*Abstract*

# Detection and Propagation of Calcified Nanostructures From Human Aneurysms

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**Background**: Mechanisms leading to vascular calcification remain incompletely understood. Nanometer-sized, mineralized structures recognized by a commercially available monoclonal antibody (8D10, Nanobac OY) are present in calcified human aneurysms. These structures were not detected by TUNEL staining, suggesting they were not apoptotic bodies. The 8D10 antibody is directed against nanobacteria, a controversial, slow-growing, and calcifying microorganism. Therefore, experiments were designed to determine whether structures from aneurysms are viable, nano-sized organisrns.

**Methods**: Aneurysms (n=3) collected as surgical waste were decalcified, sterile filtered (0.22 μm), and cultured in DMEM containing gamma-irradiated calf serum.

**Results**: In 2 of 3 cultures micron-sized particles visible by light microscopy increased in number over 4-6 weeks. The negative culture came from an aneurysm without stainable nanoparticles. With transmission electron microscopy (EM), cultured particles showed an inner core surrounded by a shell of calcium phosphate (documented via energy dispersive microanalysis). After dissolution of the shell with EDTA, spherical structures of 50-100 nm were seen by scanning EM. These cultured particles incorporated [$^3$H]uridine at a rate 2.3 times greater than control cultures of DMEM containing serum and inorganic hydroxyapatite (HA) crystals ($P<0.01$). Therefore, these nanostructures appear to synthesize RNA. Particles cultured from aneurysms also stained with the 8D10 antibody, and SDS-PAGE of extracted proteins revealed multiple distinct bands, including one ($M_r$ 47 kDa) recognized by the 8D10 antibody. The pattern of proteins extracted from inorganic HA crystals incubated with DMEM and calf serum did not contain the 47-kDa band recognized by the 8D10 antibody.

**Conclusion**: In conclusion, these results suggest that viable nano-sized organisms are present within calcified human arterial tissue. A cause and effect relationship between the presence of these organisms and development of arterial calcification remains to be determined.

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