An Overview on Clinical Implications of Nanobacteria

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ABSTRACT

Pathological calcification is becoming recognized as an important feature in the dynamics of a variety of diseases, such as renal stone, atherosclerosis, pulp stones, scleroderma and arthritis, etc. from which millions of human beings suffer in all ages. Nanobacterium sanguineum is the first calcium phosphate mineral containing nanoorganism isolated from human blood that causes pathological disease calcification in humans. Nanobacteria are the smallest cell-walled bacteria, recently discovered in human and cow blood and commercial cell culture serum. Here in the article controversy and critical role of calcifying nanoparticles as nidi and triggering factor in human pathologic calcifications are discussed.

Keywords: Pathologic calcification, Pulp stones, Biofilm.

INTRODUCTION

The formation of discrete and organized inorganic crystalline structures within macromolecular extracellular matrices is a widespread biological phenomenon generally referred to as biomineralization. Mammalian bones and dental enamel are example of biomineralization involving apatite minerals. Including humans, many multicellular organisms produce similar hard tissues, such as bones, teeth, shells, skeletal units and spicules. The molecular basis of mineralization remains largely unknown. Recently, nanobacteria have been implicated as factors in biogeochemical cycles for mineral formation in aqueous sediments.

The principal constituent of modern sedimentary rock phosphate minerals in marine sediments is carbonate (hydroxy) fluorapatite Ca. Microorganisms are capable of depositing apatite outside thermodynamic equilibrium in sea water. They can segregate Ca from Mg and actively nucleate carbonate apatite by means of specific oligopeptides under conditions pH < 8.5 and ration of Mg: Ca > 0.1. Such conditions are also present in the human body leading to pathological calcifications. These pathologic calcifications are the major health problem in humans.

Kidney stones, gallbladder stones, dental pulp stones, salivary gland calculi, chronic calculous prostatitis, testicular microliths, calcification in hemodialysis patients, atherosclerosis, malacoplakia, scleroderma (systemic sclerosis), calcinosis cutis, calcific aortic stenosis, several malignancies, some dementias, calcific tendinitis, synovitis, arthritis, diffuse interstitial skeletal hyperostosis, juvenile dermatomyositis and systemic lupus erythematosus are the most common diseases involving extraskeletal calcification.

Nanobacteria is the name of a proposed class of living organisms specifically cell-walled microorganisms with a size much smaller than the generally accepted lower limit size for life (about 200 nanometers for bacteria). Nevà et al (1992) found unusual thin slimy film developing on the culture surfaces while working on mammalian cell cultures. Using scanning electron microscope, they found 20 to 200 nanometer sized bacteria in calcified shells. As they observed nanoscale structures so named them ‘Nanobacteria’ in the year 1998.

Nanobacteria possess unusual properties, making their detection difficult with standard microbiological methods, and it can act as crystallization centers (nidi) for the formation of biogenic apatite structures.

Methods of Identification and Culture Characteristics of Nanobacteria

Nanobacteria are present in biological cells, tissues, blood and urine. Various methods of identification include immunodetection with specific microantibodies, electron microscopy and culture techniques. Since nanobacteria pass through 0.22 μm pore size filters, which exclude most common microbes, often used to clean up fluid specimens before culture for nanobacteria. It has been also shown that growth of the nanobacteria could be detected by specific methods, such as ELISA and turbidity. The identification of nanobacteria involves
its typical growth rates and optical properties, specific stainability with Hoechst fluorochrome stain (Flow laboratories) and with indirect immunofluorescence staining (IIFS) techniques.3 Von Kossa staining could be possibly used to detect specific calcifications.

Light microscopy with differential interference contrast radiography (DIC) optics revealed barely detectable nanobacteria near the bottom of the culture vessel after first week. In second week, nanobacteria appeared as groups easily visible on microscopy. After first month, many were in clumps and started to attach to the bottom of the culture vessel, and by the end of second month, most were in a white-colored biofilm visible to the naked eye.7

There are two forms of nanobacteria:
1. Calcified
2. Noncalcified

These two forms show three type of patterns as free, cell-attached, and internalized particles in mammalian cell cultures in vitro.8

Properties of Nanobacteria
Calcifying nanoparticles have the following unusual properties: Bacteria-like,9 pleomorphic, infectious,10 self replicating,11 capable of passing sterilization filters because of their small size (80-500 nm),12 resistance to heat and irradiation at doses typically fatal for conventional bacteria,13 capable of forming a calcific coating at physiologic pH and mineral concentrations,14 lacking inherent DNA,13 sensitive to certain antibiotics and capable of causing the formation of lipid compounds.15 They have not been classified in any taxonomic group in the kingdom bacteria due to limited information on their biologic characteristics.11

Nanobacteria: Fact or Fiction
Koch postulates should be satisfied if there is link between nanobacteria and pathologic calcifications. Nanobacteria satisfy Koch postulates as:
1. They are associated with pathological calcification (histopathological studies revealed bacteria induced renal tubular calcifications and various manifestations of infection).2
2. They are isolated in pure culture of lesion (nanobacteria are detected in demineralization of stones in 1N HCl).16
3. Inoculation of nanobacteria reproduce the pathologic calcification (Garcia Cuerpo et al found that translumbar, percutaneous intrarenal injection of calcifying nanoparticles that is isolated from kidney stones into rats resulted in kidney stone formation).17
4. Reisolation of nanobacteria from induced experimental animals (Shiekh et al has examined calcifying nanoparticle’s role in biocrystallization and in vivo effects on kidney pathology).18

Mechanism of Calcification by Nanobacteria
Pathologic calcification is a complicated, actively regulated process of mineralization that is similar to bone formation and remodelling.19 Mineralogists explain that all that is needed for crystal formation to start is nidi (nucleus) and an environment of available dissolved components at or near saturation concentrations, along with the absence of inhibitors for crystal formation.20 Nanobacteria or other agents producing such nidi, if present in blood and in urine, are very likely to launch and accelerate pathologic calcification,21 which is clinically important since blood contains phosphate near its saturation level.22

To initiate calcification matrix vesicles (a membranous structure derived from chondrocytes) focally concentrating calcium within their already phosphate rich structures.23 Electron microscope studies have indicated that biologic calcification occurs in the matrix vesicles in two steps, the first related to the initial deposition of hydroxyapatite within the lumen of the matrix vesicles and the second to the propagation of mineral outside the vesicles.24

Most pathologic calcifications throughout the body contain mixtures of carbonate-substituted hydroxyapatite and octacalcium phosphate. These ultramicroscopic crystals occur as snowball-like clumps which often can cause severe inflammation.25 Calcifying nanoparticles are the first calcium phosphorus mineral containing particles isolated from human blood and had been linked to pathologic calcification related diseases, such as arteriosclerosis,26,27 kidney stones,28 gall stone,29 dental pulp stone formation,30,31 prostatitis,32 Alzheimer’s disease, amyloidosis, polycystic kidney (PKD),33,34 malacoplakia3 and cancer.35,36

Life Cycle for Calcifying Nanoparticles
The ‘life cycle’ of calcifying nanoparticles is complex and includes the formation of pleomorphic cell-like nanoparticles. The Nanobacterium sanguineum is the main causative organism which is engulfed by phagocytes in the body and due to its low virulence it bypasses the body defences and starts its multiplication.37

The most peculiar feature of these bacteria is formation of slime-like biofilm, which may contribute to the ‘stickiness’ of the calcifying nanoparticle complex (cell-like vesicles and associated apatite precipitates) in human tissue.37 Biofilm links together the cell-like nanoparticles and provides anchoring to fixed structures such as arterial walls or kidney tissue. It shields the bacterium against host defences helps in colonization and provides an inherent shelter for the growth of nanobacteria.15,37 Within the biofilm the fixation of calcium and phosphorus takes place and calcium carbonate and hydroxyapatite forms which contribute to pathologic calcification (Flow chart 1).

The two important factors which regulate the calcification are amount of oxygen and protein content of the tissues. More oxygen tension favors the growth of biofilm,38 whereas the
protein content favors the reproduction of bacteria by budding or binary fission. In depleted protein environment, a special phase, i.e. igloo (large apatite crystal) formation, takes place. During which the bacterium multiply within the igloos and then released to host tissues for repetition of their life cycle.39

Nanobacteria and Systemic Diseases

Nanobacteria and Renal Stones

Nanobacteria have been found to be associated with Randall’s plaque which were soft tissue calcification found in deep renal medulla in patients with renal stones.40 On screening of demineralized kidney stones using a double staining method, scanning electron microscopy and specific culture method 93.1% stones contain nanobacteria and after compilation of all the assay results 70 out of 72 (97.2%) renal stones were positive for nanobacteria.16 Moreover, the risk of urolithiasis recurrence may be reduced with antinanobacterial therapy.41

Nanobacteria and Gall Stones

Immunohistochemical staining, transmission electron microscopy (TEM), and calcific staining revealed the association of nanobacteria and gall stones. Recent studies showed the positive rate, sensitivity, specificity, false-positive rate and false-negative rate of calcific staining were 38.7%, 58.7%, 93.1%, 6.9% and 41.3% respectively.42

Nanobacteria and Breast Cancer

Nanobacteria (NB) are novel microorganisms mediating microcalcifications in breast cancer. Nanobacteria have been shown to contribute to different benign and malignant calcifications in the form of calcium phosphate crystals and contribute to malignant calcifications in breast cancer.43

Nanobacteria and Ovarian Cancer

Nanobacteria may be involved in the formation of microcalcifications known as psammoma bodies that are found in ovarian cancers. They are found in seven microcalcified specimens by immunohistochemical staining, transmission electron microscopy, ELISA and infrared spectroscopy.2

Nanobacteria and Arterial Plaques

Nanobacteria-like particles are present in human atherosclerotic plaques.26 The previous documentation in literature suggests that the culture for calcifying nanoparticles was positive in 48 out of the 75 valves with aortic stenosis (64.0%) in comparison with zero out of eight patients (0%) for the control group (p = 0.0005). The observation of cultures by way of scanning electron microscopy highlighted the resemblance in size and morphology of calcifying nanoparticles.44

Nanobacteria and Prostatic Stones

Nanobacteria cause prostatic stone as the apatite core of 98% of prostatic stones was consistent with a nidus formed by nanobacteria. There was indirect evidence of nanobacteria on ELISA in 60% of blood and 40% of urine samples in patients with chronic pelvic pain syndrome due to chronic prostatitis.45

Nanobacteria and Oral Diseases

Ciftcioglu et al (2003) postulated that nanobacteria may be present in dental calculus which has a similar mineralization formation process as other pathologic calcification. They also concluded that nanobacteria are associated with pulp stones.46

Pulp Stones

Nanobacteria were isolated from dental pulp stones. The freshly collected 27 dental pulp stones and divided them into nine samples. Each sample contained three dental pulp stones. All samples were used for the isolation and culture of nanobacteria. Sample collection was used for the isolation and culture of nanobacteria. The results revealed that the bacterium that was isolated from dental pulp stone was similar to nanobacteria in terms of growth rate, morphology and staining properties.31

Dental Calculus

Zhang et al have found nanobacteria from gingival crevicular fluid samples from two subjects with chronic periodontitis. They emphasized that nanobacteria can be cultured and identified from dental calculus.47

Calculus is detrimental to periodontal health because it serves as a nidus for increased plaque formation and retention; thus, calculus along with local factors that causes a localized build-up of plaque, is referred to one of the most important etiology of periodontitis.48

Nanobacteria may be considered to be a risk factor for the periodontal diseases providing an efficient role in calcification
of dental calculus. On the other hand, the fact that nanobacteria are present in dental calculus may prove useful in explaining the relation between periodontal diseases and cardiovascular diseases, which is still a disputed issue.49

Remineralization of Enamel
Cracked teeth are usually found and easily ignored in clinic. If not diagnosed timely, they can lead to pulpitis and teeth fractures. The current treatments for cracked teeth include occlusal adjustments or preventive fillings, and the root canal therapy or complete crown restoration, which is decided according to the depth of cracks.50

Yixin Line (2009) has found that nanobacteria have a very unique role in biological mineralization, which can produce crystalline apatites in the conditions of physiological calcium and phosphorus concentrations. Therefore, a hypothesis is put forward that application of nanobacteria may mineralize the cracks of teeth. Then the development of cracked teeth would be completely eliminated.50

Antinanobacterial Test
Now a days, urine and prostatic fluid were tested for nanobacterial antigen in patients suffering from kidney stones and chronic prostatitis, using the rapid nanobacterial antigen test (‘Nanobac Test’ by Nanobac Life Sciences).48 But, well designed clinical studies are required in multicentric settings for the availability of the test.

Antinanobacterial Therapy
Various recommendations are present for antinanobacterial therapy but still it is an experimental approach. The treatment for systemic diseases includes 500 mg tetracycline orally, Nanobac OTC supplement (Nanobac Life Sciences) a proprietary blend of vitamin C, selenium, ethylenediamine tetraacetic acid (EDTA), coenzyme Q10, bromelain, quercetin, L-arginine, vitamins B3, B6, B9, L-lysine, L-ornithine, trypsin and papain proteinase, a rectal suppository containing 1,500 mg EDTA for a period of 3 months.48 Gallium nitrate (120 mg gallium) mixed with water making two liters of a gallium mineral water drink to treat chronic, treatment-resistant kidney stone was also hypothesized.51 Nanobac TX (combination of EDTA and tetracycline) is safely recommended in the treatment of heart diseases.52

For oral diseases, the treatment includes an antinanobacterial mouthwash or toothpaste containing bisphosphonates specifically etidronate and clodronate (1 mg/ml), gallium nitrate 14% (3.4% w/w gallium at 99.995% purity) and EDTA (1%) are recommended.49

Controversies behind Nanobacteria
The first debate about nanobacteria revolved around whether these minute particles are alive or not. To this day, critics argue that a particle just 50 to 200 nanometers in diameter cannot possibly harbor the components necessary to sustain life. Maniloff’s work suggests that to contain the DNA and proteins needed to function, a cell must be at least 140 nanometers across.34 Isolation of any kind of nucleic acid from nanobacteria has been difficult, in part because of the mineral surface that they produced during their culture period. They could not be lysed with lysozyme, proteinase K, several other proteinases, lipases, amylases, alkali, ultrasound, detergents or solvents.2

Another controversy is about the cytotoxicity of nanobacteria. Nanobacteria are cytotoxic to mammalian tissue as they release endotoxin and are internalized in host tissues either by receptor-mediated endocytosis or by a closely related pathway within 12 hours. Thus, the cytotoxicity depends on nanobacteria concentration and exposure time.53

CONCLUSION
Nanobacteria and their associated apatite precipitates and biofilms make up an extremely interesting complex found in many humans. In some ways, it might be considered to be an analogue of a possible hypothetical early primitive life form, which developed and reproduced without the benefit of DNA. However, naming an agent as nanoparticles or nanobacteria, living or nonliving but self-replicating, has relative meaning with respect to causing disease. The fundamental importance is that these self-replicating special particles that we call calcifying nanoparticles are found in blood and in pathogenic calcification and their properties of promoting ready crystallization and growth of calcium minerals are well established. The evidence that nanobacteria exist in the human body and are closely associated with many kinds of disease is now overwhelming. However, future research is required to reveal their nature and impact on health and disease.

REFERENCES


