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Covalently bound DNA on naked iron oxide nanoparticles: Intelligent colloidal nano-vector for cell transfection

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ABSTRACT

Background: Conversely to common coated iron oxide nanoparticles, novel naked surface active maghemite nanoparticles (SAMNs) can covalently bind DNA. Plasmid (pDNA) harboring the coding gene for GFP was directly chemisorbed onto SAMNs, leading to a novel DNA nanovector (SAMN@pDNA). The spontaneous internalization of SAMN@pDNA into cells was compared with an extensively studied fluorescent SAMN derivative (SAMN@RITC). Moreover, the transfection efficiency of SAMN@pDNA was evaluated and explained by computational model.

Methods: SAMN@pDNA was prepared and characterized by spectroscopic and computational methods, and molecular dynamic simulation. The size and hydrodynamic properties of SAMN@pDNA and SAMN@RITC were studied by electron transmission microscopy, light scattering and zeta-potential. The two nanomaterials were tested by confocal scanning microscopy on equine peripheral blood-derived mesenchymal stem cells (ePB-MSCs) and GFP expression by SAMN@pDNA was determined.

Results: Nanomaterials characterized by similar hydrodynamic properties were successfully internalized and stored into mesenchymal stem cells. Transfection by SAMN@pDNA occurred and GFP expression was higher than lipofectamine procedure, even in the absence of an external magnetic field. A computational model clarified that transfection efficiency can be ascribed to DNA availability inside cells.

Conclusions: Direct covalent binding of DNA on naked magnetic nanoparticles led to an extremely robust gene delivery tool. Hydrodynamic and chemical-physical properties of SAMN@pDNA were responsible of the successful uptake by cells and of the efficiency of GFP gene transfection.

General significance: SAMNs are characterized by colloidal stability, excellent cell uptake, persistence in the host cells, low toxicity and are proposed as novel intelligent DNA nanovectors for efficient cell transfection.

1. Introduction

Nanomaterials are increasingly used for biomedical purposes, such as for drug delivery, MRI contrast agents, hyperthermia treatments and cell labeling [1,]. Among proposed applications, the efficient delivery of DNA to cells by nanomaterials appears very promising [2,3], and several nanoparticles, due to their unique size-related properties, large surface to volume ratio and low cytotoxicity, showed favorable transfecting abilities in comparison with viral vectors [4]. In fact, the drawbacks correlated with viral vectors resulted in the current drive for the development and optimization of alternative nonviral DNA vectors for biomedical applications [5]. In this scenario, inorganic nanoparticles, such as silica, cadmium selenide, or iron oxide, have recently been exploited as substitutions for non-viral transfection approaches [6,7] Moreover, nanoparticles can be applied for multimodal devices (theranostics) offering the advantage of monitoring not only the therapeutic response, but also the fate of the cells treated with nanoparticles and injected in an organism or tissue [8]. Actually, long-term tracking of transplanted cell populations is of vital importance for evaluating the outcomes of cell transplantation [9]. As examples, vectors based on magnetic nanoparticles offer the advantage of generating a contrast effect in MRI measurements due to their strong magnetization resulting

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from the spin of unpaired iron electrons [10,11].

Actually, magnetic nanoparticles have been intensively explored and used for non-invasive cell tracking and magnetic manipulation in preclinical and clinical studies focused mainly on cell transplantation [12]. Thus, superparamagnetic iron oxide nanoparticles (SPIONs) were proposed by several research groups for gene delivery [13,14], as they, beside working as biomolecule carrier, behave as efficient contrast agents in MRI imaging [15]. In addition, magnetic nanoparticles were applied for 'magnetofection' approaches, namely the employment of static or oscillating magnetic fields to promote magnetic nanoparticle uptake by cells, which demonstrated to dramatically improve transfection efficacy [16]. Typically, conjugates of superparamagnetic iron oxide nanoparticles are comprised of a magnetite core and a biocompatible coating that provides functional groups for the conjugation of tumor targeting and therapeutic moieties [17,18]. Nanoparticle coatings are generally constituted of cationic lipids or organic polymers, which are capable of complexing nucleic acids by electrostatic interactions [19]. Drawbacks could arise from the coatings on iron oxide nanoparticles, as they can bind only weakly to the nanoparticle surface, and eventually desorb or exchange with bulk solution, affecting the stability of the resulting dispersions [20], not guaranteeing nanomaterial safety [21]. Furthermore, processes proposed to coat nanoparticles, are often cumbersome, time-consuming and expensive, and low yields, limiting their diffuse applications [22]. Finally, nanoparticle coatings reduce the average magnetic moment of the nanomaterial by introducing a diamagnetic shell [23–25].

In the present report, a new category of iron oxide nanoparticles (named Surface Active Maghemite Nanoparticles, SAMNs) [26,27] were used for creating a novel SAMN@DNA complex in which the intimate chemical contact between SAMNs and DNA occurs by covalent binding [28]. SAMNs present a high average magnetic moment and high water stability as colloidal suspensions without any superficial modification or coating derivatization. Because of their unique physical and chemical properties, these naked iron oxide nanoparticles were used to immobilize various biomolecules [29–31], and were proposed for applications spanning from biomedicine [32,33], to food and water remediation [34,35] and sensoristics [36–39].

The reported SAMN@DNA complex was constituted of a plasmid (pDNA), containing the expression system for the green fluorescent protein (GFP) as a reliable intracellular expression reporter [40], and was used to transfect mesenchymal stem cells. SAMNs provided DNA internalization and an optimal availability for participating at the transcription process, displaying a transfection efficiency higher than lipofectamine, even in the absence of an external magnetic field. Thus, due to their great colloidal stability in biological media and very low cyto-toxicity [32,33,41], SAMNs can be considered intelligent nanovectors responding to the fundamental requisites for DNA delivery into cells [17] and elective tools for the development of novel strategies for gene therapy.

2. Experimental section

2.1. Materials

Chemicals were purchased at the highest commercially available purity and were used without further treatment. Iron(III) chloride hexahydrate (97%), sodium borohydride ($NaBH_4$), rhodamine B isothiocyanate (RITC), were obtained from Sigma-Aldrich (Italy). Lipofectamine® was obtained from Invitrogen (cat. 15338-100, Milan, Italy). Full-length GFP cDNA clone was a kind gift of Prof. M. Brini (University of Padua, Italy). The GFP cDNA was cloned in a VR1012 plasmid (5650 bp), with kanamycin resistance gene, and amplified in XL1 blue bacteria [42]. In particular, a S65T mutant of GFP, modified at the 5′ end of the coding region to include the HA1 epitope tag (750 bp), was used. When expressed in mammalian cells, the recombinant protein shows mainly a cytosolic distribution.

Dulbecco's modified eagle medium (DMEM) with glutamine, fetal bovine serum (FBS) and trypsin solution were from Euroclone S.p.A. (Italy). Penicillin and streptomycin were from Sigma–Aldrich (Italy). Cell proliferation Kit II (XTT) was from Roche (Italy).

Buffers were prepared according to standard laboratory procedures using Milli-Q reagent grade water (Merck Millipore, Billerica, MA, USA).

2.2. Instrumentation

Optical spectroscopy measurements were performed in 1 cm quartz cuvettes using a Cary 50 spectrophotometer (Varian Inc., Palo Alto, CA, U.S.A.). A series of Nd-Fe-B magnets (N35, 263-287 kJ m^{-3} BH, 1170-1210 mT flux density by Powermagnet - Germany) was used for the magnetic driving of nanoparticles. Leica TCS-SP2 confocal laser scanning microscope (CLSM) was used for fluorescence analysis. Elisa Reader Spectracount of Packard was used to estimate the XTT colorimetric assay, at wavelength 450 nm with a reference wavelength at 650 nm.

2.3. Methods

Methods are reported in Supporting Information.

3. Results and discussion

3.1. Interaction between plasmid DNA coding for GFP and SAMNs

DNA does not possess the ability to translocate through the cell membrane, thus various approaches were employed to accomplish this task [6], including cell physical manipulations (mechanical pressure, electric shock and hydrodynamic forces [19]), as well as the use of viral and non-viral DNA vectors [43,44]. Regarding viral vectors, replication-deficient viruses, such as retroviruses, adenoviruses, and herpes simplex virus, are typically selected [19]. Furthermore, viruses can be genetically modified to be highly effective. Nevertheless, due to the high cost, practicability issues, and the biological risk they pose, alternative techniques are being examined [21]. Non-viral transfection systems include the use of cationic polymers, synthetic peptides and cationic liposomes, among which lipofectamine is widely used [22]. The proposed transfection procedures present different transfection yield, cost efficiency, cell toxicity and operational complexity.

Nanotechnology represents an attractive new frontier due to the wide spectrum of possibilities, often unpredictable, offered by nanomaterial properties.

The binding of pDNA (20 mg L^{-1}), coding for GFP (green fluorescent protein), to iron oxide nanoparticle surface (0.5 g L^{-1} SAMNs) was carried out by simple incubation overnight in water, under continuous stirring at 4 °C, as previously described [28]. The nanohybrid complex (SAMN@pDNA) was magnetically isolated and extensively washed with water. Furthermore, the amount of bound pDNA to SAMNs was assessed considering the disappearance of pDNA absorbance at 259 nm in the supernatants. The mass ratio of the pDNA shell with respect to the mass of iron oxide core resulted 20 mg g^{-1} SAMN. The SAMN@pDNA nanohybrid was stable, without any release of pDNA in solution, as checked by spectrophotometry indicating the capability of SAMN@pDNA to retain the nucleic acid in aqueous environment. The binding of pDNA on SAMNs can be explained considering the peculiar surface chemistry of the nanomaterial, and in particular considering the availability and reactivity of under-coordinated iron(III) sites on maghemite nanoparticle surface [23].

The SAMN@pDNA nanohybrid was characterized by x-ray photoelectron spectroscopy (XPS) in order to determine quantitatively the elemental composition of the nanomaterial. XPS analysis witnessed the successful binding of the nucleic acid on SAMN surface. In particular, concomitant presence of carbon (C), phosphorus (P) and nitrogen (N)