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Size-dependent transfection efficiency of PEI-coated gold nanoparticles

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ABSTRACT

Gold nanoparticles (Au NPs) are promising vectors for gene delivery applications. In order to gain insight on the influence of particle size on cell transfection, Au NPs were combined with poly(ethylenimine) (PEI) to prepare two sets of PEI-coated Au NPs having particle-size distributions centered at about 6 nm (<10 nm Au-PEI NPs) or 70 nm (<100 nm Au-PEI NPs), respectively. Au-PEI NPs were coupled to a variety of plasmids carrying reporter or suicide genes to prepare Au-PEI NPs/DNA complexes, and human osteosarcoma Saos-2 cells were used to investigate the performance of the Au-PEI NPs as transfection vectors in serum-containing media. The conjugates of DNA with both types of Au-PEI NPs were found to be negatively charged. In spite of the electrostatic repulsion that occurs between the surface of the cell and the surface of the plasmid-conjugated NPs, cell internalization was observed for both kinds of Au-PEI NPs. Cells were efficiently transfected with complexes derived from <10 nm Au-PEI NPs, but not with the <100 nm Au-PEI NPs. Large aggregates of NPs associated with DNA were found in endocytic vesicles of cells incubated with <100 nm Au-PEI NPs, while the success of the smaller Au-PEI NPs as transfection vectors was related to their lower agglomeration state inside cells and to endosomal escape of DNA.

1. Introduction

Gene therapy aims to treat human disorders by introducing genetic material into specific target cells or tissues to restore missing functionality or to eradicate pathogenic dysfunction [1]. Most of the carriers used in gene-therapy clinical trials are based on viral vectors. Delivery of nucleic acids using alternative carriers, such as polymeric micelles [2], dendrimers [3], nanotubes [4] or nanoparticles (NPs) [5], is attracting increasing attention as a consequence of the low cost, stability, ease of large-scale production and lack of immunogenicity that characterize some of these nanomaterials [6]. An additional advantage of the use of NPs as non-viral vectors for gene delivery in vitro comes from their ability to sediment on the surface of target cells, which facilitates the delivery of biomolecules [7]. Earlier studies have reported the successful use of inorganic NPs as efficient carriers of nucleic acids in transfection assays. Examples of this strategy include amino-modified silica NPs [8], iron oxide NPs [9], carbon nanotubes [10] and Au NPs [11–13]. In general, the success of the use of NPs as non-viral carriers depends on several physical variables. For inorganic NPs, size [8,14] and surface charge [11,15] are the main factors controlling the effective transport of plasmids into the cell nuclei. The weight ratio of the NPs to the nucleotide polymer [11–13,16,17] and the incubation period of the NPs/DNA complexes with the target cells [18–20] also play significant roles in the success of gene delivery.

Among the candidate nanomaterials, Au NPs stand out on account of their intrinsic properties (low cytotoxicity, low immunogenicity, biocompatibility) [21] and also because of their straightforward synthesis and the easy functionalization of their surface [16]. Chemical modification of Au NPs surfaces provides an efficient platform to maximize DNA adsorption. In particular, cationic capping favors high-affinity electrostatic interactions with nucleotide polymers to ensure an efficient gene delivery to mammalian cells [9,22,23]. Au NPs chemically functionalized with cationic amino acids [24], polyethilenimine (PEI) [12], quaternary ammonium chains [11], 2-aminoethanethiol [16] or cationic lipids [17] show improved DNA-binding which enables in vitro transfection. In addition, the particular optical properties exhibited by Au NPs may also be used to enhance the efficiency of gene delivery when exposed to near-UV irradiation [25].

Despite the knowledge gathered in recent years regarding the relationship between the properties of NPs and their efficiency as gene-delivery vectors, experimental transfection studies are still plagued by uncertainties. Even with similarly functionalized NPs, small variations in particle properties or in their state of agglomeration

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often give rather different results regarding transfection efficiency. This disparity has been associated to the different mechanisms that NPs use for cell entry and cell trafficking, which appear to hold the key in intracellular gene- and drug-delivery processes. Among the above-discussed coating agents, PEI seems to be particularly effective first at condensing DNA and facilitating cell entry, and then at engineering endosomal escape, through destabilization of the endosomal membrane via osmotic swelling [26,27]. This cell trafficking mechanism works also for drug delivery, as demonstrated recently by Roshenholm et al. [28] who labeled PEI-functionalized silica NPs to show cell entry followed by endosomal escape induced by PEI.

In this study, we have investigated the effect of NPs size on gene delivery, using Au NPs as delivery vectors in transfection assays. To this end, two sets of Au NPs combined with PEI, having particle-size distributions centered at about 6 or 70 nm, respectively, were prepared and used to transfect a variety of plasmids into human osteosarcoma cells. Cytotoxicity and cell traffic of both types of Au-PEI NPs coupled to cargo plasmids were studied, and the results observed in terms of transfection efficiency were related to the size of the NPs and the colloidal properties of the Au-PEI NPs/DNA complexes.

2. Materials and methods

2.1. Synthesis and characterization of Au-based NPs

The synthesis of <10 nm Au-PEI NPs was carried out at room temperature using PEI (~25 kDa, Sigma Aldrich, St Louis, MO, USA) as the reductant of gold(III) chloride hydrate. The tertiary amines of PEI act as reductant and stabilizer [29]. Amine functional groups bind tightly to colloidal Au by virtue of the available electron lone pairs [30]. To prepare the <10 nm Au-PEI NPs, 1 ml of a solution of 0.5×10^{-3} M PEI was added to 19 ml of a solution of 50 mg L^{-1} of $HAuCl_4$ at room temperature under vigorous stirring. To coat commercial <100 nm Au NPs (purchased from Sigma Aldrich) with PEI, 1 ml of the solution of 0.5×10^{-3} M PEI was added in 20 ml of a 1 wt.% dispersion of the commercial Au nanopowder at room temperature under vigorous stirring. The resulting Au-PEI NPs were purified by dialysis (50 kDa cut-off) against distilled water to remove the unbound PEI. The amount of PEI on the Au-based NPs surfaces was evaluated by thermogravimetric analysis (TGA) with a heating rate of 10 °C min^{-1} in a flowing nitrogen atmosphere using a Mettler Toledo TGA/SDTA 851 system.

The particle-size distribution and zeta potential of the Au-based NPs dispersions were obtained by using photon correlation spectroscopy (PCS) measurements (Malvern Zetasizer 3000 HS, Worcestershire, UK). Transmission electron microscopy (TEM) was carried out by using a JEOL 2010F field emission gun microscope (JEOL, Tokyo, Japan) which works at 200 kV and has a point-to-point resolution of 0.19 nm.

2.2. Cell culture

Human osteosarcoma Saos-2 cells (ATCC No. HTB-85) were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS), 500 IU ml^{-1} of penicillin and 0.1 mg ml^{-1} of streptomycin (DMEM-FBS). Cells were incubated at 37 °C under 5 vol.% CO_2 and 95 vol.% air in a humidified incubator.

Saos-2 pAcGFP-1-Endo cells were isolated after stable integration of pAcGFP-1-Endo plasmid (Clontech, Saint-Germain-en-Laye, France) in the genome of Saos-2 cells. pAcGFP-1-Endo is an expression vector that encodes a fluorescence fusion protein consisting of the human RhoB GTPase, which targets the surface of vesicles of the endocytic pathway, fused to the *Aequorea coerulescens* green fluorescent protein (AcGFP1). Saos-2 cells were seeded at a density of 3×10^5 cell cm^{-2} in 100 mm dishes and cultured for 24 h to allow cell attachment. Saos-2 cells were transfected with pAcGFP-1-Endo plasmid using LipofectAMINE™/Plus reagent (Invitrogen, Barcelona, Spain) and transformants were selected in culture medium containing 600 μg ml^{-1} of G418 (InvivoGen, San Diego, CA, USA). Independent clonal cell lines were isolated. A suitable cell line was selected by confocal laser scanning microscopy (CLSM) and named Saos-2 pAcGFP-1-Endo (see Supplementary data, Fig. S1A). Saos-2 pAcGFP-1-Endo cells were routinely maintained in culture medium containing 600 μg ml^{-1} of G418. To unambiguously confirm the endosome-specific labeling in Saos-2 pAcGFP-1-Endo cells, cells were seeded at a density of 3×10^4 cell cm^{-2} in 8-well chamber slides (Labtek Chamber Permanox Slide, Nunc, New York, USA) and incubated for 1 h with 10 μg ml^{-1} of AlexaFluor 594-Cholera toxin or 50 μg ml^{-1} of AlexaFluor 594-Transferrin (both from Invitrogen), which are markers for caveolae-mediated endocytosis and clathrin-mediated endocytosis, respectively. The endosome-specific signal of Saos-2 pAcGFP-1-Endo cells included clathrin-coated pits and caveolae (see Supplementary data, Fig. S1B).

2.3. Treatments with Au-based NPs

For treatments with Au-based NPs, cells were seeded at a density of 3×10^4 cell cm^{-2}, and cultured for 24 h. Immediately prior to their addition to the cells, Au-based NPs dispersions at 10 mg ml^{-1} were sonicated at maximum power for 10 min in a bath sonicator (Bransonic 12, Branson Ultrasonidos SAE, Barcelona, Spain), resuspended in culture medium at the desired final concentration and thoroughly vortexed to ensure a homogenous particle suspension. Cells were washed twice with phosphate-buffered saline (PBS, pH 7.4) and further incubated in DMEM-FBS containing Au-based NPs. For experiments involving Au-PEI NPs/DNA complexes, 1 ml of Au-PEI NPs suspensions at 0.5, 1 and 2 mg ml^{-1} in sodium phosphate buffer 50 mM, pH 7.4 (SPB), were mixed with 4, 6, 8, 10, 12 or 14 μg of DNA and incubated for 1 h at room temperature in an orbital shaker (Afora, Barcelona, Spain) to enable electrostatic binding of DNA with Au-PEI NPs. The suspensions were centrifuged at 13,400 rcf and 4 °C for 5 min and Au-PEI NPs/DNA complexes were resuspended in 1 ml of culture medium. Cells were washed twice with PBS and incubated in DMEM-FBS containing 500 μl of complexes for the indicated times. As controls, cells were subjected to the same manipulations but incubated in the absence of Au-based NPs. Immediately after centrifugation of Au-PEI NPs/DNA complexes, supernatants were recovered and subjected to a standard protocol of ethanol precipitation of nucleic acids [31]. The amount of precipitated DNA in the sample was measured by the OD 260 nm reading in a NanoDrop-100 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). DNA binding efficiency to Au-PEI NPs was calculated as percentage of conjugated DNA to total amount of DNA added.

2.4. Cell viability

Saos-2 cells were seeded at a density of 3×10^4 cell cm^{-2}, cultured for 24 h and then further incubated up to 72 h in the absence or presence of Au-PEI NPs, Au-PEI NPs/DNA complexes, or complexes of DNA and LipofectAMINE™/Plus reagent (Invitrogen). Cell viability was assessed using the alamarBlue™ reagent (Biosource, Nivelles, Belgium), which incorporates a redox indicator that changes color in response to metabolic activity. The alamarBlue assay yields results that are almost identical to those obtained by microscopic counting of viable cells using trypan blue assay, as indicated our preliminary experiments. After washing with PBS,