

## A multi-purpose platform for the assessment of the pro-oxidative potential of silver nanoparticles

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With the exponential growth of engineered Nanomaterials (NMs), there is an increasing need to find abiotic, fast, predictive and reproducible methods to test the ability of these materials to induce oxidative stress in cells. In many European projects the scientific community has studied the mechanisms of action underlying the adverse responses of the organism and has created tools to predict the effects caused by new materials already in the design phase of the material itself (Safe by Design concept). We compared abiotic colorimetric tests and Cytochrome C (cytC) tests with a cellular test (DCFDA-DH in A549 cells) to evaluate the production of Reactive Oxygen Species (ROS) due to different nanoparticle design to correlate the biological responses with the pro-Oxidative Potential (OP) assessed through the evaluation of the consumed moles of Glutathione (GSH) or P-Nitroaniline (RNO), a molecular probe specific for the detection of OH•. Different design strategies for silver nanoparticles (AgNPs)(ASINA EU project) with different biopolymeric coatings: Naked (NKD), HydroxyEthylCellulose (HEC), Polyvinylpyrrolidone (PVP) and Curcumin (CUR) has been compared. From the intracellular ROS tests, results show that AgHEC an AgCUR NPs do not induce an increase in ROS levels, while the ROS production increases in the case of AgNKD and AgPVP NPs. The same trend was also found in the case of the abiotic test with the GSH assay. As regards the tests of the intrinsic OP, the trend is reversed, with AgHEC NPs with higher intrinsic OP in comparison to the other AgNP tested. This follows what was found by the consumption test of the RNO molecule. The resulting strong correlation is very promising because provides a proof of concept for the use of abiotic tests for the evaluation of one of the main relevant mechanisms related to NPs toxicity.

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Conference presentation: this paper was presented at the Fourth Centro 3R Annual Meeting - The role of 3Rs in the age of One Health: where we are and where we're going - 13-15 September 2023, Università degli Studi Milano-Bicocca.

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