



# Small leucine-rich proteoglycans in physiological and biomechanical function of bone



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## Abstract

Proteoglycans (PGs) contain long unbranched glycosaminoglycan (GAG) chains attached to core proteins. In the bone extracellular matrix, PGs represent a class of non-collagenous proteins, and have high affinity to minerals and collagen. Considering the highly negatively charged character of GAGs and their interfibrillar positioning interconnecting with collagen fibrils, PGs and GAGs play pivotal roles in maintaining hydrostatic and osmotic pressure in the matrix. In this review, we will discuss the role of PGs, especially the small leucine-rich proteoglycans, in regulating the bioactivity of multiple cytokines and growth factors, and the bone turnover process. In addition, we focus on the coupling effects of PGs and GAGs in the hydration status of bone extracellular matrix, thus modulating bone biomechanical properties under physiological and pathological conditions.

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## Introduction

Osteoporosis and age-related bone fragility fractures are one of major health concerns for our rapidly growing elderly population. Bone mineral density (BMD) is the standard measure for the prognosis of fragility fractures. However, the prediction of bone fracture by using BMD alone lacks sensitivity to accurately evaluate the biomechanical performance of bone [1,2]. There have been reports showing the fracture risk for elderly people is much higher than that of young adults with the same BMD [3,4]. This has raised the necessity of elucidating the ultrastructural and molecular origins of bone fragility to better understand and prevent bone fragility fractures. Bone has a highly hierarchical structure, which is comprised of mineralized collagen fibrils embedded in an extrafibrillar matrix. The composition of bone includes 60% by weight mineral phase (mainly hydroxyapatite), 30% organic phase, including type I collagen fibrils and non-collagenous proteins, and 10% water [5]. Thus, the biomechanical properties

of bone are dependent on the quality and spatial arrangement of these constituents.

Previously, extensive efforts have been made to illustrate the contribution of the mineral and collagen phases to bone mechanical competence [6–10]. In addition, the non-collagenous proteins are structural proteins dispersed throughout the bone extracellular matrix, which have been found to be directly involved in the deformation and failure of bone [11–13]. As a major part of non-collagenous proteins, proteoglycans (PGs) have strong affinity to collagen and mineral crystals [14,15]. PGs are composed of core proteins with covalently attached glycosaminoglycan (GAG) chains, which are long unbranched polysaccharides [16]. These molecules are polar with a high negative charge, forming interfibrillar supramolecular assemblies that could absorb water into the bone extracellular matrix, while water has been reported to function as a critical plasticizer of bone [17,18]. Moreover, PGs and GAGs interact with a multitude of growth factors and cytokines, regulating their activity and bioavailability [19].

It has been well documented that PGs and GAGs play an important role in modulating mechanical behavior of tendon, articular cartilage, and intervertebral disks [20–22]. In this review, we mainly focus on the contribution of PGs and GAGs to the physiological and biomechanical properties of bone. We will address the function of PGs and GAGs in mediating molecular signaling pathways and bone remodeling processes involving bone formation and bone resorption. We also focus on the coupling effects of PGs and water in regulating bone biomechanical properties. Lastly, age-related changes of PGs and GAGs, and their capability in water retention will be discussed with regards to the mechanisms contributing to deterioration of bone quality during aging.

### The roles of small leucine-rich proteoglycans in bone physiology

The GAG chains of PGs consist of a series of repeating disaccharide units, which contain one of two modified hexosamines, N-acetylgalactosamine (GalNAc) or N-acetylglucosamine (GlcNAc), and uronic acid (i.e., glucuronate or iduronate) with the exception of keratan sulfate that contains galactose instead of uronic acid [23]. According to the disaccharide unit type, GAGs can be classified as chondroitin sulfate (CS), dermatan sulfate (DS), keratan sulfate (KS), heparin and heparan sulfate (HS) and can carry sulfate at various positions. In addition, HA is the only GAG that is synthesized as single chains not bound on a core protein and is not modified by sulfate groups. The sulfated GAGs are anchored to their core proteins by unique linkages. The CS, DS and HS are attached via serine residues of core proteins by way of xylose. While KS is N-glycan linked to core protein through an asparagine residue or O-glycan linked via serine or threonine [23,24]. GAGs identity relays structural and functional properties to PGs. In the bone extracellular matrix, core protein and GAG chains of PGs directly bind to collagen fibrils and regulate collagen fibrillar diameter and formation [25,26]. Additionally, PGs bind to cytokines, growth factors, and cell surface receptors, which regulate cell proliferation, matrix deposition and bone remodeling [27,28].

The most abundant type of PGs in bone matrix are the small leucine-rich proteoglycans (SLRPs), which are characterized by relatively small protein cores (36–42 kDa) harboring 10–12 tandem leucine rich repeats. Based primarily on the nucleotide and protein sequence conservation, functional properties, and intron–exon organization, the SLRP gene family has expanded to 18 members classified into five distinct classes, including canonical (classes I–III) and non-canonical (classes IV and V) [16,19]. The core proteins of SLRPs are proposed to adopt a horseshoe shaped structure, which may promote interaction with other matrix components. Biglycan (Bgn), a

class I SLRPs, is distributed abundantly throughout the bone matrix [29]. It has two Ser-Gly sites in the N-terminal region and these two sites are used to attach two CS or DS chains. Pioneering work demonstrating the direct evidence of the biomechanical contribution of PGs/GAGs in bone has been described by Xu *et al*, using the *Bgn* deficient mouse model [30]. Mice with targeted disruption of *Bgn* exhibits age-dependent osteoporosis, with less trabecular volume and thinner cortices compared to WT counterparts, due to a decrease in bone formation. At 6 months of age, the four-point bending test of *Bgn* knockout (KO) mouse bone shows reduced values of failure load and yield energy, indicating impaired bone strength and ductility, respectively. These mice also exhibit structural abnormalities in bone collagen fibrils, with a marked variation in diameter [31]. The studies of the underlying cellular and molecular mechanisms reveal that *Bgn* KO mice have significantly diminished capacity to produce bone marrow stromal cells, and these cells are metabolically defective, with reduced response to exogenous transforming growth factor- $\beta$  (TGF- $\beta$ ), reduced collagen synthesis and increased apoptosis compared to wildtype (WT) littermates [32]. In addition, *Bgn* deficiency blunts bone morphogenetic protein-4 (BMP-4)-induced osteoblast differentiation *in vitro* [33]. The absence of *Bgn* decreases BMP-4 sensitivity caused by less BMP-4 binding and is completely rescued by viral transfection of *Bgn*. Another study highlights the importance of GAG chains of *Bgn* in modulating osteoblast differentiation, showing the expression of a mutant *Bgn* lacking GAGs binding sites fail to rescue the differentiation deficiency [34]. Furthermore, *Bgn* has been found to directly bind to BMP-2 and regulate BMP-2 induced osteoblast differentiation [35]. Apart from the TGF- $\beta$ /BMP signaling, *Bgn* is shown to trigger the Wnt pathway by binding to the canonical Wnt ligand Wnt3a as well as the Wnt receptor low density lipoprotein receptor-related protein 6 (LRP6). Both glycosylated and non-glycosylated forms of *Bgn* are shown to activate this signaling pathway [36,37]. *Bgn* also plays a crucial role in ERK phosphorylation and signal transduction through the transcription factor Runx2 [38]. This activation is mediated through the GAG chains, as phosphorylation of ERK is not observed when only core protein of *Bgn* is presented. Decorin (*Dcn*) also belongs to class I SLRP, carrying a single DS or CS GAG chain. *Dcn* was named because of its ability to decorate collagen fibrils, and is considered as *Bgn*'s closest related PG [39]. *Bgn* and *Dcn* are major PGs in the mineralized bone matrix. They share 57% identity at the amino acid level and are co-expressed in bone [40,41]. In agreement with the high level of expression of *Dcn* in the dermis, mice harboring a targeted disruption of the *Dcn* gene have fragile skin with markedly reduced tensile strength [42]. Although *Dcn* deficiency also introduces changes in collagen fibril size and shape

in bone, no major changes in bone mass or architecture are observed in these mice [31]. Due to the structural similarity of Bgn and Dcn, a compensatory mechanism is likely triggered when either is ablated, leading to an increased expression of the other, which could partially be explained by clustering of SLRP family members genes on their respective chromosomes [33,37,43]. Consistently, *Dcn/Bgn* double KO mice exhibit more severe skin and bone phenotypes than either single *Bgn* or *Dcn* KO models [31]. Compared to WT controls, skin tissue in *Dcn/Bgn* double KO mice is much thinner and fragile, and bone abnormalities develop earlier and affect both the cortical and trabecular bone mass as well as cranial suture fusion. These data imply a synergistic function between Bgn and Dcn. Bgn and Dcn are critical TGF- $\beta$ -binding PGs [44,45]. The absence of Bgn and Dcn prevents TGF- $\beta$  from appropriate sequestration within the extracellular matrix, leading to increased TGF- $\beta$  signaling in bone marrow stromal cells of *Dcn/Bgn* double KO mice. This is followed by a 'switch in fate' from growth to apoptosis, resulting in decreased osteoprogenitor cell numbers and reduced bone formation [46]. Thus, Bgn and Dcn appear to be essential for maintaining a proper number of mature osteoblasts by modulating the proliferation and survival of bone marrow stromal cells. Moreover, a recent report indicates that Bgn and another SLRP, fibromodulin, are coupling components and regulate osteoclastogenesis [47]. Fibromodulin is a class II SLRP with KS side chains, and is expressed in cartilage and bone cells during fetal endochondral and intramembranous ossification [48]. Single fibromodulin deficient mice develop ectopic bone in tendon, while the effect is exaggerated when both Bgn and fibromodulin are deleted, further suggesting the synergistic activities of SLRPs [49]. *Bgn/Fibromodulin* double KO mice have markedly low bone mass, which is explained by the direct binding of both Bgn and fibromodulin to tumor necrosis factor-alpha (TNF- $\alpha$ ) as well as receptor activator of NF- $\kappa$ B ligand (RANKL) [47]. The lack of these two SLRPs leads to elevated TNF- $\alpha$  and RANKL in the cellular microenvironment, resulting in enhanced osteoclastogenesis.

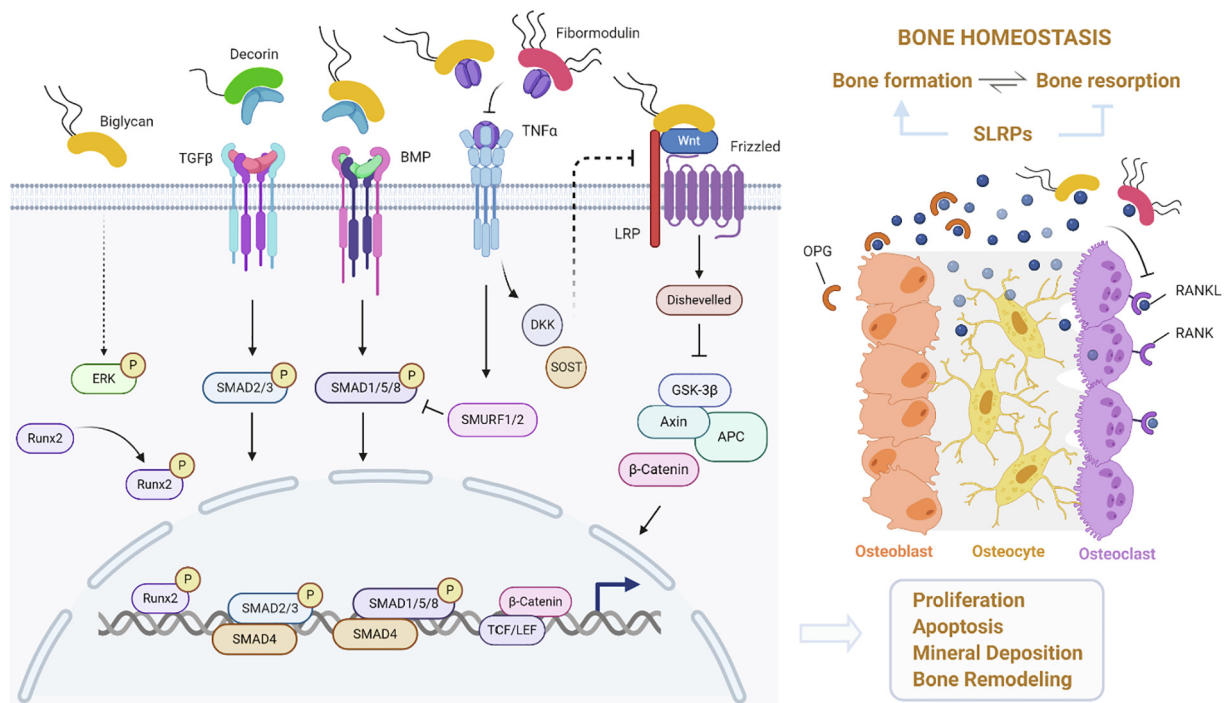
In addition to the SLRPs, heparan sulfate proteoglycans (HSPGs) can also participate in bone resorption regulation. Osteoprotegerin (OPG) is a decoy receptor for RANKL and is known to be a high-affinity HS-binding protein [50,51]. An *in vivo* study using knock-in mice with mutated OPG, which is incapable of binding to HS but binds RANKL normally, shows a severe osteoporotic phenotype similar to OPG-null mice [52]. The interaction of HS and secreted OPG immobilizes OPG at the cell surface and facilitates OPG binding to membrane anchored RANKL. In summary, the PGs in bone may regulate bioactivity and availability of multiple cytokines and growth factors, modulate bone formation and resorption,

and ultimately influence bone turnover process (Fig. 1).

### Coupling effects of proteoglycans and water in bone biomechanical properties

The negative charge of GAGs is conferred by acidic sugar residues and/or sulphate groups [24,53]. Some types of GAGs, such as chondroitin-6-sulphate, DS and KS could form homo- and hetero-aggregates [54]. These complex assemblies are stabilized primarily by electrostatic forces, hydrogen bonds and hydrophobic interactions. As a consequence, the charged matrix functions as a 'semi-permeable' membrane that attracts water to provide charge equilibrium, which makes them occupy an enormous hydrodynamic volume in solution [20,55,56]. It has been estimated that the large CS PG aggrecan may decrease 80% of their volume under dry conditions, highlighting the contributions of PGs and GAGs in absorbing water, and maintaining hydrostatic and osmotic pressure of the tissue [57]. Evidence is emerging that hydration status significantly affects bone strength and toughness [43,58,59]. Water comprises 15%-25% of the volume of bone and exists in three different compartments, including free water (also referred to as pore, or mobile water), bound water, and structural water [60,61]. Free water, which resides in the vascular space and the lacuno-canalicular network, can move according to pressure gradients that develop during movement of the skeleton. The amount of free water primarily reflects intracortical porosity, which is inversely proportional to the mechanical properties of bone [62,63]. Structural solid-like water is a part of the mineral lattice or is integrated into the tropocollagen ultrastructure [61]. Bound water may account for as much as 40%-60% of the total water in bone, arising from hydrogen bonding (collagen) and electrostatic attractions (mineral) with varying degrees of affinity, ranging from loosely to tightly bound to the matrix [64,65]. Tightly bound water is found within the collagen triple helix, which helps stabilize collagen. Loosely bound water localizes at the collagen-mineral interface. It has been suggested that water bound to the crystal surface helps orientate apatite crystals during biomineralization [66]. More importantly, loosely bound water allows load transfer between collagen and mineral, dissipating energy and reducing shear stresses at the interface, thus conferring ductility and plasticity to bone at bulk and ultrastructural levels [18,67]. Synchrotron X-ray scattering and other studies show that bound water residing in small ultrastructural spaces (<4.0 Å) dictates the mechanical characteristics of bone, and the loss of bound water correlates strongly with bone toughness deterioration [18,60,63,68].

Previous studies have used enzymatic digestion for GAG removal to investigate the influence of



**Fig. 1.** Schematic summary of the roles of small leucine-rich proteoglycans (SLRPs) in bone homeostasis. SLRPs interact with multiple cytokines, growth factors, and cell surface receptors, which are involved in key signaling pathways in regulating bone homeostasis. Decorin and biglycan are canonical class I SLRPs, with one or two chondroitin sulfate/dermatan sulfate chains attached to horseshoe-shaped core proteins, respectively. Fibromodulin belongs to class II SLRPs, with five binding sites for keratan sulfate chain. Biglycan promotes osteoblast differentiation through ERK phosphorylation and transcription factor Runx2. Decorin and biglycan bind TGF- $\beta$  and regulate the downstream SMAD2/3 signaling to control cell proliferation and apoptosis. Alternatively, biglycan binds BMP and activates SMAD1/5/8 phosphorylation to promote osteogenesis. In addition, biglycan promotes Wnt signaling and its downstream  $\beta$ -catenin to facilitate bone formation. TNF- $\alpha$  induces the expression of Dickkopf-1 (DKK-1) and sclerostin (SOST), which are inhibitors of Wnt signaling. Biglycan and fibromodulin bind TNF- $\alpha$  and RANKL, adjusting their bioavailability for bone resorption regulation by sequestering them in the cellular microenvironment. Figure created using BioRender (<https://biorender.com/>).

PGs and GAGs on the mechanical response of dental tissues. Chondroitinase ABC specifically degrades CS and DS by catalyzing the eliminative degradation of polysaccharides containing (1-4)- $\beta$ -D-hexosaminy and (1-3)- $\beta$ -D-glucuronosyl or (1-3)- $\alpha$ -L-iduronosyl linkages to disaccharides containing 4-deoxy- $\beta$ -D-gluc-4-enuronosyl groups. After GAG removal from the cementum-dentin junction using Chondroitinase ABC, the atomic force microscope (AFM)-based nanoindentation properties of the enzymatically treated specimens were compared to those from untreated tissues. Results suggest that the digestion of GAGs significantly decreases the hardness and elastic modulus of cementum-dentin junction [56,69]. A later study further verified the contributions of PGs and GAGs to the nanoindentation creep behavior of human dentin, showing their importance in the durability of dentin and likely other mineralized tissue [70]. Our previous novel findings suggest that PGs play a crucial role in toughening bone through their ability to retain water molecules in the matrix [71]. Cortical bone speci-

mens were prepared from the posterior mid-diaphyseal femur region of male human donors around 50 years old. The human cadaveric bone was treated with or without peptide-N-glycosidase F (PNGase F). The PNGase F enzyme with cleavage of N-glycosylated oligosaccharides can only enzymatically remove N-glycan of a minor subset of keratin sulfate PGs present in bone matrix. These PGs include fibromodulin, PRELP, and osteoadherin [48,72–74]. The nanoscratch test developed by Islam *et al* was utilized to measure the tissue-level toughness of bone specimens under both wet and dehydrated conditions [75]. The results show that removal of N-glycan linked GAGs has significant effects on the tissue-level toughness of wet bone samples. In contrast, the toughness of dry bone is not affected by GAGs removal, indicating that GAGs in bone play a pivotal role in sustaining the toughness of the tissue only when water is present. Since Bgn is a major subtype of PGs and is highly expressed in bone tissues [76–78], using the *Bgn*-deficient mouse model, we recently set

out to determine the specific role of Bgn in retaining bound water in bone matrix and maintaining bone toughness [79]. The amount of total GAGs and bound water determined by low-field nuclear magnetic resonance (NMR) are reduced significantly in the Bgn KO mice bone matrix, along with the reduction of bone toughness. However, such differences are diminished once the bound water is removed from bone matrix. Consistent with previous findings of the positive correlation between bound water and bone mechanical properties, a significant reduction of tissue-level toughness of bone in WT is observed after removal of bound water. These changes are significantly reduced in Bgn KO mice. These results suggest that the coupling effect of water and PGs in sustaining the toughness of bone is disrupted once Bgn is removed from the matrix.

Classically, CS is predominantly attached to Bgn and Dcn within mineralized tissues, such as dentine and bone, whereas DS is mainly associated with soft connective tissues, such as skin and ligament. It has been reported that the GAG chains of Bgn and Dcn change from DS to CS during the differentiation process of the cell proliferation phase to the mineralization phase [80]. Interestingly, treating human bone specimens with chondroitinase ABC or a protein deglycosylation mix, which could remove any type of GAGs from core proteins, results in comparable levels of GAG removal [81]. Moreover, agarose electrophoresis reveals that only CS band is identified in the mineralized compartment of human and mouse bone matrix, without noticeable presence of HS and DS [79,81]. These observations imply that CS is the major GAG in mouse bone matrix. It has been shown that CS intradermally injected *in vivo* could be incorporated into mouse tissues [82]. We have showed that supplementation of CS to WT mice increases the total amount of GAGs and bound water in bone matrix, the whole-body BMD, and the tissue-level toughness of bone [79]. However, CS administration does not improve the biomechanical properties of bone from *Bgn* KO mice. Additionally, bone histomorphometry studies suggest a decreased catabolic response in CS injected WT mice revealed by inhibited osteoclastogenesis. In fact, several previous reports have indicated the anti-catabolic function of CS by upregulating OPG production while decreasing RANKL expression, thus exerting a positive outcome for bone [83–85]. CS also has direct effects on inhibition of osteoclast differentiation, expression of TRAP and cathepsin K, and protects type I collagen from degradation by cathepsin K [86–88]. Lastly, a recent *in vivo* study using CS treatment for diabetic osteoporosis reveals an increased BMD, repaired bone morphology and decreased femoral osteoclasts [89].

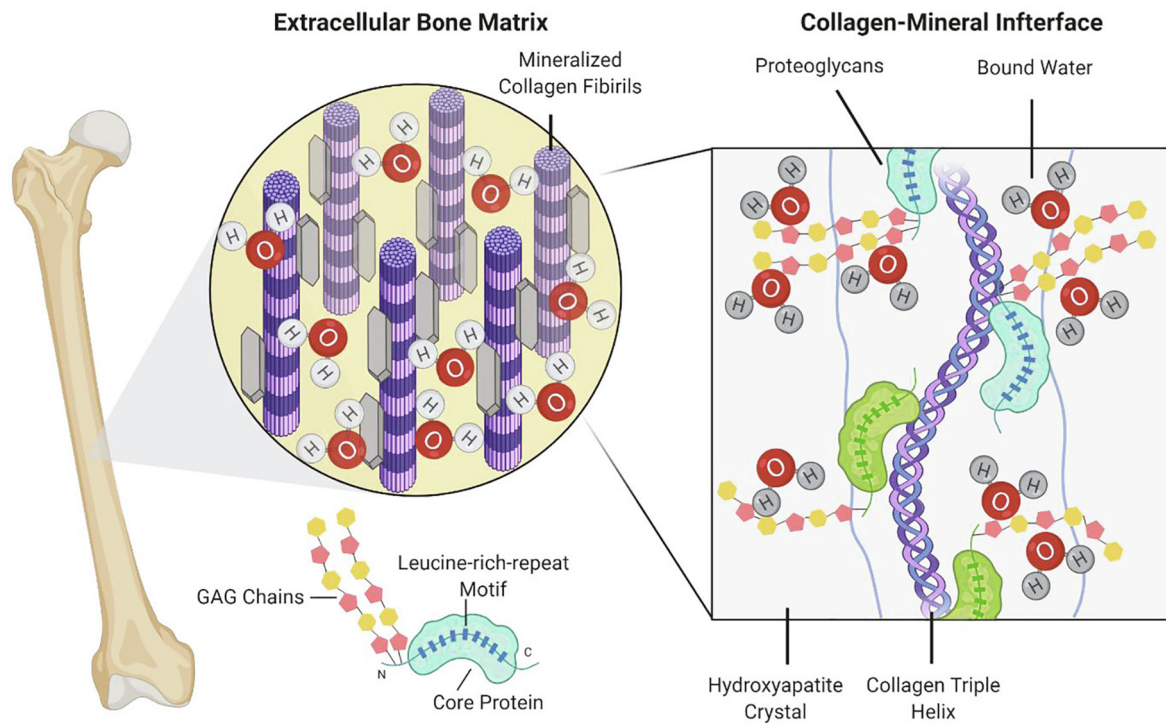
Taken together, PGs and GAGs, especially Bgn and CS play pivotal roles in retaining bound water in bone matrix, thus imparting toughness to bone

(Fig. 2). The administration of CS, possibly locally, could serve as a viable treatment strategy for improving bone quality.

### Changes of proteoglycans and water retention during aging

During the aging process, the loss of the delicate balance of bone homeostasis toward more bone resorption coupled with less bone formation leads to significant bone loss and changes in bone microarchitecture, including bone size and geometry. In addition, changes in the bone extracellular matrix material properties also occurs with aging. The loss of bone quantity and altered bone quality may weaken bones and culminate in osteoporosis with an increased risk of fractures [90].

The age-related changes in bone matrix affect different components in different ways, however, the effects in one component can have profound implications for changes of other components within the matrix, and ultimately for the deterioration of mechanical properties associated with aging [7,64]. For example, the changes of secretion and glycanation states of PGs may influence their binding to collagen. In human intervertebral discs and articular cartilage tissues, the glycanated forms of both Bgn and Dcn represent a greater proportion of the total proteoglycan population in juveniles, but the GAG-free forms become predominant with age [91,92]. A similar reduction in Dcn glycanation was further confirmed in cortical bone from aged WT mice [93]. Consistently, the GAG chains of fibromodulin are shortened with aging until the non-glycanated form becomes the predominant one [94]. These age-related changes of PGs can be a result of an increased rate of degradation of their glycanated precursors, or changes in growth factor/cytokine synthesis with aging [37,95]. In addition to PGs, the ratio of bound water and free water in bone matrix also changes during aging. The water that is bound to collagen and mineral declines with age, while free water increases with age as bone mass is lost [68,96]. There is also a re-distribution of water from the loosely bound fraction to the more tightly bound fraction [97]. These changes contribute to skeletal fragility by reducing the amount that bone can deform before fracturing, leading to age-related deterioration of bone toughness [64]. Interestingly, it has been found that loss of water in bone can initiate glycation reactions which cause the accumulation of advanced glycation end-products (AGEs). AGEs are intrafibrillar collagen crosslink-like bonds formed through a series of posttranslational modifications involving the condensation of arginine, lysine, and free sugars. AGEs increase naturally with age, and modify the physical properties of collagen fibers, including an increase in fiber diameter and stiffness, resulting in a loss of solubility and flexibility [98]. Alternatively,



**Fig. 2.** The schematic representation of proteoglycans (PGs) in attracting bound water in the extracellular bone matrix. Structurally, bone is comprised of mineralized collagen fibrils embedded in bone extrafibrillar matrix. PGs have high affinity to minerals and collagen. The glycosaminoglycan (GAG) side chains of PGs are highly negatively charged, thus play a pivotal role in retaining bound water at the collagen-mineral interface and maintaining hydrostatic and osmotic pressure of bone. The bound water allows mechanical load transfer between collagen and mineral, dissipating energy and reducing shear stresses at the interface, thus conferring ductility and plasticity to bone. Figure created using BioRender (<https://biorender.com/>).

increasing the stability of the collagen network by cross-linking could prevent the collagen from binding water [99]. Our recent findings indicate that the loss of water and reduction in toughness with age, may partly depend on changes of the PGs/GAGs in bone [81]. Human cadaveric bone specimens from male donors in three different age groups: young (aged  $26 \pm 6$  years), mid-aged (aged  $52 \pm 5$  years), and elderly (aged  $73 \pm 5$  years) were used in this study. There is a significant reduction (17%) of matrix GAGs in the elderly group compared to young human bone samples, accompanied by a 40% decrease in the overall bound water fraction and 25%-30% reduction in bone toughness. Correspondingly, we observed a decline of CS and Bgn in bone mineralized matrix with aging. Pearson correlation analysis suggests that the amount of bound water, GAGs, and the tissue-level toughness of bone are significantly correlated. This demonstrates that loss of GAGs has the potential to be one of the molecular origins of age-related deterioration of bone quality via reducing the amount of bound water. Existing evidence suggests that the receptor for AGEs (RAGE) functions as a receptor for CS GAGs, which is the major subtype of GAGs in bone [100]. Furthermore, the age-related accumulation of AGEs may negatively

modulate the synthesis of PGs [101,102], acting as a possible mechanism responsible for the loss of PGs/GAGs in the bone extracellular matrix during aging process. To summarize, the deterioration of bone toughness during aging could be through a deterministic combination of factors that include decreased GAGs/PGs, increased AGE accumulation, and the reduction of bound water, especially in the loosely bound fraction in bone matrix.

## Conclusions

Bone is a dynamic organ undergoing constant modeling and remodeling and has a highly hierarchical nature comprised of type I collagen fibrils embedded in a matrix of non-collagenous proteins, water, and a hydroxyapatite mineral phase. PGs are a group of structural non-collagenous proteins in the bone extracellular matrix. PGs and their GAG side chains form supramolecular aggregates that interconnect the collagenous network and maintain the hydrostatic and osmotic pressure of tissue. However, the participation of PGs and GAGs in biomechanics of mineralized tissues has been overlooked. The SLRPs are a family of the most abundant PGs in the bone matrix, including Bgn and Dcn. They

adopt specific structures for collagen fibril binding, and play vital roles in key signaling pathways, such as the TGF- $\beta$ , BMP-2/-4, TNF- $\alpha$ , and Wnt signaling, and modulating bone development and homeostasis. In addition, since GAGs are highly negatively charged, they are ideal candidates to attract water into the bone extracellular matrix. There is a strong correlation of hydration status with bone strength and toughness, and ablation or removal of GAGs from PGs results in loss of bound water. This is associated with reduction of bone biomechanical properties. Supplementation of CS, the major subtype of GAGs in bone matrix, increases the total amount of GAGs and bound water in the bone matrix, accompanied by improved bone toughness. Moreover, the reduction of GAGs and bound water also correlates with the deterioration of bone biomechanical characteristics during aging. In conclusion, PGs and GAGs, especially SLRPs, have molecular signaling functions that regulate bone remodeling, and are critical to the hydration status of bone, thus they contribute significantly to the biomechanical properties of bone.

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## 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.

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### Abbreviations:

ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; AS, aortic valve stenosis; BMP, bone morphogenetic protein; CVD, cardiovascular

disease; CKD, chronic kidney disease; CP, C-propeptide; CUB, complement, Uegef, BMP-1; DMD, Duchenne muscular dystrophy; ECM, extracellular matrix; EGF, epidermal growth factor; eGFR, estimated glomerular filtration rate; ELISA, enzyme-linked immunosorbent assay; HDL, high-density lipoprotein; HSC, hepatic stellate cell; HTS, hypertrophic scar; IPF, idiopathic pulmonary fibrosis; LDL, low-density lipoprotein; MI, myocardial infarction; MMP, matrix metalloproteinase; mTLD, mammalian tolloid; mTLL, mammalian tolloid-like; NASH, nonalcoholic steatohepatitis; NTR, netrin; PABPN1, poly(A)-binding protein nuclear 1; OPMD, oculopharyngeal muscular dystrophy; PCP, procollagen C-proteinase; PCPE, procollagen C-proteinase enhancer; PNP, procollagen N-proteinase; SPC, subtilisin proprotein convertase; TIMP, tissue inhibitor of metalloproteinases; TGF- $\beta$ , transforming growth-factor  $\beta$ ; TSPN, thrombospondin-like N-terminal

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