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A Preliminary Investigation into Light-Modulated Replication of Nanobacteria and Heart Disease

ANDREI P. SOMMER, Ph.D.,[1] URI ORON, Ph.D.,[2] ANNE-MARIÉ PRETORIUS, Ph.D.,[3] DAVID S. McKAY, Ph.D.,[4] NEVA CIFTCIOGLU, Ph.D.,[5] ADAM R. MESTER, M.D.,[6] E. OLAVI KAJANDER, M.D., Ph.D.,[7] and HARRY T. WHELAN, M.D.[8]

ABSTRACT

Objective: **The purpose of this preliminary study is to evaluate the effect of various wavelengths of light on nanobacteria (NB).** ***Background Data:*** **NB and mitochondria use light for biological processes. NB have been described as multifunctional primordial nanovesicles with the potential to utilize solar energy for replication. NB produce slime, a process common to living bacteria. Slime release is an evolutionary important stress-dependent phenomenon increasing the survival chance of individual bacteria in a colony. In the cardiovascular system, stress-induced bacterial colony formation may lead to a deposition of plaque.** ***Methods:*** **Cultured NB were irradiated with NASA-LEDs at different wavelengths of light: 670, 728 and 880 nm. Light *intensities* were about 500k Wm^{-2}, and *energy density* was 1×10^4 J m^{-2}.** ***Results:*** **Monochromatic light clearly affected replication of NB. Maximum replication was achieved at 670 nm.** ***Conclusions:*** **The results indicate that suitable wavelengths of light could be instrumental in elevating the vitality level of NB, preventing the production of NB-mediated slime, and simultaneously increasing the vitality level of mitochondria. The finding could stimulate the design of cooperative therapy concepts that could reduce death caused by myocardial infarcts.**

INTRODUCTION

A PRECONDITION for the majority of myocardial infarcts (MI) is atherosclerosis—a slow vascular process of unknown mechanism that is accompanied by the formation of soft and calcified plaques triggering secondary thrombosis processes. MI is the major cause of death in the Western societies. The severity of an MI depends ultimately on the degree of irreversible ischemic cell and free radical-mediated reperfusion damage. Atherosclerosis is currently known to be an inflammatory disease with circulatory infectious burden as a risk and potentially as a causative factor.[1] Calcification mechanisms in intimal plaques are unclear, but might be initiated by the deposition of crystalline apatite, as found in atherosclerotic plaques.[2] Interestingly, nanobacteria (NB) consist to a large extent of nanocrystalline apatite and other forms of calcium, similar to the calcified plaques. Structures held to be NB have been identified in plaque from calcified human cardiovascular tissue.[3] Nanovesicles isolated from human atherosclerotic aortas produced calcifications *in vitro* only in the presence of an external energy supplement.[4] NB have been detected in blood of a number of mammals (cattle).[5] The presence of bacteria in the blood is not new.[6] NB were found to be excreted from blood into urine and could be identified in 90% of human kidney stones, suggesting both their abundance in the human body (for certain diseases) and that they could nucleate kidney stones.[7,8]

[1]Central Institute of Biomedical Engineering, University of Ulm, 89081 Ulm, Germany.
[2]Department of Zoology, Faculty of Life Sciences, Tel-Aviv University, Ramat-Aviv, 69978 Tel-Aviv, Israel.
[3]National Health Laboratory Services, Department of Medical Microbiology, University of the Free State, Bloemfontein, 9300, South Africa.
[4]National Aeronautics and Space Administration/Johnson Space Center, Houston, Texas 77058.
[5]Universities Space Research Association, National Aeronautics and Space Administration/Johnson Space Center, Houston, Texas 77058.
[6]Department of Radiology and Oncotherapy, Semmelweis University of Medicine, 1082 Budapest, Hungary.
[7]Department of Biochemistry, University of Kuopio, 70211 Kuopio, Finland.
[8]Department of Neurology, Medical College of Wisconsin, Milwaukee, Wisconsin 53226,

LIGHT CONTRA STRESS

Cultured NB isolated from humans form large amounts of slime under physiological stresses. This slime promotes multicellular colony connections, where individual cells are immobilized. Slime-mediated biomineralization processes are likely to promote rapid plaque formation.[9,10] In laboratory experiments, we observed that NB responded to photonic stimulation by forming less slime. They were susceptible to stimulation with polarized white light at intensities limited by the value of the solar constant (~ 1000 kW m^{-2}) and doses around 4×10^4 Jm^{-2}.[11] Most importantly, practically equal intensities and doses of light infarct zone of the heart, have been shown to partially reverse ischemic cell death in the infarct zone of the heart, minimizing collateral damage (as demonstrated in animals irradiated with laser light[12]). Similarly, blindness in animals caused by experimental methyl alcohol poisoning could be completely reversed after irradiation of the retinal tissues with NASA-LEDs.[13] Both tissues heart and retina are rich in mitochondria.

MATERIALS AND METHODS

Irradiation of Nanobacteria

A stock culture of NB (lot 901045, Sera-Lab, Crawley Down, Sussex, U.K.) was cultured in a cell culture incubator, as previously described,[7] for 6 weeks before exposure to irradiation. A 10-mL suspension of the NB culture was diluted 1:20 in fresh Dulbecco Modified Eagle Medium (DMEM) with 10% fetal bovine serum prior to plating. Sixteen 35-mm cell culture dishes were seeded with the suspension (2 mL each). Twenty microliters (20 uCi) of titriated uridine was added to each dish immediately after plating. Cultures were incubated at 37°C in 7% CO_2, 93% moist air atmosphere. Cultures were divided into four groups of four dishes for each wavelength plus an untreated control. After 1h, LED treatment was given at one single wavelength of 670, 728, or 880 nm, all at an intensity of about 500 W m^{-2} and an energy density of 4×10^4 Jm^{-2}. Because of the moderate metabolic rate of NB, 10 days were allowed for [^{3}H]uridine incorporation. Incubations were terminated by harvesting the NB by centrifugation at 15,000g for 20 min. Pellets were washed three times with phosphate-buffered saline, pH 7.4. Radiation absorbing apatite shells were dissolved by suspending the pellets in 1 N HCl for 5 min at room temperature. After neutralization with 1 N NaOH, an aliquot from each sample was placed into an aqueous scintillation cocktail and counted. Statistically significant growth enhancement was achieved with both 670 and 728 nm wavelengths (196% and 159% over control, respectively). Results of the irradiation experiment are summarized in Fig. 1.

RESULTS

Statistically significant increase in [^{3}H]uridine incorporation was achieved with both 670 and 728 nm (196% and 159% over control, respectively). LED-irradiation revealed that the replication rate of NB depended on the wavelength of the light. Results of the irradiation experiment are summarized in Fig. 1.

DISCUSSION

NASA and DARPA have recognized the therapeutic capabilities of the light doses (670, 728, and 880nm) and are seeking development of novel technologies such as low-intensity light-activated biostimulation (LILAB) to accelerate tissue repair *in vivo* and to induce regeneration in retinal, corneal, epidermal, dermal, musculoskeletal and neuronal tissues, including strategies to counteract the severe biological imbalances experienced by astronauts in microgravity (e.g., muscle and bone atrophy), and additional photostimulation in cellular imaging via near-field optical analysis (NOA).[14–16] LILAB usually operates at moderate light energy densities in the $1–4 \times 10^4$ Jm^{-2} range, regarded as nonthermal. Reproducible biostimulatory effects require, in addition to energy densities in this range, light intensities of the order of the solar constant for linearly polarized white light, a minimum threshold intensity of about 50 W m^{-2} for NASA LED's and of about 20 W m^{-2} for laser irradia-

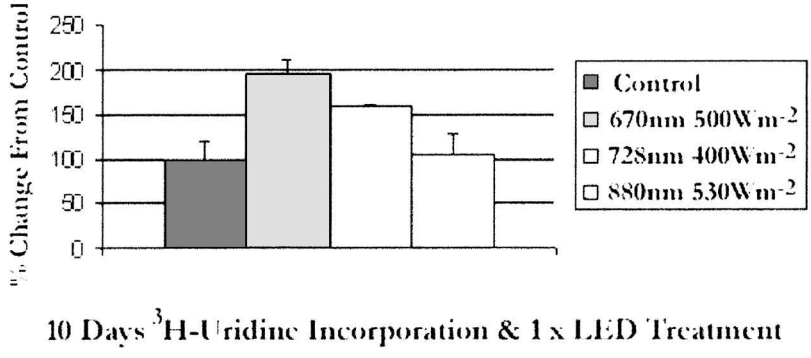

FIG. 1. LED-irradiated nanobacteria multiply faster, as indicated by the incorporation of tritiated uridine. Light treatment was given once, at the beginning of the experiment, at one single wavelength of 670, 728, or 880 nm, an intensity of 500 W m^{-2} and a dose of 4×10^{-4} Jm^{-2}. Controls received no LED irradiation.

tion, depending on the wavelength of the light.[11,16–18] The universality of the beneficial impact of practically equal doses and wavelengths of light on the vitality of cells and cell organelles, in particular on mitochondria, and the equivalence of the biostimulatory light intensities to the present value of the solar constant allowed us to propose an evolutionary model suggesting that present NB may be contemporary examples of primordial extremophiles[19,20] that may have used solar energy for proliferation and metabolic processes.[11]

In the mitochondria, photonic interaction seems to occur via cytochrome *c* oxidase.[18,21] The fact that cytochrome *c* oxidase is a key photoacceptor in the red to near-infrared range points to the importance of examining this enzyme in biological systems. The prolonged effect of a brief LED treatment implies that it induces a cascade of events leading to the stimulation of gene expression, protein synthesis, and oxidative metabolism.[18] With sizes starting below the dimensions of mitochondria, NB might have a different receptor mechanism: NB isolated from human blood have diameters between 80 and 300 nm (Fig. 2) and are coccobacillar shaped.

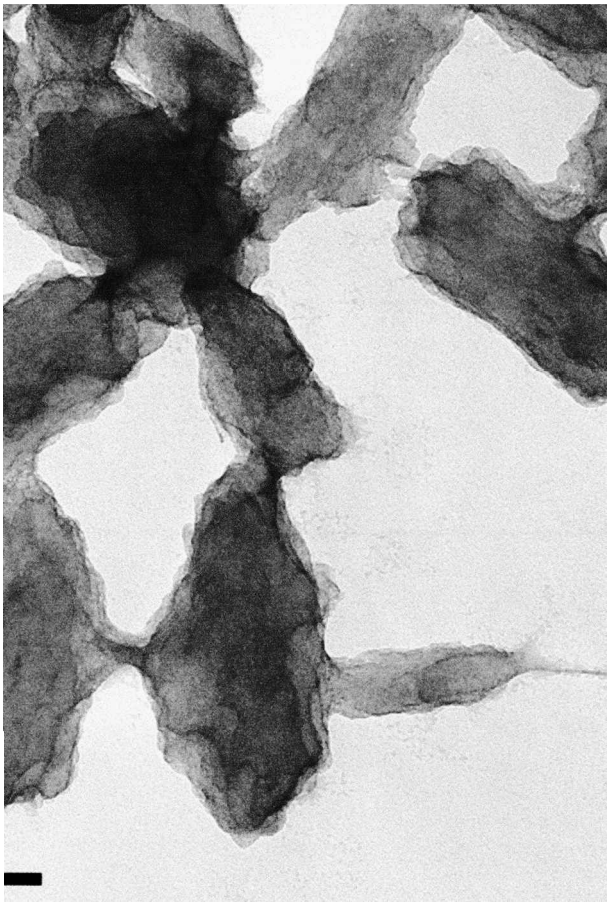

FIG. 2. Transmission electron microscopic image of slime forming nanobacteria isolated from human serum by culturing in mammalian cell culture conditions for 3 weeks. Apatite-rich nanobacteria are kept together by slime. Sample was negative stained with 2% uranyl acetate. Bar = 100 nm.

In serum-free conditions they form a protective apatite shell, containing a central cavity. The shell appears to be porous because cultured NB under stress form a slime network outside of the shell.[9] The porous nature of the shell may allow selective movement of material in solution into or out of the shell. The slime may be produced for the purpose of protecting NB from environmental stress factors (strong water currents, desiccation, inadequate nutrients, unfavorable pH or temperature, etc.). In any case, the development of slime appears to be an indicator of physiological or biomechanical stress, and minimizing that stress may, therefore, minimize slime development.

Apatite is substantially transparent to visible light. Thus, the convex architecture and the radial orientation of the nanocrystalline shell structure may allow NB to function as efficient small light collectors. Interestingly, our teeth, which consist to a large extent of apatite, have developed a microstructure suitable for transporting nutrients and collecting light: visible light is guided by the tubular "photonic crystal"-like structure of dentin directly to the pulp cavity.[22] The biological reason for this physical property of dentin is not known. Remarkably, relatively low laser light intensities (equal to 1000 W m^{-2}) induced transient liquid–vapor phase transitions in nanoscopic water films attached to translucent polymer films.[23] The observations suggest the existence of an analogous light-induced metabolic process for NB, for example, by pumping water from the central cavity across the porous apatite shell to the external environment.

Our view, based on recent clinical observations, is that NB actively participate in the nucleation of plaques deposited in the kidneys[8] and appear to play a similar key role in calcific atherosclerotic plaque-formation processes.[3] If atherosclerotic plaque results in part from stressed NB, and the stress on NB is alleviated by exposure to light, then stimulation with the proper intensity, dose, and wavelength of light may have an inhibitory effect on NB-mediated plaque by improving NB metabolism, decreasing slime production and consequently reducing the tendency of NB to form attachments and plaques throughout the vascular system. Application of light to high slime levels associated with NB and to the mitochondria-rich heart may, therefore, cooperatively reduce myocardial infarct chances and possible heart injuries related to mitochondrial damages by: (a) blocking the slime production contributing to plaque creation,[10] (b) locally reducing tissue inflammation,[24] and (c) restoring mitochondrial functions.[25] Novel treatment concepts based on combinations of light and antibiotics appear reasonable for therapeutic applications related to biomineralization processes induced by NB.[11] Indeed, *in vitro* experiments have demonstrated the susceptibility of NB to several antibiotics, bisphosphonates, and cytostatic drugs in an irreversible bactericidal way.[26]

One hundred years ago, Finsen's pioneering work on the therapy of human diseases with sunlight and artificial light led to his Nobel Prize. Since then powerful lamps, lasers, and light emitting diode array technologies have been developed, with the potential to treat cells and large volumes in the body with biologically suitable wavelengths. The recent development of LED arrays has added benefits of lower cost, virtual absence of heat, and larger arrays for treatment of large wound. In particular, red and near-infrared wavelengths have been proven to have extensive biostimulatory effects.[16,18] Near-infrared light can penetrate up to 25 cm into biological tissues. Combined

with photosensitizers (PDT) and drugs, e.g., antibiotics and/or bisphosphonates,[27] light could be efficiently used to realize complementary and synergistic actions against diseases. In the near future, diseases that previously needed open surgery or lacked any treatment may be curable noninvasively and nonpainfully by using LILAB.

The presented results predict that NB growth and slime formation could be controlled by certain wavelengths of light. Our preliminary study motivates testing of a noninvasive therapy strategy based upon the use of light to limit two major processes now believed to induce and aggravate myocardial infarcts: NB nucleated plaque and infarct fatality related to damaged mitochondria. Beneficial administrations of low level light, in particular in the $1–4 \times 10^4$ Jm^{-2} dose range, and the biological action of light intensity thresholds limited by the value of the solar constant have been ascertained *in vitro* and *in vivo* in systems as different as fibroblasts, keratinocytes, osteoblasts, neurons, retinal receptor cells, heart cells, sperm, and NB.[11,16,18] Notably, similar light doses and intensities ameliorating the vitality and elevating the survival capacity of the majority of stress exposed cells, including stem cells,[28] have been shown to sustain the vitality of living cells during extended examinations in nondestructive optical imaging.[14,15]

CONCLUSIONS

In view of the experimental evidence showing that moderate light energy densities effectively enhanced the vitality level of various stress-exposed biosystems, *in vitro* and *in vivo*,[25,29,30] and that practically the same light doses and intensities were found to affect the replication of NB,[8] responding spontaneously to their photostimulation by forming less slime (a glue recognized as the prerequisite for NB-induced plaque),[7] we feel justified to motivate further research in the preventive therapeutic use of light.

REFERENCES

1. Espinola-Klein, C., Rupprecht, H.J., Blankenberg, S., et al. (2002). Impact of infectious burden on progression of carotid atherosclerosis. Stroke 33, 2581–2586.
2. Tomazic, B.B. (2001) Physiochemical principles of cardiovascular calcification. Z. Kardiol. 90(Suppl. 3), 68–80.
3. Rasmussen, T.E., Kirkland, B.L., Charlesworth, J., et al. (2002). Electron microscopic and immunological evidence of nanobacterial-like structures in calcified carotid arteries, aortic aneurysms, and cardiac valves. J. Am. Coll. Cardiol. 39(Suppl. 1), 206.
4. Hsu, H.H., and Camacho, N.P. (1999). Isolation of calcifiable vesicles from human atherosclerotic aortas. Atherosclerosis 143, 353–362.
5. Breitschwerdt, E.B., Sontakke, S., Cannedy, A., Hancock, S.I., and Bradley, J.M. (2001). Infection with *Bartonella weissii* and detection of nanobacterium antigens in a North Carolina beef herd. J Clin. Microbiol. 39, 879–882.
6. Jacomo, V., Kelly, P.J., and Raoult, D. (2002). Natural history of *Bartonella* infections (an exception to Koch's postulate). Clin. Diagn. Lab. Immunol. 9, 8–18.
7. Kajander, E.O., and Ciftcioglu, N. (1998) Nanobacteria: An alternative mechanism for pathogenic intra- and extracellular calcification and stone formation. Proc. Natl. Acad. Sci. U.S.A. 95, 8274–8279.
8. Ciftcioglu, N., Björklund, M., Kuorikoski, K., Bergström, K., and Kajander, E. O. (1999). Nanobacteria: An infectious cause for kidney stone formation. Kidney Int. 56, 1893–1898.
9. Kajander, E.O., Ciftcioglu, N., Miller-Hjelle, M.A., and Hjelle, J.T. (2001). Nanobacteria: controversial pathogens in nephrolithiasis and polycystic kidney disease. Curr. Opin. Nephrol. Hypertens. 10, 445–452.
10. Sommer, A.P., and Kajander, E.O. (2002). Nanobacteria induced kidney stone formation: Novel paradigm based on the FERMIC model. Crystal Growth & Design 2, 563–565.
11. Sommer, A.P., Hassinen, H.I., and Kajander, E.O. (2002). Light induced replication of nanobacteria—a preliminary report. J. Clin. Laser Med. Surg. 20, 241–244.
12. Oron, U., Yaakobi, T., Oron, A., et al. (2001). Low-energy laser irradiation reduces formation of scar tissue after myocardial infarction in rats and dogs. Circulation 103, 296–301.
13. Eells, J.T., Henry, M.M., Summerfelt, P., et al. (2003). Therapeutic photobiomodulation for methanol-induced retinal toxicity. Proc. Natl. Acad. Sci. U.S.A. 100, 3439–3444.
14. Sommer, A.P. and Franke, R.P. (2002). Near-field optical analysis of living cells *in vitro*. J. Proteome Res. 1, 111–114.
15. Sommer, A.P. (2002). Near-Field Optical Analysis (NOA) via Hydrophobic Optical Elements and Low-Intensity Light-Activated Biostimulation Effect of NOA. Proceedings of the 2nd International Conference on Near-Field Optical Analysis: Photodynamic Therapy & Photobiology Effects. Johnson Space Center, Houston, TX, May 2001, NASA Conference Publication, CP-2002–210786, pp. 78–83.
16. Whelan, H.T., Smits, R.L.Jr., Buchman, E.V., et al. (2001). Effect of NASA light-emitting diode irradiation on wound healing. J. Clin. Laser Med. Surg. 19, 305–314.
17. Sommer, A.P., Pinheiro, A.L.B., Mester, A.R., Franke, R.P., and Whelan, H. T. (2001). Biostimulatory windows in low-intensity laser activation: lasers, scanners, and NASA's light-emitting diode array system. J. Clin. Laser Med. Surg. 19, 29–33.
18. Wong-Riley, M.T., Bai, X., Buchmann, E., and Whelan, H.T. (2001). Light-emitting diode treatment reverses the effect of TTX on cytochrome oxidase in neurons. Neuroreport 12, 3033–3037.
19. McKay, D.S., Gibson, E.K.Jr., Thomas-Keprta, K.L., et al. (1996). Search for past life on Mars: Possible relic biogenic activity in martian meteorite ALH84001. Science 273, 924–930.
20. Sommer, A.P., McKay, D.S., Ciftcioglu, N., Oron, U., Mester, A.R., and Kajander, E.O. (2003). Light harvesting nanovesicles—chemical and physical survival strategies of primordial biosystems. J. Proteome Res. web release date 21-Mar-2003; DOI: *10.1021/pro34005h,* papers "in print."
21. Karu, T. (1999). Primary and secondary mechanisms of action of visible to near-IR radiation on cells. J. Photochem. Photobiol. B 49, 1–17.
22. Sommer, A.P., and Gente, M. (1999). Light-induced control of polymerization shrinkage of dental composites by generating temporary hardness gradients. Biomed. Tech. 44, 290–293.
23. Sommer, A.P., and Franke, R.P. (2003). Modulating the profile of nanoscopic water films with low level laser light. NanoLetters 3, 19–20.
24. Mester, A.R., and Sommer, A.P. (2002). How it all started: Dr. Endre Mester's pioneering work. Proceedings of the 2nd International Conference on Near-Field Optical Analysis: Photodynamic Therapy & Photobiology Effects. Johnson Space Center, Houston, TX, May 2001, NASA Conference Publication, CP-2002–210786, pp. 11–13.
25. Sommer, A.P., Oron, U., Kajander, E.O., and Mester, A.R. (2002). Stressed cells survive better with light. J. Proteome Res. 1, 475.
26. Ciftcioglu, N., Miller-Hjelle, M.A., Hjelle, J.T., and Kajander, E.O. (2002). Inhibition of nanobacteria by antimicrobial drugs as measured by a modified microdilution method. Antimicrob. Agents Chemother. 46, 2077–2086.

27. Ferber, D. (2000). Osteoporosis. Cholesterol drugs show promise as bone builders. Science 288, 2297–2298.
28. Shefer, G., Partridge, T.A., Heslop, L., Gross, J.G., Oron, U., and Halevy, O. (2002). Low-energy laser irradiation promotes the survival and cell cycle entry of skeletal muscle satellite cells. J. Cell. Sci. 115, 1461–1469.
29. Mester, E., Mester, A.F., and Mester, A. (1985). The biomedical effects of laser application. Lasers Surg. Med. 5, 31–39.
30. Wollman, Y., and Rochkind, S. (1998). *In vitro* cellular processes sprouting in cortex microexplants of adult rat brains induced by low power laser irradiation. Neurol. Res. 20, 470–472.

Address reprint requests to:

Andrei P. Sommer, Ph.D
Department of Biomaterials
ENSOMA Laboratory.
Central Institute of Biomedical Engineering
University of Ulm
89081 Ulm, Germany

E-mail: samoan@gmx.net