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BIOMECHANICS (J NYMAN AND C HERNANDEZ, SECTION EDITORS)

Poor bone matrix quality: What can be done about it?

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Abstract

Purpose of the review Bone's ability to withstand load resisting fracture and adapting to it highly depends on the quality of its matrix and its regulators. This review focuses on the contribution of bone quality to fracture resistance and possible therapeutic targets for skeletal fragility in aging and disease.

Recent findings The highly organized, hierarchical composite structure of bone extracellular matrix together with its (re)modeling mechanisms and microdamage dynamics determines its stiffness, strength, and toughness. Aging and disease affect the biological processes regulating bone quality, thus resulting in defective extracellular matrix and bone fragility. Targeted therapies are being developed to restore bone's mechanical integrity. However, their current limitations include low tissue selectivity and adverse side effects.

Summary Biological and mechanical insights into the mechanisms controlling bone quality, together with advances in drug delivery and studies in animal models, will accelerate the development and translation to clinical application of effective targeted-therapeutics for bone fragility.

Keywords bone fragility · aging · disease · collagen · mineral · therapy

Introduction

Healthy bone is strong (resists inelastic deformations) and tough (resists crack propagations), and is able to adapt its architecture in response to the applied loads. Bone's unique material properties derive from its highly organized, hierarchical composite structure. Its components, primarily collagen and hydroxyapatite, but also non-collagenous proteins (NCPs) and water, are arranged at multiple length scales, and together with bone tissue (re)modeling dynamics and microdamage mechanisms, confer bone its ability to withstand loads without deformations and fractures, and to adapt to its mechanical environment. Aging, disease, and abnormal loading conditions on bone alter its composition and disrupt its hierarchical structure, changing the mechanical environment on it and increasing bone's vulnerability to fractures and deformities. Being able to directly target bone defects with therapies is critical for the development of effective treatments to prevent fractures in skeletal diseases and disorders. This article focuses on the contribution of bone matrix to resist fracture and highlights current and possible bone therapeutics targets.

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Hierarchical organization of bone and its contribution to toughness

Bone dynamically adapts its shape and structure in response to the applied loads. This mechano-adaptation process is central in maintaining bone's mechanical integrity and its ability to withstand loads, which differ with age and sex [1]. Within human bone, the matrix is organized in osteons running parallel to its longitudinal axis. Each osteon contains a central blood vessel surrounded by concentric layers of bone, the lamellae, all enclosed within thin, hypermineralized (1−5 μm) interfaces, the cement lines, generated during remodeling. Osteons orientation makes bone five times tougher to break than to split [2]. When a crack propagates, cement lines favor the formation of crack deflections and twists during bone breaking in the transverse orientation, and of crack

bridging during bone splitting in the longitudinal orientation. These mechanisms increase the toughness of the bone, shielding it against the crack propagation. Cement lines further increase bone toughness through matrix slippage at their interfaces with interstitial bone [3].

Centrally in the osteons, vascular channels, known as Haversian canals, bring blood and nutrients to bone together with the Volkmann's canals. Small mammalian bones, such those in mice, do not have osteons nor cement lines, but they have blood vessels and concentric lamellar structure around their medullar cavity. In any case, vascular porosity density within the bone matrix inversely relates to bone fracture toughness [4]. Intracortical vascular canals do not influence crack deflections, but their architecture is critical to bone resistance to fracture [5]. Many closely connected canals, as those observed, for example, in brittle osteogenesis imperfecta (OI) bone [6, 7], disrupt the continuity of bone matrix material and increase strain and stress concentrations in the bone matrix (Fig. 1A–C), thus favoring crack initiation [6] and propagation [5]. In aged human bone, the number of vascular canals, and with them of osteons and cement lines, are three times higher than in young bone. This reduces bone fracture toughness and favors the generation of microdamage accumulation in the cement lines that further facilitates bone failure [8]. Interestingly, an age-related increase in intracortical porosity has also been observed in C56BL/6 mice [9], but not in BALB/c mice, where extracellular matrix (ECM) modifications in collagen and water seem responsible for their skeletal fragility [10].

At the tissue level, bone is organized in lamellae (Fig. 1D), considered as a continuous but periodically inhomogeneous layered material with variable mechanical properties depending on the orientation of their fibers [11]. Bone tissue is composed of bundles of mineralized collagen fibers arranged in a plywood-like pattern along with disorganized periods lacking this consistent distribution of fibers [12, 13•]. By virtue of its anisotropy, the interfaces between the lamellar structure may contribute to bone toughness by acting as delamination barriers, causing crack deflections (Fig. 1E, F) and twists that can double bone fracture toughness during crack growth [5, 14, 15•]. Therefore, a larger osteonal tissue with lots of lamellar area should favor fracture resistance to crack initiation and propagation [16]. In OI bone, woven bone and lamellae coexist [17], with the greater amount of woven bone in the most severe OI variants, and with OI lamellae being shorter, thinner, less organized, and smoother than in healthy bone (Fig. 1D) [17, 18•]. This altered bone structure is accompanied by reduced fracture toughness [5, 14, 18•]. Within lamellae and woven bone, osteocyte lacunae and their canaliculi network generate another level of porosity. Osteocyte density increases with high bone (re)modeling, and lacunae become more spherical in diseases [6, 19, 20], thus generating high strains in the ECM that contribute to bone fragility [21]. Similarly, strain concentrations around canaliculi promote the initiation and growth of intra-lamellar circumferential microcracks, which are associated with the formation of shear bands, and suggest an ability of bone to develop enhanced inelastic deformations by cracking control at the mineralized collagen fibril bundles level [22].

Indeed, although toughening mechanisms shielding bone from crack growth occur at its microscale, bone plasticity, which affects both strength (ductility) and initiation toughness, arises from its nanoscale. Fibrils stretching and deformation, as well as breaking of sacrificial bonds and enzymatic cross-links, allow for fibrillar sliding, intrafibrillar dilatational band formation (i.e., 100 nm long ellipsoidal voids forming between fused mineral aggregates and two adjacent NCPs), and microcracking at their interfaces [23]. Mineralized fibrils are constituted by type I collagen molecules staggered in arrays with a 67-nm offset. Aggregates of hydroxyapatite nanoplatelets form in the intrafibrillar gaps between the collagen triple helices and in extrafibrillar loci extended along the long axis of the collagen fibrils [24]. Extrafibrillar mineral inclusions in bone also exist in the form of elongated plates tenths of nanometers long and wide, and 5 nm thick, which can be either flat or curved, wrapping around collagen fibrils and forming a distinctive visual pattern resembling rosettes [13•, 24]. Most of the mineral in bone actually lie out of fibrils in the form of mineral lamellae [13•, 24]. In the presence of collagen mutations, such as in OI, decreased intrafibrillar mineralization contribute to reduced apatite crystal alignment and thinning of collagen fibrils [25•].

During elastic bone tissue deformation, the extra- and intrafibrillar matrix stretches [26]. Damage progression and failure of the extrafibrillar matrix is responsible for initiation of bone yielding. At this point, mineralized collagen fibrils would be more involved in load bearing because the damaged extrafibrillar matrix loses its ability to carry load [26]. Mineralized collagen fibrils thus distribute the load to the nearby fibrils [27]. The load is transferred from their weaker and less mineralized overlap regions to the stronger and highly mineralized gap regions of neighboring fibrils [27].

Inside the mineralized collagen fibrils, water-mediated hydrogen bridges between hydroxyapatite and tropocollagen limit sliding between molecules and help transfer load, improving energy dissipation [28]. Loosely bound water within each fibril also contributes to bone ductility acting as a plasticizer at the interface between mineral and collagen phases in the mineralized fibril [29, 30]. When intrafibrillar water is removed, the energy dissipation of the bone is one ninth of that under hydrated conditions [30]. Structural water contained in apatite crystals has instead been suggested to boost hydroxyapatite platelets assembly and increase mineralization in bone [31•]. Hydroxyapatite significantly enhances the tensile modulus of the mineralized fibrils, reaching the highest stiffness at 2 nm thickness [32]. Thicker minerals

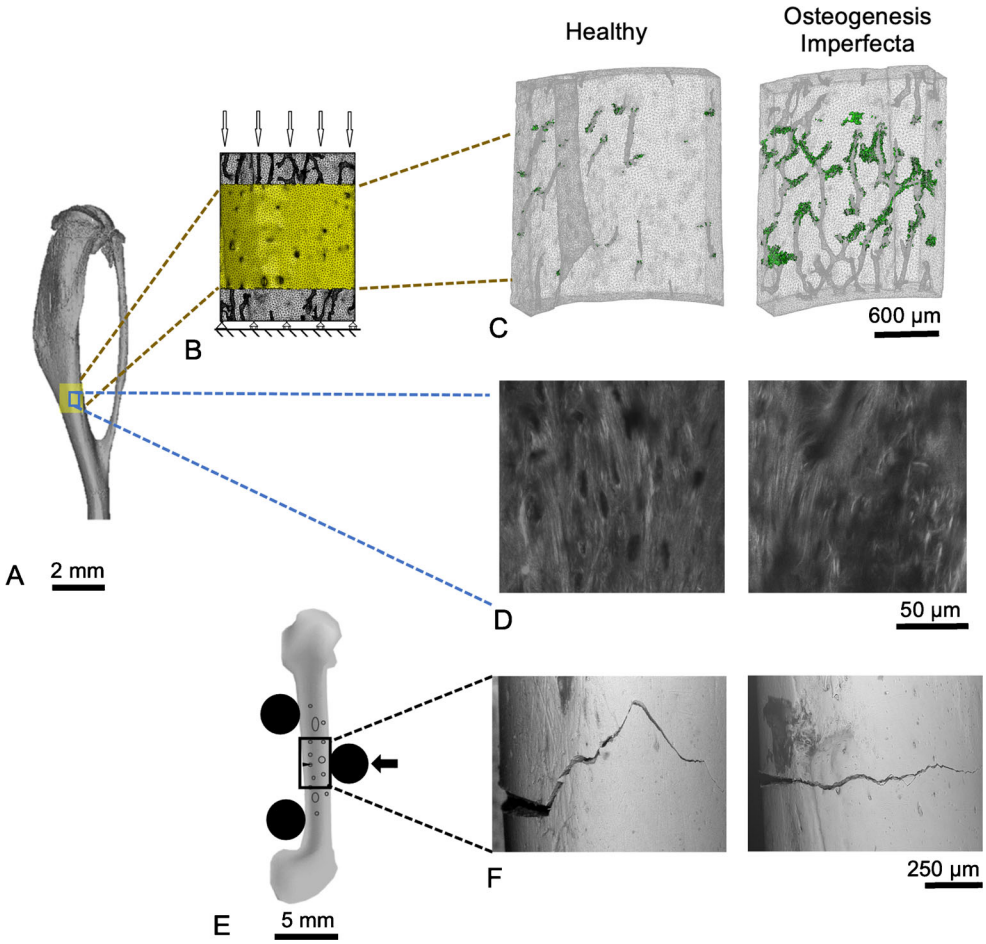

Fig. 1 Micro- and submicron-architecture changes in OI brittle bone contribute to its fragility. **A** Mouse bone reconstruction with **B** a representative cortical bone volume finite element model loaded in compression. The yellow area shows the region of interest (ROI). **C** The two finite element models of representative healthy and OI cortical bone samples show the intracortical porosity of healthy and OI cortical bone. The green sites around canals and at their intersections show the location of bone within the ROI at high risk of fracture initiation when loaded at an apparent strain of 0.4%. **D** Second harmonic generation microscopy images of collagen fiber organization in healthy and OI cortical bone in a smaller blue ROI shows lamellar structure in healthy bone and coexistence of lamellae and woven bone in OI bone tissue. **E** Schematic of a bone fracture mechanics testing of a notched mouse femur. **F** Scanning electron microscopy of the healthy and OI bone showing their crack path during fracture. In healthy bone, the crack takes deflections, while in OI bone the crack follow a straight path, corresponding to its reduced toughening mechanisms. For more information, refer to Carriero et al. [5, 6] and Docaj et al. [18•]

embrittle the fibril structure [32], while fibrils with smaller mineral particles, as in type 2 diabetes mellitus (T2DM), OI and glucocorticoid-induced osteoporosis (GIOP), have a low elastic modulus [5, 33, 34•]. Increased mineral content in overlap regions likely improves bone elastic modulus, ultimate strength, and fracture toughness [27]. Fibril deformations (stretching and sliding) vary with tissue strain and highly decrease at high strain rates, with fibril deformations being primarily stretching [35]. Large yield stress triggers fibrils debonding at their interface and reduces their plastic deformation [36]. This strain-rate stiffness effect is, however, suppressed in bone with altered mineralized fibrils nanostructure, due to low shear-transfer and shear-reinforcement of short mineral plates [34•].

The quality of collagen that constitutes bone fibrils depends on its post-translational modifications, which involve covalent and enzymatic processes, and on its relationship with minerals, NCPs, and water. Hydroxylation and lysyl oxidase-mediated modifications are important processes for hydrogen bonding and cross-linking catalysis to form stable collagen molecules and fibrils, respectively. Tightly bound water, trapped inside tropocollagen molecules, participates in their conformational stability by attaching to hydroxyl groups of hydroxyproline [29, 37]. As collagen matures, interfibrillar cross-links predominate over intrafibrillar cross-links, and loosely bound water in fibrils is substituted by mineral. Thus, water content in bone decreases with age [10, 38•], reducing its plasticity [30, 37, 39]. Inhibition of lysyl oxidase results in cross-link perturbations and decreased fracture toughness [40]. Enzymatic cross-linking varies throughout the skeleton as a function of turnover rate and mechanical environment on bone [41]. Mechanical loading on bone helps

in stabilizing the collagen matrix through divalent cross-links maturation [41]. In diabetic bone, hyperglycemia impairs the formation of divalent cross-links because collagen has increased maturity despite a reduced amount of enzymatic cross-links [42]. A type of enzymatic hydroxylysine glycosylation, namely glucosylgalactosylhydroxylysine (GGHL), is overexpressed in OI collagen as a sign of retarded triple helix formation [43] and may hinder divalent enzymatic cross-links maturation [44].

Non-enzymatic glycation (NEG) of collagen, involving the attachment of a free sugar to a collagen amino group, disrupts bone matrix quality and reduces its crack growth toughness by 35% [45]. Glycation widens intermolecular space, hampering fibril packing, and depresses lysyl oxidase-driven cross-linking as glucose carbonyls (advanced glycation end-products (AGEs) precursors) and telopeptide aldehydes (enzymatic cross-link precursors) target the same triple-helical sites [46•]. Glycated organic matrix has a lower capacity to sustain deformation and exhibits a lower creep; therefore, the ability of bone to dissipate energy is reduced and it generates longer microcracks that coalesce, resulting in longer crack length [45]. In aging and disease, bone fibril over-cross-linking by AGEs formation due to glycation, oxidative stress, or low bone turnover inversely correlates with fracture toughness [5, 8, 47]. In most cases, high AGEs content has been associated with increased resistance to fibril deformation, reduced fibrillar sliding [48], and compromised bone plasticity and resistance to fractures [8]. However, in OI, the role of AGEs remains unclear as increased deformations of the mineralized fibrils are observed in weak mineralized fibrils and brittle bone [5]. AGEs breakers, alagebrium [49•] and n-acetylcysteine [50•], did not improve bone mechanics, although they reduced bone AGEs content and oxidative stresses in rats with chronic kidney disease (CKD). Aminoguanidine and pyridoxamine tested in glucose treated cadaveric bone prevented AGEs formation and subsequent biomechanical bone degradation [51]. These studies assessed NEG as a measurement of pentosidine or fluorescent AGEs concentration. However, the amount of pentosidine is very small in bone, and carboxymethyl-lysine (CML) and glucosepane, which are not fluorescent, are found in higher concentrations (>40 times more) [52•]. Accumulation of CML in human bone strongly inversely correlates with crack propagation toughness [52•]. Further investigation is therefore needed to fully understand the impact of all NEGs on bone fracture resistance to crack initiation and growth. These studies may also lead to new diagnostic assays and therapeutic approaches for bone fragility. An age-dependent reduction in fracture resistance of cortical bone has been observed in the presence of deamidation of asparagine and glutamine residues, which disturb the affinity of collagen triple helices to bind with each other in favor of water bonds, increasing fibril diameter, and reducing bone fracture toughness [53•]. More studies are needed to better understand the contribution of deamidation to bone fragility, and how it varies in disease. This may open new avenues for targeted therapies for bone fragility.

Molecular interactions between mineralized collagen fibrils and extra-fibrillar mineral aggregates involving NCPs play a critical role in bone quality. Particularly, osteopontin (OPN) and osteocalcin (OC) at the interfibrillar interface constitute less than 2–3% of bone weight but contribute for more than 30% to its fracture toughness by a synergistic deformation mechanism of the two NCPs and bone [23, 54•]. Through strong anchoring and formation of dynamic binding sites on mineral nanoplatelets, bone nanointerface can achieve large non-linear deformation and great ductility [54•]. Whereas the rigid OC binds tightly to hydroxyapatite crystals and remains mostly stationary during deformation due to its high affinity to the mineral surface, OPN participates in the cleavage and reformation of the sacrificial bonds, detaching from one OC and rolling towards neighboring OCs in the shear direction [23, 54•, 55]. Additionally, OPN can generate new binding regions with the hydroxyapatite surface [54•]. The further stretching and unraveling leading to OPN denaturation entails dilatational bands formation, dissipating large amounts of energy [23, 54•, 56]. The one-third reduction in bone toughness in OPN and OC knockout mice has been associated with the absence of dilatational bands and subsequent diffuse damage formation, along with increased calcium variability [23, 57]. OC function can be affected by collagen glycation, forfeiting much of its energy dissipation ability [55]. Similar compromised bone toughness is experienced with loss of proteoglycans (PGs) and glycosaminoglycans, coupled with a decrease of bound water, such as in aging [38•, 58]. OPN phosphorylation adds additional negative charge and hydrophilicity that potentially eases its adhesion to mineral [59•], while its binding to free calcium ions may prevent unwanted adsorption to other components and facilitate its deposition on mineral surface [60]. The absence of NCPs triggers alterations in mineralization degree, fibrillogenesis, bone formation, and microdamage accumulation [58, 61, 62]. For example, deletion of decorin and/or biglycan results in highly disorganized collagen fibrils [63]. Thus, by targeting NCPs, or key regulatory pathways controlling their expression and activity, bone quality can be affected directly by altering its fracture toughness or indirectly by regulating mineralized collagen fibrils organization or bone cellular activity. For instance, administration of chondroitin sulfate (CS) has been shown to have antioxidant, anticatabolic, hypoglycemic, and antidiabetic effects [64], and to improve bound water amount and bone toughness [58].

Table 1 summarizes alterations in collagen and mineral structure and composition due to aging and diseases, which affect bone hierarchical structure and mechanical competences to sustain loads and resist fractures.

Table 1 Changes in bone matrix quality in aging and bone fragility diseases

Aging/bone fragility disease	Type	Changes in bone quality			
		Composition	Structure	Mechanics	(Re)modeling
Aging	Human	? Mineral-to-matrix ratio [97] ↑ Carbonate-to-phosphate ratio [65] ↑ Collagen maturity [65] ↑ AGEs [8, 52•, 59•] ↓ PGs and GAGs [38•] ↓ NCP phosphorylation [59•] ↓ Bound water [38•]	Tissue: ↑ Osteonal density [8] ↓ Osteonal spacing [8] Cellular: ↓ Lacunae density [66] ↑ Lacunae hypermineralization [66] Mineral: ↑ Crystallinity [67] ↓ Thickness [67] ↑ Length [67]	Whole bone: ↓ Stiffness [8] ↓ Strength [8] ↓ Crack initiation toughness [8] ↓ Crack growth toughness [8] Mineralized Fibril: ↑ Stiffness [8] ↓ Plasticity [8]	? Remodeling [68]
	C56BL/6 mouse	↑ Mineral-to-matrix ratio [65] ↑ Carbonate-to-phosphate ratio [65] ↓ Acid phosphate [69]	Tissue: ↑ Vascular porosity [19] Cellular: ↓ No. lacunae [19] ↓ Lacunae size [19] ↑ Lacunae sphericity [19] Mineral: ↑ Crystallinity [65]	Whole bone: ↓ Bending Modulus [19] ↓ Yield stress [19] ↓ Maximum stress [19] ↓ Maximum strain [19] Tissue: ↑ Peak and average strain [1] Fibril: ↓ Collagen strain at maximum tissue strain [19]	↓ Remodeling [19]
	BALB/cc mouse	↑ Mineral-to-matrix ratio [10] ↑ Carbonate-to-phosphate ratio [10, 70] ↑ Collagen maturity [10] ↑ AGEs [10] ↑ Asn and Gln deamidation [53•] ↓ Bound water [10]	Tissue: ↓ Cross-sectional area of the cortex [10] ↑ Cortical thickness in female [10] ↓ Cortical thickness in male [10] = Cortical porosity [10]	Whole bone: ↓ Yield stress [10] ↓ Ultimate stress [10] ↓ Initiation toughness [10] ↓ Energy to fracture [10] ↓ Post-yield toughness [10] ↓ Post-yield displacement [10]	↓ Remodeling [10]
Osteoporosis	Postmenopausal osteoporosis Human	↓ Mineral-to-matrix ratio [65] ↓ Mineral heterogeneity [70] ↑ Carbonate-to-phosphate ratio [65] ↓ Enzymic cross-links [71] ↑ Collagen maturity [65] ↑ AGEs [71] ↑ Hyl [71]	Tissue: ↓ Osteonal size [8] ↑ Cortical porosity [8, 65] ↑ Haversian canal density [8, 72] ↑ Haversian canal size [72] Cellular: ↑ Mineralized lacunae density [72] Mineral: ↑ Crystallinity [65] Mineralized Fibril: ↓ Thickness [71]	Whole bone: ↓ Bending modulus [72] ↓ Yield stress [72] ↓ Maximum stress [72] ↓ Crack propagation toughness [8] Fibril: ↓ Plasticity [72]	↑ Resorption [72]
	Estrogen depletion OVX rat	↓ Mineral-to-matrix ratio [73] ↓ Collagen maturity [73] ↑ Acid phosphate [74]	Whole bone: ↓ Cortical thickness [75] Mineral: ↑ Crystal thickness [76] ↓ Crystal maturity [73]	Whole bone: ↓ Elastic modulus [75] ↓ Ultimate stress [75] ↓ Ultimate strain [75] ↓ Energy to fracture [77]	↓ Formation [73] ↑ Resorption [73]
Osteogenesis imperfecta	Human type I - IV	↑ Mineral-to-matrix ratio [78] ↓ Collagen content [78]	Tissue: ↑ Cortical porosity [79, 80] ↑ Woven bone next to lamellar bone [17] Mineral: ↓ Crystal size [78] ↑ Crystal packing [78] ↓ Crystal organization [78]	Whole bone: ↓ Young's modulus [79, 80] ↓ Yield stress [79] ↓ Ultimate stress [79, 80]	↑ Remodeling [81] ↓ Formation [73] ↑ Resorption [73]
	oim/oim mouse	↑ Mineral-to-matrix ratio [5, 82]	Whole bone: ↓ Size [82]	Whole bone: ↓ Young's modulus [5]	↑ Remodeling [82]

Table 1 (continued)

Aging/bone fragility disease	Type	Changes in bone quality			
		Composition	Structure	Mechanics	(Re)modeling
		↓ Carbonate-to-phosphate ratio [5, 82] ↓ Intrafibrillar mineralization [25•] ↓ Enzymic divalent cross-links [82] ↑ AGEs [5]	↓ Cortical thickness [82] ↑ Vascular density and branching [5] ↑ No. Lacunae [6] ↑ Lacunae sphericity [6] Tissue: ↑ Vascular density [6] ↑ Vascular branching [6] Mineralized fibril: ↓ Organization [82] Mineral: ↑ Extrafibrillar crystal size [67] ↓ Intrafibrillar crystal size [67] ↓ Crystallinity [82] ↓ Organization [25•] ↓ Alignment [18, 82]	↓ Ultimate stress [25•] ↓ Ultimate strain [5] ↓ Work-to-fracture [25•] ↓ Crack initiation toughness [5, 6] ↓ Crack growth toughness [5] ↓ Nanoscratch toughness [25•]	↓ Osteoblast maturation [82]
Vitamin D deficiency	Human	↑ Osteoid density [83] ↑ Highly mineralized and mature regions [83] ↑ Carbonate-to-phosphate ratio [83] ↓ Acid phosphate [83] ↑ Ca concentration [83]	Whole bone: ↓ Cortical thickness [83] Tissue: ↑ Cortical porosity [83] ↑ Haversian canal volume [83] ↑ Haversian canal diameter [83] Cellular: ↑ Osteocyte lacunar volume [83] ↑ Hypermineralized lacunae [83] Mineral: ↑ Crystallinity [83]	Whole bone: ↓ Initiation toughness [83] ↓ Crack growth toughness [83]	↑ Local tissue aging [83] ↑ Formation [83] ↓ Local bone resorption [83]
Paget's disease	Human	↑ Osteoid density [84] ↓ Mineral-to-matrix ratio [84] ↓ Carbonate-to-phosphate ratio [84] ↑ Collagen maturity [84] ↓ Ca concentration [84]	Tissue: ↑ Cortical porosity [84] ↓ Haversian system alignment [84] ↑ Mosaic of lamellar and woven bone [84] Fiber: ↓ Orientation [84]	Whole bone: = Crack initiation toughness [84] = Crack growth toughness [84] = Energy dissipation [84] Tissue: ↓ Young's modulus [84]	↑ Formation [85] ↑ Resorption [85]
Type 1 diabetes mellitus	STZ rat	↓ Mineral-to-matrix ratio [86] ↑ Carbonate-to-phosphate ratio [86] ↓ Collagen maturity [86] ↑ AGEs [86]	Mineral: ↓ Crystallinity [86]	Whole bone: ↓ Elastic modulus [86] ↓ Yield stress [86] ↓ Ultimate stress [86] ↓ Toughness [86]	↑ Remodeling [84]
Type 2 diabetes mellitus	Human	↑ Mineral-to-matrix ratio [86] ↓ Heterogeneity of mineral-to-matrix ratio [87] ↓ Heterogeneity of acid phosphatase [87] ↓ Enzymic cross-links [86] ↑ AGEs [86] ↑ OPN [88•] ↑ OPG [88•]	Mineral: ↑ Crystallinity heterogeneity [87]	Whole bone: ↑ Elastic modulus [86] ↑ Ultimate stress [86]	↓ Remodeling [86]
	KK/Ay mouse	↑ Mineral-to-matrix ratio [42] ↑ Heterogeneity of carbonate-to-phosphate ratio [42] ↓ Enzymic cross-links [42]			↓ Remodeling [86]

Table 1 (continued)

Aging/bone fragility disease	Type	Changes in bone quality			
		Composition	Structure	Mechanics	(Re)modeling
	ZDSD rat	↑ Collagen maturity [42] ↑ Mineral-to-matrix ratio [89]	Tissue: ↑ Cortical porosity [89] Mineral: ↑ Crystallinity [86]	Whole bone: ↓ Elastic modulus [86] ↓ Work-to-fracture [89] ↓ Crack initiation toughness [89] ↓ Crack growth toughness [89]	
Chronic kidney disease	HBT Cy/+ rat	↓ Enzymic trivalent cross-links [47] ↓ Bound water [47] ↑ AGEs [47]	Tissue: ↑ Cortical porosity [47]	Whole bone: ↓ Stiffness [49•] ↓ Ultimate stress [47, 49•]	↑ Remodeling [47]
	LBT Cy/+ rat	↑ Enzymic trivalent cross-links [47] ↑ Bound water [47]	Tissue: ↑ Cortical porosity [47]	Whole bone: ↓ Toughness [47]	↓ Remodeling [47]

The regulators of bone matrix quality and targeted therapies for bone fragility

Mechanical loading on bone increases microdamage concentration within its matrix in the form of linear and diffuse microcracks. Linear microcracks, a micron-scale damage, boost bone remodeling by inducing osteocyte apoptosis possibly by directly cutting the osteocyte processes, or indirectly by changing the lacunar–canalicular fluid flow in the damaged area [90, 91]. Osteocyte apoptosis triggers surrounding surviving osteocytes to produce RANKL, a cytokine promoting osteoclastic bone resorption by binding to RANK and regulating osteoclasts fission into osteomorphs, bone cells able to merge back into new osteoclasts with little energy expenditure as needed during bone remodeling [92••]. In addition, hydrogen ions and cathepsin K aid in bone demineralization and collagen degradation, respectively. Mesenchymal stem cells (MSCs) are attracted to the repair site by osteoclast-secreted transforming growth factor-β (TGF-β), and osteocyte-produced insulin-like growth factor-1 (IGF-1) triggers their differentiation into osteoblasts [93••]. At the mineral level, the tissue-nonspecific alkaline phosphatase (TNAP) enzyme and the progressive ankylosis protein (ANK) regulate extracellular matrix mineralization by controlling levels of osteocyte- and osteoblast-secreted inorganic pyrophosphate (PPi), a potent mineralization inhibitor [94]. The loss of TNAP and ANK function can cause hypo- and hyper-mineralization, respectively [94, 95].

Aging and disease alter bone remodeling (Table 1). Inhibition of bone remodeling affects its extracellular matrix quality and resistance to fracture: microcracks accumulate within their tissues, and mineral, collagen maturity, and AGEs content within the fibrils increase, thus reducing fibrillar sliding and bone plasticity [8, 71]. Age- and disease-related declines in the number of osteocytes and canaliculi may reduce their mechanosensory ability to detect microdamage and the release of RANKL to initiate repair, leading to the accumulation of microcracks [96]. On the other hand, increased bone remodeling may affect bone mineralization and produce a matrix with osteoid layers next to heavily mineralized regions, as in vitamin-D deficiency [83]. Osteoclasts cannot go through thick osteoid layers, and the bone underneath continues to age and mineralize although overall bone mineral content progressively decreases [83]. In OI bone, tissue hypermineralization with reduced carbonate content (as a sign of crystals immaturity) may favor the formation of microcracks, further triggering bone turnover [5] that contributes to bone fragility.

Diffuse damage, made of submicron-sized cracks, does not cause osteocyte apoptosis nor trigger osteocytic or osteoclastic activity, but self-repairs [97] spontaneously through a physicochemical remineralization process [98••], similar to that occurring in enamel repair. It is possible that the lacunar–canalicular system of osteocytes plays a role in the maintenance of bone tissue ionic fluid and in the transport of minerals and other adhesive proteins across the matrix to sites of damage, required for the chemical repair of diffuse damage [98••]. Further studies are required to understand the actual mechanisms of this self-healing and if aging and disease alter this process, and its contribution to bone fracture risk.

Osteocytic osteolysis or perilacunar remodeling (PLR) has been suggested to have a role in determining and maintaining bone quality [99]. This process has the potential to not only regulate mineral homeostasis but also to release calcium from the matrix, affect osteocyte mechanosensation, and alter bone remodeling. Osteocyte-mediated proton release demineralizes

the perilacunar matrix and frees calcium ions, and in turn metalloproteinase-13 (MMP-13), tartrate resistance acid phosphate (TRAP), and cathepsin K remove the organic phase. Disruption of osteocyte-mediated resorption causes bone fragility in MMP-13 knock-out mice, owing to a highly porous, small cortex with disorganized canaliculi, increased collagen cross-linking, and smaller crystals with low crystallinity [95]. Compromised bone quality is also present in mice with knock-out and overexpressed tissue inhibitor of metalloproteinase-3 (TIMP-3) [100, 101], a MMP-13 inhibitor [100]. Particularly, TIMP-3 deficiency increases cortical porosity, acid phosphate levels, and heterogeneity of the collagen cross-link profile, and decreased carbonate-to-phosphate ratio [101]. Interestingly, PLR happening during lactation shows a recoverable reduction in bone tissue-level elastic modulus that has been attributed to changes in lacunar and canalicular space [102]. The extent and mechanisms of bone repair due to PLR remain to be revealed.

Microcracks, possibly due to masticatory stresses, also occur in ear ossicles and the otic capsule, where they accumulate over time due to downregulated bone remodeling and PLR. It has been suggested that mechanisms controlling bone remodeling and PLR in the otic capsule are different from those in the long bones, perhaps to preserve auditory function [103]. Increased porosity of the ear bone, as occurs in OI and otosclerosis, can further increase the formation of microcracks and bone fractures in the ears [104•]. The presence of zinc at sites of ossification in the ear seems to help prevent cochlear damage, while its deficiency in aging mice potentiate hearing loss [105]. Further studies are needed to elucidate the functional implications of biological controllers for bone matrix quality and their relationship with genetic expression at different body sites and in bone diseases with secondary disabilities, such as hearing loss.

The combination of bone remodeling, PLR, and diffuse damage healing plays an essential role in maintaining bone compositional, structural, and mechanical integrity. The interconnection between bone cells and matrix is regulated by intra- and extra-cellular molecular-signaling pathways that ultimately control bone quality. When these are disrupted, bone becomes fragile. Proteins and signaling pathways may either directly affect the mineral or organic constituents of bone ECM or have an effect on the cellular activity that regulates bone repair and (re)modeling, impairing bone mechanical properties. Endocrine, paracrine, and autocrine pathways are responsible for the cellular response to signaling in the processes regulating bone quality. This signaling may activate multiple cell populations independently or in a cascade of events with mutual cell interactions. Several growth factors regulating bone repair are likewise involved in determining bone quality. For example, TGF-β is essential for maintaining bone quality by coupling the activity of osteoblasts and osteoclasts, and human mutations in many TGF-β pathway components have been associated with skeletal dysplasia and disease [106]. TGF-β couples with other factors playing pivotal roles in bone homeostasis and cell cycle regulation, and their regulation offers positive results in terms of correcting aberrant bone remodeling. Table 2 reports the major bone proteins, signaling pathways, and transcription regulatory networks known to be implicated in controlling bone ECM material properties, offering targets for bone therapies.

Despite the advances in the field, there is a critical need to fully elucidate the effect of bone cells and biology on controlling bone quality, and particularly its fragility. This will suggest new targets for the development of therapies to prevent bone fragility. In this regard, the combination of genetically modified rodents with investigations of bone fracture toughness offers the opportunity to further understand the molecular control of bone material properties and quality. Our research and that of others on bone fracture toughness has disclosed mechanisms of bone fragility in OI mice, CKD rats, and diabetic ZDSD rats [5, 6, 14, 47, 53•, 89, 104•, 166], as well as the importance of OPN, OC, PHOSPHO1, and TIMP-3 for bone quality and toughness [23, 57, 100, 101, 162, 163]. However further research is needed in this direction, and studies must help unveil the complex relationship between bone biology and mechanics. Studies on cell activity and their signaling can shed a light on their influence on bone quality. For example, increased bone fragility in OI has recently not only related to its extracellular matrix impairment but also to altered intracellular homeostasis due to mutant collagen retention [152, 153]. Endoplasmic reticulum stress modulates the OI phenotype severity in its Brtl mouse model, and activates the unfolded protein response, autophagy, and apoptosis in human fibroblasts in dominant forms of OI as well as in some recessive OI forms characterized by altered collagen synthesis [152, 153]. Treatment with 4-phenylbutyrate (4PBA) chemical chaperone ameliorates OI cells homeostasis in vitro, and improves the OI bone phenotype in the Chihuahua zebrafish OI model by reducing intracellular misfolded protein accumulation and promoting protein secretion [152, 153]. However, more research is needed to investigate the material properties of the 4PBA-treated OI bone and enhance therapy efficacy by effectively deliver the drug to bone. Similarly, further studies on the recently discovered osteomorph genes, controlling bone structure and function, with their upregulation being associated with human skeletal diseases and osteopenia [92••], may further unveil new possibilities for bone fragility treatment. Also, bone has recently been revealed to play an important role in regulating glucose metabolism through the release of osteokines [167], with increased plasma OPN and OPG levels in prediabetes pathogenesis. While the role of OC in regulating glucose metabolism is unknown [167], its levels inversely correlated with circulating free fatty acids concentration [88•]. Future research may consider the development of novel therapies targeting these biomarkers for aging,

Table 2 Principal local and systemic factors regulating bone extracellular matrix homeostasis and mineralization as potential targets for bone fragility therapies. Signs (#, %, ^, *, &, $) indicate the same therapeutic treatment with same effect on bone matrix. OI = osteogenesis imperfecta

Bone ECM homeostasis and mineralization	Local/ systemic factor	Target in bone	Mechanisms of action and direct effect on bone quality	Therapeutic treatment	Effect of therapeutic treatment on bone quality		Aging/bone fragility disease
					Bone (re)modeling	Bone matrix	
Bone matrix homeostasis factors	Growth factors	Bone morphogenetic protein-2 (BMP-2)	BMPs are TGF-β family growth factors effective in inducing bone formation. BMP-2 plays a significant role in bone regeneration and has been used to treat fractures [107].	IGF-binding protein-3 (IGFBP-3)	Decreases osteoblastic differentiation via BMP-2 [108].		- Osteoporosis
				Recombinant Progranulin (PGRN)#	Enhances BMP-2 function and promotes bone healing, via antagonization of the inflammatory cytokine TNF-α by binding to TNFR1 [107].		
		Insulin-like growth factor-1 (IGF-1)	Stimulates osteoblasts activity and accelerates bone formation [108].	Fluoride	Increases IGF-1 and bone turnover [109].	Alters mineral crystal width and the electrostatic interactions between mineral and collagen, decreasing bone strength [109].	- Osteoporosis
				IGF-1 Enhancers (i.e., Leptin)	Increase bone formation [110].		
				IGF-1 Inhibitors (i.e., IGF-binding proteins (IGFBPs)	Suppress IGF-1 stimulated bone cell proliferation, collagen synthesis, and bone formation [111].		
		Transforming growth factor-β (TGF-β)	Released and activated by osteoclasts, TGF-β recruits mesenchymal stem cells (MSCs) to local site of repair for new bone formation [93••]. TGF-β is essential for maintaining bone quality by coupling the activity of osteoblasts and osteoclasts, and human mutations in many TGF-β pathway components have been associated with skeletal dysplasia and disease [106, 112].	TGF-β Inhibitors (i.e., inhibitors of the TBRI kinase activity, antibodies against TGF-β, inhibitors of TGF-β signaling such as TGF-β inhibitor SD-208 and 1D11)	Promote osteoblast differentiation and bone formation, while inhibiting osteoclast differentiation and bone resorption [113].	Improve elastic modulus and hardness, as well as mineral concentration [113].	- Osteoporosis - Osteogenesis imperfecta (OI)
		Vascular endothelial growth factor (VEGF)	Key factor for angiogenesis. VEGF seems to promote bone mineralization and density.	VEGF-inhibitors		Decrease bone blood flow [114].	- Osteoporosis

Table 2 (continued)

Bone ECM homeostasis and mineralization	Local/ systemic factor	Target in bone	Mechanisms of action and direct effect on bone quality	Therapeutic treatment	Effect of therapeutic treatment on bone quality		Aging/bone fragility disease
					Bone (re)modeling	Bone matrix	
	Cell surface, cell adhesion and matrix molecules	Cathepsin K	Lysosomal protease produced by osteoclasts to degrade type I collagen and hydroxyapatite [115].	Cathepsin K inhibitors, such as Odanacatib	In 2016, Odanacatib was discontinued due to increased risk of cerebrovascular accidents.	Odanacatib improves cortical thickness, reduces cortical porosity and increases compressive strength [115].	- Osteoporosis
		Matrix metalloprotein-ases (MMPs)	MMPs are enzymes degrading bone extracellular matrix proteins, also able to process a number of bioactive molecules.				
		MMP-2	Responsible for skeletal development, growth plate formation [100]. MMP-2 exhibits collagenolytic activity. Important for bone resorption. Crucial for intramembranous and endochondral ossification, bone remodeling [116]. Overexpression in bone marrow with myeloma generates osteolysis. Deficiency impairs canalicular network [117], and results in hypomineralized ECM with reduced elastic modulus and bone strength [118].	Bone seeking bisphosphonate based MMPs specific inhibitors (BMMPIs), such as ML104 and ML115 [117]	Protect against osteolysis [117].		
				Membrane-anchored inhibitor (RECK)[%]	Inhibits MMP activity and compromises ECM remodeling by targeting Notch signaling pathway important for angiogenesis [119].		
				Possible targeted therapy on TIMP-1-3			
		MMP-9	Exhibits collagenolytic activity. Promotes osteoclast activity important for bone resorption. Osteoclast-derived MMP-9 breaks down unmineralized matrix only [116].	RECK[%]			
				Possible targeted therapy on TIMP-1-3			
		MMP-13	Cleaves specific sites within fibrillar collagens. Important for coupling bone resorption to bone formation, endochondral ossification [116]. Involved	Possible targeted therapy on TIMP-2-3.			

Table 2 (continued)

Bone ECM homeostasis and mineralization	Local/ systemic factor	Target in bone	Mechanisms of action and direct effect on bone quality	Therapeutic treatment	Effect of therapeutic treatment on bone quality		Aging/bone fragility disease
					Bone (re)modeling	Bone matrix	
			in the normal distribution of mineral density in cortical bone [120]. Deficiency leads to higher nonenzymatic cross-linking, disorganized collagen, impaired perilacunar remodeling and altered canalicular formation, with compromised fracture toughness and post-yield behavior [95, 121].				
		MMP-14	Membrane MMPs Type. Exhibits collagenolytic activity. Cleaves collagen I, fragments of which undergo endocytosis. Coordinates collagen phagocytosis. Important for bone resorption and remodeling, intramembranous and endochondral ossification. Crucial for osteoclast migration [116]. Deficiency results in disrupted canalicular network [95].	RECK[%] Possible targeted therapy on TIMP-2-3.			- Osteopenia
		MMP-16	Membrane MMPs Type. Crucial for bone development in cooperation with MMP-14 [120]. Depletion represses MSc involvement in skeletal formation.	Possible targeted therapy on TIMP-2-3.			
		Nuclear factor κβ (NF-κβ)	Transcriptional factor mediating inflammatory response and bone remodeling processes in both osteoblasts and osteoclasts [122]. Mediates	OPG-thiolBP conjugate	Blocks RANK/RANKL pathway and induce active bone remodeling [93••].		- Osteoporosis
				NF-κβ inhibitors (> 800 have been reported [122], such as β-carbonile and	A RANKL inhibitor [123]. Suppresses osteoclast differentiation and activity, bone resorption and	Increases bone hardness and percentage of mineralized osteocyte lacunae [125].	

Table 2 (continued)

Bone ECM homeostasis and mineralization	Local/ systemic factor	Target in bone	Mechanisms of action and direct effect on bone quality	Therapeutic treatment	Effect of therapeutic treatment on bone quality		Aging/bone fragility disease
					Bone (re)modeling	Bone matrix	
			RANK ligand induced osteoclastogenesis.	Human monoclonal antibody (Denosumab)^).	turnover [124], increasing bone density [125].		
		Osteoprotegerin (OPG)	Secreted by osteoblasts, competes with RANK to bind with RANK-L, which can inhibit osteoclast formation and subsequent bone resorption [93••, 126]. Deficiency results in osteopenia [127].	Bisphosphonates (BPs)*	Increase OPG expression. Inhibit bone remodeling [128], and bone formation [82].	Increase mineral and collagen cross-link maturity [128], mineral homogeneity and non-enzymatic cross-links [129]. Increase ECM elastic modulus [128] and reduce bone toughness [129].	- Osteoporosis - OI - Paget's disease
				Cabozantinib	Reduces RANKL/OPG ratio in osteoblasts and inhibits osteoclastic activity [130].		
				Chondroitin sulfate (CS)&	Increases the expression of RUNX2 and OPG and reduces RANKL levels. Promotes osteoblasts maturation and osteogenesis [64].		
				Estrogen therapy	Stimulates OPG production, and suppressing RANKL levels on bone marrow cells [127], inhibits resorption		
		RANK/RANKL	Promotes osteoclastogenesis and osteoclast activation [123].	AS2676293	Small RANKL inhibitor molecule. Inhibits bone resorption and osteolysis [131].		- Osteoporosis - OI - Paget's disease
				BPs*			
				Denosumab^			
				RANK-Fc	RANKL inhibitor recombinant. Increases bone density [82].	Improves some mechanical and geometrical properties of bone, reduces fracture incidence [82].	
		Sclerostin	Glycoprotein secreted by osteocytes, reduces bone formation. Inhibits the Wnt/β-catenin metabolic pathway in bone cells.	BPS804 anti-sclerostin antibody	Increases bone formation and reduces bone resorption in adults with OI [132].	Increases bone strength in OI mice [132].	- Osteoporosis - OI - Chronic kidney disease-mineral and bone disorder (CKD-MBD)
				Denosumab^	Increases rate of cortical and trabecular bone formation [82].	Increases trabecular thickness, and strength of cortical bone. Reduces	

Table 2 (continued)

Bone ECM homeostasis and mineralization	Local/ systemic factor	Target in bone	Mechanisms of action and direct effect on bone quality	Therapeutic treatment	Effect of therapeutic treatment on bone quality		Aging/bone fragility disease
					Bone (re)modeling	Bone matrix	
						bone fragility, improves stiffness and ability to withstand higher loads before breaking [82].	
				Romosozumab		Decreases the risk of vertebral fractures [133].	
		Tissue inhibitor metalloprotein-ases -3 (TIMP-3)	TIMPs regulate MMPs activity and enhance the interactions of TIMPs with transmembrane MMPs in the control of pericellular proteolysis [134]. TIMP-3 Inhibits MMP-1, -2, -14, -15, -16, -17, -24, and -25 [100, 101] and ADAM-10, -12, -17, -28 and -33 [116]. Overexpression decreases bone remodeling and cortical thickness and increases fibril strain, which reduces bone tissue stiffness [100]. Deficiency affects remodeling, heterogeneity of enzymatic collagen cross-linking ratio and porosity, and reduces bone thickness, strength, stiffness, stress capacity and fracture toughness [101].	microRNA-222	Reduces TIMP-3 expression in rat MSCs, inhibiting their differentiation. Its inhibition instead promotes osteogenic differentiation of MSCs by enhancing TIMP-3 expression [135].		- Type 2 diabetes mellitus (T2DM)
		Tumor necrosis factor-α (TNF-α)	Pro-inflammatory cytokine triggers osteoblasts to release RANKL to bind with RANK, thus promoting bone resorption while inhibiting BMP-2-induced osteogenesis [107].	PGRN[#]			- Diabetes mellitus
		Wnt	Wnt/βcatenin signaling inhibits osteoclastogenesis and induces osteoblast differentiation [126].	Abaloparatide	A PTH analogue, stimulates bone formation [136] and accelerates fracture healing [137].	Stimulates normal bone microarchitecture [136] and reduces fracture risk [137].	- Osteoporosis - OI
				DKK1			

Table 2 (continued)

Bone ECM homeostasis and mineralization	Local/ systemic factor	Target in bone	Mechanisms of action and direct effect on bone quality	Therapeutic treatment	Effect of therapeutic treatment on bone quality		Aging/bone fragility disease
					Bone (re)modeling	Bone matrix	
					Inhibits Wnt signaling pathway, osteoblast activity and bone formation [93••].	Increases compressive strength [138].	
				Intermittent Parathyroid Hormone (iPTH)[$]	Promotes Wnt pathway signaling, osteoblast differentiation [123].	Increases cortical bone area, collagen cross-linking and decreases cortical bone crystallinity [139], improving resistance to crack formation, tissue strength and resistance to microdamage [140].	
				Romosozumab	Wnt signaling enhancer. Not approved by the FDA for osteoporosis treatment due to its cardiotoxicity [123].		
				Teriparatide	A PTH analogue, accelerates fracture healing [137].	Increases strength [82].	
	Transcription factors	Activating transcription factor 4 (ATF4)	Regulates osteoblast maturation and promotes the expression of the osteocalcin (OCN) [141]. Deficiency is associated with higher mineral-to-collagen ratio and reduced fracture toughness [141].	Possible target therapy on miR-214 in osteoblasts to regulate ATF4 expression [142].	Agomir-214 (a miR-214 agonist) decreases in vitro osteoblast activity, which is promoted instead by antagomir-214 (a miR-214 antagonist) [142].	Agomir-214 decreases in vitro matrix mineralization which is promoted instead by antagomir-214) [142].	- Aging - Osteoporosis
		Histone deacetylase-3 (HDAC3)	Nuclear enzyme controlling bone modeling by suppressing osteoclast responsiveness to RANKL and coupling to bone formation [143]. Fundamental for bone maintenance during aging [144]. Deficiency results in hypomineralized bone with reduced hardness and elastic modulus [144].	HDAC inhibitors (i.e., vorinostat, valproate etc. - drugs for cancers and neurological disorders)	Decrease bone density [145, 146].		- Aging
		RUNX2	Transcriptional regulator of both intramembranous and endochondral bone formation [147]. Promotes	Inhibitors of RUNX2 deacetylation (i.e., p300 inhibitor anacardic acid) [149].	P300 inhibitor dramatically suppresses FGF2-stimulated Runx2 activity. Disruption of Fgf2		- Osteoporosis - Chronic kidney disease-mineral and

Table 2 (continued)

Bone ECM homeostasis and mineralization	Local/ systemic factor	Target in bone	Mechanisms of action and direct effect on bone quality	Therapeutic treatment	Effect of therapeutic treatment on bone quality		Aging/bone fragility disease
					Bone (re)modeling	Bone matrix	
			proliferation of osteoblasts progenitors. Controlled by multiple posttranslational modifications (PTMs), such as phosphorylation, prolyl isomerization, acetylation, and ubiquitination, mediated by the stepwise recruitment of multiple enzymes [147]. Increases during fracture repair [147]. Overexpression generates abnormal microarchitecture [148].	RUNX2 posttranslational modification upregulators (i.e., histone deacetylase inhibitors (HDIs), such as Trichostatin A) iPTH[$] CS[&]	gene results in decreased bone formation [149]. Promote osteoblast maturation and the expression of osteoblast genes, increasing matrix mineralization [150]. Increase the expression of type I collagen, osteopontin, bone sialoprotein, and osteocalcin [150]. Stimulates bone formation [151].		bone disorder (CKD-MBD)
	Cell viability and function	The Unfolded Protein Response (UPR)	Maintains the endoplasmic reticulum (ER) under regular stress conditions and restores normal osteoblasts homeostasis [152, 153]. UPR is activated in response to unfolded protein accumulation and its failure causes programmed cell apoptosis.	4-Phenylbutyric acid chaperone (4-PBA)	Chemical chaperone enhancer of cell homeostasis. Promotes collagen secretion and reduces cell apoptosis. Facilitates the ER folding machinery impaired under stress conditions and decreases accumulation of misfolded proteins in the ER lumen [152, 153]. Improves bone mineralization and growth [152, 153].		- OI
	Immune regulation	Dipeptidyl peptidase-4 (DPP4)	Involved in the inflammatory process, interacting with adipokines and regulating bone-fat metabolism [154]. DPP4 released from marrow adipose tissue inhibits bone healing [154].	DPP4 inhibitors, i.e., sitagliptin.		Enhance bone metabolism and quality, reducing fracture risk [155].	- Osteoporosis - Diabetes mellitus
	Systemic hormones	Estrogen receptor	Key role in bone metabolism and integrity. Deficiency causes bone fragility [93••, 156].	Selective estrogen receptor modulators (SERMs, such as Raloxifene, Bazedoxifene and Lasofoxifene)		Increase tissue hydration [157] and by binding to collagen, improve strength and fracture resistance [158].	- Postmenopausal osteoporosis - Estrogen deficiency
		Glucocorticoids(GC)	Increase bone resorption, and cause lacuna-canalicular	BPs[*]			

Table 2 (continued)

Bone ECM homeostasis and mineralization	Local/ systemic factor	Target in bone	Mechanisms of action and direct effect on bone quality	Therapeutic treatment	Effect of therapeutic treatment on bone quality		Aging/bone fragility disease
					Bone (re)modeling	Bone matrix	
			network degeneration, collagen disorganization and matrix hypermineralization, thus increasing bone fragility [159].				- Glucocorticoid-induced osteoporosis
				Denosumab^			
				iPTH[$]	Counteracts bone resorption [159].	Increases cortical thickness and bone area, and improves bone biomechanical properties: strength, energy to failure, toughness, stiffness and modulus [159].	
		Parathyroid hormone (PTH) type I receptor (PTHR1)	Regulates levels of calcium and phosphate in the bone matrix. Enhances cellular activity, promoting bone formation [93••].	Selective peptide activators of the PTHR1 (i.e., Teriparatide, Abaloparatide)	Increase bone remodeling, improving fracture healing [160].	Increase cortical thickness and strength [161].	- Osteopenia - Osteoporosis
Bone matrix mineralization factors		Phosphatase orphan 1 (PHOSPHO1)	Regulates PPi availability, involved in matrix mineralization initiation. Inhibits osteocyte differentiation [162]. Deficiency increases fracture toughness [163].	PHOSPHO1 inhibitors (i.e., Lansoprazole, MLS-0038949, MLS-00390838 and MLS-0263839)		Inhibit mineralization [164].	- Osteomalacia
		Pyrophosphate (PPi)	Inhibits hydroxyapatite deposition.	BPs[*]	Act as nonhydrolyzable PPi analogs [165].		- Osteomalacia
				Possible treatments targeting tissue-nonspecific alkaline phosphate (TNAP)			
		Tissue-nonspecific alkaline phosphatase (TNAP)	Hydrolyzes and inactivates PPi. Promotes skeletal mineralization [94]. TNAP/PHOSPHO1 double-knockout mice exhibit total lack of skeletal mineralization [164].				

T2DM, and osteoporosis populations. Table 2 presents existing and possible targeted therapeutic treatments for the major known regulators of bone quality, and their effect on bone remodeling and matrix properties.

Nowadays, bone treatments are essentially still systemic, requiring mainly oral administration or injections. Their main challenges are low bone tissue selectivity, with high doses taken for the drug to reach bone, and safety, due to their adverse side effects [168]. Moreover, once the treatment reaches bone, its low permeability and reduced blood flow may further hinder the drug efficacy. Treatments for bone fragility have so far mostly relied on bisphosphonates, a group of antiresorptive drugs, that can induce atypical femur fractures by long-term low bone turnover and/or jaw osteonecrosis, as well as gastrointestinal adverse effects and cancer of the esophagus from oral treatments, and/or atrial fibrillation due to increased blood calcium level [93••]. Similarly, some bone anabolic drugs stimulate its formation by binding to the parathyroid hormone type I receptor, but they can cause post-dose hypercalcemia and increase risk of developing osteosarcoma [169]. Therefore, there is a critical need to improve drug delivery at the appropriate concentrations directly to bone. Targeted therapy delivered to either bone matrix components or cell signaling, and activation can improve efficacy of treatments, while reducing dosage and systemic toxicity-related side effects. Nanosystems, such as alendronate conjugated nanodiamonds (ALN-NDs), extend clinical exposure while reducing side effects, whereas selective estrogen receptor modulators (SERMs), such as Raloxifene, have estrogen-like resorptive actions in bone, but neutral effects in other tissues, e.g., breast and uterus, overcoming the problem of low tissue selectivity for estrogens. As opposed to antiresorptive treatment, anabolic therapy enhances bone formation rather than reducing bone resorption. When combined with the collagen-binding domain (CBD), parathyroid hormone (PTH) treatment exerts a long-lasting bone anabolic effect while preventing inconvenient undesirable effects (e.g., hypercalcemia) [93••].

New forms of drug delivery for bone-targeted therapies have been tested in laboratories that promise to improve therapeutic efficacy, control drug release, and reduce systemic toxicity. For instance, cell-infiltratable and injectable gelatin hydrogels encapsulating MSCs successfully fostered bone regeneration in an animal model of steroid-associated osteonecrosis [170], while polyurethane nanomicelles can embody and release miRNAs to osteoclasts at the bone resorption surface, improving bone microarchitecture in ovariectomized osteoporotic mice [171]. However, most non-responsive nanocarriers cannot accomplish a realistic delivery of their payload to the target site. In such a case, enzyme-responsive delivery systems exploit altered expression of specific enzymes, such as cathepsin K and certain MMPs, to drive the liberation of drugs [172].

Conclusions

With a comprehensive understanding of the biological mechanisms controlling bone quality, particularly toughness, and the development of new biotechnology for drug delivery, novel bone-targeted therapies for bone fragility will improve in the future, holding potential for their use in the clinic. A greater understanding of the physiological differences between humans and animals affecting bone mechanics, as well as advance in bone cell biology, genetics, and genomics will accelerate the translation of bone targeted-therapy to clinical application.

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Declarations

Conflict of interest All authors state that they have no conflicts of interest.

Human and animal rights All reported studies/experiments with human or animal subjects performed by the authors have been previously published and complied with all applicable ethical standards (including the Helsinki declaration and its amendments, institutional/national research committee standards, and international/national/institutional guidelines).

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- • Of importance
- •• Of major importance

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