

# Layer-by-layer deposition of cationic polymers on gold nanoparticle for non-viral gene delivery system

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## ABSTRACT

In this study, gold nanoparticles displayed spherical shapes with an average size of 12 nm were synthesized. The nano-carriers for gene delivery were subsequently developed based on DNA adsorption on the gold/cationic polymer-scaffold. The multilayer nanoparticles of gold, chitosan derivative (MPyMeChC), polyethyleneimine (PEI) and DNA were generated via layer-by-layer (LbL) depositing on gold-scaffolds. The LbL of gold-based scaffold were prepared via electrostatic interaction yielding gold/MPyMeChC/DNA (G/C/D) and gold/MPyMeChC/DNA/PEI (G/C/D/P) complexes. Size and zeta potential of DNA-bound scaffolds were examined. The results indicated that the sizes of G/C/D and G/C/D/P were less than 400 nm with positively charged on their surfaces. Atomic force microscopy confirmed the condensation of plasmid DNA on gold/polymer based scaffolds. Transfection efficiency and cell viability assay were examined on HeLa human cell lines. The result revealed that transfection via G/C/D and G/C/D/P provided transfection efficiency approximately 10- and 10<sup>3</sup>- times higher than conventional C/D polyplex. However, the cell survival decreased slightly compared to C/D transfection system. Confocal fluorescent microscopy verified the presence of DNA in Hela cell after transfection though G/C/D and G/C/D/P. This study proposes the simple and practical approach for preparation a nano-carrier for gene delivery through layer-by-layer deposition of cationic polymers on gold nanoparticle.

**Keywords:** gold, chitosan derivative, gene delivery, non-viral vector,

## 1 INTRODUCTION

Recently, non-viral gene delivery has gained much attention as an alternative choice in gene delivery [1]. A

variety of synthetic non-viral gene carriers including cationic polymer, cationic lipid and lipid-polymer hybrid system have been developed [2, 3]. The technical aspects of each approach are being investigated to achieve optimal gene transfection efficiency. With the progressive advances in nanotechnology, gold nanoparticles have been intensively used for different biomedical applications [4-8]. However, there were very few studies conducted regarding to the use of gold, for a non-viral gene delivery purpose [9-11]. In this study, the negatively surface charged gold nanoparticles were synthesized to use as the nano-scaffolds of the gene carrier. The multilayer nanoparticles of gold, cationic polymers (PEI and/or MPyMeChC) and DNA were generated via layer-by-layer depositing on gold nano-scaffolds. The study examined particle size, surface charge, DNA-binding ability, cellular uptake, transfection efficiency and cytotoxicity in a human cervical cancer cell line (HeLa). This study proposes an alternative approach for preparation a carrier in gene delivery system via layer-by-layer deposition of cationic polymers on gold nanoparticle.

## 2 MATERIALS AND METHODS

### 2.1 chemicals

A methylated N-(4-pyridinylmethyl) chitosan chloride (MPyMeChC) was synthesized with a degree of quaternization of 80% and molecular weight of 12 kDa. MPyMeChC was modified to improve water solubility and gene transfection efficiency of original chitosan [12]. The branched polyethyleneimine (PEI; molecular weight of 25 kDa) and hydrogentetrachloroaurate(III)trihydrate (HAuCl<sub>4</sub>·3H<sub>2</sub>O) were purchased from Aldrich (Milwaukee, USA). Trisodium citrate was purchased from Merck (Damstadt, Germany). The Lipofectamine 2000™ was purchased from Invitrogen (San Diego, USA). Plasmid

pGL-3-basic containing CMV promoter/enhancer and luciferase gene marker was used to monitor transfection efficiency [13].

## 2.2 Synthesis and characterization of AuNPs

Gold nanoparticles were synthesized by citrate reduction method [14]. Briefly, a 2 mL of 1.0% trisodium citrate was added into 50 mL of distilled water and refluxed for 5 min. Then, 0.5 mL of 1.0% HAuCl<sub>4</sub> solution was added into a reaction under continuous stirring and kept boiling for another 15 min. The mixed solution was then cooled and diluted with distilled water to 50 mL. Finally, the nanoparticles were prepared at concentration of 0.8 mg/mL for the subsequent experiments.

## 2.3 Preparation of gold/cationic polymer/DNA complexes:

Complexes of Gold/MPyMeChC/DNA (G/C/D) were prepared by dissolving colloidal gold in deionized (DI) water, followed by adding MPyMeChC solution. The mixture was incubated at room temperature for 20 min. One microgram of DNA was added to the mixture and incubated for 15 min at room temperature to generate G/C/D complexes. For preparation of Gold/MPyMeChC/PEI/DNA (G/C/D/P) complexes, the PEI was subsequently added into the mixture of G/C and incubated for a further 20 min resulting in the coating of PEI on the gold nano-scaffold. Finally, 1 µg of DNA was added to complex. All complexes tested in this study were G/D, C/D, G/C/D, and G/C/D/P at weight ratio of 10/1, 5/1, 10/5/1 and 10/5/1/1, respectively.

## 2.4 Characterization

The Z-average hydrodynamic diameter, polydispersity index (PDI) and surface charge of complexes were determined by dynamic light scattering (DLS) and Zetasizer Nano ZS (Malvern Instruments Ltd., UK) at room temperature. The complexes were prepared and combined to achieve a volume of 1 mL using DI water. All samples were measured in triplicate. Morphology and particle size of colloidal gold were observed with a transmission electron microscope in both normal transmission and high-resolution modes (TEM and HRTEM, JEM-2010, JEOL, Japan) at an acceleration voltage of 200 kV. A drop of sample (0.1 mg/mL) was dried on formvar-coated copper grid. Determination of surface plasmon resonance absorption was performed using a UV-Vis spectrophotometer (Perkin-Elmer Lambda 650, USA).

## 2.5 Cell cultivation and in vitro transfection assay

Human cervix epithelial carcinoma cells (HeLa) were cultured in Dulbecco's modified Eagle's medium (DMEM,

Gibco-BRL, USA) supplemented with 10% Fetal Bovine Serum (FBS; HyClone, USA). HeLa cell line was incubated at 37 °C in a humidified atmosphere at 5% CO<sub>2</sub> for 24 h and periodically subcultured prior to transfection. For in vitro transfection, HeLa cells were seeded at densities of 3 X 10<sup>5</sup> cells/well in 96-well plates. Prior to transfection, the media was removed, cells were rinsed with PBS twice, plated and incubated with serum-free medium. Cells were incubated with complexes at 37 °C for 6 h in serum-free medium then replaced with growth medium. Twenty-four hours after transfection, luciferase activity was determined in accordance with the manufacturer's recommendation (Promega, USA). Luciferase activity was quantified as relative light units (RLU) using a luciferase assay system (Promega, USA). Luciferase activity was normalized for protein concentration using the Bradford assay. Lipofectamine 2000™ (Invitrogen, USA) was used as a control carrier for gene transfection.

## 2.6 Assay of cell viability

The MTT assay was performed to evaluate cell viability after treatment with complexes. All cells were seeded and transfected following identical conditions to the *in vitro* transfection assay. The assay was performed 24 h after transfection according to the manufacturer's recommendations. Percentage cell viability was related to control untreated cells.

## 2.7 Study of cellular uptake

Plasmid DNA, pGL-3-basic containing CMV promoter/enhancer, was labeled with RITC using the Mirus labeling kit (Mirus Bio Corporation, USA). The labeled-DNA were then formed complex yielding G/C/D, G/P/D and G/C/D/P. Transfection were performed on HeLa cells seeded at density of 5 X 10<sup>5</sup> cells on cover slip in 24-well plate. The transfected cells were stained with acridinine orange. After washing in PBS buffer, cells were visualized under the confocal laser scanning microscopy (Carl Zeiss, Inc., Germany).

# 3 RESULTS AND DISCUSSION

## 3.1 Synthesis of AuNPs

The transmission electron microscope (TEM) image of the gold nanoparticles is shown in Figure 1. The gold nanoparticles displayed a spherical shape with uniform distribution. Measuring the diameter of 50 randomly selected nanoparticles in enlarged TEM images resulted in the particle size distribution histogram shown in Fig. 1b. The size distribution was found to be narrow with an average size of 12 nm ( $\pm 0.95$ ). The hydrodynamic diameter of AuNPs measured by DLS technique was 24 nm with narrow size distribution (PDI 0.27) and their surface charge was negative (-34 mV) due to citrate ion on the surface. The

surface plasmon band, centering around 520 nm, indicated the formation of gold nanoparticles, Fig. 1c. The results revealed that our synthesized colloidal gold form well-defined nanoparticles with high monodispersity, thus being appropriate for DNA delivery.

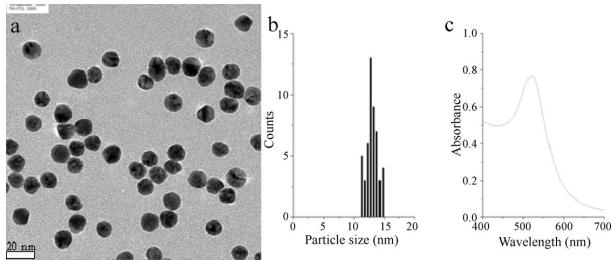


Figure 1: TEM analysis of gold nanoparticles, (a) particle size distribution histogram (b) and the surface plasmon band indicated the formation of gold nanoparticles (c).

### 3.2 Size and zeta potential analysis

The particle size and zeta-potential were determined at pH 7.4. The DNA at 1  $\mu$ g was constantly applied to form complexes with gold/cationic polymers. The C/D, G/C/D and G/C/D/P complex sizes analyzed by DLS were in the range of 310-380 nm (Figure 2). Their sizes were significantly larger than the colloidal gold nanoparticles. All complexes carried positive charges on their surfaces. This is additional evidence that shows the C/D, G/C/D and G/C/D/P are applicable for gene transfection.

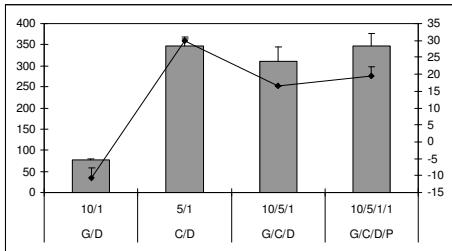


Figure 2: The size and zeta-potential of complexes

### 3.3 Intracellular delivery of gold-based complexes

Confocal microscopy verified the cellular uptake of DNA mediated gold/polymer nano-scaffolds. Gold nano-scaffold based complexes (G/C/D and G/C/D/P) were transfected into HeLa cell lines. At 4 and 24 h after transfection, the transfected cells were observed under confocal fluorescence microscope together control free cells (Figure 3). The image revealed that the complexes were found to be successfully delivered into HeLa cells.

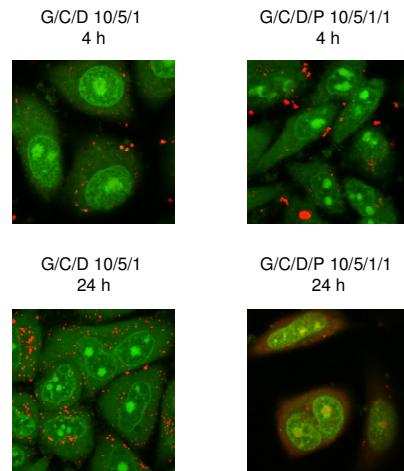


Figure 3: Confocal microscopy verified the cellular uptake of DNA mediated G/C/D and G/C/D/P at 4 and 24 h after transfection into HeLa cells.

### 3.4 Transfection efficiency

The complexes of G/P/D and G/C/D/P are an effective gene carrier providing high transfection efficiency, which is comparable to Lipofectamine 2000<sup>TM</sup> (Figure 4a). The data based on size analysis indicated that all complexes were not very much different; therefore, particle size probably was not the factor influencing differential transfection profiles via distinct types of complexes. The use of gold nanoparticle as scaffold in G/C/D and G/C/D/P complexes obviously enhanced transfection efficiency comparing with C/D polyplexes. Using colloidal gold as scaffold would improve the mono-dispersity of the gene carrier and finally facilitate gene internalization into the cells.

### 3.5 Cell viability assay

To assess the biocompatibility and cytotoxicity of the gold/polymer based transfection system, *in vitro* evaluation of the cytotoxicity was investigated using the MTT assay. HeLa cells were treated with different DNA carriers under the identical conditions to the transfection process. The result was shown in Figure 4b. The control was free cells which retained cell viability of 100%. Meanwhile, C/D polyplex and Lipofectamine 2000<sup>TM</sup> were relatively non-toxic since cell survival at 80% was evident. The viability of cells transfected with G/C/D/P was approximately 60% indicating that the cell biocompatibility was rather affected by the gold/polymer nano-scaffold transfection system. Although it is likely that using colloidal gold as a nano-scaffold may cause higher cell cytotoxicity than chitosan polyplex system, its cytotoxicity is relatively lower than PEI, another commercial cationic polymer commonly used in gene delivery. Gene transfection efficiency and cytotoxicity are the two most important factors that are required to be taken into account with regard to a gene delivery system [15-16]. Therefore, the use of gold as nano-

scaffold should be a compromise between transfection efficiency and cell toxicity.

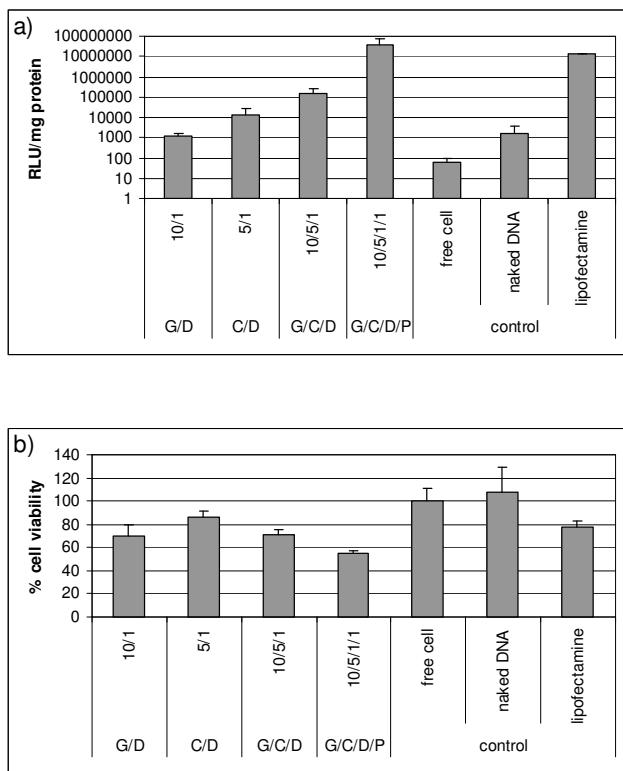


Figure 4: Transfection efficiencies (a) and cell viability assay of complexes (b) in HeLa cells. The studies were compared to non-transfected free cells, cell transfected with positive control Lipofectamine 2000™ (Invitrogen, USA) and negative control (naked DNA; plasmid pGL-3-basic containing CMV promoter/enhancer).

#### 4 CONCLUSIONS

In this study, colloidal gold was synthesized, and its physicochemical properties were examined. The well-defined gold nanoparticles were used as the nano-scaffold of the gene delivery vehicle. The gold/cationic polymer based nano-scaffolds was prepared using cationic polymer as an intermediate to adsorb DNA on nanoparticle surface yielding polyelectrolyte multilayer complexes. The chemical modification is not a required step in the complex preparation. The human cell line, HeLa, was selected to evaluate transfection efficiency. The obtained result demonstrated the potential of gold/cationic polymer-DNA (G/C/D/P) complexes in gene delivery applications. Compared with polyplex system, our system may facilitate DNA releasing at target cells resulting in higher transfection efficiency. This study purposed the alternative procedure, which is simple and practical for gene therapeutic approach.

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#### REFERENCES

- Wong, S. Y., Pelet, J. M., & Putnam, D. (2007). *Progress in Polymer Science*, 32, 799-837.
- Boas, U., & Heegaard P. M. H. (2004). *Chemical Society Reviews*, 33, 43-63.
- Pack, D. W., Hoffman, A.S., Pun, S., & Stayton, P.S. (2005). *Nature Reviews Drug Discovery*, 4, 581-593.
- Huang, X., El-Sayed, I. H., Oian, W., & El-Sayed, M.A. (2006). *Journal of the American Chemical Society*, 128, 2115-2120.
- Murphy, C. J., Gole, A. M., Stone, J. W., Sisco, P. N., Alkilany, A. M., Goldsmith, E. C., & Baxter, S. C. (2008). *Accounts of Chemical Research*, 41, 1721-1730.
- Tsai S-W., Chen Y-Y., & Liaw J-W. (2008). *Sensors*, 8, 2306-2316.
- Xia, F., Zuo, X., Yang, R., Xiao, Y., Kang, D., Vallée-Bélisle, A., Gong, X., Yuen, J. D., Hsu, B. B., Heeger, A. J., & Plaxco, K. W. (2010). *Proceedings of the National Academy of Sciences of the United States of America*, 107, 10837-10841.
- Xiulan S, Xiaolian Z, Jian T, Zhou J, & Chu FS. (2005). *International journal of food microbiology*, 99, 185-194.
- Noh, S. H., Kim, W-K., Kim, S. J., Kim, J. M., Baek, K-H., & Oh, Y-K. (2007). *Biochimica et Biophysica Acta*, 1770, 747-752.
- Zhang, Y., Liu, G., Hou, C., Chen, J., Wang, J., & Pan, Y. (2007). *Nanoscience*, 12, 49-53.
- Ow Sullivan, M. M., Green, J. J., & Przybycien, T. M. (2003). *Gene Therapy*, 10, 1882-1890.
- Sajomsang, W., Ruktanonchai, U., Gonil, P., Mayen, V., & Opanasopit, P. (2009). *Carbohydrate polymers*, 78, 743-752.
- Tencomnao, T., Rakkhitawatthana, V & Sukhontasing, K. (2008). *African Journal of Biotechnology*, 7, 2124-2127
- Jiang, Z-L, Zou, M-J, & Liang, A-H. (2008). *Clinica Chimica Acta*, 387, 24-30.
- Kraljevic S., & Pavelic K. (2005). *EMBO reports*, 8, 695-700.
- Shea, L. D., Smiley, E., Bonadio, J., & Mooney, D. J. (1999). *Nature Biotechnology*, 17, 551-554.