International Journal of Pharmaceutics 369 (2009) 144–154

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

Preparation of polyethyleneimine incorporated poly(D,L-lactide-*co*-glycolide) nanoparticles by spontaneous emulsion diffusion method for small interfering RNA delivery

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ARTICLE INFO

Article history:
Received 28 August 2008
Received in revised form 16 October 2008
Accepted 16 October 2008
Available online 1 November 2008

Keywords:
Poly(D,L-lactide-*co*-glycolide)
Polyethyleneimine
Nanoparticles
siRNA
Gene silencing

ABSTRACT

Gene therapy based on small interfering RNA (siRNA) has emerged as an exciting new therapeutic approach. However, insufficient cellular uptake and poor stability have limited its usefulness. Polyethyleneimine (PEI) has been extensively studied as a vector for nucleic acids and incorporation of PEI into poly(D,L-lactide-*co*-glycolide) (PLGA) particles has been shown to be useful in the development of gene delivery. PEI was incorporated into the PLGA particles by spontaneous modified emulsification diffusion method. Incorporation of PEI into PLGA particles with the PLGA to PEI weight ratio 29:1 was found to produce spherical and positively charged nanoparticles where type of polymer, type and concentration of surfactant could affect their physical properties. Particle size of around 100 nm was obtained when 5% (m/v) PVA was used as a stabiliser. PLGA-PEI nanoparticles were able to completely bind siRNA at N/P ratio 20:1 and to provide protection for siRNA against nuclease degradation. *In vitro* cell culture studies subsequently revealed that PLGA-PEI nanoparticles with adsorbed siRNA could efficiently silence the targeted gene in mammalian cells, better than PEI alone, with acceptable cell viability. PLGA-PEI nanoparticles have been found to be superior to its cationising parent compound; PEI polymer.

1. Introduction

Recently, the discovery of interfering ribonucleic acid (RNAi) based technology has led to the development of siRNA as potential therapeutic agents in the treatment of diseases related to aberrant gene expression such as in cancers, infectious diseases as well as in inflammation. The technology is based on the application of short double stranded RNA (siRNA) in which the administered siRNAs are incorporated into a RNA-induced silencing complex (RISC) through the natural RNAi pathway and consequently silence gene expression of the target mRNA.

Introduction of unmodified and non-vectorized siRNA in cell culture normally results in unsuccessful knock-down of the target gene, since mammalian cells appear to lack the effective dsRNA-uptake machinery that is found in other species such as *Caenorhabditis elegan* (Rozema and Lewis, 2003). Furthermore, siRNA is a polyanion and hydrophilic molecule which cannot freely cross the lipid bilayers of the cell membrane (Muratovska and Eccles, 2004). A number of strategies have been demonstrated to deliver siRNA into cells. One of the strategies is the use of physical stimuli such as electroporation. However, electroporation has been shown to potentially reduce cell viability to less than 60% although high cellular uptake and activity could be achieved (Schiffelers et al., 2004). Other strategies include incorporating siRNA into cations such as cationic liposomes or cationic polymers. Besides a significant cytotoxicity (Yamauchi et al., 2006; Zhang et al., 2006) related to liposomes, low stability of liposomes in the blood (Akhtar, 1998; Kakizawa et al., 2006; Yamauchi et al., 2006; Zhang et al., 2006) and their tendency to accumulate in the Mononuclear Phagocyte System (MPS) have led to short activity *in vivo* and reduced access to other tissues (Litzinger, 1997; Chirila et al., 2002). Furthermore, their immunotoxic effects could potentially exacerbate diseases like arthritis (Medberry et al., 2004).

PEI is one of the most successful and well-known polymers for non-viral nucleic acid delivery systems (Godbey and Mikos, 2001; Schiffelers et al., 2005). PEI is a synthetic polymer that is very soluble in water with a high capacity for positive charge (Kircheis et

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0378-5173/$ – see front matter.
doi:10.1016/j.ijpharm.2008.10.012

al., 2001; Chirila et al., 2002; Panyam and Labhasetwar, 2003) and available in both linear and branched forms (Godbey and Mikos, 2001). The branched form contains 1°, 2° and 3° amines whereby each of them has the potential to be protonated and this has been used as a standard PEI for gene delivery due to its greater success in cell transfection compared to the linear form (Godbey et al., 1999). High positive charge density of PEI, causes a strong electrostatic interaction with negatively charged polyanions such as pDNA, ODNs and siRNA to form a small and reproducible complex (polyplex) which confers protection for the nucleic acids from degradation by nuclease activity. Apart from this, it has been shown that PEI is an excellent transfection reagent which could facilitate endosomal escape after entering the cells as it acts as a 'proton sponge' during acidification of the endosome (Boussif et al., 1995; Godbey and Mikos, 2001; Dailey et al., 2004). Although PEI offers greater protection and transfection for genetic molecules, it is not the ideal transfection reagent. An overall positive charge of these polyplexes engenders numerous problems such as interaction with blood components (Kircheis et al., 1999; Kircheis and Wagner, 2000; Zou et al., 2000; Rudolph et al., 2002) and activation of the complement system (Godbey and Mikos, 2001) which result in rapid clearance of complexes from the bloodstream as well as attributing to toxic effects at a cellular level (Merdan et al., 2002).

Several strategies have been applied to circumvent these problems, for example by masking the polyplex with a protective copolymer such as polystyrene and polyethylene glycol (PEG) (Finsinger et al., 2000; Rudolph et al., 2002; Kleemann et al., 2005) which yields polyplexes with a lower surface charge and decreased polyplex aggregation, decreased interactions with protein plasma as well as decreased cell toxicity (Ogris et al., 1999; Godbey and Mikos, 2001; Kleemann et al., 2005). Woodle et al. (2001) reported that conjugation of PEG with PEI containing ODNs could enhance stability of the complexes and allows them to circulate for a longer period in the blood through reduce non-specific interactions due to stabilization by PEG.

Recently, attempts have also been made by several groups to enhance the biocompatibility as well as transfection efficiency of PEI by employing low molecular weight linear PEI (Dailey et al., 2004; Kleemann et al., 2005) biodegradable PEI (Dailey et al., 2004) and a combination of cationic lipid or polymer with the PEI to form a hybrid gene delivery system with improved biocompatibility (Brownlie et al., 2004). Moreover, PEI is incorporated into the polymeric particles either by encapsulating it within the polymer (De Rosa et al., 2003; Bivas-Benita et al., 2004) or by coating/adsorbing the surface of the polymer-based carriers with PEI (Messai et al., 2003; Kasturi et al., 2005). Such carriers have the potential advantages including reduced PEI toxicity, increased bioavailability, improved drug loading efficiency (Kasturi et al., 2005) as well as controlled release of encapsulated genetic materials from the particles which in turn could reduce the need for repeated dosing (Garcia del Barrio et al., 2003). In addition to that, adsorption of nucleic acid onto the preformed cationic particles could preserve the structural integrity of nucleic acid from the harsh environment during particle preparation. Therefore, in this study, attempts were made to develop PLGA nanoparticles incorporating PEI using a spontaneous emulsification diffusion method where the resultant particles were utilised to adsorb siRNA for *in vitro* cell culture delivery purposes. The resultant particles were subjected to physical and biological characterisations which included particle size and surface charge measurements, siRNA loading efficiency/capacity, determination and evaluation of delivery efficiency into cells as well as cytotoxicity effects in cell culture systems.

2. Materials and methods

2.1. Materials

Poly(D,L-lactide-*co*-glycolide) with a monomer ratio of 50:50 2A (14 kDa), 3A (46 kDa) and 4A (58.8 kDa) Medisorb® were purchased from Alkermes, USA. Polyvinyl alcohol (PVA, 13–23 kDa and 30–70 kDa, 87–89% hydrolysed), poloxamer-188 and -407 were from Aldrich, Germany. Tween 80® (Polyoxyethylene sorbitan monooleate) and the branched form of polyethyleneimine (25 kDa) was also purchased from Sigma, UK. siRNA targeting against pGL3 luciferase gene (sense: 5′-CUUACGCUGAGUACUUCGATT-3′, antisense: 3′-TTGAAUGCGACUCAUGAAGCU-5′) and control siRNA (non-silencing) (sense: 5′-UUCUCCGAACGUGUCACGUTT-3′, antisense: 3′-TTAAGAGGCUUGCACAAGUGCA-5′) were synthesized by Proligo, France. Reporter vectors, pGL3 control and pRL-TK which encoded firefly luciferase and the renilla luciferase gene, respectively were purchased from Promega, UK. The Dual-Glo Luciferase assay system, agarose (low melting point) and Tris–borate–EDTA buffer pH 8.3 were from Promega, UK. Lipofectamine 2000, Opti-MEM I reduced serum medium and fetal bovine serum (FBS) were purchased from Invitrogen, UK. 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) was purchased from Sigma, UK. Other reagents and chemicals used were analytical grade.

2.2. Preparation of PLGA-PEI nanoparticles with adsorbed siRNA

The preparation method of PLGA–PEI nanoparticles was a modification of the technique described by Bivas-Benita et al. (2004). Briefly, a solution of 10% (m/v) PLGA 50:50 DL 2A in dichloromethane was stirred for 30 min. A PEI solution in acetone was prepared to a final concentration of 0.1% (m/v). The PEI solution was added to the PLGA to the resultant PLGA to PEI weight ratios of 29:1 and 59:1 that equivalent to 5 mg of PEI in 295 mg of PLGA and 10 mg of PEI in 290 mg of PLGA, respectively. Tween-80® was added to a final concentration of 1% (m/v) and acetone added up to 5 ml. This organic phase was mixed and poured into an aqueous phase of 10 ml of 5% (m/v) PVA or poloxamer-188 or -407 solution in double distilled water, followed by sonication with 15% of the maximum amplitude in continuous mode (MSE Soniprep 150 with a frequency of 23 kHz, Sanyo Gallenkamp, Leicester, UK) for 4 min. The dichloromethane was then evaporated for 4 h before centrifuging (Sorvall Combi Plus, Kendo, USA) at 20,000 rpm for 30 min twice by re-suspending the nanoparticles collected as a pellet in 50 ml distilled water. After centrifuging, the particle suspension was freeze-dried using an Edward Freeze Dryer (Micro Modulo, Edwards High Vacuum, UK). Operation conditions of PLGA-PEI nanoparticle preapration have been given in Table 1. siRNA adsorption onto PLGA-PEI nanoparticles was carried out in Tris–EDTA buffer or phosphate buffer pH 7.4. A fixed amount

Table 1
Summary of formulation and operative conditions of PLGA-PEI nanoparticle preparation.

Type of PLGA 50:50 DL	PLGA 50:50 DL to PEI weight ratio	PLGA 50:50 DL/PEI composition (mg/mg)	Surfactant
2A	59:1	295/5	Poloxamer-188
	29:1	290/10	Poloxamer-188
			Poloxamer-407
			PVA (13–23 kDa)
			PVA (30–70 kDa)
	10:1	273/27	Poloxamer-188
	5:1	250/50	Poloxamer-188
3A	29:1	290/10	Poloxamer-188
4A	29:1	290/10	Poloxamer-188

of siRNA was added drop-wise to an equal volume of various concentrations of PLGA-PEI nanoparticles depending on nitrogen to phosphate ratio of PEI and siRNA (N/P ratio of 50:1 to 1:1). A mass per phosphate of 325 Da for RNA and mass per charge of 43 for PEI were used to calculate N/P ratio. The suspension was then briefly mixed and incubated at room temperature for 1 h.

2.3. Characterisation of the PLGA-PEI nanoparticles

Mean particle diameter (*Z*-average) and surface charge of the nanoparticles were determined using photon correlation spectroscopy with a Malvern 4700 submicron particle analyser system (Malvern Instruments, UK) and Zetasizer® S (Malvern Instruments, UK), respectively. The measurements of particle size were made in RNase free water at 25 °C in triplicates and no further dilution was performed for these particles. For the determination of zeta potential, samples were diluted with RNase free water to an appropriate concentration to yield a count rate per second (KCps) in the range of 2500–3500. Each batch was analysed in triplicates.

2.4. Scanning electron microscopy

The morphology of the nanospheres was investigated by scanning electron microscopy (SEM) utilising a Philips XL30 (Philips Co., Eindhoven, The Netherlands). Briefly, particles were dried with Samdri 780 critical point dryer (Tousimis Research Corp., Maryland, USA), mounted onto an SEM stub with a double-sided carbon impregnated disc. Samples were then sputter-coated with gold using an Emscope Sputtering Coater (Ashford, UK) for 2 min at 30 mA before viewing under SEM.

2.5. Determination of siRNA loading efficiency

The loading efficiency of siRNA adsorbed onto the PLGA-PEI nanoparticles was obtained from the determination of free siRNA concentration in the supernatant recovered after particle centrifugation (13,000 × g, 15 min) by absorbance measurement at 260 nm using a spectrophotometer (Cary 3E, Varian, Palo Alto, USA). Supernatant recovered from unloaded PLGA-PEI nanoparticles (without siRNA) was used as a blank. siRNA loading efficiency was expressed as adsorbed siRNA as a percentage of the total amount of siRNA added.

2.6. Gel retardation assay

The binding of siRNA with PLGA-PEI nanoparticles was determined by 4% agarose (low melting point) gel electrophoresis. A series of different N/P ratios of siRNA to PLGA-PEI nanopaticles was loaded (20 μl of the sample containing 0.2 μg of siRNA). A 1:6 dilution of loading dye was added to each well and electrophoresis was carried out at a constant voltage of 55 V for 2 h in TBE buffer (4.45 mM Tris-base, 1 mM sodium EDTA, 4.45 mM boric acid, pH 8.3) containing 0.5 μg/ml ethidium bromide. The siRNA bands were then visualised under a UV transilluminator at a wavelength of 365 nm.

2.7. Biological activity of PLGA-PEI-siRNA nanoparticles

In vitro transfection studies were performed in CHO K1 (Chinese hamster ovary) and HEK 293 (human embryonic kidney) cells. The cells were seeded in a 96-well plate at a density of 30,000 cells per well in Opti-MEM 1 reduced serum medium containing 5% of FBS without antibiotic, 24 h prior to transfection. On the day of transfection, pGL3-control (0.15 μg) and pRL-TK vectors (0.05 μg) were co-transfected to the cells using Lipofectamine 2000™ according to manufacturer's instructions. Following 4 h of transfection period, the medium was removed and the cells were washed with PBS twice, after this, media was replaced with 100 μl fresh medium containing serum. 50 μl of PLGA-PEI nanoparticles adsorbed siRNA, siRNA alone or Lipofectamine 2000–siRNA complexes (each 50 μl of sample contained 4 pmol of siRNA) in the medium without serum were then added to the cells and incubated at 37 °C with a 5% CO_2 atmosphere for 24 or 48 h. After 24 or 48 h, luciferase activities were determined using Dual-Glo Luciferase Assay System.

2.8. Cytotoxicity assay

Cytotoxicity was measured by determining cell viability of PLGA-PEI nanoparticles with adsorbed siRNA, calculated as a percentage of the cell viability of untreated/non-transfected cell samples. The general cytotoxicity test followed the method of Chen et al. (2006). CHO K1 and HEK 293 cells were seeded in a 96-well plate at a density of 30,000 cells per well in Opti-MEM 1 reduced serum medium containing 5% of FBS and grown overnight. After 24 and 48 h incubation of PLGA-PEI-siRNA nanoparticles at 37 °C, 20 μl of MTT (3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide, 5 mg/ml, m/v) in sterile PBS was added to each well and then incubated for 4 h to allow formation of formazan crystals. After 4 h, the unreduced MTT and medium was removed and the cells were washed with PBS. 200 μl of DMSO was then added to each well to dissolve the MTT formazan crystals and the plate was incubated at 37 °C for 5 min. The absorbance of formazan products was measured at 540 nm using a microplate reader (Wallac Victor^2 1420 Multilabel Counter, Beckman Coulter Inc., Fullerton, USA).

2.9. RNA preparation and reverse-transcriptase PCR (RT-PCR)

Total RNA was prepared using the TRI Reagent® (Sigma, UK). The concentration of RNA was measured by the absorbance at 260 nm using spectrophotometer (Genespec 1, Hitachi Genetic Systems, Japan), and RNA integrity was confirmed by formaldehyde-formamide denatured agarose gel electrophoresis (Bio-Rad wide Mini-sub cell G7 electrophoresis box and a Bio-Rad Power PAC200 power supply, UK). One microgram of RNA isolated from each sample was used for cDNA synthesis. First-strand cDNA synthesis was primed with Anchored oligo $(dT)_{23}$ and carried out according to the manufacturer's two steps RT-PCR instructions (Enhanced Avian HS RT-PCR Kit, Sigma, UK). The cDNA equivalent to 100 ng of total RNA was subjected to subsequent PCR analysis which was performed in the presence of firefly luciferase primers (forward: CCAGGGATTTCAGTCGATGT, reverse: AATCTCACGCAGGCAGTTCT) with an initial denaturation step of 2 min at 94 °C, followed by 50 cycles of 15 s denaturation at 94 °C, 50 s annealing at 55 °C and 1 min elongation time at 68 °C. The final extension step was performed at 68 °C for 5 min. As negative controls, an identical set of reactions was set up without the addition of the cDNA. PCR reactions for the actin control were carried out separately for 25 cycles with β-Actin Primer Pair (Promega, UK). RT-PCR products were then subjected to electrophoresis using a 4% agarose (LMP) gel.

2.10. Statistical analysis

Data are presented as means ± standard deviations. The statistical significance was determined using one-way analysis of variance (ANOVA) followed by post hoc multiple comparison tests. *P* values of <0.05 were considered significant. The statistical analyses were carried out using SPSS Base 12.0 for Windows.

Table 2
Effect of polymer type and PLGA to PEI weight ratio on mean particle size, polydispersity index and surface charge of PLGA-PEI nanoparticles ($n = 3$). In these experiments, 0.5% (w/v) of poloxamer-188 was used as a surfactant and measurement of particle size and surface charge were made in RNase free water.

Type of PLGA 50:50 DL	PLGA 50:50 DL to PEI weight ratio	*Z*-average nm ± SD	Polydispersity index ± SD	Surface charge mV ± SD
2A (14 kDa)	5:1	253 ± 15	0.15 ± 0.12	+59.4 ± 1.1
2A (14 kDa)	10:1	290 ± 22	0.11 ± 0.11	+63.6 ± 1.3
2A (14 kDa)	29:1	562 ± 50	0.24 ± 0.21	+37.6 ± 0.6
2A (14 kDa)	59:1	661 ± 37	0.30 ± 0.20	−10.8 ± 0.2
3A (47 kDa)	29:1	533 ± 42	0.23 ± 0.01	+51.9 ± 0.8
4A (58.8 kDa)	29:1	473 ± 49	0.24 ± 0.07	+55.7 ± 1.0

3. Results

3.1. Particle size

3.1.1. Effect of PLGA to PEI weight ratio

The amount of PEI added to the PLGA 50:50 DL 2A was expected to affect not only the cationic density of nanoparticles but also particle size and morphology. Therefore, the amount of PEI added was specified as PLGA to PEI weight ratio, ranging from 59:1 to 5:1 and equivalent to 5–50 mg of PEI in 295–250 mg of PLGA. For this experiment, poloxamer-188 (0.5% m/v) was used as a surfactant. Decreasing the amount of PLGA in comparison to PEI resulted in the reduction of particle size by more than 2-fold from 661 to 253 nm ($P < 0.05$) (Table 2).

A lyophilised form of these nanoparticles with a high content of PEI (more than 10 mg of PEI or PLGA-PEI weight ratio 29:1) however, could not be further analysed due to the difficulty in re-suspending them in aqueous media. This was corroborated with the SEM studies where these nanoparticles (more than 10 mg PEI) did not exhibit a regular morphology in their freeze-dried form. Therefore, only PLGA-PEI weight ratios of 29:1 and 59:1 were used for the following experiments (Fig. 1A).

3.1.2. Effect of molecular weight of PLGA polymer

In this study, three different types of PLGA 50:50 DL were studied; 2A (14 kDa), 3A (47 kDa) and 4A (58.8 kDa). These polymers were "uncapped" or contained free carboxylic acid end group and the number (2A, 3A, 4A) represents the content of carboxyl group in the polymers. To evaluate their effects on particle size as well as surface charge of PLGA-PEI nanoparticles (PLGA-PEI weight ratio 29:1), poloxamer-188 was used as a stabiliser at a concentration of 0.5% (m/v). In agreement with the previous studies, a reduction of particle size was observed with the increase of PLGA molecular weight (Murakami et al., 1997) and "uncapped" end group (Cegnar et al., 2004) as shown in Table 2 and Fig. 1B.

3.1.3. Effect of type and concentration of surfactant

Two types of surfactant have been investigated; poloxamer and polyvinyl alcohol (PVA), and both with different molecular weights. For each type and molecular weight of these surfactants, three dif-

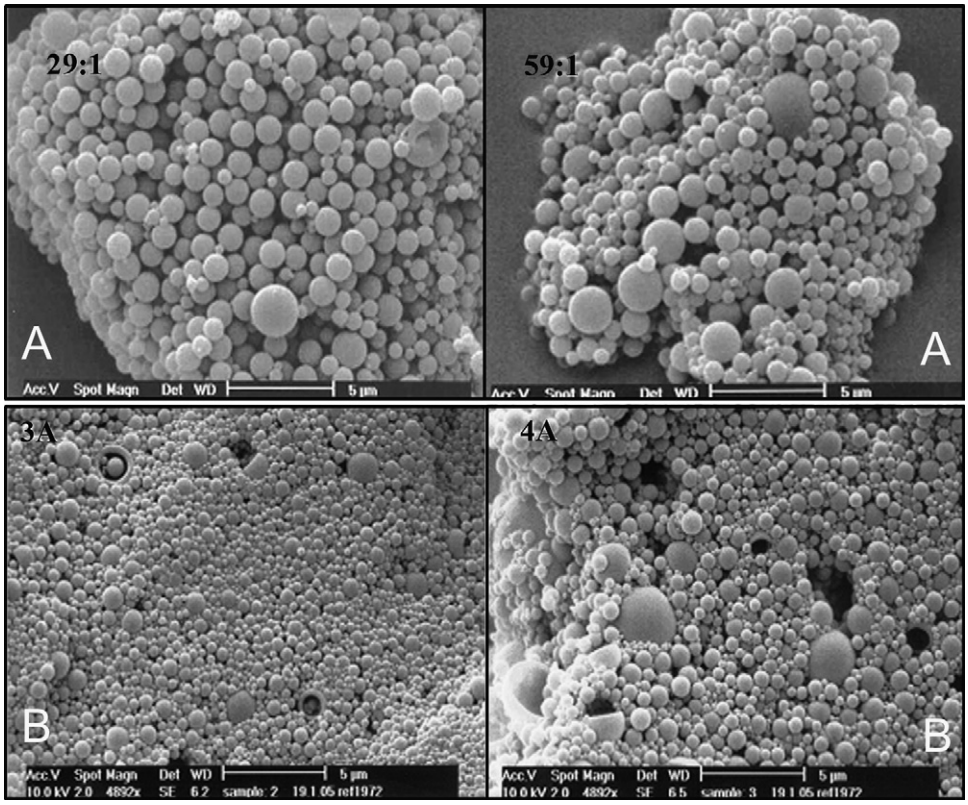

Fig. 1. (A) Effects of PLGA-PEI weight ratio 29:1 (left) and 59:1 (right) on morphology of PLGA-PEI nanoparticles. Particles were prepared by a spontaneous emulsification diffusion method using PLGA 50:50 2A and 0.5% (m/v) of poloxamer-188. (B) SEM images of PLGA-PEI nanoparticles prepared using PLGA 50:50 DL 3A (left) and 4A (right).

Table 3A
Effects of poloxamer-188 and -407 on particle size, polydispersity index and surface charge of PLGA-PEI nanoparticles ($n = 3$).

	PLGA to PEI (29:1)			PLGA to PEI (59:1)		
	Z-average nm ± SD	Polydispersity index ± SD	Surface charge mV ± SD	*Z*-average nm ± SD	Polydispersity index ± SD	Surface charge mV ± SD
Poloxamer-188 concentration %, m/v						
0.5	562 ± 50	0.24 ± 0.03	+37.6 ± 0.6	661 ± 26	0.30 ± 0.01	−30.5 ± 0.9
2.0	567 ± 36	0.40 ± 0.13	+59.7 ± 0.4	413 ± 10	0.53 ± 0.24	−27.2 ± 0.5
5.0	545 ± 23	0.65 ± 0.11	+55.1 ± 0.8	445 ± 5	0.40 ± 0.11	−10.8 ± 0.8
Poloxamer-407 concentration %, m/v						
0.5	701 ± 56	0.35 ± 0.09	+48.0 ± 1.5	1240 ± 112	0.37 ± 0.02	−32.3 ± 1.1
2.0	739 ± 44	0.48 ± 0.08	+50.3 ± 0.4	683 ± 23	0.55 ± 0.13	−25.3 ± 0.6
5.0	705 ± 34	0.73 ± 0.10	+50.7 ± 1.2	511 ± 12	0.48 ± 0.05	−19.3 ± 0.9

ferent concentrations were used in the preparation of PLGA-PEI nanoparticles which were 0.5, 2 and 5% (m/v) and only PLGA 50:50 DL 2A was utilised.

Independent of the molecular weight of the poloxamer, the increment of poloxamer concentration apparently had no effect on particle size of PLGA-PEI nanoparticles with a PLGA to PEI weight ratio 29:1, however it reduced the particle size of 59:1 PLGA to PEI weight ratio from 661 to 445 nm for poloxamer-188 ($P < 0.05$) and from 1240 to 511 nm for poloxamer-407 ($P < 0.05$). The details of their effects on particle size and size distribution of PLGA-PEI nanoparticles are shown in Tables 3A and 3B.

Particle size was found to be greatly reduced with increasing PVA concentration from 0.5 to 5% (m/v) ($P < 0.01$). In addition, PVA with molecular weight of 30–70 kDa was more effective than a lower molecular weight of 13–23 kDa in reducing particle size. Nevertheless, the results obtained showed that regardless of molecular weight, PVA was more effective in stabilising these nanoparticles than both types of poloxamers mentioned above. The details of particle size and polydispersity of PLGA-PEI nanoparticles are shown in Table 3B. Although, a significant increase in particle size of the freeze-dried form of PLGA 50:50 DL 2A-PEI nanoparticles was up to 6-fold observed. Addition of glycerol (5–35% of total polymer) was effective in prevention of aggregation/size increase (data not shown).

3.2. Surface charge of the nanoparticles

Surface charges of the PLGA 50:50 DL 2A-PEI nanoparticles at a weight ratio of 29:1 were all positive and varied from +37.6 ± 0.6 for 0.5% (m/v) of poloxamer-188 and +55.1 ± 0.8 mV for 5% (m/v) of poloxamer-188 ($P < 0.05$), respectively, while negative values of particle surface charge (−30.5 to −10.8 mV) were observed for PLGA 50:50 DL 2A-PEI weight ratio of 59:1 for all the tested poloxamer-188 concentrations. PEI was buried in the PLGA particle which attributed to the negative values of nanoparticle surface charge of 59:1 ratio. Similar results were obtained for PLGA-PEI nanoparticles made using poloxamer-407. The particle surface charge using poloxamer-407 was in the range of +48.0 ± 1.5 mV (0.5% m/v) to +50.7 ± 1.2 mV (5% m/v) for a weight ratio of 29:1 ($P > 0.05$) and −32.3 ± 1.1 mV (0.5% m/v) to −19.3 ± 0.87 mV (5% m/v) for a weight ratio of 59:1 ($P < 0.05$). In contrast to poloxamer, increasing the concentration of PVA resulted in a reduction of the particles' surface charge of PLGA-PEI nanoparticles. The surface charge of PLGA-PEI nanoparticles (PLGA to PEI weight ratio of 29:1) stabilised by PVA with molecular weight of 13–23 kDa was +46.1 ± 1.2, +40.1 ± 1.2 and +37.4 ± 1.0 mV for 0.5, 2.0 and 5.0% (m/v), respectively.

3.3. siRNA loading efficiency

3.3.1. Effect of N/P ratio

Analysis of siRNA loading efficiency adsorbed onto the PLGA 50:50 DL 2A-PEI nanoparticles with sizes around 100 nm was performed with PLGA-PEI nanoparticles prepared using the highest concentration of PVA (5% m/v, 30–70 kDa) and siRNA was adsorbed onto the particles (in phosphate buffer, 0.2 M, pH 7, pH 5.8 or distilled buffer) at different N/P ratios of PLGA-PEI nanoparticles to siRNA, ranging from 1:1 to 50:1. siRNA loading efficiency was then determined by spectrophotometry and the results showed a significantly increased siRNA loading efficiency from N/P ratio of 10:1 to 15:1 by 1.84-fold ($P < 0.05$). In agreement with the results obtained from the gel retardation assays, 100% of siRNA loading efficiency was achieved at the N/P ratio of 20:1 and at this point, siRNA was completely bound to the PLGA-PEI nanoparticles. This ratio was much higher than the equivalent N/P ratio of siRNA–PEI complexes (5:1) at which 100% siRNA loading efficiency was achieved (data not shown). This was possibly due to the partial exposure of PEI on the surface of PLGA-PEI nanoparticles; a portion of the amino groups of the PEI can be expected to interact with the carboxylic groups of PLGA or be buried inside the particles. Heparin was added to the sample suspensions to demonstrate the presence of siRNA adsorbed onto the particles. The migration of siRNA with the addition of heparin therefore confirmed the presence of siRNA on the surface of the particles as shown in Fig. 2A. In contrast to that, preliminary studies have shown that PLGA alone was unable to adsorb siRNA even though equivalent amount to the PLGA-PEI nanoparticles at N/P ratio of 20:1 were used in which the ratio could attribute to the complete binding of siRNA onto the surface of PLGA-PEI nanoparticles.

Table 3B
Effects of different molecular weights and concentrations of PVA on particle size, polydispersity index and surface charge of PLGA-PEI nanoparticles ($n = 3$).

PVA concentration %, m/v	Molecular weight of PVA					
	13–23 kDa			30–70 kDa		
	Z-average nm ± SD	Polydispersity index ± SD	Surface charge mV ± SD	*Z*-average nm ± SD	Polydispersity index ± SD	Surface charge mV ± SD
0.5	605 ± 12	0.41 ± 0.01	+46.1 ± 1.2	437 ± 10	0.50 ± 0.12	+40.6 ± 0.3
2.0	195 ± 13	0.08 ± 0.07	+40.1 ± 1.2	161 ± 9	0.08 ± 0.02	+35.1 ± 0.5
5.0	117 ± 2	0.10 ± 0.02	+37.4 ± 1.0	97 ± 5	0.08 ± 0.02	+29.4 ± 0.7

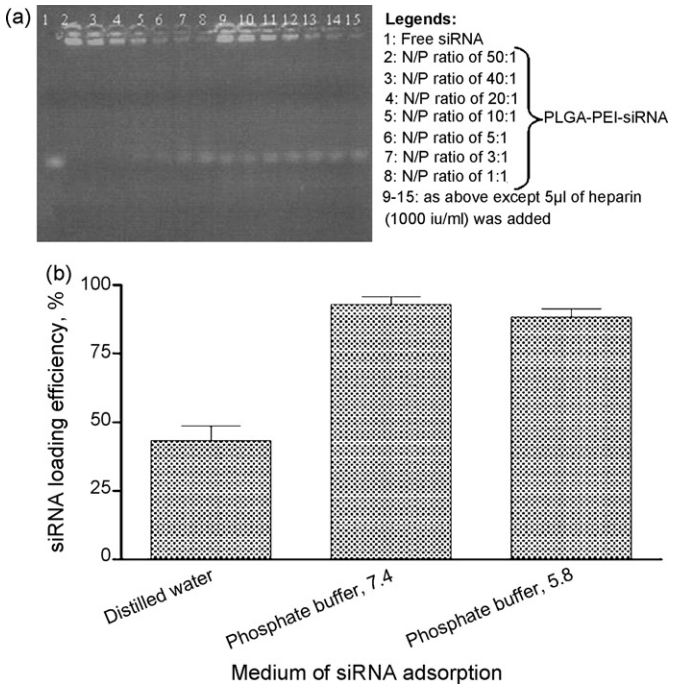

Fig. 2. PLGA-PEI nanoparticles binding efficiency and loading capacity to adsorb siRNA. (A) Effect of ratio between nitrogen of PEI in PLGA-PEI nanoparticles to phosphate of siRNA on efficiency of siRNA adsorption onto the PLGA-PEI nanoparticles made using PVA (30–70 kDa). Electrophoresis was carried out using 4% agarose (LMP) gel in TBE buffer containing 0.5 μg/ml ethidium bromide at pH 8. Each well starting from 1 to 8 contains 0.0387–2.8 μg of PLGA-PEI nanoparticles. A complete binding of siRNA to the PLGA-PEI nanoparticles was achieved at a N/P ratio 20:1. (B) Influence of adsorption medium on the siRNA loading efficiency adsorbed onto the PLGA-PEI nanoparticles ($n = 3$).

3.3.2. Effect of adsorption medium

siRNA was adsorbed onto the PLGA 50:50 DL 2A-PEI nanoparticles in phosphate buffer 0.2 M at pH 7 and 5.8 to determine the effects of pH on siRNA adsorption. A N/P ratio of 10:1 was chosen in this study as at this ratio, siRNA was not completely bound to PLGA-PEI nanoparticles in RNase free water as shown in previous experiments. A lower N/P ratio than 20:1 was used to evaluate any improvement in siRNA adsorption with different pH values since above this point, complete adsorption of siRNA was achievable even in RNase free water. The spectrophotometry results revealed that the siRNA loading efficiency with adsorbed siRNA onto the PLGA-PEI nanoparticles was greatly increased when siRNA adsorption was carried out in phosphate buffer 0.2 M regardless of the pH of the phosphate buffer (pH 7.4 or pH 5.8) instead of distilled water ($P < 0.05$) (Fig. 2B).

3.4. Transfection studies of siRNA–PEI complexes and PLGA-PEI with adsorbed siRNA

Biological activity studies of siRNA adsorbed onto PLGA 50:50 DL 2A-PEI nanoparticles were assessed in two different types of cells, namely HEK 293 and CHO K1 cell lines. In this study, only particles with a particle size around 100 nm were used and this could be achieved by preparing PLGA-PEI nanoparticles of 29:1 weight ratio using PVA as a stabiliser. PVA with either a molecular weight of 13–23 kDa or 30–70 kDa at a concentration of 5% (m/v) was shown to produce nano-size range particles of around 100 nm. Therefore, these particles were tested for their ability to deliver siRNA into the cells by varying their N/P ratio from 25:1 to 50:1 PLGA-PEI nanoparticles to siRNA.

3.4.1. Effect of N/P ratio and stabiliser on siRNA silencing

To study biological activities of siRNA adsorbed onto the PLGA-PEI nanoparticles, the cells were first transfected with reporter vectors, pGL3 control and pRL-TK and after 4 h of incubation, siRNA targeted to the luciferase pGL3 gene was introduced to the cells either adsorbed onto the surface of PLGA-PEI nanoparticles or complexed with PEI in order to evaluate silencing of the expression of the targeted gene. Analysis of the degree of downregulation of expression of the targeted gene expression was carried out by measuring the Relative Light Units (RLU) of luciferase expression using a Dual-Glo assay system kit which allows for the measurement of two types of luciferase protein, firefly and renilla luciferase to be measured. This is crucial because by normalising the expression of an experimental (firefly luciferase) to the expression of a control reporter (renilla luciferase) can help to differentiate between specific and non-specific cellular responses such as cell death, inhibition of cell growth and variable initial cell numbers. Additionally, expressing the results as a relative response ratio (RRR) permits the comparison of multiple treatments from the different experiments. However, it requires the inclusion of two sets of controls on each plate, a positive control that provides maximal luminescence (without siRNA treatment which was labelled as pGL3) and a negative control that provides minimal luminescence (Lipofectamine–siRNA complexes). Therefore, the RRR can be used to assist in determination of the effect of a new experimental treatment on reporter gene expression within the context of positive and negative control. Therefore, RRR value is in the range of 0–1, where positive value is defined as less effective than negative control (Lipofectamine 2000) and otherwise for negative value.

In HEK 293 cells, a high gene silencing effect of the targeted gene was observed at 24 h post-transfection for all of the cells treated with siRNA adsorbed PLGA-PEI nanoparticles (Fig. 3A). For the particles prepared using PVA with molecular weight of 13–23 kDa, 99% of gene downregulation of the targeted gene was observed at N/P ratio of 25:1 and the gene downregulation effect was slightly reduced to 96% and 87% for N/P ratio of 35:1 and 50:1, respectively. In contrast, particles prepared using of 30–70 kDa PVA showed an increase of gene downregulation with the increase of N/P ratio from 25:1 (28.1%) to 50:1 (97.1%) at 24 h post-transfection ($P < 0.05$). Interestingly, PLGA-PEI nanoparticles (except PLGA-PEI-siRNA nanoparticles prepared using 30–70 kDa PVA at N/P ratio of 25:1) appeared to be more efficient than PEI as a delivery system for siRNA ($P < 0.01$) because only 29% of targeted gene downregulation was observed for siRNA–PEI complexes (N/P ratio of 10:1). However, less than 30% of the gene silencing effect was measured for the cells treated with siRNA adsorbed to PLGA-PEI nanoparticles at 48 h post-transfection. On the other hand, siRNA–PEI complexes showed an increased effect of gene downregulation by 0.58-fold at 48 h post-transfection.

Furthermore, PLGA-PEI nanoparticles were comparable to the control, Lipofectamine 2000 as a transfection agent for siRNA at 24 h post-transfection. Dependent on N/P ratio of PLGA-PEI with adsorbed siRNA and molecular weight of PVA used in the particle preparation, these particles have shown a superior gene silencing effect than Lipofectamine 2000 (PLGA-PEI-siRNA with negative values) especially for PLGA-PEI-siRNA nanoparticles with N/P ratio of 35:1 (made using a lower molecular weight of PVA) as shown in the Fig. 3B ($P < 0.05$). This indicated that the formulation is better in transporting siRNA into cells than the Lipofectamine 2000 which may due to the 'proton-sponge effect' of PEI which induces endosomal escape for siRNA. Nevertheless, when comparing these gene silencing activities at 24 and 48 h post-transfection, a higher activity was obtained at 24 than 48 h post-transfection, suggesting the release of siRNA might occur within the first 24 h. Although the N/P ratio of 25:1 of the same formulation had showed a higher

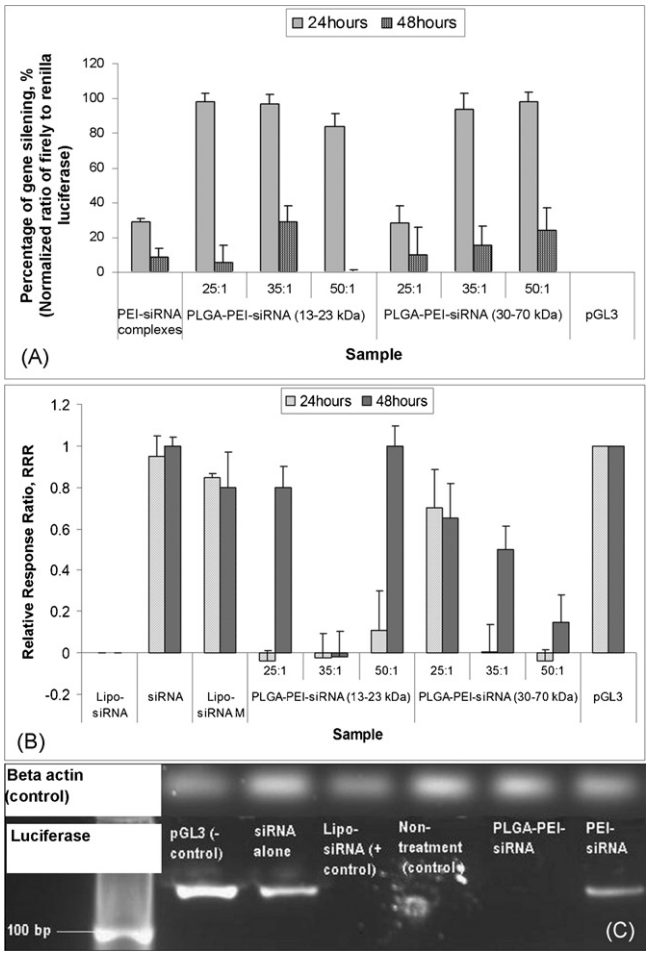

Fig. 3. (A) Percentage of gene silencing of PLGA-PEI nanoparticles with adsorbed siRNA in HEK 293 cells ($n = 9$). PLGA-PEI nanoparticles appeared to be more efficient than PEI in transfecting siRNA in cells. (B) Relative response ratio of PLGA-PEI nanoparticles with adsorbed siRNA in comparison to Lipofectamine 2000–siRNA complexes in HEK 293 cells ($n = 9$). Experiments A and B were performed on growing cells at 5×10^3 cell/well. PLGA-PEI nanoparticles showed a comparable effect with Lipofectamine 2000 (Invitrogen). *Keynotes:* Lipo–siRNA: Lipofectamine 2000–siRNA complexes, Lipo–siRNA M: Lipofectamine 2000–siRNA mismatch complexes and pGL3: control cells without siRNA treatment. (C) RT-PCR of total RNA extracted from HEK 293 cells treated with different formulations at 24 h post-transfection. RT-PCR analysis shows a reduction of mRNA level for firefly luciferase after treatment with the siRNA delivered by PLGA-PEI nanoparticles or Lipofectamine 2000 (Invitrogen) meanwhile PEI–siRNA complexes and siRNA alone show a minimal mRNA reduction. β-Actin was used as an internal experimental control. *Keynotes:* pGL3: cells transfected with pDNA encoded firefly luciferase without siRNA treatment (negative control), non-treatment control: non-treated cells either with pDNA or siRNA (negative control), Lipo–siRNA: Lipofectamine 2000–siRNA complexes (positive control), PLGA-PEI-siRNA: PLGA-PEI nanoparticles (PVA 13–23 kDa was used as stabiliser) with adsorbed siRNA (N/P ratio 35:1) and PEI-siRNA: PEI-siRNA complexes (N/P ratio 10:1).

silencing effect than 35:1 at 24 h post-transfection, but the formulation with N/P ratio of 35:1 had a sustain effect of gene silencing since the effect could still be detected at 48 h post-transfection and the silencing effect was comparable to Lipofectamine 2000.

Further investigation was performed by detecting firefly luciferase gene using RT-PCR technique from extracted total RNA of the cells treated with different formulations (at 24 h post-transfection) where the cells transfected with pGL3 and non-treated cells were used as negative controls. Lipofectamine 2000–siRNA complexes on the other hand, were used as a positive control and the experiment was further validated by detection of the β-actin gene (housekeeping gene). In this experiment, the absence of the bands for luciferase was observed for both Lipofectamine 2000–siRNA (Lipo–siRNA) complexes (positive control) and PLGA-PEI nanoparticles with adsorbed siRNA (Fig. 3C). This therefore, illustrated that the silencing of the targeted gene had occurred at the mRNA level of the cells treated with Lipo–siRNA complexes as well as PLGA-PEI nanoparticles with adsorbed siRNA which in turn supports the results obtained in the earlier experiments in this study where luciferase protein activity assays were used.

3.4.2. *Effect of cell line on siRNA silencing*

In a CHO K1 cell line, PLGA-PEI nanoparticles were less effective in transporting siRNA into cells as a lower gene silencing effect was observed compared to HEK 293 cells (Fig. 4A). At 24 h post-transfection, only 54 and 15% of gene downregulation was achieved for a N/P ratio of 35:1 and 50:1 respectively for the PLGA-PEI nanoparticles prepared using PVA with molecular weight of 13–23 kDa ($P < 0.05$), whereas 13 and 20% gene silencing was observed for PVA molecular weight of 30–70 kDa for the same N/P ratio as above, respectively ($P > 0.05$). Similar to the results obtained from the HEK 293 cells, the gene silencing effect of siRNA adsorbed onto the PLGA-PEI nanoparticles made using PVA with a molecular weight of 13–23 kDa was reduced to 32% and 11% for N/P ratios of 35:1 and 50:1 at 48 h post-transfection, respectively ($P < 0.05$). Interestingly, PVA with a molecular weight of 30–70 kDa facilitated slightly increased silencing of the targeted gene at 48 h post-transfection for both N/P ratios of PLGA-PEI nanoparticles with adsorbed siRNA as shown in the Fig. 4A. In comparison to Lipofectamine 2000, PLGA–PEI nanoparticles with adsorbed siRNA were less efficient in transfecting siRNA in the CHO K1 cells (RRR values were positive). However, for particles made using 30–70 kDa PVA, these particles showed comparable ability in silencing the targeted gene compared to siRNA–PEI complexes at 48 h post-transfection as shown in Fig. 4B. These findings therefore, indicate that the CHO K1 cell line is difficult to transfect with siRNA using PLGA-PEI nanoparticles but the efficiency of delivering siRNA into the cells as good as PEI–siRNA complexes.

3.5. *Cytotoxicity effect*

The cytotoxicity effects of PLGA 50:50 DL 2A-PEI nanoparticles as a delivery system for siRNA to the cells was investigated in cell lines, HEK 293 and CHO K1, using MTT assays. The results obtained revealed that the loss of cell viability depended on N/P ratio of PLGA-PEI nanoparticles with adsorbed siRNA and molecular weight of PVA used as stabiliser during the particle preparation. In both cells lines, 15–25% of cell loss was observed at 24 h post-incubation with PLGA-PEI nanoparticles with adsorbed siRNA. In HEK 293 cells, the loss of cell viability was increased with the increasing PLGA-PEI to siRNA ratio (Fig. 5A). However, the effect was transient as the cell viability was less affected at 48 h post-incubation and the effect was arguably more obvious for the cells treated with PLGA-PEI nanoparticles fabricated using lower molecular weight of PVA. Loss of cell viability in CHO K1 cells appeared to be insensitive to the molecular weight of PVA but more dependent on PLGA-PEI nanoparticles to siRNA N/P ratio (Fig. 5B). Approximately 25% loss of cell viability was observed for N/P ratio of 50:1 compared to less than 15% cell loss at 35:1 although the difference was not significant ($P > 0.05$). This was expected due to the increased amount of PLGA-PEI nanoparticles added to the cells (193.5, 135.5 and 67.75 μg for PLGA-PEI to siRNA ratio of 25:1, 35:1 and 50:1, respectively) which would also introduce increased amount of PEI. In line with the previous results, the percentage of cell viability in CHO K1 cells was increased at 48 h post-incubation, in comparison with 24 h, for the cells incubated with PLGA-PEI nanoparticles with adsorbed siRNA. This also illustrated that the particles had little adverse effect on cell growth.

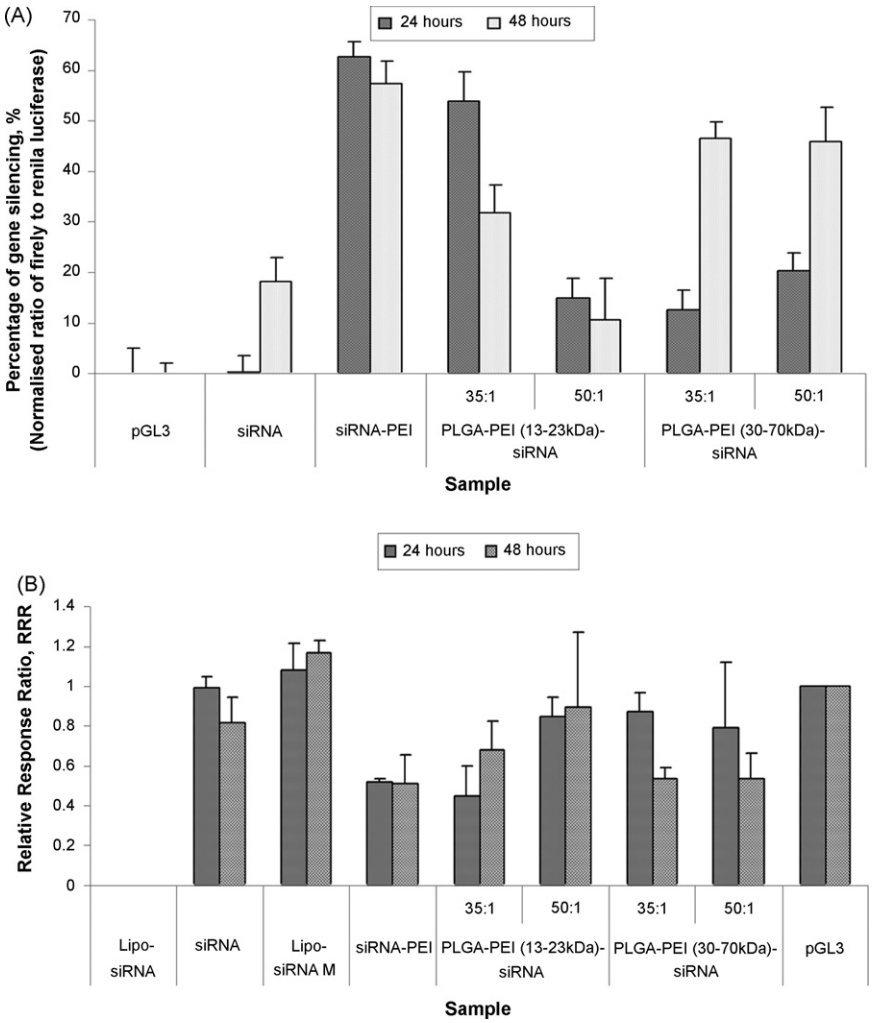

Fig. 4. (A) Percentage of gene silencing of PLGA-PEI nanoparticles with adsorbed siRNA in comparison to Lipofectamine 2000–siRNA complexes in HEK 293 cells ($n = 9$). (B) Relative response ratio of PLGA-PEI nanoparticles with adsorbed siRNA in comparison to Lipofectamine 2000–siRNA complexes ($n = 9$) in CHO K1 cells. Experiments were performed on growing cells at 5×10^3/well. *Keynotes:* Lipo–siRNA: Lipofectamine 2000–siRNA complexes, Lipo–siRNA M: Lipofectamine 2000–siRNA mismatch and pGL3: control cells without siRNA treatment.

4. Discussion

In this study, branched PEI of 25 kDa was used since PEI in its branched structure condenses nucleic acids to a greater extent than does linear PEI (Godbey et al., 1999). Branched PEI is in fact, consists of 25, 50 and 25% of primary, secondary and tertiary amines. Primary amines of PEI are reported to participate in forming complexes with nucleic acids while secondary and tertiary amines are responsible for substantial endosomal disruption after endocytosis due to their buffering effect at pHs under physiological conditions (Behr, 1997; Remy et al., 1998; Ahn et al., 2002). Therefore, the ability of PEI to deliver siRNA to the target cells was utilised in the incorporation of PEI into PLGA particles by a spontaneous emulsification diffusion method. PLGA has two functional groups; a hydroxyl and a carboxylic group at its terminal ends. Carboxylic acid end can be easily interacted with amine group of PEI via electrostatic interaction in which orientation of PEI on the surface of particles is attributed to the hydrophilicity of the PEI. Due to the use of acetone in this method, particle formation was observed immediately after mixing the organic and aqueous phases since acetone is highly miscible with water and could rapidly diffuse into the aqueous phase. Although, a smaller particle mean size was observed for a higher PLGA to PEI weight ratio, the freeze-dried form of weight ratios below 29:1 were permanently aggregated and difficult to re-suspend in aqueous media. A higher PEI amount in the particles was thought to cause inefficiency of water removal compared to the plain particles (without PEI) during freeze-drying process. As a result, the remaining water would affect the stability of the freeze-dried form of the particles due to the existence of mechanical stresses between air–water interfaces. In the emulsification diffusion method, the stabilisation of droplets by the stabiliser following diffusion process is important to avoid coalescence and the formation of agglomerates during solvent removal and polymer solidification (Hsu et al., 1999; Kwon et al., 2001). In this study, poloxamer (poloxamer-188 and -407) and PVA (13–23 kDa and 30–70 kDa) were studied as stabilisers at different concentrations. The findings obtained demonstrated that poloxamer-188 was more effective in stabilising the emulsion during particle preparation than poloxamer-407 as it produced smaller particles with narrow size distribution. A larger particle size for the PLGA-PEI nanoparticles stabilised by poloxamer-407 was also thought to be related to a longer chain length of polyethylene oxide segment of the poloxamer-407 (average number of ethylene oxide ≈200 units) compared to poloxamer-188 (average number of ethylene oxide ≈153 units) (Dunn et al., 1997). The hydrophilic polyethylene oxide can extend into solution and shield the surface of the parti-

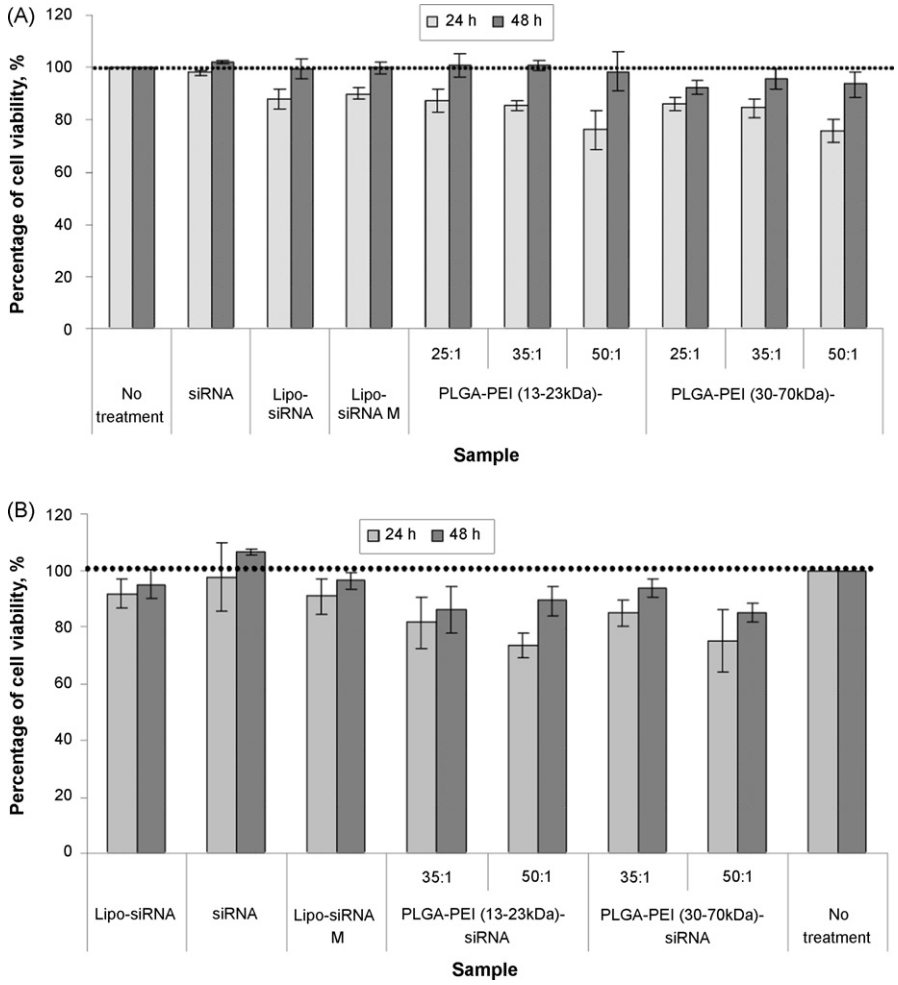

Fig. 5. Toxicity effect of PLGA-PEI nanoparticles with adsorbed siRNA in cell cultured *in vitro*. (A) Toxicity effect of PLGA-PEI nanoparticles with adsorbed siRNA in HEK 293 cells at 5×10^3/well ($n = 3$). (B) Toxicity effect of PLGA-PEI nanoparticles with adsorbed siRNA in CHO K1 cells at 5×10^3/well ($n = 3$). *Keynotes:* Lipo–siRNA: Lipofectamine 2000–siRNA complexes and Lipo–siRNA M: Lipofectamine 2000–siRNA mismatch.

cle which also potentially influences the structure of the resulting particles. However, a smaller particle size was obtained when PVA was used as a stabiliser compared to poloxamer-188 or poloxamer-407 at the same concentration. On the other hand, the decrease of particle surface charge of these nanoparticles when increasing the concentration of surfactant correlated with the increase of PVA adsorbed onto the surface of nanoparticles which shifts the shear plane outwards, resulting in a reduction of zeta potential (Dunn et al., 1997). Nevertheless, in the case of the poloxamer, the results were also indicating that poloxamer as a stabiliser had less effect on particle surface charge where nanoparticles had a PLGA to PEI weight ratio of 29:1. This was thought to be due to the presence of PEI in the PLGA particles and the orientation of PEI on the surface of particle which might interfere with the adsorption of hydrophobic part of poloxamer onto the PLGA particles. Therefore, it can be concluded that the PLGA-PEI weight ratio plays a more important role in determining the particle surface charge of these PLGA-PEI nanoparticles when poloxamer was added as a surfactant. The findings also demonstrated that particle size and surface charge of PLGA-PEI nanoparticles were affected by the types and molecular weight of PLGA polymer that were studied in this experiment. In fact, particle size has also been reported to be affected by the polymer structure which differed in polarity or molecular weight (Cegnar et al., 2004). In some cases, particle size increases as polymer molecular weight increases; due to a higher viscosity of organic solution which resistance to the shear forces and hinder the nanoparticle formation. In our study, the particle size of PLGA-PEI nanoparticles were smaller as molecular weight increased but, the difference was not significant ($P > 0.05$). This was thought to be due to the increase number of carboxylic groups which were more hydrophilic (Cegnar et al., 2004). The increase in particle positive surface charge with PLGA 3A and 4A was expected since a higher number of carboxylic groups within the PLGA molecular structure could efficiently interact with PEI amino groups once they were ionized in aqueous medium. The addition of glycerol to the particle suspension of poloxamer-188 stabilised PLGA-PEI particles was also found to prevent aggregation as no significant increase in particle size was observed in these samples following freeze-drying. The addition of glycerol also had less effect in preventing observed particle size increase after freeze-drying for the particles prepared using poloxamer-407.

The significant increase in siRNA loading efficiency in phosphate buffer may be due to the presence of phosphate salt in the adsorption medium which could allow a better conformation of PEI polymer chain to attract siRNA for binding. It was shown by Rehmet and Killmann (1999) that polymer at the particle surface was influenced by salt concentration as in the presence of salt, a loop and tail conformation of the long polymer chain was obtained due to the screening of the colloidal charge. In contrast, in the absence of salt,

the conformation of polymer mentioned to be in flat form, arising from charge neutralization (Rehmet and Killmann, 1999; Trimaille et al., 2003). Therefore, the loop and tail conformation of PEI at the surface of particles can be expected to facilitate the accessibility of siRNA to attract and interact with amino groups of PEI and form complexes. Furthermore, an increased siRNA loading efficiency in the presence of salt was also thought to be due to a higher melting temperature (T_m) of siRNA in phosphate salt (~72.3 °C) than in RNase free water (~46.1 °C). T_m is the temperature which half the oligo strands are hybridized to complimentary sequences and another half are free in solution as single strands. Therefore, siRNA is more susceptible to denature into single strands in water, thus it may require twice as many binding sites with PEI compared to in the presence of phosphate salt and this could also affect siRNA stability and efficacy in inducing RNAi. Moreover, interaction of siRNA with PLGA-PEI nanoparticles at N/P ratio of 20:1 appeared to be not affected by the increasing molar concentration of sodium chloride (0.0125–0.2 M) as determined by the gel retardation assay (data not shown). The pH of the adsorption medium however, had less effect on siRNA loading efficiency through the adsorption onto the PLGA-PEI nanoparticles as shown in Fig. 2B.

PLGA-PEI nanoparticles had a negative particle surface charge either dispersed in phosphate buffer at pH 7.4 or 5.8. The value of the particle surface charge was −3 and −1 mV for pH 7.4 and 5.8, respectively compared to +30 mV in RNase free water. The reduction of particle surface charge of PLGA-PEI nanoparticles in phosphate buffer may be due to the screening of the colloidal charge by the salt and the neutralization of the positive charge by the siRNA. This was confirmed by the increase of particle surface charge to +11 and +18 mV for PLGA-PEI-siRNA nanoparticles previously adsorbed in phosphate buffer pH 7.4 and pH 5.8, respectively upon re-suspending nanoparticle pellets in distilled water following centrifugation.

The transfection studies in mammalian cell cultured *in vitro* revealed that the ability of PLGA-PEI to transfect siRNA in cells was dependent on N/P ratio and the expression of the target gene was knocked-down significantly by a number of the PLGA-PEI-siRNA formulations at 24 and 48 h. The results also demonstrated that the ability of PLGA-PEI nanoparticles to deliver siRNA was dependent on the cell line tested, as different cell lines have different characteristics and cellular surface components which might affect cellular binding and uptake of these particles into cells. In future, we are also planning to look into particle cell trafficking and siRNA dissociation from the particles because they play important role in delivery efficiency. Although MTT assays showed a slight loss in viability of the transfected cells with the PLGA-PEI-siRNA nanoparticles compared to non-treatment cells, a partial or full restoration in cell viability was observed at 48 h post-incubation which indicated the effect was transient and this was highly dependent on N/P ratio as well as type of PVA molecular mass.

5. Conclusions

From this work, PEI has been shown to have the ability to deliver siRNA into cells. Modification of PLGA nanoparticles by incorporating PEI as well as optimisation of their preparation process (spontaneous emulsification diffusion method) has led to the formation of a better delivery system for siRNA with a higher transfection efficiency compared to PEI polymers. This system also has a relatively low cytotoxicity.

Acknowledgement

The authors would like to thank Ministry of Science, Technology and Innovative of Malaysia for the funding of this work.

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