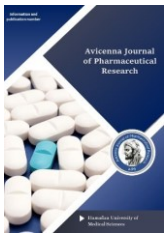
AJPR
Avicenna Journal of Pharmaceutical Research
Avicenna J Pharma Res. 2026 ;7(1): 1-11
DOI: 10.53208/ajpr.7.1.1
http://ajpr.umsha.ac.ir



Research Article

Synthesis of PLGA-Spermine Cationic Biodegradable Copolymer Nanoparticles Containing Luciferase-Silencing sirna

Elham Pakrah[1], Ali Fattahi[1], Sepehr Uromiye[2,3], Katayoun Derakhshandeh[2*]

1. Nano Drug Delivery Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran
2. Departmen of Pharmaceutics, School of Pharmacy, Medicinal Plants and Natural Products Research Center, Hamadan University of Medical Sciences, Hamadan, Iran
3. Department of Molecular medicine, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Article history:
Received: March 25, 2026
Revised: May 21, 2025
Accepted: June 08, 2026
ePublished : June 29, 2026

***Corresponding author:**
Katayoun Derakhshandeh, Professor of Pharmaceutics, Department of Pharmaceutics, School of Pharmacy, Hamadan University of Medical Sciences, Hamadan, Iran
E-mail: k.derakhshandeh@umsha.ac.ir

Abstract

Introduction: Small interfering RNA (siRNA) represents a promising therapeutic strategy for the treatment of various diseases through sequence-specific gene silencing. However, its clinical application is limited by challenges associated with intracellular delivery. Poly (lactic-co-glycolic acid) (PLGA) nanoparticles are widely investigated as biodegradable drug delivery systems, but their negatively charged surface and limited siRNA encapsulation efficiency may restrict their delivery performance. This study aimed to develop and characterize spermine-modified PLGA nanoparticles as non-viral carriers for siRNA delivery and to evaluate their gene-silencing efficiency in human lung cancer cells (H1299).

Methods: PLGA-spermine copolymer was synthesized and characterized using Fourier-transform infrared spectroscopy (FT-IR) and proton nuclear magnetic resonance (^1H-NMR). Nanoparticles were prepared using the double emulsion-solvent evaporation technique. Their physicochemical properties, including particle size, polydispersity index, and zeta potential, were evaluated by dynamic light scattering (DLS). siRNA encapsulation efficiency was determined using UV-Vis spectroscopy. Furthermore, the gene-silencing efficiency of the nanoparticles was assessed using an anti-luciferase siRNA and a luciferase assay kit with a microplate reader in H1299 cells.

Results: Successful synthesis of the PLGA-spermine copolymer was confirmed by FT-IR and ^1H-NMR spectroscopy. Optimization of the formulation and preparation parameters resulted in nanoparticles with an encapsulation efficiency of 76.7%, a mean particle size of 241.4 nm, and a zeta potential of −11.8 mV. The optimized nanoparticles achieved a gene-silencing efficiency of 54.33%. Furthermore, PLGA-spermine nanoparticles successfully delivered anti-luciferase siRNA into H1299 lung cancer cells and demonstrated greater gene-silencing efficiency than unmodified PLGA nanoparticles.

Conclusion: The findings demonstrate that spermine modification of PLGA nanoparticles can improve siRNA encapsulation and gene-delivery performance. The developed biodegradable PLGA-spermine nanoparticles represent a promising non-viral delivery platform for siRNA-mediated gene silencing and warrant further investigation for potential therapeutic applications

Keywords: Gene silencing, Luciferase, PLGA, siRNA delivery, Spermine

Introduction

While siRNA shows great therapeutic potential, multiple barriers hamper its clinical translation and must be overcome through innovative delivery approaches. Systemic administration of naked

© 2026 The Author(s); Published by Hamadan University of Medical Sciences. This is an open-access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

[Downloaded from ajpr.umsha.ac.ir on 2026-11-23]

[DOI: 10.53208/ajpr.7.1.1]

siRNAs results in rapid renal clearance and degradation by nucleases in plasma and tissues [1]. They are also negatively charged, preventing passive diffusion through hydrophilic cellular membranes and the endothelial barrier into target cells and tissues. Additional challenges include endosomal entrapment following cellular uptake, activation of immune sensors, and lack of tissue/cellular targeting specificity [2].

To improve siRNA pharmacokinetics and bioavailability, chemical modifications have been introduced, including phosphorothioate backbone linkages, 2'-O-methyl/fluoro modifications, and terminal cap structures [3, 4]. These alterations provide improved nuclease stability while minimally impacting RNAi activity. Conjugation of targeting ligands and cell-penetrating peptides also enhances cellular association and uptake [5, 6]. However, many of these modifications alone do not fully address challenges such as endosomal escape and lack of tissue selectivity, driving the need for drug delivery vehicles.

Non-viral carriers under investigation include liposomes, polymers, lipid-polymer hybrid nanoparticles, exosomes, and gold nanoparticles [7]. Cationic lipids and polymers condense siRNA through electrostatic interactions into nanoparticles of suitable size (<200 nm) for efficient uptake via endocytosis. Surface modifications can introduce targeting moieties such as antibodies, peptides, or aptamers as well as PEGylation to improve pharmacokinetics. Incorporation of cell-penetrating or fusogenic peptides helps facilitate endosomal escape into the cytosol, a key hurdle that many materials fail to overcome [8]. Viral vectors such as adeno-associated viruses offer the benefit of nuclear localization and persistent transgene expression but pose risks of immunogenicity and insertional mutagenesis [9]. Overall conjugate and formulation strategies aim to protect siRNA cargo from enzymatic destruction while optimizing delivery parameters like targeting ability, intracellular trafficking, and endosomal escape.

Leveraging the sequence specificity of RNAi, siRNAs show promise for treating diverse genetic disorders and infections once considered difficult to address with traditional drugs. Current research has highlighted progress in several key disease areas, such as Alzheimer's disease and cancer.

Poly(lactic-co-glycolic acid) (PLGA) is one of the most widely used biodegradable and biocompatible polymers approved by the FDA for various biomedical applications [10]. However, PLGA has some limitations, including its lack of cell-adhesive ligands and low gene transfection efficiency. To overcome these limitations, cationic polymers have been introduced to modify PLGA. Among cationic polymers, spermine has received considerable attention due to its natural origin and low toxicity [11].

Spermine is an aliphatic polyamine found in all eukaryotic cells. It plays important roles in various cellular processes such as gene expression, response to stress, and cell proliferation [12]. Spermine exists mainly in its protonated form under physiological conditions due to its four amino groups and has a net positive charge at physiological pH. This positive charge enables spermine to efficiently interact with negatively charged DNA/RNA through electrostatic interactions [12]. Based on these properties, spermine has been widely used to modify nanoparticles for gene and small interfering RNA (siRNA) delivery [13-15].

The PLGA-spermine copolymer is synthesized through ring-opening copolymerization of PLGA and spermine-conjugated glucose (SG). The conjugation of natural polyamine spermine to PLGA overcame the lack of cell adhesion ability and gene transfection efficiency of pure PLGA. Since then, extensive studies have been conducted to elucidate the properties and biomedical applications of PLGA-spermine copolymer. Considering the mentioned features, this study aimed to investigate the efficiency of PLGA-Spermine copolymer in encapsulating siRNA and its *in vitro* transfection efficiency.

Materials and Methods

Synthesis of PLGA-spermine nanoparticles

In order to activate PLGA (4000-15000MW), 0.10 mmol NHS, 0.10 mol EDC, together with 0.02 mol PLGA were stirred in anhydrous DMSO for 6 h. The reaction was carried out under a nitrogen atmosphere in the dark. Next, to facilitate the amidation reaction between the carboxylic acid

[Downloaded from ajpr.umsha.ac.ir on 2026-11-23]

[DOI: 10.53208/ajpr.7.1.1]

group in PLGA and the amino groups in spermine, 0.05 mmol of spermine was dissolved in 2 ml of dry DMSO and then added dropwise to the reaction mixture using a syringe. This reaction was maintained under the same conditions and stirred vigorously for 72 h. Then, in order to purify the product and remove unreacted substances and side products, it was dialyzed for 24 h using a dialysis membrane with a molecular weight cut-off of 3500 daltons. Subsequently, the reaction mixture was added to distilled water. The amount of distilled water used was 10 times the total volume of DMSO used, and after that, the mentioned mixture was centrifuged for 30 minutes at 18000 RPM and a temperature of 4°C. Finally, lyophilization was done for 24 h to dry the sediment.

Characterization of the Synthesized PLGA-Spermine Copolymer:

In order to confirm the successful synthesis of the copolymer, FT-IR and HNMR techniques were used. Briefly, the dry PLGA-spermine copolymer powder was mixed with KBr to form a transparent compressed layer.

The samples were investigated in deuterated DMSO and analyzed at the NMR facility of Zanjan University.

Preparation of PLGA-Spermine Copolymer Nanoparticles Containing Luciferase Silencing siRNA:

Nanoparticles loaded with siRNA were prepared by the double emulsion solvent evaporation technique, as follows:

First, 20 µl of PLGA-spermine with a concentration of 1.5 mg/ml was dissolved in dichloromethane (DCM), which is considered the organic phase. Then this mixture was added dropwise to the siRNA solution containing 5 picomoles of siRNA in 8 microliters of RNase-DNase-free water. In order to form an emulsion with the appropriate size, the mixture was sonicated for 90 sec to form a water-in-oil (W/O) emulsion.

In the second step, the resulting emulsion was added drop by drop to polyvinyl alcohol (PVA) solution (13000-23000MW) with a concentration of 0.025 mg/ml. It was then sonicated for 60 sec to form a water-in-oil-in-water (W/O/W) double emulsion. Dichloromethane was evaporated by stirring for 1 h.

A new method was used to evaluate siRNA loading in PLGA-spermine nanoparticles. This method involved disruption of the nanoparticle structure prepared in dichloromethane and the release of siRNA from inside the nanoparticle matrix, which enters the aqueous phase in the presence of the aqueous phase during the degradation of siRNA and can be measured by the nanodrop device. Briefly, 30 microliters of anhydrous dichloromethane were added to the precipitate and vortexed for 1 min. Then, 30 microliters of RNase-DNase-free water was added and vortexed again for 1 min. It was observed that because dichloromethane is denser than water, the aqueous phase was placed on top. Next, the collected samples were analyzed by the NanoDrop instrument.

Nanoparticle stability assessment:

The physical stability of the PLGA-spermine nanoparticles was evaluated by monitoring changes in particle size, polydispersity index (PDI), and zeta potential over 30 days. Nanoparticle suspensions were stored at 4°C and 25°C in [PBS/deionized water] and analyzed at predetermined time points (days 0, 7, 14, and 30) using dynamic light scattering (DLS). Each measurement was performed in triplicate.

***In vitro* siRNA release study**

The release profile of siRNA from PLGA-spermine nanoparticles was investigated using the dialysis bag method. Briefly, a known amount of siRNA-loaded nanoparticles suspended in 2 mL of phosphate-buffered saline (PBS, pH 7.4) was placed into a dialysis bag (MWCO: [e.g., 10 kDa]). The bag was immersed in 20 mL of release medium (PBS, pH 7.4) and incubated at 37°C under constant agitation at 100 rpm. At specific time intervals (e.g., 0.5, 1, 2, 4, 8, 12, 24, and 48 h), 1 mL of the release medium was withdrawn and replaced with an equal volume of fresh buffer. The concentration of released siRNA was quantified using [e.g., UV-Vis spectroscopy at 260 nm or a Ribogreen assay].

[Downloaded from ajpr.umsha.ac.ir on 2026-11-23]

[DOI: 10.53208/ajpr.7.1.1]

Evaluation of the effect of PLGA-Spermine on the NCI-H1299 cell line:

The NCI-H1299 cell line, originating from non-small cell lung cancer, was used as the cellular model obtained from the Pasteur Institute of Iran and was kept and incubated using RPMI1640 culture medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin in 25 cm^2 flasks in controlled conditions (37°C and 5% CO_2). The NCI-H1299 cells were subcultured twice after reaching 70% confluence for two times. Cytotoxicity of the prepared formulation of siRNA was assessed using the MTT assay, based on the reduction of tetrazolium dye to formazan by dehydrogenase enzymes. To that end, NCI-H1299 cells with a density of 10000 cells per well were seeded in 96-well plates, and after a 24-h of incubation under the specified conditions, cells adhesion was confirmed using an invert Microscope, the cells were then treated with a range of concentrations. After a 48-h incubation in a similar condition, 100 microliters of MTT Solution was added to each well, and the formed formazan crystals were dissolved by DMSO, and the solution absorbance was measured at 570 nm using a microplate reader.

Gene Silencing with siRNA-Loaded PLGA-Spermine Nanoparticles:

To account for potential variability in cell number and lysis efficiency across wells, luciferase activity (expressed as relative light units, RLU) was normalized to total cellular protein content determined by the Bradford assay for each sample. Total protein concentration was measured using Bradford reagent with bovine serum albumin (BSA) as the standard, and absorbance was recorded at 590 nm. The Bradford assay was therefore employed not as a direct measure of gene silencing but as an internal normalization control, ensuring that differences in luciferase signal reflected genuine changes in reporter gene expression rather than variations in cell viability or protein yield. Gene silencing efficiency was subsequently calculated as the percentage reduction in normalized luciferase activity (RLU/mg protein) relative to untreated control cells.

To that end, the cells were seeded at a density of 750,000 cells in the wells of a 6-well plate. For this experiment, the complete culture medium without antibiotics was used, and then, after 24 h, once cell adhesion was confirmed to the bottom of the wells, they were treated with PLGA-spermine nanoparticles containing siRNA. After 48 h of incubation at 37°C, the supernatant was removed, the wells were washed with phosphate-buffered saline, 25% of the cells were lysed with 20 microliters of lysis buffer, and finally, 5 microliters of cell lysate were mixed with 200 microliters of freshly prepared Bradford reagent and incubated at 37°C for 10 min, then the absorbance of the solution was measured at 590 nm.

Evaluation of Luciferase Activity

For appraising the activity of the Luciferase enzyme was measured based on a colorimetric method using the Kiazist Co Kit.

Statistical analysis

All quantitative data were expressed as mean ± standard error of the mean (SEM) from at least three independent biological replicates (n = 3). Comparisons among multiple groups were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons, using GraphPad Prism software (version 10.4.0). A p-value of less than 0.05 was considered statistically significant. Exact p-values were reported where applicable, and significance levels are indicated in figures as *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

Result

Characterization

In the FT-IR spectrum of spermine, the N-H group showed stretching bands at 3294 and 3421 cm^{-1}.

Figure 1 shows the comparison of FT-IR spectra of PLGA, spermine, and PLGA-spermine. Comparison of these three spectra in the range of 3250-3750 cm^{-1}, the region of the amide-bond region was observed in Figure 1.

In the FT-IR spectrum of the PLGA-spermine copolymer, the stretching bands related to the N-H group at the wavenumber of 3425 cm-1 and the amide bond at the wavenumber of 1658-78 cm-1 were appeared, indicating amidation and PLGA bonding to spermine [Figure 1].

[Downloaded from ajpr.umsha.ac.ir on 2026-11-23]

[DOI: 10.53208/ajpr.7.1.1]

In the HNMR spectrum of this compound, characteristic PLGA peaks were observed at δ equals to 5.53, 4.92, and 1.48 ppm [Figure 2].

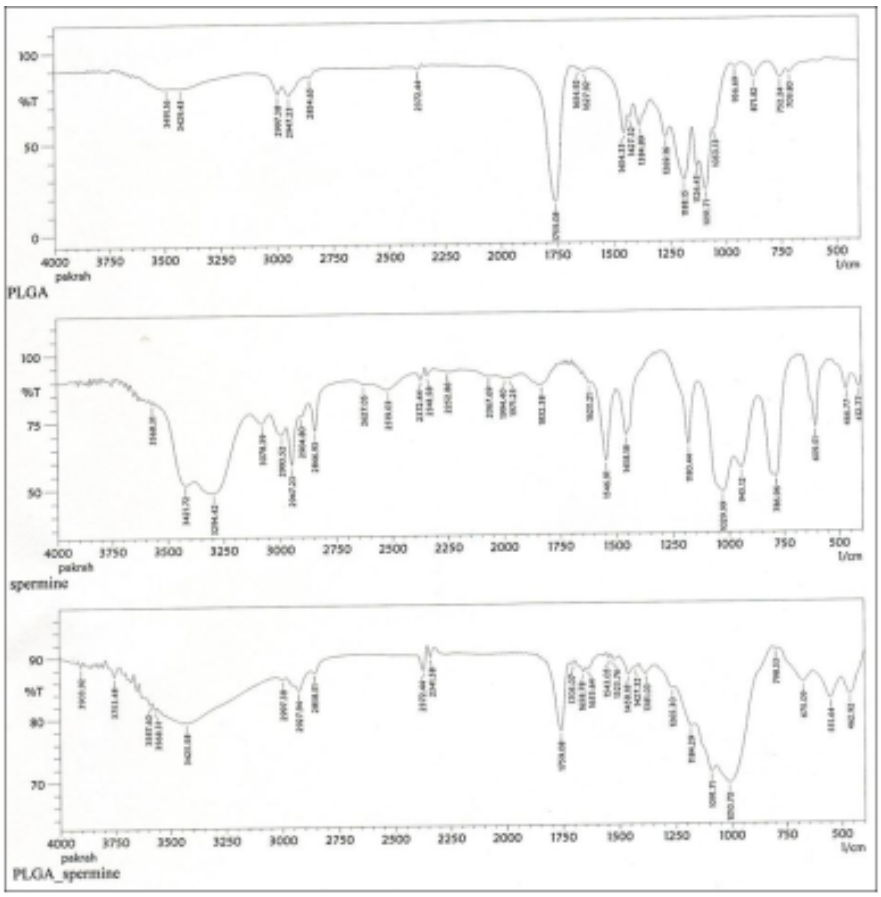


Figure 1. FT-IR spectra of PLGA, spermine, and PLGA-Spermine copolymer.

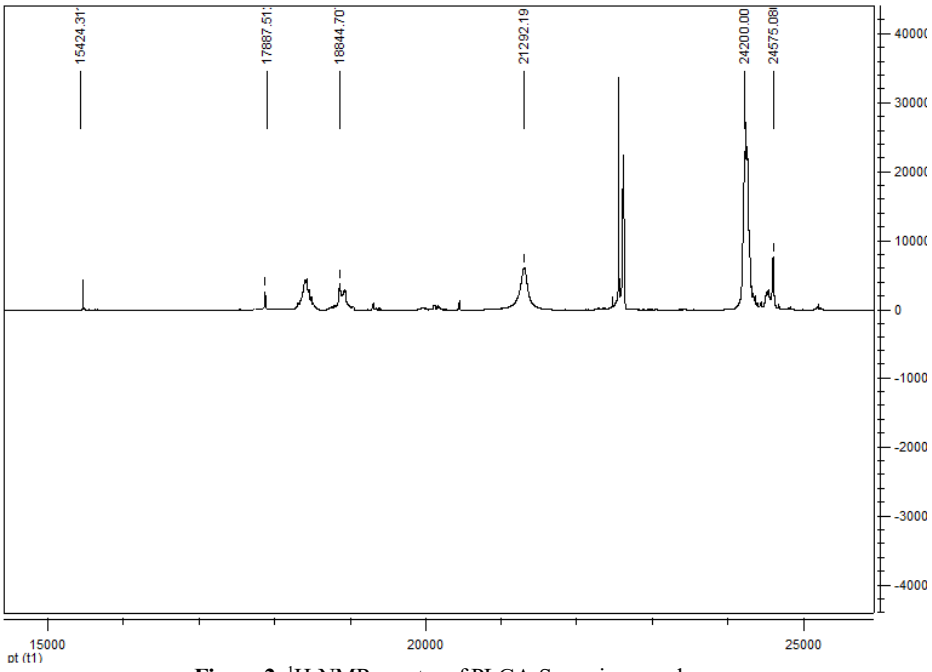


Figure 2. ^{1}H-NMR spectra of PLGA-Spermine copolymer.

[Downloaded from ajpr.umsha.ac.ir on 2026-11-23]

[DOI: 10.53208/ajpr.7.1.1]

A characteristic spermine at the δ equals 1.24 ppm, corresponding to the group CH2(-HN-CH2-CH2-CH2-NH-CO-), which is also observed in the PLGA-spermine copolymer spectrum.
The appearance of the peak at 3.34 ppm, which corresponds to the group CH2(-NH-CH2-CH2-CH2-NH-CO-), and likewise, the peak at 12.7 ppm, which corresponds to the group NH(-NH-CH2-CH2-CH2-NH-CO-), indicates amidation.

The siRNA Loading in PLGA-Spermine Nanoparticles:

The loading percentage was calculated using the following formula:

$$Loading\ percentage = \frac{The\ amount\ of\ calculated\ siRNA}{The\ amount\ of\ primary\ siRNA} \times 100$$

The results showed that nanoparticles were prepared with the appropriate loading percentage. Which equals 76.71%±2.16. The siRNA loading efficiency of PLGA nanoparticles was 48.86±0.16. Data are presented as the mean of three replicates.
Table 1 shows the effect of changing the volume of the external phase on the percentage of particle loading. As can be seen, varying the volume of the organic phase changes the amount of siRNA loading in PLGA-spermine nanoparticles. The changes were investigated in the volumes of 50, 100, and 250 microliters of PLGA-spermine, and with all other parameters held constant, the highest amount of loading in the volume of 50 microliters can be seen.

Table 1. The effect of changing the volume of the external phase on the loading percentage of particles. The results are shown as mean ± standard error. The data are the average of three replicates. Values represent the mean ± standard error of the mean (SEM) obtained from three separately synthesized nanoparticle preparations (n = 3), each analyzed under identical experimental conditions.

Formulation	siRNA pmol	PLGA-spermine (mg/ml)	emulisifier (mg/ml)	Internal phase volume (lμ)	External phase volume (lμ)	Average loading percentage in formulations
N1	5	0/6	0/025	50	300	76/71± 2/16
N2	5	0/6	0/025	100	300	68/34±1/23
N3	5	0/6	0/025	250	300	54/93±3/61

Nanoparticle Size and Surface Charge:

As shown in Figure 3, the optimal particle size was $241.4 \pm 3.2\ nm$, with a polydispersity index (PDI) of 0.187 ± 0.02, indicating a relatively narrow and monodisperse size distribution suitable for cellular uptake and endosomal escape.

The surface charge of PLGA-spermine nanoparticles is $-11.8 \pm 0.9\ mV$. Data are presented as the mean ± standard error from three replicates, reported as mean ± standard error (Table 2).

Table 2. the effect of changing the volume of the external phase on the size and surface charge of nanoparticles. The results are shown as mean ± standard error. The data are the average of three replicates.

formulation	Internal phase volume (µl)	External phase volume (µl)	Size (nm)	PDI	Zeta (mV)
N1	50	300	241/4±15/91	0/25±0/02	-11/8
N2	100	300	335/65±22/8	0/35±0/03	-21/2
N3	250	300	336/8±51/62	0/36±0/03	-18/3

Table 3 illustrates the impact of varying the organic phase volume on the physicochemical properties of PLGA-spermine nanoparticles. Increasing the volume of the internal organic phase resulted in a corresponding increase in particle size. Comparison of these results confirms that using $50\ \backslash muL$ of the PLGA-spermine solution yielded the most favorable balance of

[Downloaded from ajpr.umsha.ac.ir on 2026-11-23]

[DOI: 10.53208/ajpr.7.1.1]

minimal particle size and maximum loading efficiency.

Table 3. The level of luciferase gene expression in the NCI-H1299 cell line after 48 h of treatment

control	siRNA (free) (5pmol)	siRNA (free) (10pmol)	siRNA (free) (20pmol)	siRNA (loaded in NP) (5pmol)	siRNA (loaded in NP) (10pmol)	siRNA (loaded in NP) (20 pmol)
100	90	88	84	57	56	50
Standard Error	5	5	5	7	4	4

The surface charge (zeta potential) of the PLGA-spermine nanoparticles was -11.8 ± 0.9 mV. This shift in zeta potential, compared to pure PLGA, confirms the successful conjugation of spermine to the PLGA backbone, as spermine effectively attenuates the highly negative surface charge of the PLGA polymer. The residual negative charge is attributed to the presence of free carboxylic acid functional groups from the lactic and glycolic acid units within the polymer structure.

Stability of PLGA-Spermine Nanoparticles

As shown in Table 4, the PLGA-spermine nanoparticles exhibited high physical stability over 30 days of storage at 4°C. The average particle size showed a slight increase from 241.4 ± 3.2 nm on day 0 to 248.5 ± 4.1 nm on day 30, corresponding to an increase of approximately 3%, which is not considered significant for colloidal nanosystems. The PDI values remained low throughout the storage period, increasing marginally from 0.187 ± 0.02 to 0.205 ± 0.04, confirming that the nanoparticles maintained a narrow, monodisperse size distribution with minimal particle aggregation. The zeta potential remained relatively stable, decreasing slightly in magnitude from −11.8 ± 0.9 mV to −10.9 ± 1.2 mV, which confirms the colloidal stability of the formulation during the 30-day storage period (Table 4).

Table 4. Stability assessment of PLGA-spermine nanoparticles at 4°C over 30 days.

Storage Time (Days)	Particle Size (nm)	PDI (Index)	Zeta Potential (mV)
0	241.4±3.2	0.187±0.02	−11.8±0.9
7	243.2±2.8	0.192±0.03	−11.5±1.1
14	245.8±3.5	0.198±0.02	−11.2±0.8
30	248.5±4.1	0.205±0.04	−10.9±1.2

In vitro siRNA release profile

The in vitro release profile of siRNA from PLGA nanoparticles exhibited a biphasic pattern over 48 hours. An initial burst release of approximately 24% was observed within the first 5 h, followed by a slower, sustained release phase. Cumulative release reached approximately 32% at 12 h, 40% at 24 h, and approximately 50% by 48 h, indicating a gradual and continuous release behavior throughout the observation period (n = 3) (Figure 3).

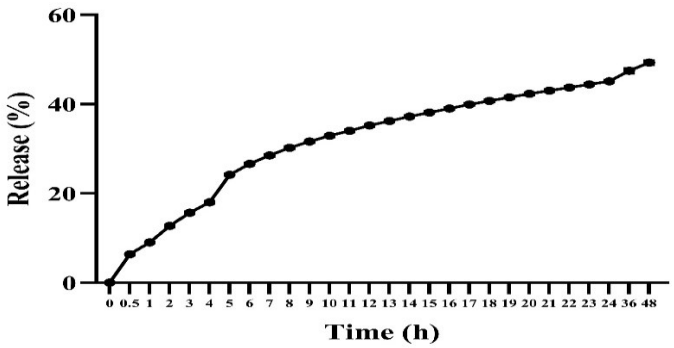


Figure 3. Cumulative siRNA release profile from PLGA nanoparticles in PBS (pH 7.4, 37°C) over 48 hours, showing an initial burst release (~24% by 5 h) followed by a sustained release phase reaching ~50% by 48 h (n = 3).

[Downloaded from ajpr.umsha.ac.ir on 2026-11-23]

[DOI: 10.53208/ajpr.7.1.1]

The effect of PLGA-Spermine on NCI-H1299 viability:

MTT assay results showed that synthesized PLGA-Spermine nanoparticles had no significant cytotoxic effect on the NCI-H1299 cell line during a 48-h treatment.

Gene silencing with siRNA-loaded PLGA-spermine nanoparticles

Treatment of the cells for 48 h with different concentrations of siRNA-loaded nanoparticles caused a significant decrease in luciferase gene expression at all concentrations, while no significant reduction was observed after cell treatment with free siRNA (Figure 4).

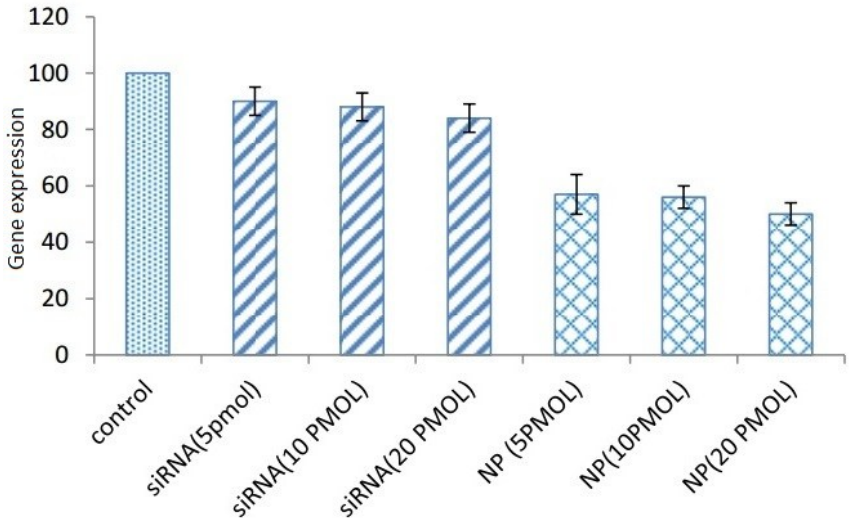


Figure 4. The effect of siRNA-loaded PLGA-Spermine nanoparticle (NP) and Free siRNA on luciferase gene expression in the NCI-H1299 cell line after 48 h of treatment. Data are the average of three replicates and are expressed as the mean ±SEM (n = 3). ****p < 0.0001.

Discussion

The effective transfer of therapeutics such as small organic molecules, peptides, and nucleic acids such as siRNAs and plasmids to target cells in living organisms faces various obstacles. Major obstacles include the extensive metabolism of drugs and their rapid clearance from the blood circulation, as well as structural degradation following cellular uptake and exposure to lysosomal enzymes. To improve siRNA pharmacokinetics and bioavailability, chemical modifications have been introduced, including phosphorothioate backbone linkages, 2'-O-methyl/fluoro modifications, and terminal cap structures [3, 4]. These alterations provide improved nuclease stability while minimally impacting RNAi activity. Conjugation of targeting ligands and cell penetrating peptides also enhances cellular association and uptake [5, 6]. However, many of these modifications alone do not fully address challenges such as endosomal escape and lack of tissue selectivity, driving the need for drug delivery vehicles.

Non-viral carriers under investigation include liposomes, polymers, lipid-polymer hybrid nanoparticles, exosomes, and gold nanoparticles [7]. Cationic lipids and polymers condense siRNA through electrostatic interactions into nanoparticles of suitable size (<200 nm) for efficient uptake via endocytosis — a finding consistent with the particle size and narrow polydispersity index (PDI) confirmed by DLS analysis in the present study. Surface modifications can introduce targeting moieties such as antibodies, peptides, or aptamers as well as PEGylation to improve pharmacokinetics. Incorporation of cell-penetrating or fusogenic peptides helps facilitate endosomal escape into the cytosol, a key hurdle that many materials fail to overcome [8]. Viral vectors such as adeno-associated viruses offer the benefit of nuclear localization and persistent transgene expression but pose risks of immunogenicity and insertional

[Downloaded from ajpr.umsha.ac.ir on 2026-11-23]

[DOI: 10.53208/ajpr.7.1.1]

mutagenesis [9]. Overall, conjugate/formulation strategies aim to protect siRNA cargo from enzymatic destruction while optimizing delivery parameters like targeting ability, intracellular trafficking, and endosomal escape. In this regard, the sustained and biphasic *in vitro* release profile observed for the PLGA-spermine nanoparticles — characterized by an initial burst release followed by a gradual sustained phase — supports the capacity of this carrier to protect siRNA from premature degradation while enabling prolonged intracellular availability.

Leveraging the sequence specificity of RNAi, siRNAs show promise for treating diverse genetic disorders and infections once considered difficult to address with traditional drugs. Here, we highlight progress in several key disease areas, such as Alzheimer's disease and cancer.

Poly(lactic-co-glycolic acid) (PLGA) is one of the most widely used biodegradable and biocompatible polymers approved by the FDA for various biomedical applications [10]. However, PLGA has some limitations, including its lack of cell-adhesive ligands and low gene transfection efficiency. To overcome these limitations, cationic polymers have been introduced to modify PLGA. Among cationic polymers, spermine has drawn much attention due to its natural origin and low toxicity [11].

Spermine is an aliphatic polyamine found in all eukaryotic cells. It plays important roles in various cellular processes such as gene expression, response to stress, and cell proliferation [12]. Spermine exists mainly in its protonated form under physiological conditions due to its four amino groups and has a net positive charge at physiological pH. This positive charge enables spermine to efficiently interact with negatively charged DNA/RNA through electrostatic interactions [12]. Based on these properties, spermine has been widely used to modify nanoparticles for gene/small interfering RNA (siRNA) delivery [13-15].

The PLGA-spermine copolymer is synthesized through ring-opening copolymerization of poly(lactic-co-glycolic acid) (PLGA) and spermine-conjugated glucose (SG). The conjugation of natural polyamine spermine to PLGA overcame the lack of cell adhesion ability and gene transfection efficiency of pure PLGA. Since then, extensive studies have been conducted to elucidate the properties and biomedical applications of the PLGA-spermine copolymer. Considering the mentioned features, this study aimed to investigate the efficiency of the PLGA-Spermine copolymer in encapsulating siRNA and its transfection *in vitro*.

The nanoparticles synthesized in this study exhibited an appropriate hydrodynamic size and narrow size distribution, as confirmed by DLS measurements, along with acceptable colloidal stability over the 30-day storage period, indicating suitable physicochemical properties for cellular uptake and further biological application. Although cationic lipid-based reagents such as Lipofectamine remain the gold standard for *in vitro* siRNA delivery due to their high transfection efficiency, their clinical translatability is limited by cytotoxicity and immunogenicity concerns. In this context, the moderate but significant gene silencing achieved by PLGA-spermine nanoparticles (54.33%), combined with the inherent biodegradability, favorable safety profile, and sustained-release behavior of this PLGA-based system, positions this platform as a promising, safer alternative for further optimization rather than a direct efficiency competitor to established lipofection reagents.

Conclusion

Gene-silencing approaches for the treatment of genetic diseases and targeted gene knockdown have advanced considerably. Diseases such as psoriasis, hypercholesterolemia, liver cirrhosis, hepatitis B, ovarian cancer, and bone cancer, etc., which are difficult to treat, can be completely cured using interfering RNA, which can turn off almost any gene in the cells. The successful clinical implementation of this method requires further developments in the field of gene transfection systems. Viral gene delivery systems, despite their high efficiency in transfection, are not considered an appropriate option due to many remaining problems, such as mutagenicity, tumorigenic properties, triggering immune responses by the host, and high cost of production. Therefore, current research largely focuses on non-viral carriers. Among these, the biocompatible and biodegradable polymer PLGA is of great interest due to its low toxicity, high cellular uptake, ability to target specific tissues, and high

[Downloaded from ajpr.umsha.ac.ir on 2026-11-23]

[DOI: 10.53208/ajpr.7.1.1]

stability. siRNA is highly susceptible to harsh conditions, such as sensitivity to organic solvents, sonication, and mechanical stress, and achieving high siRNA encapsulation in PLGA nanoparticles is challenging because of the lack of electrostatic attraction between them. Optimizing the formulation of PLGA nanoparticles using a cationic polymer improves siRNA loading and also increases cellular uptake. The synthesis of PLGA-spermine nanoparticles as siRNA carriers of the luciferase gene was performed for the first time, and their silencing effects have been investigated *in vitro*. Particle size varied according to several formulation and processing factors, including the change in the concentration of the internal and external phases, sonication time, and stirring time. The loading efficiency of synthesized nanoparticles was 27.9% higher than that of PLGA nanoparticles. In this case, the size of the particles was 241.4 nm, and their surface charge was -11.8 mV. In the last step, the amount of siRNA silencing of the luciferase gene in the H1299 cell line after 48 h was evaluated, which was 54.33±3.78%.

Therefore, the results of this study suggest that PLGA-spermine nanoparticles can be an appropriate choice to improve the physicochemical properties of siRNA and increase its therapeutic efficiency.

Acknowledgements

This work was supported by the Nanodrug Delivery Research Center of Kermanshah University of Medical Sciences.

Competing Interests

The authors declare that they have no conflicts of interest

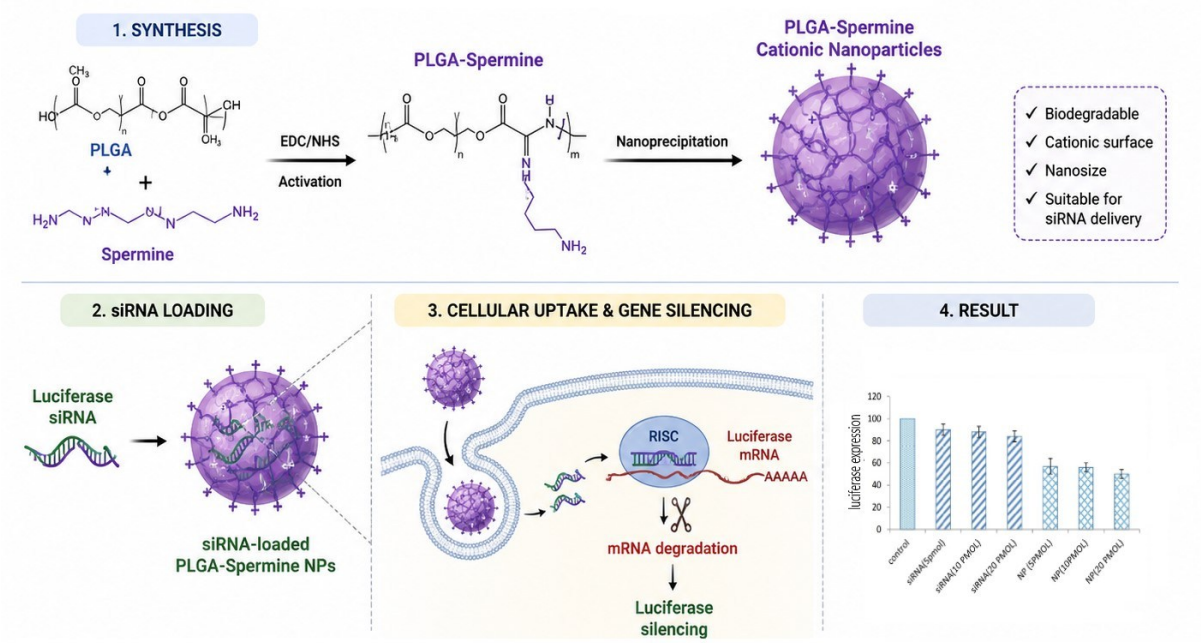
References

1. Davis ME. The first targeted delivery of siRNA in humans via a self-assembling, cyclodextrin polymer-based nanoparticle: from concept to clinic. Mol Pharm. 2009;6(3):659-668. [DOI: 10.1021/mp900015y] [PMID]
2. Whitehead KA, Langer R, Anderson DG. Knocking down barriers: advances in siRNA delivery. Nat Rev Drug Discov. 2009;8(2):129-138. [DOI: 10.1038/nrd2742] [PMID] [PMCID]
3. Manoharan M. RNA interference and chemically modified small interfering RNAs. Curr Opin Chem Biol. 2004;8(6):570-579. [DOI: 10.1016/j.cbpa.2004.10.007] [PMID]
4. Layzer JM, McCaffrey AP, Tanner AK, Huang Z, Kay MA, Sullenger BA. In vivo activity of nuclease-resistant siRNAs. RNA. 2004;10(5):766-771.
5. [DOI: 10.1261/rna.5239604] [PMID] [PMCID]
6. Domb AJ, Sharifzadeh G, Nahum V, Hosseinkhani H. Safety evaluation of nanotechnology products. Pharmaceutics. 2021;13(10):1615. [DOI: 10.3390/pharmaceutics13101615] [PMID] [PMCID]
7. Kumar S, Singhal A, Narang U, Mishra S, Kumari P. Recent progresses in organic-inorganic nano technological platforms for cancer therapeutics. Curr Med Chem. 2020;27(35):6015-6056. [DOI: 10.2174/0929867326666181224143734] [PMID]
8. Aghamiri S, Raee P, Talaei S, Mohammadi-Yeganeh S, Bayat S, Rezaee D, et al. Nonviral siRNA delivery systems for pancreatic cancer therapy. Biotechnol Bioeng. 2021;118(10):3669-3690. [DOI: 10.1002/bit.27869] [PMID]
9. Akhtar S, Benter I. Toxicogenomics of non-viral drug delivery systems for RNAi: potential impact on siRNA-mediated gene silencing activity and specificity. Adv Drug Deliv Rev. 2007;59(2-3):164-182. [DOI: 10.1016/j.addr.2007.03.010] [PMID]
10. Heller R, Heller LC. Gene electrotransfer clinical trials. Adv Genet. 2015;89:235-262. [DOI: 10.1016/bs.adgen.2014.10.006] [PMID]
11. Makadia HK, Siegel SJ. Poly lactic-co-glycolic acid (PLGA) as biodegradable controlled drug delivery carrier. Polymers (Basel). 2011;3(3):1377-1397. [DOI: 10.3390/polym3031377] [PMID] [PMCID]
12. Bianchi L, Ribeiro APD, de Oliveira Carrilho MR, Pashley DH, de Souza Costa CA, Hebling J. Cytotoxicity of adhesive systems of different hydrophilicities on cultured odontoblast-like cells. J Biomed Mater Res B Appl Biomater. 2013;101(8):1498-1507. [DOI: 10.1002/jbm.b.32971] [PMID]
13. Pegg AE. Regulation of ornithine decarboxylase. J Biol Chem. 2006;281(21):14529-14532. [DOI: 10.1074/jbc.R500031200] [PMID]
14. Thomas M, Klibanov AM. Non-viral gene therapy: polycation-mediated DNA delivery. Appl Microbiol Biotechnol. 2003;62(1):27-34. [DOI: 10.1007/s00253-003-1321-8] [PMID]
15. Pack DW, Hoffman AS, Pun S, Stayton PS. Design and development of polymers for gene delivery. Nat Rev Drug Discov. 2005;4(7):581-593. [DOI: 10.1038/nrd1775] [PMID]
16. Burgess DJ. Dynamically in the loop. Nat Rev Genet. 2014;15(7):440. [DOI: 10.1038/nrg3762] [PMID]

[Downloaded from ajpr.umsha.ac.ir on 2026-11-23]

[DOI: 10.53208/ajpr.7.1.1]

Graphical Abstract



[Downloaded from ajpr.umsha.ac.ir on 2026-11-23]

[DOI: 10.53208/ajpr.7.1.1]