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REVIEW

Clinical implications of calcifying nanoparticles in dental diseases: a critical review

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Background: Unknown cell-culture contaminants were described by Kajander and Ciftçioğlu in 1998. These contaminants were called nanobacteria initially and later calcifying nanoparticles (CNPs). Their exact nature is unclear and controversial. CNPs have unique and unusual characteristics, which preclude placing them into any established evolutionary branch of life.

Aim: The aim of this systematic review was to assess published data concerning CNPs since 1998 in general and in relation to dental diseases in particular.

Materials and methods: The National Library of Medicine (PubMed) and Society of Photographic Instrumentation Engineers (SPIE) electronic and manual searches were conducted. Nanobacteria and calcifying nanoparticles were used as keywords. The search yielded 135 full-length papers. Further screening of the titles and abstracts that followed the review criteria resulted in 43 papers that met the study aim.

Conclusion: The review showed that the existence of nanobacteria is still controversial. Some investigators have described a possible involvement of CNPs in pulpal and salivary gland calcifications, as well as the possible therapeutic use of CNPs in the treatment of cracked and/or eroded teeth.

Keywords: calcifying nanoparticles, nanobacteria, sialolith, pulp stone, enamel repair

Introduction

Unknown cell-culture contaminants were first described by Kajander and Ciftçioğlu in 1998. These contaminants were initially called nanobacteria, but were later renamed calcifying nanoparticles (CNPs).[1] The nature of these entities was unclear, raising many controversial views as to whether they are indeed nanobacteria that replicate or simply inert nanocalcification. Many theories soon emerged, with each theory having its own enduring supporters. One theory, describes CNPs as the smallest-known replicating entities of organic life on earth, while other theories held that CNPs are mineral–protein complexes unrelated to bacteria.[2–13] Sommer et al were of the opinion that nanobacteria have unique and unusual characteristics, which preclude placing them into any established evolutionary branch of life.[14] Kajander et al presented a table that compared CNPs, virus, prions, and bacteria using over 20 characteristics or properties, as shown in Table 1.[15] Despite the controversy on the true nature of CNPs, some authors have described some human diseases or conditions in which CNPs are associated as initiating or contributing agents (Table 2).

With the unresolved issue of what CNPs really are, a systematic review of informative published data on CNPs could suggest where and how to place CNPs in the scheme of things. The aim of this paper was therefore to perform a narrative systematic review of publications on CNPs since 1998 and highlight their hypothesized relationship with pulpal and salivary gland calcifications.

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Table 1 Characteristics of CNPs compared with other types of cell

Characteristics	CNPs	Viral	Prion	Bacteria
Size	50–300 nm	20–250 nm	<200 nm	>250 nm
Cell wall	CaP/atypical	No/protein layer	No	Yes
Nucleic acids	Some, atypical	Yes, atypical	No	Yes
Proteins	Yes	Yes	Yes	Yes
Carbohydrates	Yes	Yes	Yes	Yes
Self-replicating	Yes	No	No	Yes
Growth in DMEM	Yes	No	No	Yes
Resistant to γ-irradiation	~2.5 Mrad	<2.5 Mrad	>2.5 Mrad	<0.1–>6 Mrad
Resistant to boiling temp	Yes	No/yes	Yes	No
Resists antibiotics	No/yes	Yes	Yes	No/yes
Sensitive to 5-FU	Yes	No	No	Yes/no
Sensitive to CytAra	Yes	Yes	No	?
Sensitive to biophosphonates	Yes	No	No	No
Immunogenic	Yes	Yes	No	Yes
Cause inflammation	Yes	Yes	No	Yes
Lipopolysaccharide (LPS)	Yes	No	No	Yes
Host cell death	Yes	Yes	Specific	Some
Pathologic calcification	Yes	A few	No	A few
Biofilms	Yes	No	No	A few
CHD association	Yes	Some	No	Some
Stroke association	Yes	Some	No	Some
Affects blood clotting	Yes	Some	No	Some
Prothrombinase activity	Yes	No	No	Some
PDL disease association*	Yes	No/yes	No	Yes
Dental pulp stone*	Yes	No	No	No

Note: Data from Kajander et al.[15] *Added by the author (MA).
Abbreviations: CNPs, calcifying nanoparticles; DMEM, Dulbecco's Modified Eagle's Medium; 5-FU, 5-fluorouracil; CytAra, Cytarabine; CHD, coronary heart disease; PDL, periodontal ligament.

Materials and methods

Medline (PubMed) and Society of Photographic Instrumentation Engineers (SPIE) electronic and manual searches were conducted. Nanobacteria and calcifying nanoparticles were used as keywords to extend the search to all the potentially relevant articles. The search yielded 135 papers, which were screened in detail. For review purposes, 92 papers were excluded and the remaining 43 papers that were most relevant to the aim of the study were reviewed.

Results

Are CNPs living particles or physiological contaminations?

The smallest possible size reported for self-replicating life-forms is 140 nm.[16] Glass et al,[17] claimed that *Mycoplasma laboratorium* could reach even smaller sizes. Based on these reports, it would seem that size alone could not be used to determine whether CNPs are life-forms or not. Other characteristics of life-forms that have been attributed to CNPs, as shown in Table 1, support the view that CNPs are not inert nanocalcifications.

The morphological properties of CNPs, which have been examined and described in many studies, are as follows:

- diameter ranges from 80 to 500 nm[1,2]
- morphological appearance is expressed in several shapes of coccoid, coccobacillar, or bacillar[1,2,18]
- shell structure – hydroxyapatite, cellular membranous, and central cavity[1,2]
- colony formation – colonies 0.1 mm in size are grown in low-nutrient concentration environment[1,2]
- binary fission – division by binary fragmentation and gemination[1,2]
- thermoresistant biofilms – resistance to high temperature.[1,2]

Several studies have used monoclonal antibodies to detect putative specific proteins of CNPs by cross-reaction methods and their role in several diseases in medicine and dentistry, is shown in Table 2. Other investigators – Martel and Young,[9] Wu et al,[6] and Raoult et al[10] – did not obtain the same result when they used the same method. Anti-CNP monoclonal antibodies have high sensitivity and low specificity, which explains the failure to achieve cross-reactions with serum protein (albumin and fetuin-A) in other studies.

Table 2 Associations of CNPs with several human diseases

Study	Sample source	Associations of CNPs	Specialty area
Kajander et al[19]	Blood serum	Pathological calcification	Medicine
Ciftçioğlu et al[20]	Pulp stone	Dental pulp stone	Dentistry
Ciftçioğlu et al[21]	Kidney stones	Kidney-stone formation	Medicine
Hjelle et al[22]	Cyst fluid and urine from PKD	PKD	Medicine
Ciftçioğlu et al[23]	Hypothesis	Periodontitis and PAD	Dentistry
Miller et al[24]	Calcified heart tissues	Vascular calcification	Medicine
Ciftçioğlu et al[25]	Randall's plaques	Kidney-stone formation	Medicine
Zhou et al[26]	Urine from patients with prostatitis	Type III prostatitis	Medicine
Candemir et al[27]	Calcified aortic heart valves	Vascular calcification	Medicine
Hu et al[28]	Blood serum	Vascular calcification	Medicine
Jing et al[29]	Hypothesis	Repair enamel	Dentistry
Schwartz et al[30]	Calcified tissues	Arterial injury	Medicine
Yang et al[31]	Human dental pulp cells	Dental pulp stone	Dentistry
Zeng et al[18]	Dental pulp stone	Dental pulp stone	Dentistry
Hudelist et al[32]	Psammoma bodies	Psammoma body formation	Medicine
Demir[33]	Hypothesis	Periodontal diseases	Dentistry
Lin et al[34]	Hypothesis	Repair cracks on enamel	Dentistry
Shiekh et al[35]	Renal tubular calcification	Renal calcification	Medicine
Lu et al[36]	Calcified placental tissues	Placental calcification	Medicine

Abbreviations: CNPs, calcifying nanoparticles; PKD, polycystic kidney disease; PAD, peripheral artery disease.

The method detects antigen present from bacterial prions and peptidoglycans in CNP structures. Calcifying NP antigens and antibodies were detected significantly more often in CNP-containing diseases compared to controls.[2]

Although many studies[5–8,10] proposed that CNPs might have the ability to form mineral–protein complexes in normal serum in physiological conditions, in reality, Kajander et al reported that this could not be totally true.[37] However, mineral–protein complexes have been shown in a gamma-irradiated serum study model by Martel and Young.[9] The CNPs may replicate clearly in the absence of serum, as shown by Mathew et al,[38] which demonstrated that replication of CNPs could occur independently of serum protein.

Decoding of CNP genomic constitution is still under investigation. Investigations have reported positive results with different deoxyribonucleic acid (DNA)-staining techniques of CNPs (Tables 3 and 4). One author was of the opinion that nucleic acids can be attracted to the highly charged proteins and molecules (shell–mineral–protein complexes), and that these are not produced in CNPs.[3] Investigators used direct DNA-staining techniques on demineralized CNPs, which precluded a simple binding at the mineral–protein shell (Table 5). In another report, contamination by other bacteria, eg, *Phyllobacterium myrsinacearum*, was presumed possible.[13]

Ciftçioğlu et al[40] described morphological changes of CNPs caused by antimicrobial drugs under electron microscopy. In addition, they demonstrated the inhibition of CNP replications by aminocaproic acid, potassium citrate–citric acid solutions, and 5-fluorouracil.

Data from a couple of investigations have indicated the absence of bacterial protein in demineralized CNPs,[9,41] while others have shown the presence of bacterial proteins that might be due to replication, the protein-synthesis system, or bacterial metabolic process.[39,35,36]

Many findings and data oppose the hypothesis that CNPs are mineral–protein complexes. Although the formation of complexes of minerals and protein serum and other biological liquid under homeostasis was proposed by Martel and Young,[9] Wu et al,[6] Young et al,[7,8] and Raoult et al,[10] Kutikhin et al[2] believed that the presence of CNPs in an organism is clearly a pathological process. In addition to their pathogenicity, these proteins may have a specific immunological reaction in forming specific antibodies.

Table 3 RT-PCR for the detection of CNP genomic contents

Study	Test type	Result
Hudelist et al[32]	RT-PCR	Nucleic acids
Kumar et al[39]		

Abbreviations: CNP, calcifying nanoparticle; RT-PCR, reverse-transcription polymerase chain reaction.

Table 4 Indirect technique by [^{36}S]methionine and [^{3}H]L-aspartic acid confirming CNP specific protein

Study	Test type	Result
Kajander et al[37]	Indirect technique:	Protein
Puskás et al[42]	[^{36}S]methionine and [^{3}H]L-aspartic acid	biosynthesis

Abbreviation: CNP, calcifying nanoparticle.

Table 5 Direct technique test of uridine 5-^{3}H incorporation into CNP nucleic acids

Study	Test type	Result
Ciftçioğlu et al[21] Khullar et al[41] Miller et al[24] Kumar et al[39]	Incorporated uridine-5-^{3}H	Usage of 5-^{3}H in CNP nucleic acids

Abbreviation: CNP, calcifying nanoparticle.

Possible role of CNPs in dental diseases

There are significant reports in the literature that correlate CNPs with pathological calcifications in numerous human diseases (Table 2). Using scanning electron microscopy (SEM) and microanalysis by energy-dispersive X-ray spectroscopy, Ciftçioğlu et al[20,21] demonstrated similarity between the lobular mineral formation in CNPs and pulp stone, suggesting that CNPs may be implicated in the etiology of dental pulp stones. In another study, Ciftçioğlu et al[23] showed an association between CNPs and periodontal disease, their likely association with peripheral artery disease, and implications in coronary atherosclerosis. Furthermore, CNPs have been detected in high concentrations in patient serum with dental calculus and periodontitis.[20,23,43]

Demir also proposed a hypothesis that CNPs might be present in dental calculus and may have been responsible for the mineralization process ab initio.[33] Thus, the presence of CNPs could be regarded as a factor that is likely involved in periodontal disease and dental calculus formation.[43]

Yang et al[31] and Zeng et al[18] investigated possible involvement of CNPs in dental stones by several methods: immunostaining, serology, SEM observation, and in vitro cytotoxicity, taking special precautions and utilizing treatment methods to prevent contamination of CNPs. In their study, eleven of 13 tissue samples (84.6%) stained positive for CNP antigen immunohistochemically, whereas twelve (92.3%) positive samples were detected by indirect immunofluorescence staining, Moreover, extracted CNPs showed concentric circles of aggregated apatite after incubation, with morphological similarity to pulp stone under SEM.

Jing et al[29] hypothesized a therapeutic use of CNPs in enamel tooth repair in vitro. Others authors proposed that a gelatinous synthetic mix (free fluoride, calcium and phosphate ions, and CNPs) could be applied on a cracked tooth surface therapeutically to limit further propagation of the crack deeper into dentin.[34]

Conclusion

The cumulative literature evidence on CNPs since their initial description point more to the microbial nature of the particles rather than to physiological contamination. Genomic elucidation supports CNPs as living particles that do get involved positively in pathological calcifications in human organ diseases in dental pulp, salivary glands, kidneys, and arteries. Some investigators have looked into the possibility of using modified CNPs in the treatment of cracked and/or eroded teeth.[29,34]

Disclosure

The authors report no conflicts of interest in this work.

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