Cell response against water stable magnetic nanoparticles obtained by thermal decomposition procedure

María Moros, Beatriz Pelaz, Carlos Cuestas, Valeria Grazú, Jesús M. de la Fuente.
Instituto Universitario de Investigación en Nanociencia de Aragón (INA), Cerbuna 12, 50009-Zaragoza, Spain
mamoros@unizar.es

Magnetic nanoparticles offer a wide range of new opportunities including the quality improvement of contrast agents for MRI, hyperthermic treatment, and site-specific drug delivery. All these biological applications of these nanoparticles require the fulfilment of several features: high magnetization values, size smaller than 20 nm, narrow particle size distribution, simple biofunctionalization and a special surface coating to prevent nanoparticles aggregation, opsonization and toxicity effects [1, 2]. Very recently, Sun and co-workers [3, 4] had markedly improved the synthesis of monodispersed magnetite particles with size around 4 nm by thermal decomposition of iron (III) acetylacetonate in phenyl ether in the presence of oleic acid and oleylamine. As these nanoparticles are coated with a hydrophobic organic layer, they are only soluble in hexane and other non-polar or weakly polar organic solvent. However, these nanoparticles must be transfer to water to be used for biological applications. In this sense, we report here the optimization of two new procedures to prepare magnetite nanoparticles with an average size of 6 nm, a narrow size distribution and high stability in water and physiological media.

The first procedure is based in a previously reported strategy [5], which takes advantage of the hydrophobic surfactant layer of these nanoparticles to introduce an amphiphilic polymer shell. The hydrophobic nature of our nanoparticles can interact with the hydrophobic portion of this polymer. Water solubility of the nanomaterials will be ensured by the hydrophilic portion of the attached polymer. We have optimized the procedure with a different polymer (poly[maleic anhydride alt-1-octadecene]) which has a higher molecular weight than the one previously reported. Beyond the different evaluated parameters, the hydrolysis of the anhydride groups of the polymer resulted the key step for a fully solubilization of nanoparticles in aqueous media with any loss. The second procedure consists in the coating of the magnetic core with a thin gold shell following the procedure described by Wang and co-workers [6]. Then pegylated linkers modified with a thiol moiety [7] were used to drive nanoparticles into water. The water dispersed iron oxide nanocrystals obtained by both procedures were very stable in water and physiological buffers. In both cases, the presence of the outer shells was confirmed by TGA and the characterization was completed with transmission electron microscopy (TEM) and dynamic light scattering.

The influence of these nanoparticles on Hella cell line and human fibroblasts was assessed in vitro in terms of citotoxicity, morphology and cytoeskeleton organization. Standard cell viability assays (MTT and Trypan blue exclusion) demonstrated that cells incubated with all the synthesized nanoparticles remained more than 90% viable after 24 hours of incubation at concentration as high as 1 mg/ml. No difference was observed between the nanoparticles with pegylated linkers and without them, although at high concentrations (i.e 3mg/mL) nanoparticles with PEG resulted more stable. Nanoparticles conjugated with Rhodamine entered the cell, where they concentrated close to the nucleus; these data suggest some type of endocytic

internalization. To evaluate the non-specific proteins binding capacity of the synthesized nanoparticles, we used serum albumin (the most abundant blood protein) as a model. Little amount of BSA was found in nanoparticles, both with pegylated linkers and without them. This result suggests that nanoparticles will achieve long blood circulation half-lives as the removal of particle from circulation by the mononuclear phagocyte system (MPS) begins with the adsorption of plasma proteins.

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