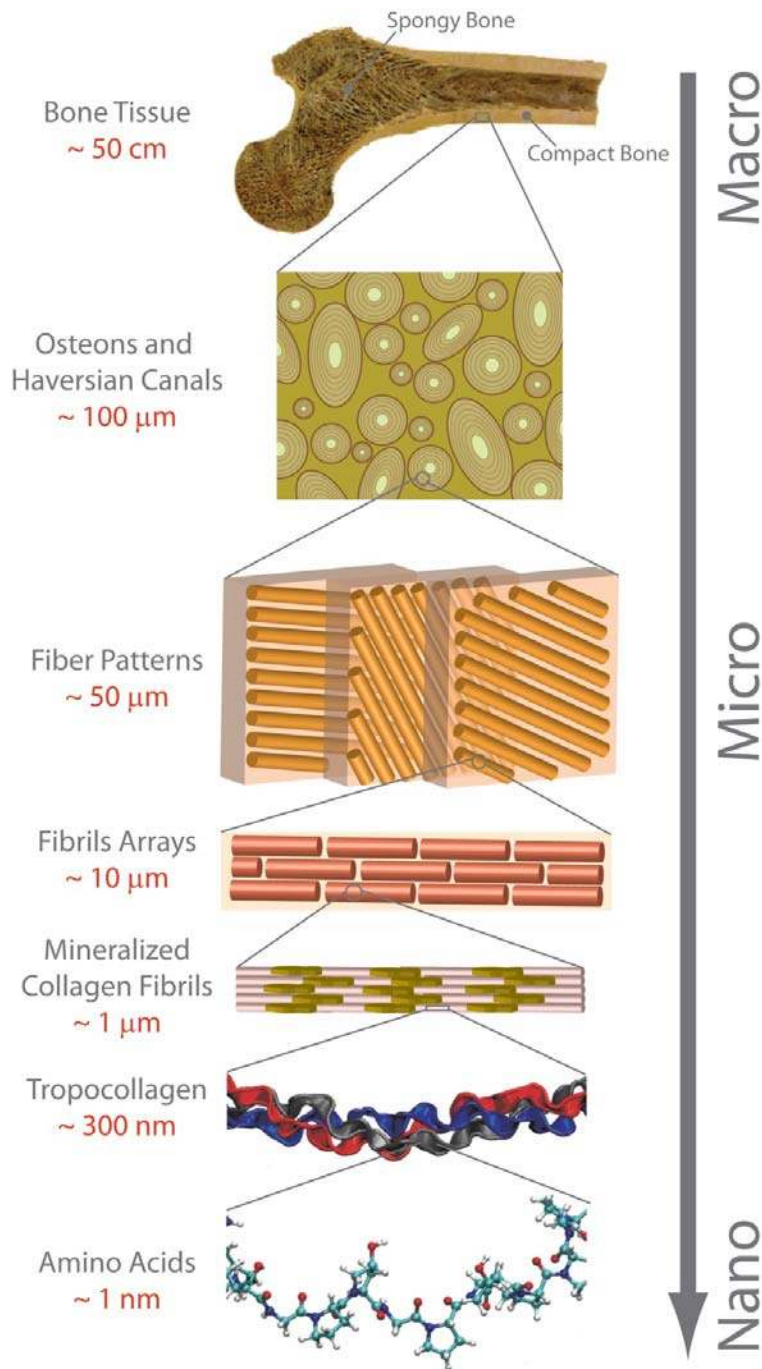
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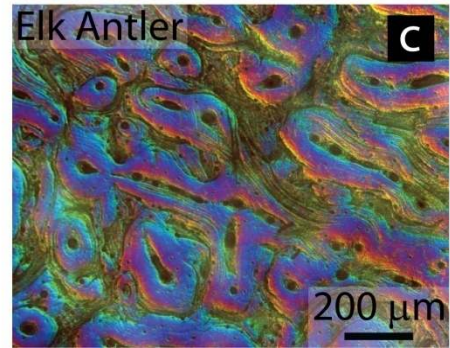
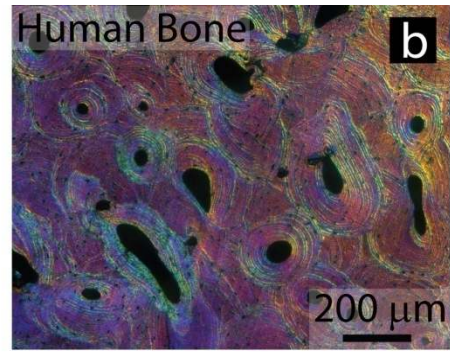
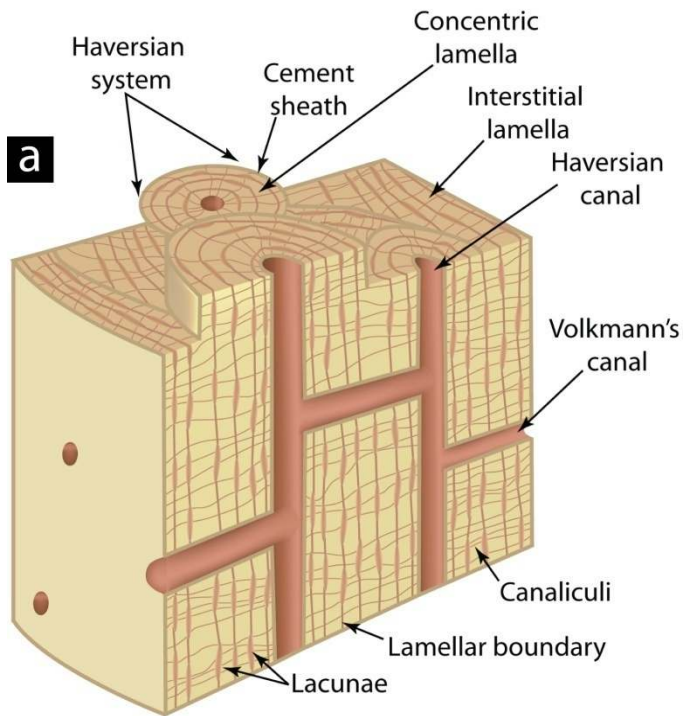
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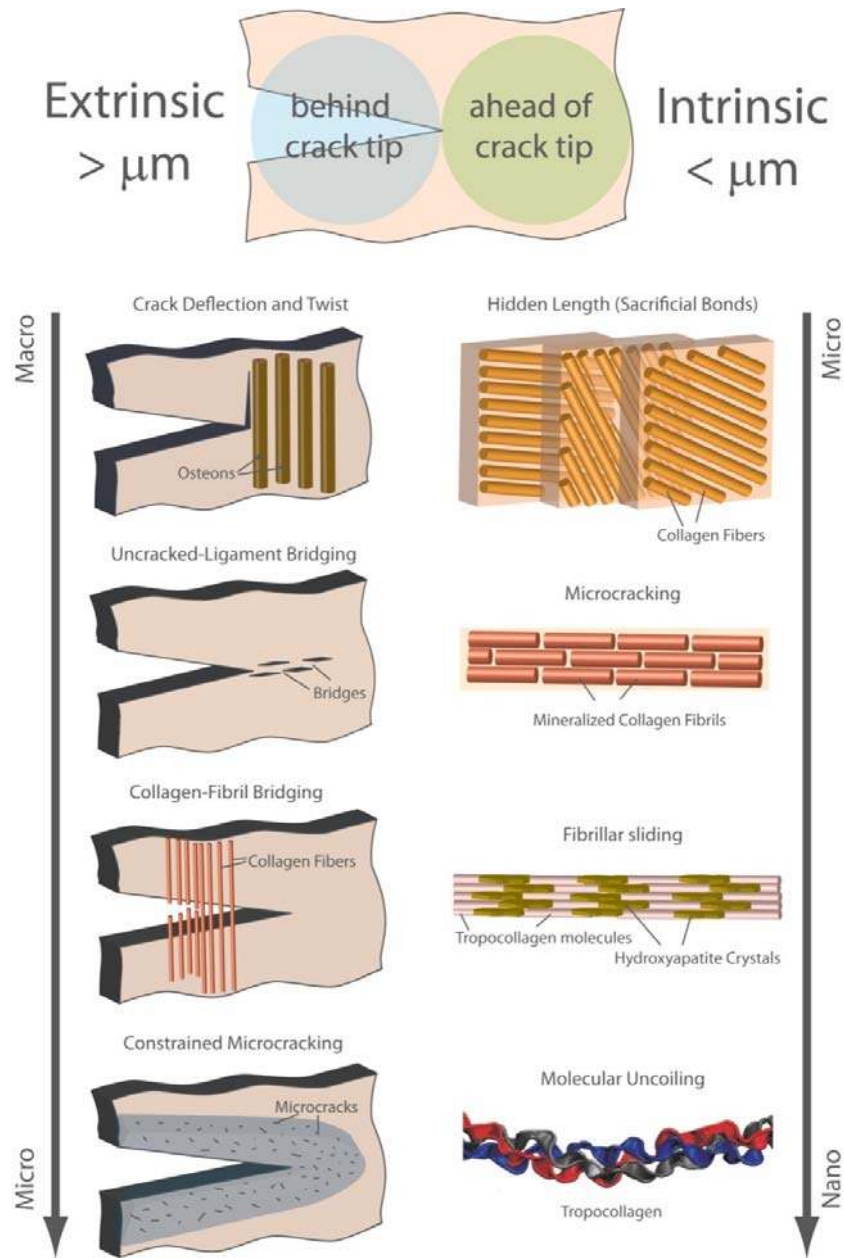
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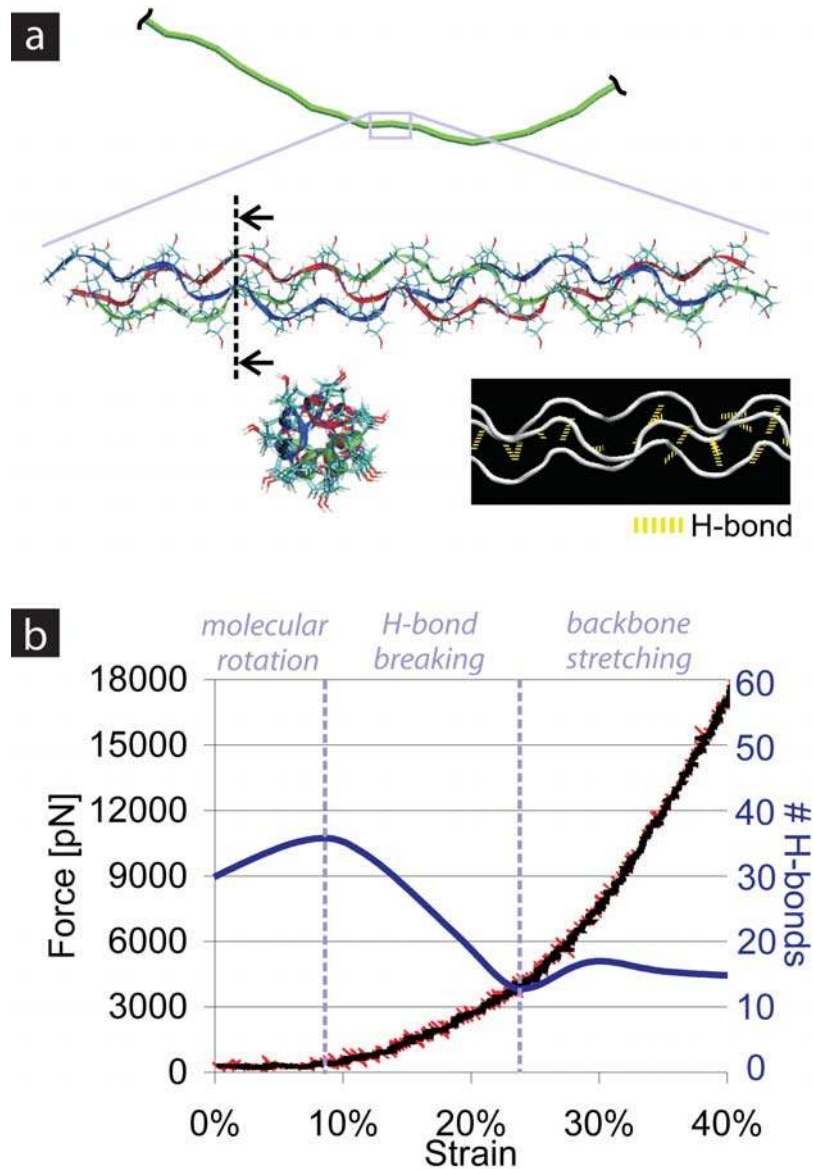
**Figure 1.** Hierarchical structure of bone showing seven levels of hierarchy. The macroscale arrangements of bones is either compact /cortical (dense material found at the surface of all bones) or spongy/cancellous (foam-like material whose struts are some 100  $\mu\text{m}$  thick). Compact bone is composed of osteons that surround and protect blood vessels. Osteons have a lamellar structure. Each individual lamellae is composed of fibers arranged in geometrical patterns. These fibers are the result of several collagen fibrils, each linked by an organic phase to form fibril arrays. Each array makes up a single collagen fiber. The mineralized collagen fibrils are the basic building blocks of bone. They are composed of collagen protein molecules (tropocollagen) formed from three chains of amino acids.



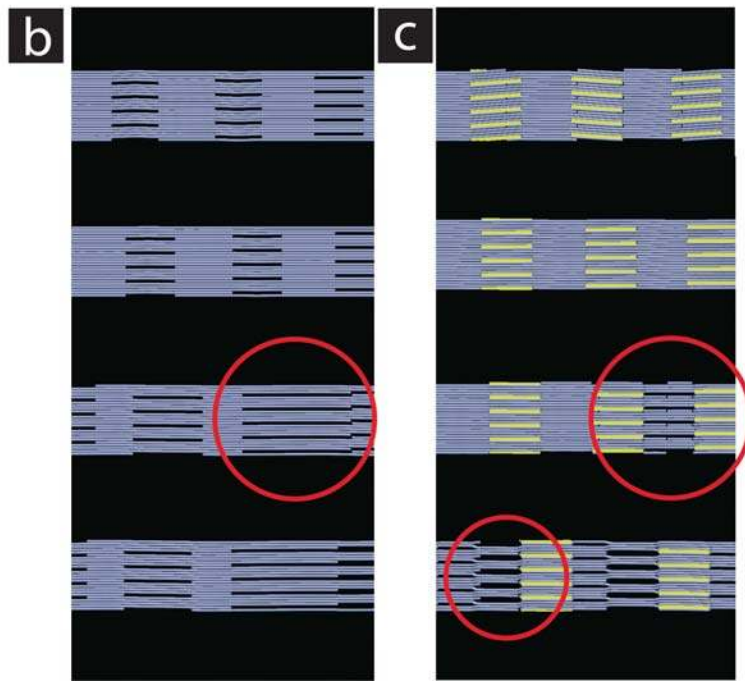
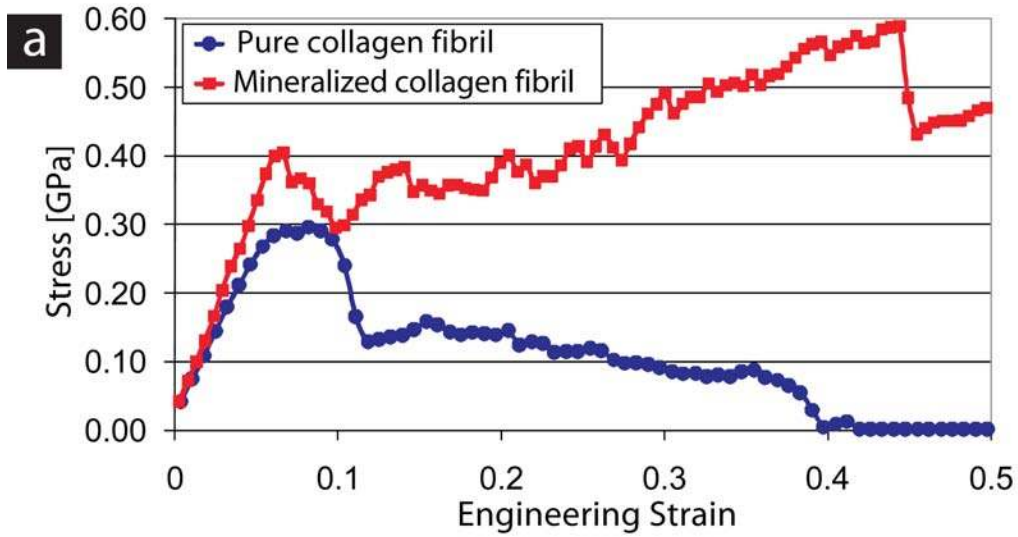
**Figure 2.** (a) Schematic diagram of the structure of cortical bone showing the pertinent microstructural features. Microstructure of (b) secondary and (c) primary bone. Differential interference contrast (Nomarski) micrograph of transverse section of compact (cortical) bone of (b) human humerus, and (c) elk antler. The main distinction between primary and secondary bone is that primary osteons do not have cement lines because they are not the product of bone remodeling; however the interfaces of the primary osteons in antler are thick hyper-mineralized regions. Primary osteons have smaller vascular channels and fewer lamellae than secondary osteons.



**Figure 3.** The toughness of bone results from a mutual competition between extrinsic (crack-tip shielding) toughening mechanisms, that predominate at length-scales above a micrometer and intrinsic (plastic deformation) toughening mechanisms that are active at length-scales primarily below a micrometer. Distinct toughening mechanisms occur at each level of hierarchy. Molecular uncoiling and intermolecular sliding of molecules are observed at the smallest level of tropocollagen molecules and mineralized collagen fibrils. Microcracking and fibrillar sliding are observed at the level of fibril arrays. At larger levels, the breaking of sacrificial bonds contributes to increasing the energy dissipation capacity of bone at the interface of fibril arrays, together with crack bridging by collagen fibrils. At the largest length-scales in the 10 to 100  $\mu\text{m}$ , the primary sources of toughening are extrinsic and result from extensive crack deflection and crack bridging by uncracked ligaments, both mechanisms that are motivated by the occurrence of microcracking.

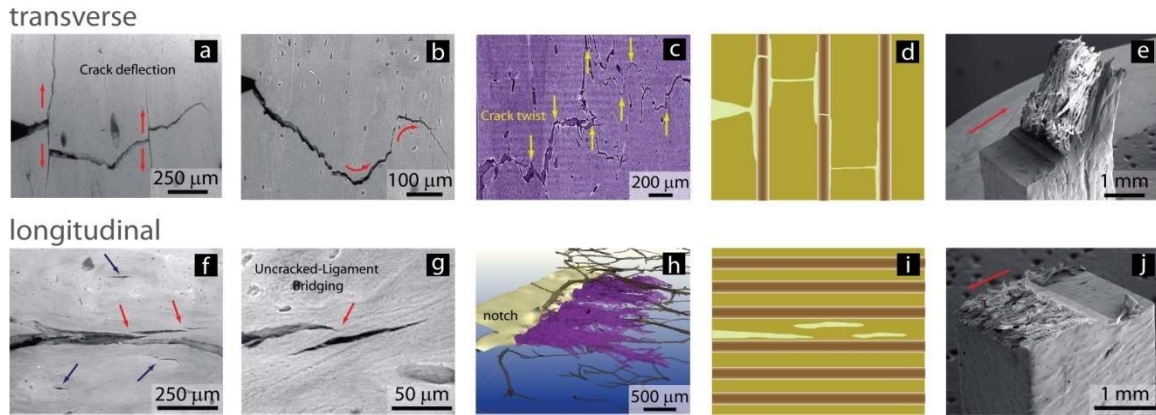


**Figure 4.** Structure and nano-mechanics of a triple helical tropocollagen molecule, the basic protein building block of bone. Subplot (a) shows the structure of this protein molecule; in the right plot the H-bond structure is depicted via the yellow thick dashed lines. Subplot (b) depicts the force-extension behavior, here shown up to 40% strain. The plot also includes an indication of the number of H-bonds (for a tropocollagen segment that is 30 amino acid building blocks long). During the first stage of deformation (less than 10% strain) the number of H-bonds remains close to 30, corresponding to approximately one H-bond for each amino acid triplet. When tropocollagen is straightened, H-bonds gradually break until 25% tensile strain is reached, where a stationary situation is attained, with a number of intramolecular H-bonds close to 15. Beyond this point the force increase is due to backbone stretching and no additional H-bonds break. In this regime no pattern for the remaining H-bonds can be recognized; H-bonds are continuously broken and reformed along the peptide, due to the fact that even if the triple-helical coiling is lost, the three chains are near enough to form H-bonds.

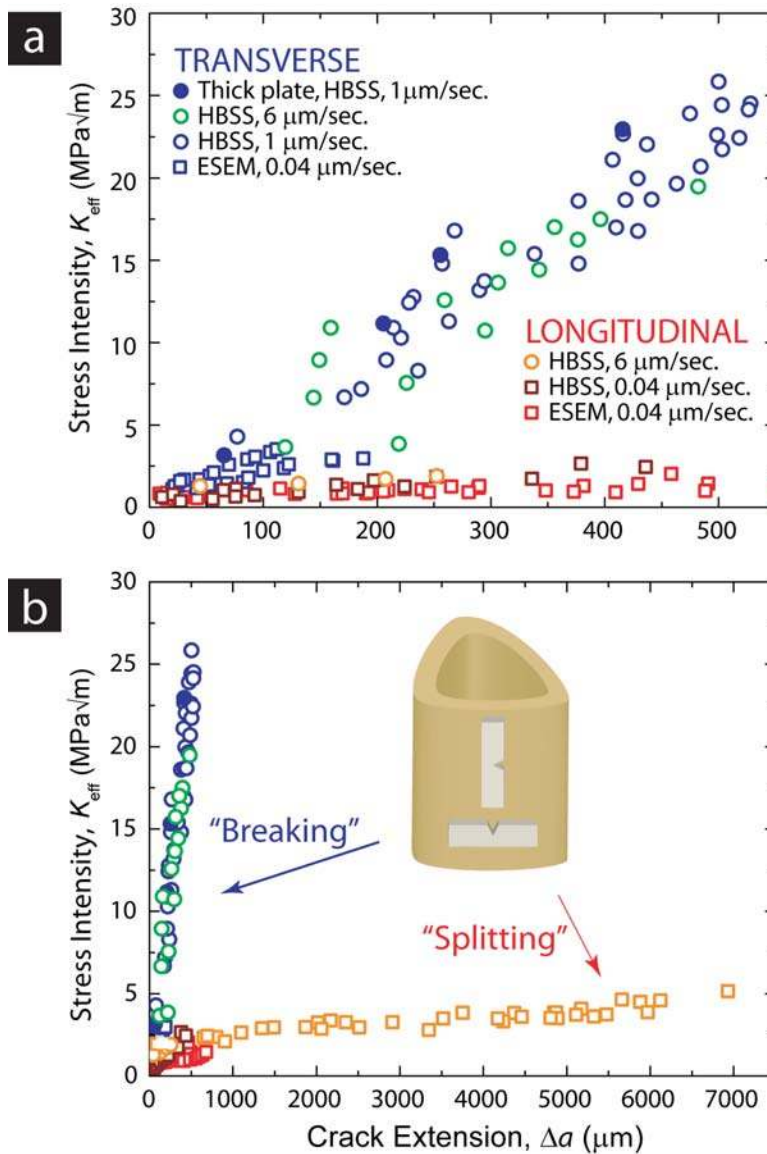


**Figure 5.** (a) Computational result of the stress-strain response of a mineralized *vs.* unmineralized collagen fibril, revealing the significant effect the presence of mineral crystals in collagen fibril has on the mechanical response (66). (b) Snapshots of the deformation mechanisms of collagen fibrils, and (c) mineral collagen fibrils. Slip initiates at the interface between mineral particles and tropocollagen molecules. Repeated occurrence of slip reduces the density, leading to formation of nano-scale voids.



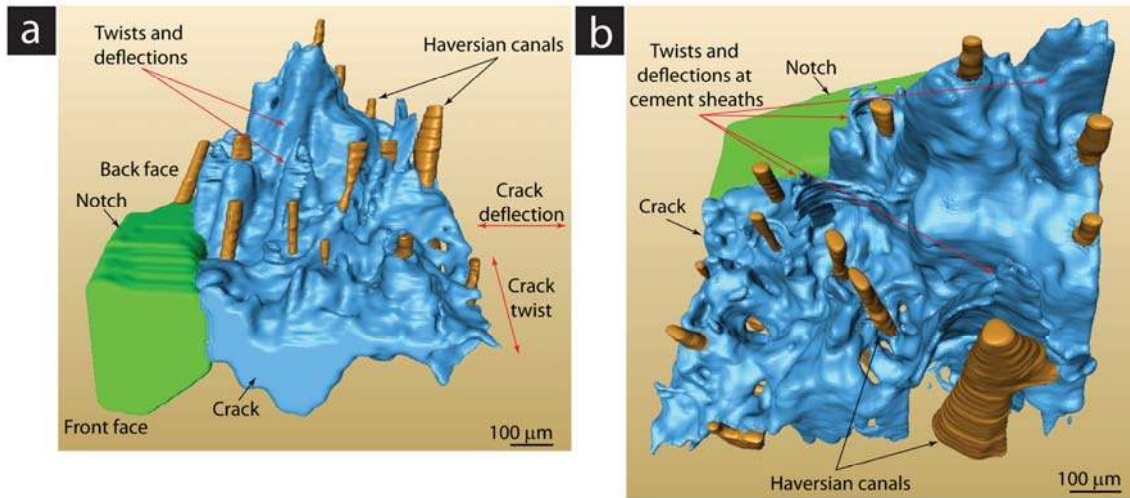


**Figure 6.** Mechanisms for stable crack propagation and toughening in the transverse and longitudinal orientations of compact bone. Environmental scanning electron microscope (ESEM) backscattered electron images of stable crack growth during *in situ* R-curve testing in the (a,b) transverse, (f,g) longitudinal orientations. Synchrotron x-ray computed micro-tomography images of the crack path in both the (c) transverse and (h) longitudinal orientations of compact bone. (e,j) SEM fractography images and (d,i) schematics of the crack trajectory for each orientations. In the transverse direction (a-e), the prominent toughening mechanisms are in-plane crack deflection and out-of-plane crack twisting. (c) A through-thickness slice from the front face to the back face near the crack tip highlighting the twists of the crack through the sample. The arrows indicate some of the major twists at  $\sim 90$  degrees. In the longitudinal (splitting) orientation (h), the crack is very planar with little evidence of deflection. The dominating toughening mechanism here is “uncracked-ligament” bridging (f,g,h,i). The red arrows indicate the direction of deflection in (a,b), direction of crack propagation in (e,j), and uncracked-ligament bridges in (f,g) involving two-dimensional uncracked regions along the crack path that can bridge the crack on opening (126). The blue arrows delineate microcracks that formed at the osteon/matrix interface along the axis of the bone. Such microcracking is essential for many of the toughening mechanisms in bone, notably crack bridging and crack deflection which predominate at micrometer-scales and above.

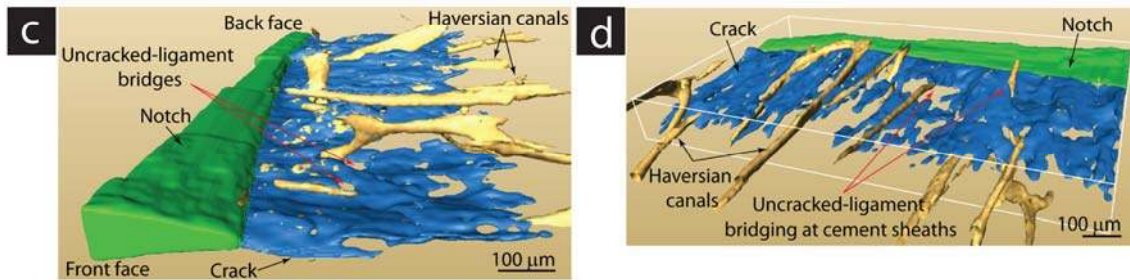


**Figure 7.** Fracture toughness resistance-curve data for the transverse and longitudinal orientations in human cortical bone. Nonlinear elastic fracture mechanics measurements of the fracture toughness of bone show that resistance to crack propagation increases much more rapidly in the transverse (breaking) direction than in longitudinal (splitting) direction, both for (a) realistically small ( $< 500 \mu\text{m}$ ) and (b) larger ( $< 7 \text{ mm}$ ) crack extensions. This results from the different primarily toughening mechanisms at play; crack deflection and twist in the transverse orientation (Figs. 6a-c) and crack bridging in the longitudinal orientation (Figs. 6f-h). This result was independent of loading displacement rate or loss of constraint. In the legend, ESEM denotes tested *in situ* in the environmental scanning electron microscope and HBSS denotes testing *ex situ* immersed in Hanks' Balanced Saline Solution; individual loading displacement rates are also given. The inset shows the orientation of the samples from the humerus. (after ref. (80)).

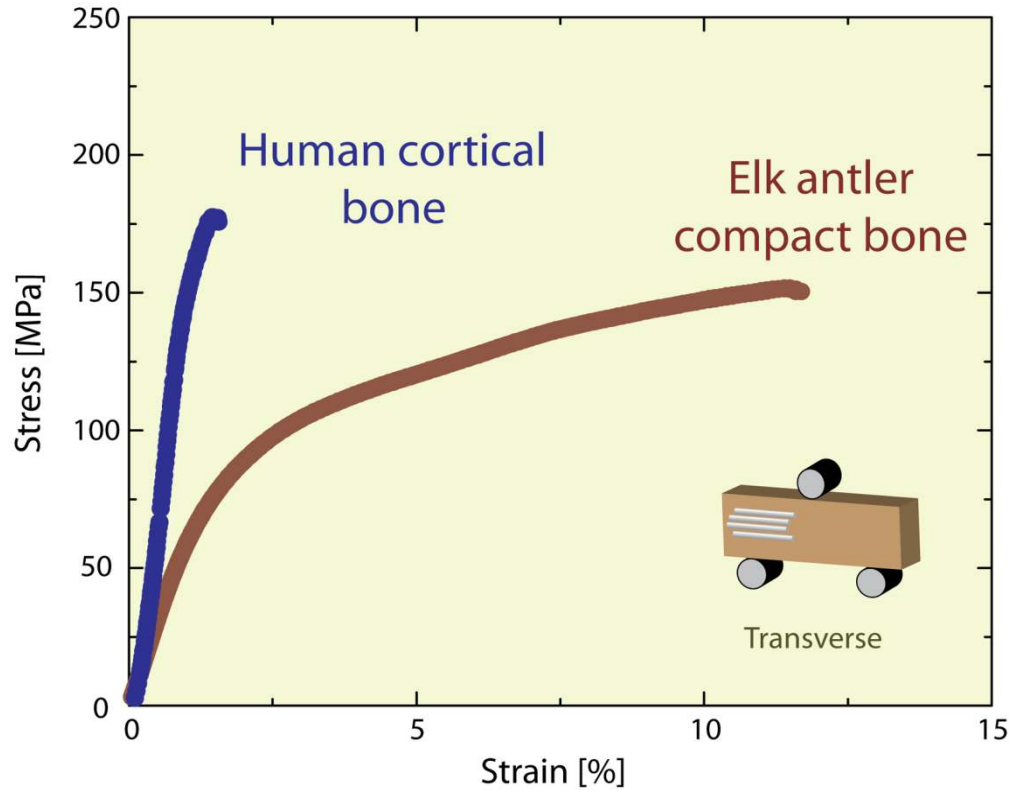
## Transverse Orientation



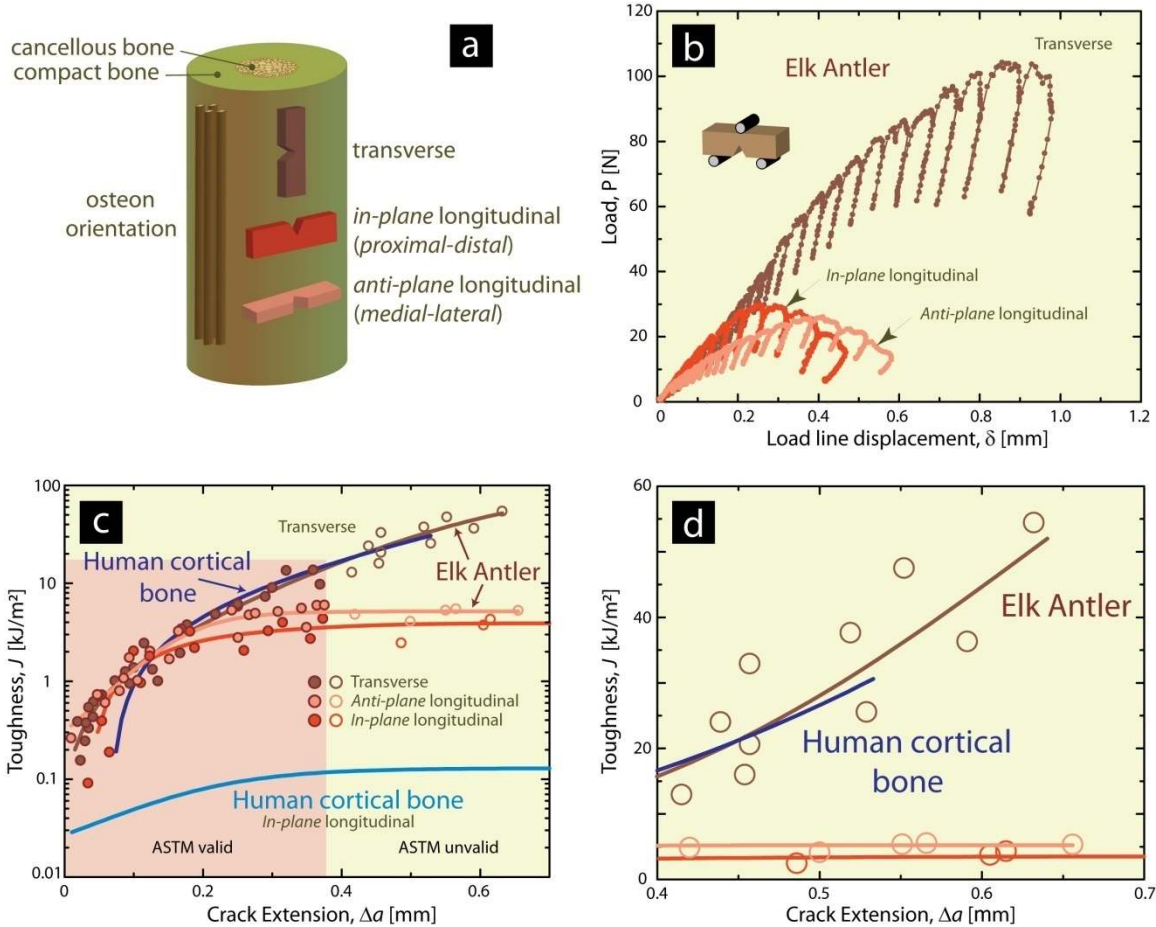
## Longitudinal Orientation



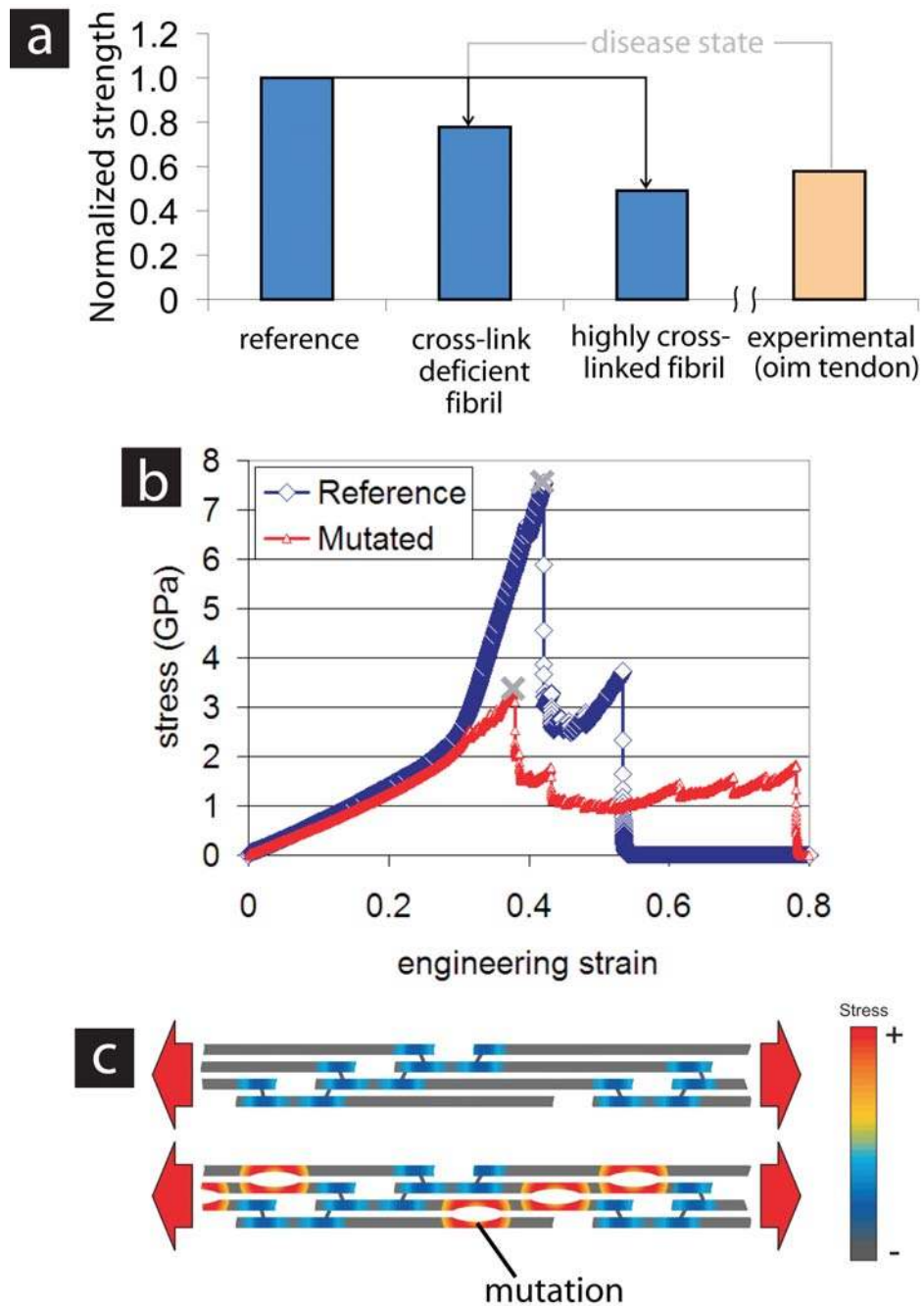
**Figure 8.** Three-dimensional reconstructions in the transverse and longitudinal orientations from synchrotron x-ray computed tomography. Both (a) and (c) show an edge on view of the notch and crack to show the shape of the crack paths. The panels (b) and (d) are oriented to highlight the dominant toughening mechanisms in the two orientations. It is clear from the reconstructions that the principal toughening mechanisms in bone are associated with the cement lines (or sheaths). In the transverse direction, crack twist and deflection dominate the toughness and occur predominantly at the cement sheaths before the crack penetrates the Haversian systems. Three-dimensional imaging in the longitudinal orientation provides novel insight into how the dominant toughening mechanism of uncracked-ligament bridging is associated with Haversian systems. As shown in (d), the crack “flows” around the Haversian system; effectively the crack does not penetrate the Haversian system but propagates along the surrounding cement sheath, leaving an intact Haversian system bridging the crack faces.



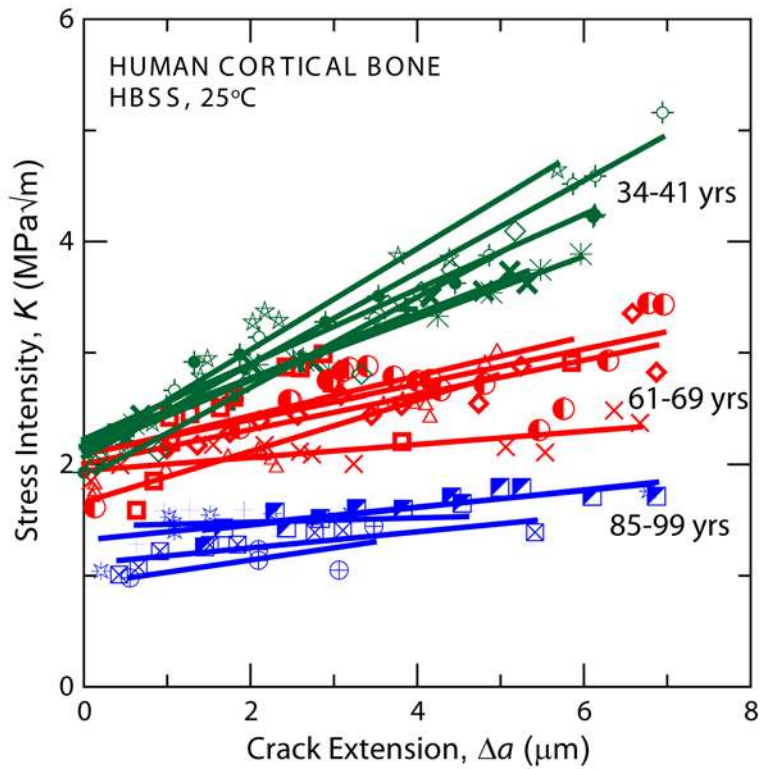
**Figure 9.** Stress-strain curves from three-point bending tests for hydrated human cortical bone and elk antler in the transverse orientation. Data for elk antler are taken from ref. (120).



**Figure 10.** The schematic (a) shows the structure of antler bone, as well as the three anatomical orientations that the specimens were taken from the compact bone. (b) Typical load *vs.* load-line displacement curve obtained during R-curve testing for the transverse, *in-plane* longitudinal, and *anti-plane* longitudinal orientation of antler compact bone. Each partial unloading event during the test corresponds to a data point in (c). (c) Full  $J_R(\Delta a)$  resistance curves for stable *ex situ* crack extension in hydrated antler compact bone tested in transverse, *in-plane* longitudinal, and *anti-plane* longitudinal orientations. (d) expansion of (c) showing  $J_R(\Delta a)$  resistance curves at larger crack extension ( $\Delta a > 400 \mu\text{m}$ ). The R-curves for short crack lengths ( $\Delta a \approx 600 \mu\text{m}$ ) are compared with data taken from human cortical bone in both transverse and *in-plane* longitudinal orientations of the humerus. Data for elk antler from ref. (91) and for human cortical bone from ref. (80). In the transverse orientation, fracture toughnesses,  $J$ , of antler compact bone were recorded at nearly  $\sim 60 \text{ kJ/m}^2$ , almost two times higher than the critical toughness value,  $J_c$ , of human cortical bone ( $J_c \approx 30 \text{ kJ/m}^2$ ). The validity of these data points is defined by the measurement capacity of each specimen in accordance with the ASTM standard (121). The circles in (c) and (d) correspond to data points for antler bone.



**Figure 11.** Effects of osteogenesis imperfecta mutations on mechanical properties of collagenous tissues, based on computational multi-scale (100,101) and experimental results (99). Panel (a) shows the effect of mutations on the strength properties of collagen fibrils and tendon. Panel (b) shows two stress-strain curves, representing the reference case without mutation and the mutated sample. A severe change in the stress-strain response can be observed. Panel (c) shows a schematic to highlight the effect of the mutation on the internal stress distribution, where the formation of nano-cracks leads to large shear stresses inside the fibril.



**Figure 12.** Crack-resistance curves for stable *ex vivo* crack extension in human cortical bone as a function of age from 34-41 years to 85-99 years (tested in Hanks' Balanced Salt Solution at 25°C). The crack-initiation toughness,  $K_0$ , is represented by the intercept of the R-curve on the Stress Intensity axis at  $\Delta a \rightarrow 0$ ; the crack-growth toughness is given by the slope of the R-curve. Note that although the crack-initiation toughness is decreased by roughly a factor of two by aging, the decrease in crack-growth toughness is far greater – by roughly a factor of ten (after refs. (79,80,83,86,87)).