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Click assembly of magnetic nanovectors for gene delivery

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ABSTRACT

Functionalization of iron oxide nanoparticles with quaternary ammonium ion-based aminooxy and oxime ether substrates provides a flexible route for generating magnetic gene delivery vectors. Using the MCF-7 breast cancer cell line, our findings show that pDNA magnetoplexes derived from the lipid-coated nanoparticle formulation dMLP transfect in the presence of 10% serum with or without magnetic assistance at significantly higher levels than a commonly used cationic liposome formulation, based on luciferase assay. The present ion-pairing, click chemistry approach furnishes Fe_3O_4 nanoparticles with lipid layers. The resultant magnetic nanovectors serve as transfection enhancers for otherwise trans-fection-inactive materials.

1. Introduction

Since the seminal report by Mah's group on the use of magnetic nanoparticles to enhance viral-mediated transduction [1], much research effort has been directed toward the development of nano-sized magnetic vectors for intracellular delivery of polynucleotides [2]. Indeed, the rising interest in 'magnetofection', the term coined by Scherer et al. [3] to denote magnet-assisted gene delivery [4,5], has led to a plethora of new strategies for functionalizing magnetic particles to promote association with negatively charged oligonu-cleotides [6], DNA or siRNA [7]. The majority of approaches have relied on coating iron oxide nanoparticles or nanocomposites with cationic polymers, such as poly-L-lysine [8] or, in particular, poly-ethylenimine (PEI) [9–11]. The high positive charge [12] of PEI effectively promotes DNA transfer into cells. Additionally, the polyamine backbone of PEI enhances escape of DNA complexes entrapped within endosomes by means of a proton sponge effect that increases osmotic pressure and causes endosome rupture [13]. Magnetic PEI-coated nanoparticles generally are prepared by mixing suspensions of magnetite (Fe_3O_4) nanocrystals with solu-tions of linear or branched PEI [14]. More recently, an in situ preparation [15] involving precipitation of iron oxide in the pres-ence of PEI and a method for covalent attachment [16] of PEI to chitosan-coated iron oxide nanoparticles have been described. Elaboration of PEI-coated iron oxide nanoparticles, such as by

addition of cationic peptide fragments [17] or single chain anti-bodies [18], is also an area under intense investigation. Irrespective of the mode of generation or subsequent functionalization, magnetic PEI-coated iron oxide nanovectors readily combine with polynucleotides on simple mixing to generate the corresponding magnetic, electrostatic charge-affinity complexes. The resultant 'magnetoplexes' (nomenclature derived by extrapolation of the established term magnetoliposome [19] and the terms lipoplex and polyplex [20]) have been used principally for gene delivery in vitro; however, successful magnetofection also has been demonstrated in vivo by externally applying a magnetic gradient to the treatment area, for example [21].

One concern that plagues applications involving PEI is the associated cellular toxicity. The high positive charge density of PEI disrupts cellular membranes [22]; thus, complications arising from vector toxicity afflict many PEI-based gene therapy approaches [23]. To overcome this limitation, a recent focus in the field of magnetofection has been to develop lipid-based magnetoplexes. This strategy has been realized in part by mixing preformed lip-oplexes (cationic lipid:helper lipid:DNA) with magnetite [24] or by adding transfection-active cationic liposome formulations (cationic lipid:helper lipid) to either oleic acid-coated [25,26] or dextran-coated [27] magnetite prior to complexation with nucleic acids to form magnetoplexes.

Given that the molecular structure of cationic lipids has a profound influence on gene delivery [28], it is surprising that few magnetofection studies have exploited structure-activity optimi-zations offered by a modular, lipid-based approach. Our program on

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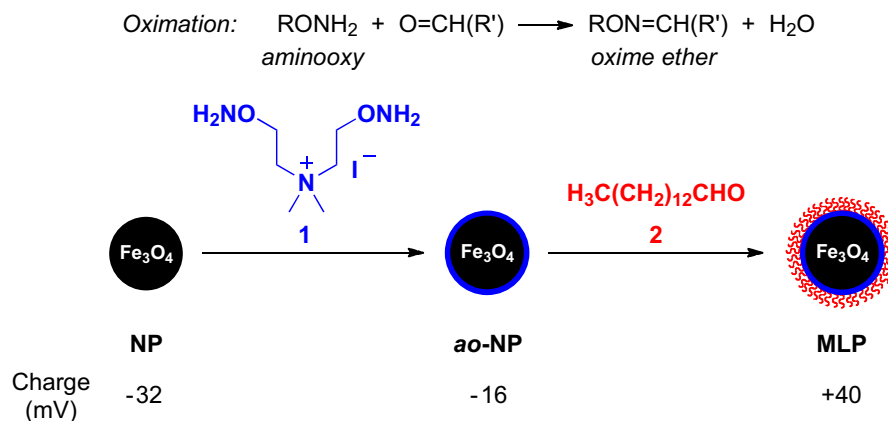
Scheme 1. Preparation of magnetic lipid-coated particles using an oximation approach. Surface charge (ζ-potential) measured in H_2O.

lipid-based transfection vectors [29] and interest in click chemistry [30] led us to consider devising a flexible method for stepwise construction of a transfection-active lipid coating directly on the surface of iron oxide nanoparticles. Without the aid of established transfection lipids and their co-lipid formulations, the use of lipid-coated nanoparticle vectors for gene transfer remains largely unexplored. Inspired by the nanoparticle 'click' conjugate work of Miller [31] and Mirkin [32], we sought to harness click chemistry [33] to attach lipid sidechains onto functionalized iron oxide nanoparticles. We report herein such an approach toward assembling transfection-active, lipid-coated magnetic nanoparticles for gene transfer applications.

2. Materials and methods

2.1. ao-NP synthesis

Iron oxide nanoparticles (**NP**) (5.0 mg), prepared according to a literature procedure [34], were suspended in ultrapure water (5 mL) by brief sonication (ca. 10–15 min, bath sonicator). To the suspension was added a solution of N,N-bis(2-aminooxyethyl)-N,N-dimethylammonium iodide (17.0 mg, 0.058 mmol) in water (5 mL). The reaction suspension was stirred at room temperature. After 12 h, the coated particles were isolated by magnet-assisted sedimentation followed by removal of the supernatant. The isolated particles then were rinsed with water, and the sedimentation procedure was repeated. After rinsing a total of three times, the resultant **ao-NP** slurry was freeze dried to afford **ao-NP** (6.9 mg) as a dry powder.

2.2. MLP synthesis

To **ao-NP** (6.9 mg) suspended in methanol (5 mL) was added myristaldehyde (20.0 mg, 0.94 mmol). The suspension was stirred at room temperature. After 12 h, the lipid-coated nanoparticles were isolated by magnetic sedimentation, washed with methanol (2×), and then dried under vacuum 5 h to yield the magnetic lipid particles (MLP; 7.8 mg).

2.3. dMLP synthesis

To **NP** (5.0 mg) suspended in methanol (5 mL) was added N,N-dimethyl-bis(2-tetradecylideneaminooxy-ethyl)ammonium iodide (**4**; 11.0 mg, 0.016 mmol). The suspension was stirred 12 h at room temperature. The resultant particles then were isolated by magnet-assisted sedimentation, washed with methanol (2×), and dried under vacuum 5 h to give dMLP (9.25 mg).

2.4. Nanoparticle characterization

Methanol suspensions of **NP** or dMLP samples were deposited on transmission electron microscopy (TEM) Cu grids coated with a carbon film. TEM microstructures and energy dispersive X-ray (EDX) spectra then were measured using a JEOL JEM 3200FS at 300 kV equipped with an EDX detector. X-Ray diffraction (XRD) patterns were obtained using a BRUKER D8 diffractometer with a CuKα radiation source ($\lambda = 0.15418$ nm). The phase identification was performed using JCPDS-ICDD 2000 software (The International Centre for Diffraction Data; ICDD).

2.5. Zeta potential measurements

The zeta potentials of **NP**, **ao-NP**, MLP, dMLP and derived magnetoplexes were measured in water using a ZetaPALS dynamic light scattering detector (Brookhaven Instruments Corporation; Model 90 Plus). The concentration of iron oxide in the aqueous particle samples was 0.1 mg/mL. Magnetoplexes were formulated at a concentration of 0.5 μg pDNA.

2.6. Cell culture

Human breast cancer cells (MCF-7) were purchased from American Type Culture Collection VA, USA. Cells were grown to 50–60% confluency in DMEM and 1% Pennstrep (Mediatech, Inc, VA) with 10% FBS (Valley Biomedical, Winchester, VA).

2.7. Luciferase gene expression

Luciferase transfections were performed in triplicate using 0.025 μg of pDNA (pCMV Luc)/well in MCF-7 cells. MCF-7 cells were seeded up to 1×10^5 cells/well in a 24-well plate to give 50–60% confluence. Magnetoplexes (MLP·pDNA, dMLP·pDNA) were prepared at nanoparticle:pDNA ratios of 30, 60, 90, 120, 180, 360 and 540 by adding the required volume of aqueous suspension of MLP or dMLP to a pDNA solution (3 μL, 0.025 μg DNA/μL). 200 μL of serum free DMEM then was added to each magnetoplex solution followed by incubation for 30 min at room temperature. The magnetoplex solutions were diluted to 600 μL with serum free DMEM, and then 200 μL of the final magnetoplex formulation were added directly to each well. For magnetofection, the cell plate was placed on top of a magnetic plate (Oz Biosciences) for 1 h at 37.5 °C. After 18 h incubation at 37.5 °C, the cells were lysed and luciferase gene expression was quantified using a commercial kit (Promega) and luminometer according to the vendor's protocol. Lipofectamine 2000 (Invitrogen) was used as a positive control. Transfections were also performed in similar manner without the assistance of a magnet.

2.8. Cytotoxicity measurements

Cell cytotoxicity and proliferation of cells treated with dMLP formulations were assessed by an alamarBlue assay. After transfection of MCF-7 cells using dMLP-derived magnetoplexes (0.025 μg of DNA/well) and incubation for 18 h, cell viability was measured according to the vendor's (Invitrogen) protocol. Fluorescence intensities were measured using a spectrofluorometer (Molecular Devices:Gemini EM) by excitation at 540 nm and emission at 600 nm.
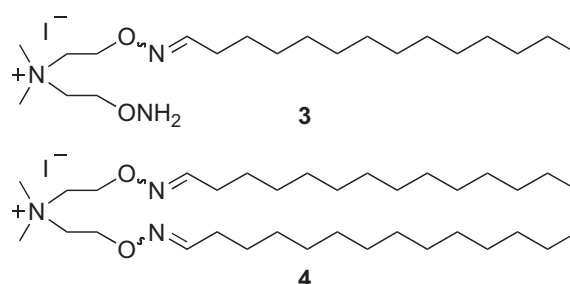
Fig. 1. Cationic oxime ether lipids formed on reaction with myristaldehyde.