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# New paradigms in cardiovascular calcification

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ABSTRACT

Out of several concurrent hypotheses that attempt to explain the development of calcific lesions in vascular tissues, the most widely accepted is based on the formation of bone-like tissue. Conversely, a study recently published on a materials science journal shed new light on the nature of cardiovascular calcification. The results have shown that cardiovascular calcification found in tissues affected by different calcific diseases is formed from the same nano/micro calcified spherical particles, which are also the first calcified structure that could be detected in vascular tissue. Moreover, cardiovascular calcification is not formed mainly by bone tissue but, surprisingly, by a not previously described, highly crystalline calcium phosphate material. By combining biology, materials science and chemistry, the new results have generated fundamental insights into the nature of cardiovascular calcification. This perspective summarizes these findings and epitomizes the scientific approach it has used to address important clinical questions.



## 1. Introduction

Calcification in the cardiovascular system is a common mineral metabolic disease that contributes to many of the approximately 17 million deaths per year caused by cardiovascular disease [1]. The burden of calcific disease is relatively larger in male individuals and increases with advancing age, diabetes mellitus, hypertension, smoking, elevated low-density lipoprotein levels, metabolic syndrome and renal dysfunction [2]. The cardiovascular tissues more commonly affected include the aorta, femoral arteries and aortic valves [3–5]. In cardiac valves, calcification is characterized by calcific lesions on the valve cusp, resulting in an impaired movement of the leaflets. Pathological mineralization in arteries can lead to stenosis through thickening and stiffening of tissues, reducing blood flow and eventually resulting in heart failure [6,7].

Calcification associated with atherosclerosis may significantly increase health concerns. Calcification nuclei have been associated to cell death and atherosclerotic intra plaque hemorrhages. The differences in mechanical properties between the mineral and the tissue can actually provoke hemorrhages. Moreover, the calcification found in the lipid core of lesions can produce micro fractures, which in turn can lead to acute thrombosis and even fatal myocardial infarction [8,9].

## 2. Existing models of cardiovascular calcification

Cardiovascular calcific disease was described as a passive degenerative process that occurs due to deterioration of tissues caused by the stress and strain of cardiac function. More recently, research associated with molecular signaling processes of vascular homeostasis has changed this view of cardiovascular calcification and redefined it as

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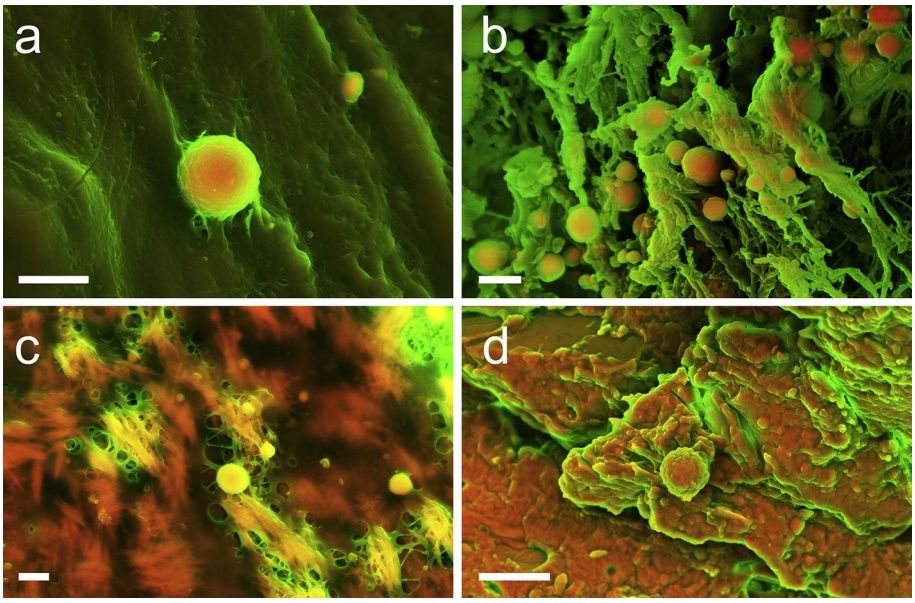


**Fig. 1.** The topographical and compositional information gathered from secondary electron and backscattering signals with SEM are correlated by color-coding and overlapping, generating density-dependent color images (DDC) that highlighted the location of nano-scale calcified features within the organic matrix. The orange color identifies denser material; structures that appear green are less dense. DDC-SEM micrographs of the aortic valve presenting (a and b) dense spherical particles (scale bars = 1 μm), (c) dense fibers (scale bar = 1 μm), and (d) compact material (scale bar = 10 μm).

an active process [5]. This active remodeling process is thought to involve inflammation and calcification due to transdifferentiation of cardiovascular cells (such as vascular smooth muscle cells and valve interstitial cells) into osteoblast-like cells [10–12]. Although cellular disease-driven processes can be targeted therapeutically, there are currently no drug therapies developed specifically against cardiovascular calcification [13]. The only treatment today is surgery and, if the aortic or mitral valve is affected, this means the surgical replacement of the valve. Regrettably, the recurrent calcification of bioprosthetic valves still carries an operative mortality of around 50% within 10 years following surgery [4,5,14].

### 2.1. New perspective

Recently, materials characterization techniques have been applied to cardiovascular calcification [15–17] in samples of aortic valve tissue, mitral valves, coronary arteries and aortas from rheumatic fever, aortic valve stenosis and atherosclerosis patients. Scanning electron microscopy (SEM) revealed the micro morphology of these samples, showing that calcific lesions (the macroscopic calcific deposits found in diseased tissue) can include three distinct calcified structures: spherical particles, calcified fibers and compact calcification (Fig. 1a–d).

Energy-dispersive X-ray spectroscopy (EDS) further revealed that all three structures are composed of calcium and phosphorus, with the spherical particles also containing magnesium (Fig. 2). The elemental composition is therefore an indication that the different structures might have different origins.

With regard to tissue morphology, even if the calcified fibers in calcific lesions displayed some resemblance to the calcified fibers in bone, spherical particles with the size reported (between 100 nm and 10 microns) have never been described in bone tissue studies. The spherical particles reported recently were found on macroscopically visible calcific lesions, in regions of aortic valves with no visible calcific lesions but where calcification was found elsewhere is the vascular system, and in aortic valves with no visible calcific lesions and where the vascular system examined was free of any calcific lesion. Unlike the calcified spheres (Fig. 1a and b), dense fibers (Fig. 1c) and compact calcified materials (Fig. 1d) were only identified in association with calcific lesions and never in non-calcified tissues.

When the calcific lesions were sectioned by Focused ion beam (FIB) and imaged by Transmission electron microscopy (TEM) [18], spherical particles were found not only on the surface of calcific lesions, but also embedded within lesions. This is an indication that this calcification process is not a clear-cut surface precipitation of calcium phosphate, but rather a more complex biomineralization process that occurs in the bulk of the tissue.

Another important piece of information provided by electron microscopy analysis is that spherical particles are also present inside the compact calcification. This means that the spherical particles keep their original morphology and internal structure while embedded in another calcified structure, suggesting in this way that calcific lesions might be formed by more than one process of biomineralization, and that the growth of compact calcification structures possibly engulfs spherical particles, which are the most conspicuous and earliest noticeable calcified structures in vascular tissue.

Interestingly, the internal structure of calcified spheres was organized in rings, similarly to the internal structure of a tree trunk. Crystallographic analyses also provided one of

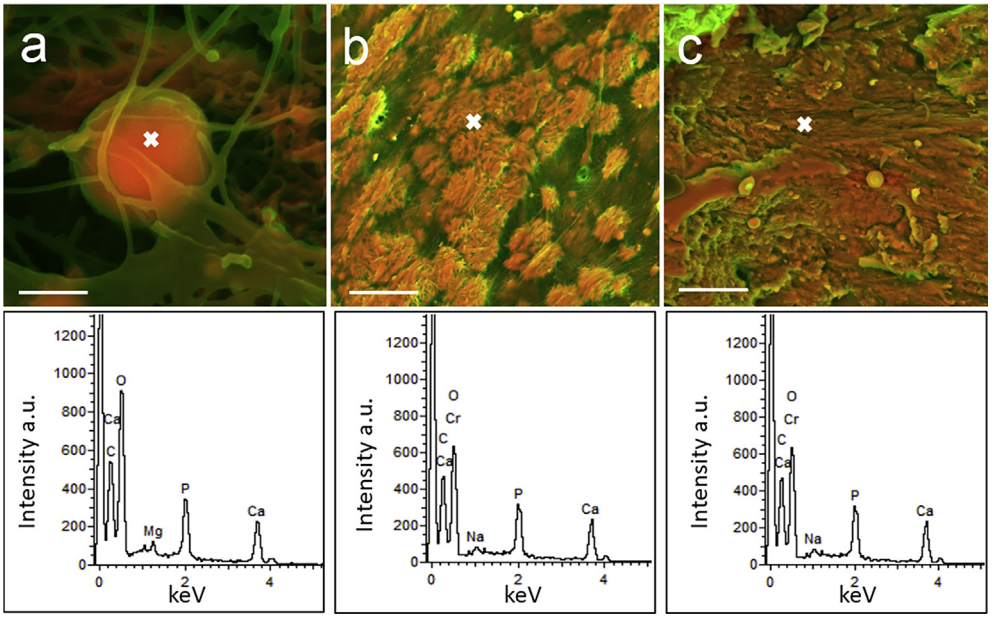


**Fig. 2.** DDC-SEM micrographs of the aortic valve presenting dense spherical particles (a), dense fibers (b) and the compact material (c). The corresponding EDS spectra collected at the marked (×) sites are indicated on micrographs a–c, respectively. Scale bars = 2 μm.

the most extraordinary results, by demonstrating that the spherical particles were formed from highly crystalline hydroxyapatite.

The fact that these spheres can have up to 5 microns and diffract as a single crystal, makes them (to the best of our knowledge) the most crystalline mineral present in vertebrates. On the other hand, the compact calcification presented a poorly crystalline apatite, which is surprisingly even less crystalline than bone mineral (Fig. 3). Once again, the results indicate there are relevant chemical differences between the structures of the material from calcific lesions and bone.

Finally, perhaps one of the most relevant findings is that the morphological and chemical characteristics of the calcific material resulting in rheumatic fever, atherosclerosis and aortic valve stenosis are roughly the same. This conclusion is supported, firstly, by the finding that the calcified material in all three diseases is formed from calcified spherical particles, calcified fibers and compact calcification. Moreover, samples from all different diseases presented roughly the same morphology, the same elemental composition and, even more strikingly, the same crystallinity. This is particularly true for the spherical particles, which present the same internal ring-shaped

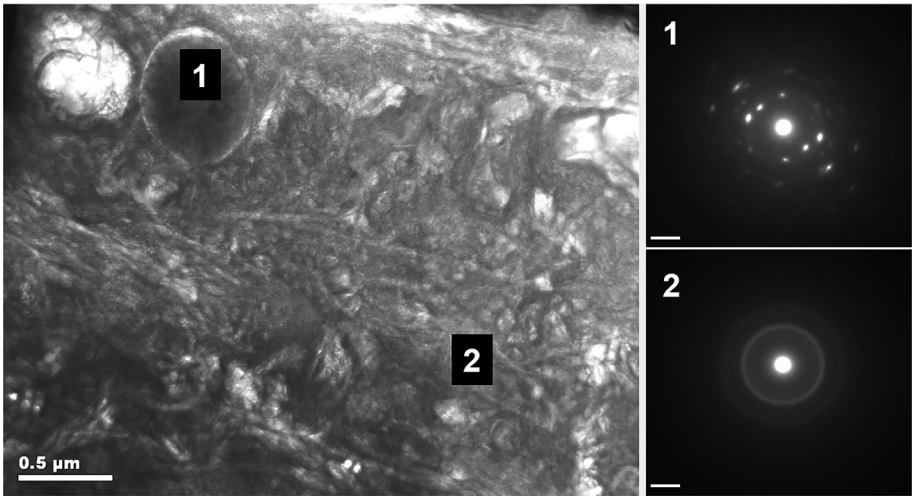


**Fig. 3.** TEM image showing spherical particles trapped in compact organic calcium phosphate matrices (scale bar = 0.5 μm). The SAED patterns obtained from different regions of the tissue section are numbered 1–2 (scale bars = 0.05 nm). Spherical particles produce diffraction patterns typical of a highly crystalline material (1), whereas the surrounding compact material shows diffraction patterns typical of a poorly crystalline material (2).

structure, the same amount of magnesium in their composition and the same electron diffraction pattern of a single crystal across samples of tissue affected by different calcific diseases.

Taken together, these findings provide strong evidence that three distinct calcific diseases (rheumatic fever, atherosclerosis and aortic valve stenosis) present a relevant commonality in their process of calcification. In this way, since all of them are formed by the same biomineral, they might even present the same mechanism of biomineralization. At this stage it is not possible to know if the origin of the three diseases is the same, but it is clear that once started, then biomineralization process produces the same material.

Even if these results are not able to provide insights into the biochemical or cellular mechanism by which the mineral is formed, they give important clues as to where further research is more likely to discover the origin of pathological calcification in the vascular system. The information provided urges the field to look for systems where it is possible to produce calcium phosphate minerals with specific morphology, composition and crystallinity.

The literature contains suggestions for the origin of calcified material found in the tissue, such as stromal cell microvesicles and apoptotic bodies[19]. Confirming these hypotheses or even finding unexpected origins of these calcified structures found in the cardiovascular system, is a necessary step to a better understanding of calcific disease. This knowledge will at the same time potentially unlock the development of new treatment and prevention methods.

## 3. Comparison to osteogenic theory of cardiovascular calcification

Considering that the typical bone mineral is present along collagen fibers, formed by poorly crystalline apatite and composed of plate-like structures with a typical size of 20 nm [20,21] whilst calcified particles from calcific lesions in vascular tissue are highly crystalline and with sizes starting at 100 nm, it is clear that the material found in vascular calcification is not bone.

Bone formation could nevertheless occur in vascular tissue either as a concomitant event or as a consequence of the formation of calcified particles, since the mere presence of hard spherical particles could trigger mesenchymal stem cell differentiation to osteoblast phenotype [22,23]. It is now well documented that in contact with practically any tissue, calcium phosphate can lead local cells to a process of transdifferentiation into mineralized phenotype [24–27].

Although the mechanism by which the presence of calcium phosphate initiates the formation of a bone-like material remains unclear, some studies provide evidence that valve interstitial cells may contribute to or even be responsible for the formation of calcific lesions [28].

This hypothesis might also explain why there are so many results in the literature demonstrating the presence of bone cells and bone proteins in calcific lesions. In this way, the presence of these cells and proteins would indeed indicate a correlation with bone formation, but does not offer proof that the process of calcification is triggered by the presence of bone cells, which could have arrived after initial stages of calcification, when the presence of calcium phosphate leads to the recruitment of bone cells or differentiation of vascular cells to bone cells in the calcific lesion.

## 4. Conclusion

To date, cardiovascular calcification has been regarded as an actively regulated process, even though the mechanisms associated with late stage mineralization remain largely unknown. New evidence shows that vascular calcification is not only a simple process of bone formation, but also includes another biomineralization process that leads to the formation of a different material hitherto unknown. More important than just shedding light onto the material that forms cardiovascular calcification, these new results urge the field to look for new ways to understand and describe vascular calcification as a process different from osteogenic formation.

Finally, the importance of an interdisciplinary approach to study biomedical questions such as vascular calcification is clear. This approach provides a stepping-stone for clinical investigation into calcific disease that will have a direct impact on the potential development of much-needed treatments for vascular calcification.

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