Monitoring nanoparticle-induced oxidative stress using cyclic voltammetry

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Introduction

The expanding field of nanotechnology has provided novel applications in many different areas including medicine and dentistry due to the unique properties exhibited by nanoparticles (NPs). However, various types of NPs have been shown to generate reactive oxygen species (ROS), a known cause of oxidative stress, and consequently toxicity. Conventional colorimetric and fluorescence-based methods used to evaluate oxidative stress have been shown to suffer from NP-induced interferences [1]. To address this problem, we hereby propose label-free impedance-based and cyclic voltammetry-based (CV) methods.

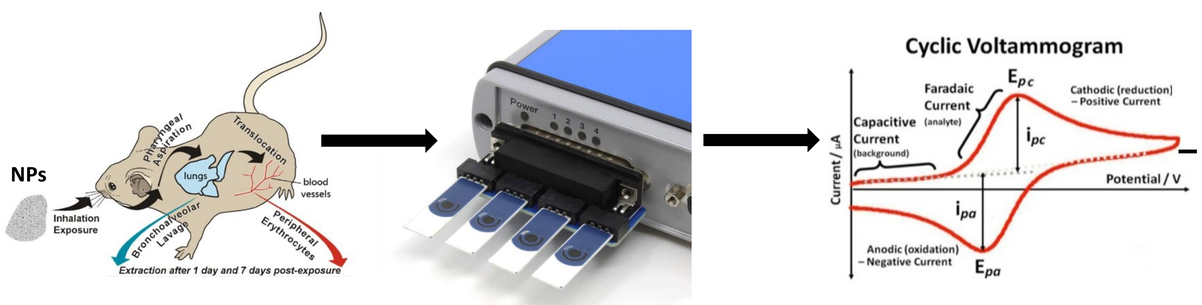

Figure 1. CV could be used for the analysis of the total antioxidant capacity of blood and other biofluids from animals exposed to NPs. The middle figure shows a multiplexed potentiostat with SPEs. The figure on the right illustrates a typical voltammogram with its components [2].

Results

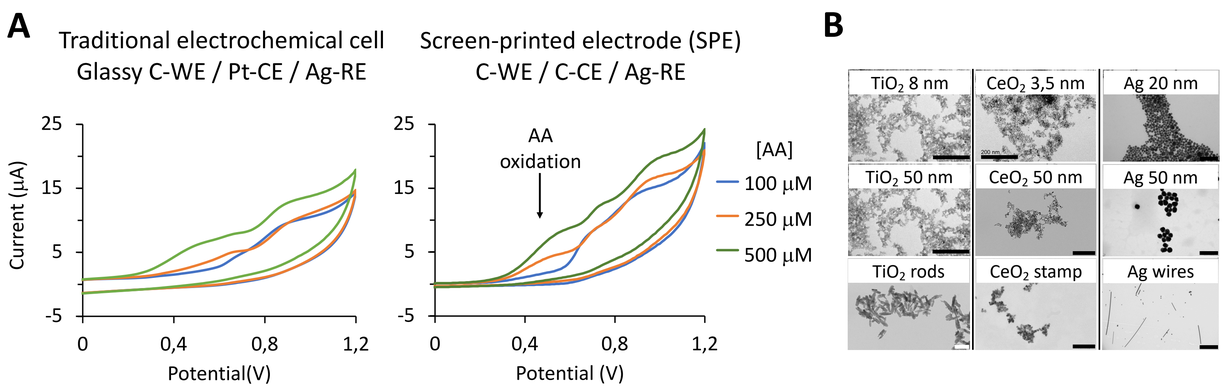

Figure 2. A) Detection of AA at different concentrations in DMEM/10% FBS using a traditional electrochemical cell and commercial SPEs. CV was performed at 100 mV/s. Only the oxidative scans are shown. Single-use SPEs eliminates washing and polishing steps between measurements and reduces sample volume for analysis. Importantly, SPEs can resolve oxidation of AA as shown by the anodic peak currents at 0,5 V. B) Transmission electron micrographs of NPs used. Scale bars: 200 nm.

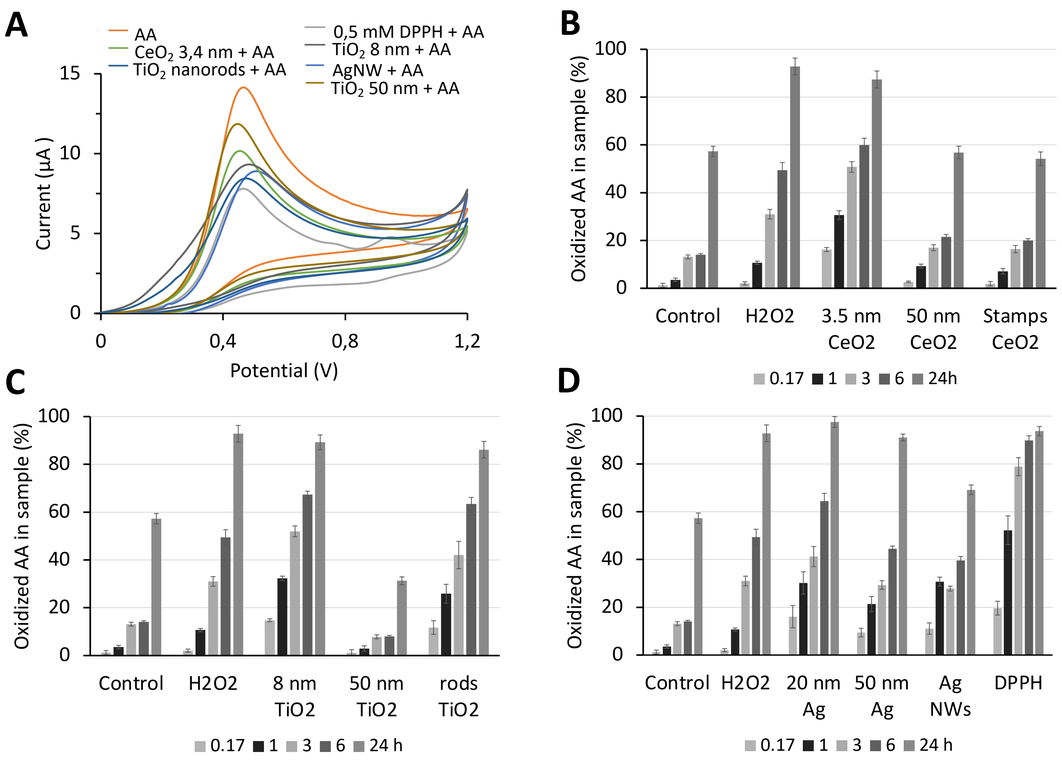

Figure 3. NP-mediated oxidation of ascorbic acid. A) Cyclic voltammograms 30 min after mixing NPs with AA. CV was performed at 100 mV/s in HBSS containing 250 µM AA and 100 µg/ml of the indicated NM. DPPH is a stable free radical. Several NMs showed reduction of the anodic peak current at 0,5 V, indicating oxidation of AA by the NPs. B-D) Peak currents were used to determine the amount of AA oxidized at different time-points when incubated with different CeO_2, TiO_2 and Ag NPs.

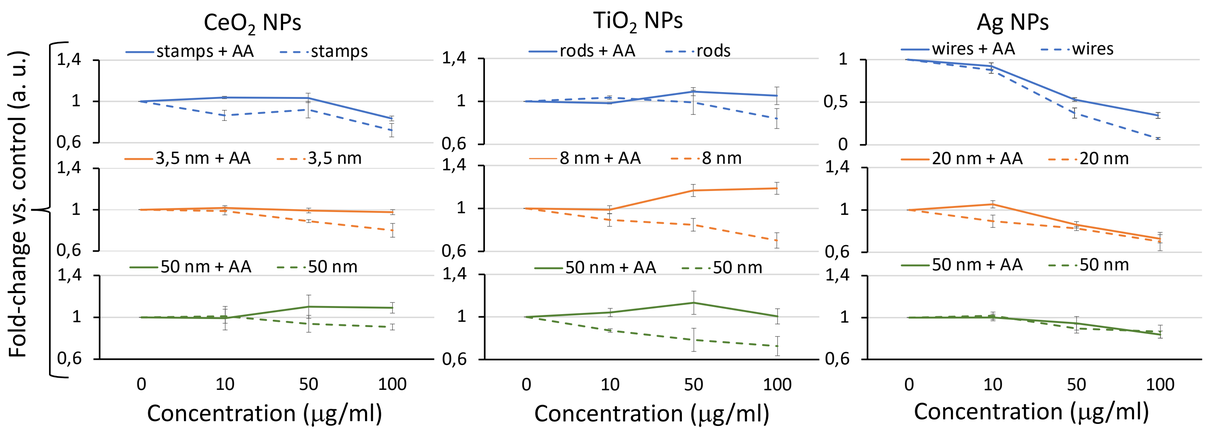

Figure 4. Viability normalized to control of A549 cells exposed for 24 h to NMs with or without 250µM AA and 1 mM 2-phospho-AA. Addition of AA diminishes the detrimental effect of some NPs.

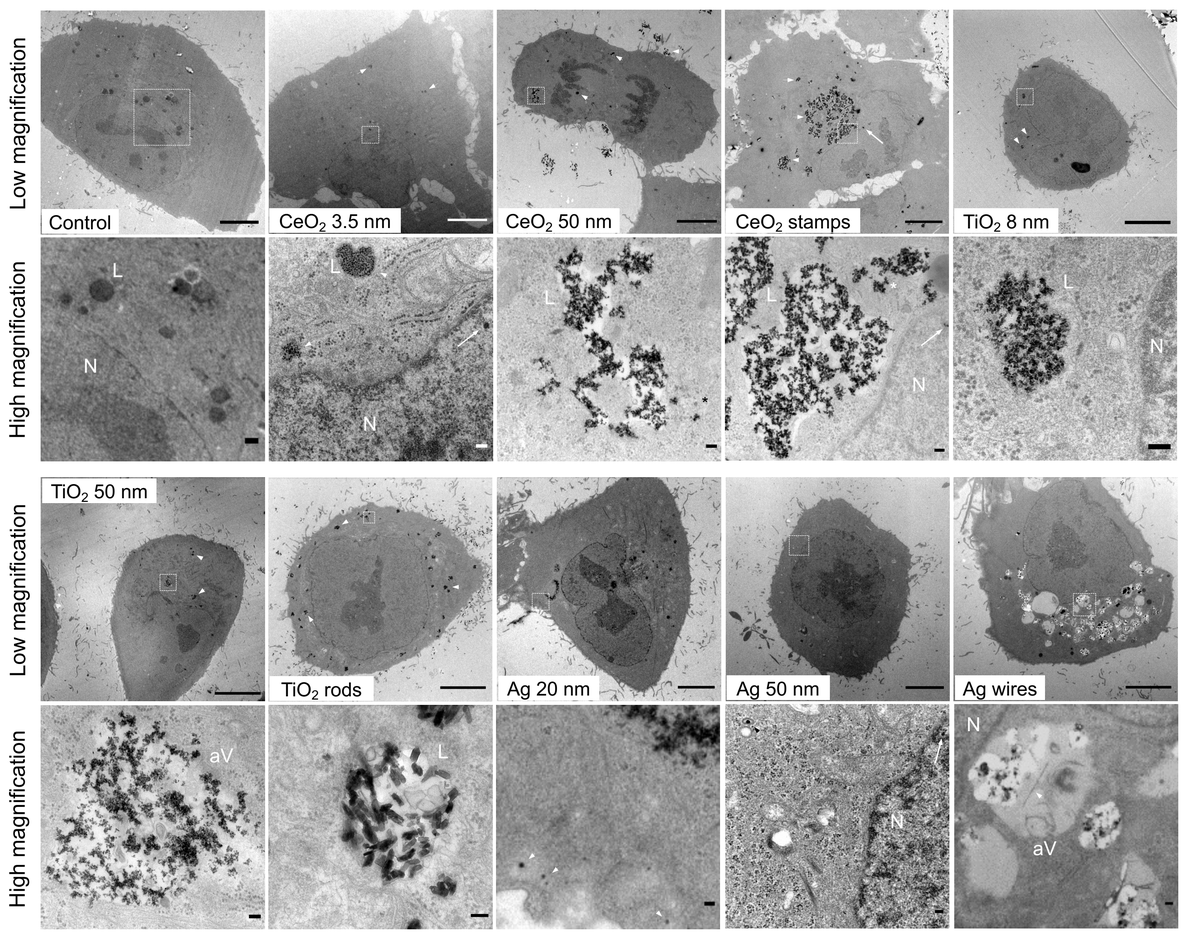

Figure 6. Transmission electron micrographs of A549 cells treated for 24 h with 50 µg/ml of the indicated NPs and untreated control. Low magnification images and high magnification of the selected area (dashed box). All NPs are readily taken up by the cells and accumulate primary in membrane-enclosed organelles. Arrows indicate examples of NP clusters. Arrowheads point to 50 nm Ag, 3.5 nm CeO_2 and CeO_2 stamps inside the nucleus. Asterisks indicate examples of non-membrane-enclosed NMs. N (nucleus), L (lysosomes) and aV (autophagic-like vacuoles). Scalebars: 5 µm in low magnifications, 100 nm in high magnifications and 400 nm in high magnification for control.

Conclusions

- CV with SPE is suitable to monitor NP-mediated oxidation of AA in HBSS.
- 3,5 nm CeO_2 and 8 nm TiO_2 caused the highest level of AA oxidation.
- The addition of AA diminished the toxic effect exerted by NPs on A549 cells.
- All NPs were readily taken up by A549 cells.

Materials and Methods

The following NMs were used: 20 nm and 50 nm AgNPs, 5-10 µm x 50 nm Ag nanowires (AgNWs), 3.5 nm and 50 nm CeO_2, 10 x 10 nm CeO_2 stamps, 8 nm and 50 nm TiO_2 and 140 x 40 nm TiO_2 nanorods (Applied Nanoparticles S. L.). A multiplexed potentiostat (PalmSens) and disposable screen-printed electrodes (SPEs) were used to assess the NPs' inherent redox properties, as well as their oxidative capabilities in the presence of 250 µM L-ascorbic acid (AA). The CV for the NPs was performed in Hank's Balanced Salt Solution (HBSS) at different time-points by applying an electrical voltage sweep and measuring the resulting current. Additionally, label-free bioimpedance (xCelligence, Agilent) was used to monitor proliferation/viability of A549 cells exposed to NPs in the presence or absence of AA and 2-phospho-AA. A549 cells were exposed for 24h to the NPs at 10, 20, 50 and 100 µg/ml.

References

[1] Ong, K. J., et. al. Widespread Nanoparticle-Assay Interference: Implications for Nanotoxicity Testing. *PLoS ONE*. 2014

[2] Elgrishi, N., et. al. A Practical Beginner's Guide to Cyclic Voltammetry. Journal of Chemical Education. 2017

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