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REVIEW

STATE-OF-THE-ART PAPER

Coronary Artery Calcification

From Mechanism to Molecular Imaging

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ABSTRACT

Vascular calcification is a hallmark of atherosclerosis. The location, density, and confluence of calcification may change portions of the arterial conduit to a noncompliant structure. Calcifications may also seed the cap of a thin cap fibroatheroma, altering tensile forces on the cap and rendering the lesion prone to rupture. Many local and systemic factors participate in this process, including hyperlipidemia, ongoing inflammation, large necrotic cores, and diabetes. Vascular cells can undergo chondrogenic or osteogenic differentiation, causing mineralization of membranous bone and formation of endochondral bone. Calcifying vascular cells are derived from local smooth muscle cells and circulating hematopoietic stem cells (especially in intimal calcification). Matrix vesicles in the extracellular space of the necrotic core serve as a nidus for calcification. Although coronary calcification is a marker of coronary atheroma, dense calcification (>400 HU) is usually associated with stable plaques. Conversely, microcalcification (often also referred to as spotty calcification) is more commonly an accompaniment of vulnerable plaques. Recent studies have suggested that microcalcification in the fibrous cap may increase local tissue stress (depending on the proximity of one microcalcific locus to another, and the orientation of the microcalcification in reference to blood flow), resulting in plaque instability. It has been proposed that positron emission tomography imaging with sodium fluoride may identify early calcific deposits and hence high-risk plaques.
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Rudolph Virchow, a 19th century pathologist, recognized that vascular calcification occurred as the result of an ossification process (1). About a century later, Tanimura et al. (2) described a stepwise process of intimal vascular calcification and alluded to the role of "extracellular matrix vesicle-like structures" in intimal calcification. Calcium in the coronary arteries has been used as a surrogate marker of coronary atherosclerosis since the 1940s, an era when cardiologists would greet their patients wearing large red googles, to maintain dark adaption so they could see coronary calcium on the fluoroscopy screens (3,4). Improvements in technology, especially high-speed multislice computed tomography (CT) scans, has allowed objective measurement of both the density and extent of coronary calcification (usually calculated by using the method of Agatston et al. [5]). Indeed, the coronary calcium score has been widely used to compute the future risk of an acute coronary event (6).

Macroscopic vascular calcification seen on clinical CT scanning evolves from nanometer foci in inflamed atheroma that are detectable at histopathology but are too small to be seen on clinical CT scanning with

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its spatial resolution of approximately 0.5 mm (7). Calcification begins within the necrotic core of the atheroma. Extracellular vesicles (8) are released by dead or dying macrophages and smooth muscle cells (SMCs). These vesicles provide the scaffolding for initiation of calcification. Control of the local concentration of calcium and phosphate are lost. In the case of phosphate, for example, enzymes such as alkaline phosphatase, adenosine triphosphatase, and reactive oxygen species (9) create free phosphate from the breakdown of larger molecules. Ultimately, the local concentration of free calcium and phosphate in autophagosomes reaches a sufficient concentration to allow formation of calcium phosphate crystals (10,11), which are visible by electron microscopy (12), via active processes that resemble osteo/chondrogenic conversion. The focus of the present review was on vascular calcification from mechanism to molecular imaging.

INTIMAL CALCIFICATION: DIFFERENT FROM MEDIAL CALCIFICATION

Intimal calcification resembles endochondral bone formation in long bones (cartilage metaplasia). Although initiation of calcification does not require participation by specific cells, progression of the lesion is likely driven by chondrocyte-like cells and associated with the expression of inflammatory factors, such as cytokines. Cytokines are produced by tissue macrophages and foam cells (13) in response to the harsh environment in atherosclerotic plaque due to the noxious effects of oxidized lipoprotein. Calcified atheroma in low-density lipoprotein (LDL) receptor–deficient and apolipoprotein (apo) E–deficient mice exhibit cartilage metaplasia and chondrocyte-like cells, which express specific chondrocytic markers such as Sox9, collagen II, and collagen X (14–16). Many disease processes, including dyslipidemia, hypertension, systemic inflammatory diseases (lupus), diabetes, and kidney disease, influence the biology of atherosclerosis, vascular remodeling, and vascular calcification (17–20).

Medial calcification has a different mechanism driven by the action of osteoblast-like cells. Bone morphogenetic protein (BMP)-2/muscle segment homeobox homologue/wingless-type MMTV integration site family member (Wnt) signaling, which is characteristic of intramembranous bone formation, occurs prominently in medial calcification. This signaling causes direct transdifferentiation of vascular cells to osteoblast-like cells, independent of Runx2/cbfa1 (21), as confirmed in an LDL receptor -/- mouse model (22). In addition, osteoblast-like cells resulting in medial calcification may also be derived from chondrocyte-like precursor cells, as has been observed in matrix γ-carboxyglutamic acid protein (MGP) -/- mice (23) and in adenine-induced uremic rats (24,25). Whether patients develop medial or intimal calcification is determined by local factors (26). Both osterix and Wnt/β-catenin signaling cause osteo-chondroprogenitor cells to evolve to osteoblast-like. Conversely, suppression of osterix and Wnt/β pathways or induction of Sox9 induces differentiation of progenitor cells down the chondrocyte pathway (27,28).

At least 4 cell types may lead to vascular calcification: 1) pericytes in microvessels; 2) pericyte-like calcifying vascular cells in the aortic intima; 3) SMCs in the media; and 4) myofibroblasts in the adventitia (29–32). In medial calcification, medial SMCs undergoing osteochondrogenic differentiation are the major contributor (33). In intimal calcification, nearly 90% of chondrocyte-like cells originate in the bone marrow (34), and transdifferentiation of local SMCs to chondrocytes contributes minimally (15).

ATHEROSCLEROTIC LESION AND DEVELOPMENT OF NECROTIC CORE

Atherogenesis is initiated by the focal retention of apo B–containing lipoproteins in the subendothelial extracellular matrix, particularly chondroitin sulfate proteoglycans (35–38). The retained lipoproteins cause local inflammation, resulting in the release of chemoattractant peptides (39). Monocytes attracted to the site enter the tissue, undergo conversion to tissue macrophages, and phagocytize/catabolize the lipoprotein cholesterol complex. In the process of catabolism, the lipoprotein cholesterol complex is aggregated and oxidized. Oxidized lipoprotein cholesterol is particularly toxic to the macrophage, and, if present in sufficient quantity, could cause death of the lipid-laden macrophage (foam cell). This macrophage cell death is initially due to apoptosis (40). However, if the atheroma has a large intensely inflamed necrotic core, normal apoptotic cell clearance mechanisms (efferocytosis) are incomplete, allowing dead cell detritus to contribute to the necrotic core, with release of toxic material into the local environment (41). T cells accelerate the noxious process by producing antibodies that recognize oxidized LDL, adding fuel to the inflammatory fire. SMCs migrate into the intima, proliferate, and

ABBREVIATIONS AND ACRONYMS

apo = apolipoprotein

BMP = bone morphogenetic protein

CT = computed tomography

FDG = fluorodeoxyglucose

LDL = low-density lipoprotein

MGP = matrix γ-carboxyglutamic acid protein

Msx2 = muscle segment homeobox homologue

NaF = sodium fluoride

OPG = osteoprotegerin

RANK = receptor activator of nuclear factor κB

RANKL = receptor activator of nuclear factor κB ligand

SMC = smooth muscle cell

VSMC = vascular smooth muscle cell

Wnt = wingless-type MMTV integration site family member

promote formation of the collagenous fibrous cap. The combination of oxidative stress, hypoxia, interferon gamma, and cholesterol overload induce macrophage cell death.

In lesions with a large necrotic core, the macrophages may die by alternative mechanisms such as explosive programmed cell death and necroptosis (42,43). More than 40% of dead cells in an inflamed atherosclerotic lesion are macrophages (44). In early lesions, the remnants of apoptotic cells, apoptosomes, are phagocytized by adjacent macrophages and SMCs. In advanced lesions, however, phagocytosis cannot keep pace with the rate of cell death, and residual vesicles remain in the lesion, lose their membrane integrity, and the toxic contents are released into the local environment. This defective efferocytosis results in further inflammation (45). Intense inflammation stimulates angiogenesis, producing fragile, capillary-like vessels that are characteristic of unstable plaque. Leaky or ruptured intraplaque vessels result in intraplaque hemorrhages, increasing free cholesterol, further increasing inflammation, and raising the likelihood of acute plaque rupture (46–51).

INITIATION OF CALCIFICATION

Four mechanisms have been proposed for initiating calcification (52):

1. death of inflammatory cells in atheroma-releasing apoptotic bodies and necrotic debris that serve as nucleating sites for calcium phosphate crystal formation;
2. matrix vesicles released locally or circulating nucleation complexes that serve as sites for calcium complex crystallization;
3. reduced local expression of mineralization inhibitors; and
4. induction of bone formation resulting from differentiation of pericytes (30) and/or vascular smooth muscle cells (VSMCs) (53–55).

Calcification is initiated in regions of inflammation where there is a local decrease in collagen fibers (56). In carotid endarterectomy specimens, matrix vesicles are especially abundant in acellular areas of plaque and along elastic fibers (8,57,58). Matrix vesicles and apoptotic bodies released during the death of macrophages and proliferative phenotype SMCs contribute to the early stages of both intimal and medial calcification (2,11,58–60). Depending on the size and type, extracellular vesicles are broadly classified as either ectosomes (microparticles 50 to 1,000 nm; rich in cholesterol and diacylglycerol), exosomes (40 to 100 nm; rich in cholesterol, sphingomyelin, and ceramide), apoptotic bodies (50 to 5,000 nm), or matrix vesicles (30 to 300 nm). These extracellular vesicles contain a variety of cargoes, including lipids, proteins, and micro-ribonucleic acid (8). Vesicle nucleation sites allow deposition of calcium orthophosphate (11,60–62), which progresses to amorphous calcium phosphate (63,64) and then to more crystalline structures such as hydroxyapatite. However, it is not clear which occurs first, the vesicle-driven formation of calcium crystals or the transdifferentiation of vascular SMCs (or pericytes [65]), to create the initial environment for calcification (8,66).

INFLAMMATION AND CALCIFICATION

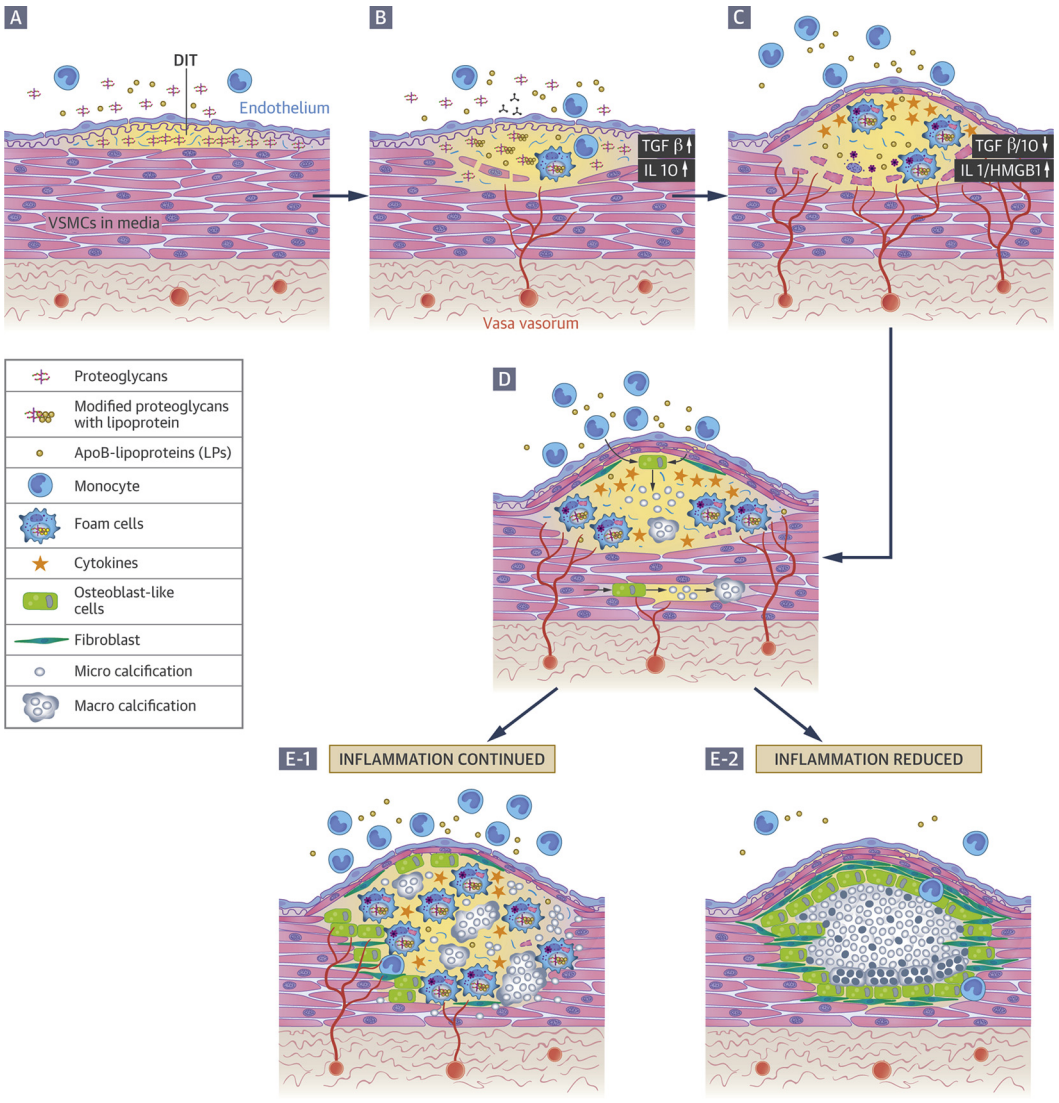
Intact macrophages and VSMCs contain calcification inhibitors, including matrix Gla protein and fetuin-A, that minimize calcification and facilitate rapid phagocytosis of the apoptotic particles (53,67,68). In the absence of these inhibitors, calcium overload leads to formation of microcalcifications.

The phenotypic function of cells in atheroma can change. For example, if local inflammation decreases, the phenotypes of T lymphocytes change from T helper 1 to T helper 2 cells, whereas macrophages change from the proinflammatory M1 subtype to the reparative M2 subtype (69–71). Surviving VSMCs promote fibrosis to stabilize the atheroma. Conversely, if pro-osteogenic conditions persist, VSMCs continue transdifferentiation toward a chondrocyte/osteoblast-like phenotype. These cells orchestrate a regulated mineralization process, ultimately leading to formation of macrocalcification, which stabilizes the plaque and acts as a barrier to the spread of inflammation (52,55) (**Central Illustration, Figure 1**).

INHIBITORS OF VASCULAR CALCIFICATION

Hypertension, dyslipidemia, and hyperphosphatemia (in patients with renal disease) are clinical risk factors for developing vascular calcification (72). Unfortunately, there is no clinically accepted therapy to reduce vascular calcification. From a biochemical perspective, there are 6 potent inhibitors of vascular calcification: 1) MGP; 2) pyrophosphate; 3) fetuin-A; 4) osteopontin (OPN); 5) Klotho; and 6) osteoprotegerin (OPG) (**Figure 2**). MGP binds to BMP, a growth factor that triggers the transformation of VSMC to osteoblast-like cells (73), and inhibits its activity (74,75). MGP needs vitamin K to be activated. Therapy with warfarin induces and accelerates vascular calcification by blocking this pathway

CENTRAL ILLUSTRATION **The Evolution of Atheroma and Calcification: Plaque Initiation, Inflammation, Microcalcification and Progression to Macrocalcification**



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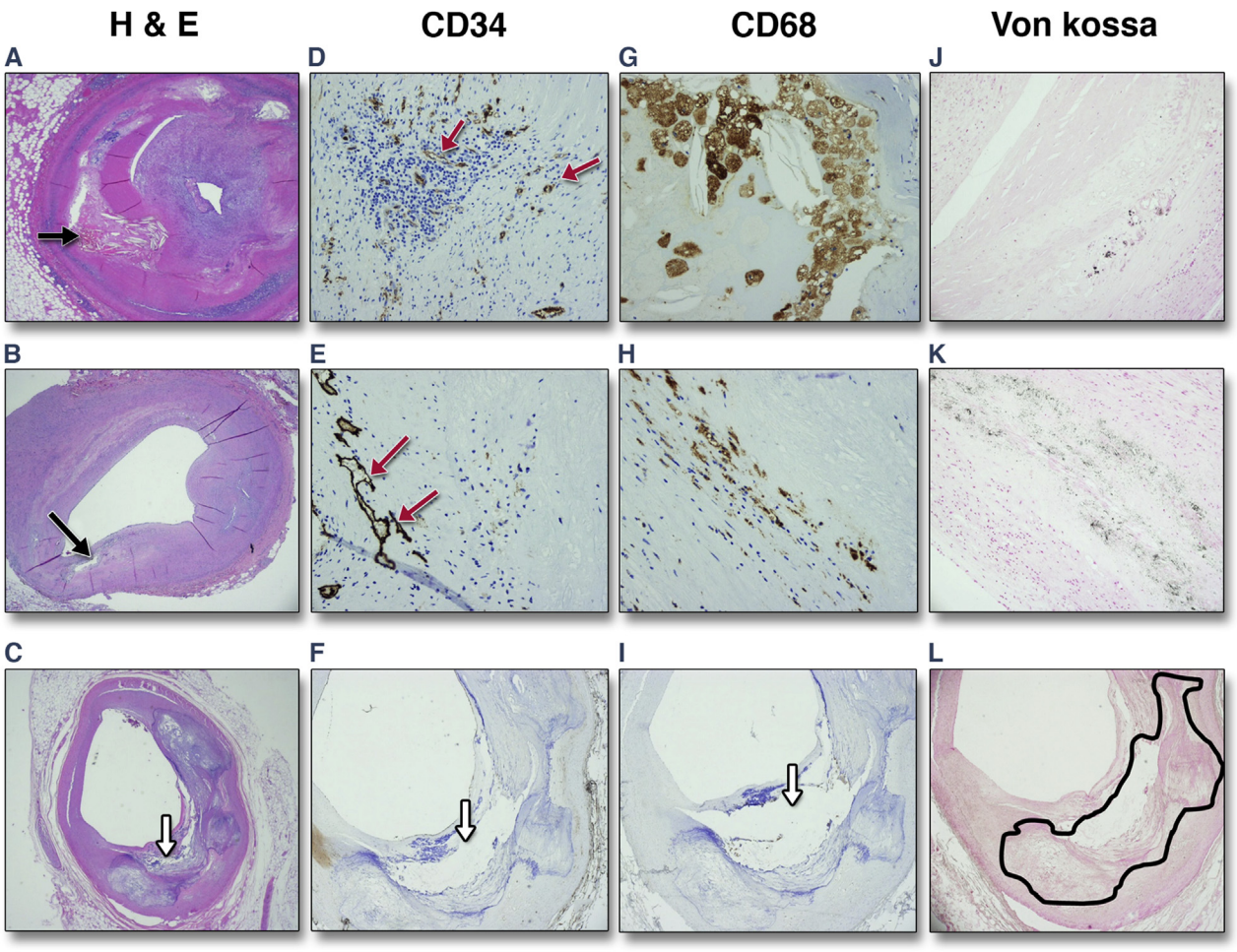
This scheme shows an overview of progression to calcification. **(A)** An atheroma is initiated by the focal retention of apolipoprotein B (ApoB) lipoproteins (LPs) in the subintima. The lesion results in diffuse intimal thickening (DIT) composed of subendothelial extracellular matrix molecules, especially proteoglycans. **(B)** Proteoglycans bind to lipoprotein cholesterol and oxidized lipoprotein cholesterol. Chemoattractant peptides are secreted, attracting circulating monocytes to the lesion. Monocytes enter the tissue, differentiate into macrophages, and ingest the retained and modified LPs to become cholesterol-laden foam cells. Vasa vasorum are stimulated to increase the local vascular supply. In this early stage, the remnants of apoptotic cells are phagocytized by macrophages that produce anti-inflammatory cytokines, such as transforming growth factor (TGF)-beta and interleukin (IL)-10, which inhibit atherosclerosis development. **(C)** In advanced lesions, phagocytosis of apoptotic detritus cannot keep up with the rate of apoptotic cell death, and instead of the orderly elimination of apoptosomes, the residual cellular components remain in the lesion, lose their membrane integrity, and the toxic content is released into the local environment (defective efferocytosis), resulting in further inflammation. Well-developed vasa vasorum also contributes to further inflammation. **(D)** M1 macrophages produce inflammatory cytokines and continue inflammation, although M2 macrophages attenuate inflammation. Associated with expression of inflammatory cytokines, calcifying cells showed osteoblast-like or chondrocyte transdifferentiation to attenuate inflammation. The extracellular vesicles serve as nucleation sites for hydroxyapatite and matrix vesicles and apoptotic bodies released in the process of oncosis from macrophages and smooth muscle cells contribute to the early stages of calcification (microcalcification). These calcifications attenuate inflammation. **(E1)** If inflammation continues, apoptosis of macrophages and vascular smooth muscle cells continues, and more microcalcifications form. Microcalcifications gather into a large mass and become spotty calcification. This type of macrocalcification will still be prone to rupture. **(E2)** If the inflammation is reduced, the lesion stabilizes. HMGB1 = high-mobility group protein B1.

FIGURE 1 Histopathological Representation of Development of Calcification From Microcalcification to Macrocalcification

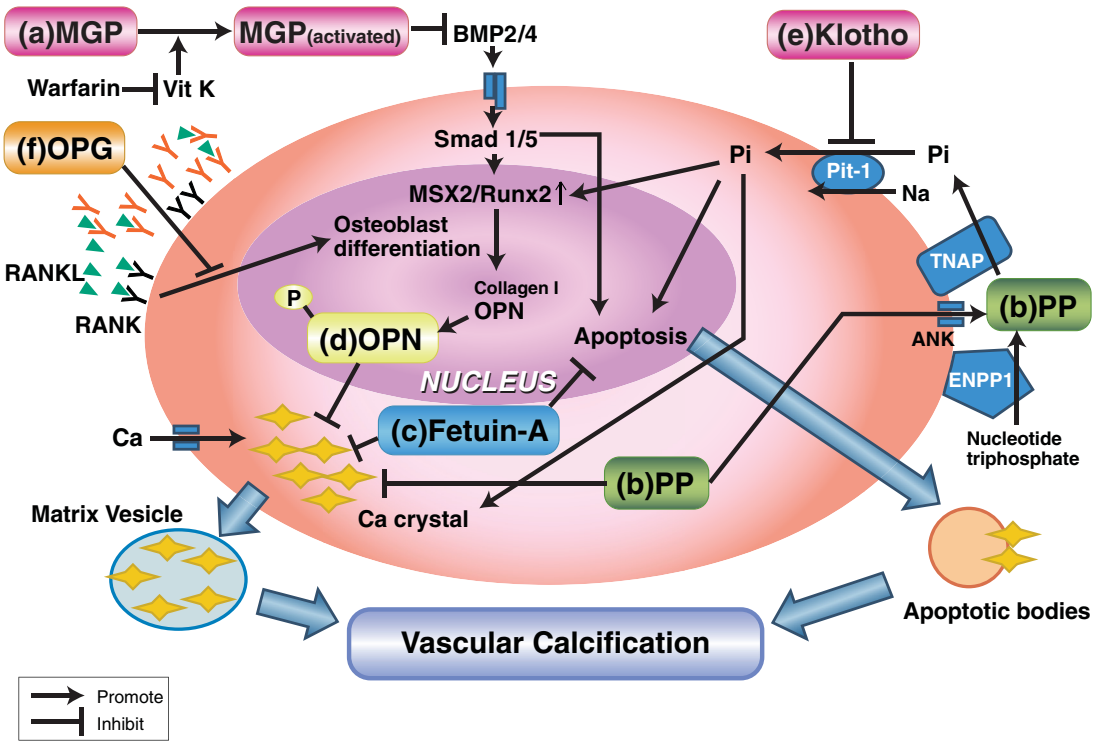


A, B, and C are images of atherosclerotic plaque with a large necrotic core, small necrotic core, and predominantly calcified plaque, respectively (**black arrows** point to necrotic cores in **A and B** and **white arrows** point to calcification in **C**). Magnification 20×. Immunohistochemical staining of the 3 plaques with an endothelial marker (CD34), macrophage marker (CD68), and special stain for calcium (von Kossa) is shown **(D to L)**. Numerous small CD34-positive vessels **(red arrows)** and macrophages are seen in plaque with large necrotic core **(D, G)** and focal granular calcium deposits seen as **black** with von Kossa stain **(J)**. The plaque with small necrotic core **(B)** has fewer vessels **(E)** and macrophages **(H)**. Von kossa stain shows the calcium deposition **(K)**. Heavily calcified plaque does not have vessels **(F)** and macrophages **(I)**. Von Kossa stain is negative, even though the calcium can be seen by hematoxylin and eosin (H&E) stain (the vessel needed to be decalcified before sectioning). The area of calcification is marked by the **black line (L)**. The empty space **(white arrow)** represents a focus from where the calcium has fallen off during sectioning. (Magnification 200×, **D, E, G, and H**; magnification 100×, **J and K**; magnification 40×, **F, I, and L.**)

(76,77). Pyrophosphate inhibits precipitation of calcium phosphate, preventing the formation of hydroxyapatite and acting to dissolve it (78). Pyrophosphate inhibits calcification in cultured injured rat aortas and in both vitamin D and renal failure models of vascular calcification (79,80). Fetuin-A (a liver-derived blood protein) binds small clusters of calcium and phosphate. Fetuin-A acts both as a systemic and local inhibitor of vascular calcification by preventing extracellular and intravesicular calcium phosphate growth and reducing calcium-induced apoptosis in VSMCs (81). OPN inhibits vascular calcification as long as it is phosphorylated (82). MGP-/-/OPN-/- mice had significantly more arterial calcification than MGP-/-OPN+/+-mice and experienced vascular rupture and early death (83).

Klotho, a gene associated with antiaging properties, directly inhibits phosphate uptake by VSMCs, in addition to enhancing phosphaturia (84,85). Klotho

FIGURE 2 An Overview of Calcification-Related Factors and Inhibitors in Vascular Smooth Muscle Cell



Calcium, phosphate, receptor activator for nuclear factor κB ligand (RANKL), and bone morphogenetic protein (BMP)2/4 contribute to calcification in the atherosclerotic regions rich in inflammation and apoptosis. Matrix γ-carboxyglutamic acid protein (MGP), osteoprotegerin (OPG), Klotho, fetuin-A, pyrophosphate (PP), and osteopontin (OPN) act at various levels in the calcification cascade to prevent calcification. PP is generated by nucleotide pyrophosphatase/phosphotransferase-1 (ENPP1) from nucleotide triphosphate or from transfer by the PP transporter ankyloses protein (ANK); PP is cleaved by tissue-specific alkaline phosphatase (TNAP). Soluble phosphate enters intracellularly via sodium-dependent phosphate co-transporter 1 (Pit-1), binds to calcium and creates calcium-phosphate crystal; calcium-phosphate crystal promotes matrix vesicles and vascular calcification. Conversely, the receptor activator for nuclear factor κB (RANK)–RANKL interaction induces differentiation and activation of osteoclasts to promote vascular calcification. Apoptosis produces apoptotic bodies and provides a nidus for vascular calcification. **(a)** MGP binds to and inhibits BMP. Vitamin K (Vit K), which is inhibited by warfarin, is required for activation of MGP and BMP2/4 inhibition. **(b)** PP inhibits precipitation of calcium phosphate. **(c)** Fetuin-A prevents calcium phosphate growth and reduces calcium-induced apoptosis in vascular smooth muscle cells. **(d)** OPN inhibits calcification when phosphorylated. **(e)** Klotho suppresses Pit1/2 expression and inhibits phosphate uptake by vascular smooth muscle cells. **(f)** OPG is a decoy for RANKL and blocks the RANK–RANKL interaction.

deficiency predisposes SMCs to transform into osteoblast-like cells and initiate mineralization in response to phosphate uptake. OPG is a regulatory factor with roles in bone turnover, inhibiting osteoclast differentiation, and serving as a decoy for the receptor activator of nuclear factor κB (RANK) ligand (86). The differentiation and activation of osteoclasts are regulated by RANK ligand binding to RANK; OPG blocks the receptor activator of nuclear factor κB ligand (RANKL)-RANK interaction. The OPG/RANKL/RANK system expressed both in clinical and experimental atherosclerosis and enhanced T-cell expression of RANKL is an important feature of unstable angina (87). OPG treatment reduced both intimal and medial calcification in LDL-/- mice and rats receiving vitamin D and warfarin (88,89).

In human specimen analysis, MGP was observed in both intimal and medial SMCs, and BMP4 was observed in early lesions (type I and II). In advanced lesions, BMP2, BMP4, and OPG in intimal SMCs located at the shoulder of fibroatheroma were observed, whereas MGP, OPN, and BMP4 were expressed in foam cells in the lipid core (90). OPG, OPN, and MGP at sites of microcalcification were observed in early coronary plaque from individuals experiencing sudden cardiac death (54). The balance

of procalcification factors and inhibitors determine whether calcification occurs.

THE NATURAL HISTORY OF CORONARY CALCIFICATION IN HUMAN ATHEROSCLEROSIS

The initial histological evidence of coronary calcifications are microscopic foci (from 0.5 to 15μm) in regions of severe inflammation (91,92). These foci are associated with expression of procalcification factors in regions of intense inflammation, typically in the lipid pool/necrotic core of atheroma, with macrophages undergoing incomplete efferocytosis (11) of apoptotic bodies (54,93–95).

Microcalcifications often aggregate into larger masses and become speckled fragments of calcification, which spread into the necrotic core, extracellular matrix (54), and the cap of the plaque. Microcalcifications are frequently observed histologically in lesions with a large necrotic core, separated from the flowing blood by a thin fibrous cap that may contain microcalcifications. Such microcalcification cannot be detected on diagnostic CT scans, which have a spatial resolution of approximately 0.5 mm. In addition to focal microcalcification of the cap, the concurrent infiltration by macrophages and T lymphocytes further reduces the integrity of the cap. Calcification in the necrotic core progress into larger sheets or plates of calcium detectable on diagnostic CT scans as "calcified plaques."

For reliable detection on CT imaging, the calcification needs to be >0.215 mm in diameter based on high-contrast phantom studies (96) and will likely have to be larger to overcome the effects of scatter and cardiac motion artifacts. If calcium nodules rise into the lumen, they may fracture the fibrous cap, causing an acute thrombus (54). Although most plaque ruptures are not associated with calcific nodules, eruption of a calcification in a proximal vessel in a hypercoagulable (vulnerable) patient (97) can be responsible for a clinically significant acute event in up to 3% to 5% of all plaque ruptures (98,99).

VASCULAR CALCIFICATION: GOOD OR EVIL?

Although macroscopic calcification leads to plaque stability, calcium in fibrotic plaque and the calcified core of coronary atheroma are associated with remodeling, suggesting that calcification might be associated with remodeling independent of inflammation (100). A clinical indication of multiple microcalcification may be "spotty" calcification on CT imaging. Motoyama et al. (101) found that spotty calcification on CT scanning is more likely to be associated with plaque rupture. The coronary artery calcium score is an independent predictor of coronary events (102–104), useful for classifying patients at low or intermediate risk of acute coronary events (104,105). Calcified atherosclerosis in other vascular beds is also independently associated with cardiovascular risk and mortality (106–108). Guideline publications, however, do not yet recommend screening asymptomatic patients for vascular calcification due to the high prevalence of calcification in asymptomatic patients (109).

Although vascular calcification is a marker of coronary atheroma, the relationship to higher acute event rate is not yet clear. Intravascular ultrasound studies showed that disease progression was slower in densely calcified plaque compared with minimally calcified plaque (110), whereas spotty calcification was associated with greater progression of atheroma volume in stable patients (111). Studies reported significantly greater calcification in stable plaque than in unstable plaques in older adults who experienced sudden death (112,113), and coronary artery calcium density on CT scanning was inversely associated with a cardiovascular event (114). Gössl et al. (115) showed that the density of vasa vasorum in human coronary arteries was significantly lower in calcified plaque compared with nonstenotic plaque or noncalcified stenotic plaque, suggesting that vascular calcification is associated with lesion stabilization (46–51). In summary, the coronary artery calcium score is not useful for identifying high-risk plaques but represents an overall risk of an acute coronary event in a population (112,113). Statin therapy, which reduces cardiovascular mortality, has been linked to increased (116), decreased (117), or no change (118) in coronary calcification. These findings could reflect a complicated situation in which statins may limit the progression of vascular calcification (119) by reducing inflammation in atheroma associated with the decrease in LDL cholesterol; statins may also increase calcification associated with plaque quiescence/healing (120). Sodium fluoride (NaF) positron emission tomography imaging (described later) may settle this controversy.

The earlier stages of spotty calcification (<1 mm) or microcalcification (<50 μm) may be associated with plaque rupture (121). Autopsy samples scanned with high-resolution microCT scans demonstrated an association of microcalcification on local tissue stress and plaque instability in three-dimensional models (94,95,122); the peak circumferential stress was increased to 396 kPa with microcalcifications from 210 kPa without microcalcifications, wherein 300 kPa is considered to be the minimum threshold for rupture.

Agglomerated or irregular microcalcification imposed higher stress than spherical microcalcifications (123), and the shape of a microcalcification was determined by the relationship with collagen and extracellular vesicles (56). In addition, the orientation of the microcalcification and proximity of adjacent microcalcification with reference to the direction of blood flow determine stress on the cap of the plaque (124,125). It has also been suggested that although microcalcification could increase the local stress in the cap during systole, the larger calcification could counteract this stress by reducing deformation (126). Clinical intravascular ultrasound studies have suggested that attenuated plaques, seen in patients with acute coronary syndromes (127), might be associated with microcalcification (128).

IMAGING OF MICROCALCIFICATION

Calcific deposits in atheroma are common. MicroCT scans, with a resolution of 2.1 μm, display nearly 35,000 microcalcifications >5 μm in size, in fibrous caps of nonruptured human atherosclerotic plaques (94). Current-generation CT scanners are unable to detect these microcalcifications (**Table 1**). An alternative to detecting inflammation in plaque is molecular imaging of vascular calcification with the bone-seeking tracer, ^{18}F-labeled NaF (129–131). NaF was first introduced in 1962 (132) for the detection of osseous metastases. NaF localizes in hydroxyapatite crystals by exchange of hydroxyl ions (OH-) in the crystals for ^{18}F-fluoride ions (133). In addition to calcium and phosphate ions, hydroxyapatite contains many positive and negative ionic species, including mono, di, and trivalent ions. For the ionic exchange to occur, the hydroxyapatite crystal must be near a capillary, allowing the fluoride ion to pass from the plasma, through the extracellular fluid space into the shell of bound water surrounding the calcium phosphate crystal. Irkle et al. (134) suggested that NaF preferentially binds to microcalcification beyond the resolution of the CT scan in the vasculature. In laboratory studies, fluoride adsorption depended on the surface area of the mineral (135). In clinical studies, 88% of vascular uptake of NaF uptake co-localized with calcification visible on CT imaging, but only 12% of all arterial calcification sites showed increased NaF uptake (129). There was an association of NaF uptake and cardiovascular risk factors, suggesting carotid NaF uptake was a surrogate measure of actively calcifying carotid plaque (130). Interestingly, only 6.5% of arterial lesions demonstrated co-localization of the inflammation marker ^{18}F-labeled fluorodeoxyglucose (FDG) with NaF, suggesting the tracers provide different information about atherosclerotic lesions (131).

In a comparison of FDG and NaF in patients with coronary disease, NaF uptake correlated with the calcium score, although 40% of patients with scores >1,000 displayed no radiofluoride uptake (136). There was no correlation between FDG and NaF uptake. Coronary NaF uptake was evaluated in a pilot study and was noted in the culprit plaque in 37 (93%) of

TABLE 1 Spatial Resolution and the Diagnostic Ability of Various Imaging Modalities for the Detection of Microcalcification and Macrocalcification

	Maximum Spatial Resolution	Invasive	Feature
CT scan	Clinical CT scan: 0.4–0.6 mm Research CT scan: 2.1 μm	No	Best modality to detect microcalcification and macrocalcification. Clinical CT imaging detects macrocalcification but cannot reliably detect microcalcification because of spatial resolution
MRI	Clinical MRI: 1.3–1.8 mm Research MRI: 250 μm	No	Research MRI can detect microcalcification; however, clinical MRI cannot reliably detect microcalcifications
IVUS	Clinical IVUS: 100–200 μm With backscatter: <40 μm	Yes	High resolution; however, IVUS does not penetrate calcification, resulting in a posterior echo shadow. IVUS tends to overestimate calcifications
OCT	Clinical OCT: 15–20 μm Research OCT: <5 μm	Yes	Very high resolution, suitable for detection of microcalcifications
NIR imaging	Clinical NIRS: 1 mm Research NIRF: 100 μm	Yes NA	Imaging agents trace molecules concerning calcifications with NIRF. NIRS is available in clinical; however, there are no clinical imaging agents for NIR imaging
Molecular imaging	Clinical PET: 3–5 mm Research PET: 900 μm	No	Imaging agents trace molecules concerning calcifications. Although spatial resolution is low, the affinity of imaging agents allows visualization inflammation, apoptosis, and early calcification

CT = computed tomography; IVUS = intravascular ultrasound; MRI = magnetic resonance imaging; NA = not available; NIRF = near-infrared fluorescent; NIRS = near-infrared spectroscopy; OCT = optical coherence tomography.

TABLE 2 Context: Vascular Calcification and Molecular Imaging

1. Vascular calcification is an active process resembling bone formation
2. Intimal and medial calcifications are caused by different mechanisms
3. Macrophages, inflammation, and apoptosis may play a crucial role in the initiation of intimal calcification
4. Dense vascular calcification generally stabilizes plaques
5. Microcalcification may be more commonly encountered in high risk plaques. Reducing microcalcification may help reduce the incidence or severity of major acute clinical events
6. PET/CT imaging with Na ^{18}F may clinically identify early microcalcification

CT = computerized tomography; Na ^{18}F = ^{18}F-sodium fluoride; PET = positron emission tomography.

40 patients with acute myocardial infarction (137); however, in patients with stable angina and advanced atherosclerosis, only 45% of patients had plaque with focal NaF uptake that was associated with microcalcification on intravascular ultrasound. Ex vivo incubation of NaF with carotid endarterectomy specimens localized at the site of macroscopic plaque rupture and were correlated with histochemical staining for tissue nonspecific alkaline phosphatase and osteocalcin. NaF uptake was seen in both CT scan-detectable calcified lesions and CT scan-negative lesions. In vivo imaging was less precise compared with the ex vivo imaging results, wherein ex vivo NaF uptake was associated with the most regions of microcalcification but only few macrocalcific few lesions. A possible explanation for this observation is the reduced density of vasa vasorum in densely calcified lesions, suggesting reduced delivery of the tracer to these lesions.

CONCLUSIONS

Vascular calcification is an established feature of atherosclerosis. Studies now suggest that the early microcalcification stage reflects a vulnerable phase in the evolution of plaque. Animal models have advanced our understanding of the development and evolution of microcalcification. Serial radionuclide fluoride images with clinical positron emission tomography/CT imaging could offer a noninvasive opportunity to determine the prognostic value of these findings. Although inflammation and macrophage activity plays a crucial role in the initiation of calcification, fluoride uptake in the coronary or carotid arteries correlates with the necrotic component of the atheroma and appears to identify a high-risk state in the evolution of the lesion. Based on this information, it is time to consider a multicenter trial of fluoride imaging to determine the prognostic significance of vascular fluoride imaging (**Table 2**).

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