Safety and internalization effectiveness of magnetic iron oxide nanoparticles

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Recent advances in nanoscience and nanotechnology have led to the development of nanomaterials for medical applications. Magnetic nanoparticles (MNPs) are attracting widespread attention because of their applications in biomedicine, in particular with the diagnostic and therapeutic purposes. However, a critical issue in nanomaterials is the clear understanding of their potential toxicity. Physical and chemical characteristics of nanoparticles (such as size, chemical composition, crystalline structure and surface properties) are proposed to determine critically their toxic potential as well as their specific accumulation in tumor cells. In this sense, quantitative study of nanoparticle uptake at the cellular level is critical for nanomedicine applications as first step to evaluate their biocompatibility before in vivo analysis.

Hence, any new magnetic nanoparticle (MNPs) formulation with potential biomedical applications should be accompanied by a detailed study that ensures both its entry effectiveness and safety. In this sense, several specific parameters and experimental protocols for assessing nanomaterial toxicity have been developed.

In this study we present a deep analysis on biocompatibility and intracellular accumulation of iron oxide nanoparticles with three different coatings: aminodextran (AD), 3-aminopropyl-triethoxysilane (APS), and dimercaptosuccinic acid (DMSA), which result in different particle charge, in HeLa (human cervical adenocarcinoma) cells. This cell line is commonly used for cytotoxicity evaluation in different research fields, including nanomaterials, and therefore it provides a good basis for comparison. Iron oxide nanoparticles were used because of its low toxicity and the existence of natural routes for its degradation. MNPs were obtained by different synthetic routes, including coprecipitation and decomposition in organic media.

Uniform nanoparticles in size and shape were observed by transmission electron microscopy. Colloidal characterization was performed by dynamic light scattering (DLS) showing hydrodynamic size below 150 nm (which assure good properties) and a Z-potential ranging from highly positive for APS coated MNPs to moderate positive and highly negative for AD and DMSA coated particles respectively (Figure 1).

Uptake of MNPs as well as their accumulation inside HeLa cells after prolonged incubation (up to 72 h), were assessed by light and scanning electron microscopy methods and quantification of intracellular iron content by ferrozine assay, thus allowing to correlate the overall cell visualization and their subcellular location with the precise amount of MNPs inside cells. These nanoparticles show excellent properties for possible in vivo biomedical applications such as cell tracking by magnetic resonance imaging (MRI) and cancer treatment by hyperthermia or drug delivery: (i) they enter into cells with effectiveness (higher in APS coated MNPs than in DMSA or AD MNPs) (see Figures 2 and 3) and are localized in endosomes; (ii) they can be detected inside cells by optical microscopy, (iii) they are retained for relatively long periods of time, and (iv) they do not induce any cytotoxicity, which was assessed by cell morphology observation, study of cytoskeleton and adhesion proteins, analysis of cell cycle, presence of intracellular ROS (reactive oxygen species), MTT(methyl thiazol tetrazolium bromide) assay and Trypan blue exclusion test [1].

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References

[1] M. Calero, L. Gutiérrez, G. Salas, Y. Luengo, A. Lázaro, P. Acedo, MP. Morales, R. Miranda, A. Villanueva. **Nanomedicine** (2014) 10, 733-43.

Figures

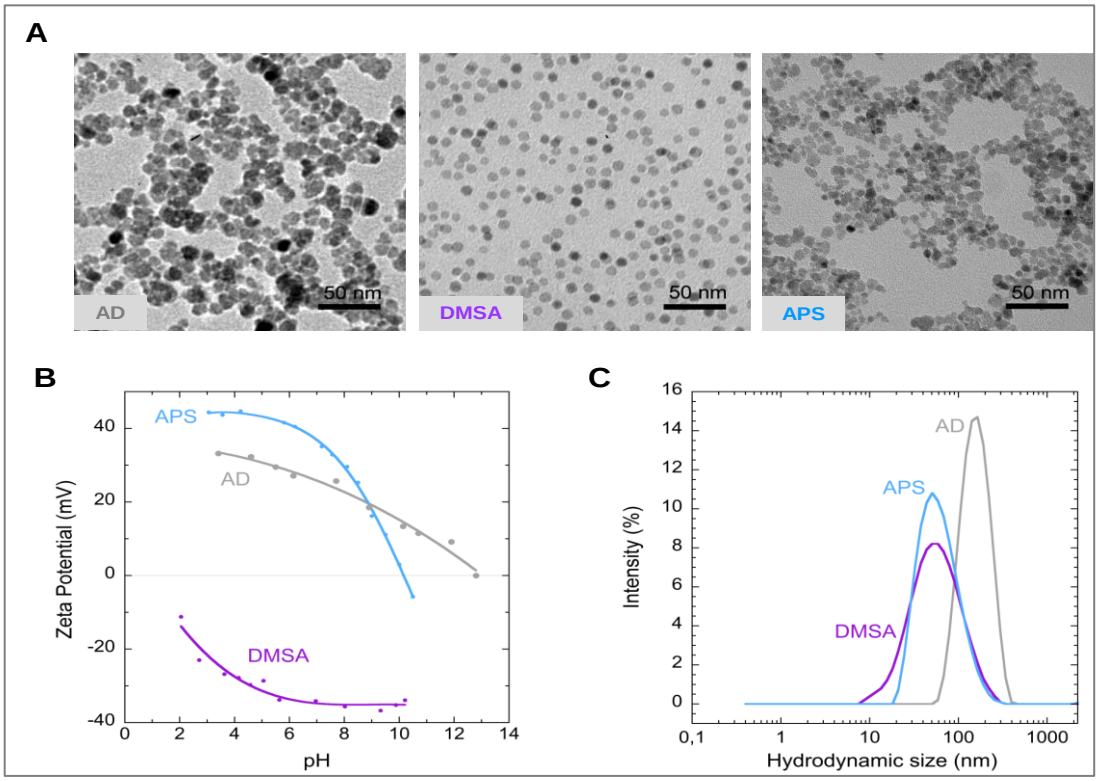

Figure 1. (A) TEM images of AD, DMSA and APS-coated magnetic nanoparticles. **(B)** Surface charge variation as a function of pH for MNPs. **(C)** Hydrodynamic size for magnetic nanoparticles.

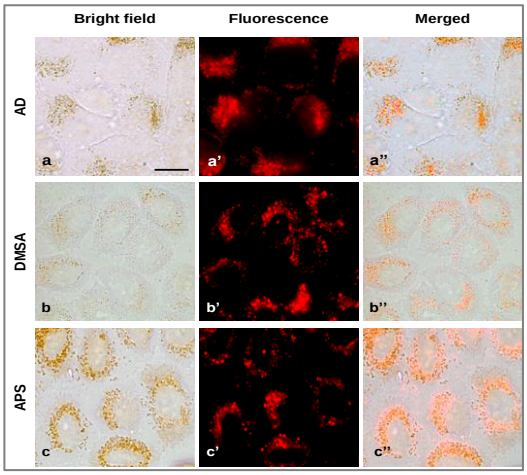

Figure 2. Subcellular location of MNPs. **(a-c)** Visualization of AD, DMSA and APS nanoparticles in living cells, respectively. **(a′-c′)** Lysosomes labeled with LysoTracker Red in the same cells. **(a″-c″)** Bright field signal merged with LysoTracker staining. Scale bar = 10 µm.

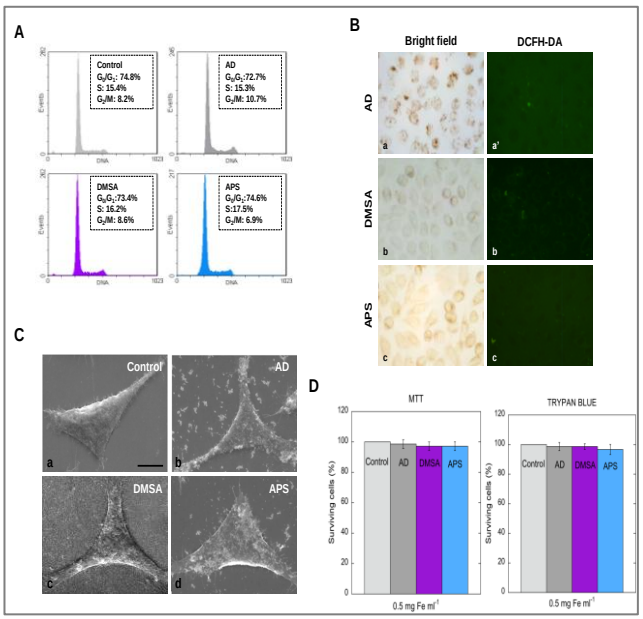

Figure 3. (A) Cell cycle of control and cells incubated with MNPs. **(B)** Qualitative characterization of ROS generation by DCFH-DA assay. **(C)** SEM pictures of HeLa cells. (a) Control cell. (b-d) Cells incubated for 3 h with 0.1 mg ml^{-1} AD, DMSA and APS, respectively. Scale bar = 10 µm. **(D)** No significant differences on cell survival, in comparison with controls, were observed 24 h after incubation with MNPs by MTT or Trypan blue assay.