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Evaluation of cationic nanoparticles of biodegradable copolymers as siRNA delivery system for hepatitis B treatment

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ABSTRACT

Cationic nanoparticles of biodegradable polymers such as poly (lactide) (PLA) have been shown to be promising carrier systems for DNA and siRNA delivery. However, the parameters which influence the transfection efficiency have not been investigated in details. In this work, four groups of cationic PLA-based nanoparticles were synthesized by the nanoprecipitation method and solvent evaporation method with polyethyleneimine (PEI) and chitosan as two types of surface coating materials. Cationic poly (D,L-lactide-co-glycolide) (PLGA)–PEI, PLGA–chitosan and methoxy poly (ethylene glycol)–poly (lactide) (mPEG)–PLA/PEI, mPEG–PLA–chitosan nanoparticles were characterized in terms of size and size distribution by laser scattering, surface charge by zeta potential measurement, and surface chemistry by X-ray electron spectroscopy (XPS). The four type pg nanoparticles were compared for their interaction with siRNA and nanoparticles mediated siRNA transfection efficiency with a hepatitis B model, where the inhibition effects of the double strand RNA (dsRNA) mediated by the four types of nanoparticles were evaluated by measuring the HBsAg expression level. The highest inhibition effect of HBsAg (the surface antigen of the hepatitis B Virus (HBV), which indicates current hepatitis B infection) expression was achieved by the mPEG–PLA–PEI nanoparticles mediated siRNA transfection. The results demonstrated that the siRNA delivery follows a size and surface charge dependant manner.

1. Introduction

Hepatitis B virus (HBV) is a DNA virus replicate within liver through an RNA intermediate. Nowadays the infection of HBV causes inflammation in liver, also called hepatitis, could be effectively prevented by the recombinant vaccines. However, there are still 350–400 million people with chronic HBV infection, facing the risk of the further development of this disease into liver cirrhosis and liver cancer (Ahn et al., 2010). Current treatments using cytokines or nucleoside/nucleotide analogues can only temporarily stop the virus from replicating without achieving the full clearance of the infection. Furthermore, the associated side effects (Song et al., 2005; Vial and Descotes, 1994) and the emergence of drug resistance (Malmström et al., 2007) hindered the successful application of these drugs.

Short interference RNA (siRNA), a new gene medicine, has emerged as a powerful tool for successful treatment of cancer (Ashihara et al., 2010) and many virus infected diseases (Martinez, 2009; Wilson et al., 2009). RNA interference (RNAi) was the process of endogenous cellular post-transcriptional gene silencing induced by double stranded RNA that is homologous in sequence to the gene being suppressed (Verma and Dey, 2004). Potent knockdown of a gene of interest with high sequence specificity makes RNAi be able to treat many gene related diseases such as cancer and virus based diseases such as HBV, hepatitis C virus (HCV) and human immunodeficiency virus (HIV). The functional siRNA delivered to the target tissue was able to deplete virus mRNA, as well as to suppress the translation of virus proteins (Ma et al., 2007). Moreover, comparing to the traditional therapeutic strategy, siRNA does not have the intrinsic property to elicit drug resistance as it targeted directly the specific gene for the diseases. There are many successful application cases of using siRNA for the treatment of HBV (Radhakrishnan et al., 2004; Wu and Nandamuri, 2004; Chen et al., 2008).

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A major challenge to develop a siRNA delivery system was the intracellular delivery efficiency and protection of its bioactivity in the delivery process. An ideal delivery system should be able to stabilize and protect siRNA against the degradation through nucleases, enhance the intracellular uptake of the siRNA so that a high level of therapeutic effect of siRNA could be achieved. In addition, the delivery vehicle used for this purpose should be biodegradable and biocompatible, without eliciting any immune response and compromising the gene silencing efficacy of the siRNA.

Both non-viral and viral delivery vectors have been reported to deliver siRNA (Guo et al., 2010). A common concern on the usage of viral vector is the immunogenicity, potential infectivity, inflammation despite its high delivery efficacy. Therefore, non-viral delivery vector emerged as an alternative using various chemical approaches, in which siRNA could either be conjugated to carrier molecules such as lipid, peptide and polymer or encapsulated into a cationic delivery system (Lee et al., 2007; Wolfrum et al., 2007). Among all these approaches, much attention was given to the application of polymeric nanoparticles because biodegradable nanoparticles can safely transport the genetic materials without exhibiting any toxicity and immune responses, which can be produced on a large scale. Poly (D,L-lactide-co-glycolide) (PLGA) nanoparticles initially were used to encapsulate the plasmid DNA after it was demonstrated to have the endo-lysosomal escaping property (Panyam et al., 2002), which achieved 57% siRNA encapsulation efficiency (Cun et al., 2010). However the efficacy of the siRNA has not been evaluated in that work. In order to enhance the loading efficiency and cellular uptake of the siRNA, the PLGA nanoparticle technique was further improved by modifying the surface of the PLGA nanoparticles with cationic coating materials, which bind negatively charged therapeutic genes through electrostatic interactions (Schiffelers et al., 2004; Hart, 2010; Xing et al., 2010). However, the excessive positive charge on the cationic complex might cause severe problems associated with toxicity and serum instability when used *in vivo*.

The aim of the present study is to develop a cationic nanoparticulate system with enhanced siRNA delivery efficacy and minimized surface charge of the siRNA loaded nanoparticles complexes. Four types of biodegradable nanoparticles were developed as the vector of double strand RNA delivery system, which consists of PLGA and methoxy poly (ethylene glycol)–poly (lactide) (mPEG–PLA) with two types of cationic materials chitosan and polyethyleneimine (PEI) used as surface coating material. The nanoparticulate systems were prepared by the nanoprecipitation method and the solvent evaporation technique, respectively. PEI and chitosan were chosen as the two surfactants due to their cationic property and the permeability enhancing properties (Hombach and Bernkop-Schnürch, 2009). The fabricated four types of nanoparticles, which are denoted as PLGA/PEI, PLGA/chitosan, mPEG–PLA/PEI, and mPEG–PLA/chitosan, respectively, were characterized by size and size distribution by the laser light scattering, surface charge by the zeta potential measurement and surface chemistry by the X-ray electron spectroscopy (XPS). Gel retardation assay was employed to evaluate the RNA binding capabilities. The PLC/PRF/5 human liver cell line was employed as a *in vitro* model of human hepatitis B as it could continuously express the surface antigen of the hepatitis B Virus (HBsAg), an indicator of hepatitis infection. Cell cytotoxicity was also investigated to evaluate the biocompatibility of the delivery system.

2. Materials and methods

2.1. Materials

Poly (D,L-lactide-co-glycolide) (PLGA), of a lactide–glycolide ratio of 50:50, HMW Polyethyleneimine (PEI 25 kDa, branched, water-free), Polyvinyl alcohol (PVA) (Mw 22,000) were all purchased from Sigma–Aldrich. Chitosan extracted from crab shells, minimum of 85% deacetylated, supplied from Sigma, was purified by re-precipitation from the filtered 1% acetic acid solution with sodium hydroxide before use. The precipitate was washed with deionized water and dried under vacuum. The purified chitosan was dissolved in 1% acetic acid with magnetic stir and gentle heating and PH was adjusted to 5.5–5.7 with sodium hydroxide. The solution was diluted to 0.02% of chitosan (w/v) and then sterile filtered through a 0.22 μm filter before use. Methoxy poly (ethylene glycol)–poly(lactide) (mPEG–PLA) copolymer was a gift from Dr. Wanyuqing of chemotherapy lab NUS with the number average molecular weight 62,500 and the weight content of mPEG is 10%. Acetonitrile and Dichloromethane (DCM) were obtained from Sigma–Aldrich.

The double strand RNA (dsRNA) was chemically synthesized by Qiagen-Xeragon (QIAGEN GmbH, German). Sequences of two types of siRNA used in this work were according to Li et al. (2004). S1 siRNA targets sites within the HBsAg ORF of the viral genome which are conserved in all the different HBV serotypes while Scr siRNA serves as the scramble control with some random sequence. The sequence is as the following:

- S1 siRNA
  5′-GGUAUGUUGCCCGUUUGUCdTdT-3′
  3′-dTdTCCAUACAACGGGCAAACAG-5′
- Scr siRNA
  5′-CUCAACCUACCAACUCCACdTdT-3′
  3′-dTdTGAGUUGGAUGGUUGAGGUG-5′

The Murex HBsAg Version 3 Kit was obtained from Murex Biotech Limited (Dartford, UK). Cell Titer 96 Aqueous One Solution Cell Proliferation Assay was purchased from Promega.

2.2. Cell culture

The PLC/PRF/5 human liver cell line was maintained in Dulbecco's modified Eagle medium (DMEM), supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 1 mM sodium pyruvate, 0.1 mM MEM nonessential amino acids, 100 U/ml penicillin and 100 μg at 37 °C in a humidified atmosphere containing 5% CO_2.

2.3. Preparation of cationic nanoparticles

Cationic nanoparticles with chitosan coating were prepared using the solvent evaporation technique (Mu and Feng, 2003). Specifically, the organic phase composed of 100 mg PLGA or mPEG–PLA in 10 ml DCM was poured to 100 ml water phase consisting of 20 mg/ml chitosan solution in 1% acetic acid and 1% PVA. The whole mixture was then sonicated at 25 W for 2 min forming a milky emulsion. The whole mixture was then evaporated with a magnetic stir overnight to remove the organic solvent. Nanoparticles formed during the evaporation process.

Cationic PLGA and mPEG–PLA nanoparticles with PEI coating were prepared by the nanoprecipitation method. The organic phase was prepared by dissolving 100 mg mPEG–PLA or PLGA in 10 ml acetonitrille. It was then drop-wise added to 50 ml 100 mg/ml PEI 25 kDa solution (filtered through 0.22 μm membrane filter). The whole mixture was then evaporated with a magnetic stir overnight to remove the organic solvent. The obtained suspension was then centrifuged for 15 min at 4 °C 12,000 rpm to remove the excessive surfactant. The pellet was then suspended and slightly sonicated to form the evenly distributed nanoparticle suspension with the final concentration of 10 mg/ml.

2.4. Characterization of nanoparticles

2.4.1. Size and size distribution

Size and size distribution property of nanoparticle play a key role in determining its internalization behavior. The particle size and size distribution of the cationic nanoparticles were measured by the laser light scattering method (Brookhaven Instruments Corporation 90 Plus Particle Sizer) at 25 °C and at a scattering angle of 90°. Nanoparticle after centrifugation was diluted with deionized water and slightly sonicated to obtain homologous suspension before measurement. Then the suspension was used to measure the mean diameter, size distribution and polydispersity.

2.4.2. Surface morphology

The formulated nanoparticles were investigated by observing the freeze-dried particles using Field Emission Scanning Electron Microscopy (FESEM, Jeol JSM 5600LV), which requires an ion coating with platinum by a sputter coater (JFC- 1300, Jeol, Tokyo) for 40 s in a vacuum at a current intensity of 40 mA after preparing the sample on metallic studs with double-sided conductive tape.

2.4.3. Surface charge

Zeta potential measured by the laser Doppler anemometry (Zeta Plus, Zeta Potential Analyzer, Brookhaven Instruments Corporation) was employed to evaluate surface charge density of the nanoparticles. Sample was prepared as the method mentioned in the size and size distribution part.

2.4.4. Surface chemistry

XPS (AXIS His-165 Ultra, Kratos Analytical, Shimadzu) was used to analyze the surface composition of the nanoparticles. Fixed transmission mode was utilized with pass energy of 80 eV for the survey spectrum covering a binding energy from 0 to 1200 eV. Peak curve fitting was carried out using the software provided by the instrument manufacturer.

2.5. Gel retardation assay

The potential of these nanoparticles as gene carrier was further studied to perform the gel retardation assay. The PLGA/PEI Nanoparticle suspensions with the concentration of 10 mg/ml were added into the RNA suspension at a concentration of 0.3 mg/ml at different ratios with range from 1:1 to 30:1 (w:w) in RNA suspension buffer and then vortexed for 30 s. RNA bound to nanoparticles forming nanoparticles–RNA complex after 30 min incubation at room temperature. The nanoparticle–RNA complexes were electrophored on the 1% agarose gel containing 0.6% ethidium bromide for 30 min at 100 mV. Images were acquired using Gel Doc 1000 Video Gel Documentation System (Bio-Rad Laboratories, Inc., Hercules, CA). All the other three types of nanoparticles were then further studied for their carrier capacity.

2.6. Nanoparticles mediated transfection with siRNAs

PLC/PRF/5 cells were seeded into 24-well plate (COS, USA) at a density of 5×10^4 cells per well and allowed to recover for 24 h before transfection. After overnight incubation, culture medium in each well was replaced with 300 μl Opti-MEM I Reduced Serum Medium. The concentration of S_2RNA we used is 0.2 μM according to Li et al. (2004). The amount of nanoparticles we used is 200 μg/ml in order to completely condense the S_2RNA. The complexes of nanoparticle and RNAi in suspension were added into each well and mixed gently. Transfection happened when the cells with the complex incubated at 37 °C 5% CO_2. After incubation for 4 h, transfection reagent in each well was removed and replaced with fresh medium. The transfection efficiency was evaluated by measuring the HBsAg expression level within the culture medium after 48 h incubation. The HBsAg level was determined using the Murex HBsAg Version 3 kit according to the protocol described in the manufacturer's manual.

2.7. Cell viability assay

PLC/PRF/5 cells were plated at a density of 5×10^3 per well in 96-well microtiter plates (NUNC, Germany). After overnight incubation, culture medium was replaced with fresh medium containing nanoparticles with the concentration in the range from 100 to 1000 μg/ml. Cell with these supplements were then incubated for 48 h. For cell viability assay, 20 μl MTS tetrazolium compound reaction solution [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] and 4.8 μl Phenazine methosulfate (PMS) were directly added to each sample. Samples were incubated with the reagent for 1 h in the CO_2 incubator at 37 °C. Absorbance was measured at 492 nm using Genios Micro plate reader (Tecan, Mannedorf, Australia). Quintuplicate determinations for each treatment were performed. The relative cell viability of sample related to control cells supplemented only with fresh medium was indicted by calculating the ratio of two absorbance values.

3. Results and discussion

3.1. Preparation of the nanoparticles and particle size

Four groups of nanoparticles were prepared with two different methods using two types of different coating materials. Fig. 1 demonstrated the successful fabrication of these nanoparticles with uniform size distribution, where aggregation of the nanoparticles was not observed. The exact particle size was measured with laser light scattering method. As shown in Table 1 PEI decoration increases the particle size for about 50–70 nm comparable to the pure PLGA nanoparticles and mPEG–PLA nanoparticles without any surfactant; while chitosan decoration increased the particle size for about 120–150 nm. Nanoparticles coated with PEI fabricated with nanoprecipitation method possessed smaller particle size than the counterpart probably because of the lower viscosity of PEI formulated water phase than the chitosan water phase. Nanoprecipitation method has also been tried to formulate the PLGA/chitosan nanoparticles and mPEG/PLA nanoparticles and the particle size are rather large at 500–800 nm (data not shown) because the water phase formed by the chitosan acetic acid solution is very sticky which might increase the water phase viscosity and tend to make the PLGA/chitosan nanoparticles aggregate. This was consistent with the results presented in previous publications that in the nanoprecipitation method the particles size not only depends on the viscosity of organic phase viscosity but also that of the water phase (Dong and Feng, 2004). The less viscous both organic and water phase tends to produce the smaller particles. As a result, the PLGA/chitosan nanoparticles and mPEG–PLA/chitosan nanoparticles were finally prepared by the solvent evaporation technique. Chitosan dissolved in the acetic solution acted as the co-emulsifier with the PVA to stabilize the formation of the nanoparticles.

The ratio of chitosan to PVA was optimally adjusted to produce uniform cationic nanoparticles because too much chitosan (more than 500 mg) added could increase the particle size of the nanoparticles significantly, while too less chitosan (less than 100 mg) could not modify the surface of the nanoparticles in a homogenous way. The polydispersity value in Table 1 demonstrated that the spherical nanoparticles formulated by this solvent evaporation technique take advantage over the traditional emulsion solvent evaporation

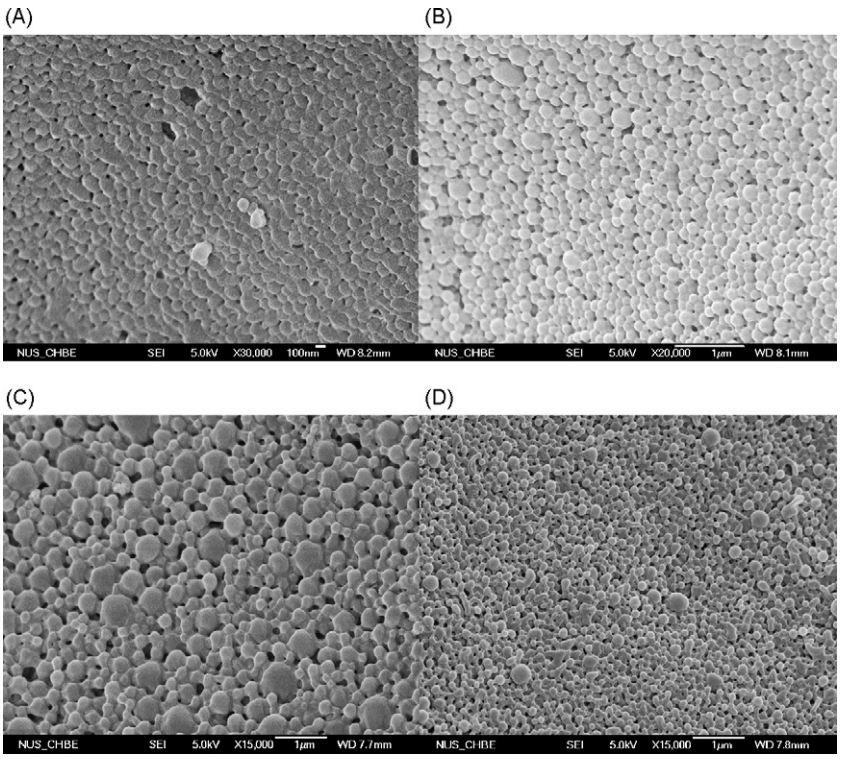

Fig. 1. FESEM images of polymeric nanoparticles. (A) mPEG–PLA/PEI; (B) PLGA/chitosan; (C) PLGA/PEI and (D) mPEG–PLA/chitosan nanoparticles.

methods produced heterogeneous size distributed nanoparticles which people previously used to load the plasmid DNA (Ravi Kumar et al., 2004).

Particle size has been demonstrated to play an important role in determining the level of cellular and tissue uptake, thus it will directly affect the transfection efficiency of dsRNA. A study of the effect of the particle size on the caco-2 uptake showed that the optimum size range for caco-2 cellular uptake of nanoparticles is 100–200 nm (Win and Feng, 2005). Another research has also been conducted to investigate the size effect on the gene transfection efficiency and PLGA nanoparticles with smaller size below 100 nm tend to gain higher gene transfection efficiency than those of 200 nm (Prabha et al., 2002).

The PLGA/PEI and mPEG–PLA/PEI nanoparticles were prepared by a nanoprecipitation method, wherein PEI 25 kDa was used to be the surfactant in the water phase to facilitate the polymers in the oil phase to form the stable nanoparticles, as well as modifying the nanoparticles into cationic surface to facilitate the loading of negatively charged dsRNA. Several concentrations of PEI 25 kDa (10–100 mg/ml) were tried and it was found that different concentrations of the surfactant PEI 25 kDa did not affect the size of the prepared nanoparticles. Excessive PEI could be removed by the rinsing process of the particles.

3.2. Surface morphology and characterization of the nanoparticles

The particles under FESEM investigation as shown in Fig. 1 showed sphere shape with smooth surface. The sub-cellular size demonstrated in this figure is consistent with the particle size values obtained by the laser light scattering technique.

The zeta potential of the nanoparticles significantly changed from negative to positive value as can be seen in Table 1 after being modified by the PEI 25 kDa and chitosan. Since the surface charge of the nanoparticle delivery system was primarily determined by the amount of the amine group (NH_2^+) of PEI 25 kDa and the chitosan coated on the surface of the nanoparticles, four groups of nanoparticles demonstrated different zeta potential indicating different charge potential of the nanoparticles (Table 1). MPEG–PLA nanoparticles demonstrated lower value of zeta potential than PLGA nanoparticles due to smaller surface and less coating of cationic polymers. PEI coating introduced more positive charge than chitosan probably because PEI possess more amine groups per molecule. In order to verify this point, XPS was further employed to quantitively analyze the nitrogen element on the surface of nanoparticles. As shown in Fig. 2, the PEI 25 kDa modified surface showed more nitrogen percentage of the nanoparticles compara-

Table 1
Size, zeta potential, of four PLGA and mPEG–PLA nanoparticles with PEI 25 kDa and chitosan surface modified.

	PLGA	PLGA–chitosan	PLGA–PEI 25 kDa
Effective diameter (nm)	147.6	260.5	200.3
Polydispersity	0.061	0.203	0.123
Zeta potential (mV)	−12.61 ± 1.27	28.68 ± 3.10	35.85 ± 0.71
	mPEG–PLA	PEG–PLA–chitosan	PEG–PLA–PEI 25 kDa
Effective diameter (nm)	88.9	226.7	164.6
Polydispersity	0.116	0.152	0.154
Zeta potential (mV)	−8.44 ± o.65	19.44 ± 0.89	25.39 ± 1.24

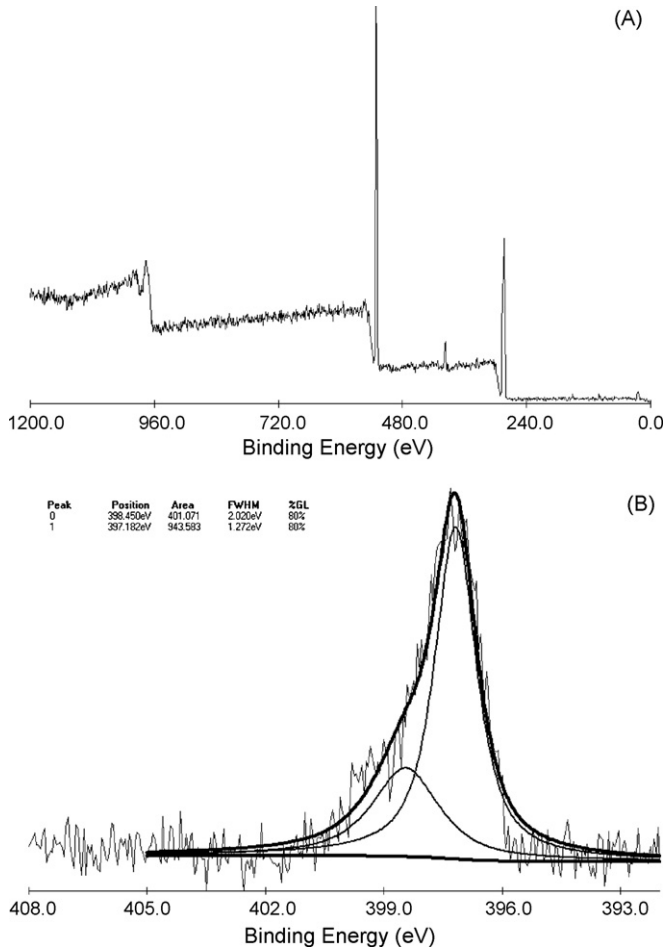

Fig. 2. XPS spectrum of PLGA nanoparticles with PEI coating. (A) The whole spectrum; (B) the nitrogen peak.

ble to the nanoparticles coated with chitosan. One possible reason might be that the nitrogen element percentage in PEI 25 kDa (C_2H_5N) (32.5%) is much higher than that in chitosan ($C_6H_{11}O_4N$) (8.69%). Another factor is attributed to the distribution of PVA on the surface of the PLGA/chitosan and mPEG–PLA/chitosan nanoparticles. As a surfactant, PVA could not be totally removed during the wash process (Zhang and Feng, 2006) and the remnant PVA might affect the adherence of the chitosan to the core of the nanoparticles. According to the element percentage in Table 2, it can be seen that the nitrogen percentage of the PLGA nanoparticle is higher than the mPEG–PLA nanoparticles indicating that PLGA attracted more cationic surfactants than mPEG–PLA. This phenomenon also leads to the fact that both PLGA and mPEG–PLA nanoparticles coating

Table 2
XPS analysis of surface element of PLGA nanoparticles and mPEG–PLA nanoparticles with PEI 25 kDa and chitosan as the surfactants.

Atomic mass concentration (%)	C	N	O
PLGA and chitosan	66.41%	1.04%	32.55%
PLGA and PEI 25 kDa	60.36%	8.97%	30.67%
mPEG–PLA and chitosan	49.47%	0.65%	49.88%
mPEG–PLA and PEI 25 kDa	55.96%	2.00%	42.04%

with PEI 25 kDa get higher zeta potential value than those coating with chitosan.

3.3. Gel retardation assay

The complexity of dsRNA with the nanoparticles was determined from the gel retardation assay. PLGA/PEI nanoparticles form complex with dsRNA when their ratio is above 6:1 (w/w) as shown in Fig. 3A. Here all of the four nanoparticles show a great dsRNA loading potential. This further confirmed the previous results that nanoparticles through polycations modification could absorb the negative charge nucleic acid and load it efficiently (Kim et al., 2005). And the charge interaction is firm enough to absorb the dsRNA at the surface of nanoparticles. This result also coincided with the zeta potential results and the XPS studies of the surface of nanoparticles. The optimum ratios of other nanoparticles for the dsRNA loading were studied in the similar way and the results are all above 10:1(data not shown). This further confirmed that the adsorption of dsRNA on the surface of the various nanoparticles largely depended on the quantity of the amine groups and also indicates that PLGA/PEI acquires better dsRNA loading capacity than all the other three nanoparticles.

3.4. Inhibition of HBsAg expression

PLC/PRF/5 cell line which would constitutively produce and secret HBsAg was used to assess the inhibition effect of S_2RNA delivered by our cationic polymers. Li et al. (2004) have demonstrated in their publication that the S_2RNA we used have been demonstrated to inhibit the HBsAg expression effectively delivered by the transfection agent lipofectamine 2000. Here we evaluated the transfection efficiency by calculating the percentage of HBsAg expression level of the treated cell well to the untreated cell well. Higher transfection efficiency also means effective treatment which is supposed to lead to lower HBsAg expression level.

As shown in Fig. 4, a significant decrease of expression of HBsAg was observed in each group of this nanoparticles mediated transfection. This suggested that the S_2RNA was efficiently delivered into the cells so that it could effectively inhibit the secretion of the HBsAg by the PLC/PRF/5 cells. Lipofectamine showed more than 80% decrease of the HBsAg expression. The

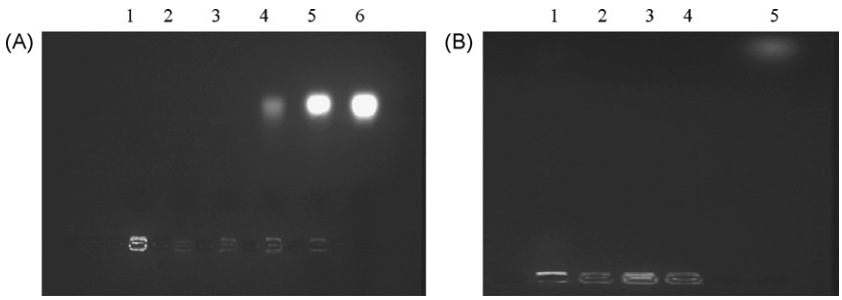

Fig. 3. Gel electrophoresis of the RNA and nanoparticles. (A) PLGA/PEI nanoparticles blending with RNA at different ratios: (1–5) 3 μg dsRNA: nanoparticle complexed with RNA (the amounts of the PLGA/PEI nanoparticles were 100, 50, 20, 10 and 4 μg, respectively). (6) 3 μg dsRNA. (B) mPEG–PLA/chitosan, mPEG–PLA/PEI nanoparticles, PLGA/chitosan, PLGA/PEI nanoparticles. (1–4) 1 μg dsRNA with 50 μg nanoparticle complex. (5) 1 μg dsRNA.

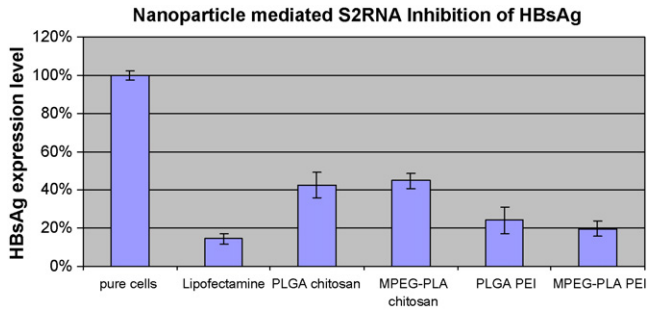

Fig. 4. Effects of siRNA carried by four nanoparticles in PLC/PRF/5 cells. From left to right: (1) pure cells untreated with siRNA; (2) cells treated with lipofectamine delivered siRNA; (3–6) cells treated with four nanoparticles delivered siRNA.

expression levels of four groups of cells treated by nanoparticles/RNA complex (PLGA/chitosan, mPEG–PLA/chitosan, PLGA/PEI, mPEG–PLA/PEI) are respectively 42.50, 44.80, 24.26, and 19.66%. All of those nanoparticles showed efficient delivery of the RNA. Among all the four nanoparticles, mPEG–PLA/PEI nanoparticles mediated RNA transfection demonstrated the best inhibition effect.

3.5. Cell viability assay

The cytotoxicity of the four nanoparticles was estimated by the MTS assay. The cell viabilities of nanoparticles were respectively 94.55, 105.93, 92.85, and 95.81%. All of the values are in excess of 90%, indicating the delivery system are more biocompatible and safer than lipofectamine comparing to the lower cell viability of lipofectamine (72.42%) as shown in Fig. 5. This result further demonstrated that the cationic nanoparticles would be of great potential to be the RNA delivery system.

Cationic nanoparticles combined the advantages of nanoparticles as a drug carrier and the complexation ability with nucleic acid in acting as the gene carrier. With better biocompatibility and easy producing property, cationic nanoparticles have gained more and more attention in the gene delivery area. Unlike the delivery of plasmid DNA, the transport of dsRNA is much easier since there is no need to deliver the dsRNA into the cell nucleus. Previous polymeric nanoparticles mediated gene delivery method tend to use double emulsion technique loading the therapeutic gene and was limited by the low encapsulation efficiency of the drug. Moreover, during the process of preparing nanoparticles through double emulsion technique, the contact of the dsRNA with a lot of organic solvent and the involvement of sonication procedure might damage or denature the dsRNA. Now more and more researchers employed the cationic nanoparticles simply because it is an easy, safe and efficient way to carry the therapeutic drug. Chitosan and PEI were both chosen as the cationic coating regents and compared

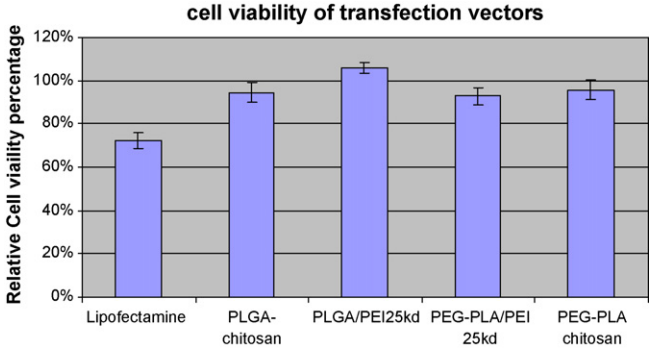

Fig. 5. Cell viability of nanoparticles in the PLC/PRF/5 cells after treatment with dsRNA/Polymer complex.

for the efficacy of RNAi delivery. Our results demonstrated that PEI 25 kDa is slightly better than the chitosan by showing higher gene transfection efficiency and higher loading efficiency of RNA. These results revealed that two factors affect the siRNA delivery efficacy of cationic nanoparticles. One factor is the property of coating polymers determined the RNAi loading capacity, thus the delivery efficacy. PEI demonstrated higher DNA loading capacity than chitosan in this system, which confirmed the previous studies (Munier et al., 2005). The other factor is the size dependence of the transfection efficiency of RNAi. Higher transfection efficiency achieved by the smaller PEI coated nanoparticles indicated that the smaller the particle is, the higher transfection efficiency could be achieved. It should be noted, however, that the cytotoxicity could be caused with increment of the amount of PEI for this application. Therefore, future work will also focus on a study of optimized transfection efficiency and minimum usage of PEI and the exploration of non-cytotoxic cationic polymers for this application.

4. Conclusions

In conclusion, the cationic nanoparticles can act as an efficient delivery system for dsRNA. PEI 25 kDa coating yielded the more positively charged nanoparticles with the higher DNA binding capacity and higher *in vitro* transfection efficiency. mPEG–PLA/PEI nanoparticles with the smallest size showed highest transfection efficiency. Of course, these results need to be further confirmed by the *in vivo* experiments.

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