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Investigating the Role of Gold Nanoparticle Shape and Size in Their Toxicities to Fungi with a Novel Synthesis Method

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With the flourishing development of nanotechnology, abundant amount of nanomaterials have been manufactured and applied in all sorts of areas in everyday life. Among which, gold nanoparticles (GNPs) possesses a certain proportion and very important status due to their fascinating properties like quantum size effects and wide applications in the fields of surface enhanced Raman scattering (SERS), chemical and biological sensing, biomedicine and so on. With such high usage in daily and industrial life, the release of GNPs into the environment is increasing in great quantities. Thus, attentions have been drawn to the effects of GNPs to the environment, especially the effects on living organisms, and ultimately the effects on human bodies and health. To achieve the such goal, the toxicity of GNPs to organisms that strongly interact with their direct environment, such as fungi and plants, is of critical importance.

Fungi, as one of the most common microbes in the environment, have vast varieties and are widely- spread. As the dominant decomposers within the ecosystem, fungi are found almost everywhere in the biosphere, especially in terrestrial, marine, and freshwater environments where the moist and nutrient promotes their growth. As decomposer, fungi recycle the energy and nutrients from dead and dying living organisms, and ensure the circulation of energy and nutrients within biosphere. With large surface of mycelia, fungi are particularly adept at bioaccumulation of contaminating heavy metals during their extraction, concentration and recycling nutrients and minerals from dead organic matter back into biosphere as decomposers. Such organisms that accumulate toxicants in the ecosystem are typically more likely to be effected at a relatively low environmental concentration. Thus, the sensitivity of common fungi to GNPs is of great importance for study, as the influence on fungi may negatively impact on the capacity to recycle materials in thebiosphere.

Unfortunately, the characteristics of the toxicology of GNPs on living organisms are still not fully understood up till now. Notably, it has been reported that the toxicity of GNPs on microbes depends strongly on the species of microbe and the physicochemical properties of the GNP, and it has also been reported that different shapes of GNPs, such as spheres, rods, triangles, hexagons, prisms, and so on, have different cellular uptake mechanisms and elicit different toxic responses. It has also been reported that the size of GNPs significantly affects the excretion ability of human body, and thus leads to a change in GNP toxicology. However, the mechanism of GNPs' toxicity still remained unclear and there are still reports that show conflict results of both toxic and non-toxic results, which could be caused by different cell viability assay, or the slight variations on the GNP properties. Thus, it is of great importance to investigate the GNP toxicity with GNPs synthesised with unified method, with no additional variables influencing theresults.

In our study, a novel bottom-up in situ synthesis method of shape and size controllable GNPs was developed. The method is based on the commonly used HEPES-reduced GNP synthesis method at the molar ratio of chloroauric acid: HEPES = 1:10, with the addition of different amount of monosodium phosphate or disodium phosphate. The shape and size of GNPs synthesised can be controlled by adjusting the kind and amount of phosphate added into the system. With monosodium phosphate added, mixtures of gold nanospheres and nanoplates sizing from ~70 nm to ~2.5 nm were synthesised by increasing the molar ratio of NaH2PO4: HEPES from 0 to 6, with higher NaH_2PO4: HEPES leading to smaller particle size and smaller size difference between nanospheres and nanoplates. By adding disodium phosphate into the synthesis system, gold nanoflowers, ranging in size from ~60 nm down to less than 1 nm could be synthesised by increasing the molar ratio of Na_2HPO4: HEPES from 0 to 40, with the amount of petals on each particle decreased as the Na_2HPO4: HEPES molar ratio increased. When the molar ratio of NaH_2PO4: HEPES was higher than 9, and the molar ratio of Na_2HPO4: HEPES higher than 50, the formation process of GNPs was completed in rather short times, followed almostinstantly by the aggregation of GNPs. Since such GNP synthesis method developed could change the shape and size of GNPs systematically with same chemicals used, the GNPs synthesised with this method could be applied in the study of GNP toxicity without introducing additional variations.

In this study, three kinds of common fungi species, which are widespread in the environment, have been chosen for toxicity assessment, namely *Aspergillus niger, Mucor hiemalis*, and *Penicillium chrysogenum*, Two kinds of GNP shape (spherical and star/flower-like shaped) with different sizes were examined. The sizes of GNPs used varied in three orders of magnitude, which were ~1-5 nm, ~60-90 nm and over 400 nm. Fungi were incubated after mixing with series concentrations of different GNP samples, of which the pH values were all adjusted to 7 in advance. The selected fungi species were exposed to the GNPs with designed size and shape, and incubated for 48 hours before survival rates were examined. Then the survival rates of fungi to GNP with different size and shape were compared. It was found that, while HEPES and phosphate promoted the growth of all 3 species of fungi to different extents, the GNPs decreased the survival rates and exhibited toxicities. Fungi species was the major influencing factor in the variation of GNP toxicities, due to the differences in accumulation capacity. To be specific, *A. niger* was the most sensitive, followed by *P. chrysogenum*, while *M. hiemalis* was only slightlyaffected.

Larger GNPs and gold nanoflowers appeared more toxic to fungi because they were able to carry more HEPES free radicals into fungi cells, causing DNA damage and mutations