Multiplicity of Nanofection: a New Index to Assess Nanoparticle Cellular Uptake

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Abstract

Engineered nanoparticles (ENPs) for biological applications are produced from functionalized nanoparticles (NPs) after undergoing multiple coupling and cleaning steps, giving rise to an inevitable loss of NPs in final compositions. Herein, we present a simple method to quantify the number of ENPs per microliter using standard spectrophotometers and volumes of up to one microliter. Light going through NP suspensions is scattered via reflection, refraction and diffraction phenomenon and the amount of the scattered light depend on the number of NPs found in suspensions. By measuring optical densities (OD) at 600 nm of different polystyrene NP suspensions of three different sizes (100 nm, 200 nm and 460 nm), linear correlations between OD600 and number of NPs were found for each NP size. These calibration curves can then be applied to estimate the number of ENP compositions of a particular NP size and material (Figure 1). To exemplify the method, we introduced the number of ENPs versus number of cells as a new parameter to report cellular uptake assays where capacities of cells to uptake beads or NPs (“nanofection”) need to be assessed. This parameter allows us to introduce “multiplicity of nanofection 50” (MNF_{50}) index, which is defined as the number of NPs per cell needed to “nanofect” 50% of a given cell type, as a measure of the capacity of a cell type to uptake certain ENPs. Three mammalian cell lines were tested with 200 nm Cy5-PEG-NPs and, following flow cytometry analysis, each of them presented different MNF50, being MDA MB 231 mammalian breast cancer cell line the one with a lower MNF_{50} and therefore with a higher uptaking capacity of these ENPs (Figure 2). Median of fluorescence intensity (MFI) of Cy5 positive cells analysis showed a linear behavior with different slopes for each cell line which is also a parameter to assess cell capacities for NPs uptaking (Figure 3). Furthermore, if we compare MFI increments (ΔMFI=MFI sample/MFI untreated) same results were obtained (Figure 4). A deeper study of ΔMFI showed a surprising data, from the closest ratio to their MNF_{50}, the increase of the ΔMFI is doubled when the NPs number are doubled, something which is not observed when ratios lowers than their MNF_{50} are used. Importantly, this effect is the same for the three studied cell lines. Therefore, when MNF_{50} is reached, the nanofection rate is constant and proportional to the number of nanoparticles used with cell lines presenting similar behavior. Nowadays the efficiency of many NPs-based delivery systems of bioactive cargoes are related to solid content (w/V) of NPs per cell. This method allows introducing a new parameter to analyze cellular uptake by reporting nanoparticle number versus cells number (*multiplicity of nanofection*). Based on these data we believe that the number of NPs per cell could be reported rather than weight of NPs per cell in any cell-based assays using NPs. The implementation of the Multiplicity of nanofection (MNF) will improve dramatically the efficiency of any nanoparticle-based devices.

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Figure 1

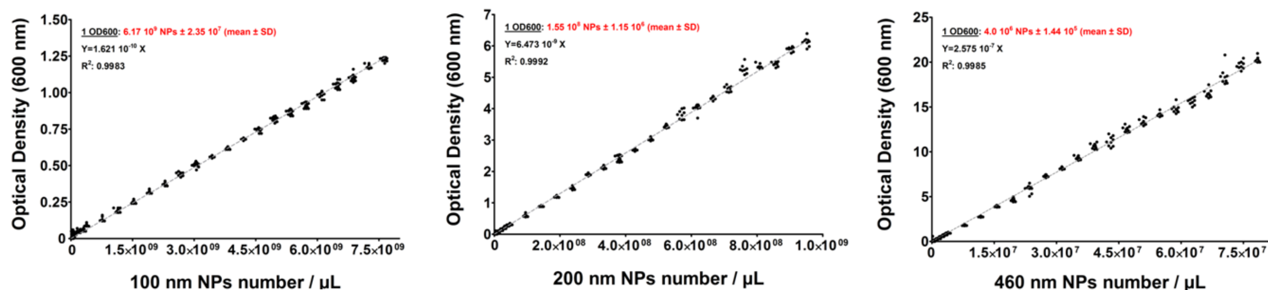

Figure 2

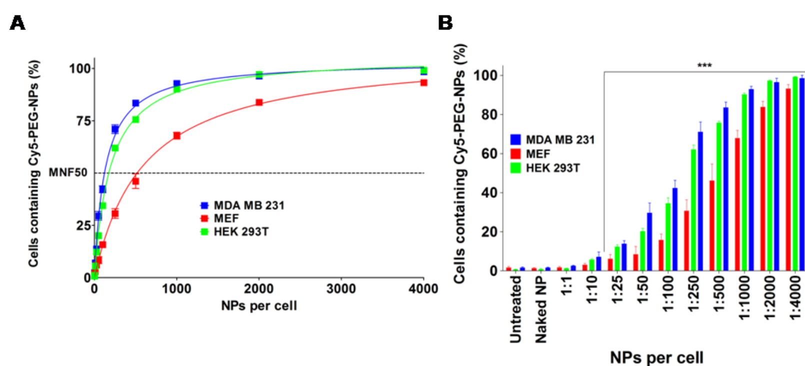

Figure 3

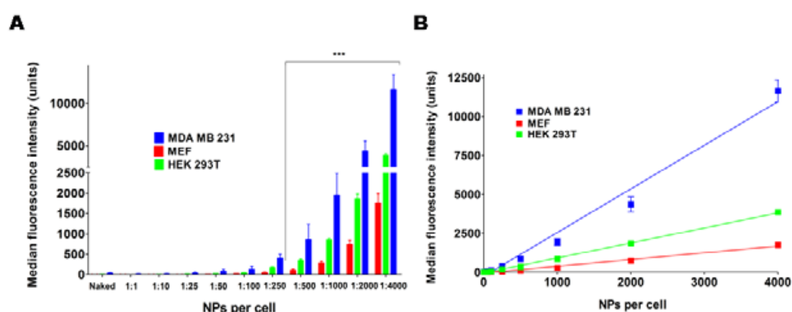

Figure 4