TRANSFORMATION STUDIES IN BACTERIAL SYSTEMS USING GOLD NANOPARTICLES

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[1,2,3]EQUAL CONTRIBUTION

1. INTRODUCTION

Nanotechnology has wide applications in biology and medicine. Nanoparticles can be made as carriers for delivering macro molecules such as DNA and proteins. Most DNA delivery systems operate at one of three general levels: DNA condensation and complexation, endocytosis, and nuclear targeting/ entry[1]. Gene delivery systems are broadly classified into two broad categories-Viral vectors and non-viral vectors. The synthetic properties of non-viral vectors gives them the advantage over viral vectors in the area of transgene insert capacity, cost, time, general safety, and delivery to cell lines grown in vitro [2-12].

Polycationic polymers modified silica nanoparticles (positively charged) with sizes of 10-100 nm are reported to efficiently transfer DNA in cultured cells. It is well known that gold nanoparticles are the most stable metal nanoparticles in solution as well as biocompatible to biological cell [13]. Conjugation of gold nanoparticles enhances polyethylenimine's transfer of plasmid DNA into mammalian cells. Branched polyethlyenimine (PEI) chains have been covalently attached to gold nanoparticles (GNPs), and the potency of the resulting PEI-GNPs conjugates as vectors for the delivery of plasmid DNA into monkey kidney cells in the presence of serum *in vitro* has been systematically investigated. The transfection efficiencies vary as a function of the PEI-gold molar ratio in the conjugates, with the best one being 12 times more potent than the unmodified polycation [14]. Rotello's group have also shown that monolayer protected gold nanoparticles could be used for transfection of mammalian cell [15].

In this paper we report that gold nanotriangles conjugated with DNA have improved efficiency of plasmid transfer as compared to spherical gold nanoparticles.

2. EXPERIMENTAL

I. Gold nanotriangles were biologically synthesized using lemongrass leaf as described elsewhere [17].

II. Tyrosine KOH reduced silver nanoparticles were synthesized as described earlier [16]. Porous gold nanoparticles were synthesized by trasmetallation reaction using silver nanoparticles.

III. Spherical gold nanoparticles were prepared by adding citric acid to boiling $HAuCl_4$ solution.

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IV. Gold nanotriangles were modified with ysine and incubated overnight at room temperature. UV-vis-NIR spectra studies were carried out for the purified gold nanotriangles and lysine modified gold nanotriangles.

V. For transformation studies, plasmid isolation was carried out by Miniprep method. Loading of plasmid into gold nanoparticles was done by incubating 10µl plasmid with 1 ml of lysine modified gold nanoparticles at room temperature, for 24 hours. Competent cells were prepared and studies were carried out to compare the efficiency of transformation between competent cells using only plasmid, plasmid bound spherical gold nanoparticles and plasmid bound porous gold nanoparticles. Samples were prepared for TEM and AFM by methods as described earlier [17].

3. RESULTS AND DISCUSSION

Figure 1 shows the representative TEM images of purified gold nanotriangles synthesized by the reaction of aqueous $AuCl_4^-$ ions by different amounts of lemongrass extract after 48 h of reaction. The purification of gold nanotriangles from spherical particles was done using centrifugation at 1000 rpm. TEM images show plane edged gold nanotriangles with an average size of gold nanotriangles is 500 nm. Spherical nanoparticles are not observed in the TEM analysis, indicating removal of spherical nanoparticles using centrifugation. The UV-vis-NIR spectrum of pure gold nanotriangles (curve 1, Figure 3) shows high intense longitudinal surface plasmon (SP) band in the NIR region of electromagnetic spectrum compared to transverse SP band at 540 nm in visible region. The high intense longitudinal SP band demonstrates the presence of only gold nanotriangles in solution, which is in good agreement with the TEM result.

Spherical gold nanoparticles were synthesized using sodium borohydride reduction methods [18]. TEM images show monodisperse spherical gold nanoparticles. The average size of gold nanoparticles is 20 nm. TEM images do not show aggregation of nanoparticles, indicating stability of gold nanoparticles in the solution.

The surface modification of nanoparticles was carried out using lysine. Biologically synthesized gold nanoparticles have a net negative charge

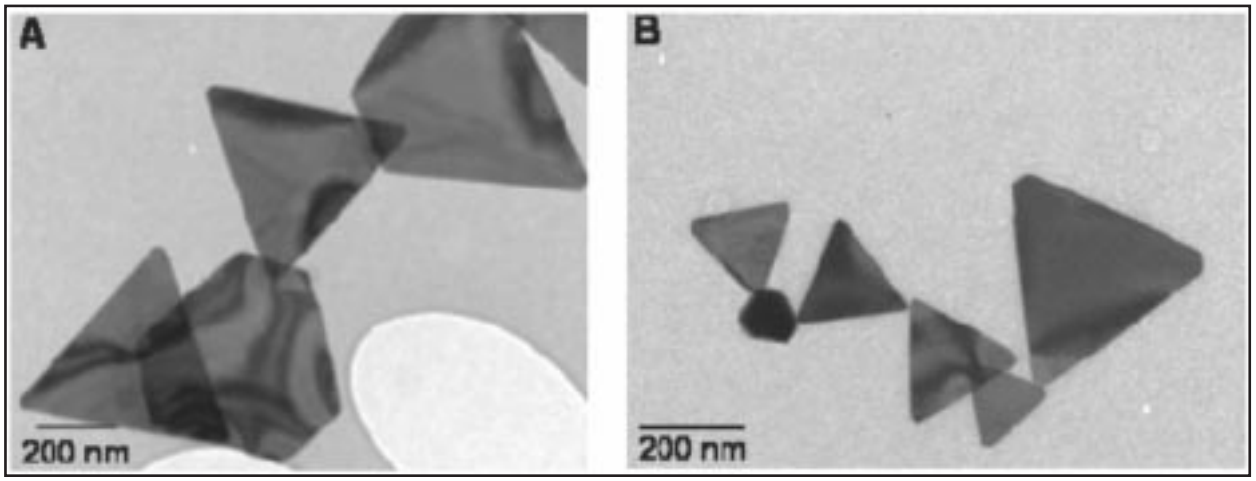

Figure 1: Representative TEM images of gold nanotriangles.

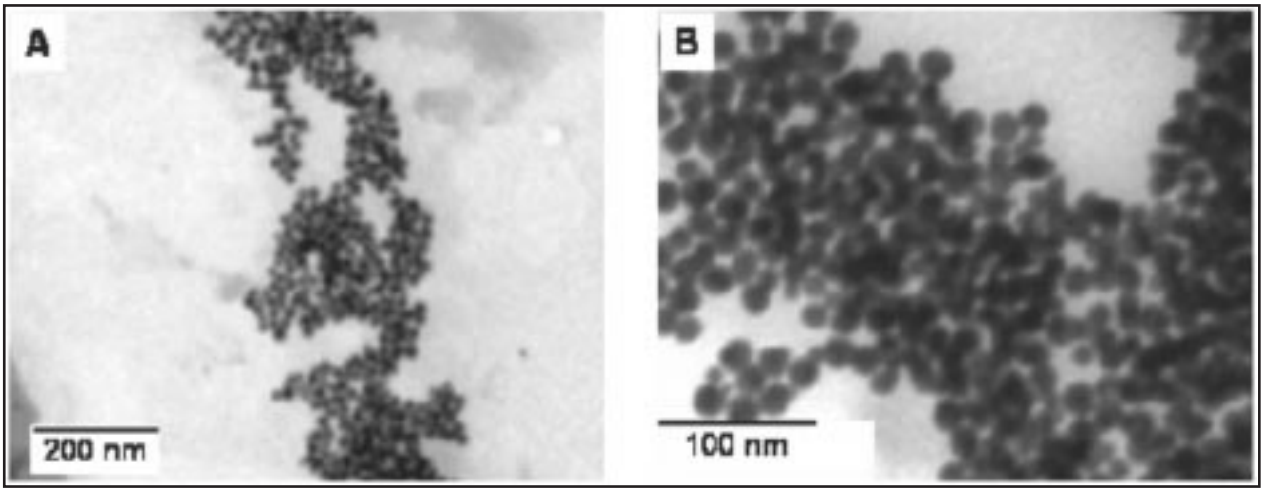

Figure 2: Representative TEM images of spherical gold nanoparticles.

because of the negatively charged biomolecules present on their surface. Similarly, chemically synthesized nanoparticles acquire their negative charge from the citric acid molecules present on their surface. Negatively charged DNA molecules and triangular gold nanoparticles repel each other so the surface of nanoparticles should be modified to positively charged for electrostatic interaction. A cationic amino acid lysine was used for surface modification of spherical and triangular gold nanoparticles. Curve 2, Figure 3 shows the UV-vis-NIR spectrum of lysine modified gold nanotriangles, which are similar to plane gold nanotriangles. This result indicates that surface modification of gold nanotriangles does not modify the morphology of gold nanotriangles.

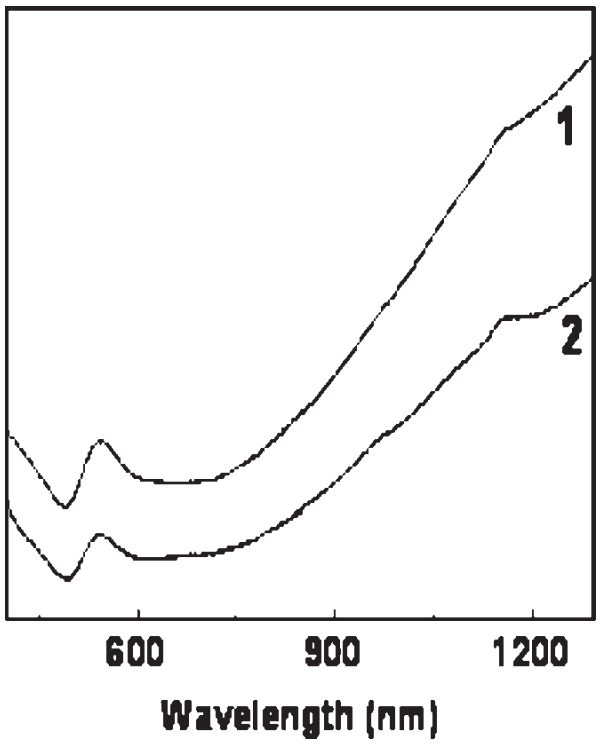

Figure 3: UV-vis-NIR spectra of purified gold nanotriangles (curve 1) and lysine modified gold nanotriangles (curve 2).

TRANSFORMATION

Standard protocols for preparation of competent cells and transformation using Ca and heat shock were used. Competent cells were tested for transformation and were found to give 80-90% transformation efficiency before carrying out experiments with nanoparticles.

Table 1

		Average± SD	Fold increase
1	Non-competent cells + plasmid	0	0
2	Competent cells + plasmid	$0.97 \times 10^3 \pm 147.90$	
3	Non-competent cells + nanotriangles attached plasmid	0	0
4	Competent cells + nanotriangles attached plasmid	$15.3 \times 10^3 \pm 1663.58$	15 - 17
5	Competent cells + Spherical gold nanoparticles attached plasmid	$8 \times 10^3 \pm 1.3$	7 - 9

Comparison of the transformation efficiency in competent and non-competent cells using plasmid bound to gold nanotriangles was done (Table 1). No transformants were observed on plates containing non-competent cells. This implies that competence is necessary for transformation. Transformation efficiency was tested with plasmids bound to gold nanotriangles, Spherical gold nanoparticles and free plasmids.

Competent cells transformed using plasmid bound to gold nanotriangles showed higher transformation efficiency as compared to that with competent cells transformed using only plasmid. The increase in efficiency was approximately 15 - 17 folds ± 2.23.

In similar studies done in mammalian cell cultures, it has been observed that mixed monolayer protected gold clusters (MMPCs) functionalized with quaternary ammonium chains efficiently transfect mammalian cell cultures, as determined through, â-galactosidase transfer and activity. The most efficient nanoparticles studied was ~8-fold more effective than 60 kDa polyethylenimine, a widely used transfection agent [15]. It has also been shown that branched polyethylenimine (PEI) chains with an average molecular mass of 2 kDa (PEI2) have been covalently attached to gold nanoparticles (GNPs), and the potency of the resulting PEI2-GNPs conjugates as the vectors for the delivery of the plasmid DNA into monkey kidney (COS-7) csells in the presence of serum in vitro has been systematically investigated. The best transfection efficiency was 12 times more potent than unmodified polycation [16].

CONCLUSIONS

We have demonstrated that gold triangular nanoparticles could serve as excellent carriers for plasmid DNA. This study has demonstrated considerable promise for the nanoparticle based gene delivery. However we need to test this delivery system in mammalian cell lines.

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