Mechanism of influence of calcified nanoparticles in the development of calcified diseases (Review)

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Abstract. Calcified nanoparticles (CNPs), also known as nanobacteria, are ubiquitously present in both natural minerals and biological systems. However, their properties remain incompletely elucidated, particularly concerning whether they represent the smallest self-replicating entities on Earth, a topic that remains highly debated. Current research has demonstrated that CNPs can be isolated from various pathological calcification conditions, including kidney stones, vascular calcification, biliary stones, and calculus oral disease. These particles have the potential to infect any tissue or cell type within the human body, forming a mineralized layer around them, which leads to pathological calcification of tissues. It is suggested that CNPs may play a significant role in these diseases by damaging cells, promoting osteogenic differentiation, and influencing metabolic processes, thereby initiating the formation of calcification cores in local tissues. Under the influence of inflammatory responses, these cores can expand further, ultimately leading to the development of calcification diseases. Therefore, the aim of the present review was to explore the roles and pathogenic mechanisms of CNPs in various pathological calcification diseases, providing new insights for in-depth research into their properties and pathogenic mechanisms, as well as identifying potential therapeutic targets for calcification diseases.

Contents

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1. Introduction

Pathological calcification, also known as ectopic calcification, refers to the deposition of mineralized complexes in soft tissues outside of bones and teeth under physiological conditions. This condition can result from genetic factors or chronic diseases acquired later in life (1). Research indicates that pathological calcification shares similarities with physiological calcification but occurs due to an imbalance between inhibitory and promoting factors, leading to abnormal mineralization in soft tissues (2). Key mechanisms include the induction of bone formation, differentiation of vascular smooth muscle cells into osteoblastic phenotypes, oxidative stress, apoptosis, mitochondrial dysfunction, calcium-phosphorus imbalance, and decreased levels of calcification inhibitors (3). Despite ongoing research, the molecular mechanisms underlying pathological calcification remain poorly understood, and effective treatments have yet to be developed. Further investigation into these mechanisms is expected to reveal more promising therapeutic targets.

In recent years, in-depth studies on nanobacteria (NB) have revealed their ability to aggregate calcium and phosphorus from the surrounding environment within the body, forming nucleation centers. These findings highlight the significant role of NB in the pathogenesis of pathological calcification diseases (4). Initially identified by Finnish scientists Kajander and Ciftçioglu (5) as contaminants in cell cultures, NB exhibit diameters ranging from 50 to 500 nm, with a peak particle size of ~130 nm. Their outer layer is covered with a dense, needle-like apatite mineralized shell, and their interior contains cavities and inclusions. The main protein component is fetuin-A, and they are Gram-negative. Their proliferation rate is ~10,000 times slower than that of bacteria. They can withstand temperatures as high as 100˚C and harsh chemical conditions, while most other bacteria cannot survive in such extreme environments, and resistance to most antibiotics (4,6-9). Tetracycline is one of the few drugs that can effectively reduce the damage of NB (7). However, debate persists regarding whether NB represent the smallest known replicating life forms or are merely mineral-protein complexes unrelated to bacteria. Recent findings indicate that NB lack definitive nucleic acid sequences, leading researchers to prefer the term ‘calcified nanoparticles’ (CNPs) (10). CNPs can accumulate calcium and phosphorus in their surrounding

environment and nucleate within the human body, especially after being internalized by cells, where they exert a direct toxic effect. CNPs can inflict damage upon cell membranes, mitochondria, lysosomes, and other cellular structures and even produce a large amount of reactive oxygen species (ROS), ultimately leading to cell necrosis and apoptosis. This process contributes to the formation of larger nucleation centers, resulting in the aggregation of calcium and phosphorus into calcified cores (11) (Fig. 1). Research has established a link between CNPs and ectopic calcification diseases and CNPs have been detected in various conditions involving ectopic calcification, including kidney stones, bladder stones, dental pulp stones, salivary gland stones, cholecystolithiasis, and testicular microstones; calcification in hemodialysis patients, atherosclerosis, scleroderma (systemic sclerosis), and several malignant tumors (7). Furthermore, fetuin-A, by binding to CNPs, enhances their solubility in circulation, thereby inhibiting calcification (12). Consequently, some researchers propose that increasing circulating fetuin-A levels may represent a promising therapeutic approach for pathological calcification diseases (13). Given these findings, the interaction between fetuin-A and NB, as well as the underlying mechanisms in calcification diseases, has emerged as a focal point of current research.

The nucleation of CNPs continues to gather calcium and phosphorus from the surrounding environment, leading to the formation of hydroxyapatite and growth of cavity-filling stones. These stones can cause tissue damage through mechanical blockage, resulting in colic and, in severe cases, organ failure. The high concentration of minerals in the excretory fluids of the body promotes the nucleation of CNPs, making the bile duct and genitourinary tract susceptible to stone formation (14). In the urinary tract, crystallization usually begins in the renal tubules. The gradual concentration of glomerular filtrate and the active secretion of calcium, uric acid, oxalate, phosphate, or drug metabolites lead to mineral supersaturation in the renal tubules. Subsequently, stones may develop in the larger renal pelvis, where calcified Randall plaques on the renal papilla are formed (15). In the bile duct, bile is rich in electrolytes, bile acids, cholesterol, phospholipids, and conjugated bilirubin, especially in the gallbladder. The concentration and storage of bile promote stone formation (14). In addition, CNPs can cause vascular calcification in circulation, which is common in the elderly and patients with uremia. This intermediate-level calcification decreases vascular compliance and increases the risk of cardiovascular disease (16). In the oral cavity, CNPs contribute to calculus oral diseases by alkalizing the environment, mediating inflammatory responses, and promoting dental plaque mineralization (10).

The aim of the present review was to explore the properties of CNPs and their mechanisms in pathological calcification diseases such as kidney stones, vascular calcification, calculus oral diseases and gallstones, offering new insights for treating these conditions.

2. Mechanism of CNPs in calcified diseases

Kidney stones. Kidney stones are caused by the abnormal accumulation of crystalline substances, including calcium, oxalic acid, uric acid, and cystine in the kidney. Among these, calcium oxalate (CaOx) crystallization is the most prevalent, making kidney stones a common and frequently occurring disease of the urinary system. Approximately 10% of the global population is affected by kidney stones, with this number continuing to rise (17). However, the mechanism of kidney stone formation is not completely clear. Currently, numerous theories attempt to explain the formation of renal calculi. These theories include renal calcification spots, supersaturated crystallization, stone matrix, crystal inhibitors, and heterogeneous nucleation. Among them, the theory of calcified plaques in the renal papilla (Randall's plaque) is a prominent explanation for renal stone formation. Alexander Randall first proposed the Randall plaque theory in 1937. Studies have shown that renal CaOx stones originate from calcified plaques in the renal papilla, with calcified plaques being almost universally present in the renal papillary tips of patients with CaOx stones (18). In addition, in-depth studies of renal papillary calcification plaques have revealed the presence of CNPs through transmission electron microscopy observations of renal sections and cultures of renal papillary calcification plaques (19,20). This finding has established a correlation between CNPs and Randall's plaque.

CNPs in the bloodstream can accumulate in the kidney by passing through the glomerular filtration membrane, thereby inflicting damage on renal tubular epithelial cells. In the blood, CNPs exist as troponin granules with a diameter ranging from 30 to 100 nm. The intercellular gaps between capillary endothelial cells, the main pore barrier of the glomerular filtration membrane, measure between 50 and 100 nm. After intravenous injection of ^{99m}Tc-labeled CNPs into rabbits, CNPs can be detected in the kidneys and urine (21). By contrast, CNPs entering the urine can adhere to renal tubular epithelial (HK-2) cells, leading to damage during urine excretion. A study involving the co-culture of CNPs with fluorescence-labeled HK-2 cells has observed that CNPs can enter HK-2 cells via endocytosis mediated by membrane invagination and accumulate around the nucleus. This accumulation results in a decrease in plasma membrane cholesterol, compromising the stability of the cell membrane and making cells susceptible to mechanical damage (22). After entering HK-2 cells, CNPs induce several adverse effects. First, CNP phagocytosis by HK-2 cells can cause mitochondrial swelling and vacuolation around CNPs, a decrease in mitochondrial membrane potential, and an overload of intracellular oxygen free radicals (such as ROS). The latter can in turn affect the stability of the mitochondrial membrane, aggravate mitochondrial damage, and lead to persistent overload of ROS. Internalized CNPs not only increase the activity of NADPH oxidase but also stimulate cells to produce a large amount of ROS (4). Second, CNPs internalized into cells can bind to lysosomes and lead to lysosome alkalinization, resulting in swelling of lysosomes and inhibition of lysosomal hydrolase activity. Alkalized lysosomes can block autophagy flux and decrease the ability of cells to digest damaged proteins and organelles through autophagy, especially the digestion of damaged mitochondria (22).

In addition, a large amount of ROS can induce inflammation in renal tubular epithelial cells. On the one hand, a large amount of ROS can activate JNK through various pathways (such as the apoptotic signal-regulating kinase 1 pathway and mixed

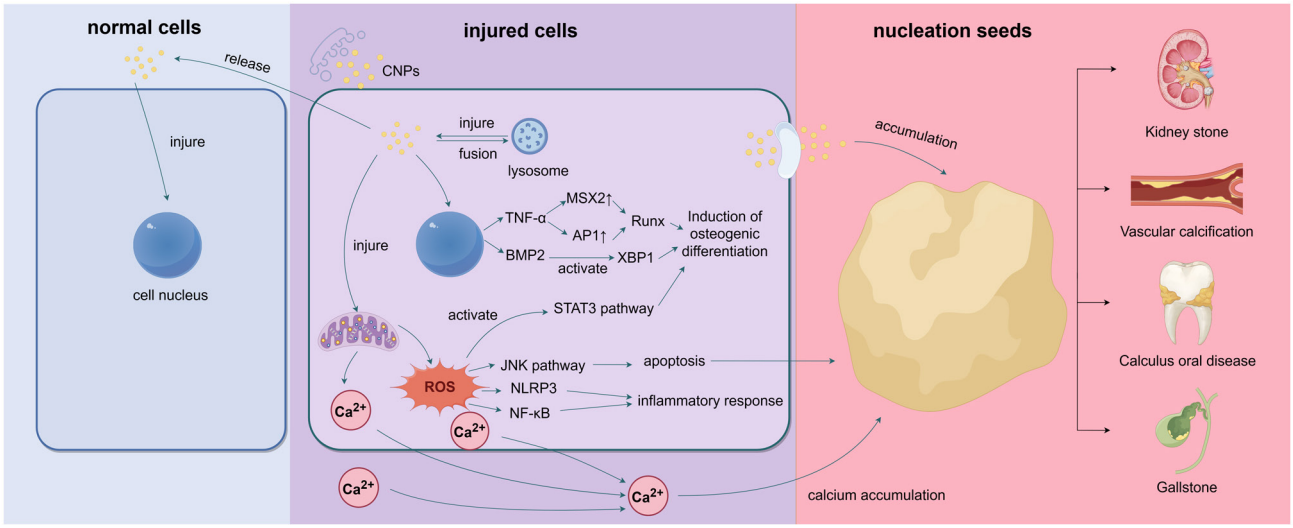

Figure 1. After CNPs enter cells through endocytosis, they induce the expression of TNF-α and BMP2. Specifically, TNF-α enhances RUNX2 expression by regulating the transcription factors MSX2 and AP-1, while BMP-2 activates the XBP1 signaling pathway, both contributing to osteogenic differentiation. Additionally, CNPs stimulate ROS production, which activates STAT3 and other pathways, further promoting osteogenic differentiation. On the other hand, CNPs can disrupt cell membrane stability, alkalize lysosomes, and stimulate mitochondria to produce excessive ROS, leading to damage in cellular structures such as the cell membrane, mitochondria, and lysosomes. The generated ROS not only activate the NLRP3 inflammasome and initiate the NF-κB signaling pathway, causing inflammatory damage, but also trigger autophagy and apoptosis via the ROS-JNK signaling pathway. During apoptosis, CNPs facilitate the aggregation of $Ca2^{+}$ from the cytoplasm, mitochondria, and extracellular matrix, forming a calcification core. Apoptotic cells release matrix vesicles containing CNPs, which damage surrounding cells, expand the inflammatory response, and promote continuous expansion of the calcification core, ultimately resulting in pathological calcification. CNPs, calcified nanoparticles; RUNX2, dwarf-related transcription factor 2; MSX2, homeobox transcription factor muscle segment homeobox 2; AP-1, activator protein 1; XBP1, X-box binding protein 1; STAT3, signal transducer and activator of transcription 3; ROS, reactive oxygen species; NLRP3, NLR family pyrin domain containing 3; BMP2, bone morphogenic protein 2.

lineage kinase 3 pathway), leading to autophagy, apoptosis, and even necrosis, resulting in local inflammation (23-26). On the other hand, ROS is the key component in activating NLR family pyrin domain containing 3 (NLRP3) inflammasomes. ROS can activate NLRP3 inflammasomes through multiple mechanisms, promoting the release of inflammatory cytokines such as IL-1β and IL-18 (27). Moreover, ROS can stimulate the secretion of various cytokines by initiating the nuclear factor-κB (NF-κB) signaling pathway, causing inflammatory damage to renal tubular epithelial cells and renal tissue (28,29).

The inflammatory injury to renal tubular epithelial cells may promote the formation of Randall plaques. Randall plaques originate from the thin ring basement membrane of Henle (30). On the one hand, CNPs entering renal tubular cells may reach the basement membrane by re-exocytosis or colonize it by inducing autophagy, apoptosis, and necrosis. During this process, CNPs release a large amount of calcium and phosphorus stored within renal tubular epithelial cells, resulting in local calcium and phosphorus supersaturation in the renal papilla. Simultaneously, inflammatory lesions further strengthen the deposition of hydroxyapatite, creating conditions for the formation of calcified plaques in the renal papilla. Furthermore, accumulating evidence indicates that extracellular vesicles (EVs) play a critical role in the pathogenesis of pathological calcification. Based on this, it is hypothesized that CNP-infected cells may facilitate the formation of calcification cores by releasing EVs enriched with CNPs into the basement membrane (31).

Colonized CNPs in the basement membrane play a role in biomineralization, aggregating apoptotic bodies and necrotic cells as the core of mineralization, gradually expanding into calcifications and promoting the formation of Randall plaques (32). In addition, the inflammatory injury to HK2 cells promotes calcium crystal adhesion to the injured cells. Tamm-Horsfall Protein (THP) increases, acting as an adhesion protein that facilitates the accumulation and growth of calcium ions, oxalic acid, and phosphate in urine. When THP polymerizes into macromolecular forms, it can inhibit or weaken the inhibition of calcium crystal aggregation in urine. Moreover, THP molecules exhibit weak binding ability to water molecules and poor molecular rigidity. Particles such as calcium salt crystals in urine attach easily, thus promoting stone formation (33).

ROS can upregulate the expression of hyaluronic acid, osteopontin (OPN), and CD44 through the p38MAPK pathway, alter the HK-2 cell adhesion to CaOx crystals and stimulate the formation of CaOx stones. Stimulated by calcified crystals, renal cells produce various inflammatory factors, which induce monocytes or macrophages to migrate to sites of stone crystal deposition via pinocytosis. Phagocytosis of stone crystals by macrophages induces the production of inflammatory factors, including TNF-α, IL-6 and IL-1β, which maintain and aggravate the inflammatory response, causing severe damage to the kidney and promoting the deposition of stone crystals (34,35).

In summary, CNPs cause severe inflammatory damage to the kidneys by damaging HK-2 cells and inducing local inflammatory responses. This inflammatory damage promotes the deposition of stone crystals, forming a positive feedback loop that enhances the formation of Randall spots.

Vascular calcification. Ectopic calcification in the cardiovascular system is a strong predictor of cardiovascular disease morbidity and mortality. This pathophysiological process

entails the deposition of minerals, mainly hydroxyapatite calcium apatite crystals, within the intima and media of vascular walls and heart valve leaflets, with calciprotein particles (CPPs) playing an important role. CPPs are mineral-protein complexes formed by the combination of CNPs and circulating proteins, dispersed in the blood as colloids (36). Circulating Gla-rich protein (GRP), matrix Gla protein (MGP) and fetuin-A are the main proteins that form CPPs. Among them, the most effective binding protein is fetuin-A. MGP and GRP contain negatively charged γ-carboxylated glutamic acid residues (37), which can bind $Ca2^{+}$ and calcium-containing compounds (38). By contrast, fetuin-A forms CPPs through negatively charged β-fold binding to calcium phosphate in the N-terminal cysteine protease inhibitor D1 (39).

Under physiological conditions, initially formed primary CPPs (CPPI) are usually harmless and contribute to the clearance of calcium and phosphate, protecting the body from extraosseous calcification (40). These CPPI are cleared by macrophages, especially Kupffer cells in the liver (41). However, under pathological conditions, especially in patients with diabetes and chronic kidney disease, the decrease of renal excretion of calcium and phosphate leads to their accumulation in the body and a relative decrease in fetuin-A. Consequently, CPPI undergo rearrangement from a spherical structure ~75-nm in diameter to larger secondary CPPs (CPPII), measuring ~120 nm in diameter. These CPPII are denser, insoluble in serum and exhibit a needle-like structure (42).

Research has demonstrated a close association between the formation of CPPII and vascular calcification. CPPII can induce vascular smooth muscle cell calcification and macrophage secretion of TNF-α *in vitro*, but CPPI cannot. Calcification of vascular smooth muscle cells has also been proven to be the result of cellular uptake of CPPII, which can be detected in calcified vascular smooth muscle cells (43). In addition, CPPs can induce macrophages to secrete IL-1β (44). Compared with CPPI, CPPII exhibit a more obvious pro-inflammatory effect, which may be related to the content of crystalline hydroxyapatite (45).

Vascular calcification is the result of two main types: Intimal and medial calcification. Intimal calcification is related to inflammation and is the main driving factor, whereas media calcification is mostly related to mineral disorders (46). Intimal calcification results from the migration of vascular smooth muscle cells during atherosclerosis, whereas medial calcification results from the transdifferentiation of medial vascular smooth muscle cells (47). CPPs play an important role in these processes.

Endothelial cells form the intima of blood vessels, the first cell type to interact with circulating CPPs. Internalization of CPPs by endothelial cells triggers lysosome and cytoplasmic calcium influx, resulting in intracellular calcium overload (48). This internalization induces significant physiological disorders in mitochondria and lysosomes, including oxidative stress, vacuolar acidification, accelerated protein degradation, and increased permeability of the mitochondrial outer membrane. In addition, the incubation of intact endothelial cells with CPP-treated endothelial cells in conditioned medium results in the release of pro-inflammatory cytokines, such as upregulation of vascular cell adhesion molecule-1 and intercellular adhesion molecule-1, increased release of IL-6, IL-8, and monocyte chemoattractant protein-1, ultimately triggering endothelial cell apoptosis (49).

CPPII also affect endothelial cell function by modulating the bioavailability and metabolism of nitric oxide (NO) (50). Endothelial-derived NO plays an important role in balancing and regulating vascular dilation and contraction mediated by vascular smooth muscle cells (51). Endothelial nitric oxide synthase (eNOS) is the principal enzyme responsible for NO production in endothelial cells (52,53). Research has demonstrated that CPPII can induce the dysfunction of eNOS, reducing NO production and bioavailability, thereby impairing endothelial function. This dysfunction affects the vasomotor regulation of vascular smooth muscle cells and eventually leads to hypertension (50). Additionally, eNOS-derived NO is an important antioxidant, which can scavenge a variety of formed free radicals (54). The inhibition of NO production aggravates intracellular oxidative stress and accelerates cell apoptosis.

Media calcification is the main mechanism of vascular calcification. Vascular smooth muscle cells internalizing CPPs can increase intracellular calcium binding and induce their differentiation into osteoblasts through various mechanisms. First, CPPs induce vascular smooth muscle cells to express and secrete TNF-α (43), enhancing the expression of dwarf-related transcription factor 2 (RUNX2) through the homeobox transcription factor muscle segment homeobox 2 (MSX2) (55) and activator protein 1 (AP-1) (56), thus triggering osteogenic dedifferentiation. Second, CPPs stimulate vascular smooth muscle cells to express and secrete bone morphogenic protein 2 (57), which can induce osteoblast dedifferentiation by increasing phosphate transport (58), resulting in endoplasmic reticulum stress and activation of osteogenic transcription factor X-box binding protein 1 (XBP1) (59). Third, CPPs induce oxidative stress in vascular smooth muscle cells (43), activating downstream signaling cascades (including Akt, p38MAPK and NF-κB), which promotes the transcriptional activation of osteochondral differentiation (60-63). Alternatively, CPPs promote the secretion of IL-6 from endothelial cells (64), which may drive the osteochondrogenic differentiation of vascular smooth muscle cells in a signal transducer and activator of transcription 3 (STAT3)-dependent manner (65). The activation of osteochondral transcription eventually leads to a decrease in the expression of contractile proteins (for example α-smooth muscle actin, smooth muscle myosin heavy chain, smooth muscle protein, and calmodulin) and an increase in the expression of osteogenic markers (such as OPN, osteocalcin, alkaline phosphatase, and collagen) (66). The ossification of vascular smooth muscle cells aggravates the damage of vascular homeostasis, promotes the formation of atherosclerotic microenvironment, and exacerbates vascular sclerosis. In addition, transformed vascular smooth muscle cells can release EVs containing CPPs. These EVs can form the core of vascular calcification together with apoptotic vascular smooth muscle cells (67).

When CPPII induce endothelial cell dysfunction through various pathways, endothelial permeability to low-density lipoprotein is increased, causing cholesterol-rich particles to accumulate in the subendothelial layer. Eventually, these particles form unstable arterial plaques, and local inflammatory stimuli further lead to plaque rupture, incorporating them

into the calcified core. In addition, CPPII induce apoptosis of vascular smooth muscle cells and macrophages, resulting in the formation of apoptotic bodies and necrotic cell fragments. These entities serve as nucleation sites within ruptured plaques, initiating microcalcification in injured sites, resulting in calcium phosphate crystal deposition (68). Furthermore, ossified vascular smooth muscle cells and macrophages release EVs containing CPPs, which promote the deposition of calcium phosphate crystals, which gradually crystallize to form hydroxyapatite crystals. Ossified smooth muscle cells also regulate mineralization by secreting osteogenic proteins. Finally, hydroxyapatite crystals continue to accumulate on the calcified core, contributing to vascular calcification.

Calculus oral disease. Calculus oral diseases include dental calculi, dental pulp stones, and salivary gland stones. CNPs may play an important role in these diseases due to their unique biomineralization properties. Some scholars have proposed that CNPs may originate from the atmosphere and can be transmitted through the air, causing calculus oral diseases (69). Kao *et al* (70) observed CNPs through sampling and imaging of salivary gland stones, whereas Zeng *et al* (71) observed and cultured CNPs from dental pulp stones. These studies have shown a large number of CNPs in the oral cavity, which may lead to the formation of oral calculi.

CNPs may induce calcification by damaging cells. Through co-culture experiments of CNPs and periodontal epithelial cells, Zhang *et al* (72) found that CNPs can internalize into these cells, leading to vacuolization of the periodontal epithelial cells. Electron microscopy shows CNPs and calcification within vacuoles, along with calcium deposition outside the cell membrane, which eventually leads to apoptosis. Moreover, CNPs can use calcium and phosphorus in periodontal crevicular fluid to form inorganic precipitates *in vivo*, which are then deposited on the tooth surface as a biomineralization centers to form dental calculus (72). In a co-culture model of human dental pulp cells with CNPs, Yang *et al* (73) found that CNPs can lead to vacuolation and mitochondrial swelling of dental pulp cells, ultimately leading to nuclear calcification and cell necrosis. Sakai *et al* (74) found that the hydroxyapatite shell of CNPs can induce the expression of IL-8 in human gingival epithelial cells through the NF-κB signaling pathway, resulting in inflammatory reactions that damage cells. Furthermore, CNPs can increase pH during mineralization, which is beneficial to the mineralization of dental plaque and the formation of stones in the neutral and alkaline oral environment (75).

Therefore, when CNPs damage human gingival epithelial cells or dental pulp cells, they induce inflammatory responses by aggregating calcium and phosphate ions both intra- and extracellularly and interacting with dental plaque, thereby forming an initial calcified core. As the inflammatory response progressively intensifies, the calcified core gradually enlarges, ultimately leading to the development of calculus-related oral diseases.

Gallstones. Cholecystolithiasis, a prevalent digestive system disorder, mainly involves cholesterol stones or cholesterol-based mixed stones and melanin stones. The cause of gallstones is extremely complex, with various factors playing a role. Any factor influencing the ratio of cholesterol to cholecystocholic acid phospholipid concentration and cholestasis can contribute to gallstone formation. Bacterial infection plays an important role in the formation and progression of gallstones (76). CNPs can be found in the bile and gallbladder mucosa of patients with gallstones (77). Additionally, animal models of cholecystolithiasis have been successfully established by injecting CNPs into the gallbladders of rabbits (78).

CNPs may interact with gallbladder bacteria to promote stone formation. Research has shown the presence of living bacteria in gallstones (79). Culturing gallstone samples from patients with cholecystolithiasis has revealed a high coinfection rate of CNPs and *Escherichia coli* (*E. coli*) in gallstones, suggesting their involvement in the formation of gallstones. Professor Kajander reported that when *E. coli* and CNPs are mixed, calcium-stained mineralized particles both inside and outside *E. coli* are composed of CNPs (80). This finding implies that CNPs can cause biological calcification of *E. coli* by directly attaching to or invading the bacteria. In addition, the number of calcium-stained positive particles found in the mixed culture of CNPs and *E. coli* is significantly higher than that in cultures of CNPs alone. Therefore, the existence of *E. coli* provides a favorable adhesion matrix and microenvironment for CNPs, promoting their reproduction and mineralization (78).

At the same time, CNPs can inflict damage on gallbladder mucosal cells. Research has revealed that after the co-culture of CNPs with human gallbladder epithelial cells, the microvilli on the surface of gallbladder epithelial cells decrease or even disappear, cytoplasmic endocrine granules significantly decrease, and mitochondria swell and vacuolate (79). Over time, cell necrosis ensues, accompanied by an increase in the mRNA levels of IL-6 and IL-1β, indicating the onset of an inflammatory reaction (79). Liang *et al* (81) found that CNPs can stimulate a decrease in the Bcl-2/Bax ratio and subsequently upregulate the expression of cytochrome *c* and activated cysteine protease (cleaved caspase-9) in gallbladder epithelial cells following co-culture with CNPs. This finding suggests that CNPs can induce apoptosis of gallbladder epithelial cells through the mitochondrial pathway (81).

Therefore, during gallstone formation, the interaction between CNPs and gallbladder bacteria may form a calcification center. Furthermore, the injury of gallbladder mucosal cells and the production of ROS to mediate inflammatory reaction aggravate the damage of gallbladder mucosal cells and the disorder of bile acid metabolism and promote the production of cholesterol stones.

3. Conclusion

In conclusion, pathological calcification diseases are a prevalent issue, and their diagnosis and treatment present significant challenges. As research on CNPs continues to advance, it is evident that CNPs plays a crucial role in various pathological calcification diseases. In-depth investigation of CNPs can further elucidate the mechanisms underlying pathological calcification, which holds substantial scientific importance. The present review provides a detailed examination of the primary mechanisms by which CNPs contribute to four common types of pathological calcification diseases. These mechanisms facilitate the formation of an initial calcification core by CNPs, which progressively expands and ultimately results in localized

calcification or stone formation. During this process, strategies such as increasing the levels of circulating calcification inhibitors, inhibiting cellular osteogenic differentiation, and mitigating local inflammatory responses may offer new avenues for the prevention and treatment of pathological calcification.

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Authors' contributions

JW and SL conceived and designed the article. SL and BB analysed the relevant literature. SL wrote the manuscript. JW and SL made revisions to the manuscript. All authors read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

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Patient consent for publication

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Competing interests

The authors declare that they have no competing interests.

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