



Article

Dual-Functional Antioxidant and Antiamyloid Cerium Oxide Nanoparticles Fabricated by Controlled Synthesis in Water–Alcohol Solutions

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Both authors (K.S. and V.H.) contributed equally to this work.

Material and Methods

Immuno-staining

Cells were fixed after the treatments with ice-cold acetone (Centralchem, Bratislava, Slovakia) for 5 min at -20°C . Cells were washed with ice-cold phosphate-buffered saline (PBS, Sigma-Aldrich, Darmstadt, Germany) and blocked in 5% bovine serum albumin (BSA, Sigma-Aldrich, Darmstadt, Germany) in PBS at 25°C for 1 hour. Subsequently, cells were incubated with primary antibodies diluted in 5% BSA with anti-giantin (ab80864, Abcam, Cambridge, UK) and anti-cathepsin B (H-5) antibodies (sc-365558, Santa Cruz Biotechnology Santa Cruz, CA, USA) for 1 hour at 25°C . After incubation, the cells were washed with ice-cold PBS. Further, the secondary antibodies conjugated to Alexa Fluor 488 (ab150077, Abcam, Cambridge, UK) or Alexa Fluor 546 (a11030, ThermoFisher Scientific, USA) and diluted in 1% BSA were applied to the cells for 1 hour at 25°C . A mounting medium with 4',6-diamidino-2-phenylindole (DAPI, ab104139, Abcam, Cambridge, UK) was utilized to stabilize the fluorescence signal and counterstain the nuclei. Fluorescence of secondary antibodies detection parameters: Alexa Fluor 488, excitation at 488 nm and emission of 500–540 nm; Alexa Fluor 546, excitation at 555 nm and emission > 560 nm; DAPI, excitation at 405 nm and emissions of 450 ± 40 nm.

Western blot

Western blot analysis of oxidative stress defense (catalase, smooth muscle actin, SOD1, TRX) and LC3B levels in U87 MG cells was performed with anti-LC3B ab221794 (1:3000) antibodies (Abcam, UK), while oxidative stress defense was analyzed with ab179843 Western blot cocktail (1:1000). Cell lysates were collected in RIPA buffer (150 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 20 mM Tris buffer saline at pH 8.0) with inhibitor cocktail (2x 1:100, Halt™ Protease and Phosphatase Inhibitor Cocktail, ThermoFisher Scientific, USA) from cells incubated for 24 h with insulin fibrils, Ce1, and Ce5. Proteins were separated via electrophoresis, transferred onto a nitrocellulose membrane, and immunodetected with the Western Breeze Chromogenic Kit (ThermoFisher Scientific, USA) according to the supplier protocol. The optical densities of visualized bands were calculated using ImageJ software



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(National Institutes of Health, USA). The level of significance was evaluated with a one-way ANOVA test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Supplementary Figures and Captions

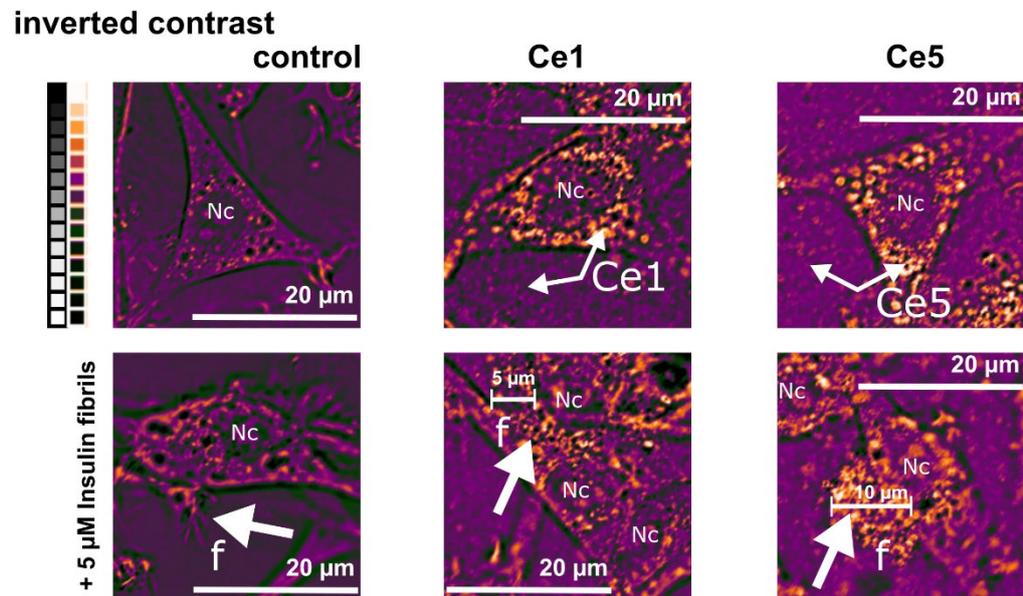


Figure S1. Representative bright-field images of U87 MG cells were subjected for 24 h to Ce1 and Ce5 nanoparticles at 150 μg/mL and with 5 μM insulin fibrils. White arrows point to nanoparticle and fibril localization. Images were converted via inverted contrast with the corresponding color code to recognize the most scattered particles inside cells. CeO₂ NPs and insulin fibrils can be identified in orange. CeO₂ NPs formed grainy surfaces in the extracellular space. Nc—nucleus; f—fibrils.

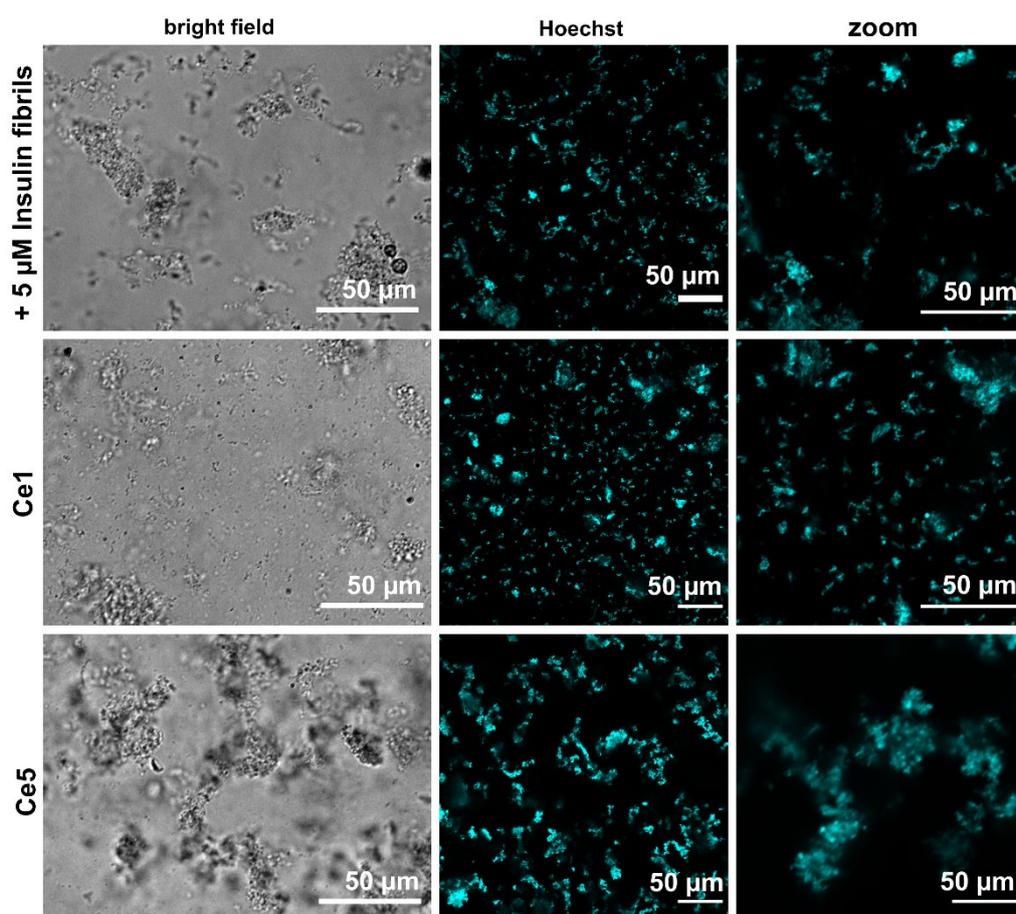


Figure S2. Representative bright-field and fluorescence images of 5 μ M insulin fibrils incubated for 24 h in cell culture medium with U87 MG cells and Ce1 and Ce5 nanoparticles at 150 μ g/mL. Cell cultivation medium was aspirated and labeled with Hoechst.

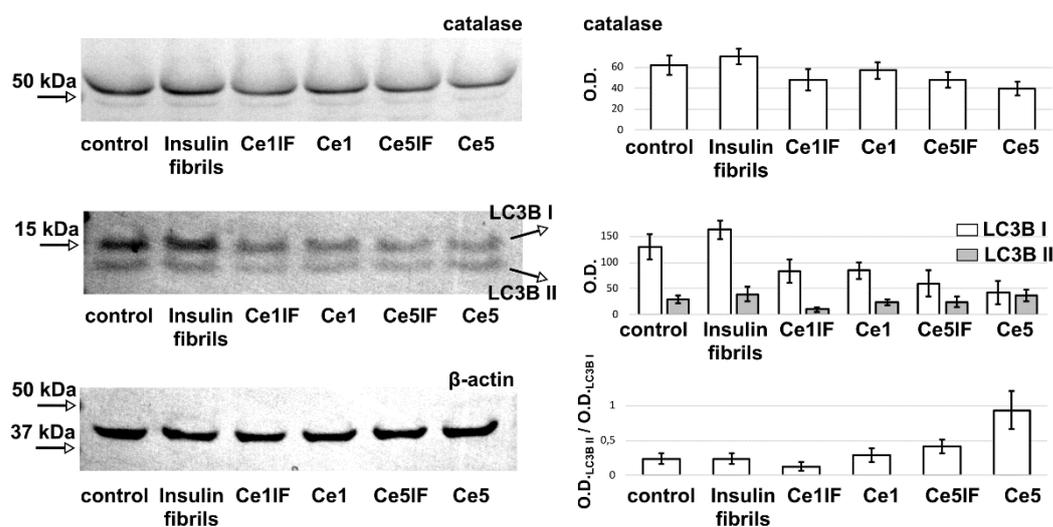


Figure S4. Western blot analysis of catalase and LC3B levels in U87 MG cells. β -Actin was detected as the housing protein. Optical densities (O.D.) are plotted as histograms. The LC3B II/LC3B I ratio is related to autophagy. The cells were subjected for 24 h to Ce1 and Ce5 nanoparticles at 150 $\mu\text{g}/\text{mL}$ with 5 μM insulin fibrils.

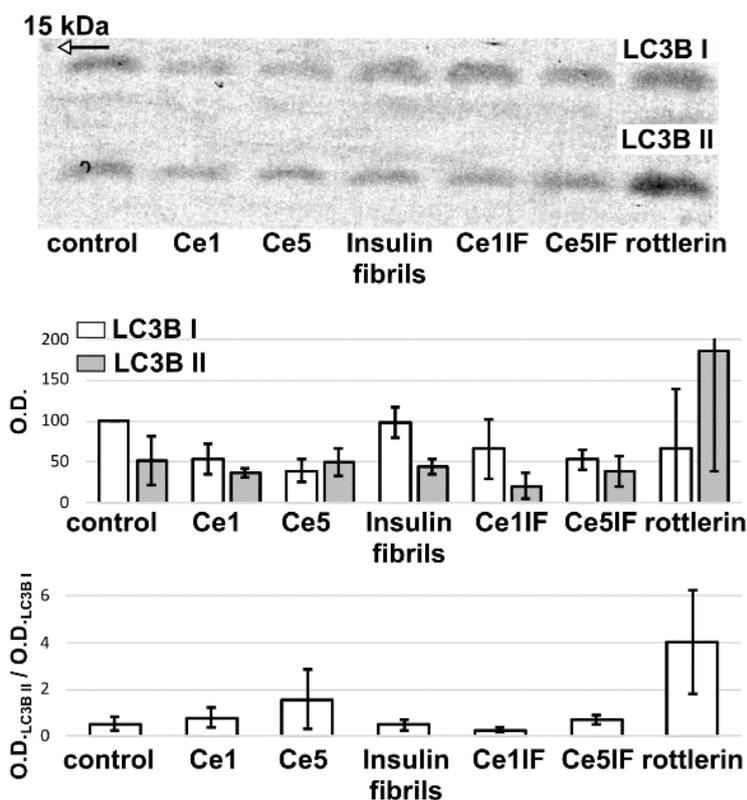


Figure S5. Western blot analysis of catalase and LC3B levels in U87 MG cells. β -Actin was detected as the housing protein (Figure 5). Optical densities (O.D.) are plotted as histograms. The LC3B II/LC3B I ratio is related to autophagy. The cells were subjected for 24 h to Ce1 and Ce5 nanoparticles at 150 $\mu\text{g}/\text{mL}$ with 5 μM insulin fibrils (IF) and 10 μM rottlerin for 1 h as a positive control to autophagy.

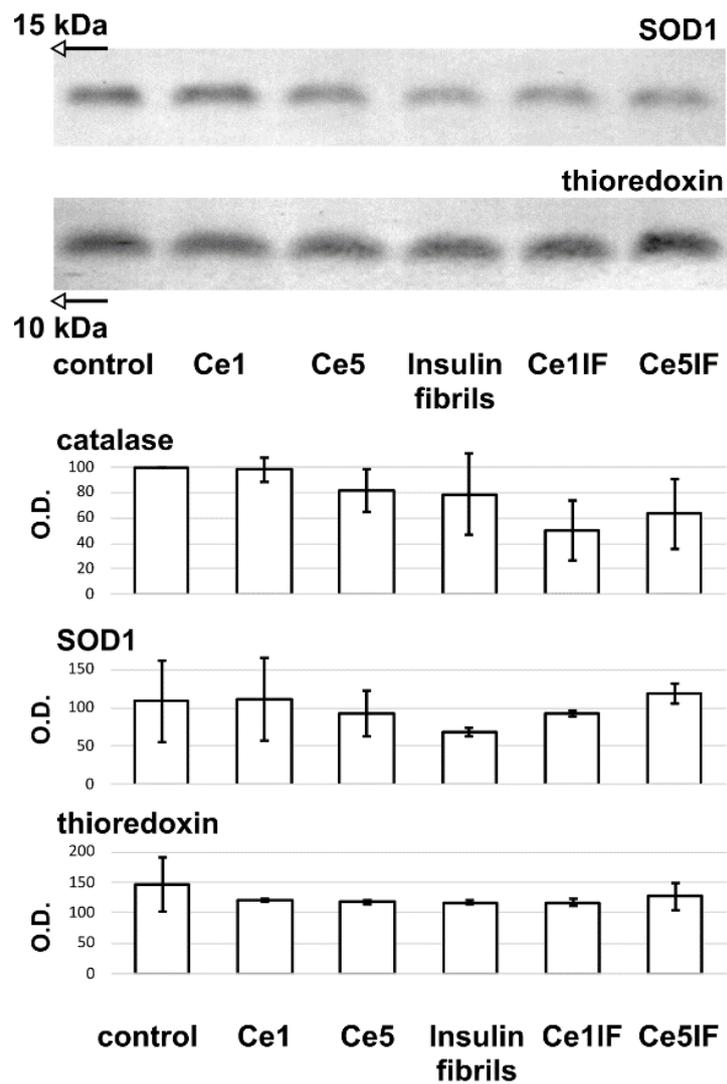


Figure S6. Western blot analysis of catalase (from Figure 5), SOD1, and thioredoxin levels in U87 MG cells. β -Actin was detected as the housing protein, as is presented in Figure 5. Optical densities (O.D.) are plotted in histograms. The cells were subjected for 24 h to Ce1 and Ce5 nanoparticles at 15 μ g/mL with 5 μ M insulin fibrils (IF).

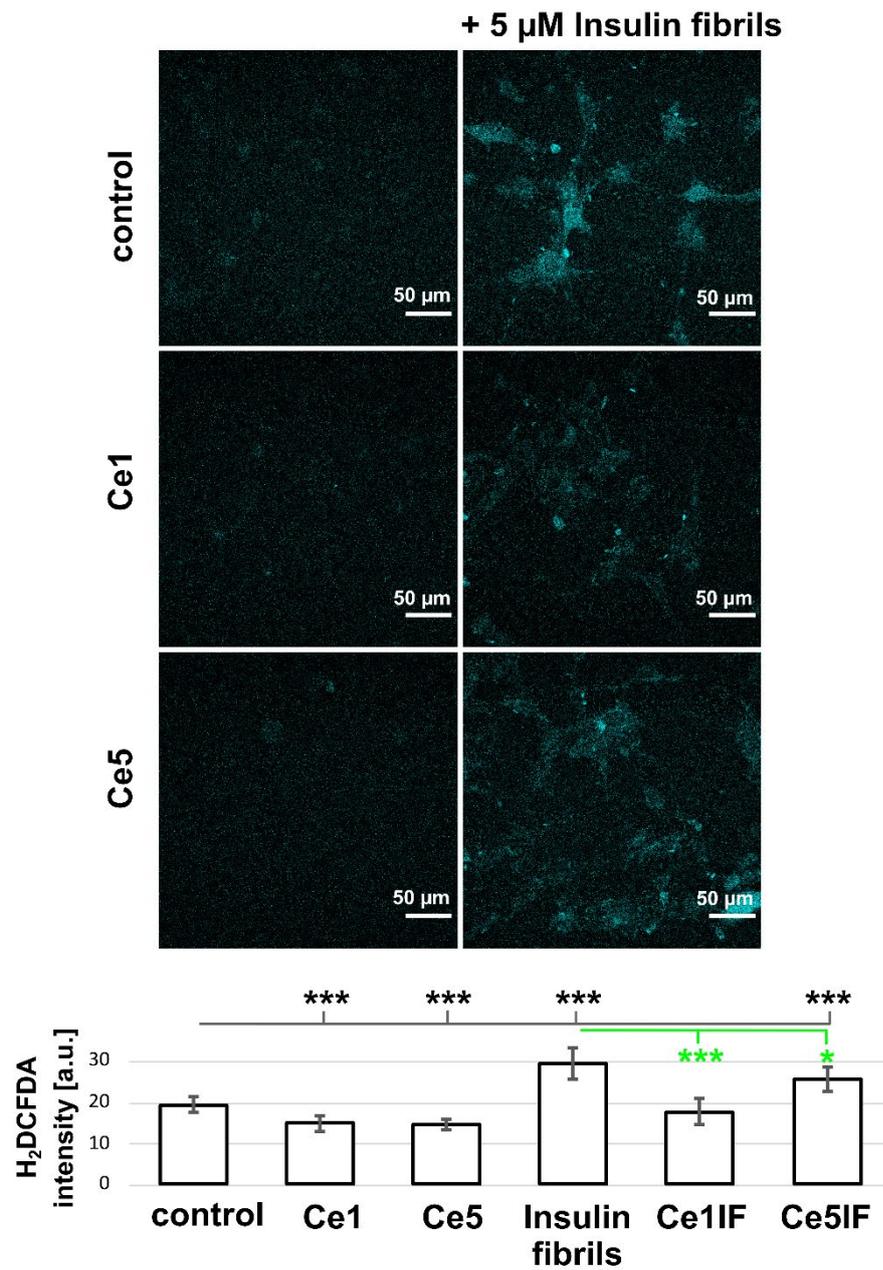


Figure S7. Representative fluorescence images of H₂DCFDA (cyan) in U87 MG cells. The cells were subjected for 24 h to Ce1 and Ce5 nanoparticles at 150 μ g/mL with 5 μ M insulin fibrils (IF). Fluorescence intensity levels of H₂DCFDA are plotted as histograms (under the images). Significant differences were calculated with ANOVA as * p <0.05, ** p <0.01, *** p <0.001.