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Establishing chitosan coated PLGA nanosphere platform loaded with wide variety of nucleic acid by complexation with cationic compound for gene delivery

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Abstract

The purpose of this paper was to establish the surface modified poly(D,L-lactide-*co*-glycolide) (PLGA) nanosphere platform with chitosan (CS) for gene delivery by using the emulsion solvent diffusion (ESD) method. The advantages of this method are a simple process under mild conditions without sonication. This method requires essentially dissolving both polymer and drug in the organic solvent. Therefore a hydrophilic drug such as nucleic acid is hardly applied to the ESD method. Nucleic acid can easily form an ion-complex with cationic compound, which can be dissolved in the organic solvent. Thereafter, nucleic acid solubility for organic solution can increase by complexation with cationic compound. We used DOTAP as a cationic compound to increase the loading efficiency of nucleic acid. By coating the PLGA nanospheres with CS, the loading efficiency of nucleic acid in the modified nanospheres increased significantly. The release profile of nucleic acid from PLGA nanospheres exhibited sustained release after initial burst. The PLGA nanospheres coated with chitosan reduced the initial burst of nucleic acid release and prolonged the drugs releasing at later stage. Chitosan coated PLGA nanosphere platform was established to encapsulate satisfactorily wide variety of nucleic acid for an acceptable gene delivery system.

Keywords: Poly(D,L-lactide-*co*-glycolide); Nanosphere; Chitosan; Emulsion solvent diffusion method; pDNA; Antisense oligonucleotide; siRNA

1. Introduction

Therapeutic agents based on plasmid DNA (pDNA) or oligonucleotide (ON) can provide a most promising way to overcome disease. To realize this idea, wide variety of vectors to deliver therapeutic genes into the desired target cells have been studied (Nishikawa and Hashida, 2002). An ideal gene delivering carrier could transport safely genetic materials without any toxicity and immune responses appeared (Smith et al., 1997). Most researches used the viral vector using either retrovirus and adenovirus or liposomes (Büning et al., 2003). Recently, many non-viral vectors which were modified with fusogenic peptides (Rudolph et al., 2003), cationic lipids (Wiethoff et al., 2004), cationic polymers (Godbey et al., 1999) and so on, have been reported. However, it was also reported that some cationic compound caused cytotoxicity (Maheshwari et al., 2000).

Polymeric nanospheres have been used to deliver medicines because of their advantages such as high stability, easily uptaken into the cells by endocytosis, and targeting ability to specific tissues or organs by adsorption or binding with ligand at the surface of the particles (Lobenberg et al., 1997). In particular, biodegradable nanospheres are available for delivering drugs and degraded after passing required specific site (Belbella et al., 1996). Among them poly(lactide) (PLA) and poly(D,L-lactide-*co*-glycolide) (PLGA) have been approved by the FDA for certain human clinical uses (Sahoo et al., 2002). The degradation time of PLGA can be altered from days to years by varying the molecular weight, the lactic acid to glycolic acid ratio in copolymer, or the structure of nanospheres. PLGA nanospheres have been suggested to be a good gene delivery carrier because of

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the safety and achieving sustained release (Prabha et al., 2002). There are two types of nanoparticle system carrying nucleic acid, i.e. DNA or RNA entrapping system (Wang et al., 1999) and surface binding system (Kim et al., 2005). Surface binding systems utilize an ionic interaction between cationic polymer and the anionic nucleic acid. Nucleic acid entrapping system is a reservoir type nanosphere system. This system has the advantages of nucleic acid protection during the drug releasing. Whereas this preparation process of PLGA nanosphere requires sonication or high shear agitation by a homogenizer causing a degradation of DNA.

We have developed the emulsion solvent diffusion (ESD) method in water for preparing PLGA nanospheres (NS) (Kawashima et al., 1998). The advantages of this ESD method are so that the nanospheres can be prepared under simple process and mild condition without using sonication. We have successfully prepared mucoadhesive PLGA nanospheres by modifying the surface of nanospheres with chitosan for improvement of peptide absorption through the mucous membrane (Kawashima et al., 2000; Yamamoto et al., 2005). PLGA and chitosan are biocompatibile, biodegradable and non-toxic polymer. Furthermore, chitosan promotes enhancing the transport of drugs across the cell membrane (Mao et al., 2001). Therefore, chitosan and chitosan derivatives might be preferentially chosen as potentially safe and useful cationic carriers for gene delivery. In this study the ESD method was applied to encapsulate nucleic acids, such as pDNA, AS-ON, siRNA and so on, into the surface modified PLGA nanosphere with chitosan. In the ESD method, the drugs to be encapsulated should be dissolved in the good solvent (e.g., acetone) for polymer used as a matrix material of nanosphere. Therefore a hydrophilic drug such as nucleic acid is hardly applied to the ESD method. To overcome this restriction, the hydrophilic properties of drug should be modified desirably to lipophilic. Preliminary we found that nucleic acid formed a complex with cationic lipid, increasing the solubility of drug in the organic solvent.

The aims of this study are to establish the preparation method of DNA or RNA encapsulating chitosan-coated PLGA nanospheres by using the ESD method with the DNA or RNA complex dissolved in the organic solvent. And the physicochemical properties (e.g., particle size, zeta potential, loading efficiency and release behavior) of DNA or RNA loaded PLGA nanospheres prepared by the ESD method was compared with those of nanospheres prepared by W/O/W emulsion solvent evaporation methods.

2. Materials and methods

2.1. Materials

PLGA (lactide:glycolide = 75:25, MW = 5000, Wako, Osaka, Japan) was used as a matrix material for the nanospheres. Polyvinylalcohol (PVA, Kuraray, Osaka, Japan) was used as a dispersing agent. D(−)-Mannitol (Kishida Chemical Co., Ltd., Osaka, Japan) was used as a cryoprorectant for the reconstitution of oligonucleotide (AS-ON or siRNA) loaded PLGA nanospheres, when redispersed in aqueous medium. Chitosan (MW 20,000; deacetylation degree 84.2%; Katakurachikkarin, Tokyo, Japan) was used as coating polymer to modify the surface of PLGA nanospheres. 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP, Sigma, St. Louis, MO, USA) and chitosan were used as a cationic complexing agent for the preparation of pDNA, AS-ON and siRNA loaded PLGA nanospheres. pDNA encoding the luciferase gene (pCMV-Luciferase) as a model pDNA was a gift from Prof. M. Hashida (Kyoto University). The plasmid was propagated in *E. coli* and purified by a EndoFree Plasmid Giga Kit (Qiagen, Hilden, Germany). Phosphorothioate antisense oligonucleotide targeting protein kinase C alpha (PKC-α, 5′-GTTCTCGCTGGTGAGTTTCA-3′, Sigma, St.Louis, MO, USA) were used as a model antisense DNA. Cy3 labeled siRNA targeting PKC-α (sense: 5′-GGACAUAUCAAAAUUGCUGTT-3′, antisense: 3′-TTCCUGUAUAGUUUUAACGAC-5′-Cy3, Dharmacon Inc.,Chicago, IL, USA) was used as a model siRNA. All other chemicals were obtained commercially at the highest analytical grade available.

2.2. Preparation of pDNA loaded PLGA nanospheres by water–oil–water emulsion solvent evaporation method

pDNA (pCMV-Luciferase) loaded PLGA NS (non-coated and chitosan-coated) was prepared by water–oil–water (W/O/W) emulsion solvent evaporation method (Wang et al., 1999). Hundred micoliters of Tris–EDTA (TE) buffer (pH 8.0) containing 100 μg of DNA was emulsified in 500 μl of chloroform containing 100 mg of PLGA by sonication (Sonifier, Branson, USA, Duty cycle 75%, Out put 2) for 10 s. The resulting primary emulsion was added to 2 ml of 10% (w/v) PVA solution and sonicated for 5, 15, 60 s to form a double emulsion. The resultant emulsion was added dropwise into 18 ml of 10% (w/v) PVA solution and agitated for 3 h at room temperature under evaporation, completely removing chloroform. The PLGA nanospheres were collected by centrifugation at 43,400 × *g*, for 10 min at 4 °C, washed three times with distilled water and freeze-dried for 3 days. For the preparation of chitosan coated PLGA nanospheres (CS-PLGA NS), chitosan (0.25%, w/v)-PVA (10%, w/v) mixed solution was used as dispersing phase for the emulsion solvent diffusion process.

2.3. Preparation of pDNA loaded PLGA nanospheres by the emulsion solvent diffusion method

Firstly, to prepare DOTAP/pDNA complex, 50 μl of TE buffer containing 10–100 μg of DNA was added to the same volume of MilliQ water containing 0–1000 μg DOTAP solution with stirring. In the case of CS/pDNA loaded PLGA nanospheres, to prepare CS/pDNA complex, 50 μl of TE buffer containing 10–100 μg of DNA was added to the same volume of acetate buffer (pH 4.4) containing 0–1000 μg CS solution with stirring. The PLGA (100 mg) and pDNA complex solution were dissolved in acetone (2 ml). The resultant organic solution was poured into 50 ml of an aqueous PVA solution (2%, w/v) and stirred at 400 rpm using a propeller type agitator with three blades. The entire dispersed system was then centrifuged (43,400 × *g* for 10 min) and resuspended in MilliQ

water (10 ml). The resultant dispersion was dried using a freeze drying method. For the preparation of chitosan coated PLGA nanospheres (CS-PLGA NS), chitosan (0.05%, w/v)-PVA (1%, w/v) mixed solution was used as dispersing phase for the emulsion solvent diffusion process.

2.4. Preparation of oligonucleotides (AS-ON, siRNA) loaded PLGA nanospheres by the emulsion solvent diffusion method

Oligonucleotides (ON) loaded PLGA NS were prepared by the emulsion solvent diffusion method in water. First, to prepare DOTAP/ON complexes, 50 μl of MilliQ water containing 5–50 μg of ON was added to the same volume of MilliQ water containing 0–500 μg DOTAP solution with stirring. The ratio of ON to DOTAP was 1:0–10 (w/w). The PLGA (50 mg) and DOTAP/siRNA complex solution were dissolved in acetone (2 ml). The resultant organic solution was poured into 25 ml of an aqueous PVA solution (2%, w/v) and stirred at 400 rpm using a propeller type agitator with three blades. The entire dispersed system was then centrifuged (43,400 × *g* for 10 min) and resuspended in MilliQ water (10 ml). The same volume of mannitol solution (5%, w/v) as a cryoprotectant was added to nanospheres suspension. The resultant dispersion was dried using a freeze drying method. For the preparation of chitosan coated PLGA nanospheres (CS-PLGA NS), chitosan (0.05%, w/v)-PVA (1%, w/v) mixed solution was used as dispersing phase for emulsion solvent diffusion process.

2.5. Stability of nucleic acids

The stability of naked pDNA and the complex of pDNA with DOTAP against various conditions exposed during manufacturing PLGA nanospheres was investigated by monitoring degradation with 1% agarose gel electrophoresis. The gels were prepared with 1% (w/v) agarose in Tris-acetate–EDTA buffer (pH 8.3). A fixed amount (1 μg) of pDNA was complexed with various amounts of DOTAP in MilliQ water. Gel electrophoresis was performed at a constant 100 V for 0.5 h. pDNA was visualized using ethidium bromide staining and gel images were obtained by using the FAS-III UV-image analyzer (Toyobo Co., Ltd., Osaka, Japan). 1 kb DNA ladder (New England Biolabs, Inc.) was used as a DNA size marker.

2.6. Analysis of physicochemical properties of nanospheres

Particle size and zeta potential measurements were performed using Zetasizer® 3000 HSA (Malvern Instruments, UK). Particle size was measured by photon correlation spectroscopy (PCS). Zeta potential determinations were based on electrophoretic mobility of the nanospheres in the aqueous medium.

The amount of pDNA or AS-ON entrapped in the nanospheres was determined after by dissolving the nanospheres (10 mg) in acetonitrile (1 ml), to which acetate buffer (pH 4.4, 0.5 ml) containing 0.1% (w/v) SDS was added to precipitate the polymer and dissolve the pDNA or AS-ON in the resultant aqueous mixture. The pDNA contents were determined by fluorescence PicoGreen Quantitation kit (Molecular Probes, Eugene, OR, USA) with excitation at 480 nm and emission at 520 nm. The AS-ON contents were determined by fluorescence Oligreen Quantitation kit (Molecular Probes, Eugene, OR, USA) with excitation at 550 nm and emission at 565 nm. The amount of Cy3 labeled siRNA entrapped in the nanospheres was analyzed by dissolving the nanospheres (2 mg) in acetonitrile (1 ml), to which acetate buffer (pH 4.4, 0.5 ml) containing 0.1% (w/v) SDS was added to precipitate the polymer and dissolve the siRNA in the resultant aqueous mixture. The siRNA contents were determined by Cy3 fluorescence measurement with excitation at 480 nm and emission at 520 nm. The loading efficiency of nucleic acid was calculated from the following equation.

$$\text{Loading efficiency}(\%) = \frac{\text{weight of nucleic acids in nanospheres}}{\text{weight of nucleic acids formulated in the system}} \times 100$$

2.7. Release studies of nucleic acid from PLGA nanospheres

The properties of pDNA or siRNA release from nanospheres were investigated in vitro. A 10 mg sample of nanospheres was dispersed in 5 ml PBS (pH 7.4) in a test tube shaken horizontally at 37 °C. At the different residence time, the buffer was separated from the nanospheres by centrifugation (43,400 × *g* for 10 min) and analyzed the amount of released pDNA or Cy3 labeled siRNA. After each determination, the nanospheres were resuspended in the fresh medium.

3. Results and discussion

3.1. Effect of ultrasonication time on the physicochemical properties of pDNA loaded chitosan-coated PLGA nanospheres prepared by W/O/W emulsion solvent evaporation method

Firstly, PLGA nanospheres were manufactured conventionally by using W/O/W emulsion solvent evaporation method (Wang et al., 1999). Effects of sonication time on the particle size and pDNA loading efficiency are shown in Fig. 1. The particle size of PLGA nanospheres could be controlled by sonication time (5, 20, 60 s.) for emulsification from 1000 to 200 nm. pDNA loading efficiency decreased with increasing sonication time because of pDNA degradation occurred by ultrasonication. Degradation of pDNA was confirmed from the band of agarose gel electrophoresis obtained after 1 min eradiation of sonication (Fig. 2). These findings suggested that on the preparation of PLGA nanospheres high shearing force application such as ultrasonication should be avoided to prevent the degradation of pDNA. By coating with CS, pDNA loading efficiency was increased. The CS coated PLGA nanospheres showed a positive zeta potential, while non-coated PLGA nanospheres being negatively charged. Therefore, the effect of coating with chitosan on loading efficiency might be caused by the ionic interaction

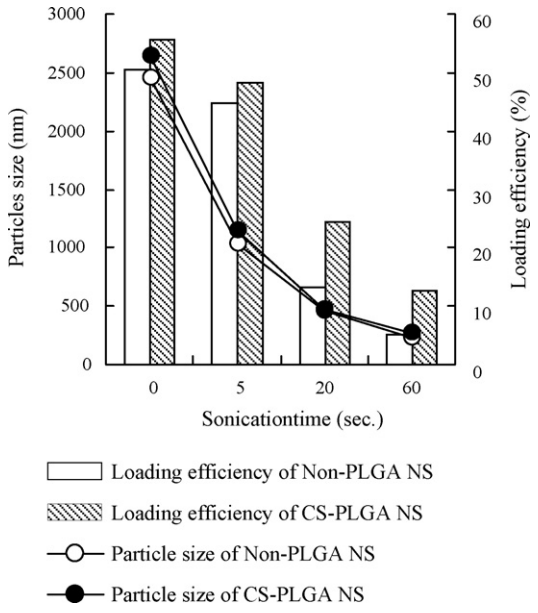

Fig. 1. Effect of sonication time on particle size and loading efficiency of pDNA loaded PLGA nanospheres prepared by W/O/W emulsion solvent evaporation method.

between pDNA and chitosan, preventing the leakage of pDNA from nanospheres during evaporation process.

3.2. Preparation of pDNA loaded PLGA nanospheres prepared by the ESD method

The advantages of emulsion solvent diffusion (ESD) method are a simple process under mild condition without sonication. However, in this method both polymer and drugs in the organic solvent is required. Therefore, the ESD method is available hardly for encapsulating hydrophilic drug such as peptide. Nucleic acid is unable to dissolve in organic solvent, e.g. acetone as shown in Fig. 3. When pDNA solution was added to acetone, pDNA was precipitated in the medium because of dehydration occurring.

In conditions such that both PLGA and a small amount of pDNA were dissolved in acetone, we tried to prepare the pDNA loaded PLGA nanospheres by using ESD method. It was found that by the ESD method with acetone the pDNA loaded PLGA nanospheres having a mean diameter of 208 nm were prepared. While, the loading efficiency of pDNA in the PLGA nanospheres

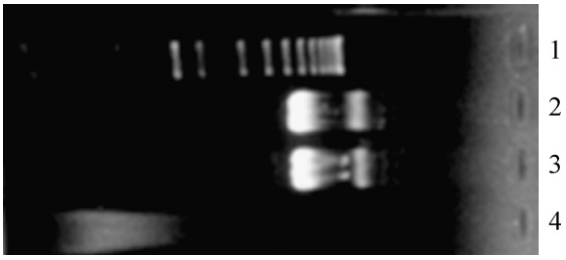

Fig. 2. Effect of eradiating time of ultrasonic action on pDNA integrity. Lane 1, 1 kb DNA ladder; lane 2, original pDNA (control); lane 3, pDNA treated by sonication for 10 s.; lane 4, pDNA treated by sonication for 1 min.

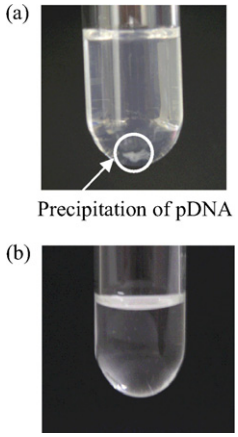

Fig. 3. Effect of DOTAP/pDNA complex on solubility of pDNA in acetone by emulsion solvent diffusion. (a) Precipitation of pDNA solution in acetone, (b) transparent solution of DOTAP/pDNA complex in acetone.

was very low (0.3%), as expected. It was also confirmed that the integrity of naked pDNA in acetone by using agarose gel electrophoresis as shown in Fig. 4. Therefore some modification for ESD process was required to improve the loading efficiency of pDNA in PLGA nanospheres.

3.3. Improvement of loading efficiency by complexation of pDNA with DOTAP or chitosan

pDNA can form easily an ion-complex with cationic compound. The physicochemical properties of pDNA can be preferably changed for dissolving in the organic solvent by transforming to complex. As expected, the solubility of pDNA in acetone increased by complexation with cationic compound as seen in Fig. 3.

Agarose gel electrophoresis was performed to assess the stability of pDNA in acetone and to confirm the polyionic complex formation of DOTAP with pDNA in acetone. As shown in Fig. 4, with increasing the amount of DOTAP, pDNA band disappeared

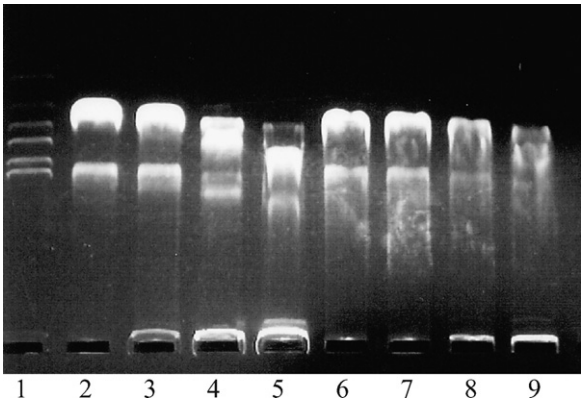

Fig. 4. Analysis of complex formation of DOTAP with pDNA at various weight ratio in the presence of acetone by agarose gel electrophoresis. Lane 1, 1 kb DNA ladder; lane 2, naked pDNA in water (Control); lanes 3–5, DOTAP/pDNA complex in water (weight ratio of DOTAP/pDNA = 1, 5 and 10, respectively); lane 6, naked pDNA in acetone; lanes 7–9, DOTAP/pDNA complex in acetone (weight ratio of DOTAP/pDNA = 1, 5 and 10, respectively).

Table 1
Effect of DOTAP/pDNA complexation on the physicochemical properties of PLGA nanospheres prepared by the emulsion solvent diffusion method

DOTAP/pDNA weight ratio	Particle size (nm)	Zeta potential (mV)
DOTAP/pDNA complex loaded PLGANS		
0	208.8	−33.4
0.1	338.9	−27.7
0.5	283.4	−30.9
1	304.5	−25.6
5	301.0	−22.8
10	298.8	−26.6
DOTAP/pDNA complex		
10	280.1	41.5

Table 2
Effect of CS/pDNA complexation on physicochemical properties of PLGA nanospheres prepared by the emulsion solvent diffusion method

CS/pDNA weight ratio	Particle size (nm)	Zeta potential(mV)
CS/pDNA complex loaded PLGA NS		
0	208.8	−33.4
1	237.2	−23.5
2	249.6	−22.7
4	250.8	−19.6
10	252.6	−21.2
CS/pDNA complex		
2	203.8	21.6

gradually to indicate the complexation with DOTAP. At DOTAP to pDNA weight ratio >5:1, the pDNA did not migrate at all, and the result showed that pDNA in acetone was stable and almost pDNA formed complexiation with DOTAP in acetone.

The particle size of PLGA nanospheres prepared by the ESD method with DOTAP/pDNA complex was about 200 nm (Table 1). With DOTAP used as cationic compound the loading efficiency of pDNA in PLGA nanospheres increased (Fig. 5). Loading efficiency of pDNA increased with increasing DOTAP/pDNA weight ratio. The neutralization of negative charge of pDNA provided some hydrophobicity to the complex dissolved in organic solvent. The alkyl chain of DOTAP interacted with PLGA to retain pDNA effectively into the matrix of PLGA nanospheres. Negative zeta potential of pDNA loaded PLGA nanospheres proved that DOTAP/pDNA complexes were loaded in the PLGA nanospheres but not adsorbed on the surface of PLGA nanospheres.

In same way, chitosan used cationic compound can form a complex with pDNA by electrostatic interaction. Chitosan/pDNA complex was found to be dissolved in the acetone solution dissolving PLGA. However, the loading efficiency of pDNA in the PLGA nanospheres decreased with increasing the chitosan concentration (Table 2, Fig. 6). The hydrophilicity of complex increased when the concentration of chitosan increased. Because of increased hydrophilicity of complex, chitosan/pDNA complex was precipitated in the acetone, leading to the decrease of loading efficiency at higher concentration of chitosan in Fig. 6. This behavior could be explained by the fact that chitosan is biodegradable, cationic and hydrophilic polymer, which is responsible for the precipitation of chitosan/pDNA complex appearing.

3.4. Effect of chitosan coating on loading efficiency of pDNA in PLGA nanospheres

Chitosan coated PLGA nanospheres could be prepared by adding chitosan solution into the outer phase with PVA solution. Zeta potential shifted to positive values by the effect of chitosan adsorbed on the surface of PLGA nanospheres (Table 3). Particle size of CS coated PLGA nanospheres increased because the molecular layers of chitosan coated on the surface of PLGA nanospheres. By coating with CS, encapsulation efficiency was increased. Effect of coating with chitosan on loading efficiency might be caused by an ionic interaction between pDNA and

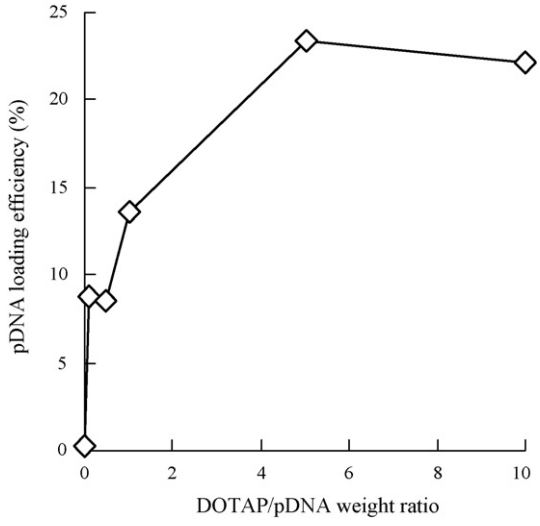

Fig. 5. Effect of DOTAP/pDNA weight ratio on loading efficiency of DOTAP/pDNA complex loaded PLGA nanospheres.

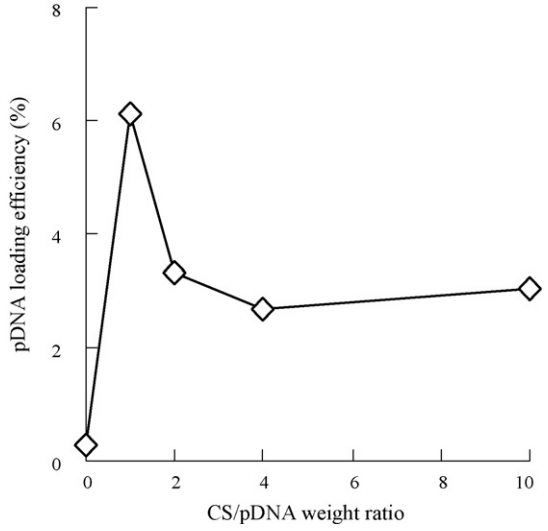

Fig. 6. Effect of CS/pDNA weight ratio on loading efficiency of CS/pDNA complex loaded PLGA nanospheres.

Table 3
Effect of chitosan coating on particle size and zeta potential of pDNA complex loaded PLGA nanospheres

Complex	Particle size (nm)	Zeta potential (mV)
Non-PLGA NS		
DOTAP/pDNA	283.4	−30.9
CS/pDNA	237.2	−23.5
CS-PLGANS		
DOTAP/pDNA	325.5	2.5
CS/pDNA	302.5	4.5

chitosan and prevented leakage of pDNA from emulsion droplet during diffusion process (Fig. 7).

3.5. Encapsulation of oligonucleotide (ON) into PLGA nanospheres by the ESD method

Novel gene based therapies using oligonucleotide (AS-ON, siRNA) represent an enormously promising approach to decrease or modulate an expression of their target molecules. As a second platform of PLGA nanosphere for gene delivery, the ESD method was further applied to encapsulate AS-ON and siRNA. It was found that these ON could be encapsulated into the PLGA nanospheres by complexation with cationic compound (DOTAP), as shown in Table 4. The particle size before freeze drying and the zeta potential of ON loaded PLGA nanospheres was about 300 nm and −30 mV, respectively. In the same case of pDNA loaded PLGA nanospheres, negative zeta potential of ON loaded PLGA nanospheres proved that DOTAP/ON complexes were loaded in the PLGA nanospheres but not adsorbed on the surface of PLGA nanospheres, and by coating with CS, encapsulation efficiency was increased. The ON loaded PLGA nanospheres were aggregated after freeze drying. Jeong et al. (2005) reported that surfactant-free nanoparticles of PLGA were aggregated after freeze-drying and this problem could be circumvented by use of sugar alcohol as a cryoprotectant. This problem could be overcome by the use of mannitol as a cryoprotectant (data not shown). Non-coated PLGA nanospheres were

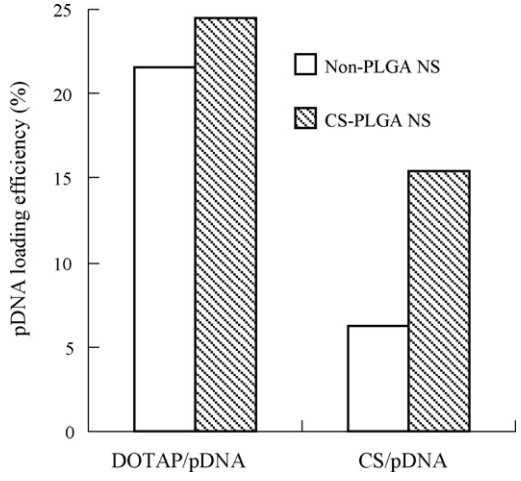

Fig. 7. Effect of chitosan coating on loading efficiency of pDNA complex loaded PLGA nanospheres.

Table 4
Effect of DOTAP/ON (AS-ON, siRNA) complex on physicochemical properties of non-coated and CS coated PLGA nanospheres prepared by the emulsion solvent diffusion method

DOTAP/AS-ON weight ratio	Particle size (nm)	Zeta potential (mV)	Loading efficiency (%)
(a) AS-ON loaded PLGA NS			
0	321.4	−35.2	12.1
1	336.3	−35.1	33.8
4	346.9	−35.2	58.1
6	380.3	−34.0	55.5
8	361.8	−35.8	85.7
10	352.5	−34.0	83.4
DOTAP/siRNA weight ratio	Particle size (nm)	Zeta potential (mV)	Loading efficiency (%)
(b) siRNA loaded PLGA NS			
0	326.3	−33.3	10.5
1	326.3	−34.0	21.9
2	332.1	−33.2	24.8
4	395.9	−33.5	29.2
6	329.8	−33.3	36.2
10	377.0	−33.1	47.1
(c) siRNA loaded CS-PLGA NS			
0	287.7	9.7	56.7
1	355.5	9.3	43.5
2	310.1	9.8	65.1
4	309.7	10.4	59.1
6	355.9	11.0	55.2
10	359.6	10.2	49.2

well reconstituted at a cryoprotectants/nanospheres weight ratio >1/1. CS coated PLGA nanospheres compared to non-coated nanospheres required much more cryoprotectants to reconstitute, such as cryoprotectants/nanospheres weight ratio >5/1.

3.6. Release behavior from uncoated and CS coated nanospheres

A sustained release profile of nucleic acid (pDNA and siRNA) from chitosan coating PLGA nanospheres was found in PBS (Fig. 8) after initial burst releasing 40% and 60% of pDNA and siRNA, respectively. This finding indicates that some of the nucleic acid was localized at the surface of the nanospheres. After the initial release burst, drug release was prolonged significantly more than 5 days, where 40% and 20% of pDNA and siRNA still remained in the nanospheres, respectively. The sustained release at later stage was caused by the diffusion of nucleic acid through PLGA matrix as well as the erosion of polymer. Coating of the nanospheres with chitosan did not alter the drug release pattern. However, the PLGA nanospheres coated with chitosan reduced the initial burst of nucleic acid release and prolonged releasing at later stage. The interaction of the pDNA with chitosan caused to remain the pDNA at the surface of PLGA nanospheres. Therefore, nanostructure design of PLGA nanospheres might be required to control desirably the release pattern. Further investigation is required to optimize the nucleic acid release rate before applying the present system to the gene therapy.

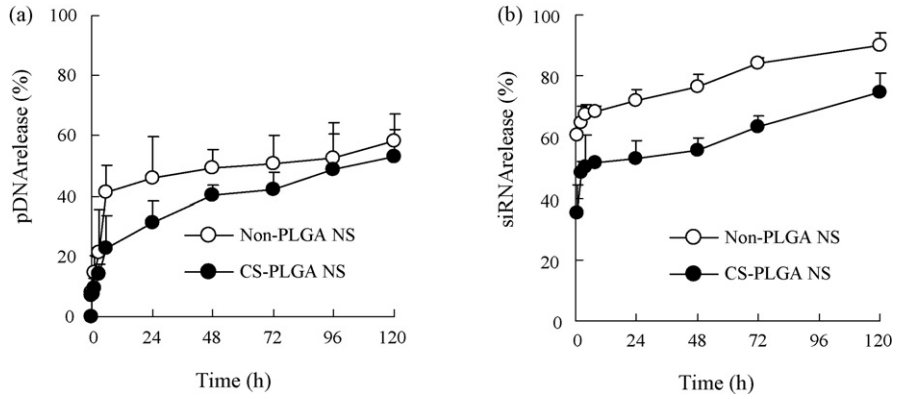

Fig. 8. Cumulative release (% of amount loaded) of nucleic acid from chitosan coating PLGA nanospheres. Release was studied in PBS (pH 7.4) at 37 °C (mean ± S.D., $n = 3$). (a) pDNA release from PLGA nanosphere, (b) siRNA release from PLGA nanosphere.

4. Conclusion

We have established PLGA nanosphere platform to encapsulate wide variety of nucleic acid for gene delivery as well as low molecular drug using the ESD method. This process is composed of simple process under mild conditions without sonication. In the present ESD method nucleic acid transformed to ion-complex with DOTAP and CS dissolve in organic solvent (acetone) is applicable to the ESD method. In conclusion, by using the ESD method, wide variety of nucleic acid such as pDNA, AS-ON and siRNA can be encapsulated in PLGA nanosphere. The required PLGA nanospheres with nucleic acid were prepared by the ESD method to coat the surface of the resultant nanospheres with a mucoadhesive polymer, chitosan. By coating with CS, loading efficiency of nucleic acid increased and the initial burst of nucleic acid significantly reduced, resulting in sustained releasing. The PLGA nanospheres also can prolong the release of nucleic acid in cytosol (Panyam et al., 2002). The present PLGA nanospheres can successfully deliver any type of nucleic acid required to target cell.

Acknowledgements

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