Atherosclerotic calcifications are related to poor prognosis and all-cause mortality in large population studies.\(^1\) Moreover, vascular calcifications are inversely associated with the potential reduction of plaque volume in regression studies during statin treatment, which suggests that more calcified lesions are less likely to undergo positive remodeling.\(^2\)

Recently, it has been suggested that calcification is a tightly regulated process of mineralization akin to bone formation.\(^3\) In plaques, the deposits consist of a nonhomogeneous composite that contains hydroxyapatite mineral nanocrystals embedded in a collagenous organic matrix, whereas at a nanoscale level, apatite crystals interact with cholesterol crystallites. In this context, mineralization may result from a basic template pattern generated by the organic matrix at a molecular level.\(^1\)

In vitro studies have documented both a potential stimulus to hydroxyapatite deposition by cholesterol crystals and a deposition of calcium-phosphorus growth on the needlelike cholesterol crystals with formations that tended to concentrate along terraces and ridges on the needles.\(^4\) However, only scarce morphological data are available on the ultrastructure of human atherosclerotic plaques, particularly with regard to calcifications. Energy-dispersive x-ray analysis is a potent analytic tool that allows characterization of biological specimens and is particularly useful in obtaining maps of elemental distribution directly associated with morphological images.

We show in the Figures the laminar pattern of calcium deposition as visualized by scanning electron microscopy (Philips XL30 FEG, Eindhoven, Nederland) in a human carotid endarterectomy specimen obtained after scheduled surgery in a 72-year-old male patient. Energy-dispersive x-ray analysis spectrometry was performed on selected areas to detect and visualize the distributions of elements, especially calcium, phosphorus, and carbon.

Figure 1 shows sheetlike crystals detectable within the media layer of the carotid artery. Calcification in the tissue evolved in a laminar pattern, with evidence of cleavage planes of the calcified layers (Figure 2). Demonstration of the laminar form of deposition of calcium phosphate is clearly shown by the elemental analysis of tissue composition in Figure 3. The energy-dispersive x-ray analysis images (upper-right panel: calcium; lower-left panel: carbon; and lower-right panel: phosphorus) of the same area shown by scanning electron microscopy in the upper-left panel demonstrate a correspondence of calcium and phosphorus to laminar deposition. The presence of carbon, related to residual organic material, is displayed in different places with respect to the laminar deposits of calcifications. Thus, coupling at a nanoscale level the morphological images and elemental analysis in a human carotid plaque shows the deposition pattern of calcium and phosphorus and their relationship with lamination sheets.

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**Disclosures**

None.

**References**

Figure 1. A, Longitudinal section of the carotid specimen visualized by scanning electron microscopy. The figure shows 2 areas of plaque formation within a thickened media layer. The arrow indicates the area shown at higher magnifications in panel B. B, Sheetlike deposits are visible within the media layer. Details are shown in the insets.

Figure 2. Section of atheromatous plaque showing laminar deposition of calcium phosphate microcrystal layers, with residual partially folded organic sheets.
Figure 3. Detail of laminar deposition of mineralization and corresponding energy-dispersive x-ray analysis maps of elemental analysis clearly shows colocalization of calcium (Ca) and phosphorus (P) within the laminations. Diffuse presence of carbon (C) indicates residual organic material displayed in different places with respect to the laminar deposition of calcifications.
Laminar Pattern of Mineral Calcium-Phosphorus Deposits in a Human Carotid Plaque: Nanoscale Ultrastructure and Elemental Analysis

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