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Opinion

Checking the Biocompatibility of Plant-Derived Metallic Nanoparticles: Molecular Perspectives

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Understanding the biocompatibility of metallic nanoparticles (MNPs) is pivotal for biomedical applications. The biocompatibility of plant-derived MNPs has been mostly attributed to capped plant molecules. This claim seems to be straightforward but lacks conclusive evidence. The capped phytochemicals and the metallic core might have decisive and individual roles in imparting the overall biocompatibility. Whether capped phytochemicals really make sense in diminishing the toxicity effect of the otherwise naked or metallic core needs further analysis. Here, we readdress the biocompatibility of plant-derived MNPs with references to contemporary cellular assays, different reactants for green synthesis, possible epigenetic involvement, and nanobiocompatibility at the molecular level. Finally, we discuss relevant *in vivo* studies and large-scale production issues.

Another Look at the Biocompatibility Issue of Plant-Derived Metallic Nanoparticles

The recent progress made in the plant-mediated **green synthesis** (**GS**, see Glossary) of different **metallic nanoparticles (MNPs)** is remarkable and this emerging green nanotechnology can now be considered to be a generalized technology platform for the synthesis of MNPs. However, the biomedical application of plant-derived MNPs has not yet been validated. Diligent testing and strict regulation will be required before introducing them into medical therapies. The first step in understanding the safe application of MNPs is to perform simple cytotoxicity tests involving cell culture studies (*in vitro*), particularly with mammalian cell lines. In the current GS methodology, the **biocompatibility** of MNPs is checked by following the well-authenticated standard protocols for quantitative and qualitative cellular assays. For quantitative evaluation, the **3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT)**, **2, 3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT)**, and **lactate dehydrogenase (LDH)** leakage assays are the most common dye-conversion assays performed in most GS-based cytotoxicity studies. For qualitative estimation, live and/or dead cell staining assays, such as the ethidium bromide and acridine orange double-staining cellular assays, are mostly used [1–5]. The action mechanisms of these quantitative (MTT, XTT, and LDH) and qualitative (ethidium bromide and acridine orange) dye-conversion assays have been well explored [6–8].

These cellular assays are used to measure the viability of cells after treatment with MNPs. However, these contemporary tools for nanotoxicological experimentation can be considered valid only for general cytotoxicity measurements. Given that live and dead are the two extreme cellular fates of MNP-treated cells, the effects of MNPs on cell organelles must be evaluated by

Trends

Recent methods for checking the compatibility of plant-derived MNPs mostly rely on live or dead cells, but damage in organelles is not analyzed.

Recent studies showed that capped phytochemicals on the NP surface can modulate cellular activities, which opens new avenues for further research on the epigenetic involvement of capped phytochemicals.

Dietary phytochemicals have been implicated in protecting against and preventing diseases, such as cancer. Capped phytochemicals can deliver these plant molecules for targeted efficacious application.

The metal homeostasis and biocompatibility of mammalian cells are influenced by the type of metal (essential or nonessential) involved. The core metal part of plant-derived MNPs is also implicated in cellular events, but its involvement warrants further studies.

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carrying out more than one cellular assay. Cytotoxicity (live or dead) and organelle damage must be tested with distinct cellular assays. In reality, this complexity is not covered by most of the biocompatibility studies of plant-derived MNPs. The results of the cytotoxicity assay (i.e., the viability) can vary according to the method adopted [8]. Cellular assays that are purposely used for general screening of the toxic agent should not lead to over- or underestimation of the MNP toxicity. To explain this result, the MTT assay, LDH leakage assay, protein assay, and neutral red assay could be considered for predicting the toxicity of MNPs. The MTT assay is a direct indication of mitochondrial activity, while the LDH leakage assay pinpoints damage to the cell membrane. The protein assay measures the protein content of viable cells, and the neutral red assay measures the lysosome activity [9,10].

It is possible that cells exposed to plant-derived MNPs suffer intracellular effects before incurring permanent cell membrane damage. Moreover, cell death (apoptosis or necrosis) is a function of both the exposure time and the MNP concentration. Mitochondrial toxicants can show early signs of inhibition of mitochondrial respiration, leading to oxygen-related cell death (e.g., resulting from reactive oxygen species). Similarly, a change in staining by propidium iodide is an indication of early nuclear damage [9]. It is obvious that the type of MNPs [e.g., gold (Au)NPs, silver (Ag)NPs, platinum (Pt)NPs, etc.], cell line selected, dose (time × concentration), and technical features (size, shape, and surface properties) of MNPs have decisive roles in their overall biocompatibility. These issues have been reviewed previously [11–18]. However, biocompatibility tests of plant-derived MNPs require in-depth analyses of different aspects of the biocompatibility issue. Specifically, the biocompatibility of plant-derived MNPs is a complex phenomenon and the overall outcome (i.e., the viability percentage) might not directly measure the MNP toxicity. Here, we navigate readers through the fine details of the biocompatibility assessments of plant-derived MNPs and discuss molecular events, the MNP synthesis effects, the probable role of phytochemicals, epigenetic concerns, and nanobiocompatibility of these compounds.

There Are Plenty of Entities to be Checked

In the contemporary methodology, plant-derived MNPs are prepared for biocompatibility tests following the sequence of curing, sterilization, and dispersion of MNPs in serum-free cell culture media (or phosphate-buffered saline) [19–25]. Thus, given the bottom-up approach of MNP synthesis with plant-derived materials (such as extracts or powder), the overall biocompatibility still needs to be checked for other individual entities. A simple and comprehensive depiction of this concept is presented in Figure 1A. The synthesis of MNPs with plant-derived reducing agents (phytochemicals) goes through the usual steps (formation of embryos, nucleation, primary NPs, and NPs) of the bottom-up approach [26]. In Figure 1A, the **surface plasmon resonance (SPR)** property of MNPs definitely shows a color change in the reaction mixture and indicates the synthesis of MNPs.

However, because the atomic economy is controlled by the reduction efficiency of the plant molecules, which, in turn, is source (plant or plant part) specific, the possibility of unreduced metallic ions (xMe^{n+}) being present in the reaction mixture is high. Moreover, not all zero valent metallic ions (xMe^{o}) may participate in the NP formation process [$x(Me^{o})_p$]. In this regard, separating the three moieties [xMe^{n+}, xMe^{o} and $x(Me^{o})_p$] from the reaction mixture and subjecting them to individual cytotoxicity tests will generate important baseline information on the metal toxicity at atomic and nano dimensions. Curing of $x(Me^{o})_p$ by high-speed centrifugation will leave the other two moieties in the supernatant. Given that cytotoxicity is often discussed in terms of the MNP size, shape, and surface properties, the present approach can further explain some of the unexplored or overlooked issues for a better cytotoxicity prediction.

Cytotoxicity to $x(Me^{o})_p$ also depends on many factors, such as: (i) the number of surface atoms; (ii) the single or agglomerated form; and (iii) the uncapped, partially capped, or fully capped

Glossary

2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) assay: a colorimetric assay used to estimate cell viability.
3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay: a colorimetric assay used to estimate cell viability.
Biocompatibility: the compatibility of a material with a living system without adverse effects.
Catalase (CAT): an enzyme found in all aerobic organisms. This enzyme protects cells from reacting oxygen species.
DNA methylation: the process by which DNA is methylated.
Dopamine: a neurotransmitter; its deficiency causes Parkinson's disease.
Epigenetic: functionally relevant changes in the genome without involving changes in the nucleotide sequence of DNA.
Fourier transform infrared (FTIR): a spectrophotometric technique used to collect the absorption or emission spectrum in the infrared region.
Glutathione peroxidase (GPx): a cellular enzyme family involve in peroxidation activity.
Glutathione (GSH): an endogenous antioxidant produced by cells.
Green synthesis (GS): a common term used to refer to the synthesis of MNPs by using plant- or animal- or microorganism-derived biomaterials as reducing and/or capping agents.
Integrated microelectrode array (IMA): a modern methodology that uses multiple electrodes for intracellular and extracellular recordings at cell electronic circuitry interface under *in vitro* or *in vivo* conditions.
Lactate dehydrogenase (LDH) assay: a colorimetric assay used for the estimation of the activity of the cytosolic enzyme LDH. Upon cell death due to damage in the cell membrane, LDH is released to culture medium and, thus, indicates the number of lysed cells.
Malondialdehyde: an aldehyde product formed during lipid peroxidation and the marker of the oxidative stress of cells.
Metal homeostasis: the maintenance of the optimal level of essential metals in cells.
Metallic nanoparticles (MNPs): a common term that refers to NPs,