Preparation and Characterization of Polyethyleneimine Modified Iron Oxide Nanocarrier for micro-RNA Delivery in Neuroblastoma Treatment

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Gene therapy has proven to be a solution to the treatment of various cancers. Neuroblastoma is responsible for around 6% of all cancers in children, with a 5–year survival rate of only 20–25%. The increased expression of MYCN is linked with poor prognosis in patients with neuroblastoma. MicroRNA–34 (miR–34) may serve as a potential target for cancer treatment, owing to its function as an oncogene and tumor suppressor. In this study, positively charged magnetic nanocarriers comprising iron oxide nanoparticles (IONPs) were synthesized through coprecipitation method and then citric acid (CA) was conjugated branch polyethyleneimine (PEI) via an electrostatic interaction to obtain PEI coated CA magnetic nanoparticles (PEI–CAIONP). These nanocarriers have the ability to penetrate the cell wall and used for the delivery of miRNA–34a into the cancer cells. The characteristic peaks at 3,380, 1,620, 2,900, and 2,840 cm^{-1} corresponded to those of NH and $-CH_2-$ groups, indicating the successful coating of PEI. The diffraction peak of synthesized magnetic nanocarriers at $2\theta = 35.44°$ corresponded to that of magnetite (Fe_3O_4) (311), consistent with JCPDS database. XANES spectra of Fe atom in IONPs and PEI–CAIONPs samples exhibited an absorbance feature (Fe = 7114 eV) of 1*s* to 3*d* transition. The EXAFS spectra showed that the standard Fe–O bond distance in IONP and PEI–CAIONP samples was 1.95 Å, with a co-ordination number of 4.30, 4.16, 4.02, 3.79, and 4.23, respectively. In addition, small angle neutron scattering (SANS) spectra showed that the alternative magnetic field (AMF) triggered core heat generation, which softened the shells. The MTT results displayed that the prepared nanocarrier did not show any significant cytotoxicity on BE–2–M17 cells. The magnetic vectors through magnetofection showed higher delivery efficiency of pcDNA3-EGFP plasmid into BE–2–M17 cells. For the LDH assay, 50% cytotoxicity were observed from the cells incubated with miR–34 for 24 h when compared with the control. After magnetofection treatment, the expression of miR–34a was raised to a 5800–fold level and MYCN expression was significantly suppressed to 0.09% when compared with the control. The IONPs–based drug delivery system would be a potential tool to enhance targeting delivery of gene/drug and MRI imaging.

Keywords: Gene therapy, Non–viral vector, Neuroblastoma, Micro–RNA, MYCN, Polyethyleneimine, Magnetic nanocarrier, Magnetofection, SANS, XANES/EXAFS.

Introduction

Gene therapy has demonstrated a remarkable potential in treating diseases that affect humans because of defective genes, such as cancers [1]. Effective gene therapy is principally reliant on the delivery of genes to cells, repair or regulate genes [2]. However, it is still difficult for non-viral vectors (RNA or DNA) to transfect numerous specific types of cells, as a result they are commonly complexed with delivery vehicles such as polymers [2, 3]. Several types of nanoparticles comprising of magnetic nanoparticles (MNPs) presents extraordinary opportunities for drug/gene delivery [4]. Iron oxide nanoparticles (IONPs) which have magnetic properties possess a promise to transform current imaging, diagnostic, and therapeutic applications. These IONPs have diameters ranging from 1 and 100 nanometer with the core containing magnetite (Fe_3O_4) or maghemite (Fe_2O_3) or a nonstoichiometric configuration of both [5].

Therefore, the aim of this study was to develop a magnetic nanocarrier made from synthesized PEI coated iron oxide magnetic nanoparticles. The prepared nonocarrier, having positively charged surfaces, would be able carry enough miR-34a and accurately deliver it to the neuroblastoma cells. Moreover, the delivery of miR-34a will help to effectively inhibit the growth of neuroblastoma.

Experiments

2.1. Preparation of polyethylenimine coated iron oxide magnetic nanoparticles

The IONPs were prepared by a coprecipitation method, whereby, 2.1 g of $FeCl_3.6H_2O$ and $FeCl_2.4H_2O$ with the ratio of Fe^{2+}:Fe^{3+} = 1:2 and 0.5 g sodium citrate were dissolved in 200 mL of deionized (DI) water and resulting solution was stirred for 1 h. The obtained nanoparticles were coated with different concentrations of PEI polymer.

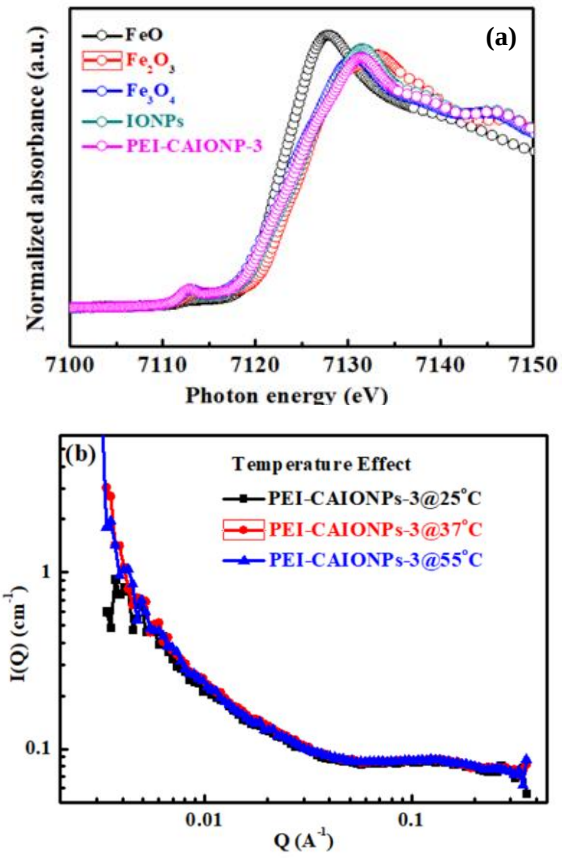
Results

In this study, the zeta potential was performed to understand whether modification of CAIONPs by PEI could increase the interfacial potential of the magnetic nanocarriers, the same values are measured by Zeta potential. The interfacial potential of pure IONPs was –24.57 mV, while the interfacial potential of CA modified IONPs is reduced to –38.62 mV. The results comparing the particle size distributions confirm that the strong negative charges promote the dispersion of CAIONPs. Furthermore, the PEI–CAIONP with different ratio of PEI had the highest interfacial potential of PEI–CAIONP–1 to PEI–CAIONP–4 of 9.39, 19.96, 32.15 and 26.43 mV, respectively. The XANES spectra of the nanocarrier and iron standards show that the main peak of the magnetic nanocarrier is located between Fe^2 and Fe^3 iron and

the curve with more fitting on Fe_3O_4 **(Fig. 1(a))**. Standard Fe–O bond distance in IONP and PEI–CAIONP samples was 1.95 Å, with a co-ordination number of 4.30, 4.16, 4.02, 3.79, and 4.23, respectively.

Discussion

The prepared nanocarrier may serve as a suitable nanovector owing to its positively charged surface that could carry negatively charged nucleic acids. Moreover, the nanovector may readily penetrate through the negatively charged cell membrane and deliver miR–34. As seen on **Fig.1 (b-c)**, PEI polymer is not very sensitive to temperature change. Primary results of AMF application highlighted the changes in temperature in response to the applied magnetic field **(Fig. 1(d)).** AMF triggered core heat generation that softened the shell. This phenomenon would enhance the gene/drug release within the tumors, leading to the inhibition of tumor growth. The possibility of integrating the AMF system and SANS seems promising. However, further *in-situ* experiments are necessary to comprehend the release mechanism of the nanocarrier after its exposure to AMF. FTIR spectra was investigated and showed characteristic peaks at 3,380 and 1,620 cm^{-1} which corresponded to NH stretching vibrations, which is an indication of the presence of PEI on the surface of IONPs. The NH groups of branched PEI chains electrostatically interacted with the carboxyl groups on the surface of IONPs to form a cationic core/shell structure. There was no evident difference in cell viability was observed between cells treated with different concentrations of PEI. Hence, the prepared nanocarrier was deemed safe as a gene nanocarrier or contrast agent to enhance gene penetration into cells. The prepared nanocarrier exhibited no significant cytotoxicity against treated cells. No evident difference in cell viability was observed between cells treated with different concentrations of PEI polymer.



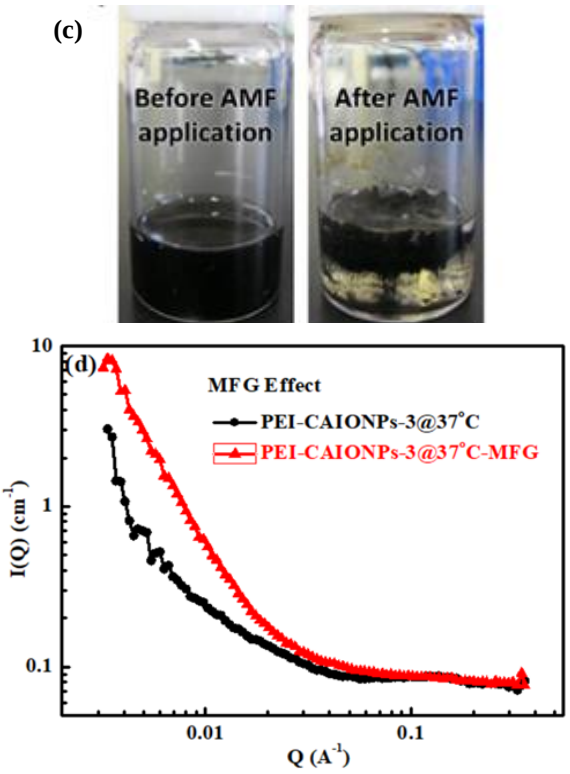


Fig. 1. (a) Fe K–edge XANES spectra of PEI–CAIONP samples and iron standards. The SANS spectra of (b) PEI-CAIONPs at different temperatures before applied 0.5 mT external magnetic field; (c) reaction of PEI-CAIONPs before and application of MFG, and (d) PEI–CAIONPs after applied 0.5 mT external magnetic field at 37°C.

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