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From Nanobacteria Controversy to OMV Innovation: Lessons for Translational Nanomedicine in Cancer Therapy

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ABSTRACT

Nanobacteria (NB), referred to as calcifying nanoparticles (CNPs), were previously identified as the smallest self-replicating entities, and their proposed role in pathological and tumor-associated calcification has generated considerable interest. However, their biological status remains a topic of considerable contention. Many studies have shown that NB can cause apoptosis in human breast cancer cells and act as nuclei for tumor calcification. This supports the idea that NB is a pathogenic nanomicrobe.

In contrast, rigorous physicochemical analyses assert that NBs are not living organisms but mineralo-protein nanoparticles, often consisting of calcium carbonate and serum proteins such as fetuin. This new way of looking at things sees NB as non-living but still biologically active parts of tissue calcification and remodeling. Bacterial outer membrane vesicles (OMVs) have also become well-known nanosystems at the same time. OMVs come from Gram-negative bacteria and have been shown to be lipid bilayer vesicles that carry proteins, nucleic acids, and other small molecules. Engineered OMVs have demonstrated significant potential as drug delivery systems, photodynamic enhancers, immunomodulators, and nanovaccines in cancer treatment.

Their intrinsic immunogenicity, scalability, and modularity highlight their translational potential. The unresolved NB debate juxtaposed with the therapeutic efficacy of OMVs illustrates a fundamental principle for nanomedicine: stringent molecular and genetic validation is essential to distinguish speculative biology from clinically applicable nanotechnology. Nanobacteria (NB) are not well understood by scientists, but outer membrane vesicles (OMVs) show how nanoscale microbial structures can be used to make new cancer treatments.

Keywords: Nanobacteria, Calcifying Nanoparticles, Outer Membrane Vesicles, Cancer Therapy, Immunotherapy, Tumor Calcification, Nanomedicine

1. Introduction

In the late 20th century, scientists found nanobacteria (NB), which were thought to be a new kind of ultramicrobacteria that could reproduce on their own even though they were very small [1,2]. These particles were linked to different calcific diseases, like kidney stones, atherosclerotic plaques, and psammoma bodies that are linked to tumors. This suggests that NB may be biological triggers of pathological mineralization [3]. But for decades, people have been arguing about who they are. Preliminary studies suggested characteristics akin to binary fission, the presence of DNA, and cytotoxic capabilities [4,5]. Subsequent analyses revealed that NB-like structures can form abiotically as calcium carbonate–protein complexes in cell-free systems [6,7]. This argument changed the name of NB to calcifying nanoparticles (CNPs) and shifted the focus from their supposed "life form" status to their physical, chemical, and pathological effects [8].

Outer membrane vesicles (OMVs) became more popular as a nanoscale biological system at the same time. These lipid vesicles made by Gram-negative bacteria are well-studied, easy to work with genetically, and biologically active carriers of biomolecules [9]. OMVs have rapidly transitioned from fundamental biology to therapeutic applications, particularly in oncology. Not so with NB.

Aim: This review methodically contrasts the NB/CNP identity discourse with the validated progress of OMVs as cancer therapies, to extract comprehensive insights for translational nanomedicine.

2. Nanobacteria and the Identity Debate

2.1 Evidence Supporting NB as "Living" or Bioactive Entities

Preliminary descriptive studies (Kajander et al.) discovered minute coccoid particles isolated from serum and tissue calcifications, which appeared to proliferate and create carbonate-apatite envelopes under prolonged culture conditions; energy-dispersive X-ray and FT-IR analyses confirmed the carbonate-apatite composition of the mineralized shell [1,3]. Some studies have shown that demineralized stones can send out antigenic signals and that

isolates can start the process of adding more minerals in a lab setting. This suggests that NB may act as a nucleus for crystallization in pathological calcification [2,11]. A substantial in vitro investigation contrasted NB and synthetic nanohydroxyapatite, demonstrating cytotoxicity and the induction of apoptosis in human breast cancer cells. This indicates that NB (or NB-like particles) may exert biologically significant effects on tumor cells and the tumor microenvironment [12]. These observations incited interest in NB as potential contributors to calcified tumor structures (e.g., psammoma bodies) and associated microenvironmental remodeling

2.2 Evidence Against NB as Life Forms (mineral / mineralo-protein complexes)

Conflicting evidence has surfaced suggesting that particles phenotypically indistinguishable from NB can be produced abiotically. Martel and Young (2008) demonstrated that uncomplicated calcium carbonate precipitates formed under specific in vitro conditions generate vesicle-like structures that are uniform in size and exhibit membranes resembling division, analogous to the structure and division-like characteristics of NB [5]. Various molecular investigations have failed to generate credible and reproducible nucleic acid signatures or comprehensive cellular biochemical markers characteristic of living organisms. The trace genetic signals identified in initial studies were subsequently determined to be the result of contamination [4,6]. Raoult et al. and other researchers have shown in experiments that fetuin-stabilized mineralo-protein complexes, which are called "nanons," can reproduce in laboratory media by repeatedly depositing minerals and adsorbing proteins. However, they do not show any signs of life, such as genomic replication machinery or ribosomal activity [6]. Reviews synthesizing the field concluded that CNPs/nanobacteria are most parsimoniously explained as mineralo-protein nanoparticles whose persistence in biological fluids and ability to nucleate apatite may nonetheless have pathophysiological consequences [4,13].

2.3 Relevance to Cancer

Even if NB/CNPs are abiotic, their consistent association with calcified deposits in tumors (psammoma bodies) and in vascular and renal calcification suggests biological relevance: mineral nucleation alters tissue stiffness, can impact cell death pathways, and may influence immune cell infiltration and tumor-stroma interactions [12,13]. The in vitro evidence indicating that NB-like particles induce apoptosis in breast cancer cells supports a mechanistic link between mineral deposition and tumor cell responses; however, the causal relationship in vivo and the clinical ramifications of these interactions remain inadequately defined. So, the NB controversy changes the question from "Are they alive?" to "How does biogenic mineralization (whether biologically produced or abiotic) affect tumor biology?"

3. OMVs as Established Tools in Cancer Nano therapy

3.1 OMVs for Combinational Therapy

Because their lipid bilayer surrounds the periplasmic content, OMVs are modular by nature. They can hold small molecules, nucleic acids, and photosensitizers. A convincing preclinical study used macrophage carriers to move OMVs that were wrapped in doxorubicin (DOX) and the photosensitizer chlorin e6 (Ce6) to triple-negative breast cancer (TNBC) tumors. The combination of chemo-photodynamic-immune stimulation resulted in total tumor regression in murine models, indicating that OMVs can synergize with various treatment modalities and overcome microenvironmental obstacles [7]. These multifunctional OMV platforms exhibit considerable translational potential: co-delivery within a single vesicle enhances local efficacy, reduces off-target exposure, and employs PAMP-driven immune activation to amplify antitumor responses.

3.2 OMVs as Immunomodulators

OMVs naturally stimulate the immune system by showing PAMPs (LPS, outer membrane proteins) that turn on innate immune pathways and antigen-presenting cells. Groundbreaking research showed that bacterial outer membrane vesicles (OMVs) given systemically tend to build up in tumors and cause immune responses that depend on interferon-γ (IFN-γ), which leads to the death of established tumors and long-lasting protection against re-challenge in mice [8]. Mechanistic analysis linked the accumulation of OMVs, the localized synthesis of chemokines (such as CXCL10) and IFN-γ, and the recruitment and activation of cytotoxic lymphocytes. This intrinsic adjuvanticity has been employed for in situ vaccination strategies and to enhance tumor sensitivity for checkpoint blockade, making OMVs attractive agents for converting immunologically "cold" tumors into "hot" ones.

3.3 OMV-Based Nanovaccines

OMVs can be engineered to display tumor antigens on their surface via genetic fusion or plug-and-display systems, or to encapsulate antigenic cargo, resulting in highly immunogenic nanovaccines. Recent research indicates that antigen-laden OMVs elicit robust $CD8^+$ T-cell responses and confer protection against tumors in preclinical models. They also have practical advantages, such as being made through bacterial fermentation for large-scale production and not needing separate adjuvants because they are already adjuvants [9,10]. The evidence collectively establishes OMVs as versatile vaccine platforms for tailored or accessible cancer vaccination strategies.

Advantages summary: OMVs combine **immunogenicity**, **biocompatibility/biodegradability**, **cargo versatility**, and **scalability via bacterial culture**, giving them translational traction unmatched by many synthetic nanoparticles.

4. Comparative Insights

Identity & Validation.

The difference between nanobacteria (NB)/calcifying nanoparticles (CNPs) and outer membrane vesicles (OMVs) shows two very different paths in nanoscale biology. Scientists still don't know much about NB/CNPs. Electron microscopy, mineral analyses, and apoptosis assays initially indicated a microbial involvement in calcification [1–4]; however, molecular evidence has yet to substantiate their status as living entities. The absence of reproducible DNA or RNA signatures, coupled with the ability to generate NB-like structures abiotically, confirmed their reclassification as mineralo-protein complexes [5–6]. In contrast, OMVs are clearly validated nanosystems: lipid bilayer vesicles that Gram-negative bacteria actively secrete, and whose proteomic, lipidomic, and nucleic acid cargo are consistently characterized [7–10]. Their molecular characterization, genetic manipulability, and consistent biogenesis provide a solid foundation for translational progress.

Translational Readiness.

Because OMVs have a clear structure, it is possible to use genetic or chemical tools to remove toxins from lipopolysaccharides (LPS), put targeting ligands on the surface, and add therapeutic payloads. Standardizing the way things are made can lead to vesicle populations that can be made again. But NB doesn't have any engineering frameworks that are the same. Due to the infeasibility of genetic or biochemical tractability, NB research is confined to observational and correlational studies. Moving things from the lab to the clinic has become harder, but OMVs are already moving into advanced preclinical pipelines as carriers, adjuvants, and vaccine scaffolds.

Scientific Lesson.

The NB story shows that claims of "new life forms" fell apart when they were closely examined and there was no molecular proof. OMVs are different because strict molecular characterization made them more quickly accepted and used in medicine. The main lesson for nanomedicine is clear: to make nanoscale observations into useful therapeutic technologies, they must be tested with omics, sequencing, proteomics, and reproducible assays.

5. Challenges and Future Directions

For NB/CNPs.

The primary challenge lies in determining their biological identity. To ascertain their composition definitively, we should employ contemporary techniques such as high-resolution cryo-electron microscopy, synchrotron X-ray diffraction, proteomics, and metabolomics. When used with strict contamination controls, sensitive nucleic acid tests like metagenomic sequencing and digital PCR may finally be able to tell if NB contains any genetic material. Even if they are proven to be abiotic, future research should not overlook them. The capacity to nucleate apatite may influence tumor rigidity, hypoxia, vascular calcification, and immune infiltration. We could use CNPs as biomarkers or targets for treatment if we can find out if they change how drugs get into cells, how likely they are to spread to other parts of the body, or how well they can avoid the immune system, even if they aren't living microbes.

For OMVs.

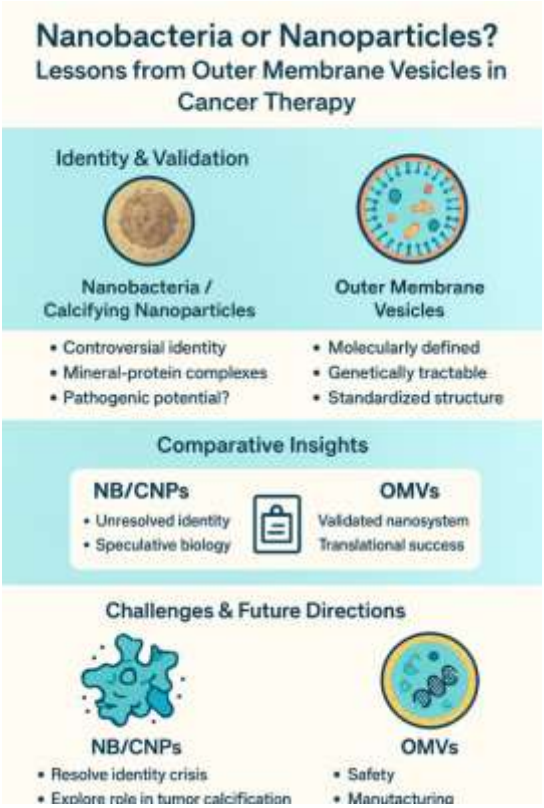
Despite their promise, OMVs face three major translational hurdles:

1. **Safety and Immunotoxicity.** LPS is present in native OMVs and can induce systemic inflammation. Detoxified bacterial strains, alterations in lipid A, and enzymatic degradation of endotoxin are all viable solutions. But it's still hard to find a balance between lowering toxicity and keeping immunogenicity.
2. **Manufacturing and Standardization.** When making GMP on a large scale, the size, composition, and cargo loading of vesicles must be the same from one batch to the next. Vesicle heterogeneity variability is a big problem for getting regulatory approval. It is very important to use advanced purification methods like tangential flow filtration and size-exclusion chromatography, as well as quality control methods like nanoparticle tracking analysis and proteomic fingerprinting.
3. **Regulatory and Clinical Pathways.** . OMVs are not quite biologics, vaccines, or nanomedicines. Regulatory frameworks for "living-derived nanotherapeutics" are not completely established, which makes it hard to move forward with clinical research. New ideas for the future.

Future Innovations.

- **Hybrid Therapeutics:** One intriguing possibility is merging insights from NB and OMVs. For example, OMVs could be engineered to carry biomolecules that regulate **pathological calcification** or to deliver factors that mimic NB-induced apoptosis, turning a controversial phenomenon into a controlled therapeutic.
- **Synthetic–Biological Platforms:** Combining OMVs with **synthetic nanoparticles, liposomes, or biomaterial scaffolds** could yield hybrid systems with tunable safety and efficacy.

- **Precision Engineering:** CRISPR-enabled bacterial strains and synthetic biology could allow for **tailor-made OMVs** with defined proteomic and immunologic profiles.



6. Conclusion

The NB/CNP debate underscores the imperative of rigorous molecular validation before ascribing biological agency to nanoscale entities; even when classified as abiotic, such particles may have considerable pathophysiological impacts. OMVs are a good example of how molecular clarity and engineering can help speed up translation. OMVs have gone from being interesting to promising cancer nanotherapeutics by defining their composition, adjusting their immunogenicity, and setting up manufacturing workflows. These strands show a way from scientific disagreement to useful nanomedicine, as long as scientists keep working on reproducible molecular characterization, safety engineering, and strong translational pipelines.

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