

## **Alleged Nanobacteria Exist and Participate in Calcification of Arterial Plaque**

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Response to November 2002 issue having Elmer M. Cranton's letter 'Alleged Nanobacteria Do Not Cause Calcification of Arterial Plaque'

Editor:

The cause of pathological calcification, including atherosclerosis, dental pulp stones and kidney stones, remains an enigma. The life-long incidence of kidney stones appears to have increased throughout the whole 20<sup>th</sup> century, and now occurs in up to 15% of the population.<sup>1</sup> Nanobacteria have been linked to human kidney stone and preliminary studies showed Koch's postulates to be fulfilled.<sup>1,2, 31</sup>  
<sup>4</sup> Calcified hard plaques are now the common form of coronary heart disease but were surprisingly a clinical rarity 100 years ago<sup>5</sup>. Calcified plaques can lead to acute myocardial infarct, because apatite (calcium phosphate mineral) exposed to blood activates a thrombotic cascade. Nanobacteria were the first (may still be the only ones) calcium-phosphate mineral containing particles isolated from human blood. Radioactively labeled nanobacteria were shown to accumulate in rabbit aorta and aortic valve, although their main elimination route was excretion via kidneys into urine<sup>6</sup>. This study already pointed to the potential role that nanobacteria could have in atherosclerosis, heart valve calcification and kidney stone formation. Nanobacteria were present and actively involved in the processes: 1. Nanobacteria were shown to be active nidi forming the right type of calcified mineral. Active nidus means a center of calcification that can mediate calcium-phosphate mineral formation under non-saturating calcium and phosphate concentrations. In fact, nanobacteria are so good in doing this that they can consume all free calcium and/or phosphate from their culture medium, whichever is first consumed to zero<sup>2</sup>. 2. Nanobacteria have and release endotoxin<sup>7</sup> and thereby stimulate chronic local inflammatory reaction in atherosclerotic plaque. 3. Nanobacteria have been shown to infect humans and infections last possibly life-long. 4. Almost 100% of atherosclerotic patients in USA and in Finland have antinobacteria antibodies in their serum, whereas in healthy blood donors antinobacteria antibodies are present in about 15% (see web pages of Nanobacteria Minisymposium held at Kuopio last year). 5. Nanobacteria have been shown to be susceptible to several antibiotics and sequestering agents<sup>8</sup>. Since nanobacteria form calcific biofilm it is clear that their eradication needs combination chemotherapy aiming at the biofilm, the calcified deposits and the agent. Such chemotherapy can be very demanding since nanobacteria grow very slowly. Thus lessons learned from the treatment of tuberculosis or leprosy should be remembered.

Dr. Cranton has not himself studied nanobacteria but has pointed out that nanobacteria do not exist and cannot cause atherosclerosis. His motivation seems to be to stop ongoing combination drug trials that aim at verifying whether nanobacteria cause atherosclerosis and how to cure this infectious process. These studies use the same principle that vindicated *Helicobacter pylori* in peptic ulcer disease: curative therapy was the evidence for the causative role of the agent. That approach led into a revolution in the therapy of *Helicobacter pylori*-mediated diseases. This was a good thing.

Nanobacteria form calcific biofilms and replicate under blood/serum conditions, as was first published by Kajander and Ciftcioglu<sup>9</sup>, a fact that has been reproduced and published by many research groups, e.g., NASA, Mayo Clinic, McGill University, Exeter University, University of Illinois, Alcala University and University of Ulm. Dr. Cranton refers only to NIH researcher Cisar<sup>10</sup>, who could also culture similar particles from serum and human saliva sources. Cisar had no positive or negative controlled controls and did not use valid published immunological control methods. They could verify our findings, realized the extreme difficulties in performing PCR, but finally suggested an interpretation that the culturable particles cannot be bacteria, since they were too small, were not inhibited with a respiratory poison, nucleic acids could not be detected with standard procedures and their protein patterns revealed only few proteins, much less than one would expect from a common bacterium. They did not sequence any proteins. They did not do successfully any DNA work besides staining with Hoechst 33258, where they got the same weakly positive result than we did. To the contrary of Dr. Cranton's claims, Cisar did not do a PCR phylogenetic analysis using 16S rDNA sequences simply because they did not get any: all their samples, including negative controls, were contaminated with *Pseudomonas* sp. This fact is clearly stated in their paper and means that they did not have any data on the putative bacterial status of nanobacteria.

We do totally agree with Cisar on that nanobacteria are not common bacteria. Nanobacterial samples may contain pieces of DNA from common bacteria, which makes phylogenetic PCR analysis using universal primers practically impossible and worthless. PCR analysis assumes that the ribosomal gene has 'universal' sequences detectable by the primers, but this is not true for all organisms. When we originally named nanobacteria in 1990, we wanted to separate them from common bacteria. Unfortunately, bacteria part of the name still lures less-well informed scientists to compare nanobacteria with *E. coli* and other common bacteria, which are 100-fold bigger and produce biomass 10,000-fold faster than nanobacteria.

As pointed out by Dr. Cranton, apatite can be formed under super-saturating concentrations of calcium and phosphate via several mechanisms. To our knowledge, nanobacteria-mediated calcification is the only mechanism to make apatite at non-saturating levels of calcium and phosphate. Cisar did not follow saturation degree analysis in his studies although saliva is known to be highly super-saturated with calcium and phosphate. Yet Cisar suggested as an alternative explanation nanobacteria to be replicating apatite mineral particles.

Naming an agent as particles or nanobacteria, living or non-living but self-replicating, has relatively little meaning with respect to causing disease, e.g., the atherosclerotic process. The fundamental importance is that these self-replicating special particles that we call *Nanobacterium sanguineum* can be found in blood and in atherosclerotic plaques. This fact was initially presented by Laszlo Puskas at Nanobacteria Minisymposium held at Kuopio last year, detected by us (unpublished data) and finally now has been verified by Mayo Clinic and University of Texas<sup>11</sup>. Macrophages in aorta and other arteries can internalise nanobacteria and stimulate a local inflammatory cascade, and eventually proceeds to nanobacteria-mediated calcification. In fact, according to Dr. Cranton, Cisar<sup>10</sup> also verified the medical importance of the nanobacteria phenomenon: he stated that submicroscopic crystals of calcium apatite, as occur in plasma, were shown to be nucleators of biomineralization. However, the presence of apatite crystals had earlier been shown only by us, but the role of apatite biofilms in blood clotting and blood vessels is well known. Thus the described presence of apatite particles could be potentially deadly, in fact a mechanism starting myocardial infarction.

Dr. Cranton is putting forward ungrounded claims on nanobacteria and on therapy trials aiming at eradicating them. The claims need short comments:

1. Nanobacteria have been shown to be a unique calcifying agent. They can be cultured and passaged in cell culture media mimicking serum in composition. Atherosclerotic plaques contain nanobacteria as detected by researchers from Mayo Clinic and the University of Texas<sup>11</sup>.
2. Administration strategy of EDTA should aim at maintaining drug blood levels on a sustained therapeutically effective level. I have personally conducted serum-EDTA levels on patients treated with NanobacTX, and this prescription combination is effective.
3. Many drugs are successfully administered rectally. In most cases rectal administration is highly effective. The efficacy of NanobacTX to deliver sustained therapeutic levels of EDTA has been determined using analytical methods developed at Kuopio University.

4. In antimicrobial therapy, administration route, dose and frequency has to be carefully considered in order to maintain high enough drug levels. In most infectious diseases, it would be unwise to administer a drug intravenously once or twice a week while knowing that the therapeutic concentrations are retained only for a short period after the administration.
5. Dr. Cranton states: 'It makes little sense to assume that calcification of artery walls are an important indicator of clinical significance of the disease state'. This statement is ungrounded and against the perspectives how myocardial infarcts develop. Additionally, the clinical & scientific literature is replete with evidence substantiating the pivotal importance of calcification processes in the development of atherosclerotic disease.
6. The purpose of NanobacTX treatment is to be effective. This means that it must decrease calcification in plaques. It is obvious that EDTA alone is not sufficient to reach this goal, because under in vitro tests<sup>8</sup> EDTA alone has no inhibitory action on nanobacteria at clinically achievable EDTA concentrations. Dr. Cranton has also stated that intravenous EDTA therapy does not decrease calcification scores. A combination therapy is more plausible to reach this aim. NanobacTX is effective in decreasing coronary artery calcification<sup>12</sup>.
7. James Roberts, MD, FACC, has reported significant reduction in EBCT scores. This is an objective way to measure the effect of therapy. Benedict Maniscaico, MD, FACC, will publish a formal study on NanobacTX therapy in *Circulation*<sup>12</sup>.
8. It is unknown at this point how nanobacteria infection affects homocysteine levels.

There is new published evidence that nanobacteria do exist<sup>8,11,12</sup> and are biological entities reacting, e.g., to light<sup>13</sup>, cause kidney stones and are found in human atherosclerotic plaques. As discussed earlier, new evidence also indicates that several drugs are effective against nanobacteria, but in-vivo eradication of nanobacterial biofilms and calcification needs combination therapy.

Administration of EDTA alone is ineffective towards this goal, since it is not killing nanobacteria at the blood concentrations achievable and has been shown, by itself, to be ineffective in reducing coronary artery calcification scores. NanobacTX, a unique prescription combinatory nanobiotic is specifically formulated to eradicate nanobacterial biofilm, calcification and the nanobacteria themselves and has been shown in validated IRB-monitored clinical studies to be uniquely effective in doing so as measured by significant decreases in coronary artery atherosclerotic plaque

burden, and other measurement parameters soon to be announced<sup>12</sup>.

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