J Med Microbiol Infect Dis, 2019, 7 (1-2): 37-43
DOI: 10.29252/JoMMID.7.1.2.37

Original Article

Enhancing HIV-1 Nef Penetration into Mammalian Cells as an Antigen Candidate

Saba Davoodi[1], Azam Bolhassani[2*], Seyed Mehdi Sadat[2], Shiva Irani[1]

[1]Department of Biology, School of Basic Science, Science and Research Branch, Islamic Azad University, Tehran, Iran; [2]Department of Hepatitis and AIDS, Pasteur Institute of Iran, Tehran, Iran

Received Jul. 19, 2019; Accepted Sep. 01, 2019

Introduction: The human immunodeficiency virus type 1 (HIV-1) Nef regulatory protein is known as a candidate for the design of therapeutic HIV DNA and protein vaccines. One of the limitations of these vaccines is the inability of DNA and protein to pass through the cell membrane. Various delivery systems have been developed to transfer DNA and protein into cells. Cell penetrating systems such as MPG and CyloP-1 are among delivery systems, which can deliver DNA and protein cargoes into the cells, respectively. **Methods:** In this study, we produced the recombinant Nef protein in *Escherichia coli* expression system. Then, the formation of CPP/DNA and CPP/protein nanoparticles was confirmed by agarose gel retardation, scanning electron microscope (SEM), Zetasizer and SDS-PAGE, and their stability was evaluated against nucleases and proteases. Finally, the delivery of the nanoparticles into HEK-293T cells was assessed by fluorescent microscopy, flow cytometry, and western blotting. **Results:** Our data confirmed the formation of stable nanoparticles through non-covalent bonds with a diameter of less than 200 nm. Moreover, the results of fluorescence microscopy, flow cytometry, and western blotting demonstrated that these CPPs could successfully deliver the Nef protein and DNA into HEK-293T cells. **Conclusion:** Our results indicated that the MPG and CyLoP-1 CPPs are suitable candidates for the delivery of DNA and protein cargoes into mammalian cells, respectively. *J Med Microbiol Infec Dis, 2019, 7 (1-2): 37-43.*

Keywords: HIV infections, Gene products nef, Cell penetrating peptides, Transfection.

INTRODUCTION

Human immunodeficiency virus type 1 (HIV-1) is the leading cause of acquired immunodeficiency syndrome worldwide. Since the AIDS epidemics in the early 1980s, nearly 70 million people have been infected with the virus, resulting in the deaths of about 30 million people [1]. Combined antiretroviral therapy (cART) has increased the life expectancy in people infected with HIV [2]. Despite the efforts, no effective vaccine is available yet. HIV encodes three primary genes, including *gag*, *pol* and *env*, and five accessory genes including *vpr*, *vpu*, *vif*, *nef*, *rev* and *tat* [3]. The Nef is a 27-34 kDa cell membrane-associated protein [4]. HIV infection progression in humans and animals has shown to be related to this protein [5]. Nef has a role in T cell signaling pathway activation [6]. On the other hand, cell-penetrating peptides (CPPs) are short peptides which can transfer their cargoes into the cells [7]. CPPs contain a large number of positive amino acids, especially lysine and arginine, which increase cell penetration [8]. Size and polarity are two essential factors that determine the mechanism for entering peptides and other molecules into the cells. Small non-polar molecules usually penetrate the cell membrane through passive diffusion [9]. Larger molecules enter the cell using two mechanisms of direct translocation and endocytosis [10]. MPG is one of the cell-penetrating peptides used to deliver DNA cargoes into the cells. Peptides such as MPG, penetratin, and CADY containing both polar and nonpolar domains, are defined as amphipathic peptides. Some of the primary amphipathic CPPs are chimeric peptides which bind to the nuclear localization sequences (NLS) via covalent bonds for effective penetration through the cell membranes. MPG is based on the NLS sequence of SV40 and HIV gp41 protein. In MPG, the hydrophobic region is separated from NLS with a linker [11].

Recently, a new cysteine-rich cationic CPP, called CyLoP-1, has been developed for the delivery of peptide and protein cargoes. CyLoP-1 was derived from the nuclear localization sequence of a snake toxin, called crotamine [12]. The presence of cysteine and tryptophan amino acids is necessary to maintain its function. Besides, the cysteine oxidation status plays an essential role in the uptake efficiency of CyLoP-1, and the disulfide-containing form plays a more active role in the CyLoP-1 uptake efficiency [13].

[*]**Correspondence:** Azam Bolhassani
Department of Hepatitis and AIDS, Pasteur Institute of Iran, Tehran, Iran, 1316943551.
Email: A_bolhasani@pasteur.ac.ir
Tel: +98 (21) 64112240 **Fax:** +98 (21) 66465132

[Downloaded from jommid.pasteur.ac.ir on 2026-11-13]

[DOI: 10.29252/JoMMID.7.1.2.37]

In this study, our goal was to produce Nef protein in a prokaryotic expression system as a candidate HIV-1 vaccine and its delivery into HEK-293T cells using two novel cell-penetrating peptides.

MATERIAL AND METHODS

Expression and purification of HIV-1 Nef protein. The pET-23a (+) prokaryotic expression vector (Novagen, USA) harboring HIV-1 Nef protein has been previously prepared by our group [14]. To produce the Nef protein, *Escherichia coli* Rosetta strain was transformed with pET-23a (+) harboring the *nef* gene. The recombinant clones were selected on LB (Luria-Bertani) agar (Sigma-Aldrich, Germany) containing ampicillin and transferred to Ty2x medium to an optical density of 0.7-0.8 at a wavelength of 600 nm. Then, 1 mM Isopropyl β-D-1-thiogalactopyranoside (IPTG, SINACON, Iran) was used at 37°C to induce the expression of the Nef protein. The cell pellet was collected, and the protein expression was analyzed by SDS-PAGE in a gel containing 12.5% acrylamide and western blot using an anti-His tag antibody (Abcam, USA). Nef protein was purified by affinity chromatography using Ni-NTA agarose column (Macherey-Nagel) under native conditions (*i.e.*, 250 mM imidazole buffer, pH=8). The concentration and purification of Nef were measured by the Bradford protein assay kit (Thermo Fisher Scientific) and NanoDrop spectrophotometer (Thermo Fisher Scientific). The expression of the recombinant (r) Nef protein was analyzed by western blot using an anti-His tag antibody (Abcam, USA).

Preparation of the CPP/Nef DNA and CPP/Nef protein complexes. The CyLoP-1 (CRWRWKCCKK) and MPG (GALFLGFLGAAGSTMGAWSQPKKKRKV) peptides were synthesized by Biomatik Corporation (Cambridge, Canada). The pEGFP-N1 eukaryotic expression vector (Clontech, USA) harboring Nef protein has been previously prepared by our group [14]. To form the MPG/pEGFP-N1-*nef* complexes, different concentrations of MPG were mixed with 2 µg of pEGFP-N1-*nef* at N/P (nitrogen to phosphate) ratios of 1, 2, 5 and 10, and incubated at room temperature for 45 min. The formation of the MPG/pEGFP-N1-*nef* complexes was investigated by gel retardation assay. DNase I was added to the complexes with different N/P ratios of 1, 2, 5 and 10, and incubated at 37°C for 1 h to verify the stability of MPG/DNA complexes against DNA nucleases. For the evaluation of serum stability, the complexes with N/P ratio of 10 were exposed to 10% serum at 37°C for 5 h [15]. To form the CyLoP-1/Nef complexes, different concentrations of CyLoP-1 were mixed with 1 µg of Nef protein at CyLoP-1:Nef molar ratios of 2:1, 5:1, 10:1, 15:1, 20:1, 30:1, and incubated for 60 min at room temperature. The formation of the CyLoP-1/Nef complexes was confirmed by SDS-PAGE in a 12.5% acrylamide gel.

SEM and Zetasizer studies. The MPG/pEGFP-N1-*nef* and CyLoP-1/Nef nanoparticles were formed with a ratio of 10, and their size and morphology were determined using a scanning electron microscope (SEM) (FEI Quanta 200 SEM, PHILIPS, USA). The charge of nanoparticles was assessed by Zetasizer Nano ZS (Malvern Instruments, UK) at 25°C.

MTT assay. The viability of the cells treated with complexes was assessed by MTT assay. Human embryonic kidney cells (HEK-293T, CRL-1651, Pasteur Institute of Iran; 1×10^4 cells/ well) were seeded in a 96-well plate (Greiner, Germany), and cultured in RPMI-1640 medium (Sigma, Germany) supplemented with 5% FBS (Fetal Bovine Serum, Gibco, Germany) and 1% Gentamicin solution at 37°C and 5% CO_2 for 24 h. The medium was replaced by fresh RPMI-1640. The MPG, pEGFP-N1-*nef*, MPG/pEGFP-N1-*nef* (N/P ratio of 10), CyLoP-1, rNef protein and CyLoP-1/Nef (molar ratio of 10) were added to the cells and cultured for 48 h. Then, the medium was removed, and the MTT solution (5 mg/mL, Sigma) was added to each well and incubated for 3 h at 37°C. After adding Dimethyl Sulfoxide (DMSO, Sigma, Germany) to each well, the absorbance was measured by ELISA reader at a wavelength of 570 nm. Untreated cells and cells treated with 70% ethanol were considered as negative and positive controls, respectively.

Transfection of HEK-293T cells with the Nef nanoparticles. HEK-293T cells (5×10^4 cells/well) were seeded in a 24-well plate (Greiner, Germany), followed by incubation for 24 h. The MPG/pEGFP-N1-*nef* complexes were prepared with the N/P ratio of 10. Then, the medium was removed, the complexes were added to cells, and the cells were incubated at 37°C for 6 h. Then, the medium was replaced with complete RPMI-1640 medium supplemented with 5% FBS, and the cells were incubated at 37°C for 48 h. The cells treated with TurboFect (Fermentas)/pEGFP-N1-*nef* complexes were considered as positive control, and the untreated cells were considered as negative control. The transfection efficiency of the cells treated with the complexes was evaluated by fluorescence microscopy (Envert Fluorescent Ceti, Korea) and FACS Calibur flow cytometer (Partec, Germany). Also, the expression of the Nef protein was evaluated by western blot analysis (Abcam, USA). The CyLoP-1/Nef complexes were prepared at a molar ratio of 10:1. Then, the medium was removed, and the complexes were added to cells. After incubation of cells at 37°C for 2 h, the cells were treated with trypsin-EDTA and harvested by centrifugation. The cells treated with TurboFect (ProJect, Fermentas)/Nef complexes were considered as positive control and the untreated cells as negative control. The transfection efficiency of the cells treated with complexes was evaluated by western blot using an anti-His-tag antibody (Abcam, USA).

Statistical analysis. Prism 5.0 software (GraphPad, San Diego, California, USA) was used for statistical analysis (Student's t-test) of cell viability and cell transfection. The value of $p<0.05$ was considered statistically significant. Each experiment was repeated two times.

RESULTS

Expression and purification of HIV-1 Nef protein. The expression of Nef protein was evaluated in Rosetta strain. The optimal expression of Nef was observed at 37°C

[Downloaded from jommid.pasteur.ac.ir on 2026-11-13]

[DOI: 10.29252/JoMMID.7.1.2.37]

and 16 h of induction. The results showed that Nef could be purified successfully under native conditions. The Nef was observed as a bright band of ~30 kDa in SDS-PAGE gel containing 12.5% acrylamide (Fig. 1). The purified Nef protein was detected and confirmed by western blot using an anti-His-tag antibody. The concentration of the recombinant peptide was between 0.6-0.8 mg/mL. According to LAL assay, contamination with LPS was less than 0.5 EU/mg (QCL-1000, Lonza).

Formation of the HIV-1 Nef nanoparticles. The MPG/pEGFP-N1-*nef* complexes were prepared with the N/P ratios of 1, 2, 5 and 10, and loaded in 1% agarose gel. The results of the gel retardation assay showed that DNA did not migrate in agarose gel at N/P ratio of 10 indicating the formation of MPG/pEGFP-N1-*nef* complexes (Fig. 2). After treatment with DNase I and serum, pEGFP-N1-*nef* (control) was degraded rapidly, while DNA in the MPG/pEGFP-N1-*nef* complexes with N/P ratio of 10 remained intact. The formation of CyLoP-1/Nef complexes was confirmed by SDS-PAGE analysis. The CyLoP-1/Nef complexes were observed as two separate bands of ~30 kDa (Nef) and ~1.4 kDa (CyLoP-1) in SDS-PAGE containing 12.5% acrylamide. This data indicated the non-covalent interactions between rNef and CyLoP-1 at 1:2, 1:5, 1:10, 1:15, 1:20 and 1:30 molar ratios (Fig. 3).

SEM and Zetasizer studies. The size and morphology of nanoparticles were studied by SEM. The pEGFP-N1-*nef* (Fig. 4a) and MPG (Fig. 4b) formed spherical MPG/pEGFP-N1-*nef* complexes (N/P ratio: 10) with an average diameter of 100-200 nm (Fig. 4c). The rNef protein (Fig. 4d) and CyLoP-1 (Fig. 4e) formed non-spherical CyLoP-1/Nef complexes (molar ratio of 10:1) with an average diameter of 100-150 nm (Fig. 4f). The charge of complexes was studied by Zetasizer at the same ratios (Table 1). The pEGFP-N1-*nef* (-9.27 mV) and rNef (-24.9 mV) had negative charges, while the MPG/pEGFP-N1-*nef* (+23.2 mV) and CyLoP-1/Nef (+4.35 mV) complexes had positive charges.

Cytotoxicity assay. Cell viability results showed that MPG, pEGFP-N1-*nef*, MPG/pEGFP-N1-*nef*, CyLoP-1, rNef protein, and CyLoP-1/rNef had no significant cytotoxicity on the cells within 48 h ($p>0.05$; Fig. 5).

Cell transfection with the nanoparticles. Transfection of HEK-293T cells with MPG/pEGFP-N1-*nef* (N/P: 10) complexes was evaluated 48 h after transfection using fluorescence microscopy, flow cytometry, and western blot analysis. The results showed that MPG was able to deliver pEGFP-N1-*nef* into the cells, which led to the expression of Nef protein in the cells. The transfected cells appeared as green spots in the fluorescent microscopy images (Fig. 6). Flow cytometry exhibited that the cellular uptake of TurboFect/pEGFP-N1-*nef* and MPG/pEGFP-N1-*nef* complexes were 73.71 ± 091 % and 28.60 ± 2.06%, respectively ($p<0.05$; Fig. 6). Moreover, the expression of Nef protein in the cells was confirmed by western blot analysis using an anti-His-tag antibody. Nef protein was observed as a clear band of ~30 kDa in the cells treated with the complexes. No bands were observed in untreated cells. Transfection of HEK-293T cells with the CyLoP-1/Nef complexes (molar ratio of 10) was evaluated 2 h after transfection by western blot analysis using an anti-His-tag antibody. The results showed that CyLoP-1 was able to deliver rNef protein into the cells 2 h after transfection and HIV-1 Nef was observed as a clear band of ~30 kDa in the cells treated with the complexes. No bands were observed in the untreated cells (Fig. 7).

Table 1. Size and charge of the nanoparticles

Sample	Size (nm)	Charge (mV)
pEGFP-N1-*nef*	100-200	-9.27
pEGFP-N1-*nef* /MPG	100-200	23.2
rNef	250-500	-24.9
rNef /CyLoP-1	100-150	4.35

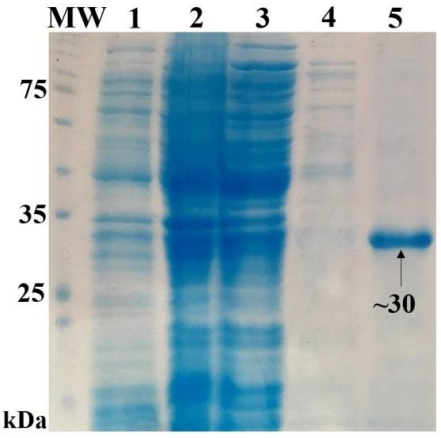


Fig. 1. Expression and purification of HIV-1 Nef protein in Rosetta: Lane 1, before induction; lane 2, 16 h after induction; lane 3, flow-through; lane 4, wash; lane 5, the purified Nef protein; MW, molecular weight marker (prestained protein ladder, 10-180 kDa, Fermentas).

[Downloaded from jommid.pasteur.ac.ir on 2026-11-13]

[DOI: 10.29252/JoMMID.7.1.2.37]

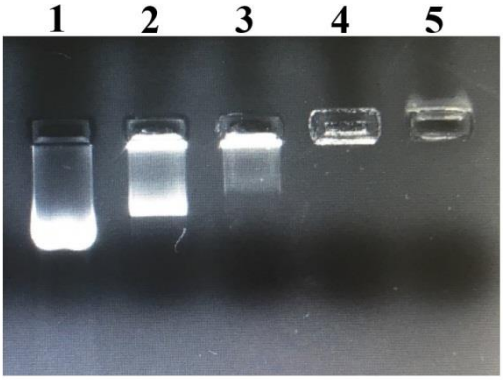


Fig. 2. Gel retardation assay of MPG/pEGFP-N1-*nef* complexes: Lane 1, pEGFP-N1-*nef* (control); lane 2, N/P:1; lane 3, N/P:2; lane 4, N/P:5; lane 5, N/P:10

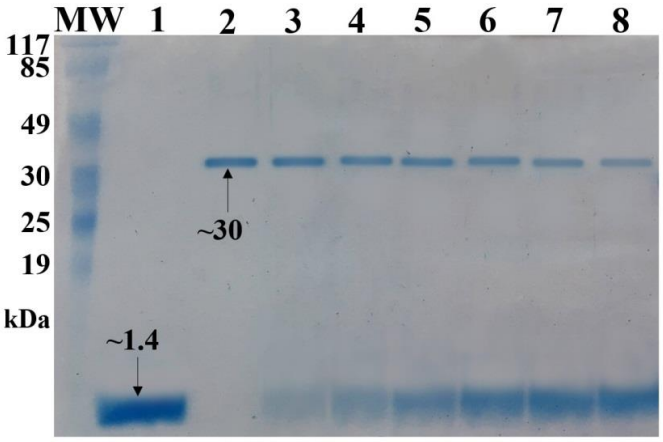


Fig. 3. Analysis of different molar ratios of the CyLoP-1/Nef complexes in SDS-PAGE. Lane 1, CyLoP-1 peptide (control); lane 2, purified Nef protein (control); lane 3, molar ratio of 1:2; lane 4, molar ratio of 1:5; lane 5, molar ratio of 1:10; lane 6, molar ratio of 1:15; lane 7, molar ratio of 1:20; lane 8, molar ratio of 1:30; MW, Molecular weight marker (prestained protein ladder, 19-117 kDa, Maxcell).

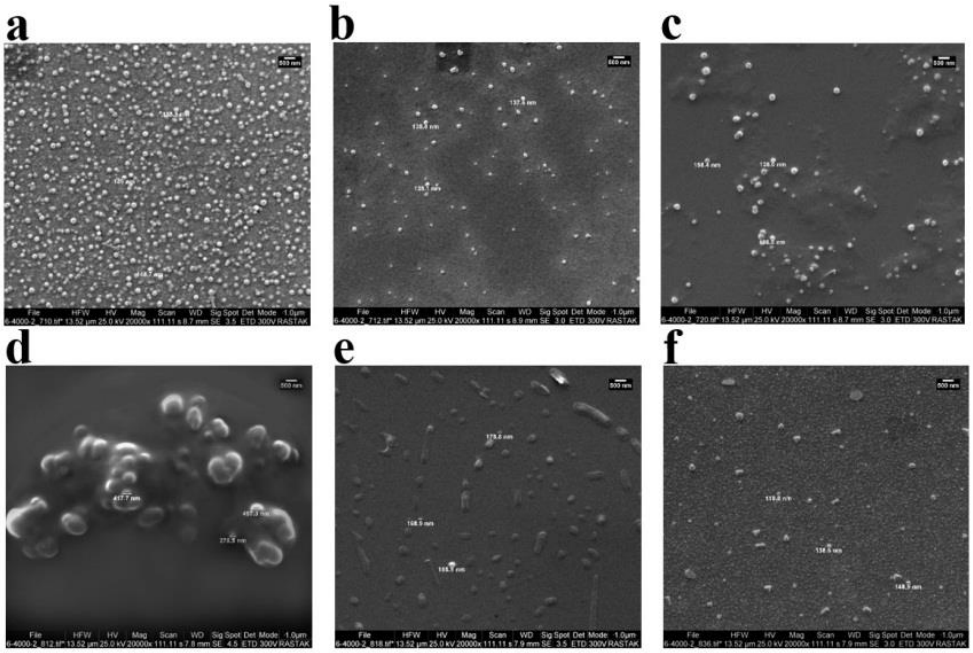


Fig. 4. The SEM micrograph of nanoparticles: a) pEGFP-N1-*nef* (~100-200 nm), b) MPG (~100-150 nm), c) MPG/pEGFP-N1-*nef* (100-200 nm), d) rNef protein (~250-500 nm), e) CyLoP-1 (~150-200 nm), and f) CyLoP-1/rNef (~100-150 nm)

[Downloaded from jommid.pasteur.ac.ir on 2026-11-13]

[DOI: 10.29252/JoMMID.7.1.2.37]

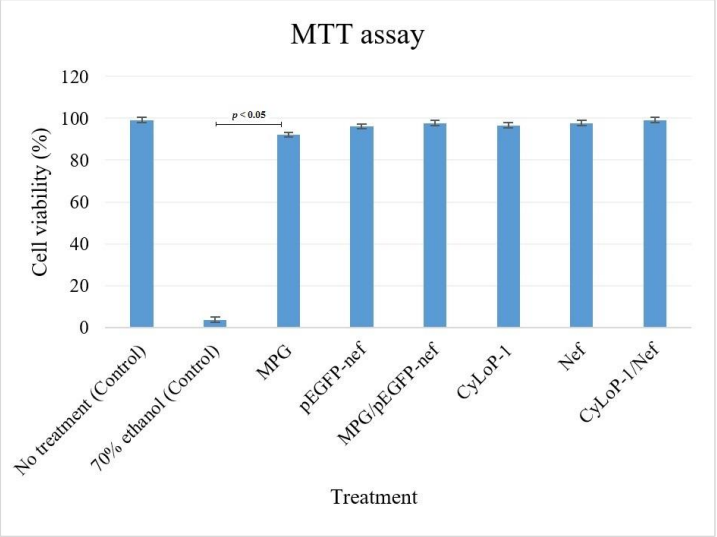


Fig. 5. MTT assay of the treated HEK-293T cells with different compounds. No significant cytotoxicity was observed in the treated cells as compared to the untreated cells (p>0.05)

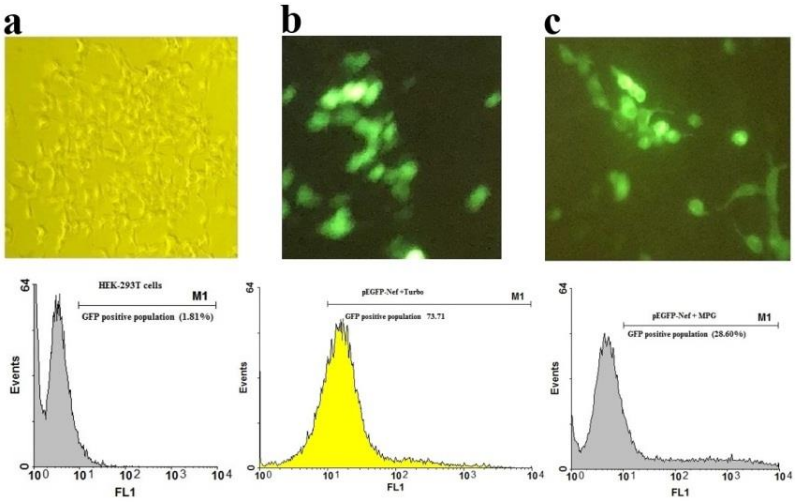


Fig. 6. Analysis of the transfected HEK-293T cells using fluorescent microscopy and flow cytometry. a) Untransfected cells (negative control), b) cells transfected with TurboFect/pEGFP-N1-*nef* complexes (positive control), c) cells transfected with MPG/pEGFP-N1-*nef* nanoparticles

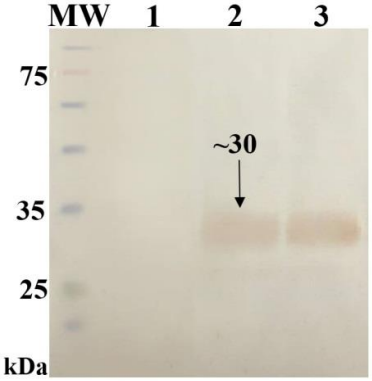


Fig. 7. Western blot analysis of the transfected HEK-293T cells: Lane 1, untransfected cells (negative control); lane 2, Cells transfected with CyLoP-1/rNef nanoparticles; lane 3, cells transfected with TurboFect/rNef complexes (positive control); MW, molecular weight marker (prestained protein ladder, 10-180 kDa, Fermentas)

[Downloaded from jommid.pasteur.ac.ir on 2026-11-13]

[DOI: 10.29252/JoMMID.7.1.2.37]

DISCUSSION

Therapeutic vaccines are considered as an approach to stimulate the immune system in people infected with HIV-1, but developing an effective HIV-1 vaccine still faces many challenges. DNA-based and protein-based vaccines are considered as successful approaches to stimulate immune responses against viral diseases [16]. The Gp120-, Gp160- and Tat-based vaccines are among the developed protein vaccines, but they are not efficient enough in stimulating the HIV-specific responses [17]. Some studies focused on Nef regulatory protein which is highly conserved and immunogenic [18]. Nef is an essential factor in the pathogenesis of the HIV-1 virus [19]. The most critical weakness of protein vaccines is their low immunogenicity [20]. Some methods such as the use of carriers (*e.g.*, CPPs) were developed to overcome this problem [16]. In this study, we used CPPs to deliver HIV-1 Nef protein and DNA into HEK-293T cells. The MPG peptide was used to transfer pEGFP-N1-*nef* into the cells [21]. Gros and colleagues (2006) studied non-covalent interactions between MPG and the plasmid DNA and showed that there is a high affinity between CPP and charged molecules [22]. In another study, Simeoni (2003) demonstrated that MPG is 80-95% efficient in delivery of luciferase gene [23]. In the present study, MPG/pEGFP-N1-*nef* complexes (N/P: 10) formed nanoparticles with a diameter of < 200 nm. Moreover, Zetasizer results indicated that the combination of negatively charged pEGFP-N1-*nef* with positively charged MPG peptides forms positively charged complexes that could pass through plasma membrane. Karjoo and colleagues (2013) suggested that nanoparticles with positive charges and a diameter of about 200 nm can pass through the membrane [24]. Our SEM results indicated that MPG/pEGFP-N1-*nef* complexes are spherical. Yoo and colleagues (2011) reported that spherical nanoparticles were delivered more efficiently than non-spherical nanoparticles [25]. In the present study, Fluorescence microscopy and flow cytometry results demonstrated that the transfection efficiency of the MPG/pEGFP-N1-*nef* nanoparticles (~ 28.60%) was less than TurboFect/pEGFP-N1-*nef* complexes (~ 73.71%). Some studies have indicated that the transfection efficiency of CPPs depends on the transfection conditions, CPP and cargo features, and cell type [26]. Our MTT assay results showed that MPG, pEGFP-N1-*nef* and the MPG/pEGFP-N1-*nef* nanoparticles (N/P: 10) had no significant toxicity for the cells, and hence, can be used *in vivo*. Moreover, we used CyLoP-1 to deliver the Nef protein into HEK-293T cells. Cysteine-rich peptides like CyLoP-1 were previously used to deliver biomolecules into cells [27]. The particles with positive charges can interact with the negatively charged membrane and cause the membrane lysis [28]. Sabouri-Rad and colleagues (2017) demonstrated that CyLoP-1 could increase transfection and decrease cell cytotoxicity [29]. In our study, CyLoP-1 was able to transfer the Nef protein into HEK-293T cells. The CyLoP-1/Nef (molar ratio of 10:1) complexes formed stable nanoparticles with a diameter of <200 nm. The SEM results showed that CyLoP-1/Nef complexes were spherical. Zetasizer results showed that the combination of negatively charged Nef protein with the positively charged CyLoP-1 peptide formed positively charged complexes that could pass through the plasma membrane. Western blot results indicated that CyLoP-1, similar to TurboFect, could deliver the Nef protein into the cells. MTT assay results showed that CyLoP-1, Nef protein and the CyLoP-1/Nef nanoparticles (molar ratio of 10:1) had no significant toxicity for the cells, and can be used *in vivo* assay.

In conclusion, CyLoP-1 and MPG peptides formed stable non-covalent complexes with rNef protein and pEGFP-N1-*nef*, respectively. They were able to deliver these nanoparticles into HEK-293T cells. Also, MTT results showed that these nanoparticles do not have significant toxicity in specific doses. However, further studies are needed to evaluate the delivery of pEGFP-N1-*nef* and Nef protein *in vivo*.

ACKNOWLEDGMENT

We thank from Elnaz Agi (Iranian Comprehensive Hemophilia Care Center, Tehran, Iran) for her technical assistance.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

REFERENCES

1. Homepage [Internet]. Unaids.org. 2017 [cited 13 July 2019]. Available from: http://www.unaids.org/en/

2. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection [Internet]. World Health Organization. 2016 [cited 13 July 2019]. Available from: https://www.who.int/hiv/pub/arv/arv-2016/en/

3. Collins D, Collins K. HIV-1 accessory proteins adapt cellular adaptors to facilitate immune evasion. PLoS Pathogens. 2014; 10 (1): e1003851.

4. Wu Y, Marsh J. Early transcription from nonintegrated DNA in human immunodeficiency virus infection. J Virol. 2003; 77 (19): 10376-82.

5. Deacon N, Tsykin A, Solomon A, Smith K, Ludford-Menting M, Ellett A, et al. Genomic structure of an attenuated Quasi Species of HIV-1 from a blood transfusion donor and recipients. Science. 1995; 270 (5238): 988-991.

6. Fackler O, Baur A. Live and Let Die. Nef functions beyond HIV replication. Immunity. 2002; 16 (4): 493-7.

7. Guidotti G, Brambilla L, Rossi D. Cell-penetrating peptides: From basic research to clinics. Trends Pharmacol Sci. 2017; 38 (4): 406-24.

8. Futaki S, Suzuki T, Ohashi W, Yagami T, Tanaka S, Ueda K et al. Arginine-rich Peptides. J Biol Chem. 2000; 276 (8): 5836-40.

9. Matsson P, Doak B, Over B, Kihlberg J. Cell permeability beyond the rule of 5. Adv Drug Deliv Rev. 2016; 101: 42-61.

[Downloaded from jommid.pasteur.ac.ir on 2026-11-13]

[DOI: 10.29252/JoMMID.7.1.2.37]

10. Kauffman W, Fuselier T, He J, Wimley W. Mechanism Matters: A taxonomy of cell penetrating peptides. Trends Biochem Sci. 2015; 40 (12): 749-64.

11. Wang F, Wang Y, Zhang X, Zhang W, Guo S, Jin F. Recent progress of cell-penetrating peptides as new carriers for intracellular cargo delivery. J Control Release. 2014; 174: 126-136.

12. Ponnappan N, Budagavi D, Chugh A. CyLoP-1: Membrane-active peptide with cell-penetrating and antimicrobial properties. Biochimica et Biophysica Acta (BBA)- Biomembranes. 2017; 1859 (2): 167-176.

13. Jha D, Mishra R, Gottschalk S, Wiesmüller K, Ugurbil K, Maier M et al. CyLoP-1: A novel cysteine-rich cell-penetrating peptide for cytosolic delivery of cargoes. Bioconjug Chem. 2011; 22 (3): 319-328.

14. Jafarzade B, Bolhassani A, Sadat S, Yaghobi R. Delivery of HIV-1 Nef protein in mammalian cells using cell penetrating peptides as a candidate therapeutic vaccine. Int J Pept Res Ther. 2016; 23(1):145-153.

15. Motevalli F, Bolhassani A, Hesami S, Shahbazi S. Supercharged green fluorescent protein delivers HPV16E7 DNA and protein into mammalian cells *in vitro* and *in vivo*. Immunol Lett. 2018; 194: 29-39.

16. Kadkhodayan S, Jafarzade B, Sadat S, Motevalli F, Agi E, Bolhassani A. Combination of cell penetrating peptides and heterologous DNA prime/protein boost strategy enhances immune responses against HIV-1 Nef antigen in BALB/c mouse model. Immunol Lett. 2017; 188: 38-45.

17. Leal L, Lucero C, Gatell J, Gallart T, Plana M, García F. New challenges in therapeutic vaccines against HIV infection. Expert Rev Vaccines. 2017; 16 (6): 587-600.

18. Mann J, Ndung'u T. HIV-1 vaccine immunogen design strategies. Virol J. 2015; 12 (1): 3.

19. Abraham L, Fackler O. HIV-1 Nef: A multifaceted modulator of T cell receptor signaling. Cell Commun Signal. 2012; 10 (1): 39.

20. Lema D, Garcia A, De Sanctis J. HIV vaccines: A brief overview. Scand J Immunol. 2014; 80 (1): 1-11.

21. Veldhoen S, Laufer S, Restle T. Recent developments in peptide-based nucleic acid delivery. Int J Mol Sci. 2008; 9 (7): 1276-1320.

22. Gros E, Deshayes S, Morris M, Aldrian-Herrada G, Depollier J, Heitz F et al. A non-covalent peptide-based strategy for protein and peptide nucleic acid transduction. Biochim Biophys Acta Biomembr. 2006; 1758 (3): 384-393.

23. Simeoni F. Insight into the mechanism of the peptide-based gene delivery system MPG: implications for delivery of siRNA into mammalian cells. Nucleic Acids Res. 2003; 31 (11): 2717-24.

24. Karjoo Z, McCarthy H, Patel P, Nouri F, Hatefi A. Systematic engineering of uniform, highly efficient, targeted and shielded viral-mimetic nanoparticles. Small. 2013; 9 (16): 2774-83.

25. Yoo J, Doshi N, Mitragotri S. Adaptive micro and nanoparticles: Temporal control over carrier properties to facilitate drug delivery. Adv Drug Deliv Rev. 2011; 63 (14-15): 1247-56.

26. Laufer S, Restle T. Peptide-mediated cellular delivery of oligonucleotide-based therapeutics *in vitro*: Quantitative evaluation of overall efficacy employing easy to handle reporter systems. Curr Pharm Des. 2008; 14 (34): 3637-55.

27. Mann A, Shukla V, Khanduri R, Dabral S, Singh H, Ganguli M. Linear short histidine and cysteine modified arginine peptides constitute a potential class of DNA delivery agents. Mol Pharm. 2014; 11 (3): 683-696.

28. Rahmat D, Khan M, Shahnaz G, Sakloetsakun D, Perera G, Bernkop-Schnürch A. Synergistic effects of conjugating cell penetrating peptides and thiomers on non-viral transfection efficiency. Biomaterials. 2012; 33 (7): 2321-6.

29. Sabouri-Rad S, Oskuee R, Mahmoodi A, Gholami L, Malaekeh-Nikouei B. The effect of cell penetrating peptides on transfection activity and cytotoxicity of polyallylamine. BioImpacts. 2017; 7 (3): 139-145.

[Downloaded from jommid.pasteur.ac.ir on 2026-11-13]

[DOI: 10.29252/JoMMID.7.1.2.37]