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Pitfalls in Detection of Novel Nanoorganisms

Drancourt el al. have reported on their attempts to isolate nanobacteria from upper urinary tract stones (3). Their findings and opinions are valuable for the nanobacteria research. However, we want to point out difficulties that any researcher will face when working with nanobacteria: lack of published data and working instructions, lack of tested commercial culture media and identification tools, and lack of readily available positive and negative controls. Novel paradigms are difficult to publish. Manuscripts on nanobacteria have so far been returned from *Nature*, *Science*, etc. Lack of publications on basic findings and methods used leads to two important consequences. (i) Scientists will waste their time trying to work with well-established routine methods, which unfortunately need modifications or must be replaced by new technologies. (ii) Negative results are obtained and accepted as such. Although the results were not properly controlled (culture media were neither pretested for growth promotion of nanobacteria nor controlled by positive test cultures), people and journals may choose the easiest way.

Any microbiological classification of tentative nanoorganisms, such as nanobacteria proposed by Kajander and Ciftcioglu (6) and nanobes proposed by Uwins et al. (8), is difficult because they are not typical bacteria. They have also virus-, fungus-, and prion-like characteristics and thus cannot fit into any existing class of microorganisms (Table 1). They should be considered as their own entity. Research tools and techniques for nanoorganisms require new attitudes and ideas. Recent findings have indicated that there are many surprises to come (4). Isolation of *Nanoarchaeum equitans*, a symbiont of hyperthermophilic bacteria, required extra efforts in characterization because standard PCR techniques failed in detecting the organism's genetic material, the presence of which was revealed with DNA stains (5). Interestingly, both nanobacteria and nanobes contain nucleic acid material detectable with DNA and RNA stains (6, 8).

It is of utmost importance to realize the limits of our current methodologies with respect to detection and culture of novel nanoorganisms, as exemplified by nanobacteria. Many so-called negative reports have been able to repeat the morphological finding of calcium phosphate self-propagating units (1, 2, 3). Nanobacteria-like organisms have been found in human atherosclerotic plaques (T. E. Rasmussen, B. L. Kirkland, J. Charlesworth, G. Rodgers, S. R. Severson, J. Rodgers, R. L. Folk, and V. M. Miller, 51st Annual Meeting of the American College of Cardiology, J. Am. Coll. Cardiol. **39**[Suppl. 1]**:**206, 2002). Atherosclerosis is a burden to billions of people. Clinical and microbiological laboratories should not take the easiest way and judge the calcium phosphate particles as artifacts. Who would like to carry self-propagating nanocrystalline apatite in their blood, blood vessels, stones, and tissues? Evaluation of nanobacteria phenomena should not be based just on routine bacteriological criteria but rather on multidisciplinary efforts by innovative and open-minded scientists.

TABLE 1. Unique properties of nanobacteria (7) with respect to those of viruses, prions, and bacteria[a]

Property	Presence and/or characterization of property in:			
	Nanobacteria	Viral particles	Prion particles	Bacteria
Size (nm)	50–300	20–250	<250	>250
Cell wall	CaP, atypical	None, protein-lipid layer	−	+
Presence of:				
Nucleic acids	Some, atypical	+, atypical	−	+
Proteins	+	+	+	+
Carbohydrates	+	+	+	+
Self-replication	+	−	−	+
Growth in DMEM[b]	+	−	−	+
Uridine incorporation	+	−	−	+
Resistance to γ-irradiation (Mrad)	≈2.5	<2.5	>2.5	<0.1–>6.0
Resistance to boiling temperature	+	−/+	+	−
Resistance to disinfectants	+	−/+	+	−
Resistance to antibiotics	−/+	+	+	−/+
Sensitivity to 5-fluorouracil	+	−/+	−	−/+
Sensitivity to cytosine arabinoside	+	+	−	Unknown
Sensitivity to bisphosphonates	+	−	−	−
Immunogenicity	+	+	−	+
Ability to cause inflammation	+	+	−	+
Lipopolysaccharide content	+	−	−	+ (gram negative)
Ability to cause host cell death	+	+	Specific	+ in some
Ability to cause pathologic calcification	+	+ in a few	−	+ in a few
Biofilm formation	+	−	−	+
Presence in atherosclerotic plaques	+	+ in some	−	+ in a few

[a] +, present; −, absent.
[b] DMEM, Dulbecco Modified Eagle Medium.

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Author's Reply

K. Aho and O. Kajander are commenting on the negative attempts in the previous study by my laboratory to grow nanobacteria. My coworkers and I tried to reproduce their technique without success. Since 1998, we have tried to obtain the strain from Kajander. Here we failed to confirm their work. The putative "*Nanobacterium*" strain is protected and not available, but they sell products to detect nanobacteria. To the best of my knowledge, nobody has reproduced this work. I would be happy to test their strain and change my mind if the data are convincing. The authors cite references on *Nanoarchaea* (1, 2), which have no correlation with this topic but the name. The main problem is that in science, the exact method and the obtained strains should be exchanged to allow other investigators to reproduce and confirm the work. Regarding my alleged reluctance to find new microorganisms, I suggest that the authors consider previous studies from my laboratory, including reports of the culture of the biggest virus (3), that of *Tropheryma whipplei* (5), and that of other microorganisms, including *Rickettsia* species, which are small bacteria (4, 6).

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