ELSEVIER

Nanomedicine: Nanotechnology, Biology, and Medicine
xx (2014) xxx – xxx

nanomedicine
Nanotechnology, Biology, and Medicine

nanomedjournal.com

Efficient and safe internalization of magnetic iron oxide nanoparticles: Two fundamental requirements for biomedical applications

Macarena Calero, MS[a,b], Lucía Gutiérrrez, PhD[c], Gorka Salas, PhD[b,c], Yurena Luengo, MS[c], Ana Lázaro, MS[b], Pilar Acedo, MS[a], M. Puerto Morales, PhD[c], Rodolfo Miranda, PhD[b,d], Angeles Villanueva, PhD[a,b,*]

[a]*Departamento de Biología, Universidad Autónoma de Madrid, Darwin 2, Cantoblanco, Madrid, Spain*
[b]*IMDEA Nanociencia, Faraday, 9, Cantoblanco, Madrid, Spain*
[c]*Instituto de Ciencia de Materiales de Madrid, ICMM-CSIC, Sor Juana Inés de la Cruz 3, Cantoblanco, Madrid, Spain*
[d]*Departamento de Física de la Materia Condensada, Universidad Autónoma de Madrid, Francisco Tomás y Valiente 7, Cantoblanco, Madrid, Spain*

Received 11 June 2013; accepted 19 November 2013

Abstract

We have performed a series of in vitro tests proposed for the reliable assessment of safety associated with nanoparticles-cell interaction. A thorough analysis of toxicity of three different coating iron oxide nanoparticles on HeLa cells has been carried out including, methyl thiazol tetrazolium bromide (MTT) and Trypan blue exclusion tests, cell morphology observation by optical and Scanning Electron Microscopy (SEM), study of cytoskeletal components, analysis of cell cycle and the presence of reactive oxygen species (ROS). We have quantified magnetic nanoparticle internalization, determined possible indirect cell damages and related it to the nanoparticle coating. The results confirm a very low toxicity of the analyzed iron oxide nanoparticles into HeLa cells by multiple assays and pave the way for a more successful cancer diagnostic and treatment without secondary effects.

Key words: Iron oxide magnetic nanoparticles; HeLa cell line; Cytoskeleton; Cytoxicity; Cellular uptake

Background

In the last decade there has been a spectacular development of magnetic nanoparticles (MNPs) for biomedical applications, such as magnetic carriers for drug delivery aided by external magnetic fields, magnetic resonance imaging contrast agents or cancer therapy compounds for hyperthermia, among others.[1-3] More recently, new multifunctional magnetic nanoparticles capable of carrying out simultaneously a dual function, cancer diagnosis and therapy, are under investigation. This promising new strategy in cancer treatment has been called theranostics.[4-8]

Magnetic nanoparticles for biomedical applications are usually formed by a mineral core of a magnetic element, such as iron, nickel, cobalt and their oxides, and an organic coating, such as dextran, polyethyleneglycol, poly(vinylpyrrolidone), streptavidin, poly-L-lysine, polyethylene imide, among others.[9-11] Due to the lower toxicity of iron oxides, the most commonly employed MNPs for biomedical applications have a magnetite or maghemite (Fe_3O_4 or γ-Fe_2O_3) core. The MNP physicochemical properties such as particle size, shape, hydrophilic nature, coating and surface charge will determine in a great extent biodistribution and biocompatibility.[10,12]

In parallel with the improvement of the nanoparticles physicochemical properties, the evaluation of their potential risk on human health has become a critical objective in nanomedicine.[13-16] In vitro analysis on cell cultures allow us to gain insights on the nanoparticle-cell interactions at both cellular and molecular levels.[17] Cell cultures have become the first indispensable step to evaluate nanoparticle effectiveness and safety and are used for pre-screening nanomaterials before in vivo analysis. New methods and modified versions of pre-existing methods have been proposed for assessing nanomaterial toxicity.[13] Unfortunately, due to the wide array of nanoparticles with or without surface modification, types of cells tested, MNPs concentrations and techniques used to address different aspects of potential cytotoxicity, biocompatibility studies on MNPs lead sometimes to conflicting results.[18] Therefore it would be

Conflict of Interest: The authors declare no competing financial interest.

*Corresponding author: Departamento de Biología, Universidad Autónoma de Madrid, Cantoblanco-28049, Madrid, Spain.
E-mail address: angeles.villanueva@uam.es (A. Villanueva).

1549-9634/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.nano.2013.11.010

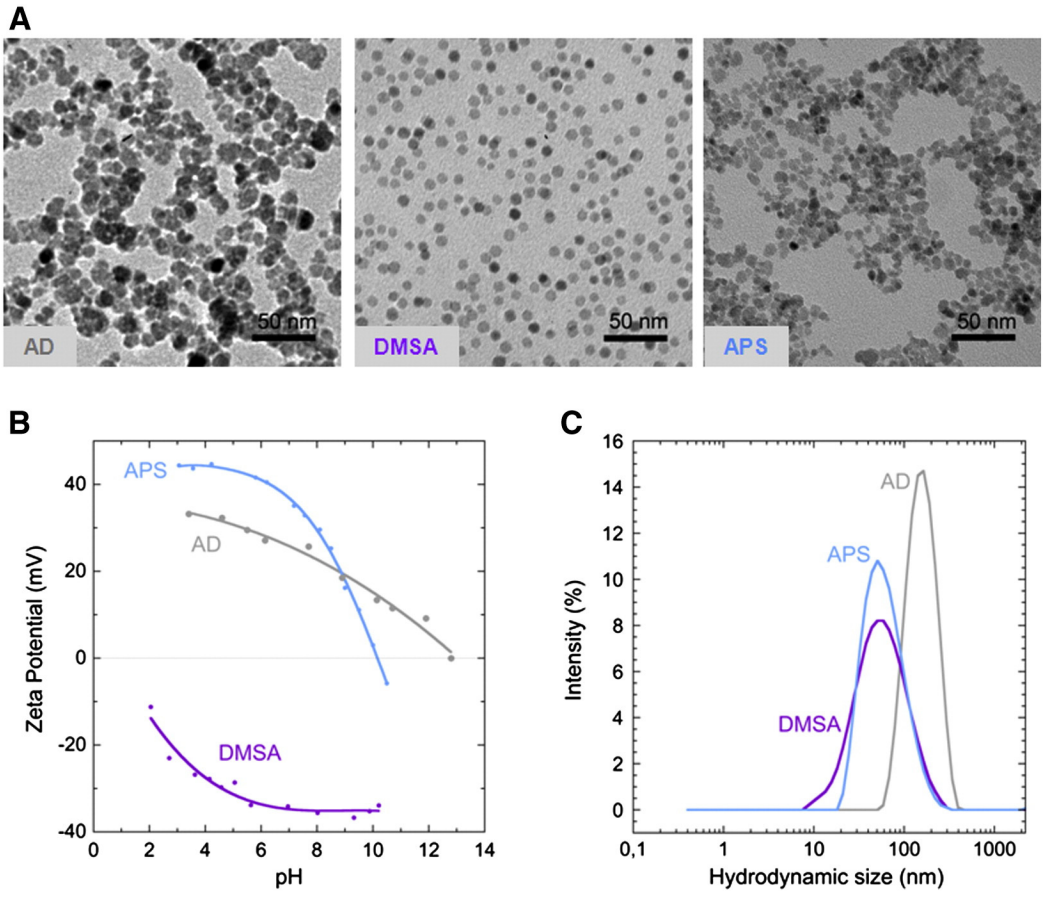

Figure 1. (**A**) TEM images of AD, DMSA and APS-coated magnetic nanoparticles. (**B**) Surface charge variation as a function of pH for AD, DMSA and APS coated magnetic nanoparticles. (**C**) Hydrodynamic size for AD, DMSA and APS coated magnetic nanoparticles.

fundamental to standardize the conditions of cell lines and MNPs concentration that are employed to evaluate the new materials cytotoxicity,[19] including the generation of reactive oxygen species (ROS).[20,21] Several studies have shown that iron oxide nanoparticles are able to induce cytotoxic effects, such as mitochondrial alteration function, DNA strand breaks and cytoskeleton alterations, via ROS generation, which ultimately would lead to cell death.[8-10,14]

In this study we present a deep analysis on biocompatibility and 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.[22] Previous studies on similar nanoparticles have proven that they do not induce any direct cytotoxicity on this cell line, but analysis about possible indirect damages at different MNPs concentrations were not performed.[12]

Methods

Particle synthesis and characterization (see details in Supplementary Material section)

Iron oxide nanoparticles where used because of its low toxicity and existence of natural routes for its degradation.[23] MNPs were obtained by different synthetic routes, including coprecipitation and decomposition in organic media (see details in Supplementary Material section). Three different compounds: (3-aminopropyl-triethoxysilane (APS), aminodextran (AD), and dimercaptosuccinic acid (DMSA) where used to coat the particles to obtain different surface charge. Particle size and shape and size distribution were measured by transmission electron microscopy (TEM). Colloidal characterization was performed by dynamic light scattering (DLS) to analyze the MNPs hydrodynamic size and Z-potential.

Cell culture and characterization (see details in Supplementary Material section)

Human cervix carcinoma HeLa cells were grown as monolayer cultures in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% (v/v) fetal bovine serum (FBS), 50 units ml^{-1} penicillin and 50 μg ml^{-1} streptomycin in an incubator with 5% CO_2 plus 95% air at 37 ºC. Subconfluent cell cultures were used. HeLa cells were incubated for 24 h or 72 h with different concentrations of nanoparticles (0.05, 0.1, and 0.5 mg ml^{-1} culture medium) depending on the experiment.

Cells were observed under bright light microscopy without being processed to avoid potential artifacts of cell fixation, in order to analyze nanoparticles internalization. Cells were also