Nanotoxicity screening using impedance-based flow cytometry

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INTRODUCTION
Nanomaterials (NMs) have gained enormous attention due to their unique properties and have found use everywhere in daily life applications. However, this widespread use resulted in an increased release of manufactured NMs and raised concerns on their adverse impact on the environment and on human health [1-2]. Unfortunately, there is a lack of standardised methods to assess the toxicity potential of these NMs as traditional toxicity assays have been shown to interfere with NMs resulting in false negatives or false positives [3]. This raises the urgent need for alternative analytical tools to study nanotoxicity [4].

AIMS
1) Assess the suitability of the AmphaZ30 impedance-based flow cytometer (IFC) for nanotoxicity screening.
2) Validation of the IFC using the Trypan Blue (TB) dye exclusion assay.

MATERIALS AND METHODS
Nanomaterials and physicochemical characterisation
Nanomaterial dispersions of a selection of eight NMs provided by the European Commission Joint Research Centre Repository and by the Fraunhofer Institute for Molecular Biology and Applied Ecology (Germany) were prepared using the generic NANOGENOTOX dispersion protocol. Particle characterisation in dispersion was carried out by dynamic light scattering (Zetasizer Nano ZSP, Malvern Instruments Ltd., UK) and transmission electron microscopy (JEM-2100, JOEL, Japan).

Cell culture
A human lymphoma cell line, U937, was cultured in 6-well cell culture plates, exposed to NMs at clinically relevant concentrations (2, 10, 20, 50, and 100 µg/mL), and prepared for toxicity screening as shown in Figure 1.

RESULTS
None of the NMs showed property-specific interferences with the IFC for all tested concentrations. Similar results for the IFC and TB was found.

CONCLUSION
The IFC appears to be an effective and reliable technique for nanotoxicity screening. None of the eight tested NMs, with variable chemical composition, size, shape, and surface coating, showed interferences with the IFC measurements. However, these physicochemical properties played a role in cytotoxicity: dissolvable particles (ZnO and Ag) were the most toxic particles under the tested conditions. SiO2 and TiO2 were non- to moderate toxic. Furthermore, we found that smaller-sized TiO2 was more toxic on U937 cells compared to its larger analogues.

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