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Suffocation of Nerve Fibers by Living Nanovesicles: A Model Simulation—Part II

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Nanobacteria may cause peripheral neuropathy by adhesion to the perineurium. This hypothesis receives support from five independent observations: (1) identification of perineurial apatite in diabetic patients with peripheral neuropathy, (2) massive presence of nanobacteria in a diabetic patient, (3) beneficial effect of lasers on peripheral neuropathy, (4) model simulation indicating that perineurial deposition and attachment of nanobacteria is encouraged by both their size and chemical nature, and (5) transient inhibition of neural function by apatite. Initial deposition of (stressed) nanobacteria is promoted by a slime thought to consist of proteins, calcium, and phosphate, and is most likely followed by an immobilization phase, mediated by a bioadhesive capacity of the apatite. Proteomics may hold the key to control both attachment processes.

Keywords: apatite • nanovesicles • nanobacteria • adhesion • peripheral neuropathy • diabetes • HIV

Cehreli et al. tested the effect of two prominent biomaterials on compound action potentials (cAPs) in nerves—titanium and apatite. Interfacial contact between the isolated sciatic nerve of rats and apatite-coated implants (hydroxyapatite) resulted in a temporary reduction of the cAPs. Surprisingly, titanium, a body-foreign material, had practically no effect on the measured cAPs.[1] The reported observation could represent the missing link, displaying one possible mechanism of progressive neural degeneration in patients with peripheral neuropathy (PN), common condition in both HIV-infected patients and diabetes mellitus. Notably, in diabetic patients with PN, the perineurium frequently contained apatite.[2,3] The nature and the origin of the mineral are not clear. Possibly, in both major manifestations HIV and diabetes, PN is caused by a layer of nanobacteria (NB) interconnected by slime, encapsulating the nerve fibers. NB have a central cavity which is protected by a predominantly spherical shell with diameters between 80 and 300 nm, consisting of self-assembled carbonate apatite.[4] The shells have a rough surface topography with some porosity allowing for bidirectional fluid flow, and apparently contain DNA,[5] The living nanovesicles seem to protect themselves by releasing slime, promoting colony formation and adhesion to tissues.[6] Observations in cultured NB indicated that slime synthesis depends on physiological and/or biomechanical stress factors.[7] Slime in combination with the rough crystalline surface structure may constitute a highly effective anchoring modality, allowing NB to attach to various biological tissues. The resulting biofilm may have a self-stabilizing capacity and could possess a cytotoxic potential, even without the internalization of individual NB into cells. The stability of the biofilm stems partly from the narrow size distribution of the NB, securing that individual NB are surrounded by a maximum number of next neighbors. Presumably, their initial perineurial attachment is further encouraged by the pronounced structural integrity and the hydrophilic nature of the perineurial tube. This was indeed suggested by the dense, biofilm-like deposition patterns formed by 60 nm polystyrene nanospheres on glass fiber surfaces, employed in laboratory experiments designed to mimic biological scenarios.[8] Simulation via 60 nm nanospheres seemed reasonable because of the dimension and the mostly spherical architecture of NB, and could be justified a posteriori by the isolation of NB of that size.[5] Apatite nanospheres may provide, however, a more realistic setting for modeling attachment of NB. The special effect of the apatite is indicated in Figure 1, showing manifest differences in the patterns produced by drops of aqueous suspensions containing 60 nm polystyrene nanospheres (Duke Scientific, Palo Alto, CA) and nonspherical hydroxyapatite particles of similar mean size (Chem. Eng. Department, Hacettepe University, Ankara, Turkey), both slowly evaporating on mirror polished titanium disks (total surface roughness <4 nm).[9] Patterns as in Figure 1 (ring vs. uniformly dense film) could help to elucidate the most prevalent interaction. Rings signify the action of a flux transporting material to the periphery of drops. The apatite nanosuspension did not form rings on titanium. However, it formed rings on Cybernox (Sitram, France), a nonstick quasicrystalline alloy, providing evidence that the apatite film on titanium resulted principally from the interaction of the nanoparticles with the substrate, and not from the attraction between the nanoparticles themselves.

Living cells need to be attached to a self-produced external meshwork known as the extracellular matrix in order to survive. Anchorage is a vital factor; without it cells undergo apoptosis.[11] Cells attach through integrins, proteins (glycoproteins) that link the intracellular cytoskeleton with the extracellular matrix. Importantly, the binding is a calcium-dependent phenomenon.

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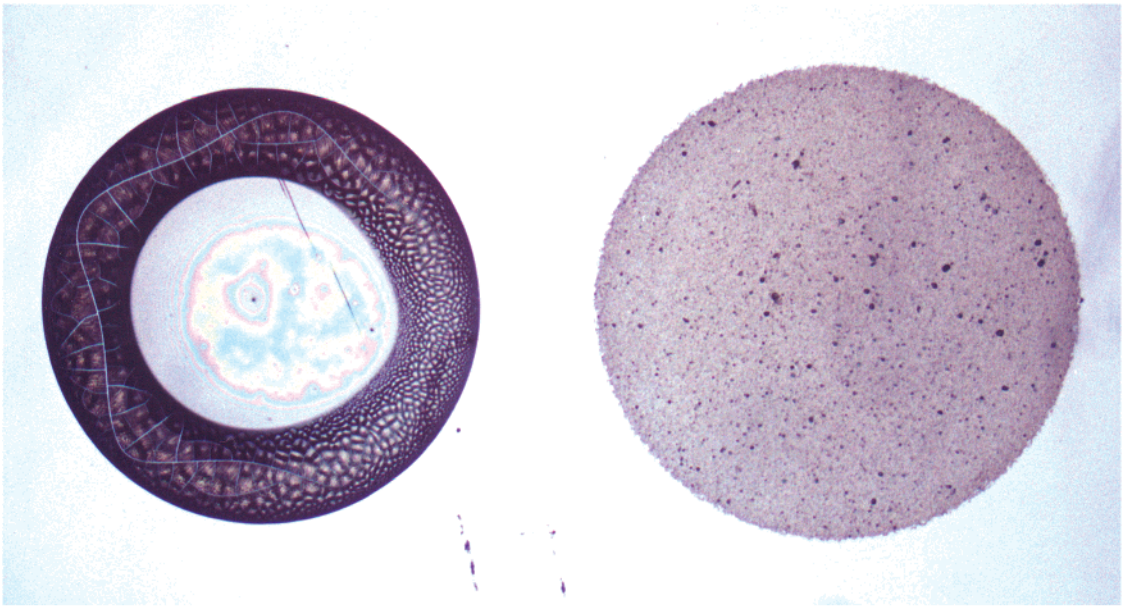

Figure 1. Light microscopy photograph showing self-organization patterns formed by slowly evaporating drops of aqueous suspensions on mirror polished titanium disk. (left) Ring formed by 60 nm polystyrene nanospheres reveals that the lateral force pulling nanospheres to the periphery of the evaporating drop is bigger than the attractive force between the nanospheres and substrate. (right) Uniform deposition pattern stemming from a suspension based on hydroxyapatite particles of same mean size exposes excessive interaction between titanium and hydroxyapatite. Patterns promise to be valuable for analyzing competing forces, and were proposed for detection of biologically active nanoparticles.[10] Rings formed by nanospheres on mirror polished titanium substrates responded sensitively to extremely small contaminations of the substrate. In proteomics, such patterns may be useful in adjusting interaction of proteins with each other or receptor-like structures on surfaces. Additives, injected into suspensions containing a known concentration of functionalized standard nanospheres, in combination with adequately functionalized substrates, recommend themselves for a simple qualitative analysis of competitive bindings: smallest deviations in ring geometry (or structure) from a pre-selected calibration standard, could clearly indicate the blocking of one of the interaction partners. Patterns formed simultaneously on same disk. Diameter of ring ~5 mm.

NB employ a self-produced slime to attach to each other and/or to surfaces. The slime is believed to be rich in calcium and phosphate (prerequisite for growth) and presumably in glycoproteins,[6] facilitating both collection of chemical components of the apatite from blood and adhesion. Attachment probably also secures the survival of individuals in the colony, and may have played a vital function in a primordial world.[12] NB can possibly exploit charge separation phenomena for attachment as well. Biofilms containing NB-like particles, deposited from wastewater onto electrodes, generated electricity.[13] And water passing carbon nanotubes was reported to produce a measurable voltage along the water flow path.[14] Establishing contact between possible light-modulated processes, pumping fluids across the porous mineral shell of NB,[12] predicted from experiments showing that the height of nanoscale water films deposited from air on substrates could be modulated by laser light intensities of the order of the solar constant,[15] these findings could indicate that NB can indeed utilize, in addition to the aforementioned chemical modalities, electrical charge for attachment.

Low level light[16] has been demonstrated to compensate various forms of environmental stress, in cells and NB.[17] The paradigm of the susceptibility of NB to low level light, and of related biomineralization processes, was first proposed in connection with bioimaging methods,[18] and was subsequently applied in the treatment of a patient presenting the severest form of painful PN. Repeatedly applied laser irradiation of the patient's feet produced dramatic systemic effects, with a reversal of all of the symptoms that could be associated to the PN, resulting in a permanent improvement in the patient's condition.[19] The extensive treatment period required for a noticeable amelioration (three months) is in accordance with the picture of an extremely stable slime-reinforced network of mineral nanovesicles coating the perineurium, and its gradual disintegration. It is likely that laser treatment did not destroy NB plaques directly, but that it affected the plaque formation rate by preventing NB freely circulating in blood from their eventual attachment to the perineurium.[20] In summary, biofilms produced by colonies of stressed NB could interact with nerves in two distinct ways, cooperatively causing PN: complete or partial metabolic suffocation of the perineurium by physically isolating it from the surrounding tissue, and transient or permanent inhibition of nerve functionality due to a possible biochemical effect specific to apatite. Relative to the extended surface area of the apatite-coated dental implants used in the sciatic nerve experiment, NB are small. Biofilms created by them would virtually adjust to all the surface irregularities on the perineurium, securing maximum interfacial contact and total encapsulation. Here, it is assumed that carbonate apatite has an inhibitory effect on nerve signal function, similar to that caused by hydroxyapatite. Reports on a contribution of NB in nucleating kidney stones,[21] and indications suggesting their active participation in PN (and possibly in HIV), are motivating challenges, stimulating the development of methods allowing the elimination of that fraction of the NB which are freely circulating in the body, and preventing those which are immobilized from growth.[22] Successful concepts would have to take into account a relationship between the size and the number of NB, and the associated infections. Atherosclerotic plaques and conglomerates representing thrombogenic potentials could be induced by NB in many ways.[6] Theoretically, local deposition of an accumulation of only a few small NB could

be sufficient for formation of minor plaques, which could eventually trigger secondary processes. Kidney stones could be practically nucleated by a small number of solitary NB, matured to giant nuclei after entrapment in the kidneys.[23] PN seems to require a substantial number of small NB, immobilized in a densely packed network on the perineurium.[8] Administration of suitable substances tolerated by the body, permitting hermetical or only partial encapsulation and shifting the surface-polarity of the NB from hydrophilic to hydrophobic, could promote their neutralization and elimination.[24] Encapsulation of the NB could be performed, in principle, in three ways: by the use of polymers with specific affinity toward NB, by nanoemulsions with a minority phase consisting of droplets smaller than NB and specific affinity to NB, or by selectively functionalized nanospheres smaller than NB. Size selective encapsulation strategies for spherical nanoparticles in general, and NB in particular, may be inspired by nanotechnology. Powerful microencapsulation models based upon self-assembly have been developed and are today employed in drug release, offering conceptual solutions for efficient inactivation of NB suspended in body fluids.[25] Starting here is a natural approach to the complex nanoencapsulation problem, which could be solved in two successive steps: identification of biologically harmless particles with a chemical affinity only to NB, and downscaling, according to principles facilitating their self-assembly around NB. In vascular and neural diseases that could be related to NB, initial attachment to surfaces seems to be important. Models using aqueous suspensions containing apatite nanoparticles of the size of NB, simulating their deposition on biological tissues, could help to explore the physical and chemical parameters controlling mineral plaque formation—in the interior of isolated blood vessels, or on top of nerve tissues. Such simple model experiments could be instrumental in identifying the key parameters promoting and inhibiting the attachment of NB to different tissues. Animal models may help evaluating possibilities to combine chemical adhesion control with laser therapy, and to optimize plaque elimination rates. By avoiding rapid plaque depletion, low level lasers operate on an ideal time-scale, and could be implemented in the therapy of PN.

The multifaceted pathogenic potential of NB received now support from the experimental side: (a) by confirming their active role in nucleating renal stones, indicated by their identification in a representative number of stones in India,[5] and (b) their identification in the heart of a diabetic patient.[26] Their implication in heart disease was indicated by some solitary observations,[27] and described by models.[6,7] Their presence in diabetes was indirectly indicated by the occurrence of PN in diabetes and the specific response of PN to low level laser irradiation.[19] Preliminary model experiments designed to mimic attachment of NB to body tissues[8] (simulated by nanostructured titanium—a biocompatible material with extreme hydrophilicity)[28] indicated that deposition and attachment of NB to body tissues are strongly encouraged by their polar nature, size and the size distribution. Titanium is tolerated by the majority of body tissues including soft and hard tissues, as well as nerves and blood vessels—in vitro and in vivo. Therefore, titanium is probably the best candidate among present-day biomaterials for simulation of the interaction of NB with tissues. Mirror polished titanium specimens are particularly suitable, since they allow exclusion of the effects of surface roughness on pattern formation. Interaction mechanisms between particles suspended in liquid (cells) and body tissues have been studied extensively.[29,30] Models could help in predicting the ample interaction modalities between NB and body tissues, targeting them in body fluids and controlling attachment.[31] Attachment inhibition could be achieved by administration of proper scavenging nanoparticles.[32]

Variations in rings deposited under controlled conditions on titanium substrates may finally help to classify relevant physical and biochemical players needed to design drugs blocking bacterial attachment to cells. This is a novel multidisciplinary challenge and requires expertise in adhesion and in proteomics. The data and the model presented may stimulate the development of a better model accounting for the interplay between the competing forces determining deposition patterns in detail, and is likely to open novel biomedical routes. Exploration of the impact of carbonate apatite on nerve functions, clarification of the precise composition of NB slime, as well as studies focusing on the nature of perineurial apatite now seem mandatory.

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