C alcium mineral deposits are radiographically evident in the majority of significant atherosclerotic lesions. These deposits generally consist of a nonhomogeneous composite containing hydroxyapatite mineral nanocrystals embedded in an organic matrix including type I collagen nanofibers. The morphometry varies over a spectrum. Amorphous deposits lack organization at the light-microscopic scale, whereas bone-like deposits have varying degrees of architectural macro-organization similar to those of developing and mature skeletal bone, which also consist of a nonhomogeneous composite containing hydroxyapatite nanocrystals embedded in a collagenous organic matrix. Based on histopathologic features, the organized, bone-like deposits appear to arise from angiogenic invasion of the amorphous deposits. This is consistent with the known sequence of events that leads to bone formation in fracture repair, embryonic intramembranous ossification, and in embryonic endochondral ossification, where bone arises from a cartilage scaffold.

The similarities between atherosclerotic calcification and osteogenesis are more than skin deep. Several investigative groups have demonstrated osteogenic features at the cellular and molecular levels, including dynamic osteogenic gene expression in vitro and in vivo as reviewed recently by Towler and colleagues. In this issue of ATVB, Duer and colleagues take this comparison to the next level—nanoscale architecture. Using solid-state nuclear magnetic resonance (NMR) techniques, they examined the mineral-organic interface in calcium deposits from atherosclerotic plaque and skeletal bone and found marked similarities. The NMR method (rotational echo double resonance) has the capacity to isolate the nanoscale features because it generates forces that act only within distances less than 1 nm. Results showed that the interface in atherosclerotic mineral is also a bonded nanocomposite rather than a simple mixture, and, interestingly, that it is enriched in glycosaminoglycans.

In bone, the organic matrix has both physical and chemical effects on the mineral. Mechanically, the protein nanofibers in skeletal bone tissue contribute to physical integrity in a manner comparable to steel in reinforced concrete or straw in adobe, one providing compressile, and the other tensile, strength. Chemically, the interaction between the inorganic mineral and organic proteins appears to be complex, with the proteins, such as osteopontin, osteocalcin, and bone sialoprotein having biphasic effects on the crystal initiation and propagation. Indeed, many of the macromolecules believed to initiate mineralization are also implicated in restricting it.

Bone proteins interact with the mineral components via electrostatic interactions between negatively charged domains (such as phosphorylated and γ-carboxylated amino acid groups) and the positively charged mineral surface, forming a biologically and chemically bonded composite, rather than a mere mixture. The forces in this bonding also allow the organic matrix to constrain the pattern of crystal formation. For example, the nanocrystal organization, which has some degrees of freedom, may be entrained to the known characteristic axial and helical periodicities of collagen I fibers. Thus, atherosclerotic calcium deposits may gain their mineral features from a basic template pattern generated by the organic matrix at a molecular or nanoscale level.

The findings of Duer et al suggest that the regulatory mechanisms of osteogenesis are recapitulated in atherosclerotic calcification and ossification. This evidence for governance in atherosclerotic calcification conflicts with the older views of the process as “dystrophic,” accidental, or maladaptive, instead suggesting that vascular calcification is no accident, but a regulated process. The body appears to have every intention of producing mineral deposits in the plaque, though the purpose is unclear. One possibility is that soft tissue mineralization evolved as an adaptive response to chronic infectious or inflammatory foci. The ultimate immune response to tuberculous infection in a wide variety of soft tissues is a Ghon focus, containment by a capsule of osseous tissue. The fact that it requires an intact immune system suggests that this shell of bone surrounding the focus is no accident. Walls of ectopic bone also form around chronic parasitic infections, foreign bodies, and tumors, including schistosomiasis, silicosis, and breast cancer. In each of these cases, ordinary cellular and humoral immune mechanisms fail.

Clinically, the presence of calcium deposits around tuberculous nodules is believed to confer stability, and the containment may explain how tuberculosis can be clinically “dormant” and recur if the wall is breached by mineral resorption. Some have suggested that calcification may protect plaque from rupture, but others suggest it may have the opposite effect.

The walling-off by bone may represent an immune response of last resort, but how the osteogenic programs are activated is not known. One possibility is that soft tissue calcification around chronic infectious foci is trig-
With lipoprotein particles via chemical "bridges." As Duer et al., droitin sulfate, not only interact with mineral but also with glycosaminoglycans from proteoglycans (GAG/PG). Interestingly, certain glycosaminoglycans, such as chondroitin sulfate, represent a nanoscale template for mineral crystallization. Thus, the glycosaminoglycans at the organic-inorganic interface are intriguing (Figure). These long repeating sugar chains, which often decorate proteins to form proteoglycans, are a signature feature of cartilage matrix, which further supports the relationship between atherosclerotic calcification and endochondral ossification. As with other bone-related proteins, GAGs have dual functionality in biomineralization: a direct role in initiating apatite nanocrystal formation and, later, a direct role in restraining nanocrystal propagation. They block crystal growth by linking via calcium ion bridges to growth sites on the crystal surface. When hydroxyapatite mineral crystallizes in the presence of chondroitin sulfate in vitro, it forms highly ordered arrays of nanocrystals.13

Thus, the glycosaminoglycans at the interface may represent a nanoscale template for mineral crystallization. Interestingly, certain glycosaminoglycans, such as chondroitin sulfate, not only interact with mineral but also with lipoprotein particles via chemical "bridges." As Duer et al. suggest, this binding of glycosaminoglycans to lipid may initiate injury and death of some vascular smooth muscle cells leading to osteogenic differentiation of others. Notably, because oxidative stress alone induces osteogenic differentiation in vascular cells, this may occur even without cell injury or death.3,15,16

Kirton and colleagues17 showed that the abundance of negatively charged GAGs in vascular cell cultures is regulated by the Wnt/β-catenin pathway, a pathway shown by Towler and colleagues to serve a central regulatory role in vascular mineralization.18 At the same time, as shown by Hirsch and colleagues, apatite crystals also interact at a nanoscale level with cholesterol crystallites both in vitro and in human atherosclerotic plaque.19 Such an interaction may further modulate the order and pattern of mineral crystals formed within the cholesterol-rich environment of atherosclerotic lesions.

In conclusion, the nanoscale evidence provided by Duer et al. in this issue underscores the macroscale evidence that atherosclerotic lesions calcify in an ordered manner similar to the highly regulated process of bone formation. Such nanotechnological approaches appear to have the capacity to answer many more questions about the fundamental physicochemical mechanisms involved in vascular calcification such as the effects of protein periodicity and cholesterol nanocrystals.

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References


Nanoscale Architecture in Atherosclerotic Calcification
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