



Integrating computational and experimental advances in bone multiscale mechanics

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A B S T R A C T

Decades of bone research have revealed the intricate hierarchical structures in bone, from the nanoscale building blocks of collagen and mineral to the complex micro-architecture and macro-geometry. Multiscale architecture confers bones their incredible toughness and strength that enables us to move through our daily lives. However, childhood and adult diseases can cause bone fragility and subsequent fractures, leading to disability, and mortality. A foundational understanding of bone mechanics across disparate scales is critical to improve the diagnosis and management of such diseases. At present, we have limited knowledge of how macroscale deformations that occur during everyday movement are transferred down to the nanoscale in order to resist fracture, especially due to historic limitations in measuring nanoscale mechanics experimentally. Recent advances in both experimental and computational tools are equipping researchers to probe the nanoscale for the first time. Here we provide a timely review of existing and next-generation experimental and computational tools and offer new perspectives on how to leverage the strengths of each approach to overcome the limitations of others. We focus on bone structure ranging from atomistic phenomena to microscale mineralized fibril interactions to build a bottom-up understanding of continuum bone mechanics and accelerate research towards impactful clinical translation.

1. Introduction

1.1. Background & rationale

The famed anthropologist Margaret Mead is said to have claimed that the first sign of a developing civilization is a healed femur—evidence that someone took the time and resources to care for an individual who could not flee danger or find sustenance

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themselves. Though this anecdote is now disputed, it indicates the well and long-understood importance of bone health and healing to human fitness and survival. Today, our understanding of this truth has progressed and continues to develop as researchers discover ties between our bones and our genetics, lifestyles, and environments. As the body's main form of structural support and a sink for both important minerals and pollutants, a foundational understanding of bone biology and management are critical to the health of the human population.

Bone health has been broadly studied for centuries [1]. Over time, research has elucidated the hierarchical structure of bone, which is considered by some to have at least 12 levels of structural organization [2], as well as the complex biological activity that governs bone growth, modeling, and repair [3]. Even so, our collective knowledge and skills have not been sufficient to effectively treat some of the most common bone morbidities. Osteoporosis, for example, is a disease that causes bone embrittlement and affects 200 million people worldwide [4], causing 8.9 million fractures annually [5]. 1 in 3 osteoporotic fracture patients die within a year, usually from complications surrounding blood loss and lung infection, and another 1 in 3 are left disabled [6]. This high morbidity and mortality costs the global economy more than \$35 billion every year [7]. Current diagnostic methods for osteoporosis only observe bone mineral density, but higher fidelity information about bone mineral quality or nanoscale tissue interactions, which are poorly understood and harder to obtain, are likely to play a crucial role in understanding bone strength and treatment. Similarly, conditions such as osteogenesis imperfecta and diabetes are known to operate at the nanoscale by causing collagen malformation [8] and impaired osteogenesis [9], respectively. These can cause drastic reductions in bone strength, demonstrating the importance of nanoscale interactions to macroscale bone behavior.

Even beyond the skeletal system itself, bone health is basal to the overall health of the human body. Bone and muscle are considered to be secretory endocrine organs, suggesting biochemical crosstalk in addition to mechanical signaling that often results in simultaneous infliction of osteoporosis and sarcopenia [10,11], which causes muscular atrophy and hypofunction. In similar fashion, bone decline has been linked to deterioration of nearly every system in the human body including cardiovascular function [12,13], cognitive function [14–16], and the immune system [17–19].

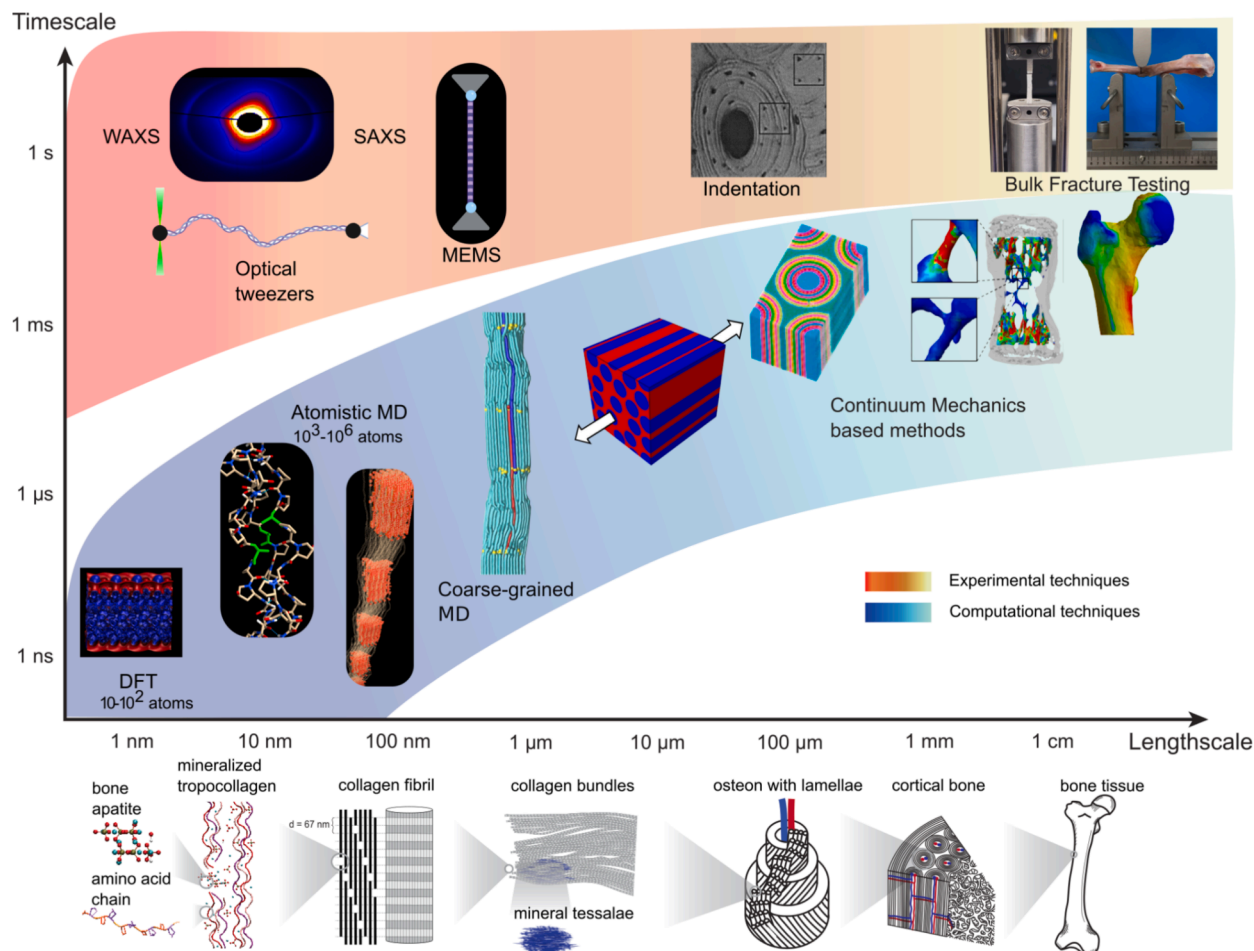


Fig. 1. Top: The variety of experimental and computational techniques that can be used to probe the mechanical behavior of bone at different length and timescales. Figures reprinted from [21–26] with permission from Elsevier. Optical tweezer and MEMS figures created using [BioRender.com](#). Bottom: a representation of the hierarchical structure of bone.

Clearly, elucidating the effect of multi-level architecture on bone function (Fig. 1) is critical to advancing the prevention, diagnosis, and treatment of bone-related morbidities. However, technological limitations have historically restricted our understanding of bone at the nanoscale level, particularly regarding how stresses and strains are transferred from the nanoscale upwards to nucleate cracks and cause large-scale fractures. As a result, many open questions that require multiscale mechanistic understanding have not yet been fully resolved. For example, where do fractures first nucleate at the smallest scales of bone structure? What is the role of non-collagenous proteins, which bind together collagen and mineral crystals, in bone deformation? What structures contribute to stopping or propagating fracture as it travels through the layers of bone hierarchy? Catastrophic bone failures, whether from chronic or acute distress, begin at the bottom of the hierarchy. Elucidating the smallest scales of bone mechanics, and their connections to clinically relevant macroscopic responses, is paramount to building a comprehensive, bottom-up understanding of complex bone behavior in health and disease.

Recent advances in both experimental and digital tools are now enabling researchers to parse nanoscale structures and interactions of bone, including with state-of-the-art imaging technologies and computational models built from biophysical understanding of proteins and materiomimetic first-principles calculations (Fig. 1). For instance, synchrotron imaging with micro-CT or X-ray scattering can resolve the microarchitecture of bone as well as collagen-mineral interactions under in situ mechanical loading [20]. Experimental findings can be corroborated with atomistic and molecular simulations, which can resolve very high-fidelity interactions, but are often restricted to very short time-scales due to computing power requirements. These tools and others can be applied in conjunction, harnessing the strengths of each to overcome limitations of others.

In this review, we intend to provide a comprehensive survey of the experimental and computational tools available to modern bone researchers, and to share perspectives on how they can be used together to probe new frontiers. In the following section, we begin by providing a brief primer on current understanding of the hierarchical structure of bone. Familiar readers may jump ahead to Chapter 2, where we consider in detail state-of-the-art experimental and computational techniques applied to each level of bone structure from the bottom-up, and discuss their strengths, limitations, and impacts. We then integrate this knowledge to provide insight into how bone fracture begins at the nanoscale and propagates up to the macroscale, addressing how some of the open questions described above are beginning to be resolved. Finally, we share a general framework for combining computational and experimental approaches, as well as perspectives on the outlook of bone research, developing technologies, and their impacts on patient treatment and prognosis.

1.2. Hierarchical structure of bone

As a prototypical mineralized biological tissue, bone is composed primarily of mineral crystals, collagen fibrils, non collagenous proteins, and water. It is generally understood that the combination of elastic organic materials (collagen, other proteins) and stiff inorganic minerals strikes a balance between density, strength and toughness that is often elusive to materials engineers. However, recent research has revealed that it is not just the constituents of bone that yield its exceptional mechanical properties, but the 3-dimensional arrangement of these constituents at multiple length scales. In some literature, a total of 12 hierarchical levels have been proposed [2], and here we will summarize the most significant and/or discrete (Fig. 1).

At the macroscale, distinguishable to the unaided eye, bone tissue can be classified into two categories: cortical bone, which is dense and stiff but relatively brittle, and trabecular bone, which has honeycomb-like porosity, can withstand higher strains, and is more metabolically active [27,28]. Cortical bone is made up of closely packed osteons, which have concentric lamellar layers of bone tissue around Haversian canals, which house blood vessels and nerve fibers that travel throughout the bone. Bone tissue lamellae, which are observable at the microscale, wrap around Haversian canals with cyclically varying pitch such that an osteon resembles nested springs, a geometry that confers mechanical advantages [29–31]. Trabecular bone is made up of lamellar plates and bars, or trabeculae, that form a network around small cavities that contain bone marrow [2,32].

Parsing the structure of lamellae, now approaching the nanoscale, reveals the organization of collagen in repeating components of “disordered” regions, which house osteocytes and their associated dendritic cell processes, and “ordered” regions where collagen forms bundles and twist around their own axis, similarly to the pitch of the lamellae [33–35]. This kind of axial twist is a common motif throughout several levels of bone structure and is thought to reduce stress-concentration in the architecture [2]. At this lamellar level, cross-fibrillar mineral tessellation is also observed. Mineral crystals are observed as ellipsoid-shaped aggregates generally aligned with the surrounding collagen fibrils, and are loosely contiguous across collagen fibrils, which likely provides a toughening mechanism [36,37].

The organized collagen bundles contain collagen fibrils, which in turn are bundles of type 1 collagen chains assembled into right-handed triple helices known as tropocollagen [38]. Collagen fibrils are 20–500 nm in diameter [39] and display a periodic banding pattern with a D-period of approximately 67 nm due to the staggering of tropocollagen molecules [40]. The mineral aggregates, meanwhile, contain crystallites with stacks of platelets and needles of crystal that merge contiguously from intrafibrillar to extra-fibrillar aggregates, forming a connected network [41,42]. Finally, at the molecular level, mineral crystals contain repeating unit cells with sub-nanometer lattice spacing, and individual collagen molecules are triple-helix chains of amino acids [2].

2. The current knowledge of bone multiscale mechanics from the nanoscale upwards

Over the past few decades, the structure and properties of the bone matrix at the nano and microscale have started to be discovered. This has involved extensive experimental and computational techniques, including solid state nuclear magnetic resonance, X-ray scattering, electron microscopy, density functional theory, molecular dynamics, and finite element modeling (Fig. 1). In this next section, we will cover the structure and properties of bone, starting from the molecular properties of the mineral crystals and collagen

molecules, moving towards mineralised collagen fibrils and finally covering macroscopic mechanical properties. We will detail the various techniques that have been used to elucidate this structure, including their limitations and how we believe the various techniques could be combined to enhance our knowledge of bone multiscale mechanics.

2.1. Bone apatite and interfacial interactions at the nanoscale

At the very bottom of the bone hierarchical structure are the bone mineral nanocrystals and the collagen proteins, as well as the fundamental interactions between these components [41]. Mineral crystals are primarily composed of calcium phosphate in an apatite-like phase which is often attributed to hydroxyapatite, the same mineral phase that composes other hard tissues in the body such as teeth. Hydroxyapatite is an ionic crystal composed of calcium, phosphate and hydroxide ions in the ratio $\text{Ca}_5(\text{PO}_4)_3\text{OH}$. The crystal structure of hydroxyapatite is generally assigned to two phases: a hexagonal $\text{P6}_3/\text{m}$ phase similar to the fluorapatite structure and a monoclinic $\text{P2}_1/\text{b}$ phase [43–46]. The primary difference between the two phases is in the ordering of the column of hydroxyl ions. In the hexagonal phase the column is believed to be disordered with half occupancy of up and down positions. Ionic substitutions such as chloride or fluoride ions may be critical to the stability of this phase, as they will permit a change in direction of the column without creating an energetically unfavorable configuration of two opposing hydroxyl ions pointing at each other. This implies that substitutions into the mineral phase are crucial in controlling the morphology of the crystals. In the monoclinic $\text{P2}_1/\text{b}$ phase alternating columns of hydroxyl ions run antiparallel to each other along the c-axis. These different crystal structures are shown in Fig. 2.

In general, bone apatite is taken to be most similar to the hexagonal $\text{P6}_3/\text{m}$ phase, although this is still an area of active debate

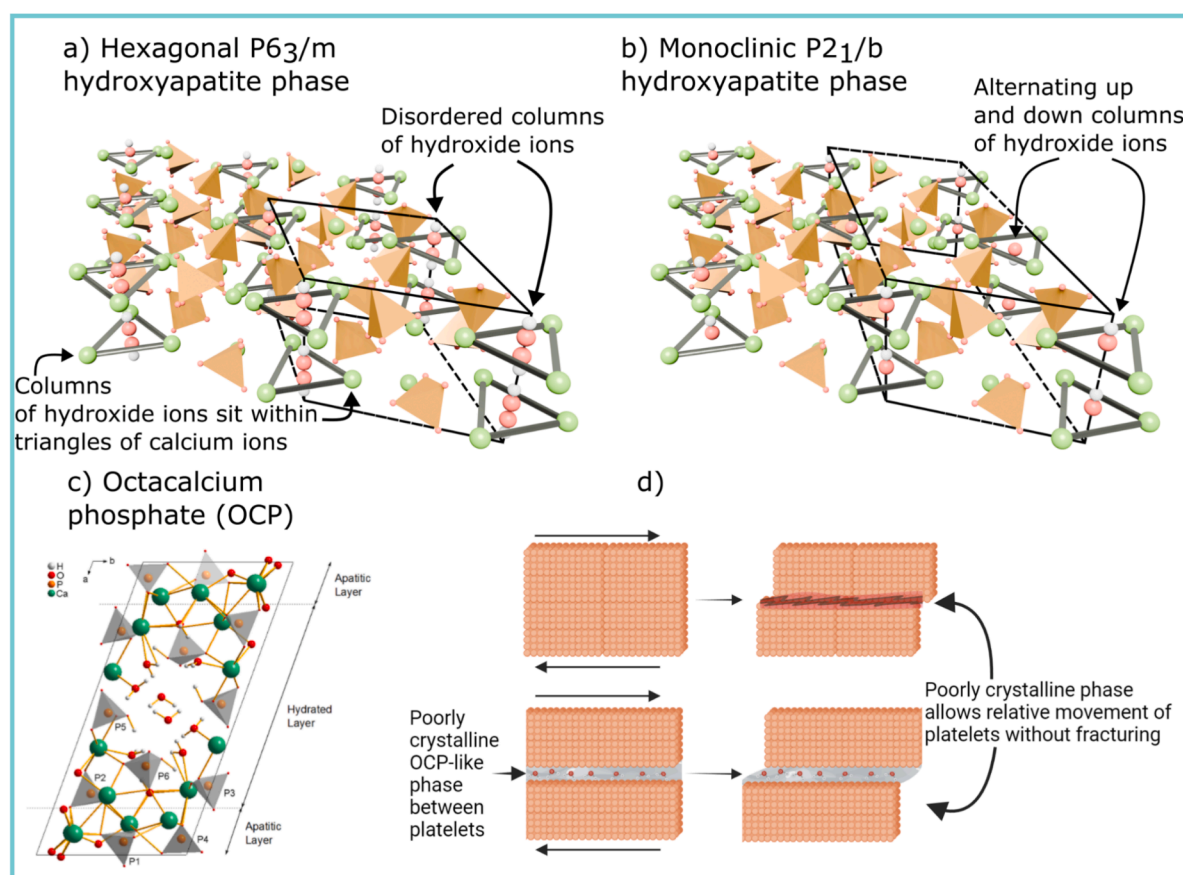


Fig. 2. 1) Images of different crystal structures relevant to bone. Oxygen atoms are red, calcium atoms are green and hydrogen atoms are white. Phosphate ions are represented by tetrahedra with the oxygen atoms reduced in size. The gray connections between the calcium ions do not represent bonding but instead show the calcium ion triangles that form the hydroxide ion channels. (a) The crystal structure of the $\text{P6}_3/\text{m}$ phase of hydroxyapatite. The disordered hydroxide ion columns can be seen. (b) The crystal structure of the $\text{P2}_1/\text{b}$ phase of hydroxyapatite. The alternating up and down columns of hydroxide ions can be seen. The alternating pattern of up and down columns leads to the unit cell for the monoclinic phase being twice as long as the unit cell for the hexagonal phase. (c) The crystal structure of octacalcium phosphate (OCP) as proposed by Davies et al [64]. The apatite layer matches well with the hydroxyapatite structure, whereas the hydrated layer has been proposed to be a good model for the water-bone apatite interface. Reprinted with permission from [64]. Copyright 2012 American Chemical Society. (d) A poorly crystalline OCP layer between mineral platelets allows for relative movement of the platelets without fracturing the mineral crystals. This layer is thought to be stabilized by the citrate ion [71]. Created with BioRender.com.

[43,47]. Bone apatite contains many alterations to the pure hydroxyapatite structure as many different ions substitute for the ions in the hydroxyapatite, in particular carbonate ions [48,49]. In addition, Raman spectroscopy and NMR experiments have indicated that bone apatite may contain significantly fewer hydroxyl ions than synthetic hydroxyapatite [50–52]. Recent computational and experimental investigations have explored the importance of these substitution ions [53–56]. Indeed, recent investigations into dentine apatite have shown a stiffening of apatite crystals upon removal of carbonate ions [56,57], further implying that mineral substitutions play a key role in controlling the mechanical properties of bone at the nanoscale.

Bone nanocrystals grow to form platelet-like morphologies, with growth occurring along the crystallographic *c*-axis [45,58–60]. This is at odds with the hexagonal symmetry of the hydroxyapatite lattice, which would imply a needle-like morphology when growth occurs along the *c*-axis. This may be evidence that the crystal forms via an alternative calcium phosphate phase known as octacalcium phosphate (OCP) as OCP crystals have a platelet morphology [61,62]. The crystal structure of OCP is a two layered structure with one layer that is virtually identical to the apatite structure and another layer that is significantly hydrated. Brown proposed that this hydrated layer is potentially a good model for the interface between bone mineral and water [44,61].

Duer and coworkers have further developed this idea using nuclear magnetic resonance (NMR) experiments [63–65]. NMR experiments measure the environments of different chemical species to determine information about their bonding states as well as the association between different groups. Using NMR, they built a model of bone mineral composed of a hydroxyapatite core, surrounded by a more disordered phase analogous to the hydrated phase in OCP. They suggested that this hydrated layer is stabilized by the citrate anion. The existence of a water-mediated disordered mineral phase shows that hydration plays a crucial role in the structure of bone apatite [66,67]. Indeed it has been shown that disorder in the mineral structure is crucial to the mechanical function of bone, with increasing crystallinity associated with poorer bone quality as animals age [68–70]. The potential role that disorder plays on mechanical behavior is demonstrated in Fig. 2.

Raman and Fourier transform infrared (FTIR) spectroscopy are alternative techniques to analyse the chemical structure of the organic and mineral phases. By analysing the vibrational spectrum, the bonds and subsequently the chemical groups present in the mineral and collagen can be obtained [72,73]. Using these spectroscopy techniques, key compositional and structural information about the mineral can be obtained, for example the degree and sites of carbonate substitution and the degree of crystallinity of the mineral [74–79]. Recent work has utilised FTIR spectroscopy to investigate the changes in bone mineral composition and structure after long-term exposure to common osteoporosis medications [80,81]. The mode of action of antiresorptive osteoporosis treatments is to reduce the rate of bone remodeling, leading to increased bone mineral density in the patient [82]. Long term treatment (5–10 years) with Denosumab was found to lead to increased mineral crystallinity and lower carbonation, indicating that changes in the rate of bone remodeling lead to modified structure and composition of bone's fundamental building blocks [80]. These changes were associated with lower microhardness measured using indentation techniques. Interestingly, treatment with another antiresorptive osteoporosis medication, Alendronate was found to be associated with lower mineral crystallinity [80,83]. Alendronate has been shown to directly bind to the bone mineral [84], potentially impacting the morphology of the mineral analogous to the role of citrate and carbonate discussed above. These studies highlight the importance of understanding the nanoscale structure in order to fully understand the long-term effects of antiresorptive bone medication. In addition, Raman spectroscopy also shows promise as a measurement technique *in vivo*, providing a potential diagnostic technique that takes into account nanoscale properties [85,86].

Building accurate computational models of these nanocrystals is very tricky to perform solely with experimental data because of the nanoscopic size and poor crystallinity. First principles thermodynamics can be used to determine structure and composition at high accuracy and therefore can be used to propose reasonable models of the bone mineral. The most widely used computational technique to model bone minerals is density functional theory (DFT). In DFT, an energy functional of the electron density is minimized to find the ground state electron density and energy for a given atomistic geometry. The solution, expressed in terms of Kohn-Sham orbitals, can be used to analyze the nature of the bonding, the quasi-harmonic Gibbs free energy, and the spectroscopic properties of the structure [87]. In general, these calculations are computationally costly, meaning they can only be performed on small systems up to a few hundred atoms. When these calculations are informed by simpler models or experimental data, the number of possible structures can be restricted and the DFT calculations can determine which of the possible structures are most likely. DFT calculations were crucial for the development of the mineral model incorporating citrate mentioned above, as they allowed the most energetically stable structure consistent with the NMR data to be found [64,71].

Along with the chemical structure, the larger scale structure and distribution of the bone mineral is also crucial. In a pioneering study in 1952, Robinson showed that the gap-overlap pattern found in unmineralised fibrils is also present in mineralised fibrils. This implies that mineralisation conserves the structure of the fibrils with mineral crystals mostly being present in the gap regions. Weiner and Traub built on this work by measuring the distribution of mineral crystals in calcified turkey tendon using an electron microscope and found parallel layers of crystal plates between the collagen molecules [88–92]. Bone mineral has been shown to exist both within the collagen fibrils (intrafibrillar mineral) and outside the fibrils (extrafibrillar mineral) [93,94]. Whilst it is generally accepted that there is mineral in both regions, the division of the mineral between these two regions and their differences in structure and morphology is still debated. Electron microscopy techniques, specifically transmission electron microscopy (TEM) have recently been utilised to try and answer these open questions [42,93,95–97]. Schwarcz et al. used dark field TEM to try and understand the structure of the mineral within the collagen fibrils [97]. By looking at the dark field TEM image from the (002) electron diffraction arc, they could look for locations of apatite crystals. Interestingly, few examples of apatite crystals within the fibrils could be found, suggesting that the calcium-phosphate found in the fibrils may not be crystalline apatite but instead an amorphous phase of calcium phosphate. TEM has also been used to investigate the morphology of bone mineral in the extrafibrillar region. Using this technique, curved mineral lamellae have been seen [98]. These lamellae form cylindrical structures around the collagen fibrils. Using dark field TEM imaging, these structures appear to be polycrystalline, being composed of apatite platelets that are misaligned with each other [42,93]. How

these platelets are held together to form the curved mineral structures is currently unknown, but these could be heavily related to the OCP-like disordered regions discussed above. It is also unknown how the formation of these cylindrical structures may be modulated by changes in the underlying chemistry and how the role these cylindrical structures have in the mechanical behaviour of the bone matrix.

Paramount to mechanical behavior in bone is the interaction at the interface between the soft protein and the stiff mineral nanocrystals. Since the mineral transfers shear stress between the protein matrix, altering the mineral-biomolecule interfacial strength will affect the point at which the structure fails via slipping [99]. Rationalizing the structure of interfaces at the molecular level is generally very difficult, which is further complicated by the compositional complexity of bone. NMR experiments have similarly been used to investigate the nature of this interface. In particular, using rotational-echo double-resonance (REDOR) experiments, it was found that glutamate residues are highly present at the interface [100]. Interestingly, collagen contains few glutamate residues suggesting that non-collagenous proteins present in the extracellular matrix, likely osteopontin or osteocalcin which contain large numbers of glutamate residues, play a significant role in the protein–mineral interface. This was further investigated by Vashishth and coworkers who performed solid state NMR experiments on genetically modified mice lacking the genes that encode for osteocalcin or osteopontin [101,102]. The REDOR spectra of these mice showed a change in the presence of the amino acids present at the interface, suggesting that the bone is attempting to compensate for the loss of osteocalcin and osteopontin at the interface. The reduced fracture toughness of the bone in these genetically modified mice indicates that this compensation is not adequate. In addition, polysaccharides have also been shown to have a significant presence at the surface of the mineral, potentially from proteoglycans in the extrafibrillar matrix [103].

Further information about the structure of the interfaces can be gleaned via DFT calculations. “Slabs” of hydroxyapatite are most often used as a model of the mineral for surface analysis. A variety of different hydroxyapatite surfaces have been analyzed using DFT [104,105]. By analyzing the electronic density at the surfaces, adsorption locations of different species can be proposed including, crucially, water and proteins [106–109]. Nanocrystals of bone apatite grow along the (001) surface leading to platelet crystals with elongated (010)/(100) faces. High-resolution transmission electron microscopy experiments (HRTEM) analyzed the (010) face of hydroxyapatite and showed a non-stoichiometric Ca-rich face to be important [110]. This non-stoichiometric termination has been analyzed using DFT, including the binding of collagen-like molecules to this surface. Using fundamental thermodynamics, the stability of different surfaces can be rationalized. By considering the crystal at equilibrium with ions in solution, Astala and Stott showed that whilst the pure 010 Ca-rich face is unstable, it becomes the most stable hydroxyapatite face when water is adsorbed and at high chemical potential of $\text{Ca}_3(\text{PO}_4)_2$ [111]. Recent DFT simulations have used these surfaces to rationalise how collagen and other peptides adsorb onto hydroxyapatite [105,108,109,112]. In particular, electrostatic interactions between C=O groups and calcium ions on the surface seem to be important for binding [109]. This shows the potential of computational techniques to rationalize and predict the morphology of bone nanocrystals at atomic resolution.

Despite significant experimental and computational research into the structure of the components at the base of the hierarchy, many significant questions still remain. For example, potential structural differences between extrafibrillar and intrafibrillar bone mineral are not well understood, and in particular, how these differences may affect the interaction between bone mineral and collagenous and non-collagenous proteins. In addition, little is understood about how changes in mineral chemistry may affect the macroscopic bone properties. Chemical changes likely play multifaceted roles where they modify both the morphology of the crystals themselves and the chemical interactions between the crystals and the other components. As mentioned previously, the poorly crystalline region between the mineral platelets is important to allow them to move laterally without breaking. Changes in chemistry that disfavor the formation of this layer and favor the formation of crystalline hydroxyapatite will change the mechanical properties of the bone and increase fracture risk. This is shown schematically in Fig. 2.

In general, analyzing bone mineral structure and interactions with experimental techniques is inherently difficult due to the nanoscopic size of the components. This is exacerbated by the sensitivity of these components to their environment, in particular hydration, reducing the number of experimental techniques that can be used. Computational tools provide much greater control than is available experimentally, meaning the crystals can be modeled in a variety of different environments. However, computational techniques such as DFT require intensive computational power which naturally limits the number of calculations that can be performed. These calculations must therefore be guided by experimental data which can provide possible candidates for a computational model to fit. Recent advances in machine learning techniques promise to transform the computational work at this hierarchical level and up. For instance, machine learning force fields such as Allegro have shown great success at reproducing high accuracy data from quantum calculations at much lower computational cost [113–115]. The use of these force fields offers the potential to test a much larger range of biomolecules interacting with the mineral, as well as scale up in size to more complex systems. This could significantly improve our understanding of the interfacial interactions and their role on mechanical integrity from the nanoscale upwards.

2.2. Understanding the molecular structure of collagen

At the center of molecular biology is the structure–function paradigm, which indicates that understanding the 3-dimensional folded structure of a protein is crucial to understanding its function. As a result, obtaining high-resolution images of folded proteins is a central tenet of molecular biology. The development of cryo-electron microscopy techniques in combination with traditional X-ray crystallography made available hundreds of thousands of high-resolution structures. However, due to the large disorder within the gap region of the collagen D-period, as well as misalignment of the fibrils, obtaining high-resolution images of the full type-I collagen molecule within the collagen fibril has not been possible [116–119]. As a result, a significant proportion of experimental research on the structure of the collagen molecule has been performed on collagen mimetic peptides (CMPs). These are small peptides that have a

triple helical fold similar to that present in collagen proteins [120,121]. Experimentally, high resolution crystal structures of these peptides are obtainable via X-ray diffraction, allowing detailed information into the molecular layout and the hydrogen bonding. The triple helical sequence of collagen structures follows a Gly-X-Y pattern with X and Y most commonly being proline and hydroxyproline [122], respectively. Therefore, collagen mimetic peptides are based on the canonical Glycine-Proline-Hydroxyproline (GPO) amino acid sequence, which represent around 10.5 % of the triplets found in the real collagen molecule [123].

By introducing changes in this canonical GPO sequence, the effect of different amino acids on the collagen structure can be observed [120,124–126]. Most commonly, one or two proline or hydroxyproline residues are substituted for different amino acids such that the effect of individual or pairs of amino acids on the collagen molecular properties can be elucidated. The X-ray diffraction

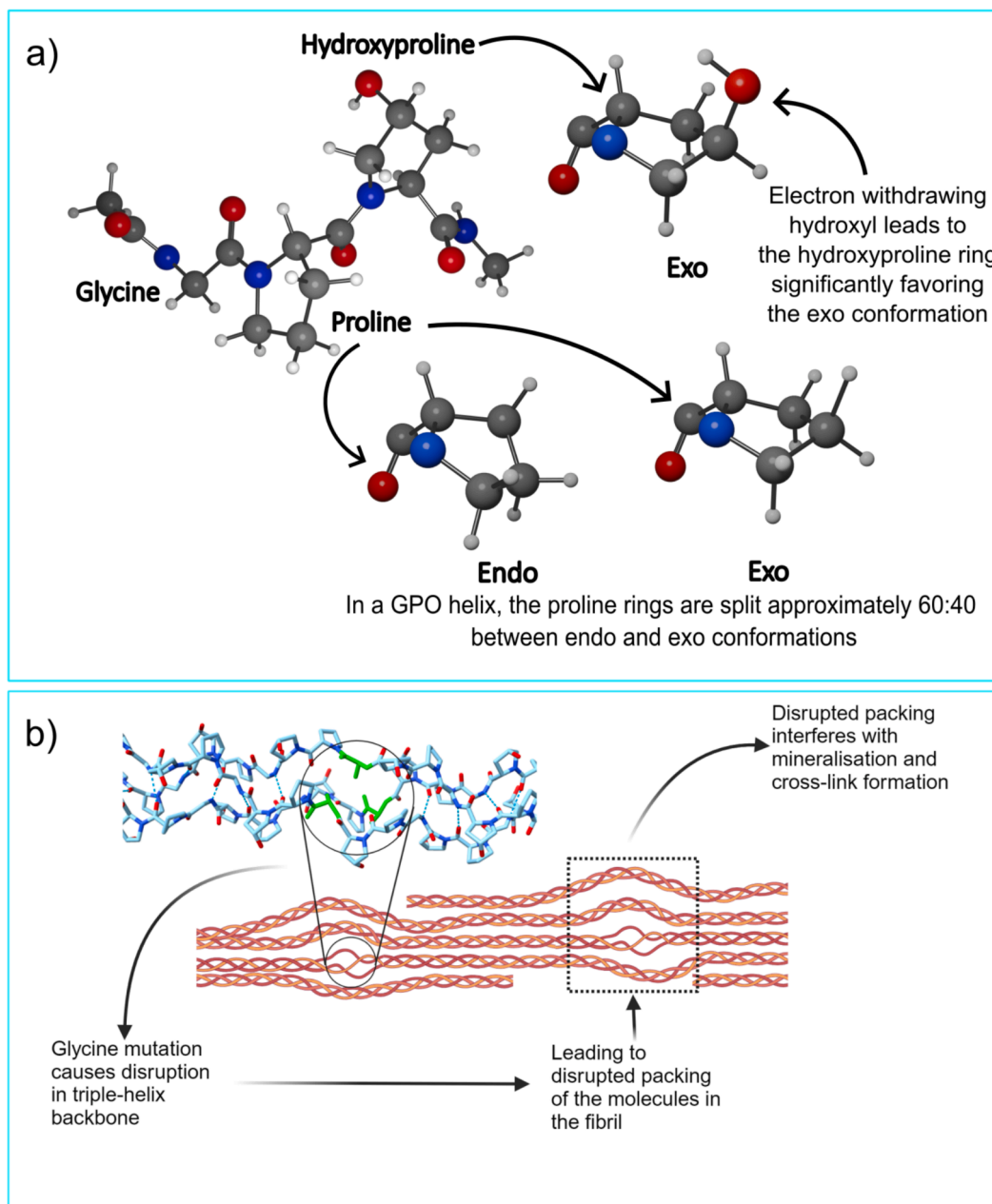


Fig. 3. (a) A GPO fragment of a collagen mimetic peptide. The electron withdrawing hydroxyl group leads to the exo conformation of hydroxyproline becoming favored over the endo conformation. This preference allows for easier formation of the triple helix as the exo conformation is well matched to the backbone conformation of the Y position in the triplet. In a GPO triplet the proline shows ambiguity between the endo and exo conformations which has consequences for the mechanical properties of these sequences. (b) A schematic showing how glycine mutations associated with Osteogenesis Imperfecta lead to widescale changes in the bone nanostructure and ultimately lead to brittle bones. Figure created with BioRender.com.

structure of a GPO sequence shows a tight 7/2 helix. Substituting the proline (or hydroxyproline) rings with non-proline residues causes a transition of the helix to a looser 10/3 helix. In addition, single amino acid substitutions away from the GPO sequence all decrease the melting temperature of the triple helix [125], suggesting a reduction in proline content leads to a destabilizing of the triple helical fold. Interestingly, pairs of charged amino acids (such as glutamate and protonated lysine) have been shown to be able to increase the thermal stability of CMPs due to stabilizing interactions between residues of opposite charge [127–129]. Using the data from x-ray diffraction structures, the effect of these deviations from the GPO sequence on the collagen triple helical structure has been encoded using a statistical method [130,131]. This method predicts the triple helical backbone structure from the protein sequence which permits the building of models of any desired CMP sequence [132]. Exciting new developments in machine learning models have built upon these statistical ideas, allowing the prediction of triple helical thermal stability of any sequence. Using these models, it is possible to design new CMPs with desirable properties [133].

A significant amount of computational work has also gone into analyzing the properties of CMPs, primarily using molecular dynamics (MD). Using computational simulations, the mechanical behavior of different CMPs as well as the mechanical interaction between CMPs can be rationalized [134–136]. Computational methods can supplement experimental work at this length scale, providing additional insight into the energetics and dynamics involved in the chemical processes of collagen. A good example of where computational and experimental work have been used in tandem is in understanding the role proline ring conformations have on both the structure and mechanical properties of the collagen triple helix. Proline and hydroxyproline rings have two dominant conformations, referred to as endo and exo conformations. The occupancy of these conformations can be calculated using NMR [137]. These conformations are shown in Fig. 3a. In a GPO triplet, it was found that the hydroxyproline is almost entirely in the exo conformation. Interestingly, the proline ring was found to be split almost equally between the endo and exo states. In comparison, the central proline in a GPP triplet shows a far greater tendency to be in the endo conformation. It was hypothesized that the conformational ambiguity of the proline in the GPO triplet gives the molecule more flexibility and greater ability to adjust to external forces. This hypothesis was tested by exploring the potential energy landscape of GPP and GPO helices [138], where it was found that the application of forces changes the conformational preference of the proline ring [139,140]. This highlights the importance of using computational and experimental techniques in conjunction to allow for a greater understanding of the collagen molecular properties.

As with bone mineral, recent experimental work has found water to have a significant impact on the behavior of the collagen molecules and fibril [141,142]. Analysis of CMP structures has found that water molecules form direct hydrogen bonds with the collagen triple helix, potentially playing a key role in stabilising the helix. These water molecules that hydrogen bond directly to the helix are often referred to as “tightly bound water”. Recently, dehydration has been shown to lead to significant contraction of collagen molecules, potentially giving the body a simple mechanism to control the tension of the extracellular matrix [141]. This water-mediated collagen contraction may play a significant role in the mechanical behaviour of bone. Recent work has identified a development of residual stresses during mineralisation putting the mineral phase under compression [143–146]. These residual stresses could be critical to bones’ impressive mechanical properties as they would allow the mineral phase to withstand significant tensile stresses. As mineral replaces water during mineralisation [147], it has been proposed that these residual stresses occur as a result of dehydration of the collagen [143,146]. The molecular origin of this contraction is still unclear, however, using experiments and simulations on CMPs, it has been shown that different amino acid sequences have different sensitivities to changes in osmotic pressure [142]. Crucially, the canonical GPO sequence shows a slight expansion under increasing osmotic pressure. Since the entire collagen molecule has been shown to contract under increasing osmotic pressure this shows the importance of the non-proline triplets on collagen structure and mechanical properties.

The effect of genetic mutations on collagen mechanical behavior has also been investigated with CMPs. Of particular interest are mutations associated with osteogenesis imperfecta, which lead to highly brittle bones in children [148–151]. Among the most common are glycine mutations, which can have particularly significant effects on the collagen molecular structure due to the importance of the glycine residue in maintaining the triple helical fold. Since the glycine hydrogen points into the center of the helix, replacement of the hydrogen with a bulkier group causes instability in the helical structure. These mutations have been investigated using both extensive experimental and computational work. X-ray diffraction experiments on CMPs with glycine to alanine mutations have shown the effect that these mutations have on the helical structure [152,153]. For instance, the presence of these mutations is observed to disrupt hydrogen bonding between the 3 strands of the triple helix, with potentially significant ramifications on the mechanical properties of the molecule. The mutation also causes a local untwisting of the helix. With computational methods, there is greater control over the amino acid sequence and the energetics involved can be directly calculated. Gautieri et al. investigated the effect of different glycine mutations over a range of severities [132,154]. In addition to altering the mechanical properties of the individual molecules, the mutations were also found to alter the interactions between adjacent molecules. With increasing severity, intermolecular interactions were found to be weaker and to have energetic minima at greater molecular separation. This has potentially significant consequences for the way molecules pack together inside a fibril, which not only directly affects the lateral interactions between molecules but may also indirectly affect the interactions via a reduction in cross-links between the molecules. This modification in packing will also modify the formation of the mineral crystals as the collagen fibrils act as templates for mineralisation. The effect that these mutations have on the bone nanostructure is shown schematically in Fig. 3b.

Experimental and computational studies on collagen mimetic peptides have provided great insight into the structure and chemical behavior of collagen. However, CMPs have shown significant promise as self-assembling biomimetic materials in their own right [155–159]. CMPs are an appealing alternative to natural collagen as biomaterials due to high control of the sequence allowing for the design of materials with a large range of properties. Here computational tools are particularly well suited as material properties can be screened in a high-throughput manner at low cost. The maturation of machine learning techniques over the past decade offers exciting possibilities for inverse design of CMPs that has only recently started to be explored [160,161]. For example, machine learning models

based upon existing experimental and computational databases can learn complicated non-linear relationships between protein sequence and material properties. Generative artificial intelligence can utilize these relationships to permit de novo design of collagen sequences with desired properties.

The investigation of collagen mimetic peptides using both experimental and computational techniques has led to large strides in the collective understanding of collagen molecular properties. However, this research has focused on sequences based around the idealized GPO sequence. The research investigating the effects of osmotic pressure highlights the importance of non-proline rings on the mechanical and chemical properties. Since non-GPO triplets make up almost 90 % of the triplets in type-I collagen, understanding the role that these non-GPO triplets play is crucial. As has been done previously, these non-proline triplets can be investigated by incorporating guest sequences into the GPO helix, however it is unknown whether this places unrealistic constraints on the behavior of the guest sequence. Investigating the role of these sequences is time consuming experimentally however they can be readily investigated using molecular dynamics. Machine learning and statistical techniques can reduce the space that has to be investigated by allowing the exploration of sequences outside the dataset. In addition, type I collagen is a heterotrimer composed of two α -1 (I) chains and one α -2 (I) chain, yet the majority of investigations into collagen mimetic peptides have been on homotrimers. This is due to the difficulty in synthesizing self-assembling heterotrimers of desired composition, because there are many different combinations of triple helix that can be formed by 2 or 3 strands [162]. The ordering of the 3 strands also affects the structure of the helix. By designing sequences where electrostatic interactions change the favorability of these sequences, heterotrimers of the desired composition can be synthesized [163]. Computational techniques offer significant promise in the design of these heterotrimers as they can account for such electrostatic interactions [164,165].

2.3. The mechanical behavior of collagen as a 300 nm long polymer

Whilst CMPs provide high-resolution insights into the collagen molecular structure, it is important to scale up and understand the properties of the whole collagen molecule, especially with regards to mechanical performance. The mechanical properties of individual collagen molecules can be experimentally determined using atomic force microscopy (AFM) and optical tweezers [166–172].

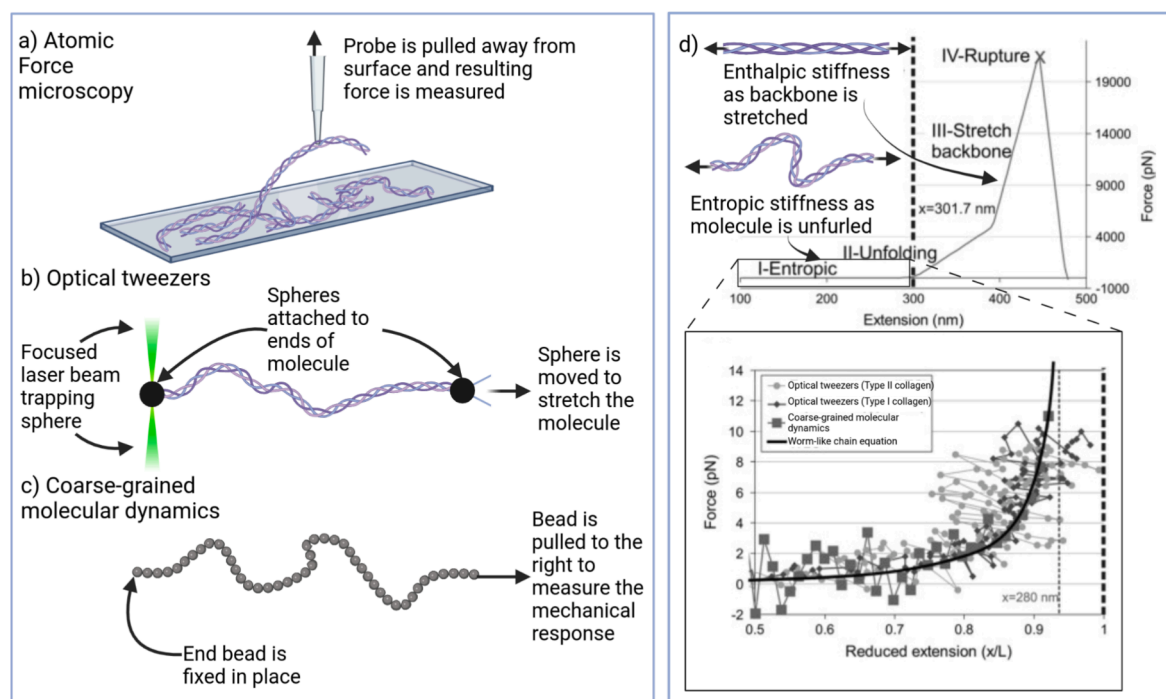


Fig. 4. Left: The different experimental and computational methods used to calculate the mechanical behavior of individual collagen molecules (a) The atomic force microscopy probe can be used to mechanically test the collagen molecule by pulling the molecules away from the surface. Figure drawn here based on the experimental setup described by Bozec and Horton [166] (b) Optical tweezers can mechanically test the collagen molecule by chemically bonding spheres to the two ends of the molecule. By trapping one sphere in the laser and pulling on the other sphere the mechanical behavior can be obtained. Figure drawn here based on the method described by Sun et al. [170] (c) The mechanical behavior can be obtained *in silico* using a coarse-grained molecular dynamics model. Here one end of the molecule is kept fixed whilst the other is pulled away at constant velocity. By measuring the force on the end that is pulled away the mechanical behavior can be obtained. Figure drawn here based on the simulation setup by Buehler and Wong [176]. (d) (Top) The force-extension graph obtained using the coarse-grained molecular dynamics model. (Bottom) Comparison of experimental and computational data on the entropic region of the force-extension curve. Bottom figure reprinted from [176], with permission from Elsevier. Figure created with BioRender.com.

Both methods show similar force-extension profiles where initially little to no force is required to stretch the molecule, indicating that kinks in the collagen molecule are being straightened out (Fig. 4d). After this initial plateau, the force increases sharply as the length of the molecule reaches its contour length, and the backbone starts to stretch. Below the contour length the mechanical response can be fit to the worm-like chain equation, which is often used for DNA and describes the entropic behavior [173]. From this fitting, the contour length of collagen molecules can be estimated. For example, using this procedure, Bozec and Horton performed AFM experiments on individual collagen molecules on top of a glass coated with gold surface. The worm-like chain model estimated the collagen length to be 206.5 ± 8.0 nm [166]. However, this is considerably less than the length of 300 nm observed in other studies, suggesting that the probe was only pulling a subsection of the molecule. These discrepancies highlight the difficulty of performing accurate experimental measurements at this length scale.

Alternatively, using computational methods, Buehler used data from molecular dynamics simulations of a GPO CMP to derive a coarse-grained molecular dynamics model of the full collagen molecule [174–176]. This model was mechanically tested using steered molecular dynamics which is a computational analog to AFM experiments where mechanical forces are directly applied to molecules. In earlier work, researchers found a response curve very similar to experiments, with an initial low force region followed by a high force region when the length of the molecule reaches its contour length [176]. The low force region was attributed to entropic stiffness as the collagen molecule is unfurling, with the high force region being attributed to enthalpic stiffness caused by stretching of the molecular backbone itself. In tendons and bone, collagen molecules are packed into fibrils which drastically reduces the free volume available to the molecules. Therefore, it is expected that in collagen fibrils, the entropic contribution is less important to the mechanical response than compared to isolated collagen molecules.

From this level of hierarchy, we can observe the importance of using computational and experimental work in tandem. There is a natural limit to the level of control available in the experiment, as shown by the difficulty in measuring the contour length using AFM. The computational work therefore acts to corroborate the experimental and provide additional mechanistic insights.

2.4. Mechanical properties of collagen fibrils

In tendons and bone, collagen molecules are packed into fibrils. The packing of the collagen molecules into the fibril is relatively regular, with a defined packing length along the z axis of the fibril. This packing length is known as the D-period which is ~ 67 nm. The length of the collagen molecule is ~ 300 nm, meaning that a single collagen molecule spans around 4.45 D-periods [122,177]. Since the collagen molecule does not span an integer multiple of the D-period, the D-period is split into two regions with differing collagen density. These two regions are known as the overlap and gap regions. The overlap and gap regions are viewed as areas of dark and light

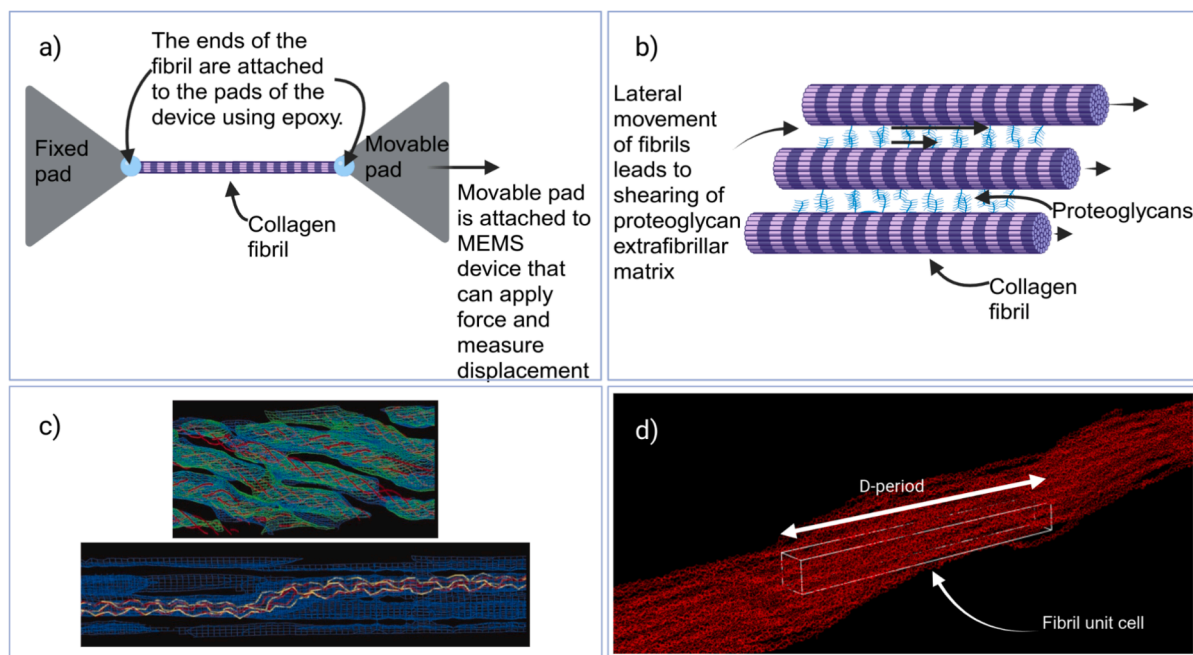


Fig. 5. (a) A schematic demonstration of how microelectromechanical systems can be used to measure mechanical properties of collagen fibrils. Based on the experimental setup of Eppell et al. and Shen et al. [184,185]. (b) Lateral movement of collagen fibrils as the material is pulled leads to shearing of the extracellular matrix. (c) Low resolution electron density maps of the collagen fibril reprinted from [116]. Copyright 2006 National Academy of Sciences. (d) A collagen fibril structure based upon the electron density map shown in (c). This structure can be used as a starting point for molecular dynamics simulations. These simulations are often performed by simulating the unit cell. Mechanical tests can then be performed by deforming the unit cell. Figure created with BioRender.com.

density in transmission electron microscopy (TEM) experiments [178].

Understanding the structural arrangement of the molecules is crucial to fully rationalizing the behavior of the fibrils. As mentioned in section 2.2, high-resolution images of this structure are not possible to obtain via X-ray diffraction. However, Orgel *et al.* were able to obtain a low-resolution trace of the electron density within the collagen fibril unit cell [116]. By fitting high-resolution structural data from collagen mimetic peptides and using the collagen amino acid sequence, a low-resolution model of the molecular layout within the collagen fibril can be devised. The comprehension of the molecular arrangement within the fibril can lead to the understanding of fibril interactions with other proteins and cells [179]. This structure is shown in Fig. 5c.

Fratzl and colleagues have determined the mechanical behavior of collagen fibrils using synchrotron light radiation [180–183]. The regular packing of the D-period leads to distinct Bragg peaks separated by $2\pi/D$ in the short angle scattering pattern. When stress is applied to a specimen (whether that be tendon or bone), the fibrils deform modifying the D-period. This leads to a change in the short-angle scattering pattern, from which the change in the D-period can be measured. This allows estimation of the Young's modulus of the fibril. When observing the scattering pattern from tendons, the regular rise of the amino acids along the length of a collagen molecule further leads to peaks in the wide-angle spectrum. Observing the changes in the wide-angle spectrum therefore allows the deformation and Young's modulus of the collagen molecule alone to be discerned. At low strains, molecular kinks in the gap region are removed, leading to a heel region in the fibril stress strain curve. When these kinks are removed, a linear region in the fibril stress strain curve is observed which is associated with side-by-side gliding of the collagen molecules. This sliding results in a change of the gap-overlap ratio in the fibril. The additional deformation processes involved with the fibril lead to the collagen molecule being stiffer than the collagen fibril. The side-by-side gliding of the molecules leads to significant forces developing in the cross-links connecting the fibrils, which shows the importance of cross-linking on the mechanical behavior of bone and tendon.

Other experimental methods have also investigated the mechanical behavior of collagen fibrils. Eppell *et al.* and Shen *et al.* used microelectromechanical systems (MEMS) to test individual collagen fibrils [184–186]. Shen *et al.* found a dependence of yield strength upon the diameter of the fibril which implies that a consideration of the geometry of the fibril is important if one wishes to model its mechanical properties. The advantage of using MEMS is that the mechanical properties of individual fibrils can be tested. However, the fibrils must be isolated meaning the mechanical properties are being tested in a relatively unnatural environment. In addition, the mechanical role of the extrafibrillar matrix cannot be elucidated.

When considering the mechanical properties of collagen fibrils, it is important to recognise viscoelastic behavior in addition to elastic behavior [135,186,187]. This has been investigated using both synchrotron radiation and MEMS devices. In both experiments, it was shown that collagen fibrils show significant viscoelastic behavior with stress relaxation occurring on the order of a hundred seconds. Molecular dynamics simulations on individual CMPs suggest that a collagen molecule has a relaxation time of a few nanoseconds indicating that the significant relaxation processes occurring in the fibril are due to interactions between the molecules such as intermolecular sliding [135]. This is corroborated by evidence that the viscoelastic behavior depends significantly on the density of cross links between individual molecules [187]. Using synchrotron radiation experiments, Puxkandl *et al.* reported that collagen from rats treated with β -APN (which effects cross-linking) had much lower stiffness and significantly greater viscous behavior than collagen from untreated rats.

Collagen fibril behavior has also been investigated using computational methods [22,175,188,189]. Buehler built a model of the fibrillar structure by packing the coarse-grained molecules derived using the GPO sequence [175]. Mechanical tests were performed *in silico* and several fundamental fibril deformation processes were observed. Two key slip mechanisms were found: homogenous slip and slip via slip-pulses. The force required for homogeneous slip grows linearly with the length of the molecules as the frictional force increases. This means that for longer fibrils slip will primarily occur via the nucleation of slip pulses. A critical length, χ_S can be defined as the length at which slip becomes more favorable via pulses than homogenous slip. Another critical length, χ_R can be defined as the length at which the force required to rupture the molecules is lower than the force required for homogeneous slip. Both χ_S and χ_R depend on the properties of the collagen molecule and the interactions between the collagen molecules. If $\chi_S < \chi_R$ and $L > \chi_S$ (where L is the collagen molecular length) then the molecule will fail via slip-pulses. However, if $\chi_S > \chi_R$ and $L > \chi_R$ then the force required to cause slip, either by homogenous slip or slip-pulses is greater than the force required to rupture the molecule and rupture becomes the primary failure mechanism. If the molecules start to rupture, then catastrophic failure of the fibril will ensue. This consideration of critical length leads to interesting design principles for collagen that nature has upheld.

These models can be extended to investigate the role of cross linking on collagen fibril mechanical properties. Cross linking in collagen fibrils can be largely divided into two separate groups: enzymatic cross links mediated by the enzyme lysyl oxidase and non-enzymatic cross links caused by glycation reactions, often referred to as advanced glycation end products (AGEs) [190–193]. Cross linking between the collagen molecules is crucial for the collagen fibril mechanical behavior as cross link deficient collagen deforms in a viscous manner, as mentioned previously. However, it is understood that increased cross linking is a significant pathogenesis of conditions such as diabetes which causes increased glycation reactions [194,195]. The effect of increased levels of cross linking can be understood using the same framework mentioned above with regards to the nucleation of slip pulses. Increasing numbers of cross links increases the stress required for slip pulses to occur and increases χ_S . This moves the system into the regime where $\chi_R < \chi_S$ and leads to the fibril failing via a catastrophic molecular rupture instead of sliding [22,175]. This causes the fibril to become brittle leading to lower toughness and potentially catastrophic failure of the fibril.

The previously mentioned low-resolution fibril molecular model developed by Orgel *et al.* [116] has been key for further developing computational models of the fibril [196]. In order to reduce time sampling irrelevant conformations, a starting structure near the global free energy minimum is required for molecular dynamics simulations. By utilizing this structure, along with higher resolution information from collagen mimetic peptides, atomistic models of the fibril have been built [188,197]. By utilizing these models, we can begin to understand the fibril behavior at atomistic resolution and gain insight into the complex relationship between the chemistry of

the fibrils and their mechanical behavior. For example, recent simulations have utilized this fibril model to understand how mechanical forces lead to preferential breakage of the bonds in the cross-links between the collagen molecules [198].

Although experimental and computational methods have been applied to collagen fibrils with success, it is important to recognise their various limitations. Recent molecular dynamics models of collagen fibrils have provided atomic-level insights into the fibril behavior. However, the experimental data shows collagen fibrils to have significant viscoelastic behavior with relaxation time scales at many orders of magnitude greater than those accessible by molecular dynamics simulations. This brings into question the validity of atomistic molecular dynamics simulations to provide realistic insights into the mechanisms involved. In addition, the compositional complexity of a biomaterial such as bone means that any computational model represents a simplification of the true system, with high-resolution molecular dynamics models only feasibly being able to test a handful of components at once. This shows the importance of considering multiple sources of both experimental and computational data as all methods have their advantages and disadvantages. It is also important to remember that in tendons, the collagen fibrils are embedded in a proteoglycan matrix which will have a significant impact on how stress is transferred between the fibrils. Only by utilizing multiscale models that integrate data from across the

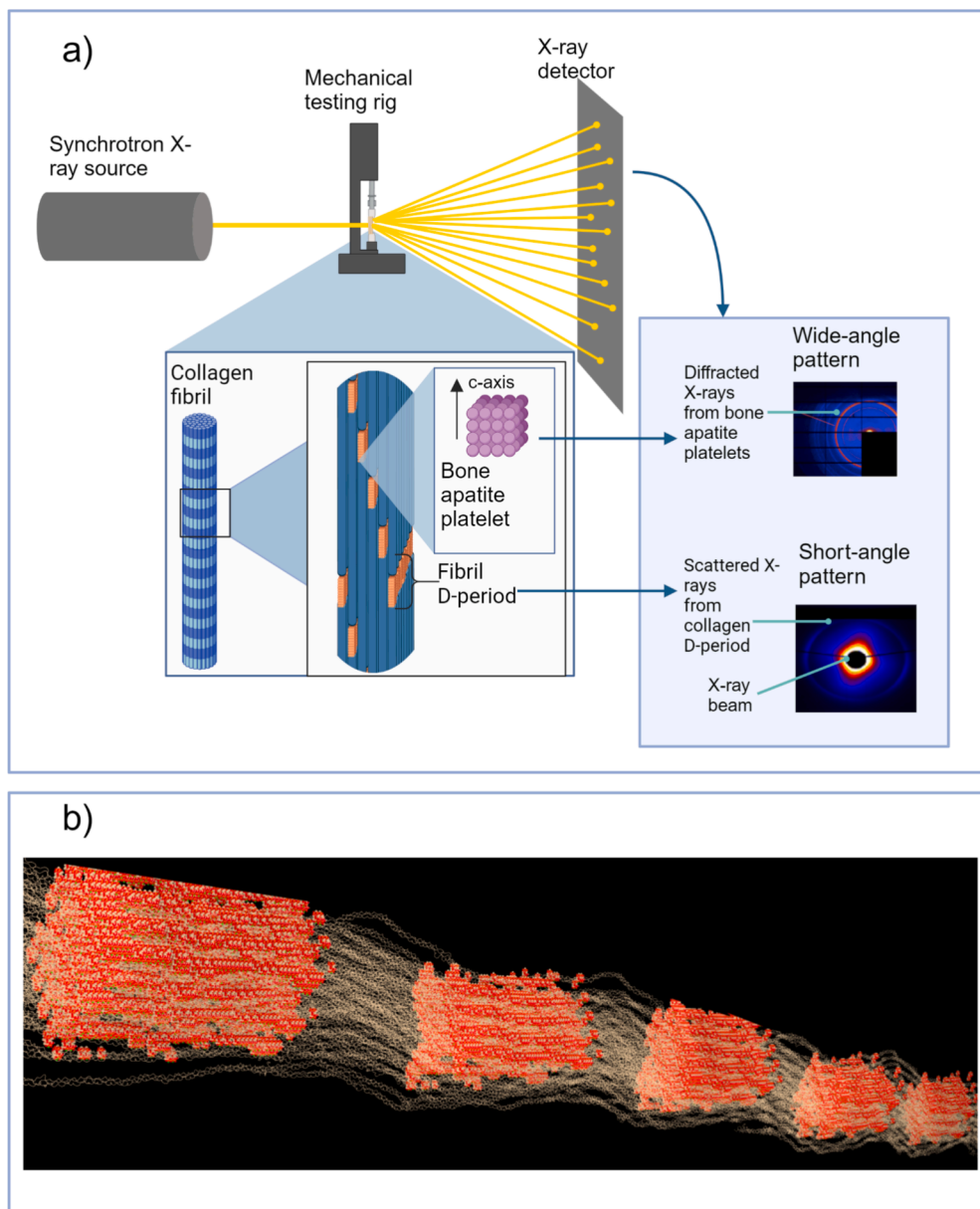


Fig. 6. (a) A demonstration of how synchrotron X-ray scattering can be used to measure fibril strain and mineral strain in bone. (b) A snapshot from an atomistic molecular dynamics simulation where the gap region has been filled with hydroxyapatite unit cells. Figure created with [BioRender.com](https://www.biorender.com/).

hierarchy will the importance of the individual components be understood.

2.5. The mechanical behavior of mineralised fibrils and the extrafibrillar matrix

Although much of the research into tendons can be carried over to bone, the importance of mineralisation on mechanical properties must be recognised. As discussed in [section 2.1](#), mineral structures are found both inside and between collagen fibrils, which are referred to as interfibrillar and extrafibrillar mineral respectively. Recent electron microscope experiments have shown the presence of complex mineral networks composed of mineral platelets [33,41,42,98,199]. These mineral networks weave through the extrafibrillar space, potentially penetrating into the fibrils [2,95].

In much the same way as tendons, X-ray synchrotron experiments can be used to investigate the deformation of mineralised collagen fibrils and the mineral itself (See [Fig. 6](#)) [200–203]. The collagen D-period structure still leads to peaks in the short-angle spectrum, which allows measurement of the deformation of collagen. Mineral deformation can be measured from the wide-angle spectrum where the change in crystal lattice c-axis can be measured as the bone sample is stretched. By correlating the deformation of the mineral with the deformation of the collagen fibrils, the mechanical behavior of each phase can be deduced. The mineral particles are found to strain significantly less than the fibrils and absorb significantly more stress. Gupta et al. have extensively tested bone samples using the synchrotron and have analyzed their data employing different constitutive equations [99,204,205]. They found experimental evidence for significant intrafibrillar plastic deformation caused by collagen-mineral debonding and associated sliding. This implies that the collagen-mineral interaction strength is a critical parameter to understanding the onset of plastic deformation at the nanoscale.

As the bone sample is stretched in the synchrotron, the strain in the whole sample can be measured, allowing the macroscopic tissue level strain to be related to the nanoscopic strain. The tissue level strain is found to be greater than the fibril strain – Gupta et al. measured the ratio of fibril strain to tissue strain to be 0.41 for bovine bone [205]. This implies that there is significant shearing between the fibrils in the extrafibrillar matrix. The chemistry and structure of the extrafibrillar matrix is not well understood but proteoglycans and non-collagenous proteins such as osteopontin and osteocalcin are believed to play significant roles. As discussed in [section 2.1](#), NMR experiments show a significant presence of osteocalcin and osteopontin at the extrafibrillar interface between collagen and mineral [100,102]. As the extrafibrillar matrix is put under shear, weak sacrificial bonds between the non-collagenous proteins and the mineral break and reform, absorbing energy and increasing the fracture toughness of the bone [206–208]. Vashishth and coworkers have proposed an additional key role for osteocalcin and osteopontin in the formation of dilatational bands [101,209]. These are voids approximately 100 nm in size that form due to the disruption of osteocalcin/osteopontin complexes. The formation of these bands results in greater energy dissipation and are a key damage mechanism that results in high bone fracture toughness. The importance of these non-collagenous proteins on bone fracture shows how fundamental physical–chemical processes such as sacrificial bond formation lead to high fracture toughness in the macroscopic material. Despite recent advances improving the understanding of the role of non-collagenous proteins, they still represent a significant challenge due to their nature as intrinsically disordered proteins [210,211]. Intrinsically disordered proteins are proteins that can fulfill multiple functions by folding into a variety of different 3-dimensional structures. This makes understanding the structure of osteocalcin and osteopontin in the extrafibrillar matrix difficult, especially as the binding of the protein to bone mineral may be critical in inducing the relevant folded state. Using molecular dynamics models to understand these structures is an active area of research as the role of mineral binding in stabilizing different folds can be understood [212].

Molecular dynamics models of mineralised fibrils have been built by integrating mineral unit cells into pre-existing collagen fibril models [213,214]. The effect of mineral nanocrystals on mechanical behavior of collagen fibrils has then been studied by deforming such models with different degrees of mineralization. These models have revealed that mineral platelets absorb substantial amounts of applied stress, stopping the collagen molecules from slipping over each other. For instance, the load transfer between collagen and mineral crystals is reduced at high strains (>30 %) and stress is concentrated back into the collagen. Corroborating our expectations, mineral density was found to be a crucial parameter. Fibrils with high mineral density were found to have higher toughness and ultimate strength as compared to fibrils with low mineral density. In particular, fibrils with higher mineral density were found to transition from ductile failure to brittle failure as plastic deformation mechanisms such as intramolecular slip were suppressed. It is important to mention that these models only account for interfibrillar mineralization, while experiments have long shown that up to 80 % of the minerals in bones are found in the extra fibrillar region [41,42,215,216]. However, only recently have molecular models that account for extrafibrillar mineralization been devised and studied [217]. They require a more complex modeling and a larger number of atoms than a microfibril with intrafibrillar mineralization only. Molecular dynamics simulations mimicking tensile tests have shown that minerals at the extrafibrillar region concentrate most stresses [218]. Thus, this can be considered an indispensable feature for future models.

Molecular dynamics models and x-ray synchrotron experiments both provide means for measuring mineral and collagen strains as the bone nanostructure is deformed. In synchrotron experiments, the measured strains are averaged over many fibrils which complicates interpretation, particularly as it is difficult to know how accurately the X-ray beam is centered on areas of the sample where stress and strain are concentrated. In addition, it is difficult to keep samples wet during the experiment and avoid damaging the small (mms to cms) samples during preparation. However, since the experiments use clinically relevant bone samples from humans and animals they incorporate all the complexity that is present in real bones. Due to this complexity, the understanding of the role that other biomolecules such as the non-collagenous proteins may play in the mechanics of mineralised fibrils is still not fully developed. Molecular dynamics models often represent a simplified view of the mineralised collagen fibril, e.g. without osteocalcin and osteopontin; however, more advanced MD simulations, in which individual components and their interactions are more accurately

described, could provide a powerful tool to explore the individual contribution of elements that cannot be easily explored experimentally. Such simulations can be adopted to shed light on the role of the mineral phase in adsorbing shear stresses between collagen molecules. Understanding the sensitivity of the interface to changes in mineral and collagen chemistry is critical to building effective multiscale models that are capable of analyzing a variety of different conditions. As highlighted in [section 2.1](#), this interface is not well understood and is likely to be modulated by other biomolecules such as non-collagenous proteins or proteoglycans. A more thorough understanding of the chemistry and structure at this level is vital to fully understand the macro fracture mechanics of bone.

2.6. The role of the nano- and microstructures in toughening bone

While strength is important for bearing loads, it is toughness that ultimately determines a material's ability to resist fracture [219–221]. A bone fracture occurs when there is a partial or complete break in the continuity of the bone, meaning a crack initiates and propagates, ultimately leading to structural failure and a breaking of the bone into two or more pieces. While external loads generate stresses that promote crack growth, the material's geometry and properties work to resist crack formation. This resistance is measured as toughness, which refers to a material's ability to absorb energy before fracturing.

Toughness is a balance between strength (the ability to withstand high stresses) and ductility (the ability to undergo plastic deformation before fracture). Toughness can also be represented as the area under a stress–strain curve—the larger and more extended the curve, the tougher the material. Toughening mechanisms are structural features, dependent on the constituents and their arrangement typically at the nano- and microscales [220,222,223], that help resist fractures by absorbing energy from external loads.

Broadly, materials can resist fracture through various toughening mechanisms that operate at different length scales. Toughening mechanisms can be categorized as intrinsic or extrinsic [224,225]. Intrinsic toughening mechanisms, commonly found in ductile materials, alter the material's inherent properties, regardless of its size. In contrast, extrinsic toughening mechanisms, typical of brittle ceramics, composites, and laminates, work to reduce the forces driving crack propagation by shielding or mitigating the crack. The differences between intrinsic and extrinsic mechanisms are described in [Table 1](#).

The toughness of bone originates from the complex hierarchical organization of its components. This multiscale structural design enables bone to effectively withstand mechanical loads while resisting fracture [219,227]. For instance, fracture resistance is achieved in bone by creating local damage in response to high loads/impacts. By creating and controlling this damage, the energy of the impact is absorbed, reducing the chance of critical failure. Importantly, bone is a living structure and so is able to repair damage [228]. Hence, damage does not pose a big problem as, given sufficient time, the damage will simply be repaired. The requirement for repair highlights a problem if pathology or other conditions, such as modified cellular activity due to hormonal changes [229], reduce bone's ability to repair. In this case, the bone will be full of pre-existing damage and vulnerable to subsequent impacts.

Notably, the many individual components that make up bone at the nano and microscales promote many interfaces, which can stop or deflect cracks before they reach a critical size. It seems that bone has evolved to optimize the strength of these interfaces, to increase the overall bone fracture toughness. By optimizing these interfaces, bone has obtained a structure of sufficient stiffness and strength to perform day-to-day tasks, but with interfaces that are sufficiently weak to absorb impact energy and blunt or deflect incoming cracks. A notable example of this optimization is antler bone, where the interfaces are weaker than in skeletal bone. Antler bone has developed under evolutionary pressure to sustain impacts. By breaking the many microscale interfaces the energy of the impact can be absorbed. Hence, antler bone becomes damaged but not broken. Accordingly, antler bone has lower material stiffness than standard bone but higher toughness [230].

The fibrous structure of bone (osteonal fibers on a larger scale and fibrils at a smaller scale) provides other types of damage-control mechanisms. One of these mechanisms is the so-called 'fiber pull-out' mechanism, which involves pulling many fibers out from the material surrounding them [231]. This process entails not just breaking the interfacial bond of the fibril and the extrafibrillar matrix, but also overcoming friction type resistance when pulling the fibril free of the surrounding material. Another fibrous damage-control mechanism is 'fiber-bridging' [225,231]. In this mechanism, energy from loading is absorbed by crack formation, but in addition, unbroken fibers span the crack surfaces preventing the crack from opening up any further. This stops the crack from progressing to critical levels.

Table 1

Comparison of Intrinsic and Extrinsic Toughening Mechanisms (crack initiation and crack opening are synonymous, as are crack growth and crack propagation) [219–221,225,226].

Characteristic	Intrinsic toughening mechanism	Extrinsic toughening mechanism
Common in Location	Ductile materials. At or ahead of the crack tip, at the nano and submicroscale.	Brittle materials. Behind or around the crack tip, interacting with the crack at the microscale and higher scales.
How it enhances toughness	Modifies material's intrinsic properties (driven by plasticity).	Shields or mitigates crack propagation.
Functionality	Act against crack initiation and propagation.	Act against crack propagation only (requires a pre-existing crack).
Dependency on crack size	Independent of crack length or geometry	Dependent on crack size and geometry
Examples in bone	Collagen uncoiling and stretching, interfacial interactions (breaking of cross links and sacrificial bonds, and mineral-collagen sliding)	Crack bridging by collagen fibrils, microcracking, crack deflection and twist in osteons cement line.

Often the widespread damage observed at the microstructural level is described as ‘micro-cracking’ or ‘diffuse damage’ and typically identified using staining techniques [232]. These microstructural observations typically encompass the earlier described mechanisms of interface failures, fiber pull-out and fiber-bridging. Diffuse damage can often be caused by microvoids which show up at the microscale when filled with stain. These microvoids could be formed by coalescence of ‘holes’ caused by collagen molecules straightening, slipping and shearing at the nanoscale, in complicated concert with the interdigitated mineral. As mentioned in section 2.5, non-collagenous protein complexes can also form voids when they are disrupted during loading [102]. Whatever the underlying mechanism leading to micro-cracking or diffuse damage, if bone can respond to impacts with significant levels of micro-damage, it will absorb more energy and avoid fracture (see Fig. 7).

Degradation in the effectiveness of toughening mechanisms due to aging and diseases such as osteoporosis, make bone more fragile and prone to fracture [220,223,233,234]. Given the critical role of toughening mechanisms in maintaining bone integrity, understanding the nano- and microscale origin of the deterioration of these mechanisms is vital. For instance, the increased incidence of bone fractures with age is partly due to a decline in bone tissue toughness, partially caused by the deterioration of the underlying collagen network [235–238]. Zimmerman et al. demonstrated that increased non-enzymatic collagen cross-linking leads to reduced fracture toughness, due to suppressed plasticity at the nanoscale [239]. They also found that increased numbers of osteons weakens crack-bridging mechanisms at the microscale, further minimizing the ability of bone to resist fracture [239]. Aging can also lead to increased tissue mineralization in certain regions of bone, accompanied by a reduction in mineralization differences between osteons and cement lines [240,241]. Importantly, research suggests that the heterogeneous distribution of mineralization is more critical to bone toughness than the overall degree of mineralization itself [241].

The accumulation of advanced glycation end products (AGEs) is one key mechanism by which collagen is modified by aging [237]. As mentioned in section 2.4, AGEs can lead to changes in the cross-linking between collagen molecules as well as changes in the packing of the fibrils [242,243]. By modifying the mechanical properties of the fibrils, the dissipation of energy at the nanoscale is reduced [22]. Type 2 diabetes mellitus exacerbates this process by inducing hyperglycemia, which promotes AGE accumulation and disrupts bone remodeling, ultimately reducing bone toughness [244–247]. While there is strong *in vitro* evidence that AGEs reduce bone toughness, *in vivo* evidence of AGE accumulation remains limited [248].

As discussed in sections 2.1 and 2.2, water plays a key role in the structure of both the collagen molecules and the mineral crystals as well as the interface between the two phases. Recent research has found that aging is correlated with a decrease in bound water within bone [249–252]. This water loss leads to lower bone toughness due to diminished intrinsic toughening mechanisms [250,253,254]. There are likely many nano- and microscale origins for loss of macroscopic toughness caused by dehydration. For example, dehydration of the collagen fibrils will lead to reduced sliding between the collagen molecules leading to reduced energy dissipation. In addition, water is critical to the structure of the mineral crystals, so dehydration will likely lead to them becoming more brittle [67].

The study of bone toughening mechanisms is fundamental to understanding bone biomechanics, as they determine its mechanical

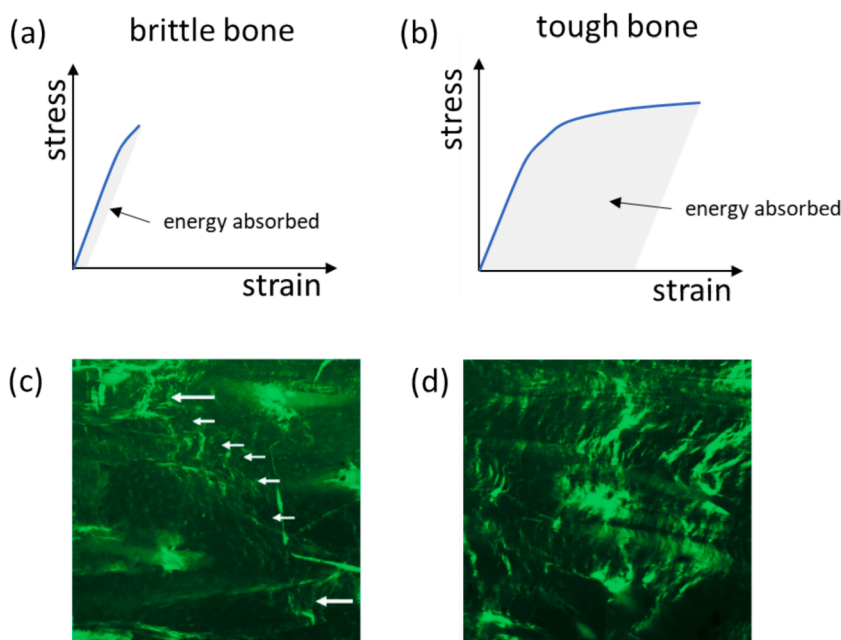


Fig. 7. Top row: illustrative stress–strain graph of bone material behavior. (a) Relatively brittle bone behavior. (b) Fracture resistant bone behavior. Size of the area under the curve (gray area) indicates the amount of energy the bone can absorb. Bottom row: fluorescein stain laser confocal images, field of view 900 μm . (c) low levels of diffuse damage associated with the relatively brittle behavior. Arrows indicate diffuse damage. (d) High levels of diffuse damage associated with fracture resistant behavior. Figure reprinted from Zioupos et al. [232], with permission from Elsevier.

fracture behavior [255]. Experimentally isolating the underlying mechanisms is challenging, if not impossible because the toughness of real bone samples is dictated by simultaneous extrinsic and intrinsic mechanisms at a variety of scales [219]. Therefore, researchers must integrate mathematical models and computational techniques to identify, isolate, and quantify individual toughening mechanisms more precisely and how they work in concert to allow bone to resist fracture.

For example, recent studies have utilized a joint experimental-computational approach to reveal the most critical microscale features of cortical bone [234]. They examined how these features were degraded by pathological processes and restored by pharmacological treatments. Thereby, improving our understanding of how microstructure and material properties influence fracture resistance. This joint experimental-computational approach can be applied to study many other clinical needs such as improving and optimizing bone implant design or developing surgical approaches to prevent fractures during surgery that cause the implant to become loose [256,257].

Another key approach to characterizing bone's fracture behavior is plotting fracture growth resistance curves (R-curves). Fracture toughness increases as the crack size grows, and experimental methods can significantly impact these measurements [226,258]. Since bone tissue exhibits a rising fracture resistance with crack extension, toughness should be evaluated as a function of crack growth rather than as a single value [226,259,260].

Concisely, to restore the behavior of diseased or aged bone from fragile to fracture resistant, it will be necessary to first understand what mechanisms lead to these different behaviors and subsequently understand how these mechanisms can be manipulated. Some of these mechanisms may be explained from micro level considerations (for example lacunae or cracks acting as stress raisers and sites of failure initiation [261]) and investigated using experiments at this scale and continuum models as described in the next section. However, if the mechanism underlying diffuse damage shown in Fig. 7 stems from collagen molecules straightening and slipping, then this understanding will need to be investigated at the nanoscale. By combining experimental and computational multiscale approaches to propagate this information from the nanoscale to the microscale, an understanding can be built about how nanoscale processes lead to damage at the microscale. Finally, once bone material behavior at the microscale has been established and understood, this behavior can be fed into the continuum models described in the next section to describe bone fracture at the macroscale.

2.7. Multiscale modelling of bone through solid continuum mechanics

Progressing from the microscale and up to the macroscale, the mechanical behavior of larger segments of bone tissue can be understood through the use of solid continuum mechanics and bulk mechanical testing. At the microscale, the mechanical properties of bone tissue can be experimentally tested through the use of e.g. indentation techniques. Macroscopic segments of bone can be mechanically tested in 3-point bending, tension and compression using single or cyclical loads [262,263]. Continuum mechanics-based methods can supplement these experimental tests by allowing the stress distribution through the material to be calculated using methods that consider the microstructure of the tissue either explicitly (microstructure-based approaches) or implicitly (using homogenisation techniques).

Solid continuum mechanics, commonly used to model bone tissue at the macro- and microscales, is a prevalent and well-established physics and mathematics-based theory for the description of deformation and failure processes [223,264–266]. It consists of solving partial differential equations and assuming a material to be a continuum, i.e., that there is no vacuum in the spatial domain. Simulations based on continuum mechanics allow the study of complex material behaviors that cannot be observed through simple experiments like tensile and compressive tests. Yet, simulations require several assumptions and information that rely on such basic experiments, which often result in the formulation of constitutive equations that can be used to describe the tissue response in an average sense. The symmetry, inhomogeneity, and constitutive behavior of the material (elastic, plastic, viscoelastic, etc), as well as some basic properties like Young's Modulus (or general elasticity tensor), toughness, and ultimate tensile strength, must be fed into continuum mechanics models for the simulation to estimate more complex behaviors. Basic mechanical properties are usually assessed from tensile and compressive tests of small samples of the bone. Symmetry and inhomogeneity are usually collected from a combination of mechanical tests and imaging techniques like CT, μ CTs, and microscopy. Several numerical methods have been developed on the basis of continuum mechanics. The most successful include the Finite Element Method (FEM) and the Boundary Element Method (BEM).

Once basic material assumptions are made, boundary conditions that describe the interactions between the domain of the body and the exterior domain through the boundary are defined and applied. Boundary conditions act as a translation of experiments or models representative of common physical trauma, e.g., compression test or a fall, respectively, into equations describing loading or constraint conditions [267–269]. These equations serve as input to a set of governing equations that describe strain and stress fields in the body. Boundary conditions require a predefined geometry which, at the macroscale, is most commonly acquired through non-destructive and non-intrusive experiments such as CT scans [270,271]. This is an advantage, for bones are patient-specific, i.e., each bone is unique in its structure and properties. However, for specific cases where *in vivo* experiments cannot reach the required resolution, the geometry is acquired from observations of invasive and destructive experiments such as synchrotron micro-CT, nano-CT, or even microscopy [20,272–275]. These high-resolution imaging techniques can capture the structure of osteons, pores, and even the propagation of submicron cracks during loading [276].

Although continuum mechanics-based methods have been mostly applied for macroscale models, they have also been used to capture the mechanical properties and behavior of bone at the micro- and even lower scales. For instance, the FEM has been used to simulate nanoindentation in an elastic-viscoplastic damage constitutive model of lamellar bone [277]; and to model mineralized collagen fibrils to study the mechanical properties of the tendon-to-bone attachment [278,279]. The material properties derived from smaller scale models and experiments serve as input for microscale FEM models, allowing the transfer of smaller scale information up

the bone hierarchy. For example, the moduli of the collagen molecules, collagen fibrils and mineral crystals, which can be determined via molecular dynamics or synchrotron experiments, can be used to build FEM models of collections of collagen fibrils and bone lamellae [21,280]. Examples of these models are shown in Fig. 1. Hellmich's group presents micromechanical representations of the bone ultrastructure based on "universal" organizational patterns and mechanical properties of the building blocks of bone (collagen, mineral and water). They published several works on multiscale modeling of bone through micromechanics, a branch of continuum mechanics [281–283]. Starting from invariant mechanical properties at sufficiently small length scales (at the collagen level), they assess the mechanical behavior of the material at large scales. A recent review by Hellmich et. al discusses in detail their bone multiscale modeling approach through micromechanics [284]. Their work has inspired other researchers to predict elastic properties of bone matrix using continuum mechanics-based methods to integrate micro and nano- experimental data [285].

Tools based on the finite element method and that use CT-data as an input can provide more accurate bone mineral density and strength predictions than standard clinical scans like dual-energy X-ray absorptiometry [286,287]. For instance, the freeware Bonemat maps elastic properties derived from CT-images onto a finite element mesh [271,288,289]. Bonemat's 4.0 update has shown that cortical bone mapping models provide accurate strain predictions to develop biofidelic failure criteria [290]. Another example is the modeling approach proposed by Risvas et al. [291] that provides a finite-element based tool that can help orthopedic surgeons make better decisions. Several other FEM-tools have been developed for different bone-related applications including: hip fracture risk assessment [292], cost-effective osteoporosis screening tools for secondary fracture prevention [293], statistical predictive modeling of trabecular bone microarchitecture [294], and investigation of the effects of intentional weight loss on bone health [295]. As an example, Fig. 8 shows a finite element model of a proximal femur used to predict bone strength in a single-leg stance condition.

Through continuum mechanics methods, models can be built based on experimental bulk mechanical testing, and used for high-throughput computational simulations that show how stress is distributed throughout the tissue [297]. Recently, regulatory agencies started receiving and accepting evidence obtained *in silico* for clinical trials and treatment, i.e., results from FE-based modeling and simulation [298,299]. For instance, the University of Sheffield and Sheffield Teaching Hospitals teamed up to develop and test a computational tool that estimates bone strength through FEM [270]. This method, especially when enriched by better integration with information obtained from the lower scales (as discussed further in Section 3.2), has the potential to provide additional quantitative data on bone health, beyond just mass or mineral density, as well as providing patient-specific predictive capabilities in terms of likelihood of fractures to occur. This is a perfect example of how experimental and computational works are teaming up to improve osteoporosis diagnosis and treatment. For further details on how to model bone as a solid continuum, see ref. [223,264–266].

3. Discussion

3.1. From nanoscale damage to macroscale failure

This paper has reviewed the findings from experimental and computational studies to provide the current understanding of bone structure and mechanics at the level of bone apatite, collagen molecules, collagen fibrils and mineralised fibrils up to the whole bone. In this next section, we will combine this knowledge to provide insight into how a bone fracture begins at the nanoscale and then

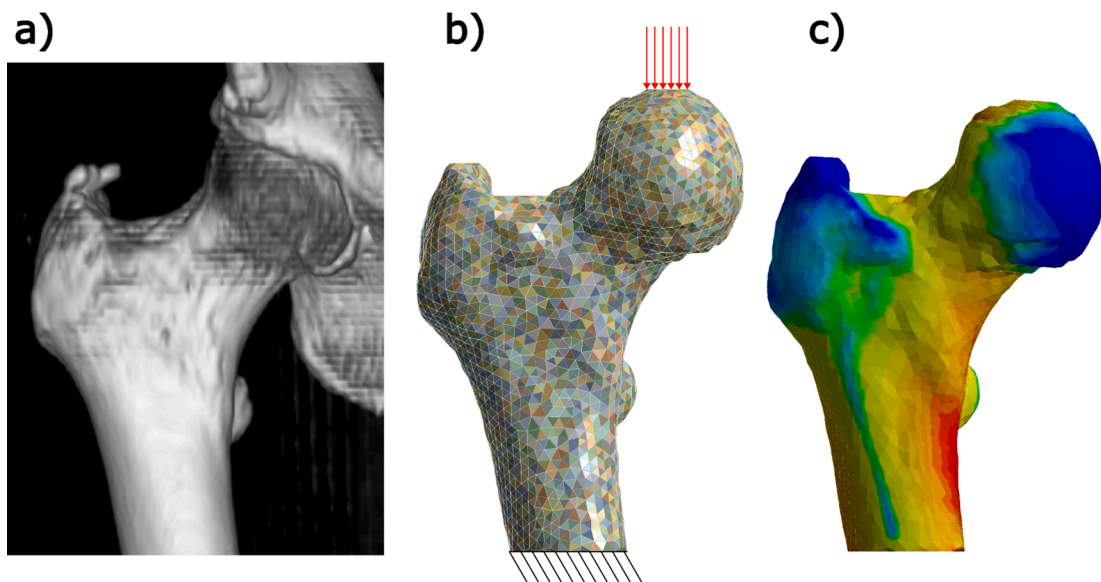


Fig. 8. Example of a continuum model of a proximal femur. a) 3D CT reconstruction; b) inhomogeneous FE-mesh and applied boundary conditions; c) stress distribution analysis. This model was created using the CT-scan provided by Bonemat [296].

propagates through the hierarchy up to the macroscale. We will also describe how the intricate hierarchical structure spanning these multiple scales gives bone its remarkable strength and toughness.

Macroscale fractures in bone originate from damage initiation at the nanoscale, particularly at the interfaces within and between the mineral and collagen phases. When an external load is applied to the bone at the macroscale, stress is transmitted down through its hierarchical structure (Fig. 1), subjecting the individual mineral crystals, collagen molecules, and their interfaces to stress concentrations. These stress concentrations can lead to plastic deformation by initiating sliding and shearing at the nanoscale interfaces in bone (Fig. 7).

Experimental techniques such as NMR and Raman spectroscopy, along with atomic resolution computational methods such as DFT calculations, have been particularly useful in elucidating the atomic structure of bones [50,71,105]. Recent studies have highlighted the importance of water and other ions such as citrate in the formation of a disordered layer between the mineral platelets. The disordered, hydrated surface layers between the apatite nanocrystals play a critical role in preventing brittle nano-fracture by allowing the crystals to deform and slide relative to one another (Fig. 2). However, it is not well understood what role disease and aging play in the formation of these layers. For instance, the loss or introduction of ions such as citrate and lactate modifies the structure of the layers [300]. In addition, loss of bound water reduces the occurrence of these layers [66]. Qualitatively, there is a good understanding of this nanoscale toughening mechanism, but quantitative data on this process is lacking, including strain and energy dissipation, because it has not been possible to image the disordered layers of mineral platelets under load. Novel experimental and modeling work is required to understand what factors control the structure and layering of the crystals and how any changes related to disease modify their ability to dissipate energy.

When the mineral crystals are put under load, shear stresses are transferred to the collagen fibrils putting the collagen molecules under stress [99]. The mechanical behavior of individual collagen molecules has been investigated using AFM and optical tweezers experiments (Fig. 4), combined with MD simulations [166,170,176]. When loaded, collagen molecules strain and uncoil, dissipating energy. In addition, they slide over each other further expending energy. Ultimately, at high stresses, the collagen molecules, or more likely the cross-links between them, can even start to rupture [301]. The combination of uncoiling, sliding and rupture mechanisms lead to nanoscale damage within the collagen fibrils [205,214]. Besides dissipating energy, collagen also plays a key role in allowing the mineral to resist damage by putting the mineral crystals under compressive stresses, a similar role to steel in reinforced concrete, where the ability of the brittle phase to withstand tension is improved by initially putting it under compression [145]. Collagen also plays a key role in providing a template for the formation of the mineral crystals. If this template is modified, the morphology of the crystals changes (Fig. 3), which compromises the fracture toughness of the mineral as discussed above. Further research needs to be done to understand how mutations in the collagen sequence and interactions with other molecules such as water affect collagens mechanical properties, and its ability to template and pre-stress the mineral component.

At the onset of fracture at the nanoscale, the mineral crystals and collagen molecules start to slide and separate, both within the mineralised fibrils and at the interface between fibrils and extracellular matrix (Fig. 5b). Sliding and separation between the mineral crystals and the collagen matrix absorbs energy and reduces the efficiency of load transfer between the two phases. NMR studies have reported an abundance of non-collagenous proteins (i.e., osteocalcin and osteopontin) at the interface between the collagen fibrils and the extracellular mineral crystals [100,102]. MD simulations along with microscopy experiments suggest that these proteins resist separation and absorb energy by breaking and unfolding the non-collagenous protein complexes and breaking sacrificial bonds [212,302]. As the loading increases, these sacrificial bonds will become overwhelmed, leading to the initiation of damage at the interface with sliding and separation between the phases. These damage mechanisms ultimately lead to the formation of voids. The absorption of energy via these mechanisms suggests that mineral-collagen separation is a key damage mechanism that leads to high bone fracture toughness. As a result, understanding the structure of the mineral-collagen interface is important to rationalizing the fracture toughness of bone. Yet the chemical structure of the interface is not fully known, nor the sensitivity of these fracture toughening mechanisms to changes in mineral composition or hydration. Furthermore, the structure of these non-collagenous protein complexes and how they may be modified by disease is yet unknown. The small scale of the components, as well as the sensitivity to hydration, means that imaging this interface is very difficult. Computational techniques such as DFT and MD simulations allow the modeling and simulation of this interface in hydrated conditions. In addition, the intrinsic disorder and nanoscopic size of the non-collagenous proteins makes establishing their role in fracture toughness difficult.

Separation at the nanoscale interfaces will initiate the formation of voids which coalesce at the microscale, giving rise to diffuse damage and microcracks (Fig. 7). As the microcracks accumulate, they can propagate through the bone tissue and coalesce into a complete fracture of a bone. Microscopy and micro-CT allow the visualization of the damage and the microcracks [232]. However, it has not been possible to simultaneously link this damage at the microscale to the underlying nanoscale processes. Advances in nano-CT imaging will allow the observation of the progression from nanoscale damage to voids, diffuse damage, and cracks under in situ loading.

In summary, the combination of experimental techniques and computational modeling have played a significant role in furthering our understanding of bone's nanostructure toughening mechanisms. Simulations allow us to bridge the gap between experimental observations and provide a theoretical understanding of the various processes, enabling us to predict and visualize the fracture process at different scales (Fig. 1). Macroscale fractures in bone are the culmination of a complex, multiscale process that begins with damage initiation at the nanoscale, involving the collagen, mineral crystals, and their interfaces. Damage accumulates and propagates across increasing length scales, leading to the formation of diffuse damage, microcracks, and eventually, macroscale fractures. Understanding this multiscale progression of fracture in bone is essential for developing strategies to predict and prevent bone fractures, as well as for designing biomimetic materials capable of mimicking bones remarkable strength and toughness. Therefore, it is essential that multidisciplinary teams of both experimentalists and modeling experts work together to fill the gaps in knowledge highlighted above.

3.2. Integrating experimental and simulation approaches to bridge bone hierarchy

To address the knowledge gaps mentioned in the previous section, the development of multiscale models that seamlessly integrate nanoscale processes with micro and macroscale mechanical behavior is crucial. While continuum mechanics models have successfully captured the macroscale response of bone, incorporating molecular-level insights, such as the role of non-collagenous proteins or the viscoelastic properties of collagen fibrils, could lead to more insightful simulations [101,186]. However, bridging the nano-to-macro scale across multiple orders of magnitude and numerous hierarchical levels of bone structure remains a formidable challenge, requiring novel computational approaches and close integration with novel experimental data.

Physics-based and data-driven methods will both have a significant role to play in solving this problem. Physics-based approaches build models from first principles and are capable of providing significant insights into the mechanisms that contribute to bones remarkable material properties. However, these methods are often limited in their scope and struggle to reach experimentally relevant scales (see Fig. 1). Data-driven approaches aim to collect large datasets in order to identify the key structural variables that lead to significant changes in the material properties [263,303,304]. A fusing of physics-based and data-driven methods, whereby the established and newly-acquired physics understanding is expertly used to distill the information held by often heterogeneous datasets, will be crucial to fully understand the role of nano and microstructure on bone macroscopic material properties. This fusion of physics-based and data-driven approaches is more relevant than ever in the age of machine learning, as is discussed in the next section.

Experimentalists and modelers need to collaborate closely to design experiments that provide the necessary data for building, fitting, and validating multiscale models of bone structure and mechanics. These experiments include high-resolution imaging techniques that are capable of capturing the smallest structures of bone, and mechanical testing methods that measure properties like viscoelasticity and fracture toughness at different scales. Experimentalists need to design mechanical testing protocols that can capture the key deformation mechanisms and failure modes predicted by simulations, allowing for direct validation of model predictions. Targeted experiments using techniques such as genetic knockout animal models with advanced imaging methods can provide new insights into specific mechanisms, guiding the development of refined computational models that incorporate these findings.

The integrated approach, combining experimental data-driven model development, computational testing of experimental hypotheses, rigorous model validation, and iterative model refinement, will be essential for filling the critical knowledge gaps in our understanding of bone mechanics and hierarchical structure. By continuing to leverage the strengths of both experiments and simulations, researchers can develop an ever more comprehensive and predictive framework for understanding bone fracture and guiding the development of new diagnostic and therapeutic strategies. New developments in machine learning can improve and accelerate our ability to integrate data from across different methods and scales.

3.3. Utilizing machine learning to accelerate bone research

Machine learning techniques have great promise in accelerating bone research by allowing us to extract more information from the literature. Specifically, recent advances in multimodal transformer architectures have shown particular adeptness at mining experimental data [305]. Because machine learning algorithms often perform well at discerning nonlinear relationships, they have shown significant potential in extracting information from large or complex datasets [133,306]. These methods have uses both in the realm of fundamental bone research and clinical diagnosis of bone disease. Machine learning techniques have already been applied to more manageable problems in bone research. These include identifying the textural features of bone from radiological images that are indicative of osteoporosis and osteopenia [307], predicting the mechanics of trabecular bone surface remodeling with osteoporotic onset [308] and fracture detection from X-Ray and MRI scans [309]. In this review, we have highlighted the importance of understanding the nanoscale in rationalizing bone fracture. New machine learning models which can incorporate nanostructural variables (such as the non-collagenous proteins) could have great potential as new diagnosis tools. Obtaining large, high quality data sets that are capable of linking smaller scale changes to macroscopic material properties is particularly challenging, due to the high cost and difficulty in experimenting at this level. This is further exacerbated by the large number of structural changes that can occur simultaneously across the bone hierarchy.

Physics-based machine learning methods show great potential in improving the effectiveness of computer models [310]. In particular, machine learning demonstrates potential in bridging the gaps between typical scales of modeling. For example, the force fields required to accurately describe biological or biochemical molecules in molecular dynamics are largely underdeveloped, especially compared to the modeling of more conventional materials such as metals [311]. By serving as universal approximators, neural networks have demonstrated the ability to predict the energy and force parameters for novel biomolecules, circumventing the need for complex or expensive quantum mechanical calculations [113,312]. By allowing the transfer of high accuracy information from models at smaller length scales to models at larger length scales, the role that these smaller length scales play can be more effectively realized. In similar fashion, unsupervised learning algorithms trained on simulation data can learn the most significant dynamical modes, which could allow crucial longer time scale biological processes, such as collagen fibril relaxation, to be captured [313–315]. In Fig. 1, the time and length scales that each simulation method can capture is shown. By employing machine learning techniques, these length and time scales can be broadened, increasing the power of these techniques and providing insight that has not previously been possible.

Finally, machine learning shows particular potential for de novo drug and materials design. Generative models have been proposed to design novel materials or geometries for bone therapeutics and engineering [316–318]. Using data-driven models for design can harness the underlying engineering principles of the nanostructure for more effective treatments and biomimetic materials.

3.4. Clinical implications: Translating multiscale research into improved patient care

Better collaboration across both experimental and modeling communities will deliver new research into multiscale bone mechanics which has the potential to significantly improve patient care, treatment outcomes, and quality of life. A key area where multiscale research can make a significant impact is in the early diagnosis and monitoring of bone diseases. By integrating experimental techniques like high-resolution imaging and nanomechanical testing with computational models that can predict bone mechanical behavior across scales, researchers can develop more sensitive and specific diagnostic tools that detect changes in bone tissue during the initial stages of disease. For example, a multiscale model that incorporates patient-specific data on bone microarchitecture, collagen-mineral interactions, and bone remodeling could provide a more comprehensive assessment of osteoporotic fracture risk than current methods based on bone mineral density (i.e. DEXA based BMD) and clinical risk factors like age (i.e. FRAX and FRAX Plus). Earlier and more accurate diagnosis would enable clinicians to start prescribing supplements or therapies at an earlier stage, which has been shown to reduce the incidence of fractures and improve patient outcomes.

Multiscale research could also inform the development of new targeted therapies for bone disease by identifying key mechanisms that contribute to bone fragility at different length scales. For instance, a better understanding of the role of non-collagenous proteins in mediating the mineral-collagen interface could lead to the development of therapies that specifically target these proteins to improve bone toughness. Similarly, insights into the nanoscale origins of microscale damage could guide the design of new materials or surface treatments that can mimic bone's natural toughening mechanisms, such as crack deflection or bridging. By targeting specific mechanisms at different scales, these therapies could be used instead of or as an adjunct to current treatments that focus on increasing bone mass alone (e.g. bisphosphonate, denosumab, romosozumab).

In addition to improving diagnosis and treatment, multiscale research could inform the development of personalized preventive strategies for bone health. By integrating patient-specific data on bone mechanics, genetics, and lifestyle factors into multiscale models, researchers could predict an individual's risk of developing bone disease and recommend tailored interventions to reduce this risk. For example, a model that incorporates data on a patient's genetic predisposition to low bone strength and fracture resistance, their level of physical activity, and their dietary habits could suggest specific exercise regimens or nutritional supplements that could optimize their bone health. Personalized prevention could reduce the incidence of bone fractures and thereby improve patients' overall quality of life by enabling them to maintain their mobility and independence as they age.

Finally, multiscale research could have important implications for the design of biomimetic materials and devices for bone regeneration and repair. By understanding the hierarchical structure and mechanical properties of bone across scales, researchers could develop scaffolds or implants that more closely mimic the natural properties of bone, promoting better integration with the surrounding tissue and reducing the risk of implant failure. For example, a scaffold that incorporates a gradient of mineral content and porosity, similar to the gradient found in natural bone, could provide optimal mechanical support while also promoting bone ingrowth and vascularization. Improved biomaterials and devices could not only enhance the success of orthopedic surgeries and implant lifespan but also improve patients' long-term functional outcomes and quality of life.

There are some barriers that need to be overcome when translating mechanistic insights from experiments and models into clinical applications. These include the complexity and heterogeneity of bone tissue across individuals. Addressing this issue is difficult because of the limited availability of high-resolution patient-specific data. This is due to the expensive and time-consuming nature of advanced imaging techniques. Clinical barriers include the difficulty in adapting complex computational models for clinical use where real-time results and user-friendly interfaces are essential. Clinicians would benefit from clear guidance to help them traverse the inherent uncertainties and assumptions involved in computational modeling that may limit the accuracy and reliability of model predictions when applied to individual patients. These barriers can all be traversed with time and coordinated research which shares data among a global community of scientists.

CRediT authorship contribution statement

James Rowe: Writing – review & editing, Writing – original draft, Conceptualization. **Sabrina Shen:** Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization. **Amadeus C.S. de Alcântara:** Writing – review & editing, Writing – original draft. **Munir S. Skaf:** Writing – review & editing, Supervision. **Daniele Dini:** Writing – review & editing, Writing – original draft, Supervision. **Nicholas M. Harrison:** Writing – review & editing, Supervision. **Ulrich Hansen:** Writing – review & editing, Writing – original draft, Supervision. **Markus J. Buehler:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Conceptualization. **Richard L. Abel:** Writing – review & editing, Writing – original draft, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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