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Review

Nanomechanics and Ultrastructure of Bone: A Review

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Abstract: In this review, a brief presentation is first given to the hierarchical structure and mechanical behavior of bone. Then, the recent advancements in nanoscale characterization of bone ultrastructure and ingredients are discussed based on an extensive quantity of references in the literature. Moreover, computational and analytical bone mechanics at ultrastructure levels are critically reviewed with the growing body of knowledge in the field. The computational and analytical models are summarized in several categories for ease of understanding bone nanomechanics and their applicability/limitations. This review is expected to provide a well-informed foundation for the researchers interested in interrogating the complex biomechanical response of bone at its nanoscale hierarchy.

Keywords: Bone ultrastructure; nanoscale; mechanical properties; simulation and modeling; strength; toughness

1 Introduction

Bone is a highly hierarchical tissue spanning from macro to nanoscale as schematically illustrated in Fig. 1. It is categorized into three different groups at the macrostructural level: i.e., long bone (such as femur, tibia, and humerus), flat bone (such as skull and pelvis), and irregular bone (such as spine column bones). At the mesoscale, bone has two basic structures: cortical and trabecular (cancellous) bone [1]. Cortical bone is a dense compact tissue that accounts for 80% of a skeletal system, while trabecular bone is the spongy network of struts and plates called trabeculae and mainly exists in the epiphyses of long bones, ribs, and vertebrates [2]. Porosity in cortical bone is approximately 5–10%, which includes vascular and lacunar–canalicular pores, whereas trabecular bone porosity is much higher (75–95%) [1]. At microstructural level, human cortical bone comprises osteons and interstitial lamellae, with an interface (cement line) between the two. Osteons, formed in modeling/remodeling processes, resemble cylindrical columns about 200–250 μm in diameter, which is made of concentric layers of lamellae about 3–5 μm in thickness, whereas the interstitial lamellae are the remnant of older osteons and initial lamellar tissues [2]. On the other hand, trabeculae in trabecular bone are typically $\sim$150 μm in thickness and are also made of lamellae [3]. At the lamellar level, bone is a natural nanocomposite, consisting of an organic phase (i.e., 90% collagen fibrils and 10% non-collagenous proteins), a mineral phase (i.e., hydroxyapatite $Ca_{10}(PO4)_6(OH)_2$ crystals), and water [4–6]. The mineral and

organic phases account for approximately 60 wt% and 30 wt% of bone, respectively [1,7], with water taking the remainder part of the tissue in void spaces, vascular network, and interfaces among mineral and collagen phases [8–10].

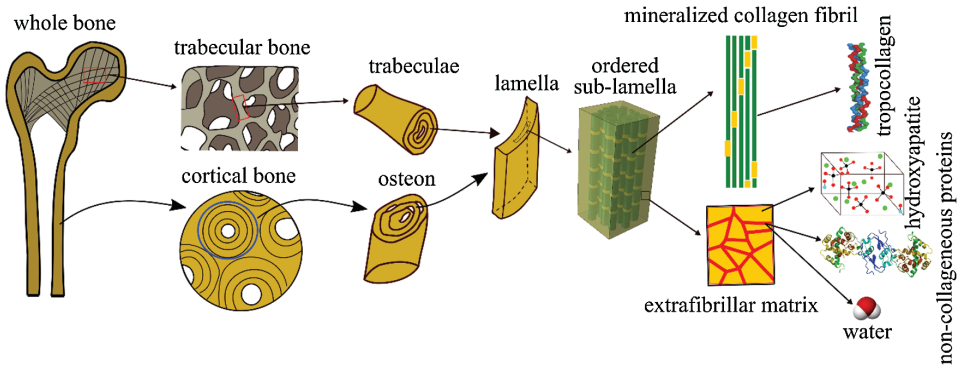

Figure 1: Schematic representation of hierarchical structure of bone spanning from whole organ to the nanoscale ingredients

Different bone hierarchies contribute to the mechanical properties of bone distinctively [11]. At macroscale, the overall size and shape control the stiffness and strength of whole bone [11]. At the mesoscale, bone architecture drives the biomechanical response of the tissue. For instance, osteoporosis may cause adverse microstructural changes (e.g., increased porosity, reduced number and size of trabeculae, and enlarged Haversian canals of osteons), thus leading to the weakening of bone. Cortical and trabecular bones are usually anisotropic, thus resulting in the mechanical anisotropy [12–20]. At the microscale, lamellae are a natural composite of mineralized collagen fibrils embedded in an extrafibrillar matrix, which itself is a nanocomposite of mineral crystals in an organic matrix made out of non-collagenous proteins. In fact, lamellae are the basic building block of bone that represents the major ultrastructural features, thus predominately dictating the material behavior of bone at ultrastructural levels. At the nanoscale, the mineralized collagen fibrils, hydroxyapatite crystals, non-collagenous proteins, and water contribute synergistically to the mechanical behavior of lamellae.

The stiffness and strength of cortical bone strongly depend on its mineral content [21,22] and the interaction between mineral and collagen phases [23,24], whereas the stiffness and strength of trabecular bone are dominated by its porosity and trabecular architecture [25]. The post-yield regime of deformation in cortical bone relies on the extensibility of collagen phase, sliding among mineral–collagen constituents, and formation of an array of small sized diffuse damage zones [26]. The fracture toughness is another critical property for bone that reflects its ability to tolerate flaws or cracks upon deformation without causing a catastrophic failure [27]. Toughness and strength is often mutually exclusive for engineering materials [28]. However, synergistic effects of hierarchical structure and unique constituents have afforded bone to be not only strong but also tough [29].

Toughening mechanisms of bone can be categorized into two general groups: intrinsic and extrinsic [29]. Intrinsic mechanisms are derived from tissue level properties of bone, defining the resistance of the tissue to deformation and fracture. They are usually activated around the crack tip to hinder crack extension by creating a plastic or damaged zone. In addition, the fracture toughness of bone is strongly dependent on organization and direction of mineralized collagen fibrils [30], showing that the toughness of bone may vary by two orders of magnitude depending

on the crack propagation direction with respect to the orientation of the underlying collagen network [30,31]. On the other hand, extrinsic mechanisms are related to the interaction of crack propagation with bone microstructure (e.g., pores) or structural units (e.g., osteons and cement lines). Understanding these toughening mechanisms is of utmost importance, as bone fragility is due to nothing but the absence of these mechanisms [32].

The mechanical competence of bone may be severely compromised by aging or skeletal disorders. Bone disorders unequivocally cause ultrastructural changes in the tissue via abnormal cellular and/or biological processes, thus consequently lead to significant reduction in bone toughness. Although bone biologists could, in many cases, figure out the biological pathways of such disorders, a large knowledge gap always exists between the biological changes and the corresponding mechanical consequences (i.e., bone fragility fractures). This paucity has significantly hindered our understanding of root causes for bone fragility fractures [33]. It is known that mechanical properties, especially intrinsic and extrinsic fracture resistance, and micro and ultrastructural structures of bone, deteriorate with aging, osteoporosis, and osteogenesis imperfecta [34–38], thus leading to bone fragility fractures. Such fractures would subsequently lead to immobility, severe pain, and increased risk of mortality for patients, thus placing a huge socioeconomic burden on the healthcare system and the society [39–41]. Therefore, it is extremely important to gain knowledge about fundamental mechanisms underlying normal biomechanical response of bone. This understanding will help invent better markers for predicting fracture risk, devise screening tools, and develop effective therapies or preventative measures to reduce bone fracture susceptibility. Beyond this, understanding bone nanomechanics can also help designing bio-inspired materials [42–45].

Understanding the ultrastructure of bone is a challenging task, which has drawn intensive interests from researchers [46–49]. In general, bone mechanical competence is dependent on two aspects: tissue quantity and quality. Traditionally, bone mineral density (BMD) is used as the measure of bone tissue quantity [50]. However, BMD alone cannot reliably predict the fracture risk in patients that are elderly or with bone disorders [51–53]. Bone quality is dependent on the composition and ultrastructure of the tissue, that may also be significantly altered by aging, bone disorders, and clinical treatments [54–57]. For instance, osteoporosis is not only associated with significant bone mass loss, but also manifested in adverse changes in bone ultrastructure [58,59]. In osteogenesis imperfecta, a so-called brittle bone disease, the root cause lies in the abnormal formation of collagen fibrils by gene mutation, which cascades to abnormalities at higher length scales [60]. In fact, clinical intervention and treatment of bone fragility are always initiated from targeting biological pathways that modulate bone formation process [57]. Therefore, bridging the gap between bone biology and bone nanomechanics has to start from the understanding of bone ultrastructure and its contribution to mechanical competence of the tissue.

The emerging technologies for material characterization and testing in the past decade have allowed us to get access to unprecedented information on bone structure and behavior at nanoscales [46,61–66]. However, among recent reviews on bone biomechanics, few were dedicated to the structure-function relationship at the ultrastructural level of the tissue [20,26,49,67–72]. Thus, a timely review is warranted to summarize and synthesize the new understanding and recent developments in the field in order to better guide the future studies on the underlying mechanisms of bone fragility fractures induced by skeletal disorders and to help developing new strategies for early prediction and prevention of such fractures. To this end, the objective of this paper is to provide a critical review on nanomechanics of bone in the experimental, theoretical, and computational fronts.

2 Experimental Observations of Bone Ultrastructure

Understanding the ultrastructure of bone needs to answer two important questions: (1) what constituents are in bone that make up the tissue, and (2) in what way the bone constituents are organized and integrated to deliver the outstanding mechanical properties. Fig. 2 summarizes in a snapshot some important experimental observations available so far on bone ultrastructure. At the lamellar scale, mineralized collagen fibrils demonstrate a twisting pattern in which the orientation of fibrils within a lamella varies smoothly with respect to the longitudinal axis of the osteon (Fig. 2a). At the sub-lamellar scale, however, bone may contain both ordered and disordered regions defined in terms of the relative alignment of fibrils (Fig. 2b). Also, the mineral phase itself is shown to display different patterns at the nanoscale including filamentous, lacy and rosette patterns (Fig. 2c). The detailed structure of mineralized collagen fibrils, including their banding pattern, is evident under atomic force microscopy (Fig. 2d) or electron mapping images (Fig. 2e). The mineral crystals, once separated from the underlying organic matrix and observed with transmission electron microscope, reveal themselves as flake-like platelets (Fig. 2f). The molecular structure of collagen fibril can be indirectly evaluated using the X-ray crystallographic techniques which might yield a highly accurate supramolecular structure (Fig. 2g). In the remaining sections, these different building blocks of bone ultrastructure are discussed in more details.

2.1 Main Ingredients of Bone Extracellular Matrix

2.1.1 Mineral Phase

Bone mineral phase is composed of carbonated hydroxyapatite crystals located both within and between collagen fibrils [64,73]. The mineral phase initially nucleates inside the collagen fibrils (referred to as intrafibrillar minerals) and gradually grows, as bone matures, to fill the space in between the mineralized collagen fibrils (referred to as extrafibrillar minerals) [74–76]. Hydroxyapatite is a hard material with some degree of elastic anisotropy at the crystal level [77,78]. The hydroxyapatite crystals in bone are tabular or plate-shaped and tend to fuse into aggregates [79]. The size of mineral crystals were estimated the to be 100 nm in length and 2–4 nm in thickness by the early transmission electron microscopy images of longitudinal bone specimens [80–84]. Because many of crystals were seen on their edges in the TEM images, they were thought to have needle-shaped structures. However, recent transmission electron microscope studies revealed that mineral structures in extrafibrillar matrix of bone were apparently polycrystalline [63,73], and the atomic force microscopy studies exhibited that they were of granular nature in shape [85–88] (Fig. 2f). Mineral crystals in bone have large surface area, a property magnifying their interaction with the collagen matrix, which is essential to increasing the strength of bone [89]. The thickness of mineral crystals is also suggested to play an important role in controlling the interfacial behavior of collagen–hydroxyapatite interface [90]. The mineral growth and remodeling of bone are intricately regulated by the underlying collagen template and non-collagenous proteins [91]. The degree of bone tissue mineralization is vital for mechanical integrity of the skeleton, its load bearing strength and its stiffness [92,93]. The elastic modulus of bone increases very steeply with increase in the mineral content, indicating that mineral crystals in fact join up end to end to form a fusion, and that they are not totally disconnected, which might explain the rapid increase in bone stiffness with the progress in mineralization [23]. However, at high mineral contents, such as in aging bone, bone tissue becomes brittle and allows cracks to propagate easily leading to a reduction in toughness and impact strength [93,94]. Therefore, there are various checks and balances in place to ensure right amount of bone mineralization essential for good mechanical properties.

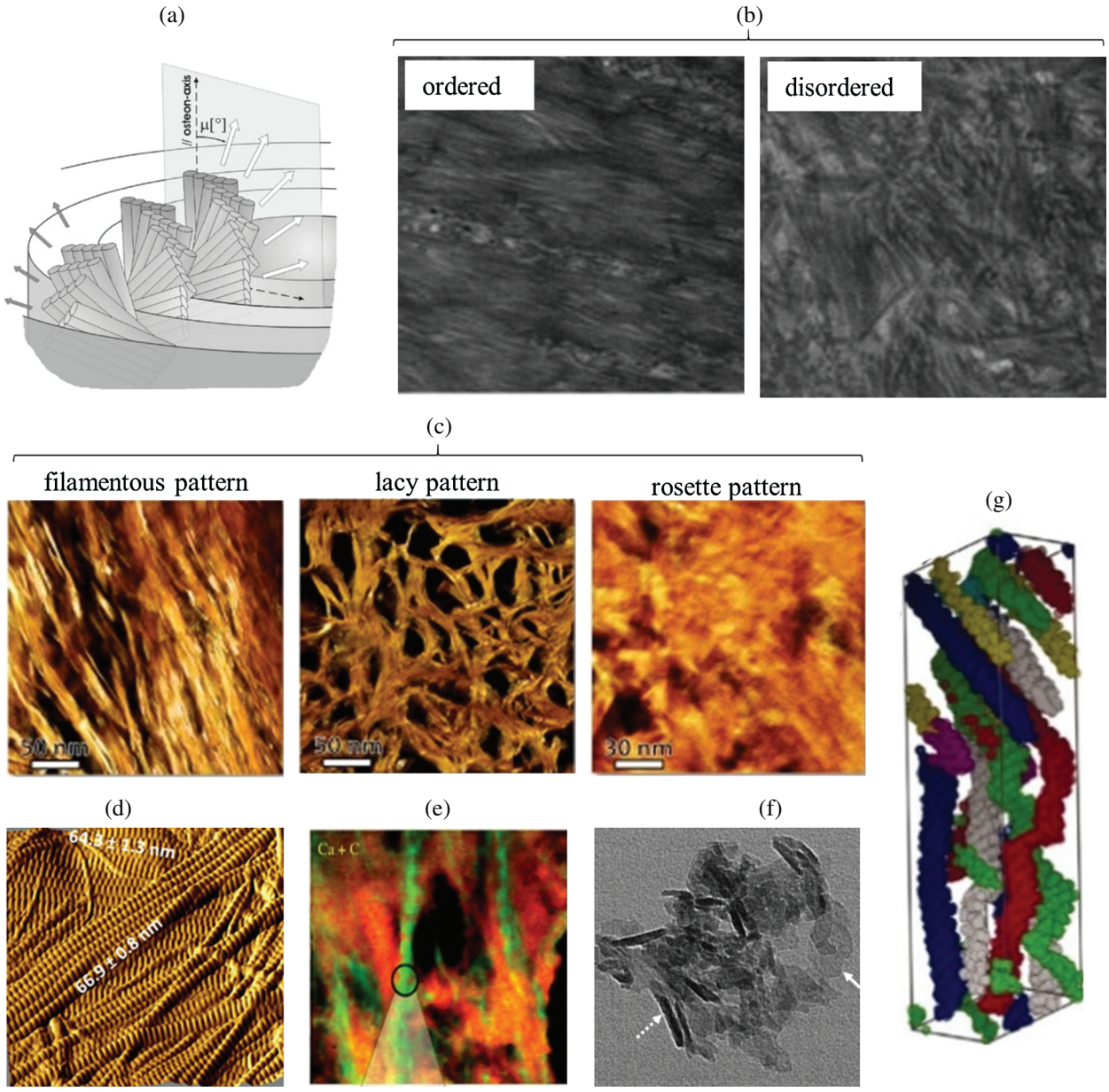

Figure 2: Architecture of bone at different length scales: (a) a spiral twisting model showing that mineralized collagen fibrils coil up with varying degrees of tilt with respect to the longitudinal axis of osteon [95], (b) ordered and disordered regions/motifs found in human lamellar bone [66], (c) three different mineral organization/patterns observed in bone at the ultrastructural scales [46], (d) the atomic force microscopy images of mineralized collagen fibrils and their D-spacing in ovine bone [96], (e) the elemental electron map images of intrafibrillar and extrafibrillar minerals crystals [97], (f) TEM image of isolated mineral crystals seen from the edge (darker rectangular features) or lateral faces (flake-like features) [63], (g) a model of supermolecular structure of type I collagen based on X-ray crystallographic experiments [98] (Copyright (2006) National Academy of Sciences, USA)

2.1.2 Collagen Phase

The collagen in bone is primarily Type I collagen whose tropocollagen molecules have a triple helix structure of three polypeptide strands, called alpha chains, stabilized by enormous hydrogen bonds [99]. These molecules are approximately 1.5 nm in diameter and 300 nm long, and secreted in the initial stage of bone formation by osteoblasts [99]. These molecules are arranged in a staggered fashion to form collagen fibrils, with a periodicity of approximately 67 nm, thus showing alternating bands of gap and overlap regions [2,96,99–101]. Collagen plays an important role in determining the strength and toughness of bone [102]. Almost all mechanical properties of the collagen and bone deteriorate with aging [36,54,103]. The relative amount of the mineral and collagen phases is also often disturbed by aging or skeletal disorders, as studied by using Fourier transform infrared and Raman spectroscopy techniques [57].

2.1.3 Water

Water is found in different compartments of bone tissue, such as in pores of lacuna–canalicular and vascular network (bulk free water), at interfaces among mineral crystals and collagen fibrils (bound water) [104], and within the molecular structure of mineral crystals and collagen fibrils [10,105,106], which takes up to 20% of bone volume all together. The water content in these different compartments could be assessed via nuclear magnetic resonance [9,10] and Raman spectroscopy techniques [107]. Water is critical for bone mechanical properties, especially the toughness of the tissue [10,105,108–110]. Water molecules readily bond to the hydrophilic groups of collagen phase (i.e., glycine, hydroxyproline, carboxyl, and hydroxylysine), the charged groups of mineral crystals (i.e., Ca^{+2} and PO_4^{3-}), and non-collagenous proteins (e.g., osteopontin and proteoglycans) in bone. Such interactions would improve the energy dissipation at the interfaces among the bone constituents [111,112], thus acting as a strong plasticizer to make bone ductile [113]. However, age-related decline in bound water reduces the capacity of bone to dissipate energy and resist fracture, contributing to the brittleness of aging bone [114–116]. Water can also affect the chemical dynamics of the collage phase (e.g., orientation, diameter, and folding patterns) [117] and the mineral structure [118,119].

2.1.4 Non-Collagenous Proteins

Non-collagenous proteins (NCPs), prominently osteocalcin, osteopontin, osteonectin and proteoglycans, account for less than 10% of the total organic phase in bone, yet play an essential role in modulating the organization of collagenous matrix, as well as regulating bone formation process by inhibiting or enhancing mineralization [91]. In addition, NCPs inflict significant effects on bone mechanical properties [120]. Having a high negative charge density, NCPs can readily bond with the positively charged ions in the mineral phase of bone or in body fluids, hence enhancing the interaction between the mineral and collagen phases and attracting water molecules in bone matrix by osmotic potentials [121]. Recently, evidence shows that proteoglycans, a sub-class of NCPs, play a significant role in attracting and retaining bound water in bone and have a remarked effect on the toughness of bone [115,116]. These supermolecules often exhibit complex modular structures, giving rise to self-healing and energy dissipating mechanisms, such as sacrificial bond breakage and re-formation while maintaining the backbone of the protein intact [122–124]. Therefore, they are often described as a natural glue to hold the bone constituents together [88,125,126]. In fact, many studies using knocking out mouse models have found that the absence of these proteins could result in significant deterioration of bone toughness [115,126–128].

2.2 Bone Lamella: A Hybrid Nanocomposite at Ultrastructural Level

Lamellae are the basic building block of osteons and trabeculae in human lamellar bone, which consist of mineralized collagen fibrils embedded in an extrafibrillar matrix, and represent the major ultrastructural features of bone. Structurally, bone lamellae is comprised of ordered regions where collagen fibrils are largely aligned with a preferential direction [66] or in a plywood-like motif [129–131], and disordered regions where the organization of collagen fibrils lacks such a harmony [66] (Fig. 2b). Important to the mechanics of bone osteon, the orientation of mineralized collagen fibrils may change from one lamella to another, spiraling around the central axis with varying degrees of tilt [95], thus showing bright or dark fringes under polarized light microscope [132]. The changes in the orientation of mineralized collagen fibrils contributes to the anisotropic elasticity of bone lamellae [133]. To elucidate the nanomechanics of lamellae, it is crucial to understand its basic building blocks: i.e., mineralized collagen fibrils and extrafibrillar matrix.

2.2.1 Mineralized Collagen Fibrils

Mineralized collagen fibrils are formed initially from self-assembly of collage molecules in a staggered manner, thus showing a periodic array of gap and overlap regions [98,134–136] (Fig. 2d). Then, hydroxyapatite minerals nucleate in the gap regions of the collage fibrils, gradually penetrate into the overlap regions [74], and extend outside of the fibrils, creating an intertwined and impenetrable composite of mineral and collagen [63]. The intrafibrillar mineral crystals are around tens of nanometers in length and widths, while about 3–4 nm in thickness [84,137,138]. The diameter of mineralized collagen fibril subunit was considered to be roughly 100 nm [139]. The interface between the mineral platelets and collagen fibrils is not directly bonded, but via a thin bound water layer, thus allowing for easy sliding between the two constituents [104,140]. In general, these mineral–collagen interfacial interactions play an important role in the mechanical behavior of mineralized collagen fibrils.

2.2.2 Extrafibrillar Matrix

As mentioned earlier, a large portion of bone mineral lies outside of the collagen fibrils, filling the space between them [47,64]. TEM images of extrafibrillar matrix exhibit different patterns of mineral phase in distinct orientations, suggesting that mineral tabulates are arranged eccentrically around collagen fibrils (Fig. 2c). A number of previous evidence suggest that extrafibrillar matrix of bone contains NCPs bounding the mineral nano-particles together [88,141–147]. A recent study demonstrated that the presence of NCPs at the extrafibrillar organic–mineral interfaces contributes to bone fracture toughness [126]. It is also observed that the presence of NCPs at the extrafibrillar organic–mineral interfaces form dilatational bands and reinforce the network of mineralized fibrils [125]. In fact, an increasing number of evidence indicate the role of NCPs, mainly located in the extrafibrillar matrix, in enhancing the strength and toughness of bone [124,148–150]. Considering the extrafibrillar matrix as a nanocomposite of hydroxyapatite crystals bounded through non-collagenous proteins, some researchers have tried to explain the nanoscale strengthening and toughening mechanisms of bone [45,151].

2.3 Nanomechanics of Bone

The bulk mechanical properties of bone are dependent on the factors at all hierarchies of bone. The stiffness and strength of bone are most likely attributed to the quantity, properties, and distribution of minerals in the mineralized collagen fibrils and extrafibrillar matrix in addition to the tissue porosity [23,93,152]. The fracture toughness of bone depends on multiple

toughening mechanisms across the different hierarchies of the tissue. At the meso and micro length scales, crack-bridging, crack path deviation, and constrained microcracking are reported to be the common mechanisms for energy dissipation during crack propagation process [13,31,153–157]. At the ultrastructural level, collagen fibrils can undergo high stretches (50%) and withstand very large strain prior to fracture [158,159], via unwinding followed by hydrogen bond breaking and re-formation. The friction between the sliding collagen molecules during stretching is to some degree controlled by a number of cross-linking agents in the collagen matrix, which play an important role in plasticity and toughness of bone at the material level [158,160–163]. In addition, the nano-sized mineral platelets embedded in collagen fibrils could significantly increase the fracture strength of bone [89], through cooperative deformation of mineral and collagen phases in bone [164,165]. Moreover, the breaking of charged interactions between molecules in the extrafibrillar matrix of bone is also described as source of plasticity in bone [166], which is most likely attributed to NCPs [124] in the form of hidden length and sacrificial bonds mechanisms [123,149,150,167]. Although plenty of information are available in the literature, the mechanistic understanding of the deformation and failure mechanisms of bone at ultrastructural levels is still debatable.

3 Ultrastructure *vs*. Nanomechanics of Bone: Modeling and Simulation

Much have been learned regarding the mechanical behavior of bone at ultrastructural levels through various modeling and simulation efforts. In the following part of the review, we intend to discuss those works based in three categories: i) finite element based models, ii) atomistic simulations, and iii) analytical models.

3.1 Finite Element (FE) Based Models

Finite element method has long been a popular and powerful method in both estimating the mechanical properties of bone and elucidating the underlying strengthening/toughening mechanisms at different length scales [168,169]. Here, we focus only on FE based modeling of the ultrastructure of bone, leaving out those modeling studies at the mesoscale or whole organ levels, for which readers can refer to Zysset et al. [170]. FE models of bone at the ultrastructural levels can be categorized into five general subsections as explained below:

3.1.1 Individual Mineralized Collagen Fibrils

In addition to the experimental studies on characterizing the mineralized collagen fibrils [171,172], FE modeling has provided a lot infights about this building block of bone. For instance, following a initially proposed structural model [136,173], Siegmund et al. [161] built a two dimensional FE model of a single mineralized collagen fibril and investigated the effect of collagen crosslinking on its mechanical behavior (Fig. 3a). They approximated the strength of crosslinks, and modeled enzymatic crosslinks as cohesion at the overlap region of collagen fibrils, and non-enzymatic crosslinks at random positions. Their results showed that enzymatic crosslinks have minimal effect on the stress–strain curve of the mineralized collagen fibrils tested in tension, while nonenzymatic crosslinks, on the other hand, may decrease bone toughness and post-yield deformability by inhibiting the relative sliding between collagen molecules. Yuan et al. [174] created both two-dimensional (2D) and three-dimensional (3D) representative volume element models of mineralized collagen fibrils, and investigated the effect of material properties and morphology of organic and mineral phases on the elastic properties of bone. Using 3D FE simulations, the effect of density and elasticity of mineral phase, and collagen crosslinks on stiffness and stress–strain response of mineralized collagen fibrils was investigated under both

hydrated and dehydrated condition [175–177]. Homogenized stiffness matrices were derived for mineralized collagen fibrils using 3D FE simulations [178], and the effect of mineral volume fraction, their staggering pattern and lateral distance on elastic properties of mineralized collagen fibrils was studied [179]. All these studies suggest that the interfacial behavior between mineral–collagen phases plays an important role in determining the overall response of the mineralized collagen fibrils. Debonding needs to be allowed between the mineral and collagen phases, which is often realized by using cohesive elements or non-linear spring elements. Perhaps one shortcoming of those models is that they often assume simple material models, such as linear elastic response for the collagen phase, while collagen has been shown to exhibit strong nonlinear behavior.

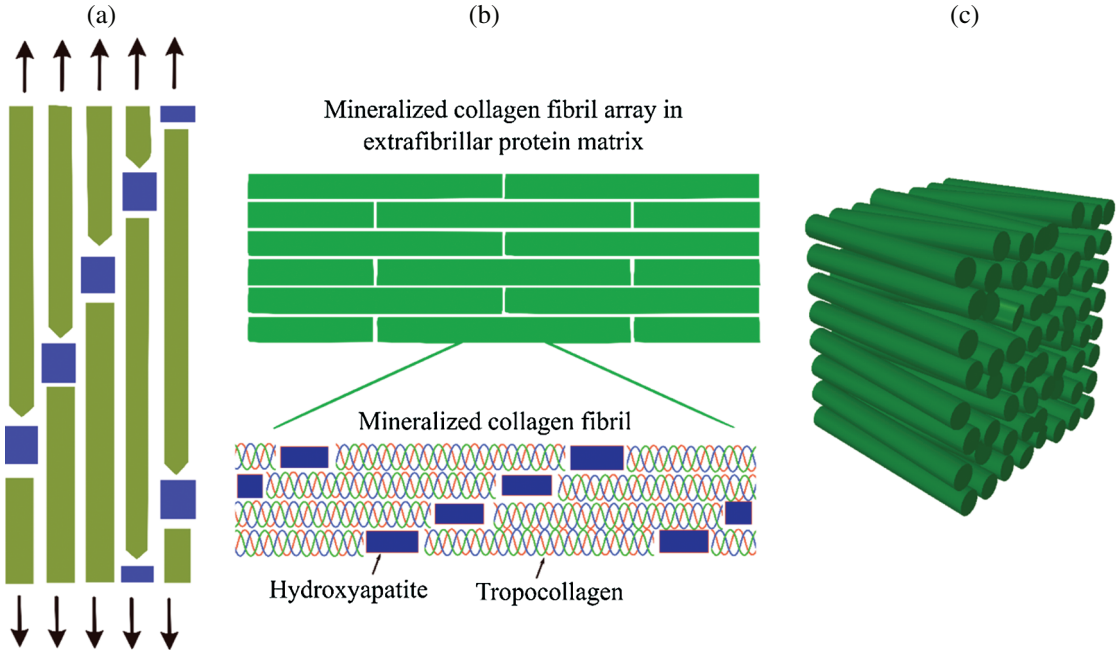

Figure 3: FE modeling of mineralized collagen fibrils: (a) a two-dimensional study considering the effect of cross-links [161], (b) a two-dimensional study considering an array of mineralized collagen fibrils [180], (c) a model investigating a three-dimensional network of mineralized collagen fibrils [181]

3.1.2 Array of Mineralized Collagen Fibrils

Some FE models are focused on studying the mechanical response of an array of mineralized collagen fibrils, rather than a single mineralized collagen fibril (Figs. 3b and 3c). For instance, Jasiuk et al. [182] considered the ultrastructure of bone at the single lamella scale as a network of mineralized fibrils aligned in a preferential direction. Fibrils were modeled as rods and oriented randomly. The elastic constants of bone were approximated as a function of mineral volume fraction. Damage was not considered in that model. Reisinger et al. [133] studied the effect of different fibril orientation patterns (orthogonal plywood, twisted plywood, and 5-sublayer pattern) on the anisotropic elastic properties of lamellar bone. In another study, Lai et al. [183] considered an array of mineralized collagen fibrils embedded within a thin layer of extrafibrillar protein matrix modeled by cohesive elements. They introduced an initial flaw in the model, from which

different patterns of interfacial damages emerged. The dependence of mechanical properties, including strength, on geometrical dimensions of mineralized collagen fibrils and material properties of organic extra-fibrillar matrix was demonstrated. The model, however, did not consider the extrafibrillar mineral phase. Wang et al. [181,184] took this modeling approach one step further by considering the 3D spatial distribution of mineralized collagen fibrils. They studied the 3D network of mineralized collagen fibrils in different anatomical directions and investigated the geometrical effect of mineralized collagen fibrils diameter and length scale, as well as some material properties on fracture response of cortical bone. In a follow up study, Wang et al. [185] used this model of 3D network of mineralized collagen fibrils to evaluate the influence of varying mineral distribution, mineral content, and interaction among the mineralized collagen fibrils on the mechanical and fracture response of bone at submicroscale. In a more recent work, a 2D array of mineralized collagen fibrils was considered with the extrafibrillar phase modeled as an elastic–plastic material without taking into account the detailed microstructure of extrafibrillar matrix [180]. In this study, cohesive elements were used to model the interface between mineralized collagen fibrils and extrafibrillar matrix. Several non-dimensional parameters were defined, such as the ratio of stiffness or strength of mineralized collagen fibrils to extrafibrillar matrix subunits. Stress–strain response and damage modes were reported, showing that the extrafibrillar matrix with high stiffness and low yield stress could enhance the toughness of the staggered array of mineralized collagen fibrils. In another study, Falco et al. [186] argued that two key structural motifs essential in explaining the hysteresis behavior observed in antler bone under cyclic loading are an axially staggered organization of mineralized collagen fibrils coupled with damageable interfibrillar interfaces. The main limitation of the aforementioned models is that they typically did not consider the detail of extrafibrillar matrix of bone, especially the mineral crystals. Nonetheless, they have been successful in exploring the role of mineralized collagen fibrils organization, geometry, and material properties on the mechanical response of lamellar bone.

3.1.3 Nanocomposite of Mineralized Collagen Fibrils and Extrafibrillar Matrix

Some studies have attempted to model the extrafibrillar matrix with more details (Fig. 4). As an example, our group [151] has previously conducted an study, in which the extrafibrillar matrix of bone was considered as a hybrid nanocomposite of HA crystals bound via a thin layer of NCPs. The model was tested in tension and compression under simulated hydrated and dehydrated conditions. The failure modes and internal mineral strains were compared against experimental observations. This study was the first attempt to model the extrafibrillar matrix of bone in detail and discerned its contribution to the bulk mechanical response of bone. We then extended the study to a sub-lamellar bone by incorporating mineralized collagen fibrils into the model as well [187]. The model was able to explain the toughening mechanisms of bone and the commonly observed damage modes in both tension and compression loading modes. Another 3D model was developed by Wang et al. [149], in which both mineralized collagen fibrils and extrafibrillar matrix were present. They used the model to explain the formation of energy dissipating dilatational bands induced by denaturation of non-collagenous proteins experiencing hydrostatic stress. In a 2D study, based on a simple geometry assumption, a unit cell of bone ultrastructure in tension loading was considered as collagen fibrils surrounded by the so-called mineral lamellae shaped as mosaics of HA crystals of 5 nm and 20–50 nm in thickness and width, respectively [71]. It was found that the mineral lamellae imparted greater stiffness and tensile strength to bone compared to the case where the mineral phase resided inside the collagen fibrils. In a recent study, Alizadeh et al. [188] combined mineralized collagen fibrils and extrafibrillar matrix subunit together to form a 3D FE sub-lamellar bone model and

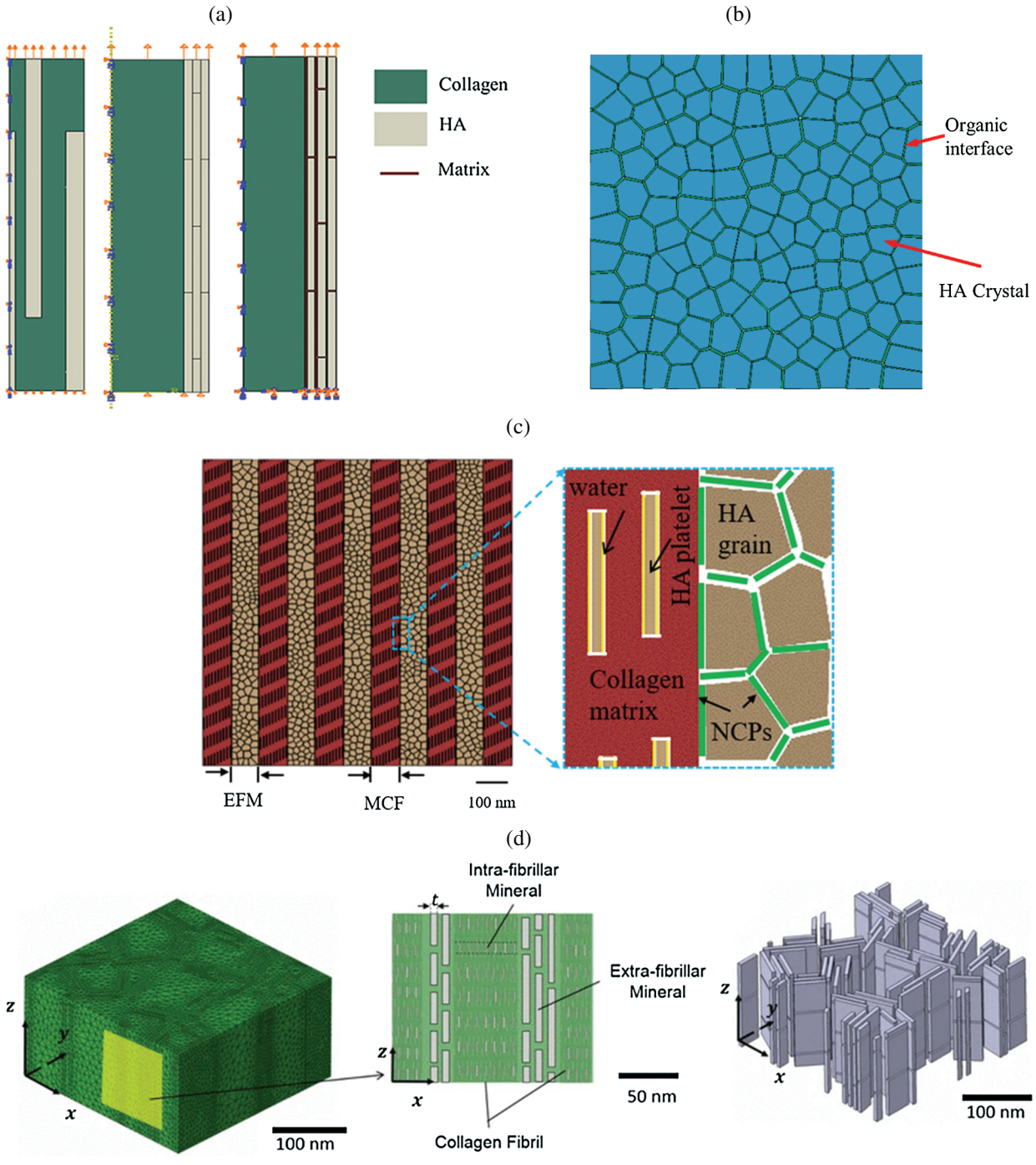

Figure 4: FE modeling of sub-lamellar bone: (a) the effect of placing mineral phase inside or outside of the fibrils was studied in [71], (b) bone extrafbirillar matrix was considered as a nanocomposite of mineral crystals bound together by thin layers of relatively soft non-collageneous proteins [151], (c) two-dimensional [187] and (d) three-dimensional models accounting for more detail composition of mineralized collagen fibrils and extrafibrillar matrix [149]

compared the anisotropic elastic properties of the model with the experimental results and various analytical predictions. They modeled the extrafibrillar matrix as simple cubic mineral particles embedded in a NCPs matrix via a thin layer, with no damage or delamination between the constituents of bone considered. The simulation results identified the shear lag mechanisms and the role of mineral volume fraction within mineralized collagen fibrils and the volume fraction of mineralized collagen fibrils in determining the elastic constants of bone at the two different length scales studied.

3.1.4 Probabilistic Mineral–Collagen Composite Models

Several researchers have used simple and probabilistic mineral–collagen composite models to study damage progression in bone (Fig. 5). It was shown that the interfacial behavior among mineral and collagen phases plays a role in determining the microdamage pattern and accumulation in bone under tension [189,190]. Strong mineral–collagen interactions led to development of a microcrack, while weaker mineral–collagen interactions resulted in formation of damage patterns reminiscent of diffuse damage observed in bone under tension. The sensitivity analysis revealed the effect of material properties and volume fraction of mineral and collagen phases on the damage formation and progression in the observed modes.

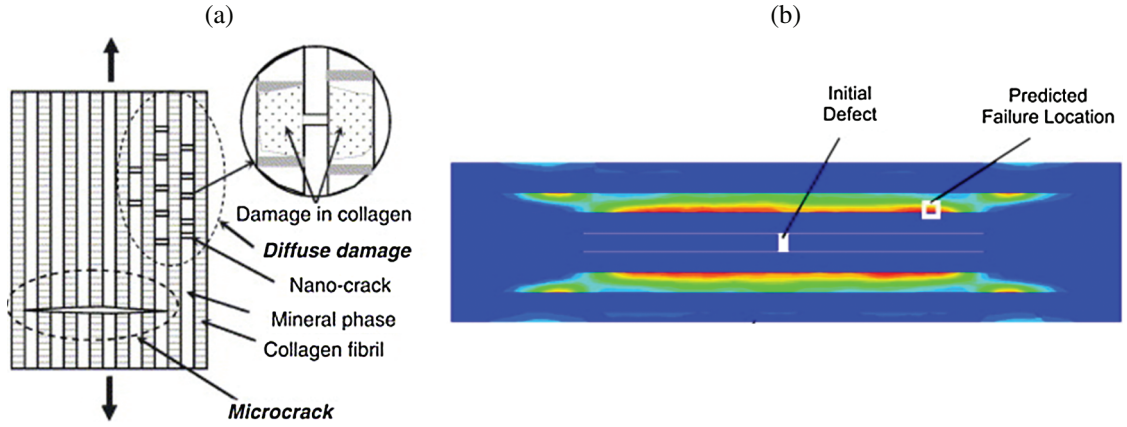

Figure 5: Probabilistic models based on simple geometries: (a) a two-dimenstional shear-lag based model built to analyze the damage mode, microcrack progression and stress concentration in neighboring mineral phases [191], (b) studying the effect of mineral–collagen interface on the damage mode and microdamage progression [189,190]

3.1.5 Multi-Scale Models

As the structure of bone is hierarchical in nature, researchers have attempted to conduct FE simulations of bone in a multiscale framework (Fig. 6). The models available in the literature are mostly hierarchical multiscale models, and not the concurrent multiscale models. In these models, the mechanical properties at lower length scales, e.g., mineralized collagen fibrils, are homogenized and incorporated into the model at higher length scales, e.g., lamellae level. Ghanbari et al. [192] created a multiscale FE model by considering microscale and macroscale hierarchies of cortical bone and investigated the effect of mineral volume fraction on the elastic modulus of bone and stress concentration patterns. Hamed et al. [193] considered a simple geometry of bone at three different scales: mineralized collagen fibrils, single lamella, and multiple lamellae, which allowed

for debonding of different constituents and presence of flaws and pores in bone. Two mechanisms responsible for the nonlinear response of the mineralized collagen fibrils were described as sliding between collagen domains and sliding between collagen and hydroxyapatite platelets at their interfaces. It was shown how damage at lower length scale could result in alteration of mechanical properties at higher length scales. In another work, Vaughan et al. [15] established a three-scale FE homogenization model of bone from mineralized collagen fibrils to the osteon, and studied the effect of mineral volume fraction, mineral aspect ratio and lamellar orientation on predicted stiffness of bone.

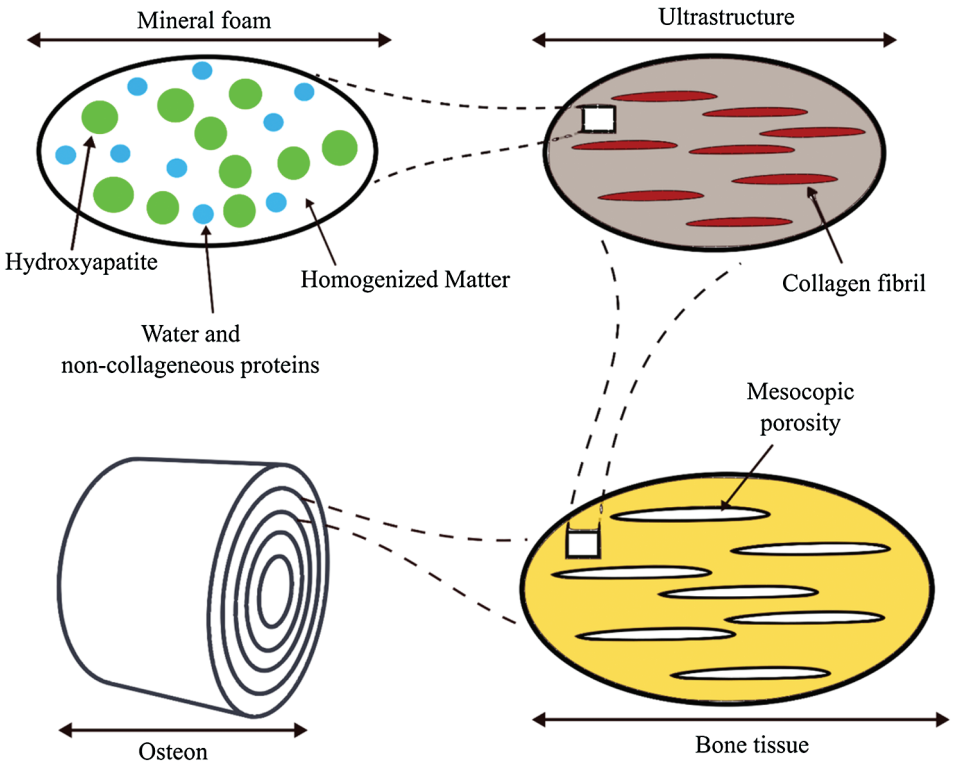

Figure 6: Multiscale micromechanical models cosidering bone as mineral foams reinforced by mineralized collagen fibrils [194]

3.2 Atomistic Simulations

There has been a tremendous improvement in our understanding of bone nanomechanics through molecular dynamics (MD) simulations. Fig. 7 contains a representative work performed in this area. Although MD models are limited by their physical size, use of first principle calculations allows them to unravel mechanisms not available for continuum-based models. One of the earliest studies in this area was conducted by Buehler [195], where atomistic simulations of a single tropocollagen molecule was performed in different loading modes, and a coarse grain mesoscale model was proposed. The results of that study showed an initial toe region followed by a stiffening behavior when tropocollagen was tested in tension, suggesting that the collagen helix is unfolded initially with increasing strain, followed by breaking free of the three polypeptide chains, and further stretched as individual strands, with the covalent bond among

atoms controlling the resistance to the external load. In another study, the unwinding response of a single collagen molecule under tensile load was studied along with an analysis of forces required to separate the strands of the triple helix structure from each other [196]. The details of interprotein interactions in collagen fibrils, including the effects of water, were also reported [197]. Moreover, a molecular dynamics study of collagen fibrils with and without mineral crystals showed that the presence of mineral platelets not only increased the stiffness of the fibril, but also enhanced its strength and toughness [89]. This was attributed to the ionic bonding at the interface between the mineral platelets and tropocollagen molecules, which allows energy dissipating by repeated glides between these two constituents (increasing toughness), and resistance against their slip (increasing strength). Later three-dimensional atomistic simulations of mineralized collagen fibrils further revealed the role of intrafibrillar mineralization in stress bearing and deformation of the mineral and collagen phases, respectively [198]. It was also shown that although intrafibrillar mineralization is crucial to compressive elasticity of bone, it is not enough to give the bone its full capacity of load bearing observed at larger length scale, thus indicating that extrafibrillar mineralization is in fact a very important contributor in providing the load bearing properties of bone [199]. Furthermore, Fielder et al. [200] demonstrated how variations in mineral and water content in mineralized collagen fibrils would lead to changes in distribution of water in collagen fibrils and its associated mechanical response to tensile load.

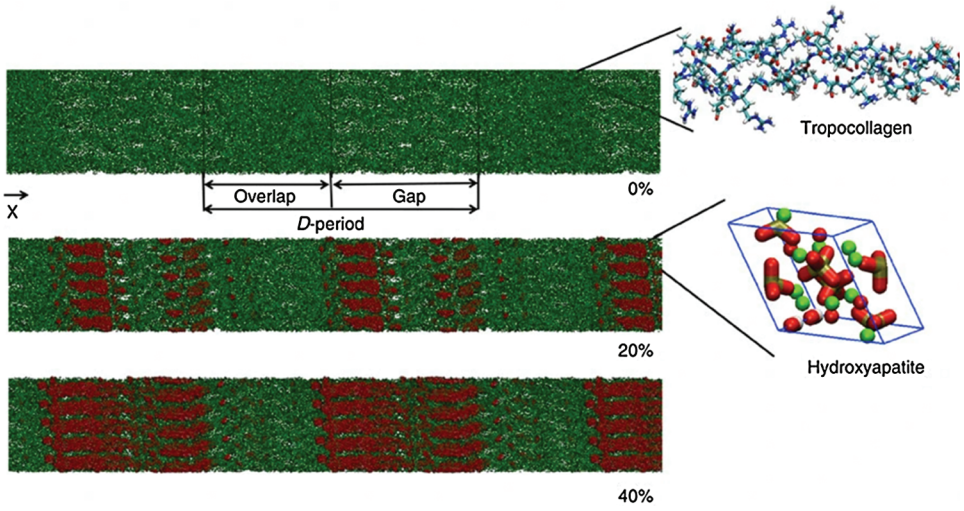

Figure 7: Molecular dynamics studies of bone using full-atomistic three dimensional study of mineralized collagen fibrils with different degrees of mineralization [198]

MD studies also become available for larger size investigations on finite size collagen fibrils (un-mineralized), which captured the rupture and sliding of tropocollagen molecules, and showed that the deformation mechanisms and mechanical properties are strongly dependent on the size of fibrils as well as the cross-linking density [159]. A more recent experimentally validated MD study, considering the hierarchical structure of collagen inclusive of collagen molecules and microfibrils, was able to extract details on the deformation mechanisms of collagen at dry and hydrated conditions with stunning accuracy [201]. This study demonstrated that collagen molecules alone could not achieve the superior mechanical properties; a hierarchical structure of collagen molecules was crucial for collagenous tissues. As aforementioned, the way in which mineral platelets are arranged

is important for mechanical integrity of mineralized collagen fibrils. To study this, Mathiazhagan et al. [202] considered mineral platelets arranged in two different manners, namely a regular and stairwise staggered, respectively, and comprehensively investigated how different mineral aspect ratios would change the stiffness, strength and toughness of a composite material akin to bone mineralized collagen fibrils.

Beside the studies on mineralized collagen fibrils, some MD simulations focused on a simpler geometry of mineral–collagen composite, and investigated in detail the mechanical role of geometrical factors or the nature of interfacial interactions between mineral and collagen phases [90,140]. In one of the studies, the interfacial interaction between bone mineral and osteopontin (a member of NCPs family) was shown to be governed by the electrostatic attraction between acidic amino acid residues in osteopontin and calcium in hydroxyapatite [203]. MD simulations have also been employed to predict the anisotropic elastic properties of hydroxyapatite [78]. In addition, some MD studies investigated the role of water in the response at mineral–collagen interfaces, showing that interaction energy between water and hydroxyapatite, and between water and collagen largely exceeds that of direct interaction between mineral and collagen phases [111,112]. Shear lag based model built to analyze the damage mode and microcrack progression in bone [191]. Stress concentration in neighboring mineral phases was studied in detail. Furthermore, the energy required to untie a collagen molecule with HA crystals in presence of water is significantly higher than that in absence of water. This is due to the successive breakage and re-forming of hydrogen bonds between water molecules, which are strongly attached to the mineral crystals [112]. In both shearing and peeling of mineral–collagen interface, the number of collagen–water hydrogen bonds was shown to increase by roughly 100% before rupture, contributing to a large increase of the mineral–collagen interfacial failure energy [204].

3.3 Analytical Models

Early micromechanical models of bone ultrastructure were mainly focused on approximating the elastic response of bone with different mineralization by employing different versions of conventional composite material models [205]. Currey [23] was perhaps the first person who tried to establish a micromechanical composite model of bone by augmenting the original model proposed by Cox [206] for general composite materials. In his model, he accounted for the shear contribution of collagen matrix, and considered the mineral crystals as short fibers where he put emphasis on the role of the aspect ratio between the length and cross section of the fibers. He was able to explain the relationship between the aspect ratio and misalignment of crystals with the stiffness of bone. Later on, Mammone et al. [207] considered a more detailed micromechanical model of bone, assuming a collagenous matrix and hydroxyapatite fillers. The prominent tensile failure mode in their model was the collagen–mineral debonding. They also studied the effect of mineral volume fraction, shape and alignment on the tensile properties of bone such as modulus and strength. Piekarski [208] compared three different elementary structural composite models namely Voigt, Reuss, and Hirsch in terms of how they predict the modulus of bone as a function of mineral volume fraction, where he found the Hirsch model performed better in comparison to some experimental data. Later, the popular micromechanical model of mineralized collagen fibrils put forward by Jager et al. [173] considered a staggered arrangement of mineral crystals within the collagen matrix, and was able to capture the increase in both elastic modulus and fracture toughness of bone as a function of increase in mineral content. Akkus [209] provided a micromechanical model of elastic response of bone by combining an Eshelby's equivalent inclusion method with the mean field theory considering mineral crystals as ellipsoidal inclusions randomly arranged within a soft collagen matrix. The model was studied under different loading

conditions, different mineral volume fractions and aspect ratios. The elastic modulus in longitudinal and transverse directions were approximated as well as the relative stress generated in mineral and organic phase. Wagner et al. [210] proposed a micromechanical model describing the Young's modulus of bone by accounting for platelet-like geometry of minerals, thin and thick lamella, and alternating orientation of crystals within the lamella.

Some analytical models assumed relatively simpler geometry model for bone nanocomposite. For instance, by using a generic mineral collagen micromechanics model it was shown that the nanometer size of mineral particles in bone is essentially selected to ensure optimum strength and maximum tolerance of flaws, making the inherently brittle mineral phase practically insensitive to cracks-like flaws [211,212]. Another detailed micromechanical analysis on a mineral–collagen composite described the stress concentration and microdamage formation in bone [191]. The spatial distribution and mechanical properties of the collagen and mineral phases were shown to have an influence on the stress concentration fields around an initial flaw in bone, which would determine the pattern of damage progression in the form of diffuse damage or linear crack. Using the simple shear-lag theory, a 2D unit cell of mineral collagen composite model was used to study the tensile stress–strain response of bone [213]. It was shown that for the model to estimate the actual properties of bone, it was necessary to consider some sort of partial debonding between the mineral crystals and the collagen matrix. In that model, it was also suggested that relative sliding of collagen fibrils could proceed the initial debonding of the mineral collagen phase. The model was used later by the same authors to consider the effect of mineralization on tensile properties of bone [214].

In addition, several approaches for multiscale modeling of bone were proposed: (i) mineral foam matrix with collagen inclusions, (ii) mineralized collagen fibrils embedded in an extrafibrillar matrix considered as an open mineral foam, and (iii) an interpenetrating network of mineral and collagen [215]. The fundamental differences among these models and their potential in predicting the mechanical properties of bone, especially its elasticity, were summarized elsewhere [215]. Based on the relationship between bone elastic constants in longitudinal and radial directions and the mineral volume fraction, an ultrastructural model of bone was proposed, in which the mineralized tissue was described as isotropic open crystal foams reinforced unidirectionally by collagen fibrils [216]. In addition, a mathematical treatment in a framework of strain energy formulation was proposed to explain this relationship. The model was later extended in a multiscale setting while considering ultrastructural water and NCPs, the volume fraction of constituents to predict the elasticity tensor of bone in different directions [217]. Multiscale models have also been proposed to explain the stiffness [194,218–220] and strength [221] of bone using some universal parameters such as mineral/collagen content, their arrangement, and different levels of microstructural porosities. These models often yield a mathematically complex treatment of bone. In a multiscale homogenization framework developed by Nikolov et al. [222], elastic constants of bone was approximated as a function of mineral volume fraction, especially the extrafibrillar mineral portion of bone. The extrafibrillar minerals were assumed as reinforcing rings coating the mineralized collagen fibrils. Although their finding suggested that no more than 30% of total mineral content in extrafibrillar, this assumption did not hold acceptable as later TEM analysis of bone suggested that extrafibrillar minerals can indeed account for more than 30% overall volume fraction [63].

Several micromechanical models are proposed to estimate the strength or toughness of bone. For instance, Schwiedrzik et al. [223] formulated a micromechanical model, based on Mori–Tanaka scheme and Ashby, in order to explain the axial splitting and fibril kinking failure modes

they observed while testing bone micropillar in hydrated condition under compression. They considered the mineralized collagen fibrils as a matrix-inclusion problem of oriented mineral spheroids in an isotropic collage matrix. They modeled the extrafibrillar matrix as a continuous mineral matrix with spherical voids. Putting these two subunits together, they then modeled the ultrastructure of bone as cylindrical inclusions of mineralized collagen fibrils in a continuous isotropic extrafibrillar matrix. The model was then used to estimate the shear strength of the extrafibrillar matrix as well as fracture toughness of bone micropillars in splitting mode. In another study [147], based on the observation that sub-micron sized bone pillars show a strong size-dependent compressive strength, a model was proposed to explain this phenomenon by considering the effect of porosity (flaws) and the extrafibrillar matrix. Tai et al. [87] applied an elastic–plastic 3D FE model with Mohr–Coulomb pressure dependent strength criterion to explore the nanoscale origins of bone strength under nanoindentation loading. Their modeling results indicated that friction between mineral aggregates could be a key player in enhancing bone compressive strength. This cohesion of mineral particles was suggested to emanate from within the collagen matrix itself, rather than the mineral–collagen bonding.

3.4 Models Based on Indirect Experimental Observations

Some researchers have examined the deformation response of bone using a diverse range of experimental techniques and have proposed different models capable of justifying their observations (Fig. 8). These models are not often directly based on available imaging techniques, but rather rely on indirect experimental evidence. For instance, based on nanoscale observations of dilatational bands forming on areas of bone damaged under cyclic loading, Poundarki et al. [125] suggested a model for ultrastructure of bone in which ellipsoidal voids, of the order of 100 nm, in between extrafibrillar mineral aggregates form as a result of damage in NCPs (primarily osteopontin and osteocalcin). The dilatational bands were described to be distinct from the submicroscopic damages often seen in diffuse damage areas of bone. In their model, the separation of NCPs binding the mineral aggregates dictates the subsequent rupture and shear of collagen fibrils. This model in fact was one of the earliest models taking into account the importance of NCPs on strength and toughness of bone at nanoscale. In another study [224], using *in situ* tensile testing of fibrolamellar bone under synchrotron X-ray diffraction the strain of bone at the tissue, fibril, and mineral particles were measured to have ratio of 12:5:2. This finding of hierarchical graduation in strain at different length scales motivated the researcher to explain this cooperative deformation mechanisms in bone by proposing a staggered model in which tensile load transfer to mineral platelets inside the fibrils was accommodated by shear deformation of collagen matrix [224,225]. Also, the load transfer between adjacent fibrils was envisaged to be caused by shearing of the extrafibrillar matrix. In an study on antler bone, using the same technique, a more detailed analysis of the two-level staggered micromechanical model was conducted beyond the initial elastic regime of deformation [226]. It was hypothesized that the macroscopic inelasticity of antler was triggered by debonding ensued between mineral and collagen within fibrils, and that the later frictional sliding at the intrafibrillar mineral collagen interfaces could explain the inelastic deformation of the tissue. Katsamenis et al. [227] studied load transfer in the osteonal and sub-osteonal hierarchy of bone during loading via atomic force microscopy measurement of stiffness. They observed that the loading will diminish the difference in stiffness between the interlamellar and lamellar regions. They proposed a model to explain this selective stiffening of interlamellar areas when loaded. They hypothesized that the interlamellar regions are in fact collagen deficient and rich in non-collagenous proteins. They captured the crack propagation in the interlamellar boundaries and highlighted the role of the non-collagenous

protein rich interlamellar areas in bone toughening. In their model the mineralized collagen fibrils are glued together via a mineralized non-collagenous protein matrix. Upon exerting tensile loading, the interlamellar areas contribute to the deformation via stretching and breaking of the modular structure of non-collagenous proteins. This reversible deformation can allow for small deformation between lamellae and contribute to the energy dissipation in bone.

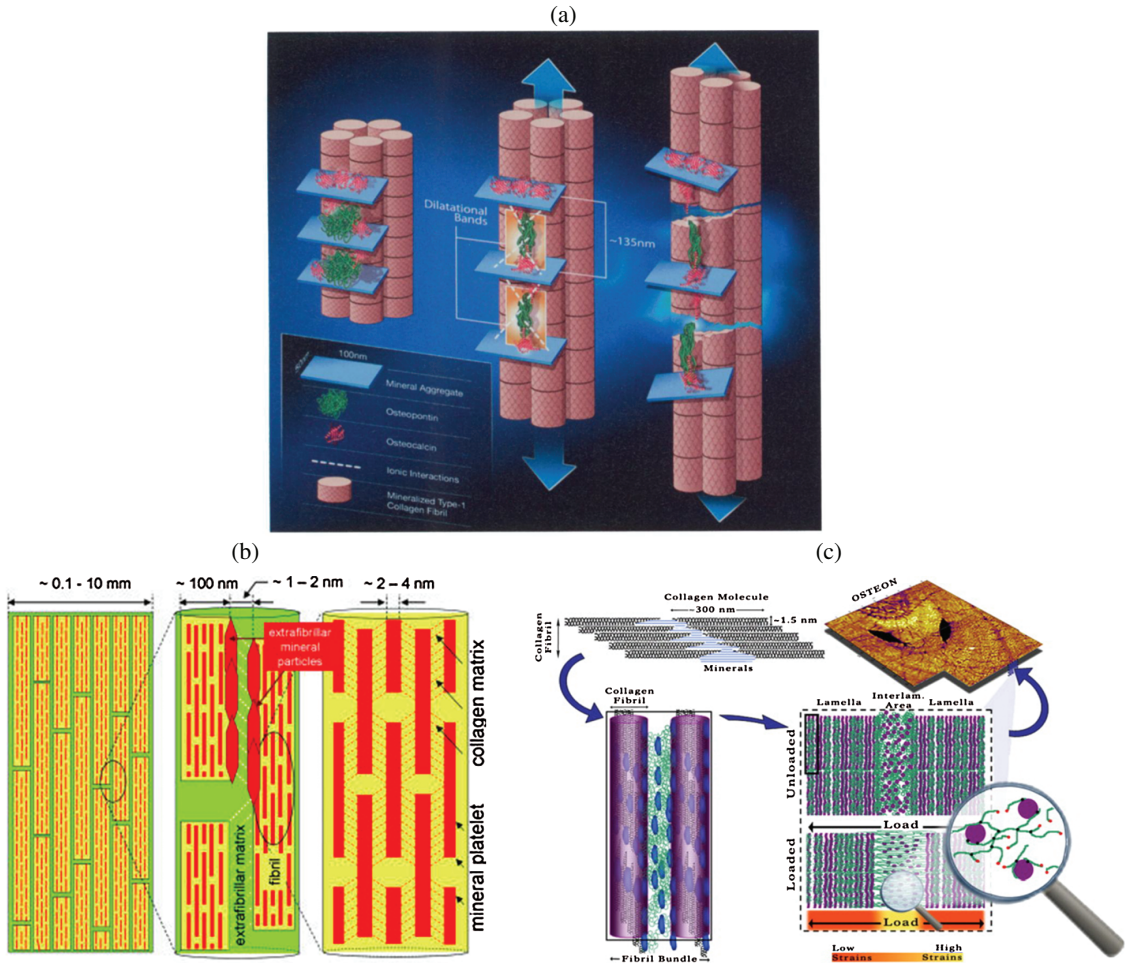

Figure 8: Models of bone ultrastructure based on indirect experimental observations: (a) a failure model of bone induced by dilatational band formation in non-collageneous protein phase and subsequent rupture of mineralized collagen fibrils [125] (Copyright (2006) National Academy of Sciences, USA), (b) a three-level structural model of fibrolamellar bone for capturing the strain in mineral, fibril, and tissue level in tension [225] (Copyright (2006) National Academy of Sciences, USA), (c) a structural model of bone for capturing the stiffening of interlamellar regions while tested in tension [227]. Non-collageneous proteins play an important role in describing the behavior of this model

4 Materials Inspired by Structure of Bone

Given the remarkable mechanical properties of bone discussed above, bone has gained a growing attention among materials science researchers who seek more efficient structural materials which are lightweight materials while sufficiently strong and tough at the same time. Prior studies have taken advantage of bone by replicating its complex internal structure at multiple length scales [44,228–233]. For instance, the design of bone-like materials by mimicking its osteonal feature [43] or lamellar characteristic using 3D printing techniques [42] has yielded enhancement of materials toughness. Despite this progress, still truly replicating the bone at the ultrastructural scales present serious challenges given the nanometer size of the ingredient and the need to simultaneously print materials within a large range of mechanical properties such as stiff mineral platelets and relatively soft collagenous matrix. These studies use different experimental techniques to manufacture materials, albeit at the laboratory scale so far, in order to employ strengthening and toughening mechanisms reminiscent to that of intrinsic and extrinsic found in bone including crack path deviation, ligament facilitated crack bridging, microcracking, interfacial-induced energy dissipation. Despite the encouraging advancements made in this bone-mimetic material design front, many challenges need to be addressed, particularly those concerning elevating the current fabrication methods and technology to enable scaled and mass scale manufacturing of these types of materials.

5 Closing Remarks

The advances in computational and experimental technologies have considerably facilitated our understanding on the ultrastructure–nanomechanics relationship of bone. More information become available regarding the nanomechanics and ultrastructure of bone. Nonetheless, the current understanding on this issue is still debatable and more efforts are still needed to further unravel the unknown ultrastructural features and the structure-function relationship of bone at ultrastructural levels. Such a pause in the field has also hindered progress in nanomechanics of bone since it is critically dependent on the accurate description and modeling of bone ultrastructure. This review intended to organize and summarize the current understanding in the field, thus giving rise to clearly defined directions for advancing this research forward with the overarching goal of translating such understanding to devising better strategies to predict bone fragility fractures and developing therapeutic treatments of such clinical complications.

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