AJPS Research Article

Development of Silica Nanoparticles as a Delivery System for Plasmid-Based Crispr/Cas9

Gözde ULTAV[1], Kübra MAÇ[2], Sena KIZILBOĞA[2], Vedat GÜNDOĞDU[3], Hayrettin TONBUL[4], Emine ŞALVA[1*]

[1] Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Inonu University, Malatya, Turkey

[2] Department of Molecular Biology and Genetics, Faculty of Science and Letters, Inonu University, Malatya, Turkey

[3] Faculty of Pharmacy, Inonu University, Malatya, Turkey

[4] Department of Pharmaceutical Technology, Faculty of Pharmacy, Inonu University, Malatya, Turkey

ABSTRACT: Clustered regular interspace short palindromic repeat (CRISPR)/CRISPR-associated system (Cas) is a promising technology for gene editing systems and genome manipulation. Transferring the CRISPR vector to cells is an important aspect of the effective use of this technology. In this study, we aimed to develop a new delivery system using silica nanoparticles (SNPs) with the CRISPR cas9 vector. SNPs were synthesized by the Stöber method. The synthesized nanoparticles were analyzed with the Dynamic Light Scattering (DLS) method and approximately 100 nm SNPs were obtained. EF1a-GFP CRISPR/Cas9 plasmid has been transfected to the Escherichia coli (E.coli) DH5α and isolated from the strain using the plasmid DNA isolation Kit. The isolated pCas-EF1a-GFP CRISPR/Cas9 plasmid was imaged by agarose gel electrophoresis. CRISPR/Cas9 plasmid (pCRISPR) attached to SNP by electrostatic interactions and obtained pCRISPR/SNP complexes were checked by agarose gel electrophoresis. Results show average particle size and zeta potential of obtained pCRISPR/SNP nanoparticles were among 146.6-272.7 nm and -20.2 - +16,9 mV, respectively and full complexation was achieved at 1/10 pCRISPR/SNP w/w ratio. Consequently, optimized silica nanoparticles can be a good candidate for the delivery of CRISPR/Cas9 plasmid.

Key Words: CRISPR/Cas9, plasmid, silica nanoparticle, delivery system, zeta potential, particle size.

1 INTRODUCTION

In the 1970s, with the development of genetic engineering (manipulation of DNA or RNA), the genome editing mechanism CRISPR-Cas system was discovered. The purpose of this genome editing experiment is to convert a targeted DNA sequence, the genome of a cell, into a new, desired DNA sequence [1, 2]. The transfer of Cas9 to cells is an important step in gene editing [3]. Therefore, choosing a suitable transfer strategy for the CRISPR/Cas9 system plays an important role in achieving efficient and precise gene editing [4]. To date, many strategies have been developed for the transfer of Cas9. These can be classified into viral and

*Corresponding Author: Emine ŞALVA
E-mail: emine.salva@inonu.edu.tr

non-viral vector-based technologies [5, 6]. In recent years, viral vectors have been widely used for the delivery of CRISPR plasmids in vitro and in vivo, largely due to their high efficiency in gene delivery and long-term stable transgene expression [7]. Although highly efficient, the risk of carcinogenesis is a disadvantage associated with viral vectors, including low packaging, immune responses, and difficulties in large-scale production [7]. The physical methods for gene transfer include microinjection, biolistic transformation, gene gun, electroporation, and nucleofection. Unfortunately, although physical approaches are often successful in the laboratory, these methods are not suitable for in vivo and clinical applications [8].

Alternatively, non-viral vectors are among the carrier systems suitable for use in CRISPR/Cas9 transmission due to their low immunogenicity, the possibility of transportation of larger genetic loads, and ease of large-scale production [8, 9]. The remarkable development of nanoparticles as non-viral carriers for the delivery of the CRISPR/Cas9 system is promising for therapeutic applications. Nanoparticles are solid colloidal particles, the size of which varies between 10-1000 nm, which release the active substance or genetic material trapped in them in a controlled manner. The desired properties of this carrier system are the controlled release of substances inside the target region and improve the stability of the substances. It has been possible to ensure that the drugs reach the target organ or tissue safely, controlled, and effectively for diagnostic imaging and/or treatment purposes, by using nanoparticles as drug carrier systems [10]. One of the most important points for nanoparticles used for carrier purposes is that their surface must be coated with an inert polymer that gives it properties that resist interactions with components of the blood flow. The Polyethylene glycol (PEG) used in the study is the most widely used "stealth" polymer due to its long-term safety history in humans [11]. Silica nanoparticles are highly resistant to external influences such as changing temperatures and pH. Another advantage of silica nanoparticles, their surface can easily modify for higher blood circulation time and delivering different types of cargo [12].

In this study, we aimed to investigate the binding efficiency of CRISPR/Cas9 plasmid to the SNP after complexation. Thus, silica nanoparticles for efficient delivery of plasmid-based CRISPR/Cas9 system were developed and characterized.

2 MATERIAL AND METHOD

2.1 Materials

All SNP synthesis materials were purchased from Sigma-Aldrich except PEG-

Silane which was purchased from Gelest. The maxiprep DNA isolation Kit was purchased from Thermo. All chemicals were purchased from Sigma-Aldrich.

2.2 Experimental Method

2.2.1 Silica nanoparticle synthesis and surface modifications.

Stöber method was used to synthesize amorphous SNPs about 100 nm in size [13]. Briefly, 1.25 mL tetra ethyl ortho silicate (TEOS) was dissolved in 9 mL ethanol (absolute) and added to 10 mL ethanol containing 1.5 mL NH_4OH solution (25% wt) under continuous stirring. After mixing overnight, nanoparticles were washed with distilled water several times and redispersed by a vortex and an ultrasonic bath.

The surfaces of the SNPs were modified by Polyethylene glycol (PEG) and N1-(3-Trimethoxysilylpropyl)diethylene-triamine (TMSPE) to improve stability and gain a positive charge, respectively. To achieve this, 250 µL PEG were added to 100 mg SNPs under continuous stirring in 6 mL. After 3 hours and 750 µL TMSPE was added and obtained solution was mixed overnight. Finally, obtained nanoparticles were centrifuged, redispersed in water, and labeled as SNP-PEG-NH_2. Nanoparticle size was measured by Dynamic Light Scattering (DLS) (Horiba NanoPartica SZ100). Nanoparticles were diluted with distilled water before measurement.

2.2.2 CRISPR/Cas9 plasmid isolation

pCas-Scramble-EF1a-GFP CRISPR/Cas9 vector was transformed using the calcium chloride method [14] into *E.coli* DH5α. Plasmid isolation was performed using the maxiprep plasmid isolation kit according to the manufacturer's instructions. Electrophoretic and spectrophotometric analyses of obtained plasmids were conducted.

2.2.3 The preparation of pCRISPR/ SNP-PEG-NH_2 nanoparticles

SNP solution was prepared at a concentration of 1µg/ul. pCRISPR and SNP-PEG-NH_2 were complexed in different weight ratios of 1/5, 1/10, 1/20, and 1/40. Complexes were vortexed and incubated for 2 hours at room temperature. The formation of pCRISPR/ SNP-PEG-NH_2 nanoparticles was confirmed by agarose gel electrophoresis. The particle size and zeta potential of nanoparticles were measured by Horiba NanoPartica SZ100 (n=3).

2.2.4 Statistics

All statistics were performed by one-way Anova by GraphPad Prism. Statistically significance was indicated with *($p<0.05$).

3 RESULTS AND DISCUSSION

3.1 Characterization of Silica Nanoparticles

The hydrodynamic size of the synthesized SNP nanoparticles before surface modification was measured as 87.4±2.5 nm. The polydispersity index (PDI) was 0.066±0.009 and zeta potentials were found -22.4±4.2. After surface modifications, size, PDI, and zeta potential values of SNP-PEG-NH_2 were found as 94.5±1.8 nm, 0.076±0.011, and +52.2±2.8 mV, respectively (Figure 1). Results, which are presented in Table 1, show that obtained SNPs were monodispersed and negatively charged with suitable particle size. These findings are in correlation with the literature [15]. After surface modifications, the size of the SNP-PEG-NH_2 did not change considerably but the zeta potential became positive as aimed. Due to the positive charge, obtained SNP-PEG-NH_2 can carry negatively charged DNA by electrostatic interaction [16].

Table 1. Particle size, PDI, and zeta potential results of silica nanoparticles.

	Particle size (Mean±SD) (nm)	PDI (Mean±SD)	Zeta potential (Mean±SD) (mV)
SNP	87.4±2.5	0.066±0.009	-22.4±4.2
SNP-PEG-NH_2	94.5±1.8	0.076±0.011	+52.2±2.8

3.2 The Characterization pCRISPR/ SNP-PEG-NH_2 Nanoparticles

In the study, SNP-PEG-NH_2 nanoparticles were used to deliver pCRISPR and different w/w ratios were analyzed to optimize the system. Different amounts of SNP-PEG-NH_2 and pCRISPR (w/w) were incubated and gel electrophoresis was performed to detect a full complexation ratio. Moreover, the size and zeta potentials of obtained systems were analyzed. Results show that the size of the nanoparticles was increased due to complexation and zeta potentials were lowered due to the negative charge of pCRISPR (Table 2).

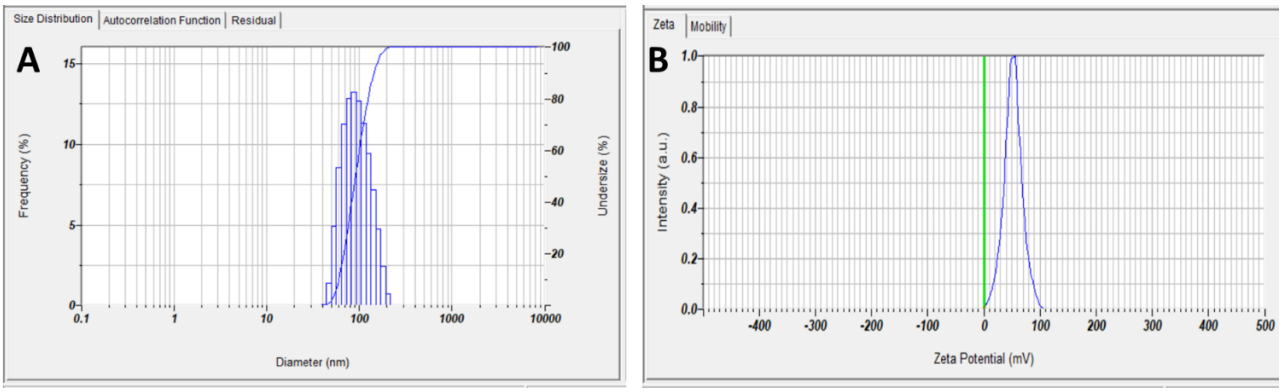

Figure 1. Particle size (A) and zeta potential (B) results of SNP-PEG-NH_2.

Table 2. Average particle size and zeta potential of pCRISPR/ SNP-PEG-NH_2 nanoparticles in different ratios.

Ratios	Average Particle Size (Mean±SD) (nm)	Zeta Potential (Mean±SD) (mV)
1/5	146.6*±27.7	-20.2±1.6
1/10	284.5±45.2	18.5±1.3
1/20	229.8±55.7	16.1±1.2
1/40	272.7±49.5	16.9±2.8

*means statistically significant ($p<0.05$)

Agarose gel electrophoresis analysis showed that SNP-PEG-NH_2 nanoparticles were fully complexed with pCRISPR at a 1/10 ratio (Figure 2). Moreover, pCRISPR/ SNP complexes after a 1/10 ratio could not be observed in wells due to tight complexation.

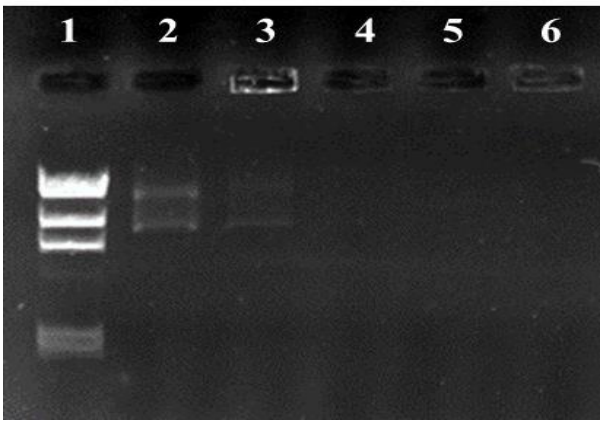

Figure 2. Gel electrophoresis results of pCRISPR/ SNP-PEG-NH_2 nanoparticles. 1. λDNA HindIII marker, 2. pCRISPR, 3-6. 1/5, 1/10, 1/20 and 1/40 pCRISPR/ SNP-PEG-NH_2 (w/w), respectively.

Results show that a proper amount of silica nanoparticles with proper surface modifications can be used in the delivery of CRISPR-based plasmids. Positive charge creation on the surface leads to complexation due to a positive-negative interaction between plasmids and silica [16]. According to gel electrophoresis results, full complexation was observed at 1/10 ratio. Particle size measurements confirm this since particle size increased dramatically with increasing ratio of 1/5 to 1/10. Although increment in particle size statistically significant between 1/5 and other ratios (1/10, 1/20 and 1/40), not significant among other ratios. Besides this, increasing zeta potential suggests that the TMSPE was incorporated on the surface to provide positive charge to SNPs. These findings show that silica nanoparticles can be used to carry and/or deliver plasmid DNA with suitable surface charges with lower NP/plasmid ratios.

4 CONCLUSION

The remarkable development of nanoparticles as non-viral carriers for the delivery of the CRISPR/Cas9 system has shown great promise for therapeutic applications. In our study, silica nanoparticles with PEG and amine functionalization were used. The most important advantage of the developed silica nanoparticles is that it is a reliable and biocompatible carrier system. As a result, we suggest that silica nanoparticles can be a promising approach for delivery of CRISPR/Cas9.

5 ACKNOWLEDGEMENTS

Special thanks to Inonu University Faculty of Pharmacy for Horiba NanoPartica device.

6 AUTHOR CONTRIBUTIONS

Hypothesis: E.S. Design: E.S., G.U. Literature Review: S.K., K.M., V.G., G.U., H.T. Data collection: S.K., K.M., V.G., G.U Analysis and/or interpretation: E.S., G.U. Manuscript writing: H.T., G.U.

7 CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

8 REFERENCESES

[1] Anzalone AV, Koblan LW, Liu DR. Genome editing with CRISPR–Cas nucleases, base editors, transposases and prime editors. *Nature Biotechnology*, 2020; **38**(7): 824-844.

[2] CRISPR's breakthrough problem. C&EN Global Enterprise, 2017. 95(7): 28-33.

[3] New CRISPR inhibitors found. C&EN *Global Enterprise*, 2018; **96**(37): 6-6.

[4] Lino CA et al. Delivering CRISPR: a review of the challenges and approaches. *Drug Deliv*, 2018; **25**(1): 1234-1257.

[5] Yip BH. Recent Advances in CRISPR/Cas9 Delivery Strategies. *Biomolecules*, 2020; **10**(6).

[6] Kretzmann JA et al. Targeted Therapeutic Genome Engineering: Opportunities and Bottlenecks in Medical Translation, in Targeted Nanosystems for Therapeutic Applications: New Concepts, Dynamic Properties, Efficiency, and Toxicity. 2019, American Chemical Society. 1-34.

[7] Wang HX et al. CRISPR/Cas9-Based Genome Editing for Disease Modeling and Therapy: Challenges and Opportunities for Nonviral Delivery. Chemical Reviews, 2017; **117**(15): 9874-9906.

[8] Li L, Hu S, Chen X. Non-viral delivery systems for CRISPR/Cas9-based genome editing: Challenges and opportunities. Biomaterials, 2018; **171**: 207-218.

[9] Buck J et al. Lipid-Based DNA Therapeutics: Hallmarks of Non-Viral Gene Delivery. *ACS Nano*, 2019: **13**(4); 3754-3782.

[10] Patra JK et al. Nano based drug delivery systems: recent developments and future prospects. *Journal of Nanobiotechnology*, 2018; **16**(1): 71.

[11] Suk JS et al PEGylation as a strategy for improving nanoparticle-based drug and gene delivery. *Advanced Drug Delivery Reviews*, 2016; **99**: 28-51.

[12] Selvarajan V, Obuobi S, Ee PLR. Silica Nanoparticles—A Versatile Tool for the Treatment of Bacterial Infections. 2020; **8**.

[13] Ultav G et al. Silica nanoparticle synthesis by experimental design for drug and gene delivery applications. *Journal of Research in Pharmacy*, 2022; **27**(1): 12-22.

[14] Chen X et al. A convenient and rapid method for genetic transformation of E. coli

with plasmids. *Antonie Van Leeuwenhoek*, 2001; **80**(3-4): 297-300.

[15] Ultav G et al. pH-sensitive chitosan-PEG-decorated hollow mesoporous silica nanoparticles could be an effective treatment for acute myeloid leukemia (AML). *Journal of Nanoparticle Research*, 2022; **24**(2): 40.

[16] Ultav G, Tonbul H, Salva E. An effective VEGF-siRNA delivery via folic acid decorated and pegylated silica nanoparticles. *Journal of Drug Delivery Science and Technology*, 2022; **76**: 103828.