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The mechanism of biomineralization: Progress in mineralization from intracellular generation to extracellular deposition

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A B S T R A C T

Biomineralization is a highly regulated process that results in the deposition of minerals in a precise manner, ultimately producing skeletal and dental hard tissues. Recent studies have highlighted the crucial role played by intracellular processes in initiating biomineralization. These processes involve various organelles, such as the endoplasmic reticulum(ER), mitochondria, and lysosomes, in the formation, accumulation, maturation, and secretion of calcium phosphate (CaP) particles. Particularly, the recent in-depth study of the dynamic process of the formation of amorphous calcium phosphate(ACP) precursors among organelles has made great progress in the development of the integrity of the biomineralization chain. However, the precise mechanisms underlying these intracellular processes remain unclear, and they cannot be fully integrated with the extracellular mineralization mechanism and the physicochemical structure development of the mineralization particles. In this review, we aim to focus on the recent progress made in understanding intracellular mineralization organelles' processes and their relationship with the physicochemical structure development of CaP and extracellular deposition of CaP particles.

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1. Introduction

Biomineralization is a distinctive and vital characteristic of the vertebrate skeletal and dental systems, playing a pivotal role in biological growth, development, and evolution [1]. It is a precisely regulated physiological process, in which stable calcium phosphate

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deposits within and outside the collagenous fibrous network, forming bone, teeth, and other hard tissue structures that are capable of bearing tremendous loads and protecting complex body structures. Biomineralization disorders can cause numerous related diseases, such as osteoporosis [2], enamel hypomineralization or hypoplasia [3], dentin hypoplasia [4], and apatite-deposition osteoarthritis [5]. Moreover, pathological calcification, including atherosclerosis and heart valve calcification, is caused by heterotopic mineralization in blood vessels and connective tissue [6].

The study of biomineralization initially focused on the observation of extracellular matrix mineral ions forming stable hydroxyapatite (HA) on collagen fibrils. However, intracellular biomineralization was not recognized until the 1960 s, when Anderson et al. [7] and Bonucci et al. [8] discovered that the earliest mineral deposits were in contact with membrane-bounded vesicles. These vesicles contained high specific activities of alkaline phosphatase and other phosphatases [9] and can also bind Ca^{2+} [10], suggesting that biomineralization occurs in vesicles initiated from intracellular structures. Nonetheless, because the initially formed mineralization is unstable, it is readily lost or misplaced during experimental sample dehydration preparation, which limits the observation of intracellular biomineralization processes. With the advancement of experimental techniques such as cryo-electron microscopy (cryo-EM) and Atomic Force Microscopy (AFM) to observe the mineral phase and its dynamic process, as well as X-ray diffraction (XRD) and Fourier Transform infrared spectroscopy (FTIR) to study crystal structure, new insights into intracellular mineralization have been obtained.

However, the physiological process of biomineralization remains incompletely understood. Firstly, there are still obstacles in combining intracellular organelle behavior of calcium phosphate (CaP) particles at different periods of mineralized physicochemical structure with their extracellular deposition. Secondly, the precise regulatory mechanisms within different organelles have yet to be elucidated, and the mechanism of selective promotion or inhibition of intracellular mineralization under different conditions has not been fully explained.

This review will focus on the step-by-step process of biomineralization, starting from mineralization intracellular formation in different organelles and ending with secretion into the extracellular matrix and further maturation. The review will be structured into several stages, including:

1. Structural changes from initial mineralization formation to stability.
2. The initial formation of minerals – from the endoplasmic reticulum to mitochondria.
3. The nucleation of minerals – from mitochondria to intracellular vesicles.
4. Further accumulation of minerals – from vesicles to extracellular environment.
5. The formation of stable mineralization – further extracellular mineralization.

2. Structural changes from initial mineralization formation to stability

Hard tissue consists of intricately intertwined organic soft tissue and rigid inorganic minerals arranged in various hierarchical multiscale structures. The principal constituent of fully developed hard tissue's inorganic minerals is hydroxyapatite, renowned as one of the most enduring forms of CaP particles [11]. Through the process of nucleation, CaP particles gradually form and become stabilized by the systematic assimilation of global calcium ions (Ca^{2+}) and hydroxide ions (OH^-). The biological state of CaP particle stability can be elucidated by the progressive crystal structure of CaP, as evidenced by selected-area electron diffraction patterns exhibiting characteristic HA (002), (004) and the merged (112), (211), (300), (202) diffraction arcs, which are systematically deposited amidst organic components, such as collagen fibers [12–14].

The fundamental aspect of the early formation of solid CaP particles during biomineralization is amorphous calcium phosphate (ACP). ACP is a soluble and highly substituted apatite with a noncrystalline texture when examined by transmission electron microscope (TEM). There is no discernible electron diffraction pattern [15], and X-ray diffraction reveals a wide-angle broad and diffuse pattern [16,17]. ACP has a Ca/P ratio of 1.5 or less [18]. The investigation conducted by Posner et al. first proposed the presence of ACP in the mineralization process, and it consists of $Ca_9(PO_4)_6$ [19], which has been further confirmed by various research [20,21]. ACP is widely present in various tissues of vertebrate organisms, such as newly formed tooth enamel of mice, fin bones of zebrafish, embryonic chicken long bones, and early intramembranous mineralization of murine calvarian tissue [22–25].

ACP is a crucial link between intracellular osteogenesis and extracellular mineralization deposition. Evidence has shown that ACP is gradually formed from calcium and phosphorus ions and transformed [26] to apatite under physiological conditions [21,27,28]. However, due to the instability and small size of these mineralization precursors, the process of ACP formation and transformation is difficult to observe precisely, and it has been a conundrum for decades.

Regarding the formation of ACP, Habraken [29] et al. provided a detailed explanation of the early physiological formation and evolution of ACP in the mineralization process. They proposed that the aggregation of Ca^{2+} and HPO_4^{2-} is the key to ACP formation. These ions first form calcium triphosphate ion-association complexes, which are called pre-nucleation complexes (cluster). Then, they aggregate into branched three-dimensional (3D) polymeric structures, which are the precursors of ACP. These precursors of ACP further nucleate to form ACP with sphere morphology [29].

Numerous studies have been conducted on the subsequent mineralization of ACP. In general, the formation of mineralized tissues is induced by the deposition of ACP on the inner and outer surfaces of fibers. Regarding the chemical molecular structure, researchers found that continued calcium uptake converts ACP into ribbon morphology octacalcium phosphate (OCP) and subsequently into elongated morphology AP, and finally into HA [29,30]. However, the existence of OCP remains controversial. Lotsari et al. and Dorozhkin et al. found that the transient metastable phase of OCP does not exist in mineralization [31,32]. The conflicting conclusion may result from either the unstable and short-lasting phase of OCP or OCP only existing in specific conditions that have not been fully understood [11]. Concerning the growth of ACP crystal structures, sphere morphology ACP first grows into the form of a steps homogeneous crystal along one orientation. And the growth front clusters incorporate in a dynamic formation-consuming-incorporation-reformation process [31]. Then, Onuma et al. observed step growth along the [1100] [14] direction via a combination of two-dimensional (2D) nucleation and stepped flow of ACP hexagonal crystal structure [33,34]. In situ study of CaP particles by atomic force microscopy (AFM) also revealed the initial elongation of CaP particles in a specific direction, followed by the observation of six distinct growth steps in segmented orientations within a 2D layer. Moreover, the stacking of certain hexagonal formations results in the formation of 3D structures [35], and the dimensions of these CaP particles initially resemble those of Posner's clusters, which subsequently undergo a transformation into stable ACP plates, and ultimately transitioning into the final crystalline HA phase [36].

Although the physical and chemical processes of mineralization have been extensively investigated, numerous obstacles still impede progress from in vitro exploration of the physical and chemical

processes of ACP mineralization to a clear understanding of the physiological mineralization process. Firstly, the subjects of biomimetic mineralization studies are mostly supersaturated solutions, with rich and diverse compositions that are compartmentalized from each other, which deviate from physiological conditions. It is undeniable that, all these investigations have resulted in the elaboration of an extensive corpus of knowledge concerning the mechanisms of CaP nucleation and growth from supersaturated solutions [29]. Significant challenges are present in the emulation of physiological mineralization environments, as the replenishment of ions such as Ca^{2+} and Pi within the body occurs in a dynamic manner. However, within an in vitro simulated mineralized environment, attaining nucleation becomes arduous without the real-time replenishment of ions [11]. Nonetheless, to replicate the intricacy of biological systems, additional research should prioritize the utilization of in vitro and in vivo models for simulating diverse physiological mineralization environments. This entails considering the concentrations of ion precursors and the physicochemical conditions involved. A device capable of real-time monitoring and controlled release of multiple ions could potentially overcome the challenge of simulating the biomineralization environment. Secondly, a better inter-subject combination of intracellular localization and dynamic transformation processes corresponding to the aforementioned mineralization precursors has not been achieved. Therefore, the intracellular process has become a key and challenging area of research in mineralization. We will elaborate on the research progress in subsequent content, with a focus on recent advances in understanding the intracellular process of mineralization and providing new ideas for future research.

3. Dynamic transfer process of minerals

3.1. Endoplasmic reticulum to mitochondria - from inorganic ions to minerals

In recent years, the initial stage of mineralization has been postulated to involve the inter-organelle communication between the endoplasmic reticulum (ER) and mitochondria. These two organelles are in close physical proximity in all eukaryotes, including yeast and mammalian cells [37,38]. Furthermore, ER exhibits a higher association frequency with mitochondria than with other organelles [39]. The endoplasmic reticulum-mitochondrial encounter structures (ERMES), a multi-protein complex that stably connects the ER and the outer mitochondrial membrane (OMM), serve as the prerequisites for ion and molecular exchange between them [40]. Through the observation of mineralized particles under an electron microscope, scholars have discovered that the formation of biomineral precursors was initiated by the gathering of calcium and phosphorus ions in the ER [41]. The transportation of calcium and phosphorus clusters from the endoplasmic reticulum to the endoplasmic mitochondria leads to the formation of these precursors [41]. This discovery can also be reflected by the research that ER-mitochondrial contact regulates osteogenic differentiation of periodontal ligament stem cells via mitofusion 2 in inflammatory microenvironment [42]. However, it remains unproven whether both calcium and phosphorus clusters and ions are transported from the ER to the mitochondria during the initiation of the mineralization process. Apart from the transport of calcium and phosphorus clusters, two potential pathways of communication between the ER and mitochondria may also be implicated either individually or in conjunction. Firstly, the regulatory function of proteins localized on the ER may govern the process of CaP particle deposition within the mitochondria [43]. In osteoblasts, the disruption of ER-mitochondria connections could trigger the initiation of the integrated stress response (ISR) by the mitochondria, consequently leading to osteogenesis imperfecta (OI) [44]. Secondly, the transportation of inorganic ions from the ER to the mitochondria could be associated with the deposition of CaP particles within the mitochondria (Fig. 1) [45].

The transfer of Ca^{2+} from the ER to mitochondria has been intensely concerning. Ca^{2+} influxes into ER via the activity of Ca^{2+} ATPases or the secretory pathway Ca^{2+}-ATPases at the expense of ATP hydrolysis [46]. sarco-endoplasmic reticulum Ca^{2+}-ATPase (SERCA) inhibitor treated osteoblasts contained reduced mineralization precursors and nodules [41]. Ca^{2+} can be released from ER through inositol 1,4,5-trisphosphate receptors (IP_3Rs), or ryanodine receptors (RyRs) [47] and induce Ca^{2+} accumulation to reach Ca^{2+} level tenfold higher than those in the bulk cytosol microdomains in ERMES [45]. At the regulation of mitochondrial Rho GTPase, increased cytoplasmic Ca^{2+} causes stable contact of ERMES, and this effect can be suppressed when MIRO is depleted [48,49]. Then Ca^{2+} is able to cross the outer mitochondrial membrane (OMM) through the voltage-dependent anion-selective channel proteins(VDACs), which mediate Ca^{2+} flux by open or closed conformation in a voltage-dependent manner [50,51]. VDACs can enhance the IP_3Rs-induced Ca^{2+} signal from the ER, facilitating Ca^{2+} entry into the intermembrane space(IMS) of mitochondria and its accumulation inside the matrix [45,52,53]. The next barrier is the inner mitochondrial membrane (IMM), which is not easy to pass through because of its high-selectivity and low-affinity [54,55]. Ca^{2+} passes the IMM mainly through the mitochondrial Ca^{2+} uniporter (MCU) complex, which relies completely on two main parameters: the mitochondrial membrane potential and the Ca^{2+} concentration in the area surrounding the channel [56–58] (Fig. 1).

In contrast to Ca^{2+}, genetic experiments in mice suggest that circulating phosphate (Pi) may play a more prominent role in the regulation of bone mineralization [59,60]. However, limited research has been conducted on the transfer of Pi from ER to mitochondria. Poly lactic-co-glycolic acid encloses the BPs, which are conducive to endocytosis of Pi, can enhance the formation of mineralization granules in mitochondria, which implies that phosphorus could be transported from the ER to the mitochondria[41]. Endoplasmic reticulum phosphate transport can be realized through the glucose-6-phosphatase(G6Pase) transport system. G6Pase enzyme is situated with its active site inside the lumen of the endoplasmic reticulum. It can hydrolyze glucose-6-phosphate to glucose and Pi [61]. Then, transport systems termed T2 can transport Pi across the endoplasmic reticulum membrane towards the cytoplasm [62]. Afterwards, Pi can easily pass through the OMM because of its high permeability to molecules < 5 kDa. Uptake of Pi by the IMM barrier is catalyzed by the phosphate carrier(PIC), which can promote Pi collaboration H^+ flow inward IMM (Fig. 1) [63].

These observations indicate a close relationship between the transportation of Ca^{2+} and Pi from the endoplasmic reticulum to mitochondria and the formation of early mineralization clusters in cells. However, the way that calcium and phosphorus clusters form on the ER membrane has not been explained, and the precise mechanism of Ca^{2+} and Pi ion and clusters transportation from endoplasmic reticulum to mitochondria has not been tightly associated with the mineralization process. Additionally, the chemical properties of mineralized particles in this process, such as whether prenucleation complexes or ACP precursors are transported from the endoplasmic reticulum to mitochondria are not yet fully understood. This requires further investigation.

3.2. From mitochondria to intracellular matrix vesicles (MVs) - nucleation of minerals

In 1964, Greenawalt et al. first discovered that mitochondria contained insoluble calcium phosphate electron-dense granules and may play a role in mineralization [64]. Subsequent investigations have indicated that mitochondria can absorb Ca^{2+} and Pi from the

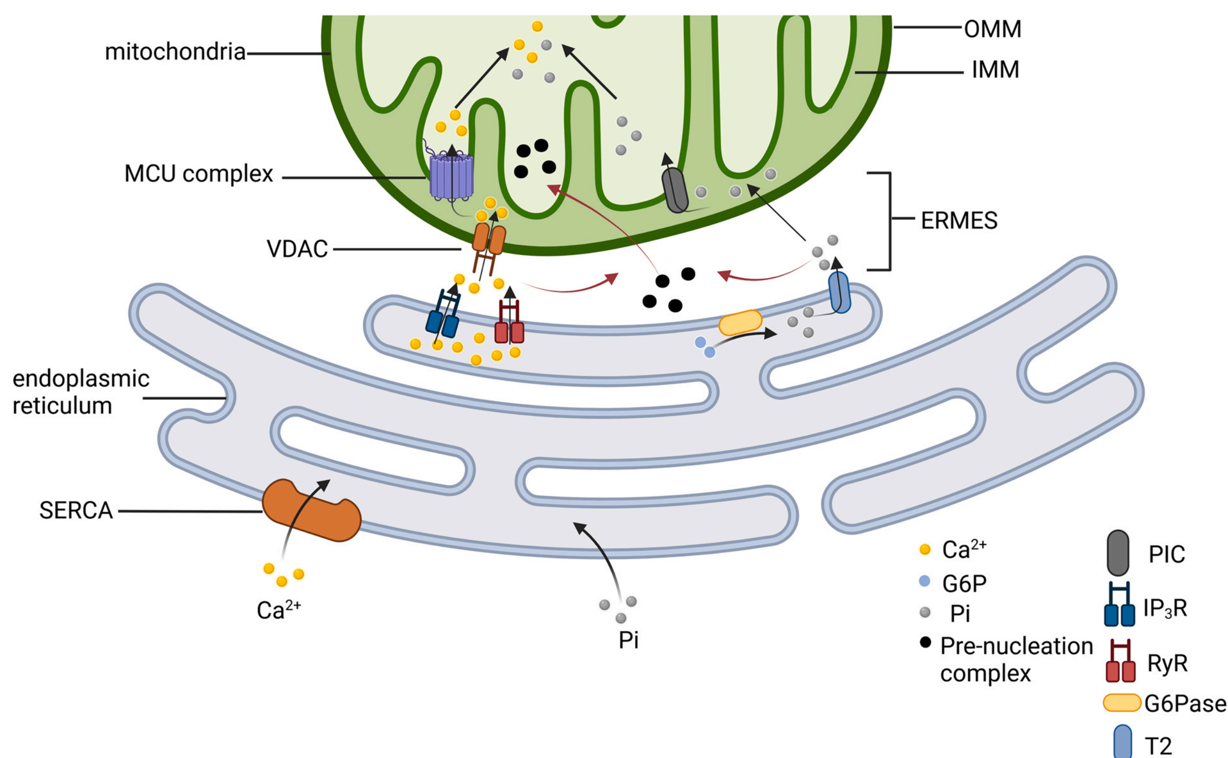

Fig. 1. Schematic illustration of intracellular biomineralization process from endoplasmic reticulum (ER) to mitochondria. The endoplasmic reticulum-mitochondrial encounter structures (ERMES) provide conditions for ions and molecular exchange between them.(1) Calcium ions (Ca^{2+}) influx into ER via the activity of sarco-endoplasmic reticulum Ca^{2+}-ATPase (SERCA), and are exfluxed from the ER through IP_3Rs and RyRs, and influx to mitochondria outer mitochondrial membrane (OMM) through voltage-dependent anion-selective channel proteins (VDACs), ultimately reaching the mitochondrial matrix via the mitochondrial Ca^{2+} uniporter (MCU). (2) Phosphate (Pi) can be transported from the ER through the glucose-6-phosphatase(G6Pase) transport system. G6Pase can hydrolyze glucose-6-phosphate to glucose and Pi. Then, transport systems termed T2 can move Pi across the endoplasmic reticulum membrane towards the cytoplasm. Subsequently, Pi can pass effortlessly through the OMM due to its high permeability. The uptake of Pi by the IMM barrier is catalyzed by the phosphate carrier (PIC). (3) Biomineral pre-nucleation complexes can also form between the ER and mitochondria with Ca^{2+} and Pi, which are then transported to the mitochondria, resulting in mineralization.

surrounding medium and promote the formation of calcium phosphate electron-dense granules [65–67]. However, it was not until 2012 that Stevens et al. reported that the observed electron-dense granules in mitochondria were ACP precursors, and could be transferred to intracellular calcium phosphate-containing matrix vesicles (MVs) via an unknown mechanism [27]. It remains unclear whether these calcium phosphate-containing vesicles correspond to exosomes, autolysosomes, or apoptotic bodies (Fig. 2).

Recently, Pei et al. and Iwayama et al. found that this unknown process can be explained by mitophagy, which provides the mechanism for the transfer of ACP precursors from dysfunctional mitochondria to autophagosomes. ACP precursors first gather in mitochondria. When ACP accumulation reaches a threshold, the mitochondria swell and lose membrane potential, resulting in PINK1 accumulation on their outer membrane. The latter recruits PARKIN from the cytosol to the dysfunctional mitochondria. PARKIN ubiquitylates mitochondrial proteins and causes the ACP-overloaded mitochondria to be engulfed by autophagosomes through P62 and LC3. And then part of these mitochondria are isolated by the bilayer membrane structure to form the autophagosome. Upon fusion with lysosomes, these autophagosomes become autolysosomes where the mitochondrial ACP precursors coalesce to form larger intra-vesicular granules, and the BMP/Smad signaling pathway is involved in this process [67,68]. However, the physical and chemical properties of ACP precursors and the mechanism of their aggregation in this process remain unclear (Fig. 2).

On the other hand, evidence suggests that apoptosis may also account for the transfer of ACP precursors from mitochondria to MVs. Ca^{2+} influx into mitochondria can also contribute to apoptosis as it is able to induce ROS generation, mitochondrial depolarization, and mtDNA damage [69]. In hyperosteosis diseases like osteoarthritis(OA) and ectopic vascular calcification, enhanced intracellular ROS generation and overloaded Ca^{2+} influx cause a significant reduction of mitochondrial membrane potential and mitochondrial dysfunction, leading to apoptotic cell death and the formation of apoptotic bodies [70–73]. This may be related to the activation of the phosphatidylinositol 3-kinase/protein kinase-B/Runx2 signaling pathway (PI3K/AKT/Runx2) or the p38/MAPK signaling pathway, thus leading to osteogenic trans-differentiation, and promoting calcification [73–75]. However, there is still a lack of direct evidence of how apoptosis participates in the process of ACP precursor transfer toward apoptotic bodies (Fig. 2).

Despite mitophagy and apoptosis potentially participating in the transportation of ACP precursors from mitochondria to intracellular vesicles, the relationship between these processes and whether other processes also contribute to the transportation of ACP among organelles requires further investigation.

3.3. *From intracellular matrix vesicles to extracellular space: enhancing mineral accumulation*

The mineral density of these ACP precursors can increase simultaneously in MVs. During this process, as nucleation and maturation of ACP precursors progress, the initial formation of the ACP particle takes place within intracellular MVs [76]. MVs contain several membrane transporters and enzymes on their plasma membranes, which provide an adequate microenvironment for calcium phosphate nucleation and subsequent growth. These factors

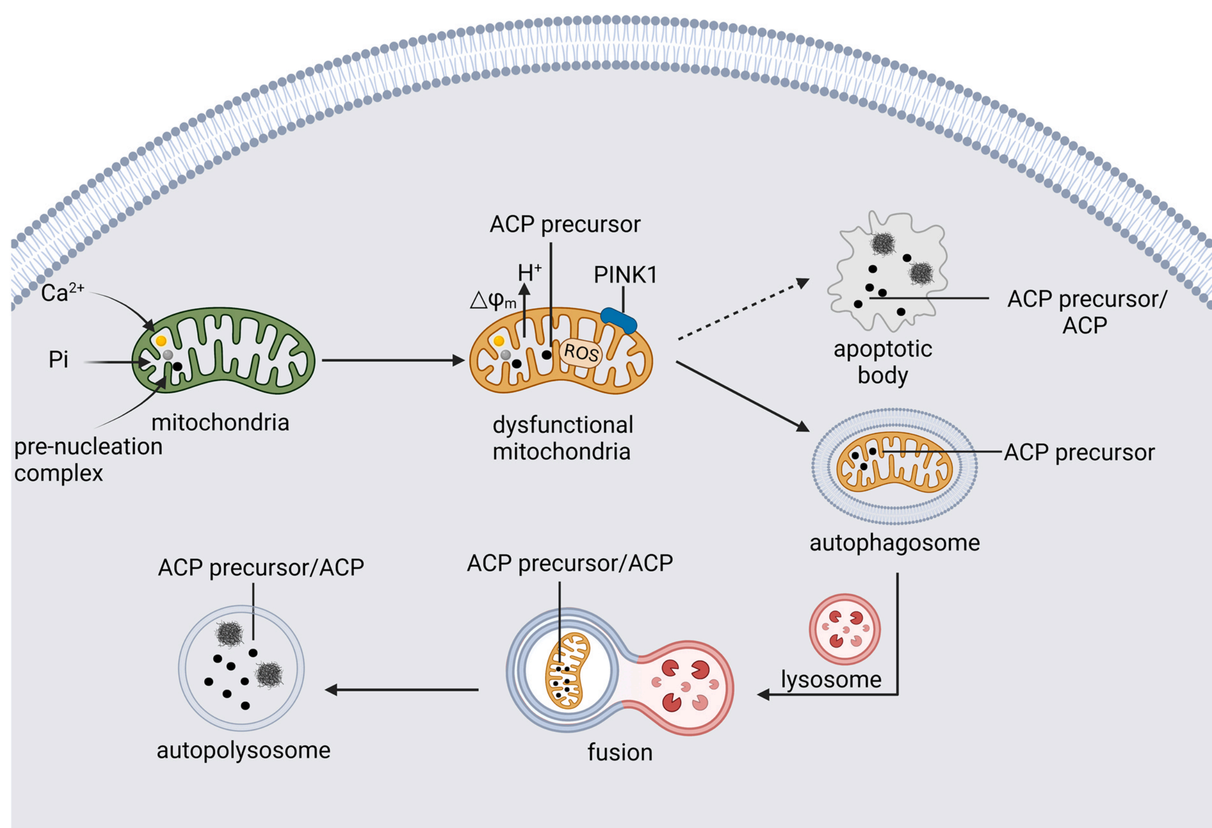

Fig. 2. Schematic illustration of intracellular biomineralization process from mitochondria to intracellular matrix vesicles (MVs). (1) Ca^{2+}, Pi, and pre-nucleation complexes can first gather in mitochondria and form ACP precursors. These mitochondria become swollen and lose membrane potential, resulting in PINK1 accumulation on their outer membrane, rendering them dysfunctional. (2) A portion of these dysfunctional mitochondria are isolated by the bilayer membrane structure to form autophagosome or apoptotic bodies. (3) The autophagosomes fuse with lysosomes and become autolysosomes where the mitochondrial ACP precursors coalesce to form larger intra-vesicular granules.

are involved in the CaP mineralization process in different ways [76]. The crystallization of Ca^{2+} is easier than that of Pi because Ca^{2+} can strongly bind to the negatively charged inner leaflet of the plasma membrane [77]. Plasma membranes consisting of phosphatidylcholine and phosphatidylserine have a substantial capacity for Ca^{2+} binding [78]. Thus, Ca^{2+} can accumulate in MVs and guide crystallization (Fig. 3).

The enrichment and crystallization of Pi are related to the concentration of Pi and the PPi/Pi ratio. Pi can bind with Ca^{2+}, forming calcium phosphate and increasing mineralization nucleation and subsequent growth. Pyrophosphates (PPi) are composed of two inorganic Pi groups joined by an ester linkage. PPi can be hydrolyzed into Pi to promote mineralization but can also inhibit mineralization by binding to nascent hydroxyapatite crystals and inhibiting alkaline phosphatase activity, thereby inhibiting crystal overgrowth [79,80]. Many membrane transporters and enzymes can regulate the Pi accumulation process. Three categories of phosphatases play an important role in the production of Pi and the PPi/Pi ratio in MVs. The first coupled enzymes are tissue-nonspecific alkaline phosphatase (TNAP) and Ectonucleotide pyrophosphatase/phosphodiesterase I (ENPP1), which can generate Pi locally by catabolism. ENPP1 is a membrane-bound glycoprotein that can catalyze ATP and generate PPi [81]. TNAP, a glycosylphosphatidylinositol anchor enzyme associated with MVs, can hydrolyze PPi into Pi to promote mineralization [82]. Secondly, some transporters of PPi or Pi can supply Pi from outside the MVs membrane. As a supplement to ENPP1, ANK, encoded by the progressive ankylosis gene (Ank), can act as a non-enzymatic PPi channel, enabling PPi to be secreted from the cell through the plasma membrane [83]. Sodium-inorganic Pi co-transporters, Pit1 and Pit2, which can sense extracellular Pi concentrations and transport Pi from the extracellular fluid to intravesicular regions of MVs through the extracellular signal-regulated kinase (ERK) pathway [84–86]. Finally, Orphan phosphatase 1 (PHOSPHO1), components of the lipid bilayers of matrix vesicles, is present within the vesicles and function inside matrix vesicles to generate Pi using phosphocholine and phosphoethanolamine [87–89]. Despite the different ways to produce Pi in MVs, all of them seem necessary for Pi supplementation. However, the combination of these different ways to accumulate Pi within MVs has not been fully understood (Fig. 3).

Regarding the secretion of MVs containing ACP precursors (or ACPs) and their role in mineralization, Weiner et al. discovered that a considerable quantity of ACP particles were enveloped in an obscure monolayer vesicle within the bone cells of mice [90] and zebrafish [91]. They also observed that ACP could be discharged via exocytosis, either through polarized budding or pinching-off processes, and subsequently participate in the mineralization of collagen fibers in the ECM (Fig. 3).

Although the process described above forms a complete chain of evidence on how ACP precursors are intracellularly formed, accumulated, and transported through the cell membrane to participate in mineralization, research combining these CaP mineral particles and the physical and chemical properties of ACP precursors is still deficient. It is difficult to characterize these electron-dense particles. Additionally, it seems contradictory that the CaP particles are grown densely and intracellularly, while intrafibrillar mineralization, which will be discussed later, needs to be small enough and arranged in a specific order to be incorporated into the gap zones of collagen fibrils. However, the balance of the sufficient size and stability of these CaP particles to achieve realistic mineralization has not been found.

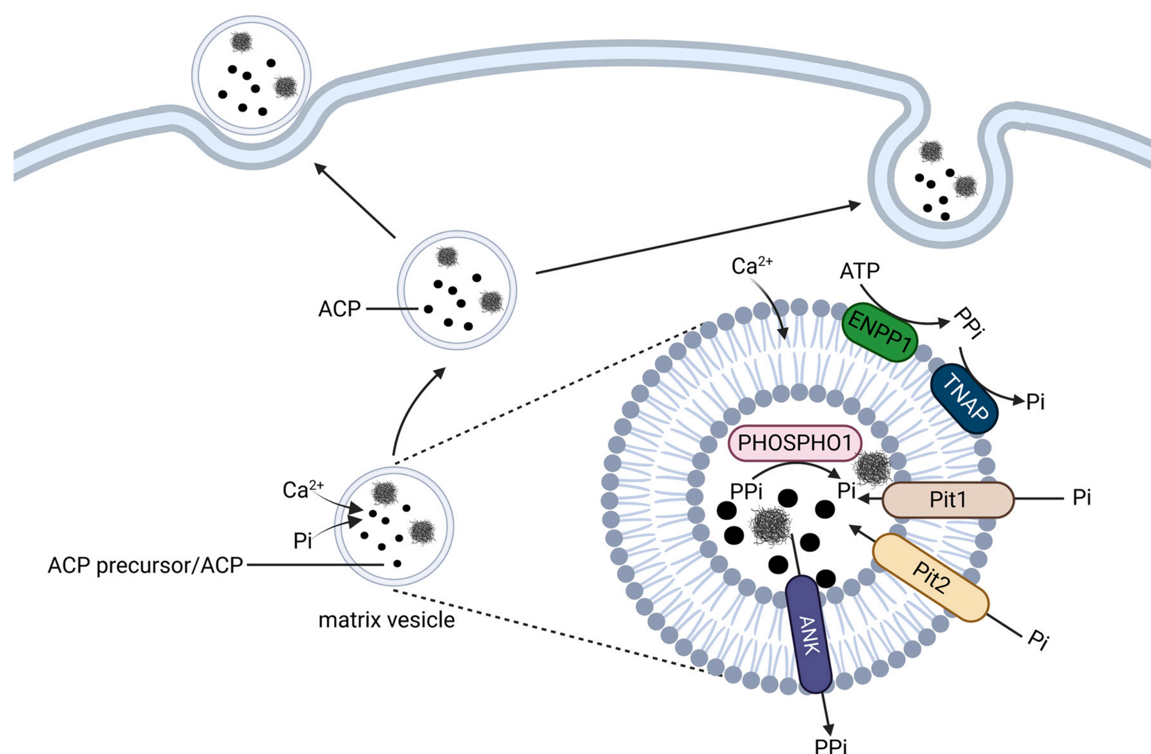

Fig. 3. Schematic illustration of intracellular biomineralization process from intracellular MVs to Extracellular Space. (1) Mineral density of ACP precursors (or ACPs) within MVs can increase through the enrichment of $Ca^{2+}$and Pi. Ca^{2+} can strongly bind to the negatively charged inner leaflet of the plasma membrane, leading to their accumulation within MVs. Pi can be enriched by several membrane transporters and enzymes. ENPP1 can catalyze ATP and generate PPi, which is then hydrolyzed into Pi by TNAP. In addition, ANK can act as a non-enzymatic PPi channel, enabling PPi to be secreted through the plasma membrane. Sodium-inorganic Pi co-transporters Pit1 and Pit2 transport Pi from the extracellular fluid to intra-vesicular regions of MVs. PHOSPHO1 is present within the vesicles and function inside matrix vesicles to hydrolyze PPi to generate Pi. (2) Intracellular MVs can either be enveloped in an obscure monolayer vesicle, and be secreted and assimilated into the collagen gap region, or be discharged via exocytosis, through polarized budding or pinching-off processes, and subsequently participate in the mineralization of collagen fibers in the ECM.

3.4. Further extracellular mineralization - the development of stable mineralization

During mid-to-late stages of osteoblast differentiation, the amorphous calcium phosphates (ACPs) or other variants of calcium phosphate present in MVs can be assimilated into the collagen gap region (~40 nm) [27]. Alternatively, they may grow in multiple directions within the MVs, culminating in the formation of needle-like crystals that pierce the plasma membrane and exit the vesicles. These crystals eventually form mineralized nodules or calcifying globules [27,76,92]. However, these needle-like crystals, which lack membranes, are less efficient at inducing mineralization than smaller MVs containing ACP [93], which the reason for remains unclear. In addition, the presence of calcium ions and Pi in the extracellular matrix and bodily fluids provides an environment conducive to the further mineralization of calcium phosphate deposition on the organic matrix in bones and teeth (Fig. 4).

The extracellular matrix (ECM) is composed of many components that provide a variety of functions in different body tissues. In osteogenic or odontogenic microenvironments, the ECM provides sufficient conditions to support and precisely regulate mineralization. Type I collagen, a right-handed triple helix typically composed of two α1 and one α2 chains, is the major organic component of ECM of the bone and dentin [94]. In dentin matrix, type I collagen is assembled at a periodic staggered array into fibrils, exhibiting a characteristic banding pattern that guides the calcium phosphate deposition within intrafibrillar and interfibrillar spaces [95,96]. In bone matrix, research has shown that the collagen matrix controls the size and the 3D distribution of apatite at larger length scales, and increased quantity and density of collagen matrices can improve bone forming ability in biomimetic mineralization environments [12]. Furthermore, mature cross-links (i.e. PYD, PYL, DPD, and DPL), a decrease in Lys hydroxylation, and an increase in enzymatic cross-link content can contribute to increased mineralization (Fig. 4) [97–100].

In addition, non-collagenous proteins (NCPs) are a type of ECM that is comprised of about 180–200 different molecules, playing a critical role in the growth of mineralization combined with collagen fibrils [101]. The most relevant and abundant family is glycoproteins, including alkaline phosphatase (ALP), osteonectin (ON), osteopontin (OPN), bone sialoprotein (BSP-2), dentin matrix protein 1 (DMP-1), and dentin sialophosphoprotein (DSPP). These glycoproteins can promote osteogenesis and odontogenesis through various mechanisms and periods, and have been discussed in detail lately (Fig. 4) [102,103].

Growth factors (GFs), an additional category of ECM regulators, play a role in osteogenesis and odontogenesis. These are a collection of proteins and peptides that are secreted by various bone tissue cells. They facilitate cell functions such as division, migration, and differentiation, thereby supporting osteogenesis and odontogenesis [102,104]. Bone regeneration-associated GFs can be primarily classified into two groups: 1) serine-threonine kinase receptors that function as high-affinity receptors, such as transforming growth factor betas (TGF-βs) and bone morphogenetic proteins (BMPs); 2) tyrosine kinase receptors that act as specific receptors for fibroblast growth factors (FGFs), vascular endothelial growth factors (VEGFs), platelet-derived growth factor (PDGF), and insulin-like growth factor 1 (IGF-1) etc. TGF-βs are potent chemotactic stimulators of osteogenesis and odontogenesis. Pertaining to bone formation, they trigger signaling for BMPs production in osteoprogenitor cells, promote osteogenic differentiation, angiogenesis, prevent osteoclast activation, and promote osteoclast apoptosis[105,106]. In dentin

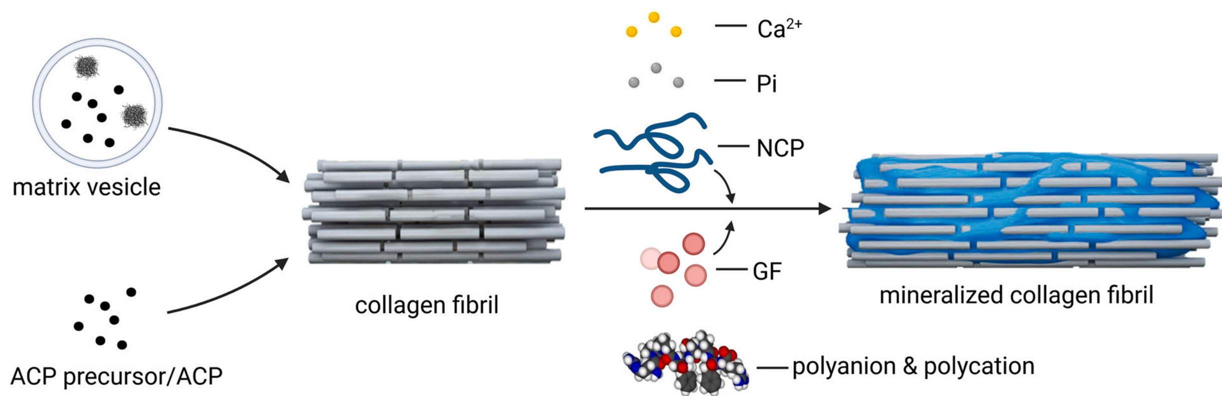

Fig. 4. The presence of Ca^{2+}, Pi, non-collagenous proteins (NCPs), growth factors (GFs), etc. in the extracellular matrix and bodily fluids, as well as artificially added agents such as polycations and polyanions, can provide an environment for further mineralization of MVs containing ACP or ACP particles, and promote calcium phosphate deposition on the collagen fibrils.

forming aspect, TGF-β1 can promote apical papilla cell proliferation and mineralization [107]. TGF-β2 can regulates tooth root dentinogenesis by cooperation with Wnt signaling [108]. However, TGF-β3 inhibits odontoblastic differentiation of DPSCs [109]. BMPs can promote cellular proliferation, migration, differentiation, and tissue matrix synthesis in bone and dentin [110,111]. In terms of osteogenesis, BMP-2 significantly increases osteocalcin expression [112], BMP-4 can induce bone and cartilage development, bone healing, and fracture repair bone mineralization[113]. BMP-7 can upregulate ALP activity and increase mineralization, causing increased osteoblast differentiation [113,114]. In terms of dentinogenesis, dysregulated BMP signaling causes a number of tooth disorders in human. Mutation or knockout of BMP signaling-associated genes in mice results in dentin defects [115]. Among them, BMP-2 plays key role in dentinogenesis, which is involved in specifying the fate of dental pulp and DPSCs differentiation into odontoblast-like cells and stimulateing tooth-related gene expression [115,116]. IGF-1 promotes the recruitment and osteoblastic differentiation of BMSCs and increases the number of functional osteoblasts [117]. FGFs support BMSCs expansion and differentiation to induce calcium deposition [118,119]; VEGFs can promote angiogenesis and vasculogenesis and thus increase bone formation [120,121]; PDGF can enhance

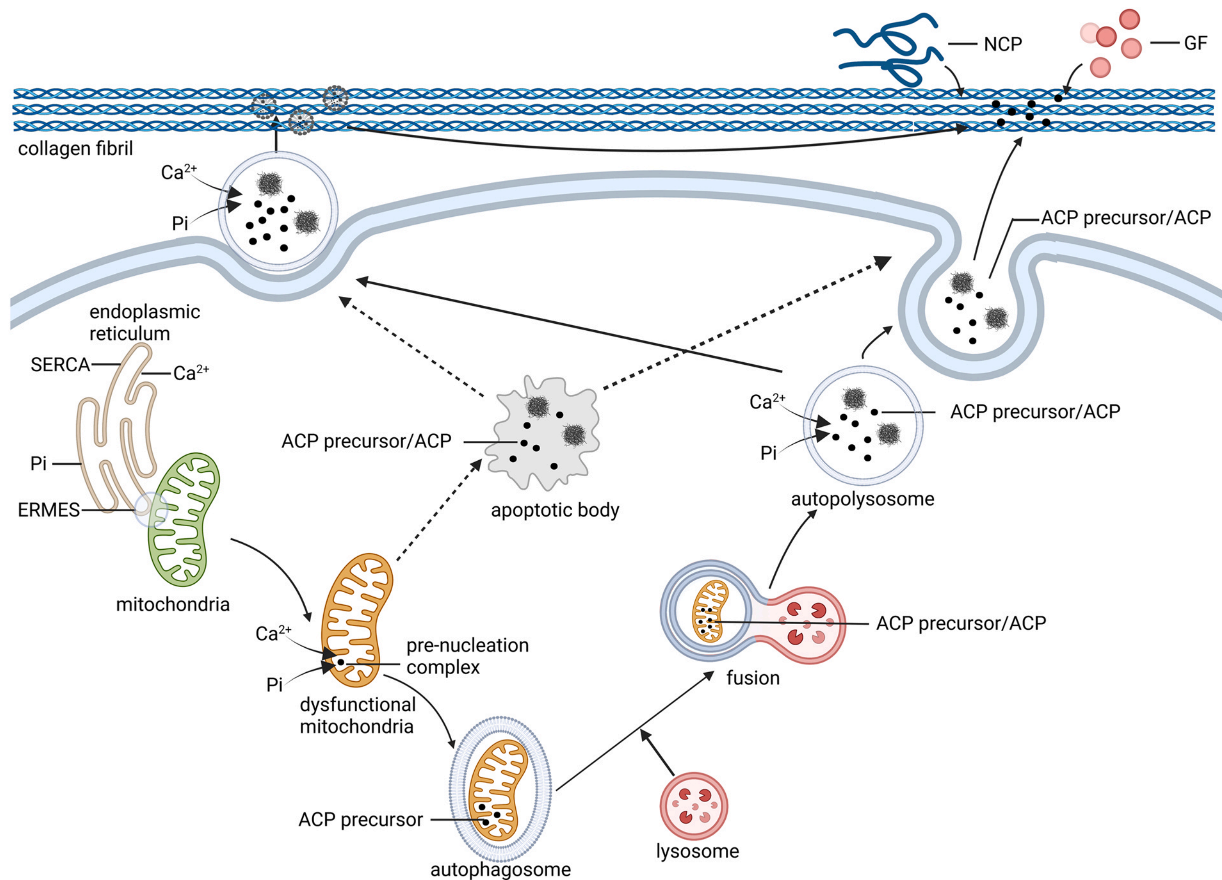

Fig. 5. Schematic illustration of current general models of biomineralization process. CaP formation proceeds via a number of cooperative/redundant mechanisms. In this picture, “ACP” is the abbreviation of amorphous calcium phosphate, “NCPs” is non-collagenous proteins, and “GFs” is growth factors, respectively. Solid black arrow: direct stimulatory modification. Dotted black arrow: tentative stimulatory modification.

angiogenesis and promote osteogenic differentiation through BMP signaling (Fig. 4) [122–124].

Considerable research has been conducted on biomimetic mineralization through agents that simulate the NCPs to guide adequate mineral ion deposition within intrafibrillar and interfibrillar spaces. Especially in the biomimetic mineralization of dentin, a variety of materials based on NCP analogue have been found to significantly improve the biomimetic mineralization of dentin. Polyanions such as polyacrylic acid[95,96] and polyaspartic acid [125] were found to increase intrafibrillar mineralization in dentin by mimicking the progressive dehydration mechanism. This occurs through replacing the free and loosely bound water within the positively charged collagen matrix by apatite crystallites via polyanion-stabilized ACP precursors and the bottom-up ACP precursor particle assembly approach. Later, it was discovered that polycation polyallylamine hydrochloride (PAH) [126] and carboxymethyl chitosan (CMC) [15,127], a type of amphoteric polyelectrolyte derived from chitosan, can also increase intrafibrillar mineralization, which is based on the balance between osmotic equilibrium and electroneutrality, by outward movement of monovalent ions and intrafibrillar water through the collagen surface occurs irrespective of the charges of the polyelectrolytes. Moreover, a novel bioactive peptide inspired by the CEMP1, in conjunction with a hydrophobic tail, can elicit dentin collagen remineralization by means of mineral interaction, collagen binding, and the initiation of intrafibrillar mineralization [128]. Regarding bone formation, polyaspartic acid [129–131] and poly acrylic acid [132] in combination with polymer-induced liquid-precursor (PILP), can facilitate intrafibrillar mineralization of type I collagen. Within this process, a very high degree of mineralization can be achieved, with compositions matching that of bone. Building upon this foundation, electrospun nanofibers composed of phosphorylated polyvinyl alcohol (PPVA) demonstrated an optimal mineralization profile. Additionally, it engendered enhanced adherence, proliferation, and osteogenic capacity in MG63 cells [133]. Further research is still needed to better explore the induction of mineralization and put it into clinical application in osteogenic and odontogenic tissue engineering and for the design of new adhering and implantable materials (Fig. 4).

4. Conclusion and perspective

A comprehensive overview of biomineralization and its physicochemical and biological processes has been presented in this review. The intracellular mineralization process comprises four steps, namely the transport of calcium and phosphorus ion/clusters (prenucleation complex or ACP precursors) from the endoplasmic reticulum (ER) to the mitochondria to initiate mineralization, the accumulation of ACP precursors in autophagosomes through mitophagy, or in apoptotic bodies through apoptosis, both of which are manifested as intracellular matrix vesicles (MVs). Furthermore, The ACP precursors mature and gather in MVs, which may transform into ACP or even become crystallized. The CaP particles containing MVs are transported through the cellular membrane through the exocytosis process. Finally, ACP or other types of CaP grow inside the MVs, penetrate the plasma membrane to exit the vesicles, and eventually form mineralized nodules or calcifying globules. These deposits occur on the organic matrix in bone and teeth, guided by collagen fibrils and extracellular matrices (ECMs), including glycoproteins, growth factors (GFs), and other non-collagenous protein mimics in vitro and in vivo (Fig. 5).

However, several questions remain unanswered. First, with regards to the diverse phenomena of intracellular mineralization process from ER to MVs, experimental studies were conducted utilizing cell lines derived from osseous and dental lineages, encompassing bone marrow mesenchymal stem cells (BMMSCs) [41], osteoblasts [27], bone cells [91], and DPSCs [67] etc. However, the in vitro investigation primarily concentrated on osteogenic markers. And the in vivo assessment of mineralization markers predominantly focused on bone forming ability in both growth stage as early as embryonic stage [41], and in continuously forming tissues such as fin bony rays [91]. There is a scarcity of evidence concerning markers associated with dentin formation, necessitating further investigation. In addition, the behavior of organelles during different mineralization structure periods has not been clarified. Additionally, little is known about the chemical structure and physical morphology of these intracellular CaP particles. Furthermore, the conflict between the intracellular growth of CaP particle diameter and the demand for small particles to fit the gap zone of collagen fibril extracellularly has not been reasonably explained. We speculate that there may exist a balance between the sufficient size and stability of these CaP particles to achieve realistic mineralization, which requires further research to verify. Finally, while biomineralization is universally recognized to be a precise process regulated by many factors, the precise regulation mechanism within different organelles has not been elucidated yet. A better understanding of the intracellular mineralization process, regulation, and the combination of intracellular mineralization process with CaP particles' structure and extracellular mineralization process will be crucial to understanding the integrated mineralization process. This will also be necessary for the development of therapies that modulate intracellular mineralization processes in bone/teeth defects or bone metabolism-related diseases.

Conflicts of interest

None.

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Declaration of Generative AI and AI-assisted technologies in the writing process

None.

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