Nanotoxicity testing using electrical impedance and cyclic voltammetry

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Introduction

Nanomaterials (NMs) can be purposefully designed to provide significant benefits, however, they may also cause undesired effects on humans and the environment. Most of the methods used to evaluate NM-induced oxidative stress are colorimetric and fluorescence-based, which can be affected by NM-caused interferences [1]. We hereby propose the use of Cyclic Voltammetry (CV), which is less prone to interferences. To assess cell death and proliferation, we used electrical-based impedance (EI), which is also label-free and has the advantage of monitoring adherent cells in real-time.

Method

CV measurements. The NMs used in the oxidative stress assessment by CV were: 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, Barcelona) at 100 µg/ml.

The negative control consisted of Hank's Balanced Salt Solution (HBSS) + 250 µM ascorbic acid (AA), while the positive control, consisted of a 500 µM compound that contains peroxide (H_2O_2), which generates ROS. A portable multiplexed potentiostat (PalmSens) with CCAg SPE at 0.3V/s was used in the CV testing. The measurements were recorded at 0, 10, 30, 60, 120, 180 min, 6h and 24h after exposure to NMs immersed in HBSS with 250 µM pure AA. An electrical voltage sweep was applied and the current resulting from the electrons that were transferred in the redox reaction was measured. The linear scan was swept from 0 V to 1.2 V, then further to -1.0 V and back to 0 V (Figure 1).

Impedance-based measurements. The impedance-based measurements were performed using an xCELLigence RTCA SP instrument (Agilent, Santa Catalina, CA, USA) equipped with 96-wells for label-free, real-time monitoring of cell proliferation, adherence, and viability under static exposure conditions. The impedance at the gold-plated electrodes of the wells on which the cells were seeded was recorded at a 15 min interval from cell seeding until the end of the exposure to engineered nanomaterials (ENMs). The cell index (*CI*) was calculated from the impedance measurements. The RTgut and ZF4 cells were exposed to ENMs after an initial cell seeding and proliferation for 24 h in the E-plates. In each experiment, the cells were seeded at the starting point and left to attach to the electrode surface and proliferate for 24 h before being exposed for 24 h to five different concentrations of ENMs: 2, 10, 20, 50, 100 µg/ml. The *CI*s were normalized to the *CI*-value measured right before the cells' exposure to the ENMs, i.e., at 24 h after seeding.

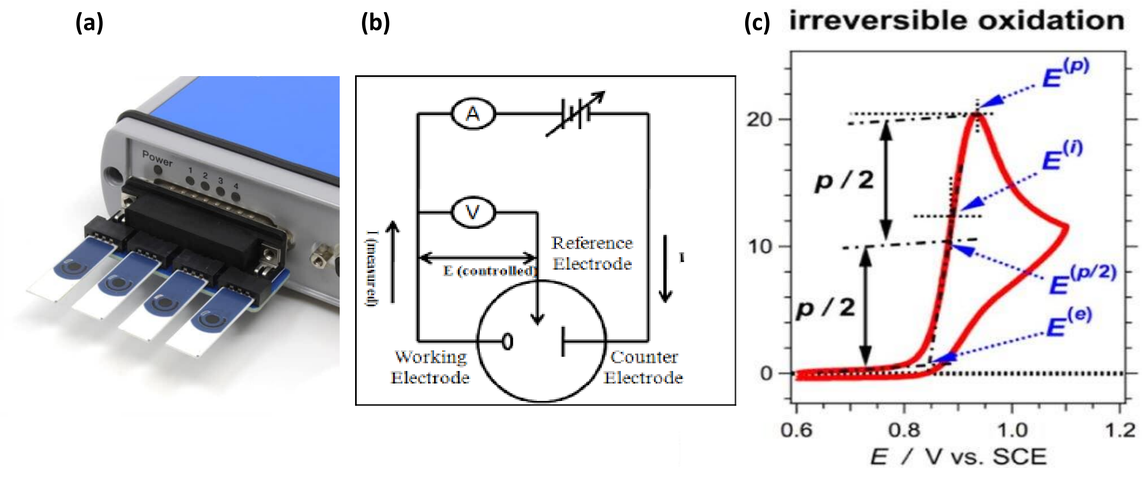

Figure 1. (a) The electrode multiplexer of the potensiostat used in CV measurements with commercial electrodes. These CV tests were performed using screen-printed electrodes that avoid washing and polishing between measurements. **(b)** A simplified electrical circuit used in CV tests **(c)** Typical CV voltammogram characteristic for irreversible oxidation processes.

Results

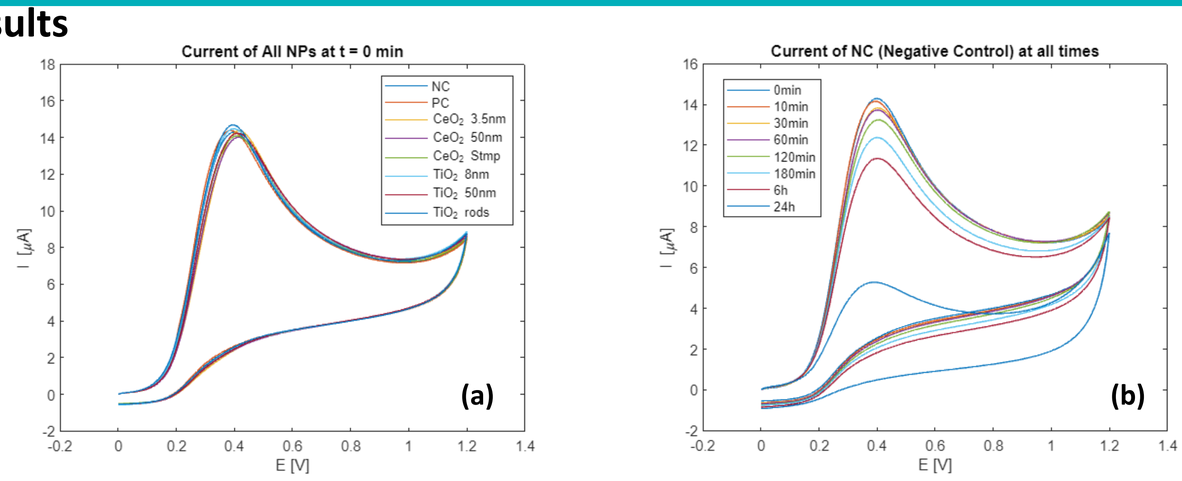

Figure 2. (a) Typical CV tests of all NPs at *t* = 0 sec., showing identical anodic current *I* for all NMs. **(b)** Cyclic voltammograms of negative control (NC) at all times, used as reference for AA consumption.

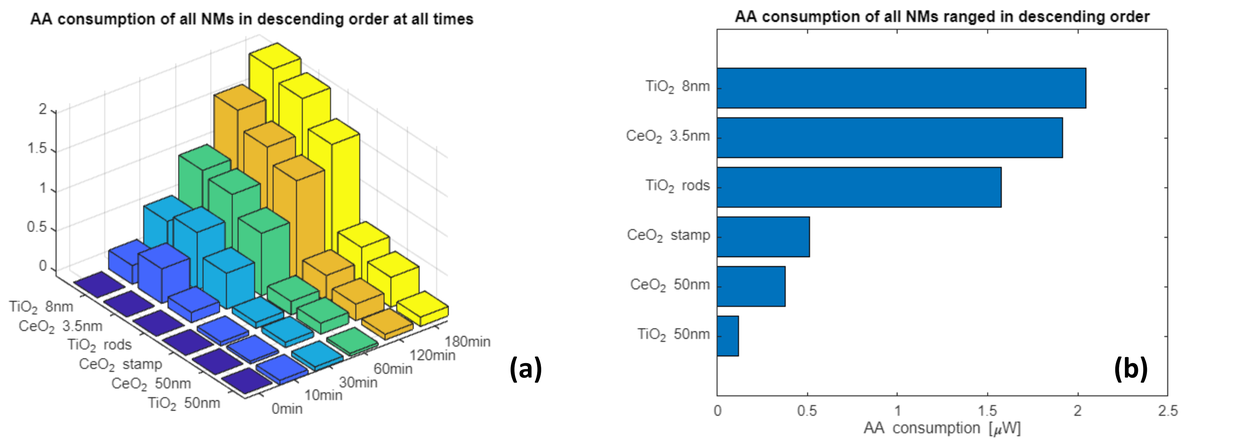

Figure 3. AA consumption of all NMs **(a)** at all times **(b)** at end point ranged in descending toxicity order.

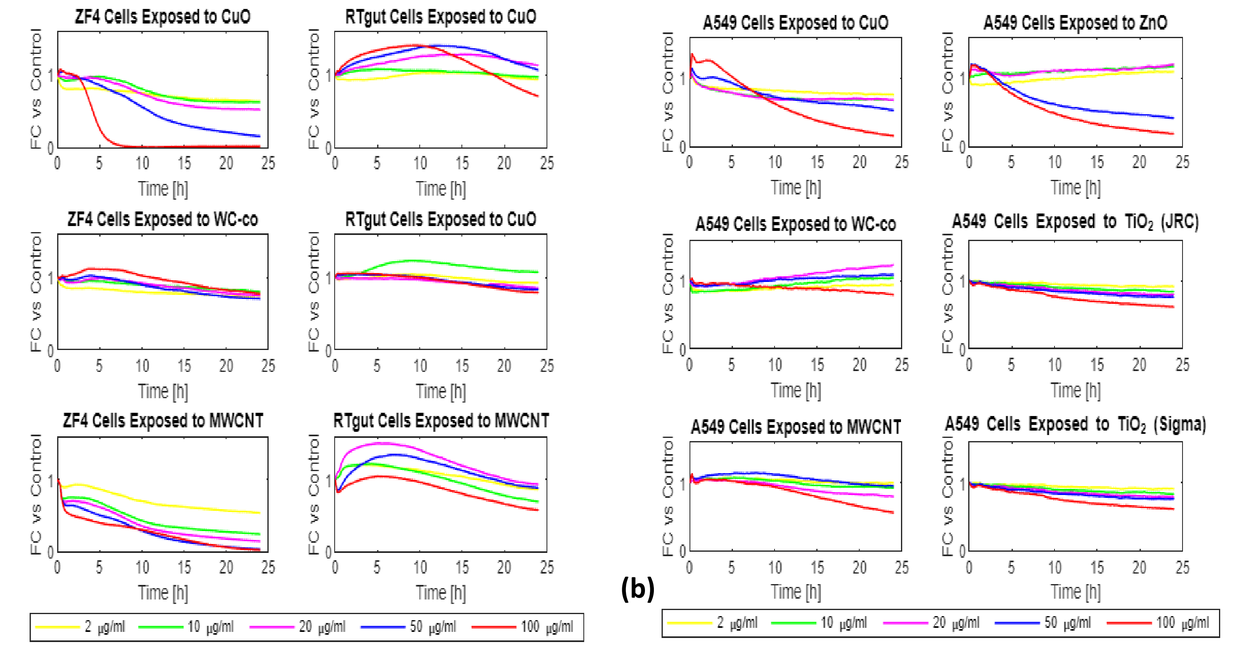

Figure 4. (a) The EI measurements of ZF4 and RTgut exposed to CuO, WC-co and MWCNT in round-robin 1 (RR1) (Task 6.2). **(b)** The EI measurements of A549 cells exposed to all NMs used in Task 5.1 RR1 & RR2.

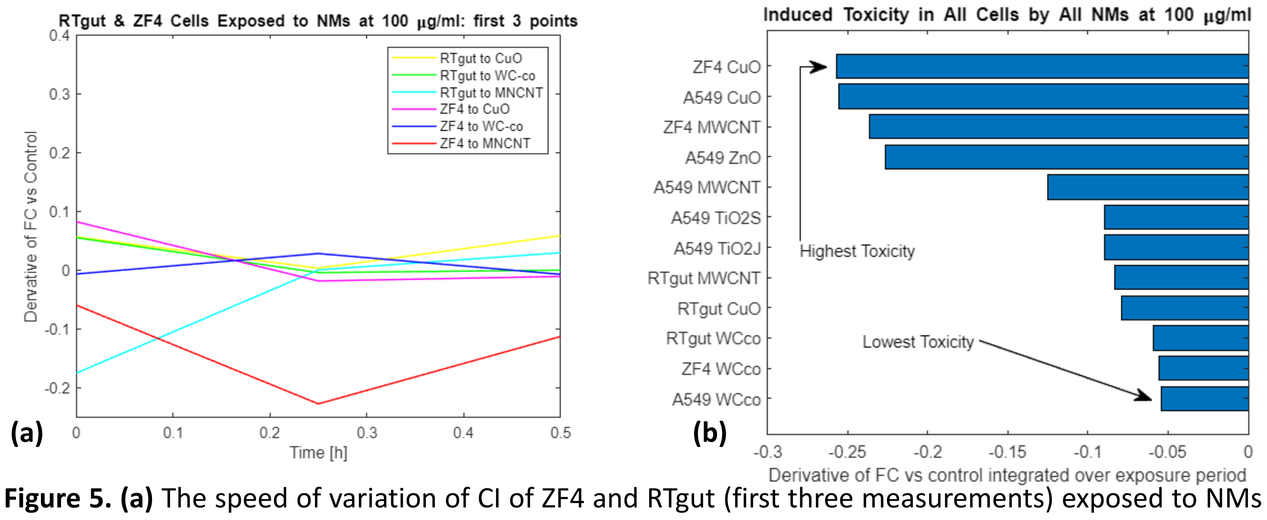

Figure 5. (a) The speed of variation of CI of ZF4 and RTgut (first three measurements) exposed to NMs in RR1 showing the CuO as the most toxic NM for ZF4 cells. **(b)** The induced nanotoxicity in all cells by all NMs ranged in descending order of nanotoxicity.

Conclusions

- The 8 nm TiO_2 and the 3.5 nm CeO_2 caused the highest AA consumption in CV tests.
- The CuO NMs induced the highest toxicity for ZF4 and A549 cells in xCELLigence tests.
- EI and CV are promising methods for cell death /proliferation and oxidative stress assessment, respectively, due to the advantages of being label-free and thus less prone to interferences and more environmentally friendly.

References

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