Optimization of Nanoparticle Delivery to Plants: Do Nanoparticle Properties Affect Cellular Internalization?

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Abstract:

To minimize damage to the ecosystem and enhance sustainability in farming practices, intervention of nanotechnology in agriculture has increased in recent years. The purpose of this work was to study the effects of various gold nanostructures of 30 to 80 nm delivered by foliar application on uptake, translocation, and accumulation in a watermelon plant. Cellular uptake and translocation of gold nanoparticles from leaf to root was confirmed by ICP-MS. Accumulation and transport of nanoparticles depends on nanoparticle shape and application method.

Introduction:

Gold nanoparticles (AuNPs) have been extensively studied in recent years due to the abundance of their potential applications to biological systems; being biologically inert, AuNPs are less toxic to cells than nanoparticles of other materials [1]. Because they also adsorb DNA easily, AuNPs can be used in agricultural biotechnology for delivery of genetic material to plant cells [2] and detection of plant viruses [3]. Researchers have asked the questions: How do we measure the ecotoxicity of these emerging technologies, and how do we make these technologies safe and sustainable? To answer these questions, it is essential to understand the fundamental mechanisms of morphology-dependent cellular uptake of gold nanoparticles and their subsequent fate in a plant system.

Experimental Procedure:

Gold Nanoparticle Synthesis and Characterization. AuNPs of spherical, cubic, rhombic dodecahedral (RD), and rod morphologies were chemically synthesized using seed-mediated methods [4,5] and characterized for physical diameter, hydrodynamic size and zeta potential using transmission electron microscopy (TEM) and dynamic light scattering respectively, and surface plasmon resonance determined with UV-visible spectrophotometry. Finally, concentrations of nanoparticles in suspension were analyzed using inductively coupled plasma-mass spectroscopy (ICP-MS).

Application of Nanoparticles to Plant. Foliar aerosol delivery of nanoparticles (NPs) may help to reduce environmental harm by increasing uptake by plants and limiting contamination of the soil environment [6]. Consequently, we employed a foliar application of AuNPs that delivered them through the stomatal openings, avoiding direct contact with the soil ecosystem. The phloem subsequently transports NPs from shoot to root [7]. Watermelon plants (*Citrullus lanatus*) were chosen for this experiment due to their large stomata and vessel size, which may facilitate NP translocation [6]. In this study, drop-cast and aerosol based foliar applications were compared to determine the effect of applied droplet size on AuNPs-plant interactions. The drop-cast method uses an auto pipette to render 800 ± 175 µm droplets that may contain soft agglomerations of nanoparticles. An aerosol method, the atomizer, breaks up agglomerations, producing tiny droplets in the mean size range of 250 ± 50 nm. Real-time applied particle size was monitored using scanning mobility particle size (SMPS) measurement.

Nanoparticle Uptake Analysis. Plants were harvested 48 hours after applying the nanoparticles and washed to remove soil. Roots, stems, and leaves of each plant were separated, dried, and digested. The resulting concentration of elemental gold in each plant section was analyzed with the aid of ICP-MS.

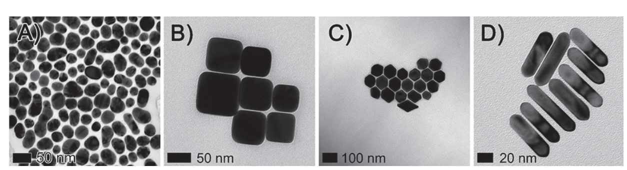

Figure 1: TEM images of gold (A) spheres, (B) truncated cubes, (C) rhombic dodecahedra, and (D) rods.

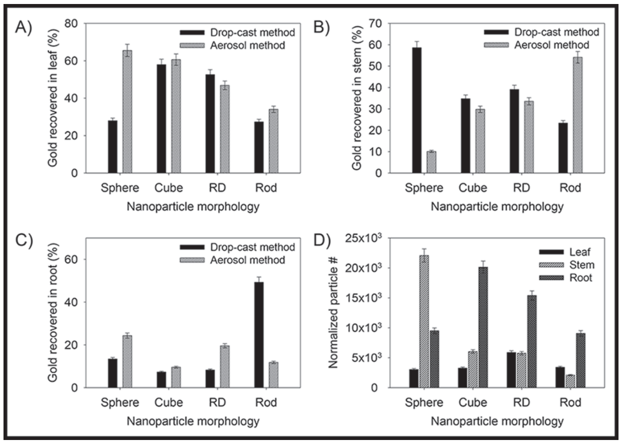

Figure 2: Percent of total gold in plant that was recovered by ICP-MS in (A) root, (B) shoot, and (C) root sections of treated plants, comparing results of drop-cast and aerosol methods. (D) Number of particles recovered in sections of aerosol-treated plants, normalized to number concentration of particles.

	Particle Size[a] (nm)	Hydrodynamic Size[c] (nm)	PDI [c,d]	λ_{max}[e] (nm)	Zeta Potential[c] (mV)	Concentration, Au (ppm)
Sphere	35	61.0±31.6	0.457	527	-40.5	2.29×10^3
Cube	70	99.2±25.1	0.034	563	56.7	1.12×10^4
Rhombic Dodecahedra	65	99.8±30.4	0.066	550	64.3	1.18×10^4
Rod	20, 60[b]	114.1±52.1	0.296	816	43.9	1.44×10^3

[a] Mean geometric diameter, obtained from TEM images (Fig. 1)
[b] Diameter, length
[c] From DLS, intensity particle size distribution
[d] Polydispersity index
[e] Surface Plasmon Resonance, obtained from maximum UV-vis peak

Table 1: Characterization data for nanoparticles in aqueous suspension.

	Method	Amount in leaf (%)	Amount in stem (%)	Amount in root (%)	Percent difference, drop-cast vs aerosol
Sphere	Drop-cast	28.0	58.6	13.4	10.9
	Aerosol	65.5	10.1	24.4	
Cube	Drop-cast	57.9	34.8	7.3	2.3
	Aerosol	60.6	29.8	9.6	
Rhombic	Drop-cast	52.6	39.1	8.3	11.3
Dodecahedra	Aerosol	46.8	33.6	19.6	
Rod	Drop-cast	27.4	23.4	49.3	37.4
	Aerosol	34.0	54.1	11.8	

Table 2: Results of elemental analysis of gold in plant sections by ICP-MS.

Results and Conclusions:

All characterization data is depicted in Table 1. Images confirming nanoparticle geometric diameters and morphologies were obtained with TEM (Figure 1). Uptake and distribution of AuNPs throughout the plant system was confirmed by ICP-MS. The measured concentrations of elemental gold in each sample were normalized by the dried mass of the plant section, and this data was processed to determine the percent of recovered gold in each plant section (Figure 2).

Comparing the two application methods, the aerosol method enhanced the transport of low aspect ratio NPs (Sphere, Cube, RD) while the larger droplet size applied by the drop-cast method improved the transport of high aspect ratio, rod-shaped NPs. For sphere, cube, and RD morphologies respectively, the increase in translocation rate of the aerosolized versus drop-cast nanoparticles was 10.9, 2.3, and 11.3%, whereas a 37.4% decrease in translocation rate was observed for the nanorods (Table 2).

Among drop-cast NPs, a trend in the efficacy of translocation (percent of recovered gold accumulated in roots) was observed as: rod (49.3%) > sphere (13.4%) > RD (8.3%) > cube (7.3%) (Table 2). In order to account for any discrepancies in the number of particles applied by the aerosol method, the aerosol results were normalized by the acquired concentration of gold obtained by our SMPS number concentration measurements.

From these normalized results (Figure 2D), a trend in the efficacy of translocation (number of particles recovered in root) was observed as: cube (2.0×10^4) > RD (1.5×10^4) > sphere (9.5×10^3) > rod (9.0×10^3), a trend that again suggests the aerosol application method results in improved translocation of low aspect ratio particles. Further experiments are needed to confirm these results, as well as to study the effects of other nanoparticle properties on their uptake and fate.

Importance of the Study:

From the morphology-dependent trends in nanoparticle translocation, it can be concluded that accumulation and transport of nanoparticles depends on nanoparticle shape. Our evidence also suggests that different application methods may be optimal for delivery of different morphologies of nanoparticles to plants.

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