OVERVIEW OF BIOMINERALIZATION AND NANOBACTERIA N. Ciftcioglu[1] and D. S. McKay[2], [1]Nanobac Life Sciences, NASA JSC, 2101 NASA Parkway, Mail code KA, Houston, TX 77058. E-mail: Neva.Ciftcioglu1@jsc.nasa.gov, [2] NASA JSC, 2101 NASA Parkway, Mail code KA, Houston, TX 77058. E-mail: david.s.mckay@nasa.gov

Introduction: Biomineralization is a frequently used term in nanotechnology, astrobiology, geology, and medicine. In the process of biomineralization, a living organism provides a chemical environment that controls the nucleation and growth of unique mineral phases. Often these materials exhibit hierarchical structural order, leading to superior physical properties, not found either in their inorganic counterparts or in synthetic materials. Biomineralization is widespread in the biosphere and hundreds of different minerals are produced or assisted by a variety of organisms from bacteria to humans. Teeth, bones, kidney stones, and skeletons of algae, mussels, and magnetotactic bacteria are all examples of biomineralization. We do not fully understand the control mechanism of biomineralization either in primitive or in developed organisms. The presence of organic molecules, among other characteristics, can influence the coherence length for X-ray scattering in biogenic crystals [1]. Control over biomineral properties can be accomplished at a myriad of levels, including the regulation of particle size, shape, crystal orientation, polymorphic structure, defect texture, and particle assembly. In the latter case, cellular processes enable control in both the spatial and temporal domain in such a way that hierarchical composite structures can be built which increase the toughness and durability of the material, which is invaluable for load-bearing materials such as bones, teeth, mollusk shells, etc. Durability of biominerals produces remarkably preserved bacterial and cyanobacterial microfossils from billions of years-old samples [2]. The differentiation between microffossils and nonbiogenic artifacts has been a lively discussion subject in astrobiology especially in the last decade [3-5]. Clearly, more detailed information on the mechanism of biomineralization, and the effect of organic matter on crystal formation/fossilization would help focus such discussions.

Recent investigations in geomicrobiology have demonstrated the ability of living organisms to control crystallization through a biomineralization process that involves application of specialized macromolecules which enable nucleation and growth of crystalline structures of carbonates, phosphates, oxides, oxalates, silicates, and other inorganic materials [6]. Some of these processes have been demonstrated in both 1g and analog microgravity conditions [7]. Although some researchers believe that microgravity does not affect crystal formation and/or crystal organic matter interraction, it has been shown that long periods in a microgravity environment does affect the biomineralization process *in vivo*, e.g., loss of bone, and enhanced kidney stone formation-like disorders in astronauts [8]. The reasons are probably multi-factorial. In order to elucidate the fundamental factors, the mechanisms of biomineralization must be better understood.

Study of calcium-based biomineralization processes creates new approaches for studies of space- and nano-medical technology. Bone loss is one of the major physiological problems in planning the 30-month manned mission to Mars and/or other planets. Joint Russian-American studies show that bone loss from lower vertebrae, hips and femur occur at an average rate of about 1-1.5 percent per month depending on the specific bone site. If the rate of loss continues during longer missions, e.g., a mission to Mars might result in 30 % decrease in bone density. This is a major cause for concern since the loss of bone elevates sera and urinary calcium levels and could potentially cause kidney stones and calcification in soft tissues during the mission. Some bone loss may be irreversible, and if so, astronauts who have been in long duration missions may be more prone to broken bones after flight or experience early onset of osteoporosis [8].

Since biomineralization seems to be an important phenomenon in topics ranging from space science to medicine, and from biology to geology, additional focused research on this process is clearly required. While bone and teeth formation and alteration, as well as sea shell formation have been the focus of many investigations of biomineralization, research on the mechanism of biomineralization at the microbial level lacks a good model. A simple biomineralizing microorganism is needed as a model for understanding the environmental, physical, chemical and even immunological effects on biomineralization/calcification mechanism, since they do not have a complicated metabolic pathways.

Nanobacteria (NB) are the smallest known self-replicating agents, recently discovered in blood, kidney stones, atherosclerotic plaques, and some other pathologic calcification ("bad biomineralization") related patient samples [9-11]. We have determined by TEM with energy-dispersive X-ray microanalysis and Fourier transform IR spectroscopy that all growth phases of NB produce biogenic apatite on their cell surface [9]. Although biological characterization of NB is still incomplete, the precipitation and growth of calcium phosphate readily occurs in systems containing trace amounts of NB, but not in identical control systems lacking NB. Due to their specific macromolecules, NB can produce apatite very efficiently in media mimicking tissue fluids; available calcium and phosphate are rapidly precipated on NB surfaces. This can be also monitored by ^{85}Sr incorporation and provides a unique model for *in vitro* studies on calcification [10].

We have performed a pilot research study on NB multiplication and their biomineralization processes in rotary bioreactor culture systems. Such experiments have been shown to mimic the effects of reduced gravity [12, 13]. We found that apatite-forming NB increase their multiplication rate in rotary bioreactors without changing their size. However, the thickness of apatite formation on their cell surface was less compared to cultured NB in stationary flasks (1g) [14]. An understanding of the fundamental changes that occur to apatite crystals under simulated microgravity conditions would yield important information that will help in preventing or minimazing astronaut bone loss. To our knowledge, there are only a few studies of apatite crystal formation in bioreactors. None of those studies were made in biological-fluid mimicking media. These previous studies all used nonphysiologic super saturated conditions for initiating the crystal formation. Because NB form apatite crystals under physiological conditions, we think NB can be an improved *in vitro* model for further studies related to biomineralization/calcification. Additionally, these studies may help to understand another space-science related research branch; early life on earth.

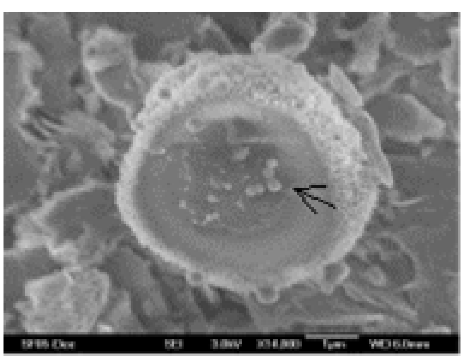

Figure. SEM image of a NB-associated precipitate obtained by culturing NB in serum-free media, DMEM. We detached this structure by scraping the culture vessel. EDS analysis of this structure shows strong Ca and P peaks. Arrow shows the free NB particles. Bar: 1 micrometer.

Interestingly, microstructures similar to NB have also been observed in rare sedimentary rocks and martian meteorites; studies are underway to investigate whether these microstructures might represent a primitive early form of life.

Biologically-induced fossilization (crystallization) processes related to hundred nanometer-sized organisms has not been well described. NB research findings may be important to the general understanding of the biomineralization process and the development of bio-inspired 'smart' materials.

References:

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