

SUPPLEMENTARY MATERIAL

Supplemental Methods

High resolution histopathology: Two animals dosed with 0.5 mg/kg of each type of AgNP and the respective controls had the left lobe fixed in Karnovsky's fixative to preserve cellular ultrastructure and allow examination of high resolution histopathology for intracellular localization of silver and evidence of cytotoxicity. Left lobes were fixed with Karnovsky's fixative (0.9% glutaraldehyde/0.7% paraformaldehyde in cacodylate buffer, adjusted to pH 7.4, 330 mOsmol/kgH₂O) for 1 h at 30 cm of pressure. Fixed lungs were sliced and embedded in Araldite 502 resin. Blocks were sectioned at 1 µm. Sections were etched with 10% hydrogen peroxide for 10 minutes and stained for silver as described above except that incubation was for 30minutes. Sections were counterstained with methylene blue azure II stain.

Silver Nitrate Treatment: Animals were dosed with either 0.5 mM or 0.05 mM silver nitrate (AgNO₃) solution at 1 ml/kg body weight and necropsied 1 day after treatment. Left lung lobes were fixed, sectioned and stained for silver as described in the methods section. There were two animals per dose. The 0.5 mM dose represents a dose, at 1 ml/kg equal to 0.5 mg/kg AgNPs dose that completely dissolved. The 0.05 mM dose is based on a ~5-6% dissolution of the AgNPs.

Supplemental Results

Animals: Rats averaged 360 g at time of treatment and at one day timepoint, ranging from 330 g to 400g. At 7 days post treatment animals weighed 410 g to 445 g (mean 425 g) for vehicle control treated and 390 g to 430 g (mean 415 g) for AgNPs treated. Finally, at 21 days post exposure animals weighed from 395 g to 470 g (mean 426 g) in the control groups and 390 g to 460 g (mean 421 g) in the AgNPs treated groups.

Silver Nitrate Treatment: Silver nitrate solution, 24 hours after treatment, produced a spotty and diffuse pattern of staining in lung tissue (Figure S6). The high (0.5 mM) dose (Figure S6A) produced a greater number of spots than the low (0.05mM) dose (Figure S6B).

Supplemental Table 1. Scoring system used to determine silver staining in terminal bronchioles.

Score	Silver
0	No silver staining present in tissue
1	Silver stained macrophages present. No tissue staining of terminal bronchioles.
2	Mild tissue staining of terminal bronchioles and stained macrophages.
3	Moderate staining of the terminal bronchiole and staining of the surrounding parenchyma.
4	High saturation of the terminal bronchiole regions. High presence of silver in the surrounding parenchyma. Silver saturated macrophages.

Supplemental Figures

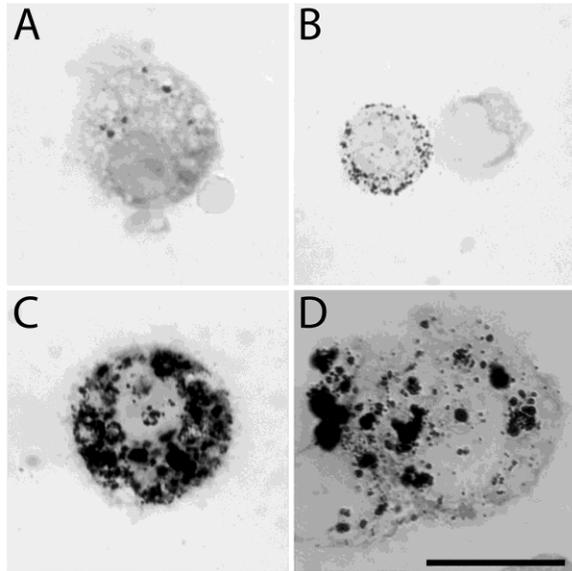


Figure S1. Autometallography (dark staining) of silver in lung macrophages recovered from BALF demonstrates the variety of profiles and amount of silver incorporated in macrophages at 1d after exposure. (A) Macrophage with little silver positive staining, (B) macrophage with moderate staining in the cytoplasm, (C) macrophage with intense staining and (D) macrophage with intense staining and foamy appearance. Bar = 20 μ m.

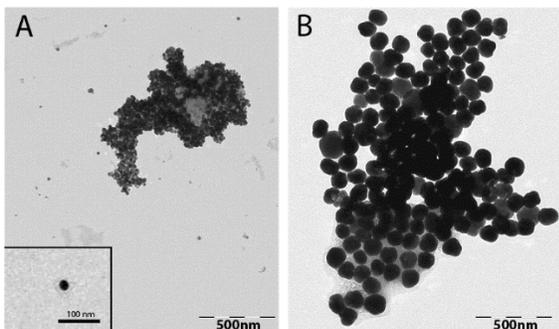


Figure S2. Transmission electron micrographs of (A) 20nm AgNP and (B) 110nm AgNP. PVP particles shown.

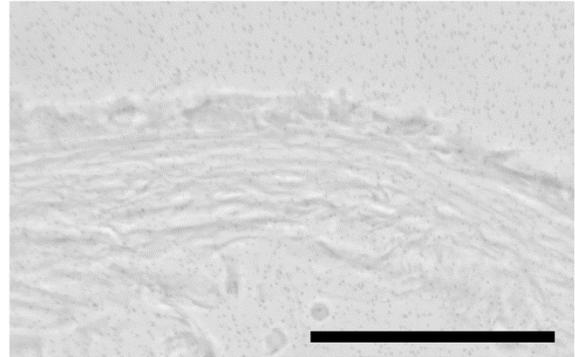


Figure S3. Autometallography of a representative proximal airway showing a lack of staining at 7 day post exposure. Bar = 50 μ m.

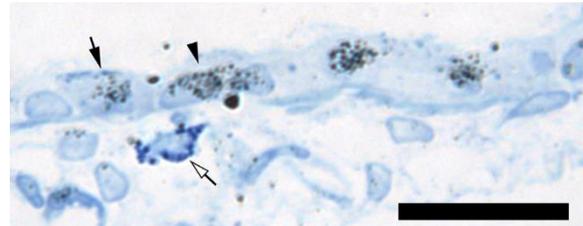


Figure S4. Autometallography (dark staining) of silver internalized by distal airway cells at 7 day post exposure to 0.5 mg/kg 110 nm citrate AgNP. Silver staining was present in ciliated cells (arrow) and Clara cells (arrowhead). Macrophage (open arrow). Bar = 20 μ m.

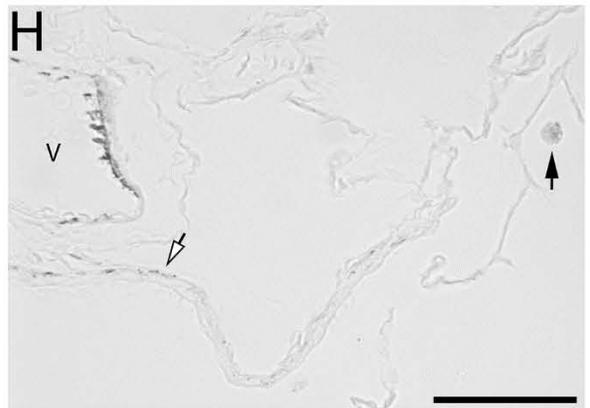
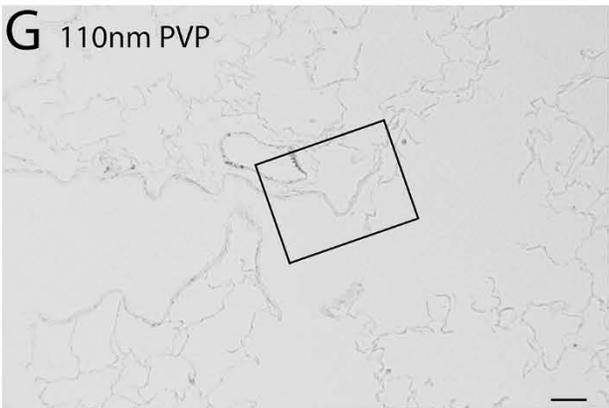
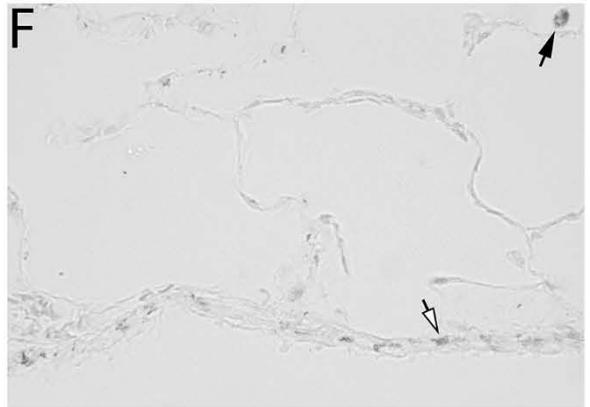
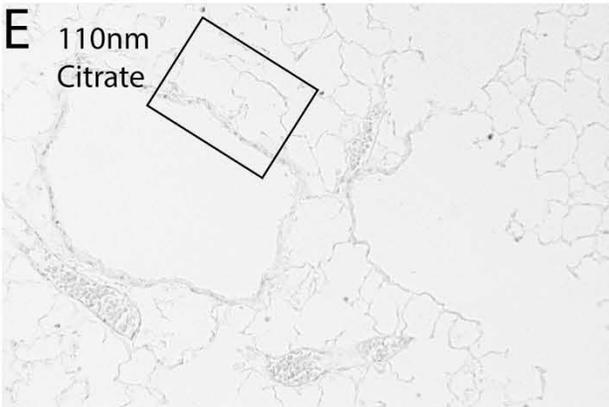
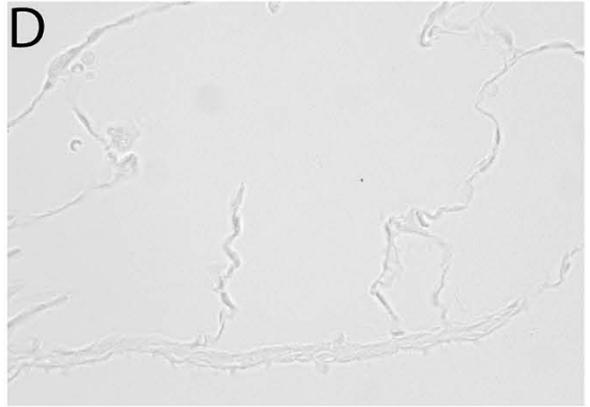
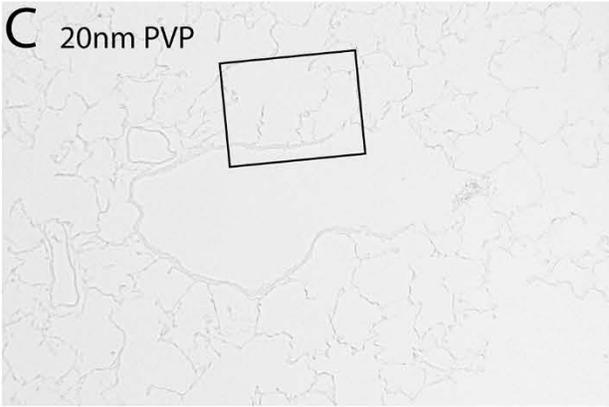
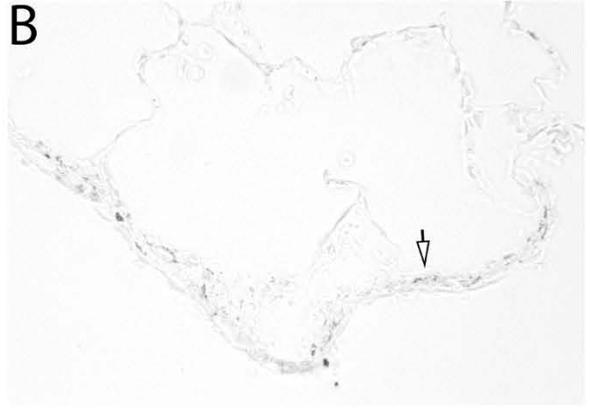
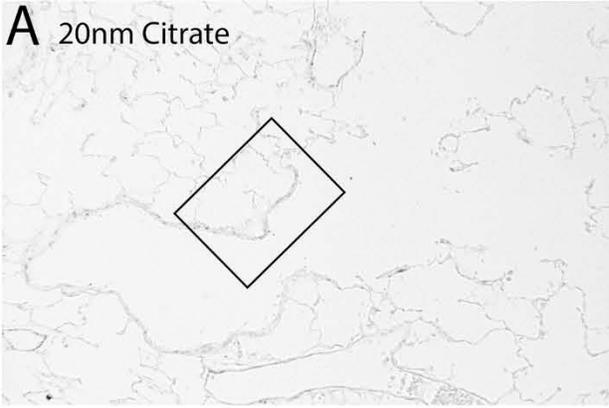


Figure S5. Autometallography (dark staining) of silver localization to the terminal bronchiole alveolar duct junction at 21 days post exposure to 1.0 mg/kg AgNPs. (A, B) 20nm citrate, (C, D) 110nm citrate, (E, F) 20nm PVP and (G, H) 110nm PVP. At 21 days post exposure, silver was still detectable in focal areas of the terminal bronchiole/alveolar duct region (open arrows) and associated macrophages (arrows) as well as some vessels (V) but was greatly diminished in all groups compared to the pattern at 1 and 7 days post exposure. Bars = 50 μ m.

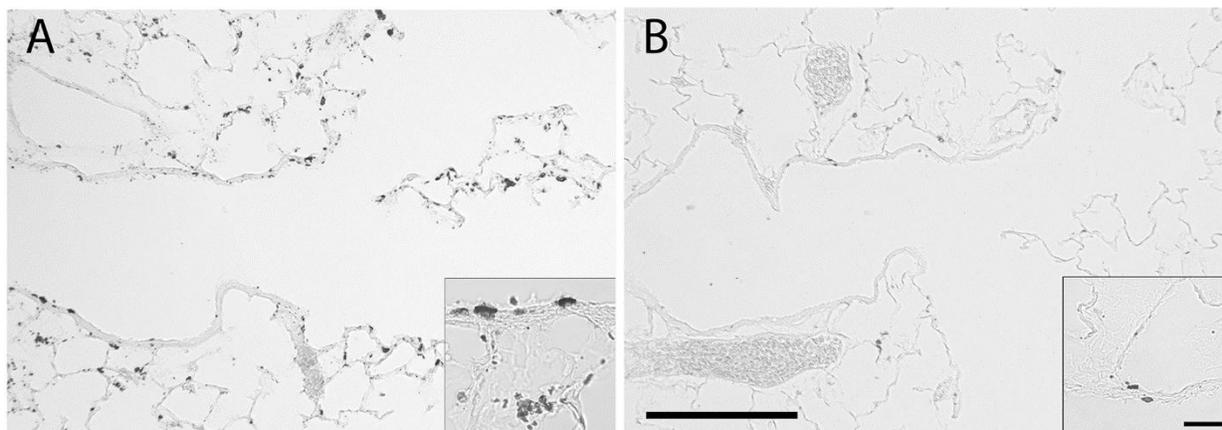


Figure S6. Autometallography (dark staining) of silver localization to the terminal bronchiole alveolar duct junction at 1 day post exposure to either (A) 0.5 mM or (B) 0.05 mM AgNO₃ solution. (Low mag bar =50 μ m, High mag bar = 20 μ m)