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Validation of an Impedance-Based Flow Cytometry Technique for High-Throughput Nanotoxicity Screening

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Abstract : Background: New reliable and robust techniques to assess biological effects of nanomaterials (NMs) in vitro are needed to speed up safety analysis and to identify key physicochemical parameters of NMs, which are responsible for their acute cytotoxicity. The central aim of this study was to validate and evaluate the applicability and reliability of an impedance-based flow cytometry (IFC) technique for the high-throughput screening of NMs. Methods: Eight inorganic NMs from the European Commission Joint Research Centre Repository were used: NM-302 and NM-300k (Ag: 200 nm rods and 16.7 nm spheres, respectively), NM-200 and NM- 203 (SiO_2: 18.3 nm and 24.7 nm amorphous, respectively), NM-100 and NM-101 (TiO_2: 100 nm and 6 nm anatase, respectively), and NM-110 and NM-111 (ZnO: 147 nm and 141 nm, respectively). The aim was to assess the biological effects of these materials on human monoblastoid (U937) cells. Dispersions of NMs were prepared as described in the NANOGENOTOX dispersion protocol and cells were exposed to NMs at relevant concentrations (2, 10, 20, 50, and 100 µg/mL) for 24 hrs. The change in electrical impedance was measured at 0.5, 2, 6, and 12 MHz using the IFC AmphaZ30 (Amphasys AG, Switzerland). A traditional toxicity assay, Trypan Blue Dye Exclusion assay, and dark-field microscopy were used to validate the IFC method. Results: Spherical Ag particles (NM-300K) showed the highest toxic effect on U937 cells followed by ZnO (NM-111 ≥ NM-110) particles. Silica particles were moderate to non-toxic at all used concentrations under these conditions. A higher toxic effect was seen with smaller sized TiO2 particles (NM-101) compared to their larger analogues (NM-100). No interferences between the IFC and the used NMs were seen. Uptake and internalization of NMs were observed after 24 hours exposure, confirming actual NM-cell interactions. Conclusion: Results collected with the IFC demonstrate the applicability of this method for rapid nanotoxicity assessment, which proved to be less prone to nano-related interference issues compared to some traditional toxicity assays. Furthermore, this label-free and novel technique shows good potential for up-scaling in directions of an automated high-throughput screening and for future NM toxicity assessment. This work was supported by the EC FP7 NANoREG (Grant Agreement NMP4-LA-2013-310584), the Research Council of Norway, project NorNANoREG (239199/O70), the EuroNanoMed II 'GEMN' project (246672), and the UH-Nett Vest project.

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