

Supplementary Materials

to the paper: *Diminished amyloid- β uptake by mouse microglia upon treatment with quantum dots, silver or cerium oxide nanoparticles* by Sikorska et al., 2019

Materials and Methods

Cell metabolic activity assay (MTT assay)

MTT assay, which measures activity of cytosolic and mitochondrial dehydrogenases that roughly corresponds to cellular viability, was used to evaluate toxicity of the nanoparticles. In brief, the cells were seeded in 96-well microplates (TPP, Switzerland) at a density of 4×10^3 cells/well in 100 μ L of culture medium. Next day, AgNPs, CdTeQDs or CeO₂NPs (5, 10, 25, 50 and 100 μ g/mL) were added and incubated for 24 h at 37°C in CO₂ incubator. After incubation, 20 μ L of MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide), 5 mg/mL stock solution in PBS (pH = 7.0) was added to each well and incubated for 4 h at 37°C. The formed formazan crystals were dissolved in 120 μ L of DMSO:SDS (10%) 1:1 overnight in the CO₂ incubator. Finally, absorbance of the formazan was measured in a plate reader spectrophotometer (Infinite M200, Tecan, Austria), at a wavelength of 570 nm. The cell metabolic activity was expressed as a percentage of the absorbance of the treated cells in relation to control. IC₅₀ value was calculated using GraphPad Prism 5.0 software (GraphPad Software, Inc., USA).

Table S1. The metabolic activity of microglia after 24-h treatment with NPs (MTT assay).

AgNPs (μg/mL)	0	5	10	25	50	100
% of metabolic activity	100	72	71	57	46	28
CdTeQDs (μg/mL)	0	5	10	25	50	100
% of metabolic activity	100	48	47	25	10	11
CeO₂NPs (μg/mL)	0	5	10	25	50	100
% of metabolic activity	100	76	68	60	57	46

Table S2. Impact of A β on the NPs uptake by microglia.

A

Sample	CdTeQDs Fluorescence (arbitrary units)*
control	50 \pm 7
A β	40 \pm 1
CdTeQDs	2072 \pm 105
A β + CdTeQDs	1936 \pm 137

B

Sample	SSC value (arbitrary units)*
control	37 \pm 3
A β	35 \pm 2
AgNPs	169 \pm 18
A β + AgNPs	168 \pm 19
CeO ₂ NPs	91 \pm 11
A β + CeO ₂ NPs	86 \pm 7

* As measured by flow cytometry, BV-2 microglial cells were seeded into 6-well plates and grew overnight to reach 1.35×10^5 cells/well in average. The cells were incubated with A β (1–42) labelled with HiLyte™ Fluor 488 (AnaSpec Inc., Belgium) (0.1 μ M), AgNPs (50 μ g/mL), CeO₂NPs (100 μ g/mL) or CdTeQDs (10 μ g/mL) for 2 h. Subsequently, the cells were detached by trypsinization, spun down (200 x g, 4°C, 5 min), washed once with the culture medium containing 10% FBS, resuspended in the culture medium containing 2% FBS and analysed by flow cytometry (LSRFortessa, BD Biosciences). The AgNPs or CeO₂NPs uptake was assessed by change in a side scatter (SSC) value. The uptake of CdTeQDs was measured as an increase in fluorescence at 655 nm.

Table S3. Impact of NPs (AgNPs, 50 $\mu\text{g}/\text{mL}$; CeO₂NPs, 100 $\mu\text{g}/\text{mL}$; CdTeQDs, 10 $\mu\text{g}/\text{mL}$) on HiLyte Fluor 488-A β (0.1 μM) fluorescence in RPMI medium.

Sample	Fluorescence (arbitrary units)*
RPMI medium	10394 \pm 106
A β	38480 \pm 414
AgNPs	9371 \pm 361
CeO ₂ NPs	10318 \pm 226
CdTeQDs	9929 \pm 173
A β + AgNPs	40174 \pm 535
A β + CeO ₂ NPs	44089 \pm 833
A β + CdTeQDs	40380 \pm 1234

* As measured by microplate fluorescence reader Infinite M200 (Tecan, Austria), set to excitation at 503 nm and emission 528 nm. The values are means \pm SD. All solutions were prepared in RPMI medium without fetal bovine serum.

Table S4. Cell cycle distribution of BV-2 population after treatment with A β and/or NPs.

Cell cycle phase, % of whole cells population				
Sample analysed	G1 phase	S phase	G2 phase	Apoptosis/debris*
control	45.3 \pm 2.2	50.0 \pm 3.3	4.7 \pm 1.7	0.3 \pm 0.3
A β	46.5 \pm 1.9	48.9 \pm 3.5	4.5 \pm 1.9	0.5 \pm 0.7
CeO ₂ NPs	39.4 \pm 1.7 ^a	55.6 \pm 0.5 ^a	4.9 \pm 1.6	0.9 \pm 0.8
AgNPs	39.6 \pm 2.4 ^a	58.8 \pm 2.1 ^a	1.4 \pm 0.2 ^a	25.2 \pm 7.2 ^a
CdTeQDs	52.4 \pm 1.2 ^a	42.5 \pm 2.3 ^a	5.1 \pm 1.2	12.8 \pm 2.2 ^a
A β + CeO ₂ NPs	40.7 \pm 0.6 ^a	53.9 \pm 0.6 ^a	5.4 \pm 0.7	0.8 \pm 0.7
A β + AgNPs	37.3 \pm 3.8 ^a	61.9 \pm 3.8 ^a	0.7 \pm 0.7 ^a	25.3 \pm 5.6 ^a
A β + CdTeQDs	55.1 \pm 3.1 ^a	40.3 \pm 1.6 ^a	4.6 \pm 1.6	16.0 \pm 2.0 ^a

BV-2 cells were treated with 0.1 μ M A β (1–42) and/or AgNPs (50 μ g/mL), CdTeQDs (3 μ g/mL), CeO₂NPs (100 μ g/mL) for 48 h. The percentage of cells in respective cycle phase was calculated using ModFit LT v 5.0 Software (Verity Software House Inc., USA). Results are expressed as mean \pm SD, n = 3, 'a' denotes statistically significant difference versus control group, $P < 0.05$, by t-test. The level of apoptosis/debris was indicated as % of whole cell population.