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In vivo formation of Ce-phosphate Nanoparticles following Intratracheal Instillation of $CeCl_3$: Subcellular sites, Nanostructures, Precipitation Mechanisms and Nanoparticle 3D-Alignment

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We demonstrate the *in vivo* formation of nano-particulate Ce-containing structures in lungs instilled with 5 mg/kg $CeCl_3$ using high resolution electron microscopy (HRTEM), energy loss spectroscopy (EELS), and elemental mapping (EDS). The observed high lung retention of Ce after instillation of $CeCl_3$ (75-92% retained at 28 days) is unexpected since metal ions are usually readily transported across the air-blood barrier. The binding of cerium ions to lung constituents has been suggested [1], and the formation of cerium phosphate has been shown previously, but without discussions on the mechanisms involved in nanoparticle nucleation and growth [2]. Determining the form of Ce after lung exposure to $CeCl_3$ has been challenging due to the difficulties in distinguishing ions from particulate forms when using radioactivity or ICP-MS. We have identified cerium nanoparticles at the sub-cellular level in lung macrophages after $CeCl_3$ instillation. This observation provides insights on the cell structures and components that will help distinguish which cellular areas are the sites of *in vivo* nanoparticle formation.

Lung tissue samples were fixed by vascular perfusion with 2.5% glutaraldehyde, 2% paraformaldehyde in 0.1M HEPES buffer. The samples were processed for electron microscopy at 2 hours, 7 days and 4 weeks post-instillation. High-resolution imaging revealed that nanoparticles had formed at all of the selected time-points and were located in lysosomal regions, along membrane surfaces and inside mitochondria. The nanoparticles formed in a narrow particle size range (~1-5 nm) and were bundled together in 3D-agglomerates up to several hundred nm (Fig. 1a). Individual nanoparticles appear self-aligned into rod-shaped structures or needles that reach up to 10 nm in length and 2-5 nm in width, with some structures arranged in a spider-like form (Fig. 1b). The typical crystalline structures of individual ultra-fine nanoparticles are shown in Fig. 1c. EELS analyses demonstrate co-localization of Ce and P for individual nanocrystals (Fig. 2). The *in vivo* nucleation and growth of cerium phosphate nanoparticles in the observed large agglomerates are demonstrated by the EDS elemental maps obtained in the STEM mode (Fig. 2). EDS maps and corresponding spectra indicate that cerium ions formed cerium phosphate nanoparticles. Furthermore, the elemental map also reveals the presence of abundant Fe, which may correspond to the ultrafine ferritin nanoparticles (~5nm) that formed in the vicinity of the cerium phosphate nanoparticles (Fig. 2). Inflammatory responses have been previously shown to activate abundant ferritin formation in localized regions with invader nanoparticles [3]. The current study shows for the first time that the same process occurs after *in vivo* delivery of ions, followed by their transport, and precipitation.

References:

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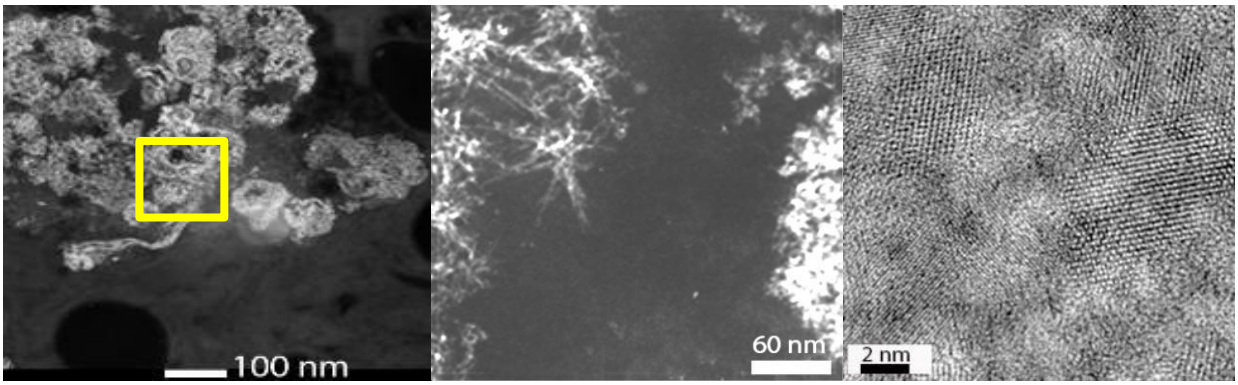
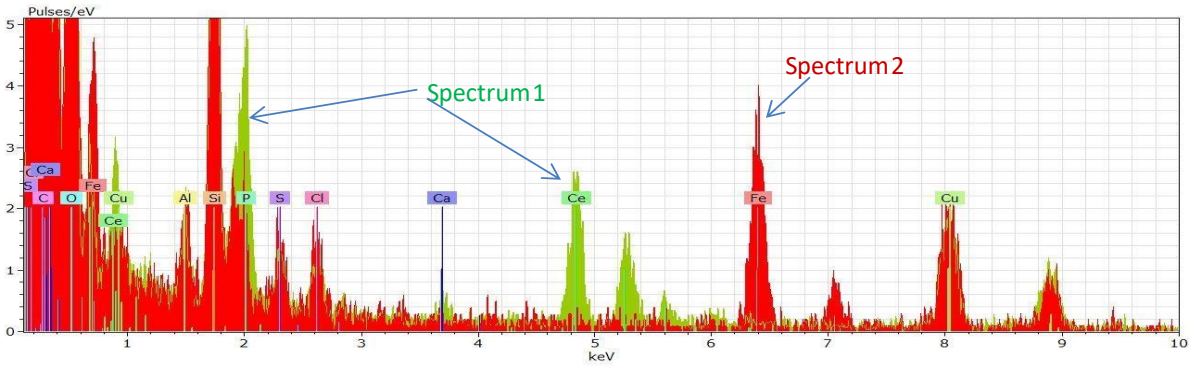


Figure 1. STEM images of (a) *in vivo* formed nanoparticle agglomerates in lung; (b) self-aligned nano needles from insert; (c) TEM image showing crystalline structures of nanoparticles.



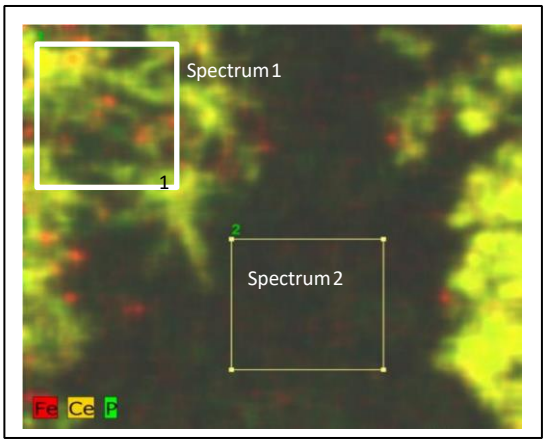


Figure 2. (Upper) EDS spectra of selected areas show distinct elemental content in location 1 (in green) and location 2 (in red); (lower) elemental maps showing distribution of elements Ce (yellow) and P (green) which correspond to cerium phosphate nanoneedles and Fe (red) which marks the location of ferritin nanoparticles.