

Evaluation of flow cytometry to detect micronucleus induction by different nanoparticles in BEAS 2B cells

Liliya Kazantseva¹, Alba Garc3a¹, Alba Hern3andez^{1,2}, Ricard Marcos^{1,2}

¹Grup de Mutag3nesi, Departament de Gen3tica i de Microbiologia, Facultat de Bioci3ncies, Universitat Aut3noma de Barcelona, Cerdanyola del Vall3s, Spain.

²CIBER Epidemiologia y Salud P3blica, ISCIII, Spain.

Nowadays the use of nanoparticles can be seen in many goods of daily use. As the use of these compounds is increasing, the human exposure does the same. Currently, there is no clear indication of the adverse effects produced by nanoparticles, as there is high heterogeneity between studies. To evaluate the genotoxic potential of these compounds an *in vitro* micronucleus (MN) assay by using flow cytometry was performed. This is an automated approach replacing the classical method of MN scoring by microscopy. The advantages of using flow cytometry are: i) high-throughput technology that enables to analyze more than 5000 cells in several minutes; ii) there is no need to use cytochalasin B that can interfere in the analysis; iii) it is a sensitive method that has multiparametric measurements; iv) is able to determine relative cell survival, percentage of apoptotic/necrotic cells and provide information on cell cycle; and finally v) it was observed to be useful in different cell lines, such as CHO, V79, L5178Y and CHL.

Thus, the aim of this study was to evaluate the applicability of flow cytometry to determine the genotoxicity of different nanoparticles since there are few reports using this method. For this, BEAS-2B cells were treated for 48 hours with 6 different nanomaterials: titanium dioxide (NM100 and NM101), zinc oxide (NM110), multi-walled carbon nanotubes (NM401), cerium oxide (NM212) and silver (NM300K), in the frame of an EU project (NanoReg). The nanomaterial's morphology and size was obtained by using transmission electron microscopy (TEM) and dynamic light scattering (DLS). The range of concentrations to be tested was selected based on previous cytotoxicity assays. To be able to perform flow cytometry a sequential staining was applied: ethidium monoazide bromide (EMA) added before cell lysis to show apoptotic/necrotic cells and SYTOX Green added in the lysis solution for staining of MN and nuclei. Each concentration was tested in duplicate.

Results indicate that both titanium dioxide and cerium oxide were not able to induce MN formation, while zinc oxide, multi-walled carbon nanotubes and silver induced significant increases in the frequency of MN. Our conclusion is that scoring MN induction by flow cytometry is a useful tool for rapid screening of potential genotoxicants that should be combined with other methods from a battery of assays.