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# Proteins in Calcium Phosphates Biomineralization

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## Abstract

Biomineralization is a process of creating crystalline structures under biological control. The process takes place in hard tissues, such as bones, cartilages, and teeth. Biominerals are a combination of a crystal phase deposited onto an organic matrix. Inorganic components are mainly responsible for the biomineral hardness, while the organic matrix controls the shape, size, and polymorph of the crystals. Within the organic matrix, proteins exhibit a special biomineralization activity. Among them, one can distinguish insoluble collagen and soluble noncollagenous proteins. It is particularly noteworthy that noncollagenous proteins are intrinsically disordered proteins. High flexibility, acidic nature, and susceptibility to modifications make them especially adapted to the biomineralization control. This review paper is dedicated to the proteins which are involved in biomineralization of bones and teeth.

**Keywords:** biomineralization, bones, teeth, SIBLINGs, intrinsically disordered proteins, collagen

## 1. Introduction

Biomineralization is a process of formation of an inorganic solid within the biological system [1]. Biominerals are organic-inorganic composites, which fulfill various biological functions. In vertebrates, hard tissues provide body support, take part in tearing food, protect organs, and are reservoirs of calcium and phosphate. Understanding of molecular basis of biomineralization is essential to obtain new biomaterials.

Human hard tissues are formed of calcium orthophosphates [2]. Among them, most important are amorphous calcium phosphate, octacalcium phosphate, calcium hydrogenphosphate dihydrate, and calcium-deficient apatite and hydroxyapatite (HA) [3]. In organisms, calcium orthophosphates occur mainly in the form of poorly crystallized nonstoichiometric sodium-, magnesium-, and carbonate-containing HA—so-called biological apatite [2–5].

Biomineralization is a multistep process, which requires using the structures of extracellular matrix vesicles, numerous enzymes and glycoproteins. Due to strict control, biominerals differ from pure chemical minerals [6]. In contrast to geological minerals, biominerals are composite materials comprised of both inorganic and bioorganic components. The mineral constituent gives tissues hardness and resistance to mechanical damage. Stiffness of the tissue depends on the amount of inorganic components and organic phase [7]. About 70% of bone tissue is made up of mineral structure, while the rest is water and organic substances [1]. Moreover,

Protein name	Accession number	pI	Mass (kDa)	Overall percent disordered—PONDR
Osteopontin	AAA59974.1	4.4	~34	70%
Bone sialoprotein	AAA60549.1	4.1	~35	59%
Matrix extracellular phosphoglycoprotein	AAK70343.1	8.6	~58	64%
Dentin matrix protein-1	AAC51332.1	4.0	~56	90%
Dentin sialophosphoprotein (preproprotein)	NP_055023.2	3.6	~131	88%
Ameloblastin	AAG35772.1	4.9	~48	58%
Amelogenin	AAC21581.1	6.5	~22	65%

Bioinformatic predictions of a disordered structure in proteins were done with PONDR predictor (<http://www.pondr.com/>) [13].

**Table 1.**  
*Proteins involved in bone and teeth biomineralization.*

having formed *in vivo* under well-controlled conditions, biomineral phases often have properties, such as shape, size, crystallinity, isotopic, and trace element compositions, quite unlike their inorganically formed counterparts [2].

The key factors, which determine the size, shape, internal structure and properties of biominerals, are proteins, which control the nucleation and growth of the crystals. Biomineralization involves protein-protein interactions and interactions between proteins and inorganic fraction. Among them, two major groups can be distinguished. Scaffold for a growing mineral phase provides insoluble collagen. In human bones, collagen makes up to 20–30% wt. Nucleation and crystal growth regulation are controlled by soluble, noncollagenous proteins. They are usually highly acidic [8, 9], undergo extensive posttranslational modifications [10], and frequently belong to the group of intrinsically disordered proteins (IDPs) [11]. IDPs are dynamic, flexible, heterogeneous populations of molecules without a well-defined folded structure. The highly charged character, along with the low content of hydrophobic amino acid residues, results in strong electrostatic repulsion and the lack of a well-packed hydrophobic core [12]. High content of IDPs’ carboxyl and phosphate acidic groups involved in biomineralization results in high calcium binding capabilities. Examples of proteins engaged in bones and teeth biomineralization are shown in **Table 1**.

## 2. The basic division of proteins involved in the formation of calcium phosphate biominerals

Bone and teeth biomineralization takes place within the extracellular matrix (ECM), outside the cells. ECM is a highly organized and complex structure, unique to the specific organ type. It is an organic, noncellular fraction of mineralized tissues, composed of structural and functional proteins [14]. The content of the organic fraction varies depending on the type of tissue and constitutes 30% of bone, 20–25% of dentin, and only 0.5% of mature enamel. The basic division of proteins involved in the formation of calcium phosphate biominerals includes insoluble matrix like collagen and soluble noncollagenous proteins (NCPs).

### 2.1 Collagenous proteins of ECM

Among organic phase components, there are insoluble substances, which include structural macromolecules, creating a scaffold for the growing mineral.

They give shape to the resulting crystal and can act as initiators for the creation of crystallization roots. An example of such substance is collagen. Animal tissues primarily contain collagen type I or collagen type II [15]. Collagen is the major ECM molecule that self-assembles into cross-striated fibrils, provides support for cell growth, and is responsible for the mechanical resilience of connective tissues. The term “collagen” defines a whole family of glycoproteins. The most common motifs in the amino acid sequence of collagen are repeating sequence [Gly-X-Y]<sub>n</sub>, both with and without interruptions. The X and Y positions are occupied by proline and its hydroxylated form, hydroxyproline, respectively. The right-handed triple helix is formed from three left-handed polyproline  $\alpha$ -chains of identical length, which gives collagen a unique quaternary structure [16]. Hydroxylation of the rest of the proline in collagen is necessary for the stabilization of the triple helix [17]. Collagen chains create interactions leading to the organization of chains in a four-stage array [18]. Collagen fibers are also cross-linked via lysyl residues [19]. Electron microscopy techniques allow to observe successive regions of overlap and gaps, resulting from the mutual arrangement of collagen fibers [20].

## **2.2 Noncollagenous proteins (NCPs) of extracellular matrix (ECM)**

While structural collagen is the main protein of ECM, NCPs are important for the regulation of biomineralization. NCPs are usually acidic, capable of binding large amounts of calcium ions. Proteins, which directly influence biomineralization, act as nucleators and regulators of crystal growth and orientation [21]. NCPs are frequently posttranslationally modified, e.g., phosphorylated, glycosylated, or proteolytically processed. A large number of noncollagenous proteins have disordered secondary and tertiary structures and belong to a group of intrinsically disordered proteins [11].

## **3. Intrinsically disordered proteins in calcium phosphate biomineralization**

IDPs are a group of proteins that have gained a special interest of researchers for over 20 years [12, 22, 23]. Their discovery was especially challenging for traditional protein structure paradigm, stating that protein function depends on a fixed tertiary structure. The IDP group includes proteins with altered secondary and tertiary structures from total random coil structure to some intrinsically disordered regions. They are characterized by considerable plasticity, flexibility, and high conformational dynamics. IDP features are high net charge and low hydrophobicity, which is not conducive to the formation of the hydrophobic core. They often bind low molecular ligands and macromolecules such as ions and proteins. By interacting with ligands, IDPs can undergo local or global structuring. They can also fulfill their functions while remaining completely disordered. Other characteristics of this group are multiple amino acid repeats and susceptibility to posttranslational modifications. An open structure and lack of a packed core increase the availability of potential phosphorylation, glycosylation, and proteolytic cleavage sites [24].

Extended, flexible structure, highly acidic nature, susceptibility to modifications and especially the ability to interact with many different partners make these proteins particularly adapted to the biomineralization control. Many NCPs belong to IDP group. Family of small integrin-binding ligand N-linked glycoprotein (SIBLING) is the example of IDPs engaged in HA formation and is presented below. In addition to SIBLINGS, enamel matrix proteins such as ameloblastin, amelotin,

and enamel also have disordered structures [25]. More information about the proteins involved in HA biomineralization belonging to IDPs can be found in reviews [11, 25, 26].

### 3.1 SIBLINGs

An example of NCPs is the family of small integrin-binding ligand N-linked glycoprotein (SIBLING). It is a group of proteins identified in bone, dentine, and cementum, which includes osteopontin (OPN), bone sialoprotein (BSP), dentin matrix protein-1 (DMP1), dentin sialophosphoprotein (DSPP), and matrix extracellular phosphoglycoprotein (MEPE) [27]. It is believed that they all evolved as a result of gene duplication from extracellular calcium-binding phosphoprotein family. Although proteins differ in composition of amino-acid sequence, SIBLINGs share common features [28]. Their genes are located in a 375 kb region on the 4q21 human and 5q mouse chromosomes. They display a similar exon structure. Their sequence contains an Arg-Gly-Asp (RGD) motif, which mediates cell attachment/signaling by binding to cell-surface integrins [28]. SIBLINGs belong to IDP group and are highly acidic. They share numerous sequence repeats, which are often observed in the case of IDPs [26]. Particularly important for their biomineralization activity may be acidic serine- and aspartate-rich motifs (ASARM), which are involved in the phosphate administration [29, 30]. SIBLINGs are frequently posttranslationally modified. Some of their biological functions depend on phosphorylation, glycosylation, proteolytic processing, sulfonation, or transglutaminase cross linking [31].

#### 3.1.1 Osteopontin

Osteopontin (OPN), also known as secreted phosphoprotein 1 (SSP1), is a highly phosphorylated and glycosylated sialoprotein. It is a multifunctional protein expressed by several cell types including osteoblasts, osteocytes, as well as osteoclasts or odontoblasts [32]. OPN undergoes many posttranslational modifications such as phosphorylation, glycosylation, sulfonation, or proteolytic processing, and modifications vary depending on a protein role and localization [31]. In bones and dentin, osteopontin is located at the site of biomineral formation. Synthesis of OPN is stimulated by calcitriol, physiologically active form of vitamin D, which is known as a trigger for bone destruction and remodeling [33, 34].

Osteopontin was originally identified as a bridge between the cell and HA in ECM of bone [35]. It is known that OPN increases the adhesion of bone cells by concentrating in mineralized collagen matrix during bone formation and remodeling [36, 37]. The protein is highly produced by developing osteoblasts and osteoclasts, and it has been shown to regulate both cell type adhesions.

Another role of OPN during biomineralization is modulation of osteoclastic function. OPN binding to integrin  $\alpha\beta3$  is essential for regulation of osteoclastic activity and is necessary in the formation of a sealing zone [34]. Through CD44-associated cell signaling, OPN stimulates osteoclast migration [38].

As a highly acidic phosphoprotein, OPN binds to the surface of hydroxyapatite crystals through the electrostatic interactions between crystals and carboxyl and phosphate groups. Furthermore, disordered structure of OPN can promote the binding of calcium ions. The results of *in vitro* and *in vivo* studies suggest that osteopontin has an inhibitory effect on HA formation and growth [39, 40]. OPN knock-out mice display increased mineral content and size [41]. Therefore, it is suggested that OPN is one of the proteins whose role is to prevent crystal formation in soft tissues. Peptides of OPN obtained after proteolytic processing differ in a biomineralization effect. ASARM peptide of OPN binds to HA crystals and consequently



inhibits ECM matrix mineralization [42]. Its inhibition activity is dependent on serine residues' phosphorylation. N- and C-terminal fragments of OPN from milk promote *de novo* HA formation, but at the same time, the central fragment showed an inhibitory effect [43].

### 3.1.2 Bone sialoprotein

Bone sialoprotein (BSP) is one of the most abundant NCPs of bone. In contrast to OPN, BSP is only localized in tissues that undergo mineralization: bone, dentin and mineralizing cartilage, and cementum [44]. The protein is produced by osteoblasts, osteocytes, osteoclasts, as well as by chondrocytes. Disordered structure of BSP was shown by NMR, CD, and SAXS studies [28, 45–47]. BSP is highly glycosylated especially at C-terminal fragment, and to a lesser extent also phosphorylated and sulfonated [31]. N-terminal region of the protein contains collagen-binding motif. *In vivo* and *in vitro* studies indicated that BSP can be involved in initial stages of hydroxyapatite biomineralization [48]. BSP is intensely expressed by osteoblastic cells in sites of primary bone formation. The protein is present at mineralizing boundaries of bone, dentin, and calcifying cartilage tissues. On the other hand, BSP increases osteoclastogenesis, and in that way, it can initiate bone remodeling. BSP knockout mice are characterized by short, hypomineralized bones with high trabecular bone mass [49, 50]. Overexpression of BSP leads to dwarfism, decreased bone mineral density, and decreased trabecular bone volume [51].

It was shown that BSP has high affinity to hydroxyapatite and acts as a *de novo* nucleator of HA crystals *in vitro* [52]. The protein also binds to type I collagen, and by interaction with collagen, BSP could regulate HA nucleation [53]. Moreover, control of mineral formation is highly associated with the state of phosphorylation, sulfonation, and glycosylation of BSP [27, 54, 55].

When creating skull bones, including the mandible, alveoli, and skull vault, the osteoprogenitor cells secrete a collagen extracellular matrix—osteoid. Skull bones are formed by intracerebral ossification. BSP affects biomineralization of the matrix in the ossification process, hence, the formation, shaping, and growth of hydroxyapatite crystals. In the absence of BSP, bone formation is delayed and osteoblast activity is impaired [56].

### 3.1.3 Matrix extracellular phosphoglycoprotein

Matrix extracellular phosphoglycoprotein (MEPE) is located in mineralized tissues as bone and dentin, but was found also in nonmineralized organs. The protein is primarily expressed by osteoblasts and osteocytes that are embedded within the mineralized matrix in bone and by odontoblasts during odontogenesis [57]. Similar to osteopontin, MEPE seems to be a multifunctional ECM protein. Results of *in vivo* and *in vitro* studies suggest that MEPE is important for bone mineralization, Pi homeostasis and cell attachment [58–60]. The protein was originally identified as interacting with PHEX. PHEX by binding to MEPE protects the protein from proteolytic cleavage by cathepsin B. Cleavage by cathepsin B leads to the release of the ASRAM peptide. The ASRAM motif is a functional domain of MEPE responsible for its inhibitory activity [61]. The peptide may be responsible for phosphate and calcium regulation during the mineralization process. ASARM peptide inhibits hydroxyapatite mineralization by binding free calcium with high avidity, and their inhibitory effect is highly dependent on serine residues phosphorylation. AC100, another MEPE fragment, containing the integrin binding (RGD) and glycosaminoglycan-attachment (SGDG) motifs stimulates new bone formation *in vitro* and *in vivo* [62].

### 3.1.4 Dentin matrix protein-1

Dentin matrix protein-1 (DMP1) was the first molecule identified by cloning from the dentin matrix. Besides dentin, the protein is located also in bone and cementum as well as in other nonmineralized tissues [63–65]. It is remarkably acidic and is a hydrophilic protein with many serine, aspartate, and glutamate residues. The protein is characterized by disordered structure that can aggregate in the presence of calcium ions [66].

It was shown that DMP1 is essential for both odontoblasts and osteoblasts maturation. The protein controls odontogenesis, osteogenesis, and Pi homeostasis. The effect of DMP1 on HA biomineralization depends on posttranslational modifications. It was shown that phosphorylated DMP1 inhibits HA formation and growth, while the dephosphorylated form acts as a HA nucleator [67, 68].

It was demonstrated that DMP1 is proteolytically processed into N-terminal 37-K fragment and 57-K fragment from the C-terminal region, and it is likely that full-length DMP1 is a precursor to these functional fragments [69]. It seems that the 57-K fragment plays a key role in the biomineralization process. The 57-K fragment contains 41 phosphate groups, while the 37-K fragment has only 12 phosphate groups. In addition, the 57-K fragment contains many functional sequences and domains such as the RGD motif [70], the ASARM peptide [71], and the peptide functioning as nucleator [72, 73]. In contrast to the full-length DMP1, the 57-K fragment promoted HA nucleation and growth [66]. Furthermore, it was shown that DMP1 N-terminal fragment is able to stabilize the metastable amorphous calcium phosphate. Due to high aspartic acid residual content, the 37-K fragment can bind calcium ions very strongly favoring formation and stabilization of the amorphous nuclei [72]. It was shown that the DMP1 57-K fragment also controls calcium carbonate mineralization *in vitro* [74].

### 3.1.5 Dentin sialophosphoprotein

Dentin sialophosphoprotein (DSPP) was discovered by cDNA cloning using a mouse odontoblast cDNA library and was the first believed to be connected only with dentin. Subsequent studies have revealed that the protein is expressed also in bone, cementum, and some nonmineralized tissues [75, 76]. Analysis of *DSPP* knockout mice and mutation in the *DSPP* gene indicated a special role of protein in dentin mineralization. In humans, a mutation in the *DSPP* gene causes dentin hypomineralization and significant tooth decay, named dentinogenesis imperfecta [77]. Additionally, studies of *DSPP*-null mice suggest that DSPP is crucial in the initial mineralization of bone as well as in the remodeling of the skeleton and therefore on bone turnover [78].

It is suggested that DSPP is a precursor protein activated after proteolytic processing. Cleavage of DSPP by zinc metalloproteinase bone morphogenetic protein-1 (BMP1) results in two protein fragments: dentin phosphoprotein (phosphophoryn) (DPP) and dentin sialoprotein (DSP) [79]. Subsequently, cleavage of C-terminal of DSP by matrix metalloproteinase 2 (MMP2) and MMP20 leads to the release of the third fragment called dentin glycoprotein (DGP), which has a strong affinity to hydroxyapatite [80].

Highly acidic DPP is the most abundant NCP of ECM in dentin. Amino acid sequence of DPP is composed mainly of aspartic acid and serine residues, whereas ~80% of serine residues are phosphorylated and phosphorylation is crucial to its function. The protein is also glycosylated [81] and contains ASARM peptide. Due to its amino acid composition and phosphorylation, DPP binds large amounts of calcium ions with high affinity. The protein is involved in nucleation and control of

the formation and growth of HA crystals during dentin mineralization [82, 83]. DPP binds to collagen fibrils and is present in front of dentin mineralization [84, 85]. There is a hypothesis that in front of mineralization, DPP is promoting the formation of initial apatite crystals [86]. When predentin is converted to dentin, DPP with other proteins binds to the growing HA faces and inhibits or slows down crystal growth [86–88].

## **4. Other proteins specific to teeth or bones**

### **4.1 Tooth**

Dentin, enamel, and cementum are calcified tissues and are major components of teeth. Dentin is usually covered by enamel on the crown and cementum on the root and surrounds the entire pulp. About 70% (wt) of dentin consists of the mineral phase, 20% (wt) is an organic material, and 10% (wt) is water. Enamel is the hardest substance in the human body and contains 96% (wt) of mineral phase. Enamel and dentin are created by two layers, namely odontoblasts and ameloblasts. Dentin is characterized by a high content of collagen I comprising ~90% of the dentin organic matrix [89].

#### *4.1.1 Enamel*

The organic part of enamel consists mainly of hydrophobic proteins, known as amelogenins and anionic proteins, which include ameloblastin, enamelin, and tuftelin [90]. Enamel also contains sialophosphoproteins and enamel proteases such as matrix-20 metalloproteinase (MMP-20, matrix metalloproteinase-20, also known as enamelysin) and kallikrein 4 (KLK4). At the end of the crystallization, enamel loses almost entire organic matrix, which is degraded and replaced by hydroxyapatite crystals [90]. Residues, small peptides, and amino acids account for only 1% of enamel and do not resemble the original matrix [90].

Amelogenins are a group of heterogeneous proteins present in odontoblasts and identified in enamel of maturing teeth. They are the main proteins of the organic enamel matrix, constituting over 90% of the pool of proteins secreted by ameloblasts into the intercellular matrix during the formation of enamel. These are hydrophobic proteins rich in proline, glutamine, leucine, and histidine. Amelogenins isolated from various organisms are characterized by high homology [91]. Amelogenins belong to IDP groups; conformational analysis by CD and NMR spectroscopy showed that recombinant porcine amelogenin rP172 exists in an extended, unfolded state in the monomeric form [92]. The extended, labile conformation of rP172 amelogenin is compatible with known functions of amelogenin in enamel biomineralization, i.e., self-assembly, associations with other enamel matrix proteins and with calcium phosphate biominerals, and interaction with cell receptors [92].

Amelogenin controls the organization and growth of enamel crystals, and its presence is critical for normal enamel formation. Defects in amelogenin sequence lead to defective enamel crystal formation and organization [93].

Ameloblastin, also known as amelin, is a tooth-specific glycoprotein. Unlike amelogenin, ameloblastin is located close to the cell surface [94]. Ameloblastin accounts for about 5–10% of all proteins present in the enamel, and it is the second most abundant protein among the intercellular matrix of the enamel [95]. Ameloblastin is also synthesized in dentin and cement, but its role in these tissues is not determined. Ameloblastin is a molecule of cell adhesion required to maintain a



diversified state of ameloblasts [96]. Bioinformatic analyzes and molecular modeling of the protein structure suggest that it may belong to IDPs [97], which was confirmed by the CD spectra of recombinant mouse protein [98].

The N-terminal fragment of ameloblastin comprising the self-assembly motif was shown to colocalize with amelogenin across the entire growing enamel, indicating the role of the two proteins in the organization of the linear growth of HA crystallites [99–101]. Both proteins self-assemble into higher order structures from monomeric subunits, similar to type I collagen, the predominant matrix protein of bones, and dentin [102, 103]. Full-length amelogenin undergoes hierarchical step-wise assembly, first forming oligomers, which in turn assemble into higher order structures and stabilize mineral prenucleation clusters, and organize them into parallel arrays of linear chains, yielding the formation of crystallite bundles [102]. There is a hypothesis that the higher order structures of the self-assembled ameloblastin (or most likely its N-terminal moiety) contribute to the oriented growth of the linear chains of amelogenin in the 3D space [104].

#### *4.1.2 Dentin*

Dentin forms a support for hard tissues of a tooth. It covers both crown and root structures and is responsible for its shape. The formation of dentin is closely related to bone. Both bone and dentin are composed of a collagenous matrix and a mineral phase with hydroxyapatite plate-like crystals. Mineralization of dentin and bone extracellular matrix is initiated with the aid of matrix vesicles and later involves secretion of families of a specialized matrix protein [105]. In contrast to bones, dentin is not remodeled and does not participate in the regulation of calcium and phosphate metabolism. The tooth formation takes place throughout the lifetime of a tooth. Unlike enamel, dentin mineral content increases with age due to continuous mineral deposition either as physiological secondary dentin or as tertiary dentin following injury [105].

Dentin is composed of a collagen I, which accounts for about 92% of organic components. In addition to collagen, there is also a group of noncollagen proteins, which include dentin-specific phosphoryls (DPPs) and dentin sialoproteins (DSP), sialic acid, osteopontin, dentin matrix proteins (DMP1, DMP2, DMP3), BSP, acidic bone-75 glycoprotein, osteonectin, and proteins rich in  $\gamma$ -carboxyglutamic acid. Among organic components should also be mentioned proteoglycans, growth factors, phospholipids, and enzymes [106, 107]. Inorganic dentin components make up to about 70% of the tissue mass, making the dentin harder than the bone. The mass of a single hydroxyapatite crystal is about 10 times greater than in bone, but many times less than in enamel, and its size is about  $35 \times 10 \times 100$  nm [108].

#### *4.1.3 Cementum*

Cementum protects the dental root dentin with a very thin layer. In many respects, it is very unique: it is not vascularized and is not innervated, it does not undergo constant remodeling like a bone, but it grows all the time. Cellular and acellular cement are distinguished based on the presence of cementocytes in its structure. The structure and composition of cement resembles bone tissue. Inorganic constituents make up to about 65% of tissue mass and consist mainly of hydroxyapatite crystals [109, 110].

Cementum provides contact between roots of the teeth and periodontium ligaments [111]. hrCEMP1 (recombinant human cementum protein 1) is an isolated form of cementum human  $\beta$ -sheet protein. It has been showed that hrCEMP1 forms clusters of 6.5 nm diameter [111]. hrCEMP1 is an inducer of specific

biomineralization proteins. It stimulates the differentiation of osteoblasts and cementoblasts. It is suggested that hrCEMP1 plays a significant role in octacalcium phosphate biomineralization and helps its binding to hydroxyapatite even without posttranslational modifications. X-ray diffraction measurements showed that hrCEMP1 is an inducer of polymorphic crystals [111].

## 4.2 Bone

The matrix of bone consists of type I collagen molecule self-assembled into a triple helix consisting of two  $\alpha 1$  and one  $\alpha 2$  chains (ca. 300 nm in length and 1.5 nm in diameter) and hydroxyapatite ( $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ) nanocrystals (plate-shaped, 1.5–4 nm in thickness) deposited along collagen fibrils [112, 113]. The collagen fiber does not undergo spontaneous mineralization in the presence of phosphate and calcium ions [20], and the involvement of noncollagen proteins is necessary. The majority of these proteins belong to the SIBLING family described above [27].

Bones and dentin are characterized by a similar composition and the mechanism of their formation [112]. Their organic matrix consists of type I collagen and the mineral matrix built of hydroxyapatite. Osteoblasts and odontoblasts, cells involved in osteogenesis and dentinogenesis, first secrete a nonmineralized matrix—a bone osteoid, and in case of dentin—predentin and then with fibers of collagen I form matrix for biomineralization [112]. Calcium and phosphate ions are dislocated from the vascular network into the mineralization matrix. Osteoid and predentin contain numerous noncollagenous proteins called NCPs. On the basis of mutation experiments and suppression of NCP genes, it is suggested that they stimulate the nucleation and growth of hydroxyapatite crystals [112].

Bones and dentin differ significantly. Bone is a dynamic tissue because it is constantly remodeled, while dentin is a rather static tissue [112]. Osteoblasts produce matrix components as a result of controlling growth factors to form a bone. Hydroxyapatite crystals grow above vesicles, thus creating mineralization centers. Bones have a lot of vessels, and they store calcium ions. The reason for bone resorption is the action of lysosomal enzymes produced by osteoclasts and lowering the pH in extracellular bone matrix.

Osteoclasts are responsible for the attachment, bone degradation, and subsequent tissue resorption. It was found that this is possible due to the strong polarization of osteoclasts. They have both frontal and posterior abdominal polarization. On the underside of the osteoclasts, there are structures responsible for the degradation of the mineral surface. The adhesion zone is located in the sealing zone [114]—a ring made of actin and podosomes in which extracellular resorption pit is segregated and is used to retain heavy substances (minerals) formed during bone resorption near the bone surface [43, 114]. Podosomes are dynamic structures built of actin. They perform important functions, including adhesion, destruction of the bone and its matrix, and recognition of the appropriate medium [115]. Podosomes contain a large number of V-ATPase molecules that pump protons and secrete proteases, which promote bone degradation [114].

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## **Conflict of interest**

Authors declare no conflict of interest.

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