NANNOBACTERIA AS A BYPRODUCT OF ORGANIC TISSUE DEGRADATION BY BACTERIA.
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Introduction: Tiny spheroidal objects in the size range from 40-150 nm are common in sedimentary rocks, and have been interpreted as the fossilized remains of minute life forms [1,2,3]. Because etching and mineral precipitation can produce oval shaped artifacts in the size range of nannobacteria, there was always some scepticism in this regard. However, when similar looking objects were discovered in Martian meteorite ALH 84001 and interpreted as possible evidence of extraterrestrial life [4], what once was just a curiosity for some geologists was now scrutinized extensively by astrobiologists, early life researchers, and biologists. In a 1998 workshop convened by the National Academy of Sciences, the general consensus was that the lower size limit for microorganisms should be in the 200-300 nm range [5]. This would suggest that features previously desribed as nannobacteria are not actual living entities [6] or their fossilized remains [1].

Nonetheless, there are nannobacteria culture experiments with human and cow sera [6] that have been construed as proof for the actual existence of nannobacteria. Research in other labs, however, suggests that the supposedly biogenic hydroxyapatite mineralization associated with nannobacteria was actually caused by the nucleating activities of self-propagating microcrystalline centers [7], and that proteins most likely mediate their nucleation, growth, and morphology [8]. In this contribution we report on tissue decay experiments that produced abundant proteinaceous spheroids in the size range of nannobacteria (described as nannoballs in the remainder of this paper). Subsequent mineralization of these spheroids may be a common formative process for nannobacteria observed in the rock record.

Tissue Decay Experiments: In an effort to examine early diagenetic iron sulfide deposition, we buried different types of tissue samples (bean, beef, squid) in a clay layer at the bottom of a tank filled with sulfate saturated water. Samples were inoculated with microbes from pond muck and produced black iron sulfide coloration of the clay within days.

Frequent sampling allowed us to track the decay process over several weeks, until essentially all the original organic matter had been consumed by microbial degradation. When removed from the tank, organic tissues were fixed with Gluteraldehyde and Osmium Tetroxide. After critical point drying the samples were coated with gold/palladium (Au/Pd) and examined with an SEM.

While our SEM examinations showed the successive degradation of tissues and tissue elements very nicely, at high magnifications (30,000-50,000) we also noticed the widespread occurrence of masses of spheroids in the size range of nannobacteria (informally referred to as nannoballs, Fig. 1). We observed these regardless of tissue type or tissue element that was undergoing decay, and surmised that these features might be a byproduct of bacterial degradation rather than nannobacteria.

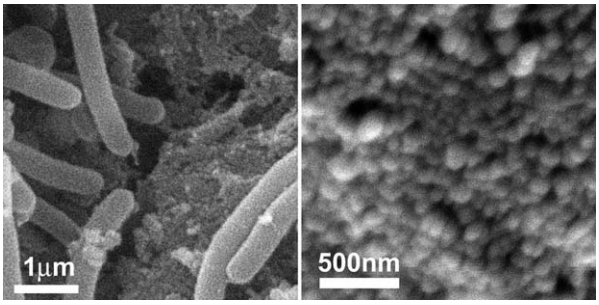

Figure 1: Bacterial decay of squid muscle tissue. At left we see abundant rod-shaped bacteria on muscle tissue that is already taking on a granular appearance. At right we see a close up of a granular area from the photo at left. We see that the granular areas consist of spheroidal bodies ranging in size from 50 to 120 nm.

Enzymatic Breakdown of Tissues: Because bacteria break down organic tissues with a variety of enzymes, we decided to test this hypothesis by exposing the same types of samples (bean, beef, squid) to a range of commercially available purified enzymes. After one week these samples were fixed and prepared for SEM observation in the same way as the samples in the previous decay experiments. Examination of fixed specimens again revealed at high magnification the widespread occurrence of nannoballs in these samples (Fig. 2).

Results: The observations presented here suggest that when proteinaceous organic tissues are exposed to protein degrading enzymes, either through bacterial activity or via immersion into purified enzyme solutions, nannoballs are a natural byproduct of the ensuing degradation process. In both cases the enzymes act as catalysts or chemical "knifes" that break up large complex molecular structures, such as cell walls and mus-

cle fibers, into smaller, simpler pieces. Although ultimately all soft tissue including the nannoballs should be consumed in this process, we know from the fossil record as well as from laboratory experiments that under favorable circumstances partial preservation of soft tissue structures, e.g. via calcium carbonate and calcium phosphate mineralization, occurs.

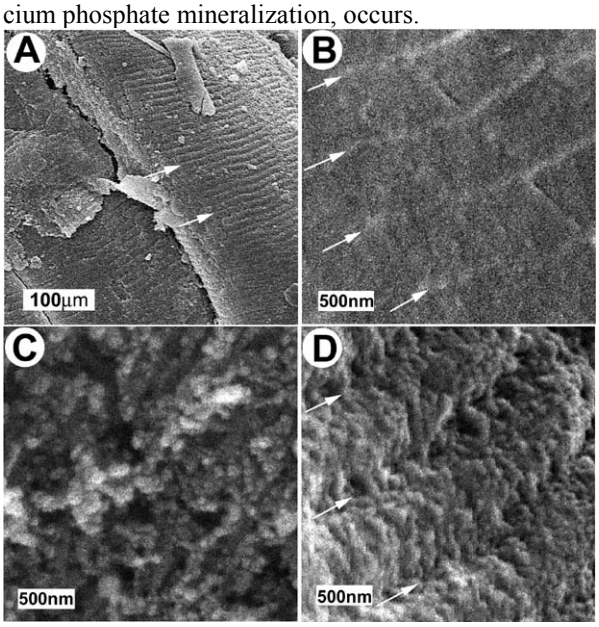

Figure 3: (A) low magnification view of beef muscle fibers with characteristic striations (arrows). Muscle fibers extend from upper left to lower right corner of field of view. (B, C, D) comparing beef muscle tissue of control sample (B), with the effects of bacterial degradation (C), and enzyme digestion (D). (B) Close up view of muscle fiber in control sample. Orientation of striation (arrows) same as in (A). Note comparatively smooth surface. (C) Surface of muscle fiber exposed to bacterial decay processes. Note formation of granular masses of nano-spheroids. (D) Surface of muscle fiber exposed to enzyme solution (proteinase). Orientation of muscle fiber and striations (arrows) the same as in previous pictures. Note granular nature of muscle fiber when compared to (B), as well as formation of nano-spheroids of comparable size as in (C).

The ubiquitous occurrence of balled-up nanoscale sub-units is possibly related to elastic forces inherent in larger structures (such as muscle fibers or cell walls) that are composed of coiled and folded up proteins or crystalline and non-crystalline zones of cellulosic fibers. One might consider as a possible analog an interconnected network of deformed springs that may appear like a flat layer when intact and viewed from a distance. When the structure is cut into smaller subunits, however, now unbalanced forces cause the subunits to deform and contract into sphere-like structures.

Conclusion: We conclude from our research that the fossil record of diagenetic mineralization related to microbial decay should have prolific preservation of nannoballs, and that most if not all of the purportedly nannobacterial structures in geologic specimens are more likely a byproduct of bacterial degradation of organic matter than evidence for the former existence of minute life forms. Yet, while fossilized nannoballs can not serve as direct evidence for the former existence of living entities, they may nonetheless be a good indicator of bacterial enzyme action on organic tissues. In a way they could be considered as a visual "proxy" for microbial activity.

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