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Evaluation of CTAB coated gold nanoparticles as a potential carrier for gene delivery

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Abstract

Gold nanoparticles (AuNPs) exhibit a variety of attractive physical, chemical, optical, thermal, and biological properties, making them potential candidates for non-toxic drug and gene delivery carriers. The surface modifications of AuNPs vastly enhance their circulation, minimize aggregation rates, and increase their targeting capability. In this investigation, cetyltrimethylammonium (CTAB) coated AuNPs were prepared and characterized for potential application in gene delivery. This surface modification can lead to the improvement of dispersibility and stability in aqueous solution, and surface charge density. In this study, CTAB coated AuNPs were complexed with plasmid DNA (pUMVC3-hIL-12) via electrostatic interaction and resulted in the formation of nano-sized CTAB-AuNP/plasmid DNA complexes with the size of 84.7±9.8 nm. The zeta potential of these complexes was surface +4 mV at carrier: plasmid (C/P) ratio of 10. These complexes could condense the pDNA at C/P ratios of 8 and 10 and protect it against nuclease enzyme at C/P ratios of 4, 8, and 10. This study suggests that CTAB coated gold nanoparticles can be tested for potential applications in nucleic acid delivery.

Keywords: Gene delivery, gold nanoparticles, cetyl trimethyl ammonium bromide, pUMVC3-hIL-12.

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1. Introduction

Gene delivery has emerged as a promising technology for the treatment of diseases resulting from abnormal gene expression. It includes the delivery of exogenous nucleic acid materials to target cells with the different carrier such as viral and non-viral delivery systems. Viral vectors are highly efficient gene carriers with flexible chemistry. However, immune responses may occur after their administration leading to several concerns regarding their wide clinical application in humans. Non-viral vectors consist of various materials including polymeric systems, liposomes, ceramic particles, carbon nanotubes, and metal nanoparticles. These gene carriers have shown less immunological concerns rather than viral vectors. However, their transfection efficiency is lower than viral-based systems. Enormous studies are underway to improve their gene transfer efficiency through various types of modifications (1-3). Among various non-viral vectors, great attention has been directed

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to gold nanoparticles (AuNPs) (4). The system has been used for various purposes including photomedicine, targeted drug delivery, tissue engineering, biosensors, optical contrast agent, surface modification agent, as well as antibacterial and theranostic agents (5). The major advantages of AuNPs are stability, uniformity, and biocompatibility with a unique electronic structure (3). Since AuNPs can be prepared in a scalable manner with minimal size dispersion and in multifunctional monolayers, they have been suggested as a potential gene carrier. (6). The disadvantages of present technologies for gene delivery include the poor stability of traditional medications and genes in biological fluids, their enzymatic breakdown, and challenges in assuring their penetration through some barrier or nucleus of cells. The possibility of enhanced control and therapeutic efficacy is offered by loading gold nanoparticles with nucleic acid compounds (7).

Cetyltrimethylammonium bromide (CTAB) is a quaternary ammonium surfactant with positive charge density, CTAB coated AuNPs have several advantages such as improvement of dispersity and stability of AuNPs in aqueous solutions. On the other hand, CTAB may induce cell toxicity due to its positive charge (8, 9). CTAB is a hydrophilic macromolecule that non-covalently binds to the surface of gold nanoparticles and increases its solubility (9). Utilizing CTAB coating makes DNA more compact at the surface of gold nanoparticles (7). Hence, they facilitate endocytosis and prevent DNA destruction in the endosome owing to its high buffering capacity through proton sponge effect (10). It seems that the relatively high transfection efficiency of gold nanoparticles with CTAB coating is due to high amine density and buffering capacity of the carrier (10, 11). In the present study, CTAB coated gold nanoparticles were prepared and their biophysical characteristics was evaluated. Since we have been working on various non-viral carriers for efficient delivery of plasmid encoding IL-12 gene for local delivery of nucleic acids for melanoma therapy (12-14), we decided to evaluate the preliminary potential of CTAB-coated AuNPs for gene delivery. The results of this investigation are used for further evaluation of such system towards preparation of IL-12 plasmid delivery system for local gene therapy.

2. Materials and methods

2.1. Materials

CTAB coated gold nanoparticle purchased from Sigma-Aldrich (Munich, Germany), Plasmid pUMVC3-hIL-12 (human interleukin-12 under control of the cytomegalovirus enhancer/promoter) was obtained from Aldevron (Madison, Wisconsin). FavorPrep™ Plasmid extraction Maxi kit was purchased from FAVORGEN (National Biotechnology Park, Taiwan). KBC power load dye was obtained from Kawsar biotech company (Tehran, Iran), DNase I and DNA ladder 1kb were purchased from Cinnagen company (Tehran, Iran).

2.2. Transformation and amplification of pUMV3-hIL-12 plasmid

The plasmid pUMVC3-hIL12 was transformed into Escherichia coli bacteria strain DH5α. Then the plasmid was amplified in Luria-Bertani (LB) medium with ampicillin. The amplified plasmid was extracted by Favorprep™ maxi kit according to the manufacturer's instructions. The purity and concentration of the plasmid were measured by UV spectrophotometer.

2.3. Polyplexes formation

In order to form polyplexes, gold nanoparticles were prepared at different concentrations in HBG buffer (HEPES-buffered-glucose solution; 20 mM HEPES, 5% glucose, pH 7.2). Gold nanoparticles were mixed with the same volume of plasmid solution (40 µg/ml), then the mixture was incubated for 20-30 min at room temperature. The polyplex composition is defined based on C/P (w/w) ratio, in which C demonstrates the weight of carrier (gold nanoparticle) and P represents the weight of pDNA.

2.4. Gel retardation assay

In order to demonstrate the condensation of plasmid with gold nanoparticle, 20 µl of each polyplex solution with C/P ratios ranging from 0.5, 2 and 4 were mixed with 4 µl KBC power load dye and the mixtures were loaded on the 1% agarose gel (w/v). Electrophoresis was run for 1 h at 50 V and the location of bands was visualized with UV

illuminator.

2.5. Resistance of pDNA against nuclease degradation

DNase I was utilized for evaluating the protection effect of gold nanoparticles against enzymatic degradation. Briefly, polyplexes were prepared at different C/P ratios (0, 0.5, 2, 4, 8, and 10) as described earlier. Then, DNase I was added to polyplex solution. The same formulations were treated with PBS (as negative control) and incubated at 37oC for 30 min. In order to inactivation of DNase I, EDTA (100 mM) was added to the samples. Finally, sodium dodecyl sulfate (SDS) in NaOH (1M) was added and mixed to separate the plasmids from the polyplexes. Then, the samples were run on agarose gel for 1 h at 50 V. The location of plasmid bands was visualized using UV illuminator.

2.6. Particle size and surface charge density measurement

Particle size and zeta potential were measured by dynamic light scattering (DLS) and Laser Doppler Velocimetry (LDV), respectively. DLS measurements were carried out using a Nanotrack particle size analyzer and LDV measurements were done by Microtrack Zeta Check@ equipment HoribaSz-100 (Japan). Desirable amounts of gold nanoparticles were dissolved in 125 µl HBG buffer and added to the equal volume of HBG buffer containing the plasmid. These measurements were carried out at C/P ratio of 10, and the results were reported as mean ± SD (n=3).

3. Results and discussion

3.1. pDNA condensation ability

One of the main factors for successful gene delivery by the polycationic compounds is the ability of these positively charged materials in condensation of pDNA and formation of nanostructures (15). The formation of CTAB-AuNPs/pDNA complexes is the result of electrostatic interaction of surface positive charge of CTAB AuNPs with negatively charged pDNA phosphate backbone. (4). Gel retardation assay was utilized for evaluating the ability of CTAB AuNPs to condense the pDNA. The mobility of pDNA in agarose gel is an indicator of the strength of electrostatic interaction between the carrier and pDNA. The results of gel retardation assay (Figure 1.) demonstrated that CTAB-AuNPs couldn't condense the plasmid at C/P ratios of 0, 0.5, 2, and 4 while at higher C/P ratios of 8 and 10 full condensation was occurred. With increasing the C/P ratio, more positive charge was provided and subsequently higher condensation ability for CTAB-AuNPs was observed. The effect of the formation of loose or tight complexes has been discussed in several literatures. There are some reports indicating that the formation of tight complexes outside the cells is a prerequisite step for successful gene delivery. These tight complexes are able to protect nucleic acid materials and form smaller nanoparticles. On the other hand, there are some investigations demonstrating the impact of loose complexes on transfection efficiency. The higher transfection efficiency by loose complexes could be the result of efficient vector unpackaging inside the cell nucleus. Since the expression of genes needs the dissociation of plasmid from its carrier, loose complexes may provide easier access to the transcriptional machinery of the cell. Altogether, the association of plasmid and carrier outside the cell is crucial to form nano-sized complexes while the dissociation of plasmid from car-

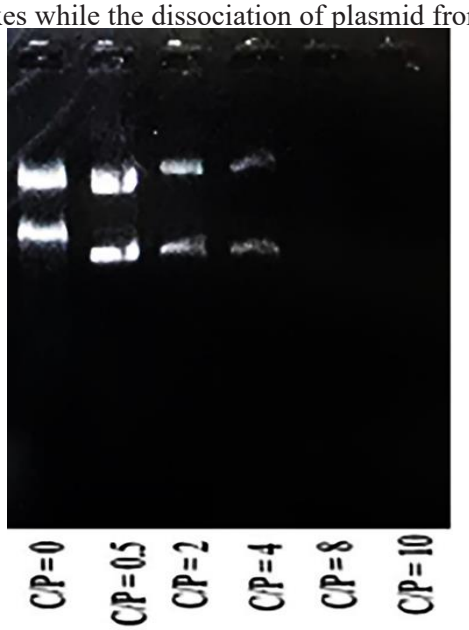

Figure 1. Gel retardation assay. pDNA condensation by CTAB-AuNPs evaluated by gel retardation assay at various C/P ratios ranging from 0 to 10.

rier provide opportunity for efficient transcription of the transgene (16).

3.2. Protection of plasmid against DNase I digestion

The condensation of plasmid with CTAB-AuNPs not only forms nanoparticles with favorable charge density but also protects the plasmid from enzymatic degradation. In order to demonstrate the protective effect of CTAB-AuNPs against enzymatic digestion, DNase I was utilized as a model. Agarose gel electrophoresis demonstrated the protective effects of CTAB-AuNPs against nuclease digestion. As illustrated in Figure 2. all the complexes at all C/P ratios remained intact without the enzyme treatment, while the naked pDNA was completely digested by the nuclease enzyme. According to our results, the protection effect was significantly increased with higher C/P ratios. CTAB-AuNPs could protect the plasmid from digestion at C/P ratios of 4, 8, and 10 whereas enzymatic digestion was occurred at C/P ratios of 0.5 and 2. Although, there are some investigations showing that higher condensation ability and surface charge density do not necessarily lead to more protective effect against nuclease digestion (17, 18). According to several studies, the binding affinity of polycationic structure to plasmid cannot necessarily lead to efficient gene delivery. Loose binding affinity may lead to digestion and destruction of the nucleic acids by enzymes present in the cellular matrix microenvironment, resulting in lower gene transfer efficiency. On the other hand, strong binding may lead to weak dissociation of the nucleic acid payload from its carrier at the target site leading to low gene delivery (13, 19) . A full condensation would not always provide significant protection against degrading enzymes. This behavior could be associated with the others polyplexes physicochemical features, such as particle shape, size, and charge. These properties are likely to lead to the creation of nanoparticles with plasmid parts on their surface areas, increasing the sensitivity of exposed pDNA segments to nuclease

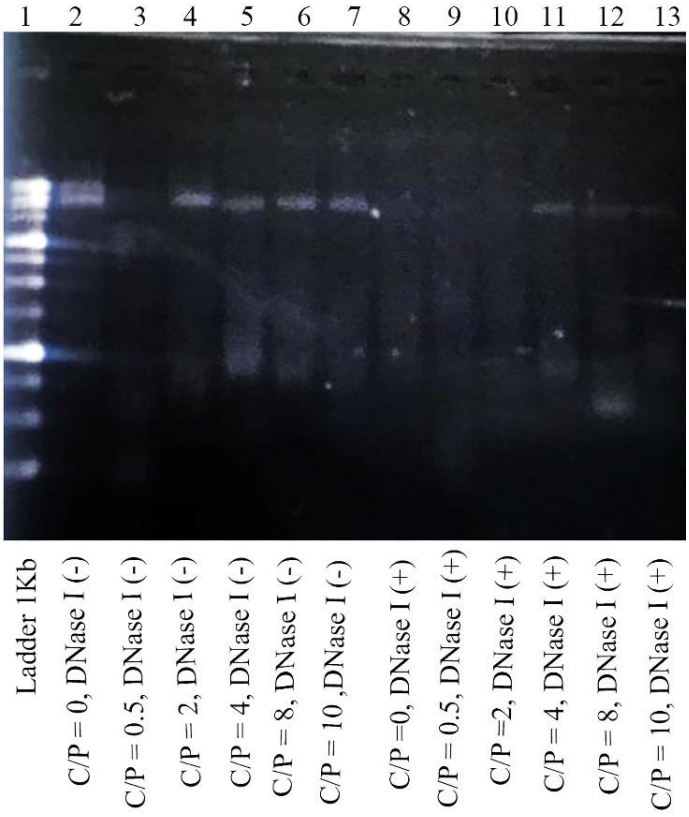

Figure 2. Protection against DNase I digestion. Polyplexes at various C/P ratios were tested with and without DNase I, to assess the protection of plasmid.

digestion (20).

3.3. Measurement of particle size and zeta potential

One of the critical characteristics of complexes for effective gene delivery is their size and surface charge density. These two parameters are determining factors for biodistribution in the body and the mechanisms of cell entrance (21, 22). The particle size and zeta potential of CTAB-AuNPs and its complex with pDNA at C/P of 10 were measured. As demonstrated in Figure 3, the particle size of CTAB-AuNPs was 10±4 nm while the size of its complexes with pDNA at C/P ratio of 10 was 84.7±9.8 nm. The zeta potential of CTAB-AuNPs was +30 mV, while it was reduced to +4 mV following the formation of complex with pDNA at C/P ratio of 10. This reduction of zeta potential is the result of the interaction between the positive charge of CTAB-AuNPs and the negative charge of the phosphate backbone of pDNA. The neutralization of the surface positive charge of CTAB-AuNPs leads to the formation of larger complexes due to the weaker repulsion between complexes. It has been reported that endocytosis in many mammalian cells is limited to particles smaller than 150 nm. Generally, the size of 50-100 nm is the optimal size range for the polyplex to reach the cells (9).

The particle size of the complex has a critical role in gene delivery (23). According to previous studies, the particle size of gold nanoparticles without coating has been estimated to be around 89 nm (24). According to a study on the effect of coating of different materials on the properties of gold nanoparticles (2011), it was shown that gold nanoparticles coated with citrate, Arabic gum, and starch have particle sizes of 20, 23, and 3 nm, respectively (24). Porcaro *et al.* (2016) showed that the size of glucose-coated gold nanoparticles is about 35-40 nm (25). In another study, the particle size of the citrate-coated gold nanoparticles complex was 15.5 nm (26). In a study carried out by Zhang *et al*., (2018) using cystamine-coated gold nanoparticles, the particle size of the complex was estimated around 13 nm (27). Hameed *et al.* (2020) conducted a study on the effect of different amino acid coatings on gold nanoparticles for drug delivery (28). The result of this study showed the particle size of complexes following the coating with tyrosine, tryptophan, and cysteine was 27.2, 14.6, and 8.6 nm, respectively. In the present study, particle sizes at two ratios of 8 and 10 were not significantly different and both ratios were in the optimal size range for gene delivery purposes.

The surface charge of the gene delivery system is another crucial factor that affects tissue distribution and efficient gene delivery. According to previous studies, the ideal surface charge density for transferring polyplex into the cell is in the range of ±10 mV (28). In a study conducted by Kumar *et al.* (2015, they found that the surface charge of hyaluronic acid co-functionalized gold nanoparticle complex was -33.2 mV (29). In another study, it was reported that the glucose-coated gold nanoparticles complex, had a surface

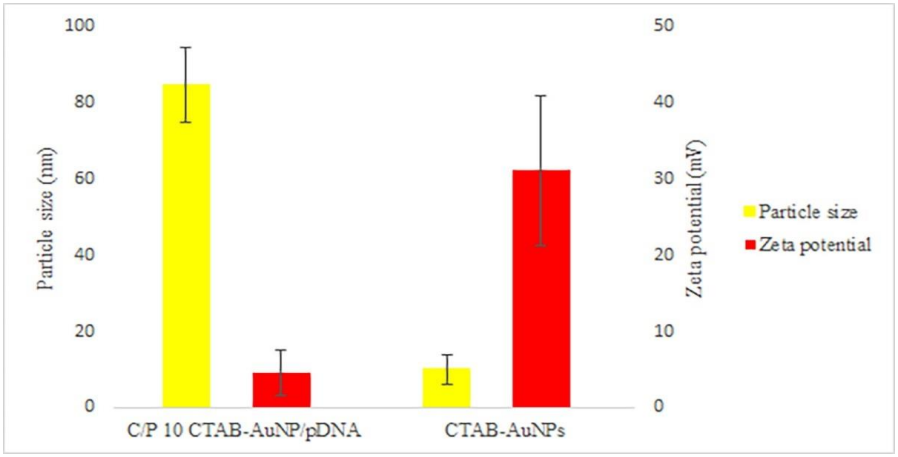

Figure 3. Particle size and zeta potential measurement. Size and zeta potential of CTAB-AuNP and its complex with pDNA at C/P ratio of 10 were assessed in HBG buffer.

charge of +30 mV (Porcaro *et al.*, 2016) (25). Trigo *et al.* (2018) worked on the citrate-coated gold nanoparticles and the results showed that the complex had a zeta potential of -45.7 mV (26). In another study reported by Park *et al.* (2019), it was found that the zeta potential of this complex is -26.1 mV (30). According to our results, the electrostatic interaction between the CTAB coated AuNPs and pDNA resulted in the formation of nanoparticles with the zeta potential of around +4 mV. The positive surface charge can lead to toxic effects on the cells. It has been shown that the polyplexes with high positive charge density result in the induction of toxic effects on the cells by various mechanisms including apoptosis and necrosis (31). The reduction of positive surface charge has been suggested as an efficient strategy to decrease the toxic effects of polycationic compounds (32). In this study, the reduction of zeta potential following the formation of nanoparticles can be considered as a promising result which may lead to lower toxicity on the cells.

4. Conclusion

In this study CTAB coated AuNPs were prepared and characterized in terms of biophysical properties. The complexes showed suitable condensation ability and protection against nuclease digestion. Also, they were able to form nanoparticles at optimal size and charge. The results of current study are basis for further evaluation of this gene carrier candidate *in vitro* and *in vivo*.

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Conflict of Interest

None declared.

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