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# Biomineralization Induced by Stressed Nanobacteria

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**ABSTRACT:** Animal experiments provided direct evidence that myocyte apoptosis may be a causal mechanism of heart failure, suggesting that inhibition of this cell death process may constitute the basis for novel therapies. The data suggested that even inhibition of a small fraction of cardiac myocyte apoptosis could be instrumental in preventing cardiomyopathy. Here we analyze the mechanisms by which nanobacteria (NB) are expected to contribute to the inhibition of cellular functions in the heart. NB are protected by a nanocrystalline apatite shell. Under environmental stress, they produce a slime providing ideal conditions for individual mineralization and rapid formation of giant thrombogenic assemblies. We establish a model based upon a possible synergistic impact of physical and chemical stimuli on NB, exposing the principles of a novel preventive strategy promising to inhibit formation of NB clusters in the circulation.

Light-induced biomineralization processes,[1] first proposed to exist in nanobacteria (NB),[2] have attracted much attention, from the biomedical side,[3,4] as well as from the astrobiological side.[5] Importantly, the irradiation parameters which have been shown to affect the vitality level of NB were equivalent to those demonstrated to modulate the thickness of nanoscopic water films on polymer surfaces,[6] and also to those compensating stress in a large body of biosystems.[7] Stress-compensating effects of low level light (light energy densities identified as biologically effective, causing no noticeable thermal effects in biological tissues) have been ascertained both in vitro and in vivo, in an extensive number of cells including stem cells, fibroblasts, keratinocytes, osteoblasts, neurons, and retinal receptor cells. The parameters employed for irradiation with low level light (dose, intensity, wavelength) were virtually equal for all these cell types. The impact of light on the vitality level of cells and cell organelles, in particular, on heart cells and mitochondria in vivo,[8] and on nanobacteria in vitro,[1,3] has stimulated the postulation of a cooperative model,[3] suggesting that the formation of cardiovascular plaque networks, created by stress-exposed NB, could be prevented by light—practically the same light that has been shown to elevate the vitality level of mitochondria in vivo.[8] The stress affecting NB could have three principal environmental sources: physical (radiation, temperature), physiological (nutritional limitation, pH), and biomechanical (fluid shear stress, contact pressure, hydrostatic pressure).[5] The interplay between NB and mitochondria seems of paramount interest in possible therapies of myocardial infarcts.[3] NB exposed to physiological stress produced large quantities of slime in a considerably short time.[3,5] As in ordinary bacteria, the slime released by NB seems to consist to a large extent of multifunctional glycoproteins, substances facilitating colony formation, serving as protection and nutrition to the NB. It is reasonable to assume that plaque formed by stressed NB is mediated by rapid production of slime: Once produced, the slime-field surrounds the nanocrystalline apatite shell protecting the NB. Collision of one potentially stressed NB with other (stressed or nonstressed) NB could induce formation of larger conglomerates. In this way, a number of NB circulating in the blood vessels (circulation in blood vessels is likely to represent a form of biomechanical stress) could collect other NB, ultimately immobilizing them in compact colonies, a dynamic collection process similar to the way hydrometeors are collected by graupel pellets in clouds. By arriving into the cardiovascular system, compact crystalline assemblies could spontaneously trigger thrombogenic processes inducing myocardial infarcts.[9] The severity of an infarct is thought to depend primarily on the number of irreversibly damaged mitochondria in the low perfusion ischemic area of the mitochondria-rich heart. According to recent animal experiments, the inhibition even of a very small fraction of cardiac myocyte apoptosis could provide a near-complete prevention of the cardiomyopathy.[10] These observations underline the need for a new definition of normal and pathological cardiac homeostasis in which the balance between myocyte death and renewal are essential factors for the maintenance of cardiac function.[11] Similarly, in neurodegenerative diseases, apoptotic cell death has been invoked as the underlying basis for neuronal loss, but the data are not clear.[12] However, there too, apoptosis mediated by mitochondria could be dramatically reversed by light.[13] Unexpectedly, mechanisms and practicable routes allowing minimal inhibition of cell death could become of vital interest. In view of this new development, recent work that has demonstrated that laser light, if timely used, could potentially reverse mitochondrial damage in the infarct zone of the heart of animals (rats, dogs) receives an imminent clinical significance.[3,14] The heart of the rats was irradiated noninvasively, through the chest muscle between the ribs. The heart of the dogs was irradiated at the open thorax, using an optical fiber positioned at 4−5 cm from the beating heart. Infrared laser irradiation (804 nm) was used to achieve maximum possible penetration into the targeted tissue.[14] Irradiation time was adjusted to preselected biostimulatory standards (local intensity and dose) published in the literature.[7] Notably, visible irradiation (delivered by light-emitting diodes LED operating at 670 nm) has been found to have a pronounced biostimulatory effect on both NB in vitro[3] and mitochondria in vivo.[13] This result indicated the possible beneficial potential of 670 nm laser light (applied externally at intensity levels tolerated by the body) for amelioration of heart disease in humans

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by noninvasively stimulating both mitochondria and NB (if present) simultaneously. Such therapy concepts could become relevant in emergency scenarios, e.g., for the self-treatment of astronauts in extreme situations that could occur during extended space missions. Targeting mitochondria-rich cells seems logical: The number of cell organelles varies among different cells, depending in large part on the metabolic requirements. Thus, skin fibroblasts contain only a few hundred mitochondria, whereas neurons may contain thousands, and cardiomyocytes tens of thousands.

Psychophysiological stress may provoke massive physical and chemical changes within the cardiovascular system (e.g., transient variations, triggered by a critical imbalance between supply and demand of blood oxygen).[15,16] NB passing the stressed heart may respond to the variations of their environment by subsequent slime release, activating avalanches of synergistic effects stimulating individual biomineralization and formation of slime-interconnected conglomerates. Under favorable conditions, slime-interconnected populations of collectively stressed NB, each surrounded by a nutrient-rich milieu, would start growing by rapid mineralization of the apatite shell.[5] Growth of the nanovesicles immobilized in the interior of the heart could take place via Ostwald-ripening. ATP released from apoptotic disintegration of nearby mitochondria may serve as supplementary energy source, triggering additional mineralization and growth of NB locally. Indeed, laboratory experiments have clearly revealed that NB-like nanovesicles isolated from human atherosclerotic aortas started to mineralize only in the presence of ATP.[17] Once trapped in the heart, NB may continue to grow by metabolic processes between differently sized proximal nanovesicles via free energy reduction by molecular interface crossing (FERMIC).[18] From the above model, it is clear that light may prevent plaque formation, and partly reverse mitochondrial damage potentially caused by stressed NB: Light energy densities about $4 \times 10^4$ J m$^{-2}$ and intensities on the order of the solar constant have been described to alleviate slime production in NB, which respond with secretion of slime to various environmental stimuli,[3] to simultaneously elevate mitochondrial vitality and to reduce inflammatory processes[19] related to atherosclerosis,[20] preventing release of apoptotic decomposition products which may nurture NB.

## Conclusions

The treatment of the ischemic heart with low level light doses[21] was found to be beneficial in animal cardiomyocytes, and may be useful for inhibition of thrombogenic effects in humans, associated with plaque formation induced by NB.[3] Progress in this field requires an understanding of biomineralization in primitive biosystems in general,[22] and in NB[23] in particular: interaction between NB (collision, adhesion, and crystallization) and between NB and light. Of special interest could be the possibility of light administration combined with chemical substances selectively targeting inhibition of NB functions, or destruction of the slime barrier. The reported irreversible inhibition of NB activities by several antibiotics[24] demonstrated the promising potential of the chemical route in blocking a possible contribution of NB to atherosclerotic plaque formation.

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