Development of a protocol to assess cell internalization and tissue uptake of nanoparticles by AC Biosusceptometry

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Several applications of nanoparticles rely on the internalization and accumulation of nanocarriers in specific cell compartments and tissues. The internalization process has been extensively explored for multiple particles and conjugations. However, the methods currently employed for characterizing such processes, although well described, are time-consuming and do not provide in vivo information, which is a crucial barrier towards translational applications. Here, we hypothesize that the AC Biosusceptometry technique can be employed to assess cell internalization of magnetic nanoparticles, with possible applications in screening assays to track specific biomarkers and cell types. The ACB system is a biomagnetic tool, extensively employed for magnetic nanoparticles detection and quantification in animal models. We tested this approach as a simpler and easier alternative to quantify cell internalization and to track specific cell-types in mixed cell cultures. Such application might offers considerable advantages, such as extrapolation to tissue accumulation after perfusion. We utilized citrate coated, manganese ferrite nanoparticles and evaluated the internalization process in mouse macrophages cells (P347), for protocol validation, and in an embryonic neural stem cells (E145) after differentiation in astrocytes and neurons, to assess internalization specificity. The differentiation was performed by EGF (Epidermal Growth Factor) removal. Respecting the particles toxicity limits, we tested different concentration of particles, in different incubation times. Throughout the experiments, we tested doses of 100-5000 particles per cell, incubating the cells with particles for 0.5-24 hours. We assessed particles toxicity by quantification of cell death by trypan blue assay. Sequentially, we imaged the cell cultures to confirm internalization and nanoparticles localization, labeling nucleus and cell body to assure that the particles were inside the cells and not simply attached to the well or to the cell membrane. For particle quantification, we trypsinized the wells and collected the cells to a volume controlled vial for further ACB analysis. Employing the ACB system for quantification, we were able to quantify particles in all doses and with all incubation times, indicating low toxicity of the nanocarriers and good sensitivity of the ACB system for cell internalization. Our results showed a non-linear pattern of accumulation indicating a saturation process after 1000 particles per cells approximately. These positive results suggest that this approach may be the initial step towards a new nanoparticle internalization assay, that offers faster analysis, that demands considerably less time for sample preparations, providing good signal to noise ratio and sensitivity.