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Laboratory formulated magnetic nanoparticles for enhancement of viral gene expression in suspension cell line

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Abstract

One factor critical to successful gene therapy is the development of efficient delivery systems. Although advances in gene transfer technology including viral and non-viral vectors have been made, an ideal vector system has not yet been constructed. Due to the growing concerns over the toxicity and immunogenicity of viral DNA delivery systems, DNA delivery via improve viral routes has become more desirable and advantageous. The ideal improve viral DNA delivery system should be a synthetic materials plus viral vectors. The materials should also be biocompatible, efficient, and modular so that it is tunable to various applications in both research and clinical settings. The successful steps towards this improve viral DNA delivery system is demonstrated: a magnetofection system mediated by modified cationic chitosan-coated iron oxide nanoparticles. Dense colloidal cationic iron oxide nanoparticles serve as an uptake-enhancing component by physical concentration at the cell surface in presence of external magnetic fields; enhanced viral gene expression (3–100-fold) due to the particles is seen as compared to virus vector alone with little virus dose.

Keywords: Magnetofection; Gene; Viral vectors; Magnetic nanoparticles; Chitosan; Iron oxide

1. Introduction

Gene therapy is a potential theraputic modality that requires effective gene delivery into lining cells (Giannoukakis and Thomson, 1999; Pfeifer and Verma, 2000; Hunt and Vorburger, 2002). To the date the most common methods for delivering genes into cell *in vitro* include cells treated with viruses such as retrovirous and adenovirus, calcium phosphate, liposome, particle bombardment, fine needle naked DNA injection, electroporation, or any combination of these methods (Giannoukakis and Thomson, 1999; Pfeifer and Verma, 2000; Hunt and Vorburger, 2002; Bergelson et al., 1997). Although viral methods are powerful methods to deliver the engineered gene into cells with high efficiency in different cell line but not successfully deliver in suspension cell line especially leukemia cells (Fields, 1996). And also high doses of viral gene delivery system causes greatest two potential dangers like oncogenesis and inflammation. So there is urgent need of alternative method of viral gene delivery system or improvement of this system by reducing the viral doses, time of infection and eventually reduce the viral side effect and applied it in variety of cell line for gene delivery vector.

Focusing above burning issue, a combined chemical and physical method was applied to improve the viral gene delivery system. Among different chemical or physical method, gene delivery by the use of magnetic forces, so-called magnetofec-

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tion, has been shown to enhance transfection efficiency of viral and non-viral systems up to several 100-fold (Luo and Saltzman, 2000). Using magnetofection, the efficiency of synthetic gene carriers has been enhanced up to several 100-fold. In particular, the duration of the transfection procedure has been reduced to minutes (Scherer et al., 2002). Moreover, an unrestricted use of magnetofection and its benefits it would be advantageous to gain more insights into the cellular uptake mechanism of the magnetofectins (magnetic nanoparticles complexed with DNA).

The main objective of the present work is to develop of a synthetic formulation to enhance adenoviral-mediated gene transduction from the goal of reducing adenoviral vector dosages *in vitro*. Previous report already investigated the hydrophobic modification of natural chitosan and stabilization of magnetic iron oxide nanoparticles (IOPs) (Bhattarai et al., 2007a). In this paper, previously reported a novel modified chitosan-coated magnetic nanoparticles (*Nac-6-IOPs*) was used to enhance of adenoviral gene delivery in suspension cell line (K562) via magnetofection as a result little doses of virus and short periods of physical contact between the vector and target cells significantly improved for efficient gene transfer will be discussed.

2. Materials and methods

2.1. Magnetic nanoparticles (Nac-6-IOPs) and physiochemical properties

The formulation procedure of *Nac-6-IOPs* was taken from previous publication (Bhattarai et al., 2007a). The *Nac-6-IOPs* were resuspended in triply distilled water by sonication as above for 20 min and centrifuged at 1000 rpm for 20 min at 7–11 °C to remove any large aggregates. The supernatant containing *Nac-6-IOPs* was collected, re-diluted in phosphate buffer at pH 7.4 and stored in 4 °C at a final stock concentration (11.2 mg/ml) for further application. The size and morphology of IOPs and *Nac-6-IOPs* were observed by transmission electron microscopy (TEM) (JEM-1230, JEOL, Japan). The average hydrodynamic diameter and the ξ-potential of the particle was determined by dynamic light scattering (DLS) and electrophoresis light scattering (ELS) (Zetasizer ZEN 3600, Malvern, UK), respectively. The results were the mean values of two experiments using the same sample. The sample for TEM analysis was obtained by placing a drop of *Nac-6-IOPs* suspension diluted by distilled water onto a copper grid without any staining at room temperature. All DLS measurements were done with an angle detection of 90° at 25 °C after diluting the dispersion to an appropriate volume with water. Magnetic measurement was done using superconducting quantum interference devices (SQUID) magnetometer (MPMSXL-7, Quantum Design, USA). Magnetization curves were recorded for a solid sample of *Nac-6-IOPs* at 27 °C with an applied magnetic field up to 10,000 Oe.

2.2. Preparation of adenovirus

First-generation adenoviral vectors expressing *Escherichia coli* β-galactosidase (Ad/LacZ) under the control of a CMV promoter were amplified in the 293 cell line using a modification of established methods. Virus was purified from cell lysates by banding twice on CsCl gradients followed by desalting on Econo-Pac 10 DG disposable chromatography columns (Bio-Rad, Hercules, CA) equilibrated with each respective formulation. Concentration of the virus was determined by UV spectrophotometric analysis at 260 nm. All experiments were performed with freshly purified Ad/LacZ stock. The number of plaque forming units (p.f.u.) per ml of stock was determined.

2.3. Cell line preparation

K562 cells was used for transient magnetofection experiments and cytotoxicity, and grown at 37 °C under 5% CO_2 atmosphere. Dulbecco's modified eagle's medium (DMEM) (Gibco) with 10% (v/v) fetal calf serum (Gibco) was used. For all media, penicillin (100 U/ml) and streptomycin (100 μg/ml) was used.

2.4. Adenovirus infection studies

K562 cells (ATCC CCL 185, human leukemia cells) were seeded at a density of 1×10^4 cells/well in 12-well culture dishes for all infection studies. When cells were confluent, medium was removed and 0.1 ml formulation with Ad/LacZ at an MOI of 20 was added. After incubation at 37 °C for 2 h, formulation was removed and replaced with 2 ml culture medium. After 48 h, transduction efficiency was assessed by X-gal staining method as described in our previous report (Bhattarai et al., 2006, 2007b).

2.5. Evaluation of cytotoxicity

Evaluation of the cytotoxicity was performed by the MTT assay in K562 cell. Briefly, cell suspensions containing 1×10^4 cell/well in DMEM containing 10% FBS were distributed in a 96-well plates, and incubated in a humidified atmosphere containing 5% CO_2 at 37 °C for 24 h (Bhattarai et al., 2006, 2007b). The cytotoxicity of *Nac-6-IOPs* was evaluated in comparison with control cells. Cells were incubated for additional 24 h after the addition of defined concentration of *Nac-6-IOPs*. The mixture was replaced with fresh medium containing 10% FBS. Then, 20 μl of MTT solution (5 mg/ml in 1× PBS) were added to each well. The plate was incubated for an additional 4 h at 37 °C Next, MTT-containing medium was aspirated off and 150 μl of DMSO were added to dissolve the crystals formed by living cells. Absorbance was measured at 490 nm, using a microplate reader (ELX 800; BIO-TEK Instruments, Inc.). The cell viability (%) was calculated according to the following equation:

$$\text{Cell viability (\%)} = \left[\frac{\text{OD 490(sample)}}{\text{OD 490(control)}}\right] \times 100$$