Reviews

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# Vascular Calcification: Where is the Cure?

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## ABSTRACT

With the progress of aging, the incidence of vascular calcification (VC) gradually increases, which is correlated with cardiovascular events and all-cause death, aggravating global clinical burden. Over the past several decades, accumulating approaches targeting the underlying pathogenesis of VC have provided some possibilities for the treatment of VC. Unfortunately, none of the current interventions have achieved clinical effectiveness on reversing or curing VC. The purpose of this review is to make a summary of novel perspectives on the interventions of VC and provide reference for clinical decision-making.

**Key words:** vascular calcification; clinical; pathophysiology; therapeutic strategies; novel findings

## INTRODUCTION

The prominent manifestation of vascular calcification (VC) is the ectopic deposition of calcified plaques caused by the disorder of mineral metabolism, which is associated with senescence and renal dysfunction. Ectopic calcification usually occurs in intima and media of the artery of communications and micrangium as well as in the heart valves and calciphylaxis. VC is common in chronic kidney disease (CKD), especially in patients with end-stage renal disease (ESRD). Coronary arterial calcification (CAC) significantly increases the risk of cardiovascular events, including myocardial infarction, heart failure, and cardiac death in patients with CKD[1]. Clinical studies have shown that current attempts on reversing VC are insufficient to obtain satisfactory effects. The mechanism of VC is so complex that effective prevention and treatment strategies remain limited.

The review outlines strategies to treat VC and its pathogenesis, emphasizing the importance of managing secondary hyperparathyroidism and cardiovascular risk factors. Inhibition of vascular smooth muscle cell transformation and calcium deposition are crucial drug targets. Stem cell technology and drugs like SNF472 show potential as therapies, offering new insights into VC research and treatments.

## PATHOPHYSIOLOGY OF VASCULAR CALCIFICATION

Previous studies have manifested that VC is caused by hyperphosphatemia and other risk factors including oxidative stress, inflammation, apoptosis, and lipid deposition[2]. VC refers to the pathological deposition of hydroxyapatite in the vascular wall, which is closely attributed to the imbalance of calcium and phosphorus metabolism. In addition, calcified blood vessels continue to mineralize, similar to the ossification process in bones. Therefore, even the calcium and phosphorus disorders have been restored, the existing calcification will continue to progress, which is the main challenge in calcification treatment. *In vitro* primary vascular smooth muscle cells (VSMCs) are possible to be induced to undergo phenotypic transformation expressing osteogenic markers and also lose their specific markers.

Intimal calcification is mainly caused by metabolic diseases, which is characterized by the deposition of lipids and lipoproteins in the intima of large and medium arteries, leading to luminal stenosis, which is similar to the pathogenesis of atherosclerosis. Medial calcification is the main manifestation of VC in CKD patients, which is characterized by the linear deposition of hydroxyapatite in the arterial media,

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resulting in decreased vessel wall compliance, increased pulse pressure, and secondary ischemia, heart failure, and stroke. Heart valve calcification is also common in CKD patients. Studies have shown that calcium deposition in the heart valves is increased in dialysis patients, mainly involving the aortic and mitral valves, which significantly increases the risk of cardiovascular events in CKD patients. Calciphylaxis, also known as calcific uremic arteriopathy, is commonly seen in patients with ESRD. It is a lethal condition characterized by ischemic necrosis of tissues and organs due to calcification, intimal hyperplasia, and thrombosis in systemic small arteries[3]. The disease is mainly characterized by extremely painful skin ulcers, and wound eschar formation can also occur in lung, digestive system, penis, and other organs, manifested as unexplained recurrent dyspnea and gastrointestinal bleeding[4,5]. At present, no unified diagnostic criteria for calciphylaxis have been available. Due to its insidious onset and lack of clinical understanding, calciphylaxis is often diagnosed at an advanced stage, so the treatment is difficult and the prognosis is extremely poor.

Senescence-related mitochondrial damage or dysfunction leads to insufficient energy required for physiological operation of all cells, which is widely recognized as a profound mechanism of VC. More than two-thirds of patients over 70 years of age have extensive VC, and more than 96% of these elderly individuals exhibit CAC[6].

## THERAPEUTIC AGENTS FOR VASCULAR CALCIFICATION

### Vitamins

#### *Vitamin D*

Vitamin D is a lipid-soluble vitamin that can promote the absorption of calcium by the small intestinal mucosa to increase the circulating calcium and phosphorus and regulate bone health. Vitamin D also promotes cell growth and differentiation, regulates immune system, and affects inflammatory response. 1,25-hydroxyvitamin $D_3$ ($1,25(OH)_2D_3$, or calcitriol), the active form of vitamin D, can induce the synthesis of calcium-binding protein that is required in calcium absorption in the intestine and promote the renewal of calcium and osteogenesis[7]. Furthermore, calcitriol also promotes the reabsorption of calcium and phosphorus in renal tubules[7].

The effect of vitamin D supplementation on VC remains controversial. Numerous studies suggest that vitamin D excess increases the risk of VC. Other studies indicate that vitamin D deficiency also leads to VC, and long-term vitamin D supplementation is recommended to prevent calcification. The inconsistent conclusions may stem from two aspects: on the one hand, vitamin D increases circulating concentrations of calcium and phosphate by promoting intestinal and renal absorption. Animal experiments have demonstrated that vitamin D significantly accelerates arterial calcification, but vitamin D doses in these experiments tended to be very high (100,000 to 500,000 IU/kg/day) which is close to toxic dose[8]. Vitamin D, on the other hand, may have anti-inflammatory effects, which have potential benefits for vascular diseases[7]. In several large clinical and epidemiological studies, vitamin D deficiency was significantly associated with cardiovascular and non-cardiovascular mortalities[9]. One clinical study showed that patients with either low or high vitamin D had greater carotid thickness and calcification than patients with normal levels[10]. Due to the rarity of vitamin D excess in the population and the ethical violation of inducing vitamin D toxicity in patients, the relationship between vitamin D excess and VC cannot be conducted in clinical studies. Based on the current evidence, the effect of vitamin D excess on promoting calcification may be less prominent than that of vitamin D deficiency.

#### *Vitamin K*

Vitamin K is a fat-soluble vitamin that has two natural analogues: vitamin $K_1$ (chloroquinone) and vitamin $K_2$ (mequinone, MKs). Vitamin K can be obtained from diets, synthesized by intestinal bacteria and artificial supplements, and transported through the lymphatic system. Studies indicate that, compared with vitamin $K_1$, vitamin $K_2$ has a more comprehensive effect on human body[11]. A total of 17 vitamin K-dependent proteins (VKDPs) have been discovered, which play important roles in regulating mineral metabolism, osteogenesis, VC, and inflammatory responses. The primary function of vitamin K is to serve as a coenzyme of coagulation factor (gamma-carboxylase) and an essential substance for the synthesis of clotting factors Ⅱ, Ⅶ, Ⅸ, and Ⅹ[12]. Vitamin K deficiency may prolong blood clotting time and generate severe bleeding.

Matrix Gla protein (MGP) is mainly synthesized in chondrocytes and VSMCs, which are the primary suppressant of calcinosis, assisting to prevent VC[13]. After translation in the cytoplasmic endoplasmic reticulum, MGP is rapidly carboxylated by gamma-glutamyl carboxylase (GGCX). This process requires vitamin K as a key cofactor to form the carboxylated MGP. Secreted by osteoblasts, osteocalcin (alternative name is bone gla protein) is a component that promotes calcium deposition into bone[14]. Growth arrest-specific protein 6 (Gas6), a component of the tyrosine kinase receptor family, inhibits VC by suppressing apoptosis of endothelial cells and VSMCs[15]. Gla-rich protein has been regarded as a new type of VKDP in sturgeon cartilage, which contains many Gla residues and may be a potential inhibitor of VC[16]. Therefore, vitamin K can inhibit the progression of VC by facilitating the activities of VKDPs.

Vitamin K alleviates lipopolysaccharide-induced inflammatory responses by intercepting the transduction of nuclear factor κB (NF-κB) signaling pathway[17]. Vitamin K level is suggested to have a negative association with certain inflammatory markers such as interleukin-6 and C-reactive protein[18]. However, a randomized trial recruiting 379 healthy subjects receiving vitamin $K_1$ supplements for three years failed to observe any changes in inflammatory biomarkers[19]. A multicenter trial enrolled 365 elderly men with an aortic valve calcification (AVC) score >300 arbitrary units (AU) manifested that two-year MK-7 plus vitamin D supplementation made no difference on AVC progression[20]. A phase II trial to evaluate the influence of vitamin K supplementation over one-year follow-up on vascular stiffness or calcification in kidney transplant recipients also received a disappointing result[21].

## Statins

Statins are the acknowledged basic drugs for the prevention and treatment of coronary heart disease by competitively inhibiting the rate-limiting enzyme hydroxy methylglutaryl coenzyme A (HMG-CoA) of endogenous cholesterol synthesis, blocking intracellular cholesterol synthesis, and effectively reducing total cholesterol and low-density lipoprotein. In recent years, studies reveal that statins have a variety of other non-lipid-lowering properties, including stabilizing atherosclerotic plaques, treating osteoporosis, anti-inflammation, anti-oxidation, anti-tumor, and anti-Alzheimer's disease. A series of imaging studies have shown that high-intensity statin therapy is associated with plaque volume reduction by reducing fatty and necrotic plaque ingredients and increasing plaque calcification. Statins have anti-inflammatory and antioxidant properties in addition to their cholesterol-lowering effects. Ivanovski *et al.* [22] first demonstrated that simvastatin could reduce oxidative stress (OS) markers and aortic calcification in apolipoprotein E deficient mice with CKD, independent of cholesterol changes. Further studies indicated that pravastatin was capable of decreasing OS level through inhibiting farnesyl transferase in calcified VSMCs[23]. Statins could increase atherosclerotic calcification by disinhibiting the Rac1-IL-1β signaling axis of macrophage[24]. Statins and vitamin $K_2$ can inhibit apoptosis-mediated VSMC calcification by activating Gas6/Axl-PI3K/Akt pathway[25,26]. After five years of follow-up, a population-based study implied that statin treatment was independently correlated with CAC progression[27]. An analysis of two randomized controlled trials comparing the influence of high-dose statin therapy (atorvastatin 80 mg) and low-to-moderate-intensity statin therapy (pravastatin 40 mg or atorvastatin 10 mg) on CAC failed to demonstrate any difference in Agatston score or CAC volume within one year[28]. However, the available data have not reached a uniform conclusion about the relationship between statin use and VC progression.

## Matrix Gla protein

Matrix Gla protein (MGP) is a vitamin K-dependent calcium-binding protein synthesized by osteogenesis and VSMCs. It is a carboxyglutamic acid translated in bones, cartilage, heart, and kidneys. This protein acts as an inhibitor of vascular mineralization and plays a role in bone tissue. Keutel syndrome is a rare autosomal recessive disorder characterized by severe calcification of soft tissues, which is thought to be caused by mutations in the gene encoding for MGP[29]. The possible mechanisms that MGP inhibits calcification can be summarized in two aspects. First, MGP has a high affinity for calcium ions and hydroxyapatite crystals, which can be used as a chelating agent[30]. Second, MGP reduces the ability of osteoblast differentiation by binding bone morphogenetic protein-2 (BMP-2) [31]. The ability of MGP inhibiting calcification is not limited in bone metabolism but also in atherosclerotic calcified plaques and calcified lesions of vascular media[32,33]. Therefore, MGP is considered to be a crucial suppressant of the intima and media of VC. However, there is no clinical evidence of MGP for the treatment of VC.

## Mitochondrial function modulators

Nicorandil is the first clinically used mitochondrial ATP-sensitive $K^+$ channel activator with abilities to prevent intracellular $Ca^{2+}$ release and stimulate $K^+$ internal flow, thus maintaining membrane integrity and energy metabolism[31]. As impaired mitochondrial function is partially related to VC in patients with CKD, nicorandil can relieve VC in rat models of CKD. Nicorandil reversed mitochondrial function in adenine-induced CKD rats by enhancing mitochondrial depolarization and energy metabolism, and reducing mitochondrial swelling[34,35]. In addition, nicorandil has been proved to facilitate antioxidant capacity and inhibit reactive oxygen species (ROS) production[35], resulting in reduced aortic calcification[34,35]. Clinical studies have confirmed that nicorandil is suitable for all types of angina pectoris and can significantly reduce the risk of cardiovascular events. However, the protective effect of nicorandil against myocardial reperfusion injury was not present in potentially calcified rat hearts[36].

Mitochondrial autophagy is an evolutionarily conserved biological process of mitochondrial degradation and recycling. Under the stimulation of ischemia and hypoxia in mitochondria, the activity of electron transport chain is weakened, resulting in the obstruction of ATP production, the disappearance of mitochondrial membrane ion gradient and $Ca^{2+}$ overload, and finally the opening of mitochondrial membrane permeability conversion pores and the entry of cytochrome c and various mitochondrial proteins into the cytoplasm, thus inducing cell apoptosis. Activation of mitochondrial autophagy can prevent the accumulation of damaged mitochondria and protect VSMCs from oxidative stress, inflammatory response and apoptotic cascade reaction. However, excessive mitochondrial autophagy can lead to repeated degradation of normal mitochondria, affect energy production, lead to cell death, and eventually cause serious damage to tissues and organs. Autophagy activation in the presence of cell damage is thought to be protective, while autophagy defects in normal cells lead to apoptotic activation. Therefore, it is of great significance to regulate proper mitochondrial autophagy to ensure its cell-protective function. As a key regulator of mitochondrial division, mitochondrial dynamin-related protein 1 (Drp1) plays an important role in triggering mitochondrial autophagy. Failure of mitochondrial autophagy can activate Drp1-mediated apoptosis pathway, promote vitamin $K_2$-induced osteoblast differentiation and bone mineralization, and eventually affect VC[37]. The interaction between mitochondrial autophagy and VC is complex and can be influenced by disease progression, anatomical location, and surrounding microenvironment. While the clinical role of Drp1 in VC is still unclear, inhibiting Drp1 is a potential target to treat VC.

### Hydroxyapatite inhibitors

The phosphate binder sevelamer[38] and the cinacalcet[39], for the treatment of hyperphosphoremia and secondary hyperparathyroidism, reduce the potential risk factors for VC in ESRD, but they can hardly resolve the biological issues responsible for this complex pathological phenomenon. Myo-inositol hexaphosphate (IP6, phytate), a substance from fiber-rich foods such as grains, nuts, and legumes, has been applied to treat cardiovascular calcification[40,41]. The mechanism of IP6 is completely different from that of the above-mentioned drugs. IP6 binds to hydroxyapatite in a concentration-dependent manner, preventing the formation and aggregation of hydroxyapatite crystals and fundamentally inhibiting the formation of calcification products. Although IP6 has a natural vascular protective effect, its clinical application is limited by poor oral bioavailability[42].

SNF472 was developed as an intravenous formulation of IP6 to intervene calciphylaxis and VC in hemodialysis patients, and it can be administered via the hemodialysis line to achieve direct inhibition of hydroxyapatite aggregation without being easily cleared by dialysis[43,44]. SNF472 can inhibit the occurrence and development of ectopic calcification, thereby preventing the formation of hydroxyapatite in blood vessels and treating VC. A phase I clinical trial proved that in hemodialysis patients, treatment with SNF472 9 mg/kg inhibited the hydroxyapatite by 80% (compared with 9% in the placebo group) without affecting the serum calcium level and did not cause clinical symptoms related to hypocalcemia[45]. Valve replacement is the only approach that has been proven to improve membrane function. There is no effective drug that can delay the progression of valvular disease. CaLIPSO (Cal for calcium and IPSO meaning the item itself)[46] is the first multicenter clinical trial to compare progression of CAC between 52-week administration of SNF472 and placebo. Results showed that SNF472 significantly alleviated CAC and aortic valve calcification in hemodialysis patients apart from fundamental treatments. The CaLIPSO further evaluated the changes in bone mineral density (BMD) of the total hip and femoral neck and found that BMD decreased in all groups during the follow-up period, but there was no correlation between changes in the volume of CAC and changes in BMD at other locations, and fracture events were rare[47]. SNF472 also had no significant effect on serum alkaline phosphatase, calcium, magnesium, phosphate, and intact parathyroid hormone, which are related to bone metabolism. Preliminary findings from studies of SNF472 in animal models of cardiovascular calcification and in patients undergoing dialysis have led to the advancement of this compound into phase II clinical trials for the treatment of calciphylaxis[48]. However, the study was a single-arm, open-label trial with a small sample size that was not sufficient to provide a clinically significant result. SNF472 has entered phase III clinical trial, and preliminary results suggested that it might become the first drug approved to cure VC or calciphylaxis[49].

### Antioxidant therapies

Oxidative stress (OS) is a vital inducement of cellular aging, which may cause severe damage to cellular organelles and nucleic acids, proteins, and cytomembranes. ROS production in calcified VSMCs may cause telomere shortening, mitochondrial DNA damage, and gene mutations, resulting in VSMC senescence and VSMC transformation. These sequence of changes will activate osteoblast transcription[50].

Coenzyme Q10 is one of the substances involved in the electron transport chain and aerobic respiration in eukaryotic cell mitochondria. It can promote oxidative phosphorylation and protect the integrity of cytomembranes. Coenzyme Q10 is widely used in the treatment of viral myocarditis, chronic heart failure, hepatitis, and cancer, with an ability to reduce the adverse reactions caused by radiotherapy and chemotherapy[51]. In animal models with CKD, coenzyme Q10 was found to suppress ROS production and reduce apoptosis, thus alleviating VC[52]. Moreover, administration of coenzyme Q10 could reduce aortic stiffness in the elderly by alleviating mitochondrial ROS, compared with placebo[53].

Alpha-lipoic acid (ALA) is a natural antioxidant that has multifarious functions. Additionally, ALA is suggested to enhance mitochondrial function and inhibit cell apoptosis[54]. ALA can be used in the clinical treatment of acute and chronic hepatitis, liver cirrhosis, hepatic coma, fatty liver, diabetes, skin aging, and other diseases. In *in vivo* models of CKD, ALA attenuated VC by inhibiting ROS and enhancing mitochondrial function through regulating the Gas6/Axl/Akt pathway to reduce apoptosis[55]. Nonetheless, treatment with ALA 600 mg/d for two to six months failed to change the OS or inflammatory markers in both nondialysis and hemodialysis CKD patients, compared with placebo[56,57].

Sodium thiosulfate (STS) has antioxidant capacity and direct chelation of calcium that commonly used to treat VC[58]. STS is found to inhibit BMP-2 synthesis and promote MGP function in calcified VSMCs[59], suggesting that STS might be involved in the formation of VC. In adenine-induced CKD rats, STS reduced VC without improving mitochondrial function or OS[35]. STS combined with other antioxidants or mitochondrial function modulator may provide a synergistic effect in VC intervention. STS combined with nicorandil effectively restored mitochondrial metabolism, improved antioxidant capability, reduced ROS production, and ultimately alleviated VC[35]. With the help of fat cells, STS could alleviate arterial calcification to a greater extent[60]. These results suggest that the role of STS in reducing VC may be attributed to direct chelation of calcium instead of antioxidant capacity. Moreover, STS treatment has been proved to reduce arterial stiffness, stabilize VC, and prevent heart valve calcification in hemodialysis patients[61,62].

Quercetin, a natural and dietary source of flavonol, has antioxidant and antiinflammatory activities by inhibiting mitogen-activated protein kinase (MAPK) and NF-κB pathways[63]. An optimized dose of quercetin can reduce VC through inhibiting ROS, enhancing ATP synthesis, and alleviating apoptosis in animal models with CKD[64]. Quercetin mitigates aortic calcification and chondrogenesis through suppressing the expression of transglutaminase 2 and β-catenin[65]. Additionally, quercetin can partially decrease aortic calcification through enhancing the level of superoxide dismutase 2 and activating nitric oxide synthetase (iNOS)/MAPK pathway[66]. Nevertheless, the phase Ⅱ clinical trial manifested that quercetin combined with sodium nitrite failed to optimize vascular function in predialysis CKD patients compared with placebo, including biomarkers of endothelial function (*e.g.*, vascular adhesion molecule, e-selectin, and von Willefamer factor), inflammation, and OS[67].

### Anti-inflammatory therapies

Systemic and local inflammation play a key role in the occurrence and development of VC. In addition to mineral metabolism imbalance, the oxidative stress, inflammation, and cell signal transduction abnormal factors are also involved in VC. Anti-inflammatory therapies contribute to reduce the production of inflammatory cytokines (*e.g.*, IL-6 and tumor necrosis factor alpha [TNF-α]) and C-reactive protein during inflammation, which promotes the transformation of VSMCs into osteoblast-like cells. The use of anti-inflammatory drugs, including non-steroidal anti-inflammatory drugs (NSAIDs), glucocorticoids, or more targeted biologic agents, can reduce the production of these inflammatory mediators and slow the calcification process. The accumulation and activation of inflammatory cells such as macrophages and lymphocytes at the site of VC can exacerbate local inflammatory responses. Anti-inflammatory therapies can inhibit the activation and migration of inflammatory cells and reduce inflammation of vascular injuries. Receptor activator of nuclear factor-kappa B ligand (RANKL)/receptor activator of nuclear factor-kappa B (RANK)/osteoprotegerin (OPG) passway associated with bone remodeling also plays a key role in VC. Certain anti-inflammatory drugs may slow down the process of calcification by inhibiting VSMC ossification through regulating bone formation correlation factors. Denosumab, a specifically targeted monoclonal antibody against RANKL, reduces disease progression in patients with aggressive fibrous dysplasia of the bone and treats osteoporosis. Clinical studies have shown that denosumab has the potential in reducing VC[68], but not in another trial[69]. Given the small sample sizes, no definite conclusions have been available.

Monoclonal antibodies, particularly those targeting specific inflammatory factors, provide a new insight for VC treatment. For instance, the TNF-α inhibitors (infliximab or adalimumab) reduce inflammation through direct combination with excessive TNF-α, which may be beneficial to controling VC. Animal studies have shown that the infliximab could block the aorta BMP-2, Msx2, Wnt3a, and Wnt7a signaling pathways and significantly reduce the aortic calcium accumulation[70]. However, there is almost no relevant clinical research[71]. IL-6 is an essential mediator of inflammation and autoimmune diseases. The inhibitor (tocilizumab) can effectively reduce inflammation, which may be conducive to inhibiting VC. With a deeper understanding of the inflammatory mechanisms of VC, more biological agents targeting specific inflammatory pathways such as IL-1β and IL-17 are being developed, aiming to intervene in the inflammatory process more precisely. Although the anti-inflammatory and immune therapies have shown certain potentials, their actual efficacy, long-term safety, and optimal timing of use in VC treatment warrant more studies.

### Mineral metabolism regulators

The balance of calcium and phosphorus metabolism is extremely essential for the organs and cells to maintain normal physiological functions. CKD patients often also have metabolic disorders of minerals such as calcium, phosphorus, and magnesium. Severe disturbances in calcium and phosphorus homeostasis, particularly hyperphosphatemia, are recognized as pivotal contributors to the pathogenesis of arterial calcification and the onset of other cardiovascular complications in CKD individuals. Phosphate is related to vascular inflammation and can induce VSMCs to produce a variety of proinflammatory cytokines such as tumor necrosis factor, IL-1β, and IL-6. Oxidative stress induced by inflammation is a strong inducer of VSMC osteogenic transformation and VC. The increase in calcium and phosphorus shows a synergistic effect in inducing VC. Calcium-induced VC may be achieved by promoting the release of VSMCs matrix vesicles and cell apoptosis. In addition, hypercalcemia is an independent risk factor for aortic calcification in CKD patients. Secondary hyperparathyroidism is also involved in the occurrence of VC. A rat model of uremia demonstrated the relative effect of high blood phosphorus and high parathyroid hormone on VC. It was found that high parathyroid hormone had a direct effect on VC and could also enhance the VC induced by high phosphorus. Stabilization of calcium and phosphorus levels in patients with CKD is particularly critical for preventing VC progression and prolonging life. Servilam, a calcium-free phosphate binder, reduces treatment-associated hypercalcemia, but its effects on reducing VC and all-cause mortality have not been conclusively concluded[72,73]. Calcimimetics is used as a quasi-calcium agent that can reduce serum parathyroid hormone, phosphorus, and calcium levels in patients with secondary hyperparathyroidism[74,75].

In addition to traditional calcium and phosphorus, magnesium may be associated with the incidence of cardiovascular diseases in patients with CKD. Magnesium, a cofactor of various metabolic reactions in human body, is involved in regulating ion channel currents. It plays a momentous role in regulating cell growth and maintaining the excitability of myocardium and skeletal muscle. Recent evidence suggests magnesium is involved in many cardiovascular function-regulating processes, and disruption of magnesium homeostasis can precipitate a spectrum of cardiovascular pathologies including hypertension, arrhythmia, atherosclerosis, arterial calcification, and vascular aging. Magnesium ions can activate the expression of calcium-sensitive receptors and are considered to be natural physiological calcium blockers. Magnesium deficiency promotes VC. Combined with the use of quasi-calcium, magnesium supplementation can reduce the deposition of minerals in blood vessels[76]. In animal studies, magnesium supplementation has been observed to repress the expression of osteogenic genes, thus supressing the occurrence of calcification[77]. It has also been proved that magnesium can regulate calcification by affecting calcium and phosphorus metabolism, lipid deposition, oxidative stress, inflammation, and other processes. Magnesium supplementation may be a new therapeutic approach for delaying or alleviating VC. However, multiple randomized controlled trials have shown that magnesium supplementation alone is inadequate to delay the progression of VC, and other treatment strategies are badly in need to reduce the risk of cardiovascular disease in patients with CKD[78,79].

## POTENTIAL TARGETS IN VASCULAR CALCIFICATION

### Iron homeostasis

Some new molecular targets provide new perspectives and possibilities for the treatment of VC. Iron is a pro-oxidant that can induce ROS productions, and its overload can aggravate oxidative stress and VC[80]. Ferroptosis is a type of cell death characterized by the accumulation of supersize oxidative stress products. Iron overload is associated with a variety of diseases, including cardiovascular diseases. Ferroptosis occurs in VSMCs during calcium-phosphate-induced VC, and inhibiting iron death significantly reduces osteogenic differentiation and calcification of VSMCs. Regulating iron homeostasis, such as lowering excess iron in the body through chelating agents, may help slow or prevent the process of VC. Deferoxamine chelates free or protein-bound 3-valent iron ($Fe^{3+}$) to form a stable, non-toxic, water-soluble iron-amine complex that is excreted via urine. Primarily, it is used for relieving acute iron poisoning. Animal studies have shown that deferoxamine can reduce aortic calcification in rats with CKD. Therapeutic strategies targeting specific iron metabolism in VC are still under investigations[81].

### Epigenetic modification

The transdifferentiation of VSMCs from contractile type to osteoblasts is the key molecular event of VC. Epigenetic modification is involved in the regulation of target gene expression through DNA methylation, histone modification, and non-coding RNA regulation without changing the DNA coding sequence. It can regulate the transdifferentiation of VSMCs and participate in the regulation of VC. Specific epigenetic modification enzymes such as demethylase alkB homolog 1 (ALKBH1) are involved in the regulation of VC[82]. Upregulation of *ALKBH1* gene leads to decreased demethylation level, and *ALKBH1* overexpression aggravates the progression of VC. Targeting *ALKBH1* may be a therapeutic approach to reducing VC burden in CKD patients[83]. Sirtuin 6 (SIRT6), a member of the Sirtuin family, is a class III histone deacetylase and a key epigenetic regulator. SIRT6 inhibits osteogenic transdifferentiation of VSMCs by down-regulating human runt related transcription factor 2 (RUNX2) and plays a role in alleviating VC. Thus, SIRT6 may be an attractive therapeutic target for VC[84]. Non-coding RNAs, such as miRNAs, affect the osteogenic differentiation of VSMCs and regulate VC by affecting the expression of osteogenic factors such as RUNX2 and alkaline phosphatase. However, relevant clinical evidence is lacking. Enhancing or inhibiting specific epigenetic modifications to restore normal function of VSMCs may provide new pathways to reduce calcification. More research in these emerging fields is in need of transitioning from basic research to clinical applications.

### Stem cell technology

The role of stem cell technology in VC is a cutting-edge and highly concerned research field. Stem cells, especially mesenchymal stem cells (MSCs), have multidirectional differentiation potentials and strong paracrine effect. They can secrete a variety of growth factors and anti-inflammatory factors, which help to promote vascular repair and reduce macrophage-mediated damage[85]. Stem cells may be involved in remodeling damaged vascular tissue by inducing differentiation into vascular smooth muscle cells or endothelial cells[86]. Autologous or allogeneic stem cells can be injected into diseased vascular region with the expectation that they would differentiate into functional vascular cells to alleviate or reverse calcification. In addition to direct transplantation, stem cells might also be used as a vector carrying anti-VC gene to achieve more precise therapeutic effect.

Ectoblastic cells expressing specific adhesion molecules of stem cells, including Sca1 and/or CD34, has the potential to differentiate into VSMCs[87]. Mesenchymal stem cell (MSC)-like cells, specifically those positive for Gli1 expression, express a number of known vascular progenitor cell markers, including CD34, Scal, and platelet-derived growth factor receptor beta (PDGFRβ). Gli1+ cells are located in the outer membrane of the artery and are progenitors of VSMCs, contributing to intimal recovery and regeneration after acute injury. Gli1+ cells are the main source of osteoblasts during VC. Gene modification of Gli1+ cells prior to renal injury significantly reduced the degree of VC[88], suggesting that Gli1+ cells are vital adventitial progenitor cells in vascular remodeling after acquired injuries, and they might be the most promising therapeutic targets for alleviating VC. However, these theories are based on animal experiments, and there is no evidence for clinical application.

The current drugs related to the treatment of VC and the relevant clinical and preclinical studies are list in **Table 1**.

## INTERVENTIONAL THERAPIES IN VASCULAR CALCIFICATION

CAC lesions bring great difficulties to interventional therapies, which increases the risk of surgical failures (e. g., inadequate balloon or stent dilation), complications (e. g., coronary dissection, coronary perforation, or balloon rupture), and perioperative mortality[89]. A variety of techniques have been developed to address the diverse challenges posed by calcified lesions, including various balloons, rotational, orbital and laser atherectomy, and intravascular lithotripsy (IVL)[90].

Balloon angioplasty (BA) is an interventional cardiovascular treatment technique that is mainly used to treat stenosis or occlusion of coronary artery, carotid artery, renal artery, lower extremity artery, and other vessels. The procedure involves making a small incision in the skin, delivering a catheter with an inflatable balloon to the site of the diseased blood vessel, and then using pressure to expand the balloon to squeeze and flinch the atherosclerotic plaque on the inner wall of the blood vessel, thereby restoring blood flow. Non-Compliant (NC) balloons are often applied in the treatment of moderate CAC to achieve more ideal stent dilation that can withstand high pressure and achieve more uniform balloon dilation. BA with NC balloon is an auxiliary therectomy performed after therectomy to ensure sufficient plaque modification prior to stent releases[89]. High-pressure balloon is a NC balloon catheter that can withstand high pressures (up to 35 atm) without significantly changing the diameter inside the balloon[91]. Cutting balloon is another NC balloon catheter with the blades making shallow incisions in calcified plaques to facilitate stent implantation. The microsurgical blades are mainly applied in ostial or in-stent restenosis (ISR) lesions[92]. Scoring balloon is surrounded by sharp scratch elements, allowing concentrated force into calcified plaques throughout the expansion process. Compared with cutting balloons, scoring balloons reduced the risk of vascular wall injury and coronary dissection[93].

Intravascular lithotripsy (IVL) is a novel technique for treating severe CAC. The shock waves produce positive pressures (up to 50 atm) to selectively rupture the surface and deep layers of the calcified regions without affecting the surrounding soft tissues[94]. IVL is administered safely without causing coronary dissection, slow flow phenomenon, embolism, or coronary perforation[95-98].

Rotational atherectomy (RA) mechanically ablates hard calcified stenosis with a rotating diamond tip while deflecting flexible non-calcified tissues. High-speed burr rotation enlarges lumen, smooths plaque surface, and increases balloon predilation[89]. Current guidelines indicate that RA improves surgical success in fibrosis or severe CAC (class 2a, evidence B)[99].

Orbital atherectomy (OA) utilizes a diamond-coated crown to generate targeted force for the ablation of non-flexible calcified plaques. The OA crown is suggested to be performed with a slow and continuous motion in areas that need repetitive ablation. OA generates less slow flow and minor thermal damage during crown orbiting, which is possible to decrease slow flow events and thermal injuries during interventional procedures[100].

Laser atherectomy modifies plaques by photoablation. This technique produces short wavelengths with high-energy ultraviolet light that causes water

**Table 1. Summary of studies on current drug interventions of vascular calcification**

| Intervention | Study model | Inclusion | Number | Follow-up | Conclusion |
|---|---|---|---|---|---|
| Vitamin D | Animal, human | Men from the community with AVC[20] | 365[20] | 24 months[20] | Controversial[9], negative[20] |
| Vitamin K | Human | Men from the community with AVC[20], kidney transplant recipients[21] | 365[20], 90[21] | 24 months[20,21] | Negative[20, 21] |
| Statin | Human | Subjects from the HNR study without CAD at baseline[27], hypercholesterolemic women and men[28] | 3,483[27], 852[28] | 5 years[27], 24 months[28] | Negative[27,28] |
| Matrix Gla protein | Molecular, cellular | - | - | - | Positive[29,31] |
| Nicorandil | Animal | - | - | - | Controversial[34,36] |
| SNF472 | Animal, human | Adult patients with ESRD receiving hemodialysis[46] | 274[46] | 52 weeks[46] | Positive[44,46] |
| Coenzyme Q10 | Animal, human | Healthy older adults (60-79 years) with impaired endothelial function[53] | 20[53] | 6 weeks[53] | Positive[52,53] |
| Alpha-lipoic acid | Animal | - | - | - | Positive[55] |
| Sodium thiosulfate | Human | Haemodialysis patients with abdominal aorta calcification[61] | 60[61] | 6 months[61] | Controversial[61] |
| Quercetin | Animal, human | Patients with predialysis CKD[67] | 70[67] | 12 weeks[67] | Positive[64], negative[67] |
| Denosumab | Human | Hemodialysis patients[68,69] | 58[68], 30[69] | 2 years[68], over 2 years[69] | Positive[68], negative[69] |
| Infliximab | Animal | - | - | - | Positive[70] |
| Servilam | Human | Patients with CKD[72], adults with CKD not on dialysis or post-transplant[73] | 2,802[72], 2,498[73] | -(34 RCTs)[72], median 9 months (20 RCTs )[73] | Positive[72], negative[73] |
| Calcimimetics | Cell | - | - | - | Positive[75] |
| Magnesium | Animal | - | - | - | Positive[77] |
| Deferoxamine | Animal | - | - | - | Positive[81] |

AVC: aortic valve calcification; HNR: Heinz Nixdorf Recall; CAD: coronary artery disease; ESRD: end-stage renal disease; CKD: chronic kidney disease; RCTs: randomized controlled trials

evaporation, carbon bond dissociation, and molecular vibrations, contributing to plaque elimination and lumen expansion[101]. It can be used to treat balloon lesions that cannot span or dilate, chronic complete occlusions, debulking vein graft disease, and calcific non-dilatable in-stent restenosis (ISR)[102,103].

## SURGICAL TREATMENTS IN VASCULAR CALCIFICATION

Surgical bypass or endarterectomy represents the rescue treatment for severe vascular stenosis caused by heavy VC. Endarterectomy is a surgical procedure that is mainly used to treat the disease of intimal lipid plaque deposition, luminal stenosis, or even occlusion of the large and middle arteries due to atherosclerosis. This procedure mainly targets severe stenoses in the carotid, iliac, and femoral arteries. In endarterectomy, the surgeon will cut open the outer tissue of the diseased vessel, directly expose and remove the hardened plaque and thickened intima on the inner wall of the vessel, so as to restore blood flow and reduce the risk of cardiovascular and cerebrovascular events caused by poor blood flow.

A study comparing percutaneous coronary intervention (PCI) and coronary artery bypass grafting (CABG) in patients with severe CAC within ten-year follow-up showed that the calcification was an independent predictor of mortality; however, there was no difference in prognosis between PCI and CABG treatment[104]. Clinical evidence demonstrated that anatomic factors of VC would affect the risk of carotid angioplasty and were associated with 30-day risk of stroke or death in patients with symptomatic severe carotid stenosis undergoing endarterectomy or angioplasty[105].

As an invasive treatment, surgery can be associated with perioperative and long-term complications and therefore is not the preferred choice for calcification intervention.

## CONCLUSIONS

Based on the possible mechanisms of VC, the therapeutic strategies focalize in the regulation of bone and mineral metabolism disorders. The management of secondary hyperparathyroidism is essential for rectifying the dysregulation of bone and mineral metabolism, which encompasses conditions such as hyperphosphatemia, hypocalcemia, hypercalcemia, and disturbances in parathyroid hormone levels. Oral administration of phosphate binders, calcitriol, antioxidants, and $Ca^{2+}$ mimics can be helpful. Given the association between intimal VC and atherosclerosis, the management of pertinent risk factors — such as dyslipidemia, hypertension, diabetes mellitus, tobacco use, obesity, and physical inactivity — constitutes a pivotal therapeutic strategy for the prevention of cardiovascular incidents.

Currently, no efficient therapy is available to prevent or reverse vascular or valvular calcification. Inhibition of VSMC phenotypic transformation and calcium deposition are the two main strategies for reversing VC. Stem cell therapy is the most promising intervention, and SNF472 has been applied as an orphan drug to treat peripheral artery disease in ESRD.

The advent of multi-omics technologies, epigenetics, single-cell sequencing, and bioinformatics has furnished novel methodologies for elucidating the underlying mechanisms, pinpointing potential therapeutic targets, and repurposing existing medications for the treatment of vascular and valve calcification.

## ARTICLE INFORMATION

### Competing interests

The authors declare no conflict of interest.

### Authors' contributions

Liu WW and Liu ML wrote, reviewed, and edited the manuscript before submission. Both authors confirm the final version of the manuscript.

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