Supporting Information

Characterization of Engineered Cerium Oxide Nanoparticles and Their Effects on Lung and Macrophage Cells

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Dynamic light scattering (DLS)

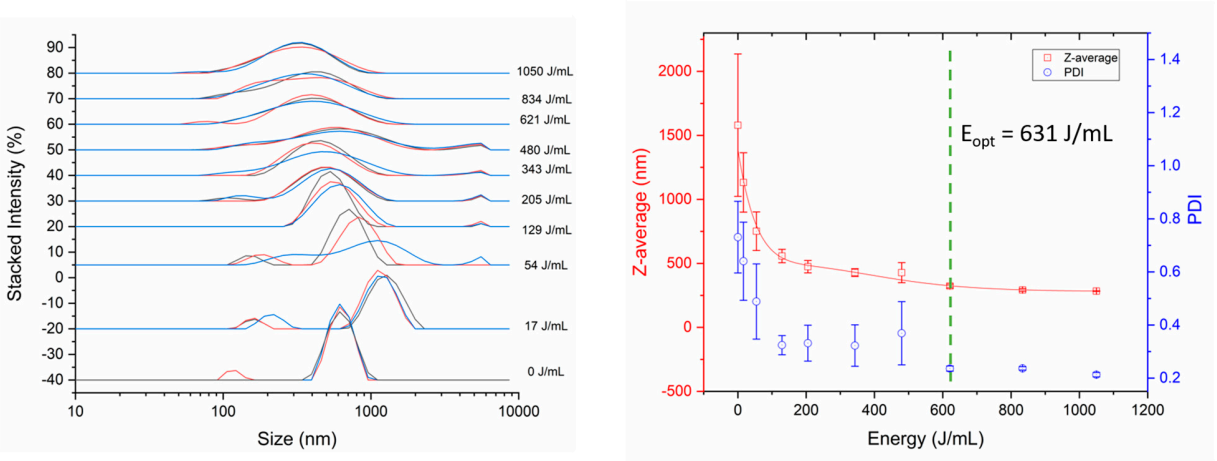

Figure S1. Ce-03 sonication optimization results. (Left) Stacked DLS spectra for a Ce-03 sample sonicated at the specified energy. Each DLS experiment comprised of three trials, shown in black, red and blue. Note that the logarithmic x-axis can make visual comparisons between the sonication energies challenging. (Right) The corresponding Z-average (red boxes) and polydispersity index (blue circles, PDI) values for each sonication energy. The determined optimal sonication energy (E_{opt}) is marked with a green dashed line and the red curve is a visual aid representing an exponential fit to the hydrodynamic diameter data.

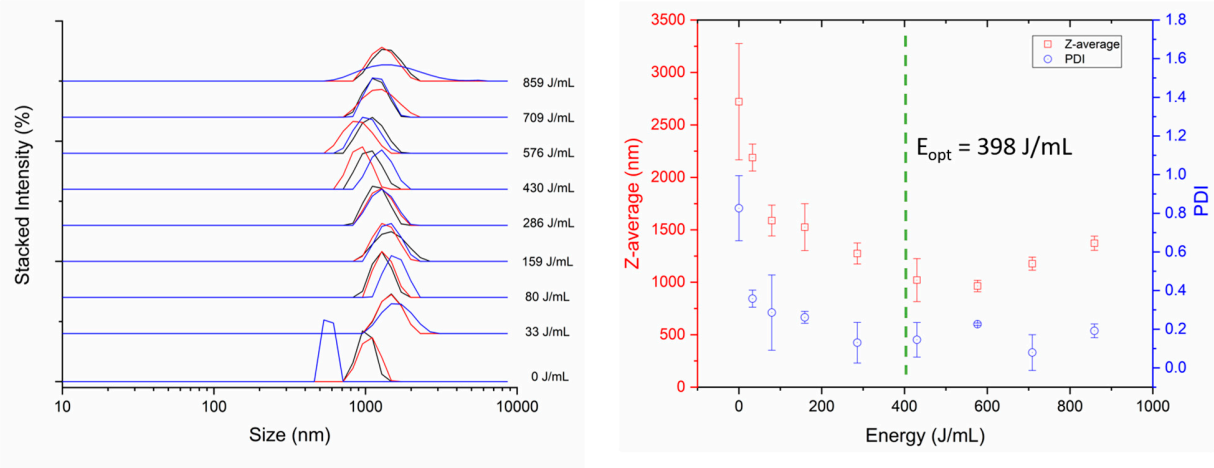

Figure S2. Ce-05 sonication optimization results; note that this sample was dispersed in ethanol as it was coated in stearic acid. (Left) Stacked DLS spectra for a Ce-05 sample sonicated at the specified energy. Each DLS experiment comprised of three trials, denoted as black, red and blue. Note the large size at every sonication energy. (Right) The corresponding Z-average (red boxes) and polydispersity index (blue circles, PDI) values for each sonication energy. The determined optimal energy (E_{opt}) is marked with a green dashed line.

Zeta potential

The zeta potential of a nanoparticle solution represents a measurement of the surface charge of the particles in solution. Ideally, a large surface charge promotes electrostatic repulsion between particles, which should reduce aggregation and agglomeration in the dispersion. The surface charge of a material can be adjusted by changing the pH, as protonation or deprotonation will impact its surface charge. For this work, well-dispersed solutions that were biologically compatible were preferred. This will allow the same dispersion protocol to be applied for the physical characterization and biological studies. Zeta potential was measured using a Zetasizer Nano ZS (red) (Malvern) for samples dispersed in deionized water. The solutions were then diluted to 0.01 w% (approximately 100 µg/mL) in water and placed in a bent capillary DLS cuvette (DTS1061, Malvern). The pH is based on triplicate measurements for the 100 µg/mL suspensions. The DLS and zeta potential measurements for the samples are summarized in Table S1. The effect of pH was examined for Ce-06, demonstrating that zeta potential values increased to values between 0 and + 10 mV below pH 4.

Table S1. Characterization of cerium oxide nanoparticle suspensions dispersed with the optimized sonication energy. The optimized sonication energy, DLS data (Z-average and polydispersity index, PDI) and zeta potential data are summarized for each sample.

Sample[1]	Optimized sonication energy (J/mL)	Z_{avg} (nm)	PDI	pH	Average Zeta potential (mV)
Ce-01	380	190 ± 11	0.32 ± 0.04	4.4	-19.6 ± 0.9
Ce-02	32	232 ± 12	0.42 ± 0.03	4.3	-25.8 ± 0.3
Ce-03	621	324 ± 15	0.24 + 0.01	4.6	-29.5 ± 0.6
Ce-04	593	368 ± 5	0.25 ± 0.01	6.4	27.0 ± 0.3
Ce-05[2]	398	964 ± 54	0.22 ± 0.01		-12.1 ± 0.6
Ce-06	32	~400	>0.5[3]	4.5	-17.8 ± 2.5
Ce-07	49	~430	>0.5[3]	4.8	-30.9 ± 1.5
Ce-08	50	420 ± 60	0.43 ± 0.03	4.7	-31.9 ± 0.6

[1]The concentration of the nanoparticle samples was 0.01 w% for both DLS and zeta potential measurements.

[2]Ce-05 was dispersed in ethanol, and DLS and zeta potential measurements were performed in ethanol.

[3] Samples with PDI values above ≈ 0.4, have high polydispersity and are not suitable for cumulants analysis. Therefore, only approximate Z-average values are provided for these samples.

Thermogravimetric Analysis

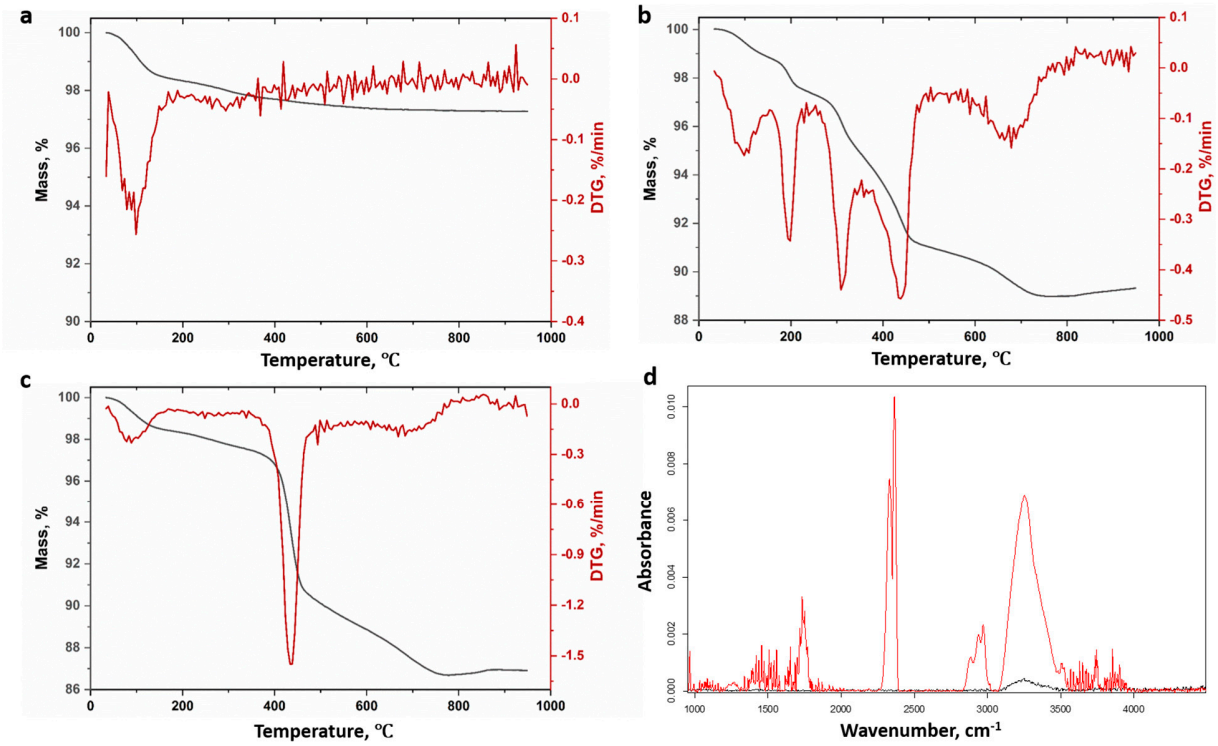

Figure S3. TGA results showing % mass loss (black) and derivative curves (DTG, red) for Ce-03 (a), Ce-05 (b, stearic acid) and Ce-04 (c, PVP). (d) The FT-IR spectrum of evolved gases at 430 ℃ for Ce-04 shows a carbonyl signal at ~ 1770 cm^{-1}, providing confirmation of the expected surface coating. Note that the broad signal between 3000 cm^{-1} and 3500 cm^{-1} is due to ice condensing in the detector. Carbonyl signals have also been observed for stearic acid-coated metal oxides nanoparticles in previous work.[1]

1. Kunc, F.; Gallernault, M.; O. Kodra, A. B.; Lopinski, G. P.; Johnston, L. J. Surface chemistry of metal oxides nanoparticles: NMR and TGA quantification. *Anal. Bioanal. Chem.* **2022,** *414* (15), 4409.

Table S2. Hydrodynamic diameter (Z_{avg}) and polydispersity index (PDI) of cerium oxide samples diluted into cell culture medium at 10 µg /mL over 72h. Values for Z_{avg} over 1000 nm and PDI values greater than 0.4 are noted and reasons for these values are discussed in the text. Attempts to improve the PDI values was not performed as the intention was to treat all samples the same way and not try to optimize samples in order to get a better quality measurement.

Sample	**Start** Z_{avg} / PDI (errors in brackets)	**24h** Z_{avg} / PDI (errors in brackets)	**48h** Z_{avg} / PDI (errors in brackets)	**72h** Z_{avg} / PDI (errors in brackets)
Ce-01	229(6) / 0.30(0.01)	236(8) / 0.32(0.01)	293(5) / >0.4	246(5) / 0.39(0.03)
Ce-02	300(30) / >0.4	340(50) / >0.4	400(70) / >0.4	350(30) / >0.4
Ce-03	377(5) / 0.26(0.02)	393(7) / 0.28(0.02)	401(9) / 0.34(0.06)	760(10) / >0.4
Ce-04	357(8) / 0.25(0.04)	348(4) / 0.27(0.04)	366(6) / 0.28(0.01)	383(6) / 0.34(0.03)
Ce-05	>1000 / >0.4	>1000 / >0.4	>1000 / >0.4	>1000 / >0.4
Ce-06	>1000 / >0.4	>1000 / >0.4	>1000 / >0.4	>1000 / >0.4
Ce-07	>1000 / >0.4	>1000 / >0.4	>1000 / >0.4	>1000 / >0.4
Ce-08	>1000 / >0.4	>1000 / >0.4	>1000 / >0.4	>1000 / >0.4

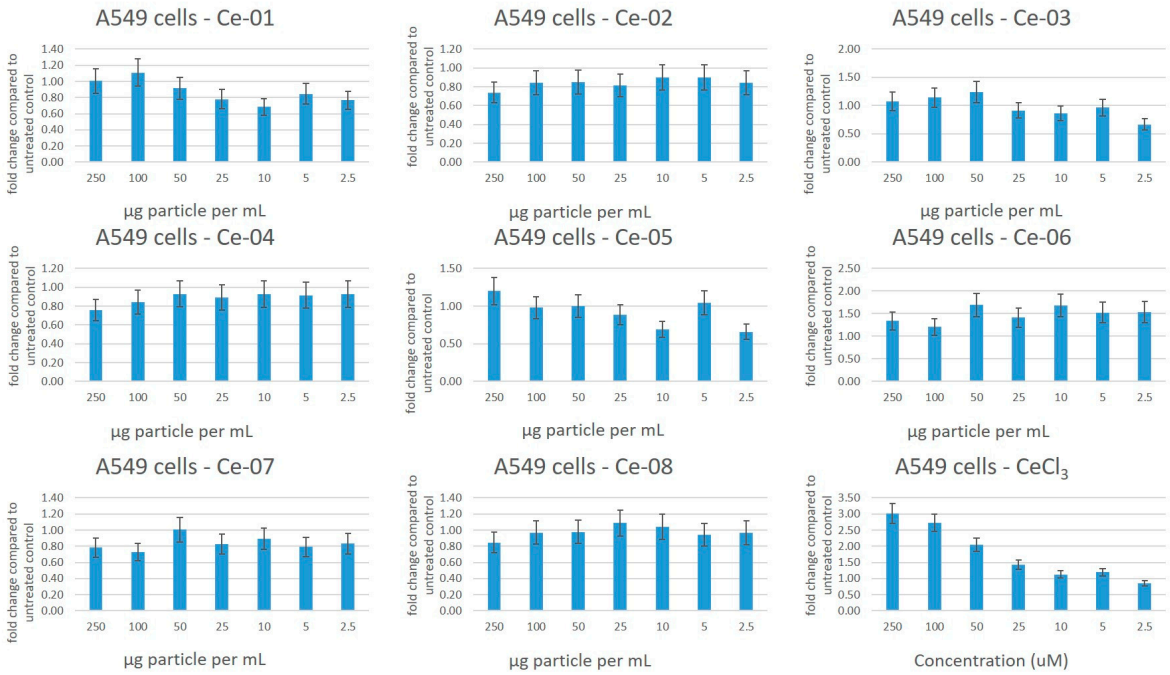

Figure S4. Results of the DCFDA assay performed at 7 different concentrations, measured at 24h for 8 different cerium oxide nanoparticles and $CeCl_3$ as an ionic control in A549 cells. Results are reported as 'fold change' where a sample treated with just cell culture media was used as a blank control. A value of 1.0 means that the increase in fluorescence over 24h was the same as for the control sample. Values

above 1 indicate an increase in ROS production, while values below 1 indicate a reduction in measured ROS after 24h.

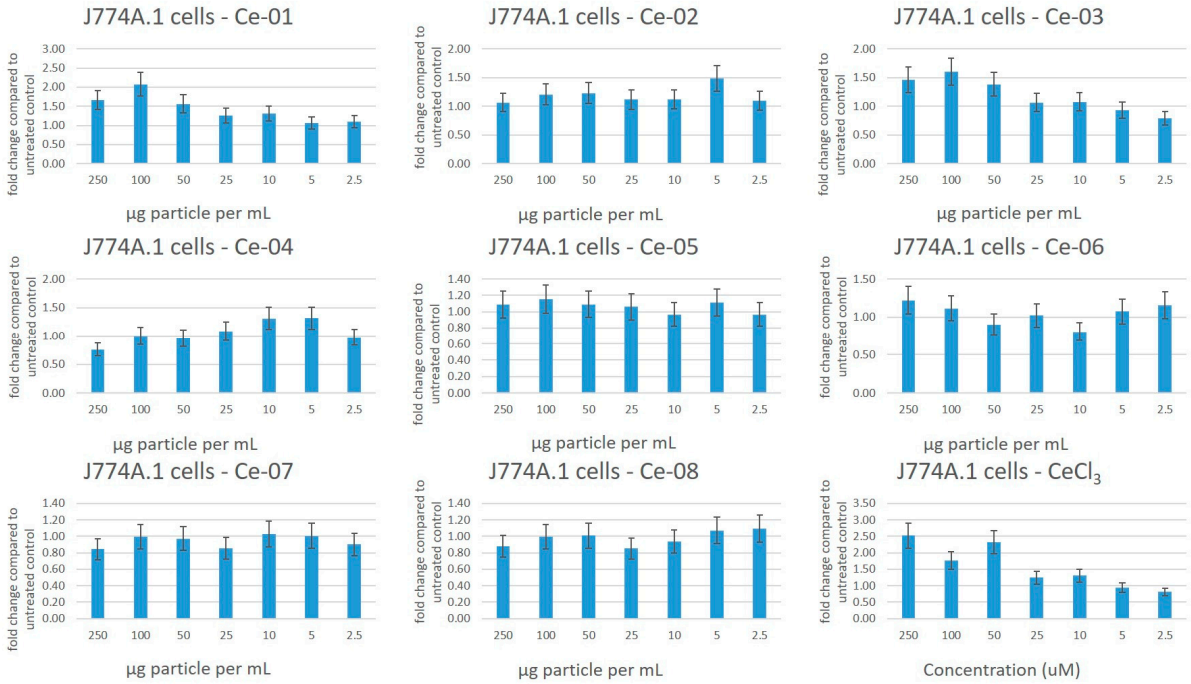

Figure S5. Results of the DCFDA assay performed at 7 different concentrations, measured at 24h for 8 different cerium oxide nanoparticles and $CeCl_3$ as an ionic control in J774A.1 cells. Results are reported as 'fold change' where a sample treated with just cell culture media was used as a blank control. A value of 1.0 means that the increase in fluorescence over 24h was the same as for the control sample. Values above 1 indicate an increase in ROS production, while values below 1 indicate a reduction in measured ROS after 24h.