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Modified nanoprecipitation method to fabricate DNA-loaded PLGA nanoparticles

Xiuming Niu[1,*], Weiwei Zou[2,*], Chunxi Liu[2], Na Zhang[2] and Chunhua Fu[1]

[1]*Department of Pharmacy, Shandong Medical College, Ji'nan, Shandong Province, PR China and* [2]*School of Pharmaceutical Science, Shandong University, Ji'nan, Shandong Province, PR China*

Abstract

Objective: The objective of this study was to formulate DNA-loaded poly(D,L-lactide-co-glycotide) (PLGA) nanoparticles by a modified nanoprecipitation method. *Methods*: DNA-loaded PLGA nanoparticles were prepared by the modified nanoprecipitation method and the double emulsion/solvent evaporation method. The characterizations of DNA-loaded nanoparticles such as entrapment efficiency, morphology, particle size, zeta potential, structural integrity of the loaded DNA, and stability of the loaded DNA in PLGA nanoparticles against DNase I, in vitro release, cell viability and in vitro transfection capability were investigated. *Results*: The resulted PLGA nanoparticles by the modified nanoprecipitation method had uniform spherical shape, narrow size distribution with average particles size near 200 nm, negative zeta potential of −12.6 mV at pH 7.4, and a sustained-release property in vitro. Plasmid DNA could be efficiently encapsulated into PLGA nanoparticles (>95%) without affecting its intact conformation using this modified nanoprecipitation method, which was superior to the double emulsion/solvent evaporation method. The PLGA nanoparticles were much safer to A549 cell compared to commercial Lipofectamine 2000 and could successfully transfer plasmid-enhanced green fluorescent protein into A549 cells. *Conclusion*: In conclusion, the modified nanoprecipitation method could be applied as an efficient way to fabricate DNA-loaded PLGA nanoparticles instead of the conventional double emulsion/solvent evaporation method.

Key words: *Double emulsion/solvent evaporation method; gene therapy; modified nanoprecipitation method; nonviral gene delivery system; PLGA nanoparticles*

Introduction

Gene therapy is an emerging therapeutic modality for the treatment of genetic and infectious diseases. The development of safe and efficient gene transfer vectors is of crucial importance for successful gene therapy. Various kinds of gene delivery systems based on viral[1,2] or nonviral[3,4] vectors have been devised; however, none of them seemed to be completely satisfactory. Viral vectors account for about 75% of the clinical protocols currently in operation because they provide higher transfection efficiency than nonviral gene vectors[5]. However, there are some serious drawbacks associated with viral vectors such as immunogenicity, triggering severe inflammation, and potential oncogenicity[6]. Nonviral vectors have received considerable attention lately because they hold several advantages over viral vectors employed for gene delivery, in terms of improved and predictable safety profile, a high DNA-carrying capacity and increased versatility, and the ease of large-scale production and quality control[7,8]. Nevertheless, their efficiency is currently falling behind that of viral systems[9]. Therefore, to develop a safe and effective nonviral vector system is an urgent matter. Nanotechnology offers the possibility to break through this bottleneck because it holds many advantages such as high stability, easy uptake into cells by endocytosis, and the targeting ability to specific cells or tissues modified with ligand materials at the surface of the particles[10,11].

Polymeric systems for gene delivery have attracted much attention due to their favorable physicochemical characteristics. Recently, biocompatible and biodegradable

*Co-first author (equally contributed)
Address for correspondence: Professor Na Zhang, School of Pharmaceutical Science, Shandong University, 44 Wenhuaxi Road, Ji'nan 250012, Shandong Province, PR China. Tel: +86 053188382015, Fax: +86 053188382548. E-mail: zhangnancy9@sdu.edu.cn

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poly(D,L-lactide-co-glycotide) (PLGA) nanoparticles have been suggested as one of the most successful gene delivery systems because of the possibility of achieving a safe and sustained action[12]. The degradation time of PLGA can be altered from days to years by varying the polymer molecular weight, the lactic acid to glycolic acid ratio in copolymer, or the structure of nanoparticles. And recent studies demonstrated rapid escape of PLGA nanoparticles from the endolysosomal compartment into cytosol following their uptake[13]. Therefore, PLGA nanoparticles might be a promising and suitable candidate for gene delivery application.

The water-in-oil-water (W/O/W) double emulsion/solvent evaporation method to encapsulate DNA into PLGA nanoparticles is an efficient strategy that was conventionally used for controlled release of plasmid DNA[14]. This method, however, cannot guarantee the integrity of DNA under ultrasonication or high-speed homogenization, which was necessary in the encapsulation process to obtain smaller particle size[15]. It has been demonstrated that the high-shear forces were very harmful to the integrity of plasmid[16] and caused the conversion of supercoiled DNA into open circular or linear topologies, both of which were known to be of much lower transfection efficiency than the supercoiled one[17].

In comparison, the nanoprecipitation technique (or solvent displacement method) developed and patented by Fessi and coworkers[18] is a mild and easily performed method, which does not require extended shearing/stirring rates, sonication, or very high temperatures. This technique does not rely on shear stress to produce nanoparticles but rather on differences in the interfacial tension, a phenomenon also designated as Marangoni effect[19]. It takes place at the interface of the solvent and the nonsolvent and results from complex and cumulated phenomena such as flow, diffusion, and surface tension variations[20]. This technique is mostly suitable for hydrophobic compounds that are soluble in ethanol or acetone but display very limited solubility in water. Consequently, reduced or even zero drug leakage toward the outer medium led to nanoparticles, with entrapment efficiency values reaching almost 100%[21]. However, this method is typically not an efficient technique for the direct encapsulation of water-soluble drugs. Recently, several groups have tried to modify the traditional nanoprecipitation method, and encouraging results have been provided with water-soluble drug incorporation. Yoo et al.[22] have carried out experiments by slightly modifying the original concept in order to encapsulate lysozyme. Briefly, they successfully effected the diffusion of a dimethylsulfoxide (DMSO) solution containing both the protein and the polymer (PLGA) into an aqueous solution of poloxamer 407. This work provided evidence that nanoprecipitation could also occur with solvents other than acetone or ethanol and thus that an accurate solvent and nonsolvent selection (e.g., by screening) can also lead to nanoparticle formation and possibly extend the use of nanoprecipitation to more hydrophilic drugs.

In this respect, this study essentially attempted to encapsulate plasmid DNA into PLGA nanoparticles using the modified nanoprecipitation method. Firstly, the physicochemical properties (e.g., morphology, particle size, surface charge, plasmid loading efficiency, and the integrity of loaded plasmid DNA) of DNA-loaded PLGA nanoparticles prepared by the modified nanoprecipitation method were evaluated and compared with those of nanoparticles prepared by double emulsion/solvent evaporation method. Secondly, the resistance stability to nuclease as well as the DNA release behavior of PLGA nanoparticles prepared by the modified nanoprecipitation method was studied in vitro. Finally, in vitro cytotoxicity and transfection efficiency of PLGA nanoparticles in lung cancer cell line A549 were also investigated to verify the feasibility and suitability of the modified nanoprecipitation method on fabrication of DNA-loaded PLGA nanoparticles.

Material and method

Material

PLGA (50:50, Av. M_W 25,000) with carboxylic end group was purchased from Shandong Institute of Medical Instrument (Shandong, China). Pluronic F127 and MTT (3-[4, 5-dimethyl-2-thiazolyl]-2, 5-diphenyl-2H-tetrazolium bromide) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Plasmid DNA (pEGFP-N_1) was provided by Zhejiang University (Hangzhou, Zhejiang Province, China). PicoGreen dsDNA Reagent was purchased from Molecular Probes (Invitrogen, Eugene, OR, USA). Goldview was obtained from Beijing Saibaisheng Biological Engineering Co. (Beijing, China). Lipofectamine 2000 was from Invitrogen. Refrigerated plasma was provided by Shandong Blood Center (Shandong, China). A549 lung cancer cell line was obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). DNase I enzyme was obtained from Beijing Yinfeng Century Scientific Develop Co., Ltd. (Beijing, China). All other chemicals were of analytical grade.

Preparation of DNA-loaded PLGA nanoparticles

Preparation of DNA-loaded PLGA nanoparticles by the modified nanoprecipitation method

The DNA-loaded PLGA nanoparticles were prepared by the modified nanoprecipitation method. Typically,

accurately weighted (20 mg) PLGA was dissolved in 1 mL DMSO containing 200 μg of plasmid DNA. The resulting clear solution was slowly (30 mL/h) injected by a microsyringe pump (KDS 100, KDS, Garden Grove, CA, USA) into 20 mL magnetically stirring 0.5% (w/v) Pluronic F127 aqueous solution (600 rpm, RCT basic stirrer; IKA, Staufen, Germany) and agitated for 5 hours at room temperature. The DNA-loaded PLGA nanoparticles formed instantaneously. The entire dispersed system was then centrifuged (Beckman, Fullerton, CA, USA) at 24,975 × *g*, 4°C for 30 minutes and resuspended in phosphate-buffered saline (PBS, pH 7.4) solution followed by filtering through 0.45-μm nitrocellulose membrane (Millipore, Billerica, MA, USA) filter. The supernatant was recovered for determining the entrapment efficiency of nanoparticles. The nanoparticles were characterized and used directly after preparation.

Preparation of DNA-loaded PLGA nanoparticles by the double emulsion/solvent evaporation method

In comparison with the modified nanoprecipitation method, the DNA-loaded PLGA nanoparticles were also prepared by the double emulsion/solvent evaporation method[23]. In brief, 200 μL Tris–EDTA buffer (pH 8.0) containing 200 μg DNA was emulsified into 1 mL ethyl acetate containing 20 mg PLGA by sonication (Sonifier, HS 3120, Tianjin, China; output high) for 1 minute. The resulting primary emulsion was added to 5 mL of 1.0% (w/v) Pluronic F127 aqueous solution and sonicated for 1 minute to form a double emulsion (water-in-oil-in-water). The resultant emulsion was then poured into 15 mL magnetically stirring 0.5% (w/v) Pluronic F127 aqueous solution (600 rpm) and agitated for 5 hours at room temperature under evaporation, completely removing ethyl acetate. The PLGA nanospheres were collected by ultracentrifugation (Beckman) at 24,975 × *g*, 4°C for 30 minutes and resuspended in PBS (pH 7.4) solution followed by filtering through 0.45-μm nitrocellulose membrane (Millipore) filter.

The entrapment efficiency of DNA-loaded PLGA nanoparticles

The amount of DNA loaded into PLGA nanoparticles was calculated by measuring the difference between the total amount of DNA added during the preparation process and the amount of free DNA remaining in the aqueous suspension after ultracentrifugation. The content of the free DNA was determined using PicoGreen™ dsDNA quantitation reagent (Molecular Probes) according to the manufacturer's procedures. The fluorescence was measured by fluorescence spectrophotometer (Hitachi 850; Hitachi, Tokyo, Japan) at excitation and emission wavelengths of 480 and 520 nm, respectively. The amount of DNA was calculated according to the linear calibration curve of DNA ($F = 0.0002 \times C + 0.0042$, $R = 0.9998$). The entrapment efficiency was calculated from the following equation:

$$\text{DNA entrapment efficiency (\%)} = \frac{\text{total DNA content} - \text{free DNA content}}{\text{total DNA content}} \times 100.$$

Morphology, particle size, and zeta potential of DNA-loaded PLGA nanoparticles

The morphology of DNA-loaded PLGA nanoparticles was examined under transmission electron microscope (JEM-1200EX, JEOL, Tokyo, Japan). Samples were prepared by placing a drop of nanoparticle suspension onto a copper grid and air-dried, following negative staining with one drop of 3% aqueous solution of sodium phosphotungstate for contrast enhancement. The air-dried samples were then directly examined under the transmission electronic microscopy.

The average particle size, size distribution, and zeta potential of the nanoparticles were measured by photon correlation spectroscopy using Zetasizer 3000 (Malvern Instruments, Malvern, UK). All measurements were carried out in triplicates. The average particle size was expressed in volume mean diameter and the reported value was represented as mean ± SD ($n = 3$).

Structural integrity analysis of the loaded DNA in PLGA nanoparticles

The integrity of the loaded plasmid was analyzed by agarose gel electrophoresis analysis[12]. For this, PLGA nanoparticles were diluted in 500 μL TE buffer (Tris–HCl 10 mM, EDTA 1 mM, pH 7.5) followed by adding 500 μL chloroform. The mixture was rotated end-over-end to facilitate the extraction of DNA from the organic phase into the aqueous phase. After centrifugation at 24,975 × *g* and 4°C for 10 minutes, the water phase was collected and concentrated when necessary. Samples of control and recovered DNA were applied to a 0.8% agarose gel in 20 mL TAE buffer (40 mM Tris, 40 mM acetic acid, 1 mM EDTA, pH 8.5) containing 2 μL goldview. Adequate separation of the bands for supercoiled, linear, and open circular plasmid DNA was obtained by running the gel electrophoresis for 25 minutes at 90 V. Images were obtained using UV transilluminator and Multimage™ Light Cabinet (Alpha Imagers EC; Alpha Innotech Corporation, San Leandro, CA, USA).

Stability test of the loaded DNA in PLGA nanoparticles against DNase I

Protection of plasmid DNA from nucleases is one of the most important properties for efficient gene delivery both in vitro and in vivo. To test whether PLGA nanoparticles can protect the loaded plasmid DNA from nuclease digestion, the results of DNase I-mediated digestion were evaluated using agarose gel electrophoresis[24,25]. In brief, DNA-loaded PLGA nanoparticles containing 1 μg DNA were, respectively, incubated with different amounts of DNase I (0.4, 0.2, and 0.1 U/μg DNA) in DNase I/Mg^{2+} digestion buffer (50 mM, Tris–HCl, pH 7.6, and 10 mM $MgCl_2$). Naked DNA (1 μg) was treated with DNase I at 0.1 U/μg DNA as positive control. The suspension was incubated in shaking water bath (100 rpm) for 30 minutes at 37°C. After that, the enzymatic digestion reaction was terminated with EDTA solution (0.5 M, pH 8.0). The nanoparticles in the system were collected by centrifugation and were dissolved in chloroform followed by adding an equal volume of TE buffer for the extraction of DNA. The samples were then centrifuged at 24,975 × *g* for 30 minutes. The configuration of plasmid DNA in the supernatant was analyzed by gel electrophoresis with untreated naked DNA as a reference. The samples were applied to a 0.8% (w/v) agarose gel in TAE buffer as described above.

In vitro release studies of DNA-loaded PLGA nanoparticles

The in vitro DNA release from PLGA nanoparticles was performed in 0.1 M sodium phosphate (pH 7.4) buffer over 30 days using separate aliquots for each time point. Typically, an aliquot of PLGA nanoparticles (equivalent to 2.5 μg DNA) was diluted in 0.5 mL sodium phosphate buffer in Eppendorf® tubes and shaken in water bath at 37°C and 100 rpm. At predetermined time intervals, the tubes were withdrawn and centrifugated at 24,975 × *g* for 30 minutes, the supernatants were collected for analysis. The amount of the released DNA was evaluated by the PicoGreen fluorimetric assay mentioned above. Background readings were corrected using the centrifugation supernatants from blank nanoparticles.

Cell viability test of DNA-loaded PLGA nanoparticles

The cytotoxicity of DNA-loaded PLGA nanoparticles was evaluated by MTT method in A549 lung cancer cell line (ATCC). Briefly, the cells were seeded into a 96-well microtiter plates at a density of 8×10^3 cells per well in 0.2 mL of RPMI 1640 supplemented with 10% fetal bovine serum (FBS) and antibiotics in 5% CO_2 incubator at 37°C overnight. After that, the culture medium was replaced by 200 μL fresh serum-free RPMI 1640 medium with different concentrations of the nanoparticles (expressed as PLGA concentration, 5, 10, 25, 50, 100, and 250 μg/mL) and Lipofectamine 2000 in comparison. After incubating for 24 hours, 20 μL of MTT stock solution in PBS (5 mg/mL, pH 7.4) was added into each well with a final concentration of 0.5 mg/mL MTT. The plate was then incubated at 37°C in 5% CO_2 for 4 hours. The medium was removed and 150 μL DMSO was added to dissolve the formazan crystals. The plate was read spectrophotometrically at 570 nm by microplate reader (model 680; Bio-Rad, Benicia, CA, USA). The cell viability (%) was calculated and compared with the untreated control (100%) according to the following equation:

$$\text{Cell viability (\%)} = \frac{\text{OD}_{570(\text{samples})}}{\text{OD}_{570(\text{control})}} \times 100.$$

$\text{OD}_{570(\text{samples})}$ represented measurement from the cells treated with samples and $\text{OD}_{570(\text{control})}$ from the untreated cells.

Statistical analysis

All measurements were collected in triplicates and experiments repeated three times. Values were expressed as mean ± SD. Unpaired Student's *t*-test was used to assess statistical significant differences ($P < 0.05$) between the group means.

In vitro transfection experiments

The transfection capability of plasmid enhanced green fluorescent protein (EGFP)-loaded PLGA nanoparticles was evaluated on A549 cell lines. The cells were seeded into 24-well plates at a density of about 1×10^5 cells per well in 1 mL of RPMI 1640 with 10% FBS, 24 hours prior to transfection. At a confluence level of 70–80%, cells were washed twice with PBS and incubated with 500 μL serum-free media containing DNA-loaded PLGA nanoparticles (1 μg DNA) at 37°C. Lipofectamine 2000 (Invitrogen) was used as positive control, and the formulation of Lipofectamine/DNA complexes was carried out according to the manufacturer's protocol. Naked DNA was used as negative control. After incubation for 4 hours at 37°C in 5% CO_2 incubator, the cells received 1 mL complete medium and were incubated sequentially until 24 hours post transfection. Detection of expression of EGFP was carried out using an inverted inversion fluorescent microscope with an attachment for fluorescent observation (Olympus ZX71; Olympus, Tokyo, Japan) and the picture was captured using a 400× objective. Transfection experiments were performed in triplicates.

Results and discussions

Preparation of DNA-loaded PLGA nanoparticles

Encapsulation of hydrophilic drug into hydrophobic biodegradable polymers such as PLGA on a nanosize scale has been one of the most interesting topics in nanotechnology research[26,27]. Ternary systems, such as emulsification/solvent evaporation methods and double emulsion/solvent evaporation method, were commonly employed[28,29]; however, high energy input was necessary to achieve small particle sizes. This shear stress was usually not well tolerated by sensitive drug molecules such as proteins or DNA[30,31]. Therefore, many efforts have been expended to modify the mild conventional nanoprecipitation method though it is typically not an efficient technique for direct encapsulation of water-soluble drugs[32–34]. In our study, we tried to modify the mild nanoprecipitation method to entrap plasmid DNA into PLGA nanoparticles. DMSO, the so-called alcahest, was applied to replace acetone to cosolve PLGA and DNA followed by the injection into aqueous stabilizer medium. The formation of nanoparticles is based on the Marangoni effect, a process of droplet formation arising from the rapid diffusion of DMSO into the larger aqueous phase. The rapid diffusion creates interface turbulences and results in small droplet formation without use of shear forces to create nanoparticles. As a result, the plasmid DNA was successfully encapsulated in PLGA nanoparticles with high entrapment efficiency up to 97.6%. It was remarkably higher than that of the double emulsion/solvent evaporation method (65.3%).

Physicochemical characterization of DNA-loaded PLGA nanoparticles prepared by different methods

The morphology of the DNA-loaded PLGA nanoparticles formulated by the modified nanoprecipitation method and double emulsion/solvent evaporation method was shown in Figure 1. It was shown that the obtained nanoparticles by the two methods exhibited similar uniform spherical shape with smooth surface and separated from each other. Table 1 summarized physicochemical parameters of DNA-loaded PLGA nanoparticles prepared by the two different methods. It could be seen that the modified nanoprecipitation method in this study produced nanoparticles with smaller particle size but a higher DNA entrapment efficiency than those of the double emulsion/solvent evaporation method ($P < 0.05$). Figure 2 showed a representative size distribution of the nanoparticles. The polydispersity index of both nanoparticles formulated by the two methods was less than 0.10, especially in the case of the modified nanoprecipitation method (0.03), which demonstrated a relatively narrow particle size distribution. In terms of surface charge, the resultant DNA-loaded PLGA nanoparticles showed that both negative zeta potential and absolute value were higher than that of blank PLGA nanoparticles without loaded DNA (zeta potential of blank PLGA nanoparticles formulated by the modified nanoprecipitation method and double emulsion/solvent evaporation method was −7.58 ± 0.7 and −8.74 ± 0.9 mV, respectively). The negative surface charges of blank PLGA nanoparticles might come from the carboxylic end of PLGA while the even negative surface charges of DNA-loaded PLGA nanoparticles might be attributed to the incompletely encapsulated DNA, which was bound to or inserted into the PLGA nanoparticles.

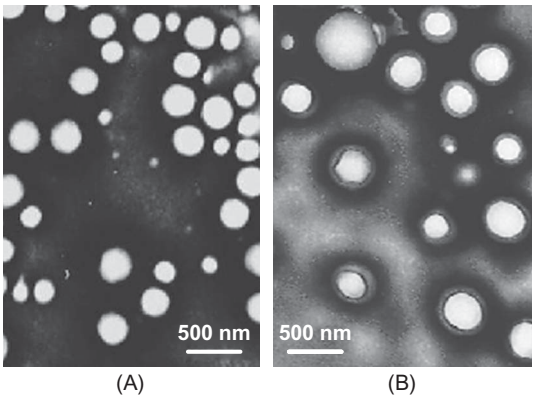

Figure 1. TEM imaging of DNA-loaded PLGA nanoparticles formulated by modified nanoprecipitation method (A) and double emulsion/solvent evaporation method (B).

Structural integrity of DNA encapsulated in nanoparticles

Plasmid DNA extracted from PLGA nanoparticles was analyzed for structural integrity by agarose gel electrophoresis compared with nontreated control DNA. As

Table 1. The physicochemical parameters of DNA-loaded PLGA nanoparticles prepared by different methods (mean ± SD, $n = 3$).

Nanoparticles prepared by different methods	Average particle size[a] (nm)	Polydispersity index	Zeta potential (mV)	Entrapment efficiency(%)
Modified nanoprecipitation method	197.0 ± 4.2*	0.03 ± 0.002*	−12.6 ± 0.9	97.6 ± 0.7*
Double emulsion/solvent evaporation method	260.5 ± 6.3	0.07 ± 0.003	−10.8 ± 0.9	65.3 ± 0.8

*$P < 0.05$ compared with that of double emulsion/solvent evaporation method; [a]The size distribution is weighted by volume.

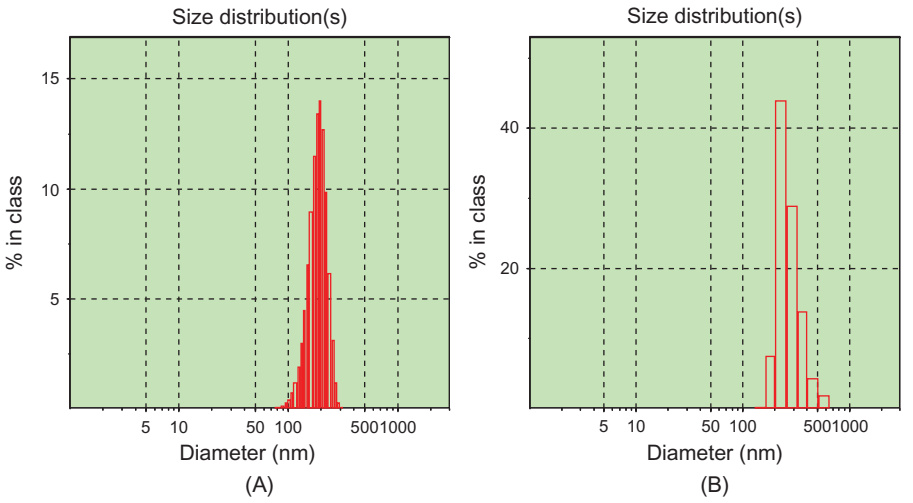

Figure 2. Size distribution profile of DNA-loaded PLGA nanoparticles formulated by modified nanoprecipitation method (A) and double emulsion/solvent evaporation method (B).

shown in Figure 3, the original plasmid DNA (lane 1) was predominantly supercoiled, although a small amount of open circular plasmid DNA was visible. And the extracted DNA in PLGA nanoparticles formulated by modified nanoprecipitation method (Figure 3A, lane 2) was exactly the same as that of the control plasmid DNA. It was implied that preparation conditions of the modified nanoprecipitation method for PLGA nanoparticles in our study was mild enough so as not to affect the structural integrity of the encapsulated DNA. However, the commonly used double emulsion/solvent evaporation method was observed to be detrimental for plasmid integrity, resulting in the conversion of supercoiled DNA into open circular and predominantly linear topologies, and which have much lower transfection efficiency than the supercoiled one. In this respect, the modified nanoprecipitation method held advantage than the common double emulsion/solvent evaporation method.

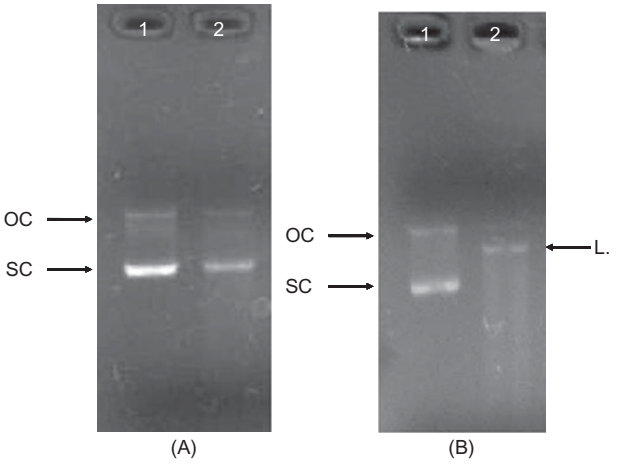

Figure 3. Agarose gel electrophoresis of DNA extracted from PLGA nanoparticles formulated by modified nanoprecipitation method (A) and double emulsion/solvent evaporation method (B). Lane 1 corresponds to control plasmid DNA. Lane 2 corresponds to DNA extracted from PLGA nanoparticles. The lower band is supercoiled (SC) DNA, the intermediate band is liner (L) DNA, and the upper band is open circular (OC) DNA.

Protection from DNase I

Degradation of DNA by nuclease, such as DNase I, is a major barrier for gene delivery both in vitro and in vivo[35]. To test whether PLGA nanoparticles can protect encapsulated plasmid DNA from nuclease digestion, the nanoparticles were exposed to DNase I. Figure 4 showed that naked plasmid DNA (Lane 5) was completely digested

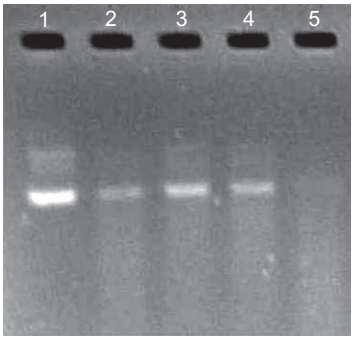

Figure 4. Agarose gel electrophoresis of plasmid DNA extracted from PLGA nanoparticles after treatment with DNase I. Lane 1 corresponds to control nontreated DNA. Lanes 2–4 correspond to the extracted DNA in PLGA after incubation with DNase I at 0.4, 0.2, 0.1 U/μg DNA, respectively, for 30 minutes. Lane 5 corresponds to naked plasmid DNA after incubation with DNase I at 0.1 U/μg DNA for 30 minutes.

by DNase I at 0.1 U/μg DNA within 30 minutes of incubation. Although DNA encapsulated in PLGA nanoparticles remained intact in the present concentrations of DNase I within 30 minutes of incubation (Lane 2–4), the amount of DNA detected decreased in the case of PLGA nanoparticles treated with DNase I at 0.4 U/μg DNA. These results demonstrated that PLGA nanoparticles could protect the loaded DNA from degradation by DNase I at the selected concentrations for 30 minutes, and the structures of DNA maintained integrity.

In vitro release studies

The in vitro drug release property is one of the important characteristics of nanoparticles. Figure 5 showed the accumulative release (expressed as percentage of loaded DNA) versus time curve of DNA from PLGA nanoparticles during 30 days in sodium phosphate buffer. Release profile of DNA from PLGA nanoparticles exhibited an initial burst of release of about 45% in the first day and then followed by slow and continuous release of about 85% in 30 days.

Evaluation of the cytotoxicity of PLGA nanoparticles

One of the limiting factors in gene therapy is the toxicity of vectors including viral vectors, cationic liposomes, and polymeric cations, which most often limits the dose of DNA that can be delivered[36]. It was necessary to investigate whether the obtained PLGA nanoparticles would damage the cultured cells in vitro. In this study, the cell viability was measured by the MTT assay. Briefly, the exponentially grown A549 cells were treated with various amounts of PLGA nanoparticles ranging from 5 to 250 μg/mL and using a commercial transfection reagent, Lipofectamine 2000 as control. As shown from Figure 6, average cell viabilities of PLGA nanoparticles at different concentrations (5–250 μg/mL) were between

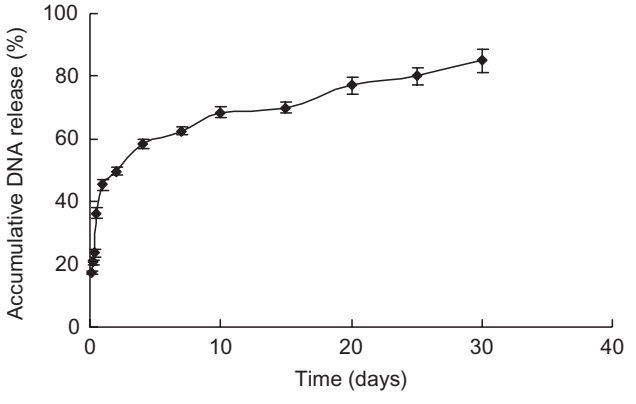

Figure 5. The release profile of DNA from PLGA nanoparticles in sodium phosphate buffer at 37°C. Points indicate mean value ± SD, $n = 3$.

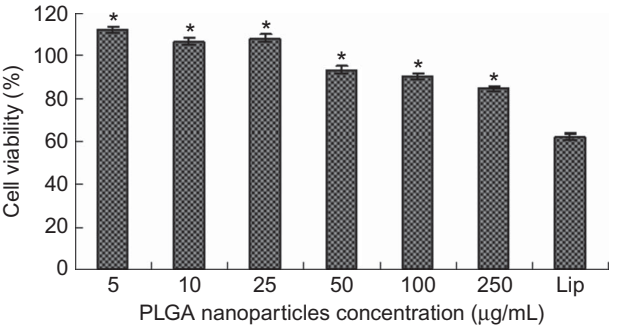

Figure 6. Cell viability of PLGA nanoparticles with different concentrations and Lipofectamine 2000 (Lip) against A549 cell line by MTT assay, 24 hours post treatment ($n = 3$). *$P < 0.05$ compared with Lip.

80% and 120% compared with control cells, which were all substantially higher than that of Lipofectamine 2000 ($P < 0.05$). These results suggested that the PLGA nanoparticles at the concentrations studied were nontoxic and safe to the A549 cell compared with Lipofectamine 2000, which might be attributed to the biocompatibility and negative surface charge of PLGA nanoparticles. Thus, the PLGA nanoparticles could be a safer candidate as gene delivery vector and might overcome the cytotoxicity of cationic nonviral vectors.

Gene transfection in vitro

The transfection efficacy of PLGA nanoparticles in A549 cells was qualitatively analyzed by fluorescent microscopy with the plasmid DNA-EGFP as a reporter gene. The commercial cationic liposome-based reagent, Lipofectamine 2000, well known to provide high transfection efficiency and high level of transgene expression in a range of mammalian cell types in vitro[37] was chosen as positive control in our study. As shown in Figure 7, PLGA nanoparticles may successfully transfer DNA into A549 cells, and the gene can encode the green fluorescent protein. It was noticeable that PLGA nanoparticle was a much more efficient carrier than the EGFP plasmid

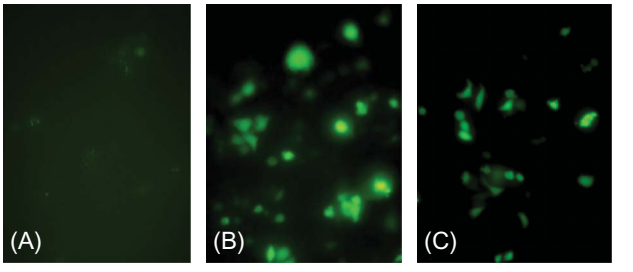

Figure 7. Fluorescent microscopy picture of A549 cells transfected by EGFP gene with (A) naked plasmid DNA, (B) Lipofectamine 2000, (C) and PLGA nanoparticles after 24 hours post transfection.

alone, which could barely transfect A549 cell. It could be attributed to the enhanced uptake of DNA-loaded PLGA nanoparticles via endocytosis. The macroscopic fluorescence intensity of A549 cells transfected with PLGA nanoparticles was not comparable with that of commercial cationic Lipofectamine 2000. That could explain that the EGFP-loaded PLGA nanoparticles were net negatively charged, which may be unfavorable to access the cells because cell surface is generally negatively charged. However, the fact that PLGA nanoparticles did not perform as well as the commercially available Lipofectamine 2000 in vitro did not mean that it would not be a good DNA delivery system in vivo because the negative surface charge of nanoparticles could improve its stability in vivo such as the transporting through blood circulation. More experiments will be carried out in the future to optimize the formulation and further evaluate the in vivo performance of the PLGA nanoparticles as a gene delivery system.

Conclusion

Gene therapy offers a promising approach for the treatment of genetic disorders, vaccine development, and tissue engineering. The success of gene therapy is largely dependent on the development of safe and efficient gene delivery systems. The ideal system must carry DNA efficiently and protect DNA from degradation both in vitro and in vivo, allow it to enter the targeted cell, and successfully realize the genetic expression. Nanoparticles used as a gene transport vector are nonimmunogenic, noninflammatory, and quite applicable in a variety of gene therapy protocols.

In this study, the biodegradable and biocompatible PLGA nanoparticles were established using a modified nanoprecipitation method. This method is easily performed and scaled up, most importantly, which is free of harsh preparation conditions such as ultrasonication or high-speed homogenization that are detrimental to DNA. Results showed that plasmid DNA could be efficiently encapsulated into PLGA nanoparticles (>95%) without affecting its intact conformation using this modified nanoprecipitation method, which was superior to the double emulsion/solvent evaporation method in this respect. The obtained DNA-loaded PLGA nanoparticles with a negative surface charge and a small particle size (<200 nm) showed sustained release of DNA in vitro within 30 days. It was nontoxic and safe to A549 cell compared with commercial Lipofectamine 2000. And it could successfully transfer plasmid EGFP into A549 cells, though it was not comparable with Lipofectamine 2000. Our further study will focus on the optimization of the formulation for higher transfection efficiency and the in vivo performance of the PLGA nanoparticles as gene delivery system will also be conducted.

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Declaration of interest: The authors report no conflicts of interest.

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