Electronic Supplementary Material (ESI) for Analyst.

Electronic Supplementary Information

Combined Raman and AFM detection of changes in HeLa cervical cancer cells induced by CeO_2 nanoparticles – molecular and morphological perspective

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AFM and TEM images of SPRT NPs

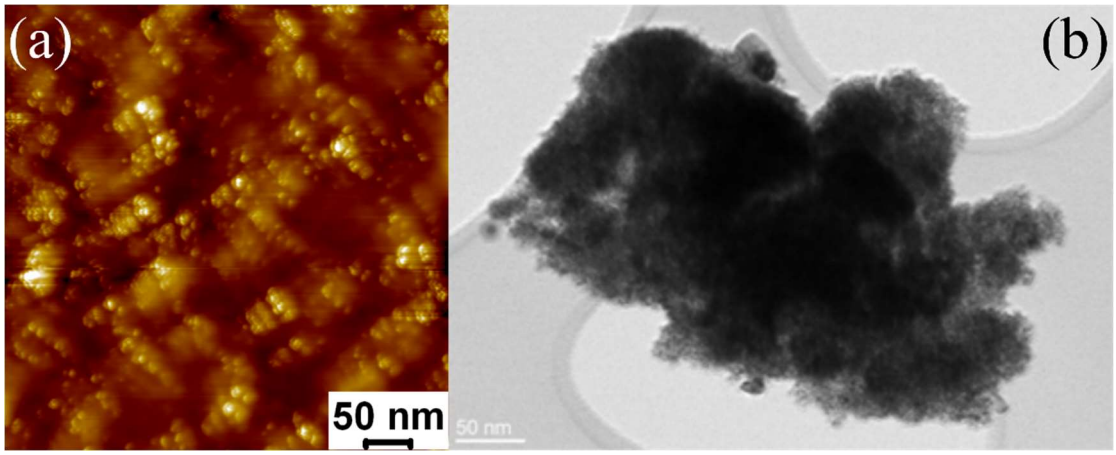


Figure. S1. a) High-resolution AFM image of SPRT CeO_2 NPs in powder. b) TEM image of dispersed SPRT CeO_2 NPs.

UV-VIS spectrometry measurements

In the UV-VIS spectrum obtained from SPRT CeO_2 there is a peak positioned at ~340 nm belonging to O 2p - Ce 4f ^{0} (Ce^{4+}) charge transfer transition[1,2]. The spectrum obtained from CD CeO_2 contains the same peak at blueshifted position of ~300 nm, blueshift occurs due to quantum size effect[2]. It also contains a new peak, positioned at ~250 nm, observed in reduced CeO_2[1] and CeO_2 NPs of different morphologies[2]. This transition can belong to charge transfer transition but also overlaps with Ce^{3+} 4f – 5d transition[2]. This can imply that Ce^{3+} is more abundant in CD CeO_2 compared to SPRT CeO_2. Nanometric CeO_2 is known to be nonstoichiometric and the nonstoichiometry increases with decreasing crystallite or particle size, leading to higher content of vacancies and Ce^{3+} ions in CD NPs than in SPRT CeO_2 which, although it possesses similar crystallite size forms larger grains and even clusters of several tens of nanometers.

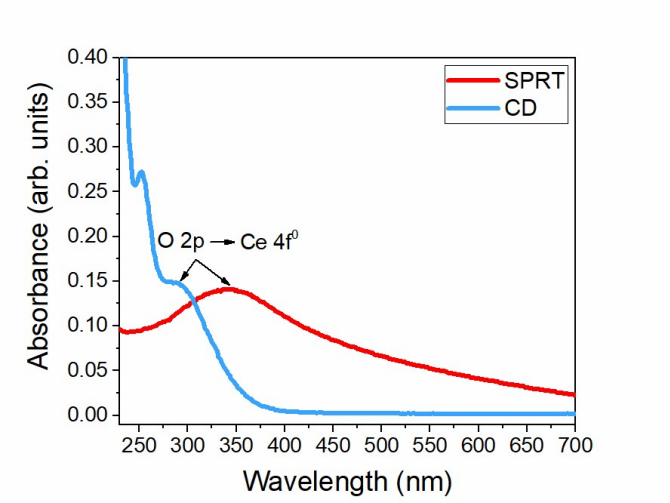


Figure S2. UV-VIS absorbance measurements of SPRT and CD CeO_2 nanoparticles

Testing the doses of NPs and exposure times

In order to choose optimal concentration of NPs and exposure time, i.e. such conditions that will cause significant reduction of cell growth, we tested the cytotoxicity of both types of CeO_2 NPs. For this purpose, we used sulforhodamine-B (SRB) test, which evaluates total amount of proteins in the cell culture as a measure of relative change in cell growth (see Materials and methods, 2.6). NPs were applied in three different concentrations and for three different exposure times in Hela cell culture. The results are shown in the Figs.S3 and S4.

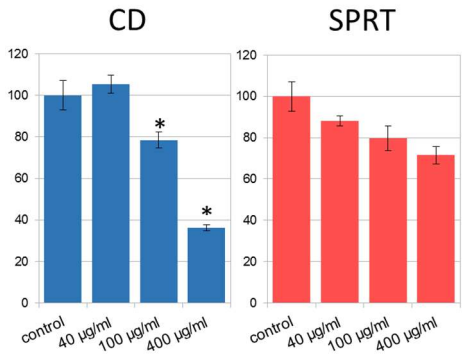
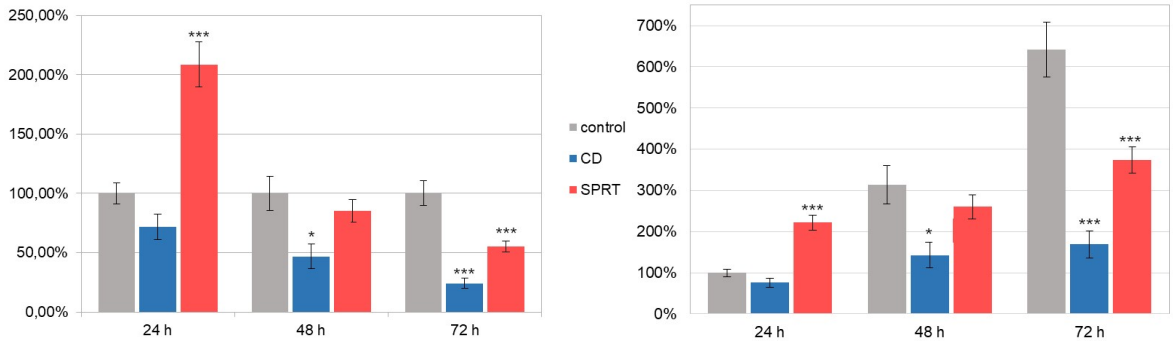


Figure S3. *Changes in the growth of HeLa cells caused by three different concentrations of CD CeO_2* **(a)** *and three different concentrations of SPRT CeO_2* **(b)***, evaluated by SRB test. Exposure time was 48 hours for all groups. Data presented as mean ± standard error, N=5. Mean of control (untreated) group is given as 100 %, while values for treated groups*

*are expressed related to the mean of control. *P<0.05 vs. control group, estimated by non-parametric one-way ANOVA.*

Based on the experimental data for cytotoxic dose range of CeO_2 NPs given in the literature [3-5], we firstly tested effects of 40 μg/ml, 100 μg/ml and 400 μg/ml of CeO_2 NPs, incubated with cells for 48 hours. Results are shown in the Fig. S3(a) and (b). For SPRT NPs, there was no statistically significant change in cell growth, although slightly decreasing trend with increasing concentration is registered. For CD NPs, doses of 100 μg/ml and 400 μg/ml significantly reduced cell growth. Higher dose had a stronger effect. In order to assure detection of induced changes, we chose the highest dose of 400 μg/ml to be used in further experiments. Then, for the chosen dose, three different exposure times: 24 hours, 48 hours and 72 hours were tested (Fig. S4).



(a) (b)

Figure S4. *Changes in the growth of HeLa cells caused by CeO_2 NPs, incubated in cell culture for 24, 48 and 72 hours, evaluated by SRB test. Concentration of NPs in the culture medium was 400 μg/ml for all groups. Data presented as mean ± standard error, N=8. Statistical significance legend: *P<0.05, ***P<0.001, vs. control group, estimated by one-way ANOVA. Untreated control: gray bars, CD CeO_2-treated: blue bars, SPRT CeO_2-treated: red bars.* ***(a)*** *Mean of control group is given as 100 %, while values for treated groups are expressed related to the mean of control.* **(b)** *Mean of control group after 24 hours is given as 100%, while all other samples, including controls after 48/72 hours, are expressed related to the mean of control for the first day.*

After 24 hours, SPRT CeO_2 NPs stimulated the growth of HeLa cells, while CD CeO_2 NPs did not cause statistically significant changes in cell growth. Since we wanted to investigate cytotoxic effects of NPs by Raman spectroscopy, exposure of 24 hours was considered inadequate. On the other hand, 72 hour-exposure induced significant reduction of cell growth for both types of NPs, but at the same time it induced overgrowth of control cells. It is particularly obvious from the Fig. S4(b), when compared to the first day control. By simple cell counting, we confirmed that the number of control cells in the culture 72 hours after treatment (i.e. 96 hours after seeding) was 2-3 times larger than the

highest recommended number for homeostatic growth. In order to avoid possible negative effects of overgrown controls and to keep at the same time sufficient number of CD-treated cells for further experiments, we chose exposure time of 48 hours as optimal.

High-resolution AFM images of cell membrane

In order to get further insights into structural changes of cell membrane due to interactions with NPs, high-resolution AFM images were recorded on small scan areas of 500×500 nm^2. 3D topographic images are depicted in Fig. S5(a) for the control, in Fig. S5(b) for CD-treated and in Fig. S5(c) for SPRT-treated cell. As can be seen, the membrane surface of the control cell is smooth and grainy. On the other hand, such grainy structure disappears on the membranes of cells treated with CD and SPRT NPs. Furthermore, these membranes are characterized with local depressions represented with a dark contrast in AFM images and denoted by arrows in Figs. S5(b) and S5(c). The lateral size of the depressions is around 50-100 nm. Therefore, the main effect of the interaction with NPs is vanishing of the membrane structure and appearance of depressions.

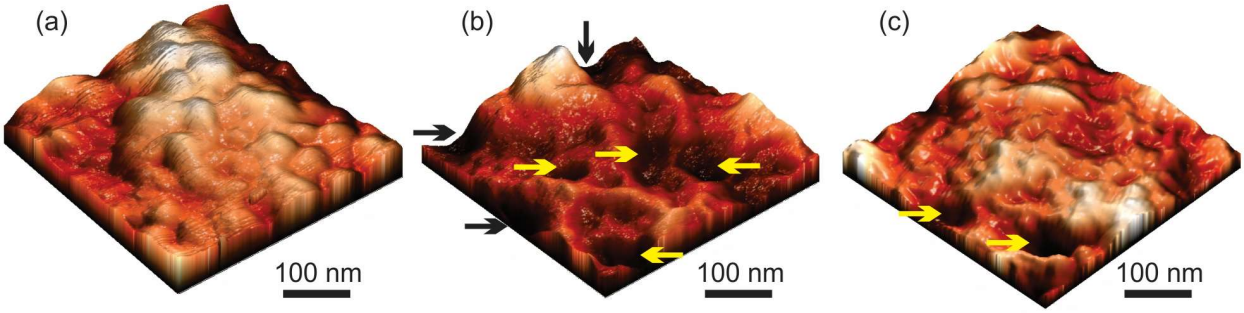


Figure S5. 3D topographic images of (a) control, (b) CD-treated, and (c) SPRT-treated cell. Heights scales are 20 nm, 16 nm, and 15 nm, respectively. Local depressions in the treated cell are marked by arrows.

PCA of cell Raman spectra

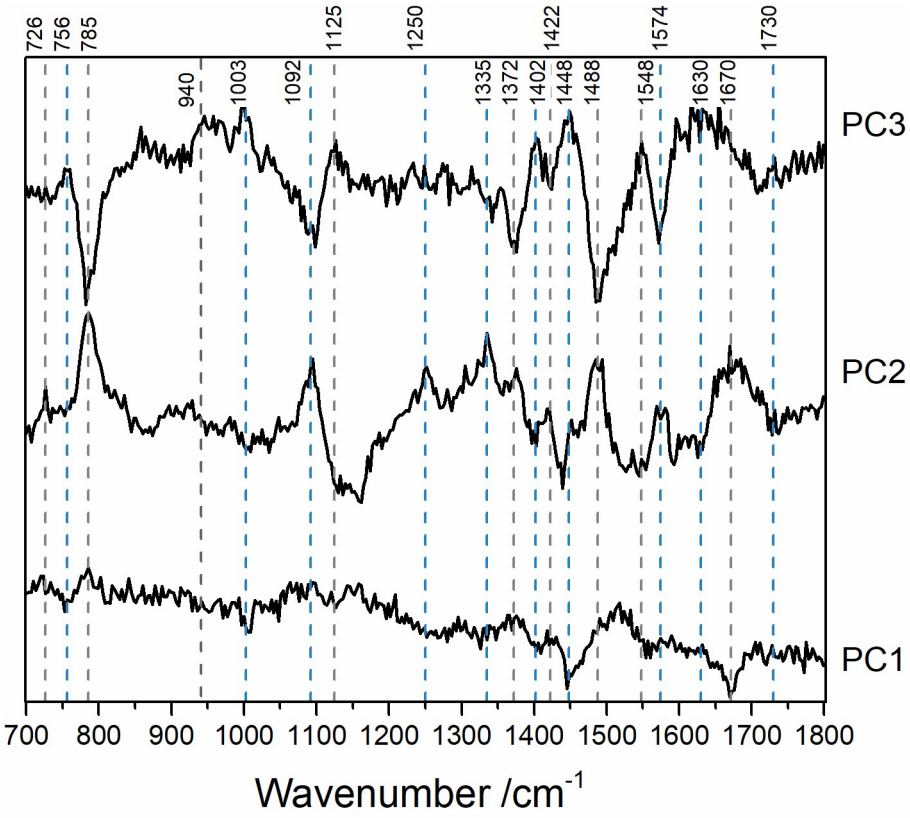


Figure S6. First three PC loadings in the spectral region (700-1800) cm^{-1}

References

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