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Design and characterization of a magnetite/PEI multifunctional nanohybrid as non-viral vector and cell isolation system

Raúl Megías[a], Maialen Arco[b,c], Jesús Ciriza[b,c], Laura Saenz del Burgo[b,c], Gustavo Puras[b,c], Margarita López-Viota[a], Ángel V. Delgado[d], Jon P. Dobson[e], José L. Arias[a,f,g], José L. Pedraz[b,c,*]

[a] Department of Pharmacy and Pharmaceutical Technology, Faculty of Pharmacy, University of Granada, Granada, Spain
[b] NanoBioCel Group, University of the Basque Country (UPV/EHU), Vitoria-Gasteiz, Spain
[c] Biomedical Research Networking Center in Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Vitoria-Gasteiz, Spain
[d] Department of Applied Physics, Faculty of Sciences, University of Granada, Granada, Spain
[e] Department of Biomedical Engineering and Medical Physics, Institute for Science & Technology in Medicine, Keele University, Staffordshire, United Kingdom
[f] Institute of Biopathology and Regenerative Medicine (IBIMER), University of Granada, Granada, Spain
[g] Biosanitary Institute of Granada (ibs.GRANADA), Andalusian Health Service (SAS), University of Granada, Granada, Spain

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ABSTRACT

It is described the reproducible formulation and complete physicochemical characterization of nanohybrids based on magnetite (Fe_3O_4) cores embedded within a polyethylenimine (PEI) matrix. Particle size, surface electrical charge, X-ray diffraction and Fourier transform infrared spectroscopy (FTIR) analyses, and magnetic field-responsive behaviour characterizations defined that the 4:3 (Fe_3O_4:PEI) weight proportion led to the best production performances of magnetically responsive nanocomposites in which the magnetic nuclei are completely covered by the polymeric shell. Agarose gel electrophoresis assays demonstrated the capacity of the Fe_3O_4/PEI particles to condense, release, and protect the DNA against enzymatic degradation. *In vitro* assays were performed to evaluate the transfection efficiency (up to 4.5% of transfected HEK-293 cells at a 10/1 PEI/DNA ratio), iron absorption by D1-mesenchymal stem cells (D1-MSCs, high values after only 15 min of magnetic incubation), influence on metabolic activity (negligible effect up to 44 μg nanocomposites/10^5 cells), and cell isolation capacity of the core/shell particles (significant increase in the retention of D1-MSCs transduced with green fluorescent protein). The Fe_3O_4/PEI nanohybrids hold promising characteristics suggestive of their capacity for transfection and cell isolation applications.

1. Introduction

Superparamagnetic iron oxide nanoparticles (NPs) have proved a great potential for biomedical applications, *e.g.* drug delivery (Arias et al., 2001, 2009, 2011), contrast agents in magnetic resonance imaging (Ahmad et al., 2015; Ma et al., 2015), hyperthermia (Munoz de Escalona et al., 2016; Sadhasivam et al., 2015), gene therapy (Arsianti et al., 2010; Ma et al., 2011; Plank et al., 2011), or cell isolation (Abdel Fattah et al., 2016; Lokmic 2016) to cite just a few. Among the iron oxide particulate systems, magnetite (Fe_3O_4) is classically described as a referent on the design of biocompatible and biodegradable magnetic nanocarriers, since it is a well-known material with relative low toxicity and well tolerated (Müller et al., 1996; Okon et al., 1994; Reddy et al., 2012). The crystalline structure of Fe_3O_4 has been described to be responsible for the superparamagnetic behaviour and the more than acceptable magnetic responsiveness (Reddy et al., 2012). Surface functionalization of these iron oxide NPs can help in preventing the stability problems associated to such a nano-sized system and in optimizing the control of their *in vivo* fate (Laurent et al., 2008; Reddy et al., 2012).

Branched polyethylenimine (*b*PEI) is a biocompatible polymer characterized by its content in repeated units of primary,

* Corresponding author at: NanoBioCel Group, University of the Basque Country (UPV/EHU), Vitoria-Gasteiz, Spain.
E-mail address: joseluis.pedraz@ehu.es (J.L. Pedraz).

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secondary, and ternary amine groups (Suh et al., 1997). First report describing the potential applications of the polymer was devoted to the prevention of flocculation in colloids (Thiele and von Levern, 1965), while its significant possibilities in Biomedicine were described later (Jagur-Grodzinski, 1999). Probably one of the most important applications of *b*PEI can be found in gene therapy given its strong deoxyribonucleic acid (DNA) condensation capacity at physiological pH (pH 7.4), high uptake by cells, proton-sponge potential, and intrinsic endosomal activity (Lungwitz et al., 2005); despite the promising possibilities of the polymer in the synthesis of star polymers (Cao et al., 2008) or in the formulation of drug delivery systems (Qiu and Bae, 2007). Thanks to the positively charged amine groups, *b*PEI can interact with negatively charged cell membranes, thus facilitating endocytic processes and accumulation in the cytoplasm of cells (Rejman et al., 2006). Unfortunately, the safe use of *b*PEI has been hindered by controversy on its cytotoxicity associated to cell membrane disruption and induction of apoptosis (Kawakami et al., 2006).

In this work, attention is given to the development of a reproducible methodology for the formulation of Fe_3O_4/PEI (core/shell) nanocomposites for gene delivery and cell isolation applications. Commonly nanocarriers based on magnetite have been either studied by their gene delivery applications or by their cell isolation properties but both applications have rarely or never been tested in the same system, envisioning future new applications such as the concentration of transfected cells. The detailed physicochemical characterization of nanoparticles included the selection of the optimum Fe_3O_4:PEI weight proportion for the preparation of the nanohybrids, along with the characterization of the geometry, chemical structure, and magnetic field-responsiveness. *In vitro* studies on human embryonic kidney-293 (HEK-293) cells helped to evaluate transfection efficiency of Fe_3O_4/PEI NPs, qualitatively by fluorescence microscopy and quantitatively by flow cytometry. Finally, iron absorption, viability, and cell enrichment assays were carried out in D1-mesenchymal stem cells (D1-MSCs).

2. Materials and methods

2.1. Materials

All chemicals were of analytical quality from Sigma-Aldrich Co. (Spain). Before use, water was deionized and filtered with a Milli-Q® System (Merck Millipore Co., Germany).

2.2. Methods

2.2.1. Synthesis of Fe_3O_4 nuclei and Fe_3O_4/PEI NPs

Colloidal Fe_3O_4 was prepared by chemical co-precipitation, as previously described (Munoz de Escalona et al., 2016). Then, the Fe_3O_4 NPs were re-dispersed in a 0.1 N citric acid solution, sonicated for 40 min, and finally the dispersion was adjusted to a pH 7 with 0.5 N NaOH.

To obtain Fe_3O_4/PEI NPs, a *b*PEI (25 kDa) aqueous solution was added drop-wise to an iron oxide aqueous dispersion under mechanical stirring (2000 rpm). After 5 min, the dispersion was neutralized to pH 7 with 0.5 N HCl. In order to define the effect of the relative amounts of Fe_3O_4 and *b*PEI on the characteristics of the nanomaterial, the formulation of the nanohybrid was done with Fe_3O_4:*b*PEI proportions ranging from 1:4 to 4:1. Finally, the core/shell particles were magnetically cleaned by repeated separation of the NPs from the liquid medium by a permanent magnet (0.4 T), and re-dispersion in water until the conductivity of the supernatant was $\leq$10 μS/cm. Production performance (yield, %) of the preparation conditions was determined [(nanocomposites obtained (mg)/sum of materials used in their preparation (mg)) × 100].

2.2.2. Geometry and electrophoretic characterization

The mean hydrodynamic diameter (and polydispersity index, PdI) of the colloids was determined by dynamic light scattering (Zetasizer Nano ZS, Malvern Instruments Ltd., UK). The measurement of the aqueous dispersions (≈0.1%, w/v) was done in triplicate at room temperature. To qualitatively confirm these determinations, the particles were observed by scanning electron microscopy (SEM, SUPRA™ 40VP field emission scanning electron microscope, Germany). Before visualization, drops of the NP dispersion (≈0.1%, w/v) were placed on carbon-coated copper grids which were then dried at 25.0 ± 0.5 °C in a convection oven.

Electrophoretic measurements in water and in the presence of KNO_3 0.1 mM were accomplished for checking the characteristics of the *b*PEI coating onto the iron oxide cores when the Fe_3O_4:*b*PEI ratios ranged from 1:4 to 4:1. Measurements were performed at room temperature (Zetasizer Nano ZS, Malvern Instruments Ltd., UK), after 24 h of contact under mechanical stirring (50 rpm). The surface electrical properties of the colloids were further investigated by evaluating the influence of pH on the zeta potential (ζ) of the particles in the presence of 10^{-3} M KNO_3. ζ was also determined as a function of KNO_3 concentration at a constant pH ≈5.

2.2.3. DNA complexation capacity

Fe_3O_4/PEI/DNA nanocomplexes were prepared by mixing during 15 s an appropriate volume of pmax-GFP (1 mg/mL stock solution) with Fe_3O_4/PEI NPs (of 4:3 weight proportion). Size and ζ determinations were done at room temperature in dilute aqueous NP dispersions (≈0.1%, w/v) (Zetasizer Nano ZS, Malvern Instruments Ltd., UK).

An agarose gel electrophoresis assay was performed to evaluate the ability of the core/shell NPs to condensate, release, and protect the DNA (*i.e.* pmax-green fluorescent protein plasmid, pmax-GFP, Lonza Group Ltd., Switzerland) against enzymatic digestion. To that aim, Fe_3O_4/PEI/DNA ternary complexes (4/3/1 weight ratio, 20 μL containing 200 ng of pmax-GFP) were loaded to an agarose gel (0.8%, w/w) which was immersed in a tris/acetate/ethylenediaminetetraacetic acid (EDTA) (TBE) buffer and exposed for 30 min to 120 V. DNA bands were stained with GelRed™ (Biotium Inc., USA) and images were obtained (ChemiDoc™ MP Imaging System with Image Lab™ software). Sodium dodecyl sulphate (SDS, 3.5%, w/v) and the enzyme DNase I (1 unit of enzyme/2.5 μg DNA) were added to the samples to evaluate the DNA release and protection, respectively. Integrity of the DNA in the samples was compared to controls.

2.2.4. X-ray diffraction and fourier transform infrared spectroscopy (FTIR) analyses

Characterization of the internal structure of the iron oxide nuclei, and the nanohybrids was done by X-ray diffractometry (Philips PW1710 diffractometer, The Netherlands). Fourier transform infrared spectrometry (JASCO FT/IR-6200 FTIR spectrometer, UK) data were used to complete the chemical characterization of the NPs.

2.2.5. Magnetic responsive behaviour

Hysteresis cycles of the Fe_3O_4 and Fe_3O_4/PEI NPs were determined at 25.0 ± 0.5 °C (Manics DSM-8 vibrating magnetometer, France). Their field-responsive behaviour was qualitatively analysed by optical visualization of a 0.3% (w/v) aqueous dispersion under the influence of a 0.4 T permanent magnet placed close to the glass vial containing the NPs.