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.

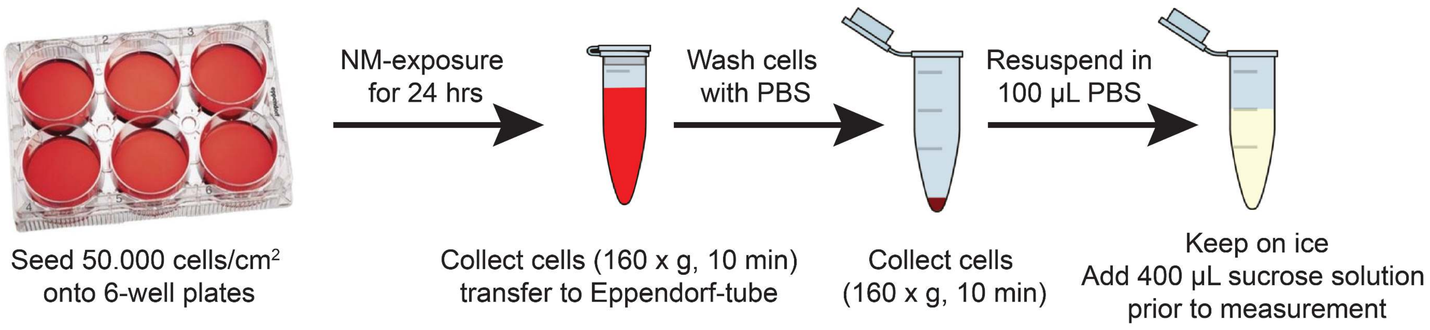

Figure 1: Illustrating the experimental setup and sample preparation for IFC measurements.

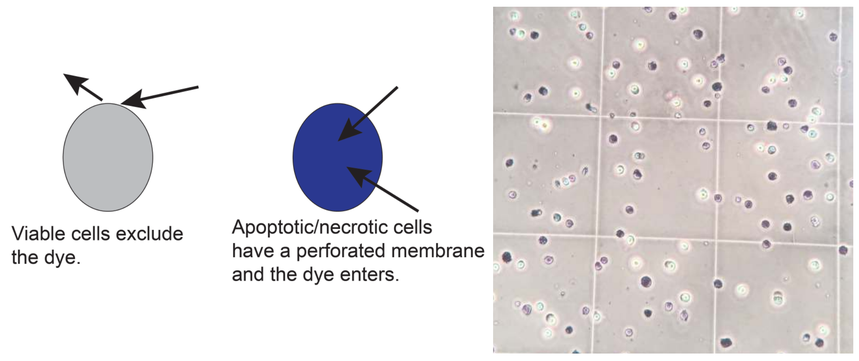

Figure 2: Trypan Blue assay. Cells with disrupted membrane take up the dye (blue cytoplasm); viable cells (clear cytoplasm) exclude it.

Toxicity screening

Impedance measurements using the AmphaZ30 (Amphasys AG, Switzerland) were carried out at four frequencies (0.5, 2, 6, and 12 MHz) using a microfluidic chip with a 50 µm x 50 µm-sized channel and following settings: Trigger level 0.02 V, Modulation 3, Amplifier 3, Demodifier 2, Pump 100 rpm. A total of 20,000 cells per sample were measured. Additionally, cell viability was assessed by a traditional viability assay (TB assay) using an automated cell counter (Countess ®, Invitrogen™, USA) (Figure 2).

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.

PHYSICO-CHEMICAL CHARACTERISATION

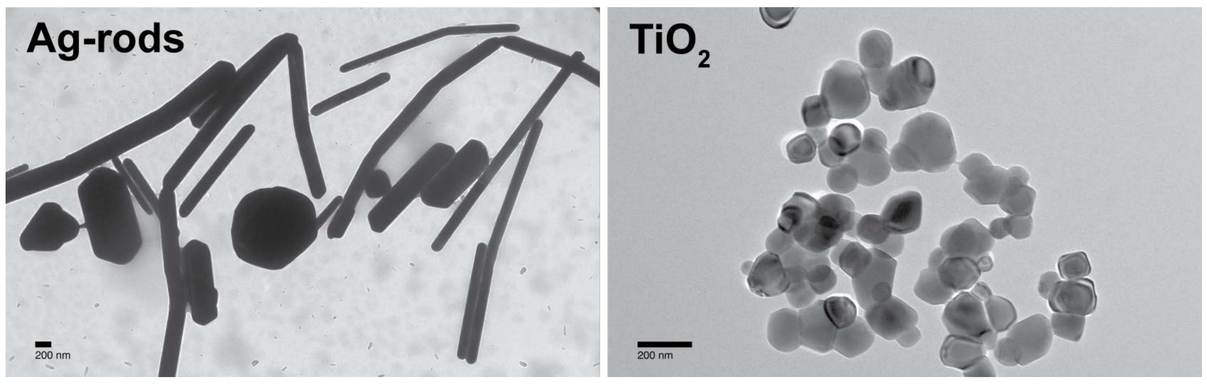

Figure 3: Transmission electron micrographs of Ag-rods and TiO_2 particles dispersed in 0.05% w/v BSA-water.

NANOTOXICITY SCREENING - ANALYSIS

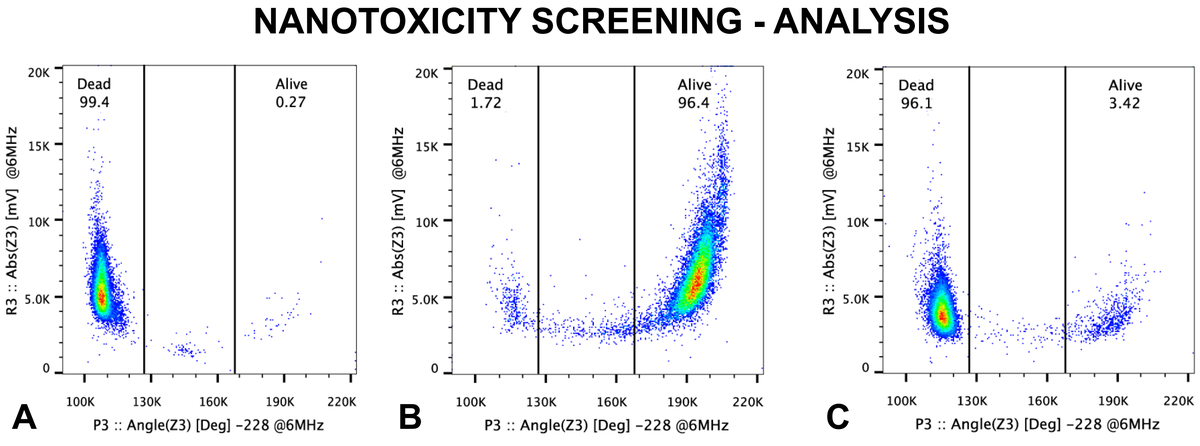

Figure 4: Impedance-based measurements of U937 cells exposed to spherical Ag particles with controls: (A) necrotic cells, (B) viable cells, and (C) cells exposed to Ag particles. Applied settings: Trigger level 0.02, Modulation 3, Amplifier 3, Demodifier 2, and pump speed 100 rpm.

METHOD COMPARISON

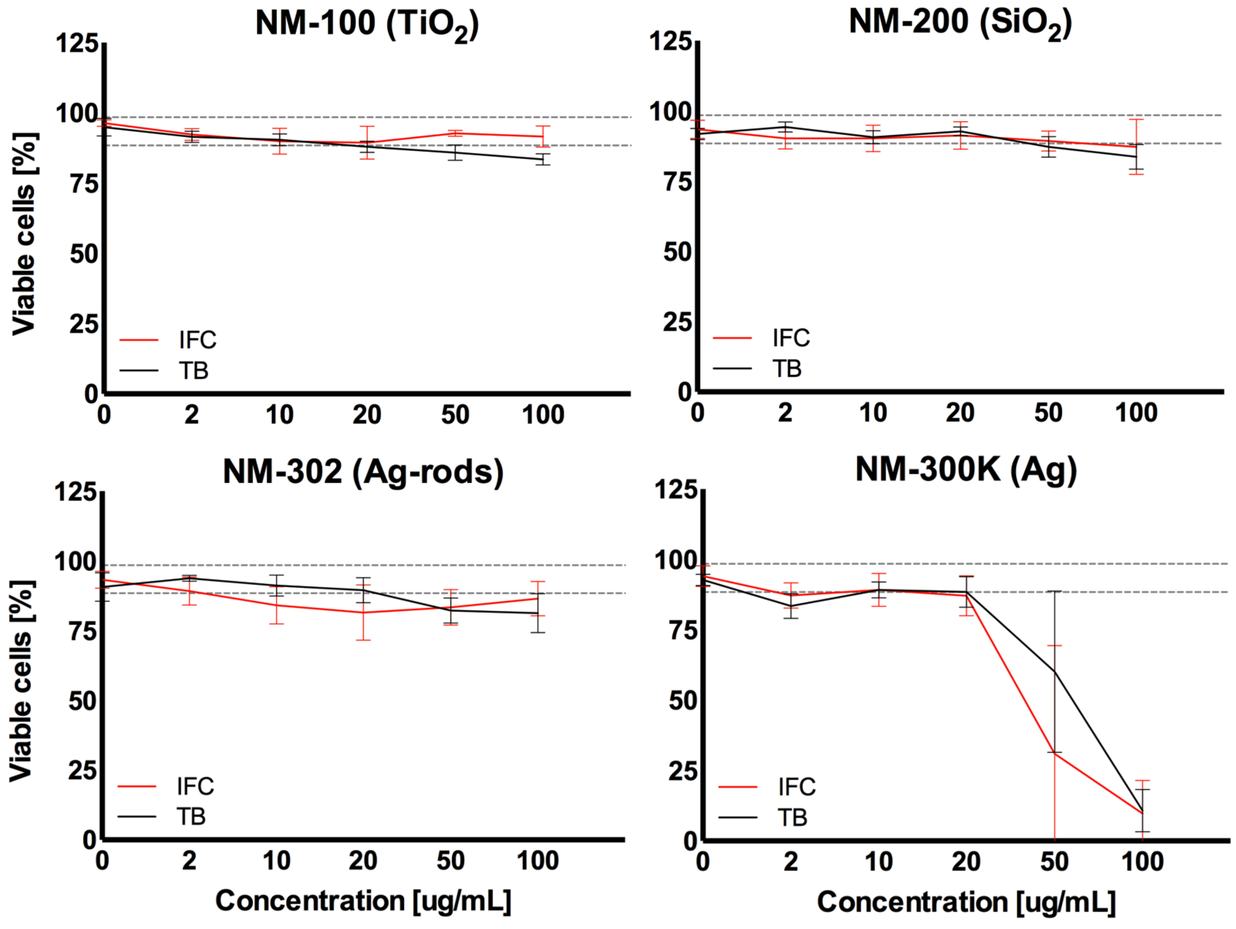

Figure 5: Dose-response curves of TB (black line) and IFC (red line) viability data. The dotted grey line represents the mean ± standard deviation of viable cells for all negative controls (TB and IFC together) in percentage (%).

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. SiO_2 and TiO_2 were non- to moderate toxic. Furthermore, we found that smaller-sized TiO_2 was more toxic on U937 cells compared to its larger analogues.

ACKNOWLEDGEMENTS

This work was supported by the EC FP7 NANoREG project (Grant Agreement NMP4-LA-2013-310584), the Norwegian Research Council, project NorNANoREG (239199/O70), Meltzer foundation, the EuroNanoMed II “GEMNS” project (246672), UH-Nett Vest, and the Department of Clinical Dentistry, Faculty of Medicine and Dentistry, University of Bergen.

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