Electron Microscope and Immunological Evidence of Nanobacterial-Like Structures in Calcified Carotid Arteries, Aortic Aneurysms and Cardiac Valves

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**Background:** Definitive mechanisms causing vascular calcification are unknown. Experiments were designed to evaluate explanted calcified human vascular tissue for the presence of nanometer-scale objects hypothesized to be a type of bacteria associated with calcified geological specimens and human kidney stones (Folk RL; J Sed Petrology 63:990-999, 1993; Kajander EO, et al., PNAS 95:8274-9279, 1998).

**Methods:** Sections (5 microns) were cut through calcified human carotid plaques (n=2), aortic aneurysms (n=7) and cardiac valves (n=2) collected from patients during surgery. Adjacent sections of all tissues stained for calcium phosphate and a commercially available antibody directed toward nanobacteria were examined by light microscopy; another section was scanned by electron microscopy.

**Results:** Heterogeneously distributed, positive staining for calcium phosphate was identified in all sections of diseased tissue. Positive immunological staining for nanobacteria was also heterogeneously distributed both in areas showing positive and negative staining for calcium phosphate. Electron microscopic analysis of areas showing positive immunostaining revealed spheres ranging in size from 30-150nm frequently forming chains. Using High Energy Dispersive Spectroscopy, some spheres show no pattern; some show only a pattern of calcium and others show calcium and phosphorus. On a mitral valve, there were discrete colonies of finger-growths consisting of spheres of 100-150nm ranging up to finger-like rods 500nm long length similar to the “nanobes” cultured from Australian sandstone (Uwins PJR et al., Am Mineralogist 63:1541, 1998).

**Conclusions:** These immunohistochemical and anatomical characteristics provide evidence of nanometer-scale structures in calcified human cardiovascular tissue. These structures are similar to nan(n)obacteria described and isolated from human kidney stones and geological specimens. These observations fulfill one criterion for Koch’s postulate to suggest that a calcifying “nanobe” could participate in calcification of vascular tissue.