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Design, engineering and preparation of a multi-domain fusion vector for gene delivery

Faranak Sadeghian[a], Saman Hosseinkhani[b,*], Abdolali Alizadeh[c], Arash Hatefi[d]

[a] Department of Nanobiotechnology, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran
[b] Department of Biochemistry, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran
[c] Department of Chemistry, Faculty of Sciences, Tarbiat Modares University, Tehran, Iran
[d] Department of Pharmaceutics, Rutgers, The State University of New Jersey, Piscataway, NJ, USA

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ABSTRACT

Peptide based gene carriers are among the most promising non-viral vectors for gene delivery to eukaryotic cells. We have engineered a new fusion peptide using recombinant technology with the purpose of overcoming the cell barriers to gene delivery. A His- tagged multi-domain peptide was expressed in *Escherichia coli* BL21 (DE3) pLysS and purified using Ni-NTA resin. The fusion peptide is composed of two repeats of truncated histone H1 peptide to condense pDNA, a fusogenic peptide to disrupt endosome membranes and a nuclear localization signal to enhance translocation of pDNA towards nucleus. The results demonstrated that the vector can effectively condense plasmid DNA into nanoparticles with average sizes of 200 nm. The fusogenic peptide in the vector structure also showed membrane disruptive effect in the endosomal pH. Overall, the transfection efficiency of the vector demonstrated that it holds great promise as a nontoxic and effective non-viral gene carrier.

1. Introduction

Gene therapy is one of the new therapeutic approaches with the aim of correcting genetic defects or to express gene products that are therapeutically useful (Gabor and Rubany, 2001). Although gene transfer methods based on viruses have shown partial success, challenges such as, low capacity for genetic materials, immunogenicity (Herz and Gerard, 1993), toxicity (Simon et al., 1993) have to be overcome. The alternative method for gene delivery is to use non-viral systems (Mintzer and Simanek, 2009). The most common are cationic lipids (Wheeler et al., 1999; Samadikhah et al., 2011), cationic polymers (Neu et al., 2005; Mohammadi et al., 2011), and cationic peptides and proteins (Mahat et al., 1999; Sloots and Wels, 2005; Mangipud et al., 2009; Kim et al., 2010). Despite their low transfection efficiency in comparison to viruses, they are extensively investigated because of their low toxicity. Peptide and peptide-based vectors have advantageous over other non-viral strategies in that they are able to be prepared as monodisperse material with exact molecular weight either by solid phase synthesis or genetic engineering methods (Cappello et al., 1990; Chow et al., 2008). While the large-scale production of viral vehicles remains a limiting step (Wu and Ataai, 2000), the synthesis of therapeutic amounts of recombinant peptides for gene therapy can be accomplished more easily. In addition, using peptides as a gene carrier provides a unique situation to overcome the cell barriers in a single construct with precise control over designing in the molecular level (Martin and Kevin, 2007; McCarthy et al., 2011; Canine et al., 2011). Considering the advantages of peptide based vectors and knowing the intracellular cell trafficking, in this study a genetically engineered fusion peptide with different domains was prepared in order to overcome the barriers of the cell as a gene carrier.

Here, two repeats of a 16 mer peptide, derived from C-terminal tail of H1 histone was used as a DNA binding domain. KSPKKAKK sequence present in many H1 subtypes but just a tandem repeat of this peptide called 16 mer (ATPKKSTKKTPKKAKK) which is found in the C-terminus of rat histone H1 is able to condense DNA (Khadake and Rao, 1997). So, two repeats of this sequence were used to improve DNA packaging as a part of fusion peptide in our construct.

Following internalization of the gene delivery systems *via* the endocytic pathway, endosomal entrapment and subsequent lysosomal degradation are major bottlenecks that limit the efficiency of gene delivery. So it is critical for gene vectors to be able to escape from endosome. One strategy is use of fusogenic peptides to facilitate endosomal release. Several peptides are characterized which

* Corresponding author at: Department of Biochemistry, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, 14115-175, Iran. Tel.: +98 21 8288 4407; fax: +98 21 8288 4407.
E-mail addresses: saman_h@modares.ac.ir, shosseinkhani@yahoo.com (S. Hosseinkhani).

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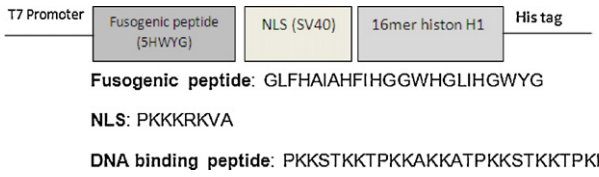

Fig. 1. The schematic image of the fusion peptide with related sequences.

can enhance the endosomal release (Li et al., 2004; Wyman et al., 1997). H5WYG (Midoux et al., 1998) is a synthetic pH responsive fusogenic peptide which is effective in transfection (Canine et al., 2009; Moore et al., 2008). Also the simian virus 40 (SV40) large T-antigen NLS (Adam and Geracent, 1991; Xavier et al., 2009) was used as a nuclear localization signal and suppose to nuclear addressing of the peptide. It has a short positive sequence which not only use as a nuclear localization signal but in some studies play a role in condensing with DNA (Morris et al., 1997).

In this study, above-mentioned peptides were used to design a single chain recombinant peptide as a fusion vector (Fig. 1). Different ratios of the vector with pGL3 plasmid were prepared as nanoparticles and their abilities in transferring genes into mammalian cells were evaluated.

2. Material and methods

2.1. Materials

Escherichia coli BL21 (DE3) pLysS was obtained from Invitrogen, pET21b vector (Novagen), fetal bovine serum (FBS), Dulbecco's modified Eagle's medium (DMEM), purchased from Gibco, chloroquine and MTT 3-(4,5-s-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide were obtained from Sigma–Aldrich, restriction enzymes were purchased from fermentase, plasmid extraction kit and Ni-NTA from Qiagen. High molecular weight (25 000 Da), anhydrous branched PEI were obtained from Sigma–Aldrich.

2.2. Methods

2.2.1. Synthesis and cloning of the fusion peptide

The gene encoding peptide was designed and synthesized by Eurofins MWG Operon Germany with *NdeI* in N-terminal and *Xho1* in C-terminal which was provided in pCR®2.1. The synthesized gene was double digested with *NdeI* and *Xho1* restriction enzymes and cloned into a pET21b expression vector. Vector containing the desired fusion peptide was sequenced using an automatic sequencer (MWG) by T7 promoter and T7 terminator universal primers.

2.2.2. Over expression and purification of the recombinant peptide

The expression vector was transformed into *E. coli* BL21 (DE3) pLysS (Novagen). 5 ml of LB medium containing 100 μg/ml ampicillin and 34 μg/ml chloramphenicol was inoculated by a fresh bacterial colony harboring the expression plasmid and grown at 37 °C overnight. 250 μl of pre-cultured bacteria was used to inoculate 250 ml TB medium and grown at 37 °C with vigorous shaking until the OD_{600} reached 0.6. The mixture was induced by IPTG to a final concentration of 1 mM and incubated at 30 °C for 4 h in 250 rpm shaking. The cells were harvested by centrifugation at 3500 rpm for 20 min at 4 °C. The cell pellet was resuspended in lysis buffer [100 mM NaH_2Po_4, 500 mM NaCl, 8 M urea, 5 mM imidazol, and 1 mM PMSF; (pH 11)] and incubated at room temperature for 1 h with rotational shaking. Centrifugation was done in 13 000 rpm at 4 °C for 40 min to pellet insoluble fractions. The supernatant was used for peptide purification. Binding was performed in the batch form with 0.5 ml Ni-NTA resin while incubating at room temperature with rotational shaking. After 2 h the mixture was loaded into the column and washed by 50 ml wash buffer with increasing imidazol concentration [100 mM NaH_2Po_4, 1000 mM NaCl, 7 M urea, 20 mM, 40 mM, 50 mM imidazol, 1 mM PMSF; (pH 8)]. The desired peptide was eluted from the column by elution buffer [100 mM NaH2Po4, 250 mM NaCl, 5 M urea, 250 mM imidazol, 1 mM PMSF; (pH 8)]. The final yield was typically 2 mg from a 1000 ml culture where the peptide was greater than 95% homogeneous, as determined by 15% sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS-PAGE). Western blotting of this purified peptide was carried out using monoclonal anti His-tag antibody against the 6XHis-tag of fusion peptide followed by binding to the secondary antibody.

Purified peptide was desalted as well as buffer exchanged by centriplus filter (Millipore YM-3 MW cut-off: 3000D), in this case fractions with purified peptide was pooled, HEPES buffer (pH 7.4) added and centrifugation was done in 3500 rpm. Peptide stock was prepared for further studies.

2.2.3. Gel retardation assay

DNA-binding ability is a prerequisite for an ideal gene delivery vector. To confirm whether the fusion peptide can condense DNA, 1 μg plasmid harboring the gene encoding luciferase was mixed with increasing amounts of the fusion peptide in several charge ratios (nitrogen to phosphate (N/P) ratio) and incubated at room temperature for 30 min. 1% Agarose gel was used to investigate the electrophoretic mobility of each complex followed by visualization by UV illumination (UV tech, Germany).

2.2.4. Serum stability assay

Without adequate protection, therapeutic DNA may not survive its journey from the site of administration to its site of action. Serum has a very high protein concentration, and some of those serum components could interact with the complexes to change their ability to protect DNA from degradation so analyzing their ability to prevent digestion of the DNA by serum nucleases are essential. In the present study we tested the serum stability of peptide/DNA by exposure of the complex to 10% serum at final concentration. The complex (N/P:12) was incubated for 60 min at 37 °C and visualized by ethidium bromide staining followed by 1% agarose gel. To dissociate plasmid from peptide 10% SDS was used and incubated overnight at 60 °C and plasmid DNA was recovered by phenol/chloroform and precipitated by ethanol and checked by agarose gel. A naked DNA control was included in the test to ensure that serum nuclease activity was effective.

2.2.5. Particle characterization

Zeta potential and size of particles play key roles in transfection efficiency. So it is important to know about particle characteristics. The average particle size and the particle size distribution of the complexes were determined by dynamic light scattering using photon correlation spectroscopy. Zeta potential is normally used for surface charge determination. Zetasizer Nano ZS instrument (Malvern Instruments, UK) was used to measure both particle size and zeta potential at 25 °C. In order to do these measurements different N/P ratios of pDNA (1 μg) and fusion peptide were prepared as described previously and measurements were done in the final volume of 100 μl deionized water.

2.2.6. Cytotoxicity assay

CHO cells were seeded in 96-well plates for 5000 cell per well and incubated for 24 h at 37 °C in humidified incubator with 5% CO_2 atmosphere. Different concentration of fusion peptide was added to the cells and left for 48 h without exchanging the media. In the

parallel assay different N/P ratios prepared and added to the cells, after 4 h the media was exchanged with fresh media. The control well was without treatment and PEI was used as a positive control for comparison. Cell viability was assayed using MTT after 48 h. Briefly, 10 μl MTT (5 mg/ml) was added to each well and the cells were incubated at 37 °C for 4 h. The formazan product was dissolved in 100 ml dimethyl sulfoxide (DMSO). Color intensity was measured using a micro plate reader (ELx800, Biotek, USA) at 570 nm.

2.2.7. Hemolysis assay

Hemolysis due to exposure of red blood cell with membrane destabilization agents is used to show the endosomal escape of a system (Plank et al., 1994). In the present study hemolysis assay was used to investigate the fusogenic peptide H5WYG's capability to disrupt the membrane in different pH. Briefly whole human blood was collected in EDTA-containing vacutainers and the red blood cells (RBCs) were separated from the plasma by 2 min centrifugation in 13 000 rpm. RBCs were washed three times with phosphate buffer saline (PBS). After the final washing, the RBCs were resuspended and 10 times diluted in PBS at pH 7.4 and 5.5 which yielded a solution with 10^8 RBCs per 200 μl. This solution adjusted to 1 ml with PBS at appropriate pH. Different concentrations of fusion peptide added to the solution and then incubated for 1 h at 37 °C. The tubes were centrifuged for 5 min at 13 000 rpm and the absorbance of the supernatant was measured at 450 nm. 1% TritonX-100 was used as the positive control, and buffer alone at the appropriate pH was used as the negative control.

2.2.8. Transfection assay

CHO (Chinese hamster ovary) cells were cultured in DMEM, high glucose, supplemented with 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin in humidified atmosphere of 5% CO_2 and 37 °C. Cells were seeded in a 24-well cell culture plate for transfection studies.

CHO cells were seeded in the density of 2×10^5 per each well and in the time of transfection they were 80% confluent. Transfection was done in the absence of serum. In this case 4 h before transfection the cells media were changed with the DMEM media and various N/P ratios of nanoparticles were added to investigate transfection efficiency. After 4 h the media completely removed and cells were washed with PBS and complete media supplemented with10% FBS was added to the cells and incubated for another 48 h in humidified atmosphere of 5% CO_2 and 37 °C. PEI was used as the positive control. Transfection procedure is similar to reported procedure for similar compounds (Choosakoonkriang et al., 2003). In another study chloroquine was used at the time of transfection. In this case 100 μM chloroquine was added to the cells just before adding nanoparticles.

In this assay firefly luciferase in pGL3 plasmid was used as a marker gene. The backbone of the pGL3 luciferase reporter vectors contains a modified coding region for firefly (*Photinus pyralis*) luciferase that has been optimized for monitoring transcriptional activity in transfected eukaryotic cells. It was chosen because the assay of this genetic reporter is rapid, sensitive and quantitative. The luciferase activity (indicator of transfection) was measured according to a procedure as reported earlier (Nazari and Hosseinkhani, 2011). To analyze luciferase activity, the transfected cells in each well were gently washed twice with PBS and the cells were lysed with 100 μl of cell culture lysis buffer (Promega, WI, USA). The luciferase activity in each sample was indicated as the relative light unit (RLU/sec) in presence of luciferin, ATP and Mg^{2+} as the firefly luciferase substrates with a luminometer (Berthold detection systems, GmbH).

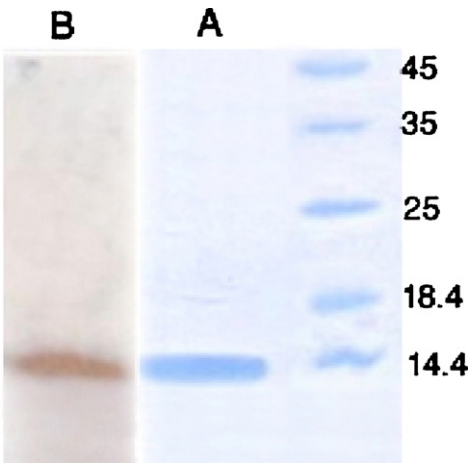

Fig. 2. (A) The coomassie blue stained purified peptide in 15% SDS-PAGE with affected molecular weight of 14 kDa; (B) western blot with anti-6XHis antibody against His-tag of the fusion peptide.

3. Results and discussion

3.1. Cloning and peptide expression

Structure of the fused peptide and amino acid sequences of each domain are shown schematically in Fig. 1. As shown in the scheme, His-tag is in the C-terminal and the whole construct is under the T7 promoter control. After the sequence of the plasmid which encoding the fusion peptide was confirmed, it was expressed in *E. coli* Bl21(DE3) pLysS and was purified using Ni-NTA resin under denaturing condition as described in Section 2.2.2. The purity of the peptide was more than 95% as confirmed by SDS-PAGE 15% (Fig. 2A). Although the calculated molecular weight of the peptide was 8425 Da, a single band around 14 kD was observed which is higher than the expected MW. This noticeable increase in molecular weight is related to the high density of the positive charge on the peptide due to several lysine in the sequence as the same effect has been shown in some other studies (Becker-Hapak et al., 2001; Rajagopalan et al., 2007). In this case charge neutralization by SDS is not sufficient so peptide movement is partially retarded. Anti His-tag antibody was used to confirm the presence of the His-tag in the peptide and a single band in the appropriate region was detected using the secondary antibody (Fig. 2B).

3.2. Investigation of DNA binding capability by agarose gel

The ability of recombinant peptide to bind plasmid DNA is the first step to investigate the gene delivery capability. In this case gel retardation assay was performed to show the mobility of the plasmid. Fusion peptide which dissolved in HEPES buffer pH 7.4 was mixed in different molar ratios with pGL3 plasmid which encodes firefly luciferase. N/P ratio was calculated according to number of basic amino acids (positively R-group) in the sequence which can neutralize 1 μg of plasmid DNA. As shown in Fig. 3, with increasing the N/P ratio the plasmid mobility in agarose gel was retarded and it was stopped completely in N/P above 8.0. As the molar ratio of peptide (N/P ratio) increased up to 14 and 16 accessibility of plasmid to ethydium bromide was limited and less DNA can be observed in the related wells. In this experiment the naked plasmid DNA was used as a control.

3.3. Stability of nanoparticles against serum nucleases

Mobility of the plasmids on 1% agarose gel was used as an indicator of stability of peptide/plasmid complexes in 10% serum. N/P

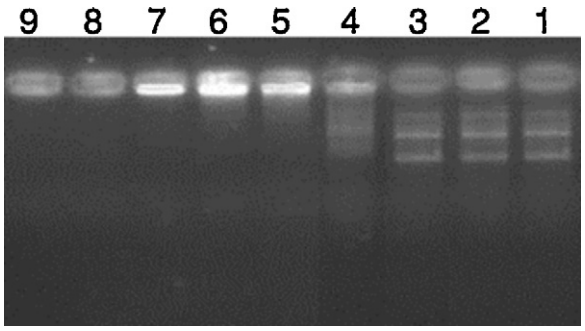

Fig. 3. Agarose gel electrophoresis (1%) indicates the mobility pattern of different N/P ratios of fusion peptide and pGL3 plasmid DNA; lane 1: naked plasmid, lane 2: N/P = 2, lane 3: N/P = 4, lane 4: N/P = 6, lane 5: N/P = 8, lane 6: N/P = 10, lane 7: N/P = 12, lane 8: N/P = 14, lane 9: N/P = 16.

12 was chosen in this assay, because not only it has proper particle characteristics and transfection but also it was clearly visible in agarose gel in comparison with upper N/P ratios (Fig. 3). Results showed that produced nanoparticles retarded the plasmid movement on the agarose gel in the presence of serum as well as in the absence of serum. On the other hand, plasmid DNA is completely protected against serum nucleases and the recovered plasmid DNA remained intact (Fig. 4). Therefore, it may be suggested the produced nanoparticles can remain intact *in vivo* condition. However, further animal experiments will be required to support stability of the nanoparticles under *in vivo* conditions.

3.4. Evaluation of particles characteristics

Particle parameters including size and surface properties influence the delivery processes in a complex manner. The size and the charge of particles made by cationic carriers/DNA are dependent on their ratio and needs to be optimized for each system separately. For tumor targeting, particles less than 250 nm (Yoo et al., 2011) are preferred owing to a higher probability of crossing the leaky endothelium. Furthermore particles larger than this are susceptible to elimination through the splenic filtration. So it is essential to have a particle with suitable size. On the other hand positively

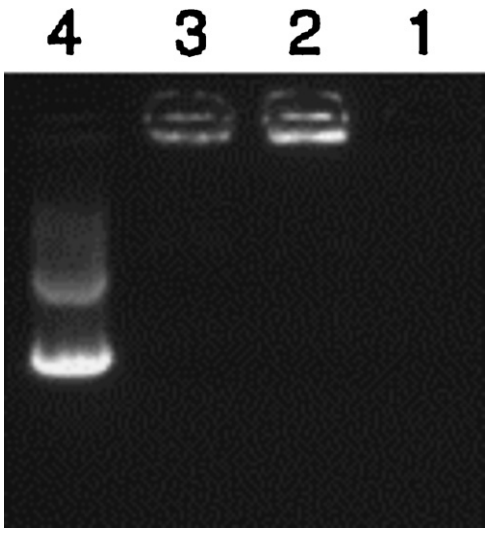

Fig. 4. Serum stability assay, lane 1: naked plasmid DNA in the presence of 10% serum which is completely degraded by the nucleases. lane 2: peptide/plasmid complex (N/P 12) in the presence of serum, lane 3: peptide/plasmid complex (N/P = 12) in the absence of serum, lane 4: recovered plasmid DNA from the complex. Samples were electrophoresed on 1% agarose gel in TAE buffer and visualized by staining with ethidium bromide.

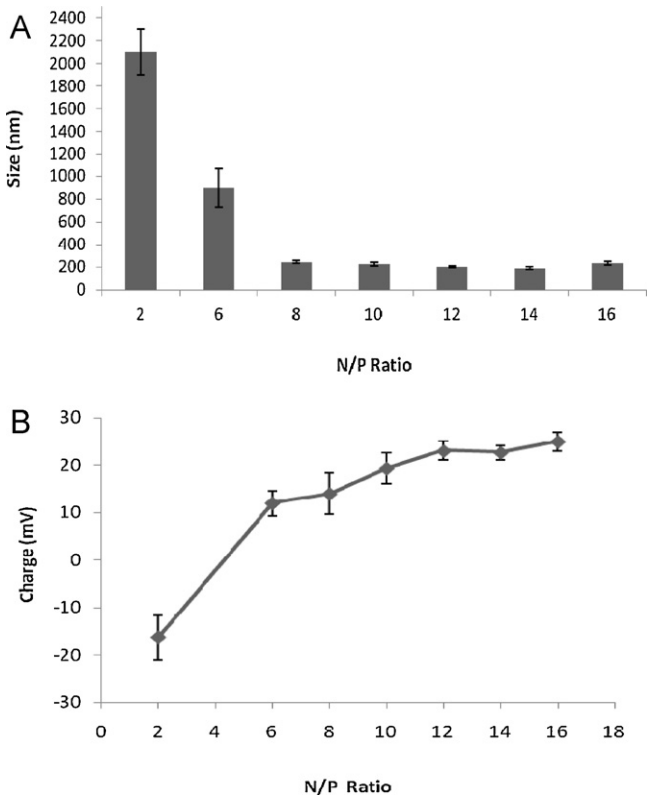

Fig. 5. (A) Size determination using dynamic light scattering for different N/P ratios of peptide/DNA complexes. (B) Measurement of zeta potential in different N/P ratios of peptide and plasmid DNA.

charged particles have a higher tendency to attach and internalize into the cells compared to negatively or neutrally charged particles due to negatively charged cell surface constituents. In respect with these knowledge, various N/P ratios of peptide and pGL3 plasmid DNA were prepared and the size and zeta potential of the complexes were measured. According to Fig. 5A increasing the ratio of the fusion peptide to plasmid DNA decreased nanoparticle sizes to about 200 nm. As we expected from the gel retardation results the low N/P ratios have very big sizes which show that peptide is not able to condense DNA completely. The same pattern was found for their zeta potential (Fig. 5B). Highest N/P ratios showed more positive zeta potential. All particles have shown suitable polydispersity about 0.2 or less (data not shown). According to particle characteristics, suitable nanoparticles were selected for transfection studies. It should be noted the size effect on cellular uptake of nanoparticles in receptor targeting complexes is more important as compared to non-targeting particles. This is due to electrostatic interactions between non-targeting complexes and the cell surface which may promote endocytosis by sedimentation for *in vitro* studies. So, based on this knowledge it can be concluded that decrease in nanoparticles size may not increase the transfection efficiency.

3.5. Evaluation of nanoparticles toxicity

One of the most challenging areas in designing of a vector is its toxicity. For example, PEI as the most known polymeric vector although has a good transfection efficiency but its use *in vivo* is limited because of its high toxicity. So it must be modified or replaced with other non-viral vectors which are nontoxic. In this study MTT assay was used to investigate the toxicity of the fusion peptide either in the form of nanoparticles with the plasmid DNA or by different concentrations of the peptide alone. CHO cells were incubated with various amounts of fusion peptide (12–21 μg) for

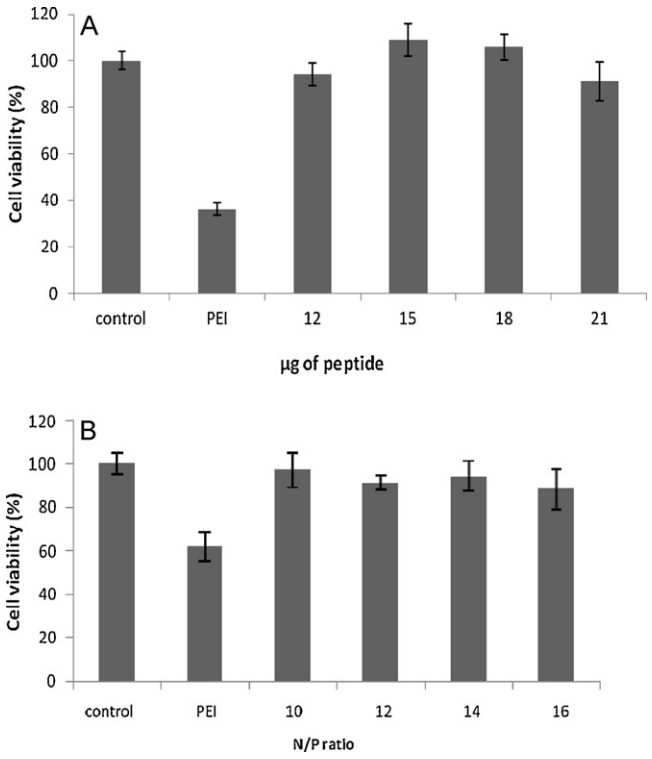

Fig. 6. (A) CHO cell viability was examined using MTT assay in the presence of various amounts of peptide after 48 h. (B) Different N/P ratios of peptide/plasmid prepared and cells were treated by them for 4 h. Media was changed after this time and cells were incubated at 37 °C for another 40 h and MTT assay was done to determine the percentage of viable cells. In both experiments we assume untreated cells as 100% viability and PEI as a negative control.

48 h to examine the toxicity of the peptide during longer exposure to the cells. Different N/P ratios from 10 to 16 were also used to study the nanoparticles toxicity. Fig. 6 indicates that no significant toxicity was observed, neither for fusion peptide nor for nanoparticles in comparison with untreated cells, PEI was used as a positive control. Results have shown even after 48 h treatment by the fusion peptide more than 85% of cells were alive but in the case of PEI the cell viability reduced to 30%. These results show that this fusion peptide can be used as a non-toxic gene carrier.

3.6. Hemolysis assay

As it is expected from the H5WYG, it could penetrate into the membrane and disrupt it at pH lower than 6. So if the fusion peptide is a membrane lytic, the RBC membrane will be disrupted and hemoglobin release into the solution. This hemolysis behavior can be corresponding to the endosome disruptive activity of the peptide. According to Fig. 7 fusion peptide disrupted 35% of the RBC membrane in the concentration of 20 μg at pH 5.5 but this effect was not observed when 5 μg of fusion peptide was used. At the pH of 7.4 this peptide was not disruptive and no significant hemolysis was detected. It can be concluded that H5WYG show a disruptive role at higher concentration so it can help the whole cargo to escape from the endosome when the pH is decreased in the late endosome. Moreover, as usually these kinds of vectors injected intravenously and hemolysis assay of this peptide was positive in acidic pH without apparent hemolysis effect at physiologic pH, it may be concluded, the first barrier (blood cells) against intravenous injection of those vectors is safe but it will not guarantee eventual use of the prepared samples *in vivo*.

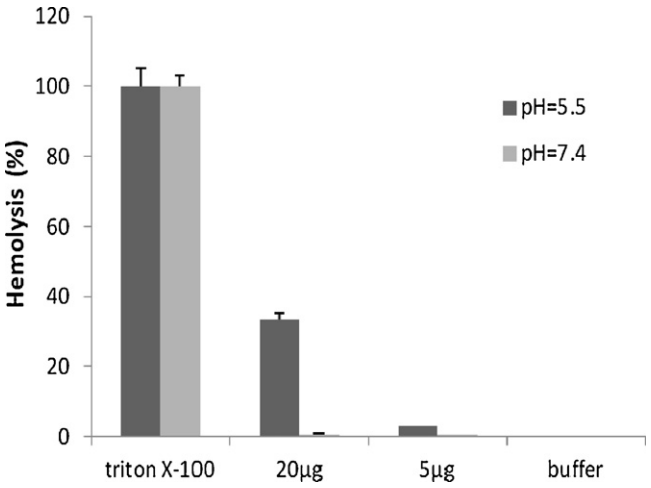

Fig. 7. Hemolysis assay, according to the figure the fusion peptide capable to disrupt the RBC membrane in higher concentration in pH 5.5 but in lower concentration this disruptive activity was not significant. In the physiological pH 7.4, fusion peptide cannot lyse the RBC membrane so no hemoglobin was released and the absorbance in 541 nm was almost zero. In this experiment triton-100 was used as a positive control and the lytic activity of peptide was compared to that. Buffer only was used as the negative control.

3.7. Cell transfection

According to results obtained from the gel retardation assays and particle characterization the best nanoparticles for transfection assays were selected from N/P 8 to 16. Luciferase activity was measured 48 h post-transfection, as detailed in Section 2. No significant difference was observed between 4 h and 6 h of incubation time, so we chose 4 h as incubation time for our transfection studies. Branch PEI (25 kD) with N/P ratio of 5 and naked plasmid DNA were used to compare the transfection efficiency of our new construct. It should be pointed out that maximum transfection efficiency for PEI seems to occur near N/P ratio 6 in CHO cell line (Choosakoonkriang et al., 2003). In our experiments N/P 5 is the most suitable ratio. So this ratio was used in transfection studies. As seen in Fig. 8, although all nanoparticles can efficiently transfect the CHO cells but the highest transfection efficiency was obtained with N/P 14. It indicates that using this ratio of fusion peptide to plasmid DNA can make the most suitable nanoparticles which can uptake by cells and overcome the cellular trafficking more efficiently. Almost no transfection was observed by cells treated with naked pGL3 plasmid.

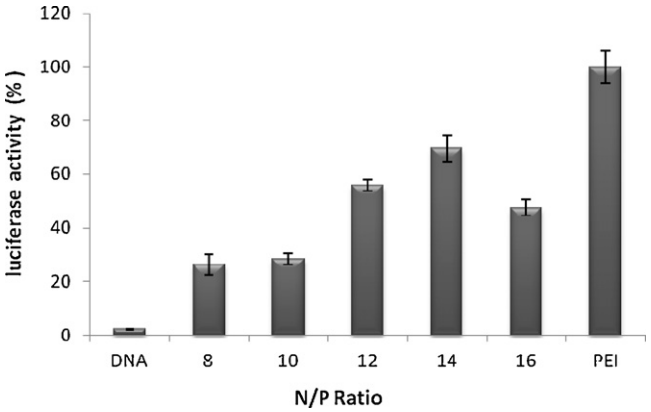

Fig. 8. Transfection of pGL3 plasmid using different N/P ratio of peptide/plasmid complexes. Luciferase activity is indicator of transfection efficiency. Transfection with different ratios of peptide/DNA clearly shows that all complexes can efficiently transfect the CHO cell lines and the most efficient N/P ratio was found to be 14 which has the highest transfection.

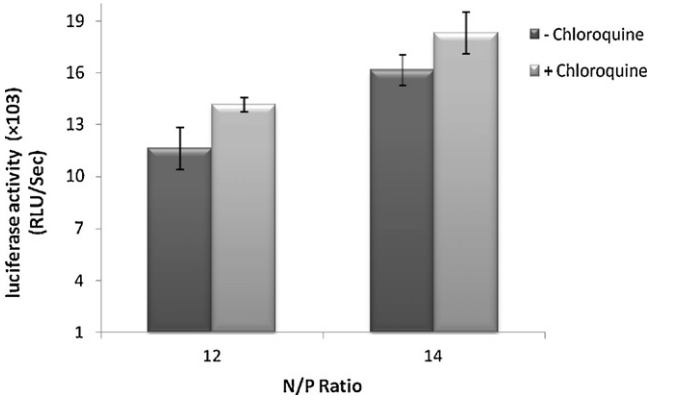

Fig. 9. Effect of chloroquine (100 μM) on transfection efficiency of two different N/P ratios of peptide/plasmid complexes. Transfection efficiency doesn't show big changes in the presence of chloroquine. Addition of 100 μM chloroquine in the time of transfection can increase transfection efficiency enormously, if the gene delivery vector is entrapped into the endosome and cannot release to the cytoplasm.

3.8. Effect of chloroquine on transfection efficiency

Several studies have shown that transfection efficiency can be improved significantly by adding lysosomotropic agents such as chloroquine. Chloroquine is an agent that neutralizes the lysosomal compartment and prevents the degradation of cargo by enzymatic reactions which are activated in the acidic conditions. H5WYG was used as a fusogenic peptide in our gene delivery construct and we expected that this peptide can help the whole nanoparticle to escape from the endosome to the cytoplasm. To investigate the capability of this domain, transfection assay was done in the presence of chloroquine in the final concentration of 100 μM.

As Fig. 9 indicates, slight increase was observed after adding chloroquine which demonstrates that the fusogenic peptide can efficiently penetrate the endosomal membrane and release the cargo to the cytoplasm.

4. Conclusion

The aim of this study was to achieve a safe and efficient vector for transferring the genes into eukaryotic cells. For designing this vector, the main bottlenecks in the cells which are the cell membrane and the endosome have been considered. Most of the time, overcoming these barriers can lead to high transfection efficiency. With this respect a fusion peptide including different domains for packaging DNA, endosomal release and nuclear targeting was developed. Recombinant DNA technology was used to prepare this peptide rather than solid phase peptide synthesis as it is easier and cost effective. Results from gel retardation assay and serum stability study show that the DNA binding motif of 16mer histone H1 not only can efficiently condense plasmid DNA into nanosized particle, but also completely protect plasmid DNA from nucleases attack and prevent degradation.

In order to overpass the endosome as the most important barrier in the cell after up taking the particles by endocytosis, we propose H5WYG as pH dependent fusogenic peptide. This peptide tends to change the conformation in low pH due to Histidine protonation and this new conformation can disrupt the endosome and release the contents into the cytoplasm. The efficiency of this domain was followed by the hemolysis assay and transfection in the presence of chloroquine. Both studies confirmed that H5WYG still retained the disruptive activity when used as a part of fusion peptide and finally transfection to the CHO cell line was done efficiently using fusion peptide. However, it may be suggested from size point of view, the current nanoparticle may be suitable candidate for gene delivery and tumor targeting.

In conclusion, according to the results present in this manuscript, it may be concluded a new multi-domain fusion peptide carrier based on biomimetic properties designed and were successfully used in cell transfection.

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