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Gold/cationic polymer nano-scaffolds mediated transfection for non-viral gene delivery system

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ABSTRACT

Gene delivery scaffolds based on DNA plasmid condensation with colloidal gold/cationic polymer were developed. The synthesized gold nanoparticles displayed spherical shape with an average size of 12 nm under observation with a transmission electron microscope. Gold/cationic polymer nano-scaffolds were formed though electrostatic interaction yielding gold/polyethyleneimine (PEI), gold/chitosan and gold/chitosan/PEI complexes. Luciferase-encoding plasmid DNA was subsequently added and adsorbed on the prepared scaffolds to be used as a non-viral gene carrier. Physicochemical properties of DNA-binding scaffolds were examined including size and zeta potential. The results indicated that the sizes of gold/polymer based scaffolds were generally less than 400 nm, and they carried positive charge on their surfaces. Gel retardation assay and atomic force microscopy confirmed the condensation of plasmid DNA on gold/polymer based scaffolds. Confocal fluorescent microscopy verified the presence of DNA in the cell using gold nano-scaffold as a carrier. Transfection efficiency assay using A549 and HeLa human cell lines revealed that gold/polymer based nano-scaffolds provided transfection efficiency approximately 10 times higher than polymeric-based gene carriers. This study proposed simple and practical approach, having the potential for use as an alternative gene carrier in non-viral gene delivery system.
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1. Introduction

Gene therapy is one of the most promising strategies for treating genetic defective diseases. Since the use of viral gene delivery system causes an increased risk of cytotoxicity and immune response, there is a need for a safer gene carrier. Recently, non-viral gene delivery has gained much attention as an alternative choice in gene delivery (Wong, Pelet, & Putnam, 2007). A variety of synthetic non-viral gene carriers including cationic polymer, cationic lipid and lipid–polymer hybrid system have been developed (Boas & Heegaard, 2004; Ewert, Evans, Bouxsein, & Safinya, 2006; Pack, Hoffman, Pun, & Stayton, 2005). 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 including sensing, photothermal therapy, tracking and drug delivery (Huang, El-Sayed, Qian, & El-Sayed, 2006; Murphy et al., 2008; Tsai, Chen, & Liaw, 2008; Xia et al., 2010; Xiulan, Xiaolian, Jian, Zhou, & Chu, 2005). However, there were very few studies conducted regarding to the use of gold, for a non-viral gene delivery purpose (Noh et al., 2007; Ow Sullivan, Green, & Przybycien, 2003; Zhang et al., 2007).

Here, we demonstrated the use of two different non-viral gene carriers in combination. The study investigated the roles of gold-bound cationic polymers for gene delivery into human cell lines. Gold nanoparticles have the advantages of easy preparation, and the possibility of chemical modification of surfaces for further targeting purposes (Gang, Martin, & Rotellao, 2006; Ow Sullivan et al., 2003). Furthermore, colloidal gold is considerably as a biocompatible material (Shukla et al., 2005). Meanwhile, cationic polymers such as polyethyleneimine (PEI) and chitosan derivatives have been developed so far as gene carrier molecules (Boussif et al., 1995; Lv, Zhang, Wang, Cui, & Yan, 2006). PEI is considered as the effective cationic polymer for gene delivery due to its buffering capacity via proton sponge effect leading to osmotic swelling, membrane disruption and eventually DNA escape. Chitosan is recognized as a biodegradable polymer with biocompatibility, low cytotoxicity and low immunogenicity. Therefore, chitosan deriva-

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tives have been continuously synthesized for many applications in medical uses, especially for regenerative medicines including gene therapy (Illum, 1998; Shi et al., 2006; Singla & Chawla, 2001; Varshosaz, 2007). Regarding our previous study (Sajomsang, Ruktanonchai, Gonil, Mayen, & Opanasopit, 2009; Sajomsang, Tantayanon, Tangpasuthadol, & Daly, 2008), a methylated *N*-(4-pyridinylmethyl) chitosan chloride (MPyMeChC) was successfully synthesized with a degree of quaternization of 80% and molecular weight of 12 kDa. This chitosan derivative was modified to improve water solubility and gene transfection efficiency. *In vitro* transfection efficiency and cytotoxicity of MPyMeChC were previously determined in the human hepatoma cell (Huh 7). The experiment showed high biocompatibility with the Huh 7 cell (Sajomsang et al., 2009). In addition, the combination of MPyMeChC, PEI and DNA forming polyplexes demonstrated the synergistical effect in gene transfection in the human lung adenocarcinoma epithelial cell line (Hela), the human cervical cancer cell line (A549) and the neuroblastoma cell lines (SH-SY5Y) (Rakkhithawatthana et al., 2010).

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 the particle size, surface charge, DNA-binding ability, cellular uptake, transfection efficiency and cytotoxicity in a human lung adenocarcinoma epithelial cell line (A549) and 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 (Sajomsang et al., 2009). The branched polyethyleneimine (PEI; molecular weight of 25 kDa) and hydrogentetrachloroaurate(III)trihydrate ($HAuCl_4{\cdot}3H_2O$) 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 (Tencomnao, Rakkhitawatthana, & Sukhontasing, 2008). The plasmid was propagated in *Escherichia coli* DH5α which was grown in Luria–Bertani broth (Molekula, UK) supplemented with ampicillin (100 μg/mL) under shaking condition of 250 rounds/min at 37 °C. The plasmid was extracted using PureLink™ Hipure Plasmid DNA Purification (Invitrogen, USA). The extracted plasmid purity and concentration were determined by measuring light absorbance at 260 and 280 nm using a SpectraMax M2 microplate reader (MDS, Inc., Canada).

2.2. Synthesis and characterization of AuNPs

Gold nanoparticles were synthesized by citrate reduction method (Jiang, Zou, & Liang, 2008). 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% $HAuCl_4$ 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 the 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, P/D, G/P/D and G/C/D/P at the weight ratios of 10/1, 5/1, 10/5/1, 1/1, 10/1/1 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. Gel retardation assay

A constant amount of plasmid (1 μg) was incubated with different weight ratios of complex for 15–30 min at room temperature. After the complexes were formed, loading dye was added and mixed before loading into a 1% agarose gel. Electrophoresis was carried out at 100 V 60 min. Agarose gels were stained in 1 μg/mL ethidium bromide. The presence of DNA was visible under UV transilluminator (Syngene, UK). The shifted bands, corresponding to free plasmid, were determined.

2.6. Atomic force microscopy analysis

The AFM images of polyplex were obtained by using a dynamic force microscope (DFM) mode (Seiko SPA4000, Japan). All samples were prepared by dropping the polyplex solution on a mica surface and left for air drying. All images were obtained with scanning speed 1.0 Hz over a 2 μm × 2 μm area.

2.7. 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). Human lung adenocarcinoma epithelial cells (A549) were grown in Rosewell Park Memorial Institute (RPMI)-1640 (HyClone, USA) containing 10% FBS. Both cell lines were incubated at 37 °C in a humidified atmosphere at 5% CO_2 for 24 h and periodically subcultured prior to transfection. For *in vitro* transfection, HeLa and A549 cells were seeded at densities of 3×10^5 and 2×10^5 cells/well in 96-well plates, respectively. Prior to transfection, the media were removed, cells were rinsed with PBS twice, plated and incubated

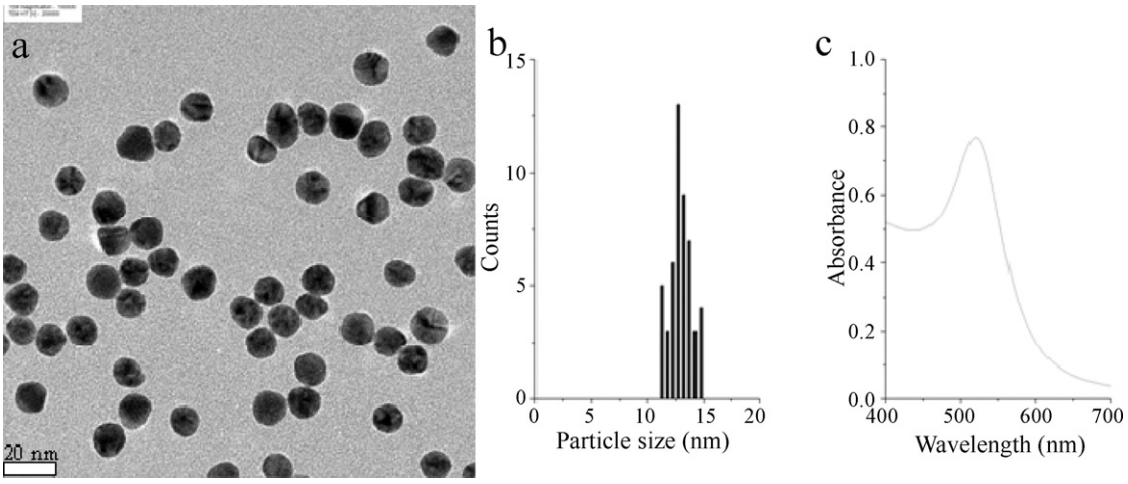

Fig. 1. TEM analysis of gold nanoparticles, (a) particle size distribution histogram (b) and the surface plasmon band indicated the formation of gold nanoparticles (c).

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 manufacture'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.

2.8. 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.

2.9. 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 5×10^5 cells on cover slip in 24-well plate. The transfected cells were stained with acridine 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 Fig. 1a. 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 (±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.

3.2. Complex formation

Layer by layer coating using gold nanoparticles as the core of G/D, C/D, G/C/D, P/D, G/P/D and G/C/D/P complexes were prepared as described in Section 2. The approach for generating gold nano-scaffold-based DNA carriers is illustrated in Fig. 2. Cationic polymers, PEI or MPyMeChC, were absorbed on gold nanoparticle via electrostatic interaction resulting in the formation of gold/polymer cores, G/P or G/C. DNA strands were then bound electrostatically on the G/P or G/C cores to yield G/P/D or G/C/D, respectively. For G/C/D/P complex, PEI was added to condense DNA strands via electrostatic interaction on G/C/D complex surfaces. The G/C/D/P complex occurred in accordance with the hypothesis that PEI was probably coated on the surface of G/C/D complex through ionic interaction between the positive charge of PEI and the negative charge of DNA on G/C/D complex (Kim, Kim, Akaike, & Cho, 2005; Rakkhithawatthana et al., 2010). The adsorption of DNA was examined by AFM analysis (Fig. 3). Complexes of C/D were loosely formed, the DNA strand still remain around complex surfaces (Fig. 3a). The attachment of plasmid DNA on G/C surfaces at the complex formation of G/C/D at the weight ratio of 10/5/1 was confirmed and the strands of DNA condensed on the G/C scaffolds were clearly observed (Fig. 3b). Although the total surface charge of G/C/D was positive, but DNA was not well condensed. Therefore, the strands of DNA can be interacted with positive polymer such as PEI to generate G/C/D/P complexes (Fig. 3c). The size of G/C/D complex estimated under AFM analysis was approximately 90 nm in diameter under the dry condition, and it was slightly increased when PEI was supplied to coat on the particles to yield G/C/D/P. The result indicated that DNA-polymer coated gold nano-scaffold showed potential for use in non-viral gene delivery.

3.3. DNA binding affinity assay

To determine DNA binding affinity and stability of complexes, a gel retardation assay was performed (Fig. 4). One microgram of DNA was applied to prepare the complexes with gold/cationic polymer based nano-scaffold at different weight ratios. Compared to free DNA (Lane 1), the result indicated that DNA could not be adsorbed on gold by directly mixing colloidal gold and DNA because the electric repulsion between the negative charge of both gold (−34 mV)

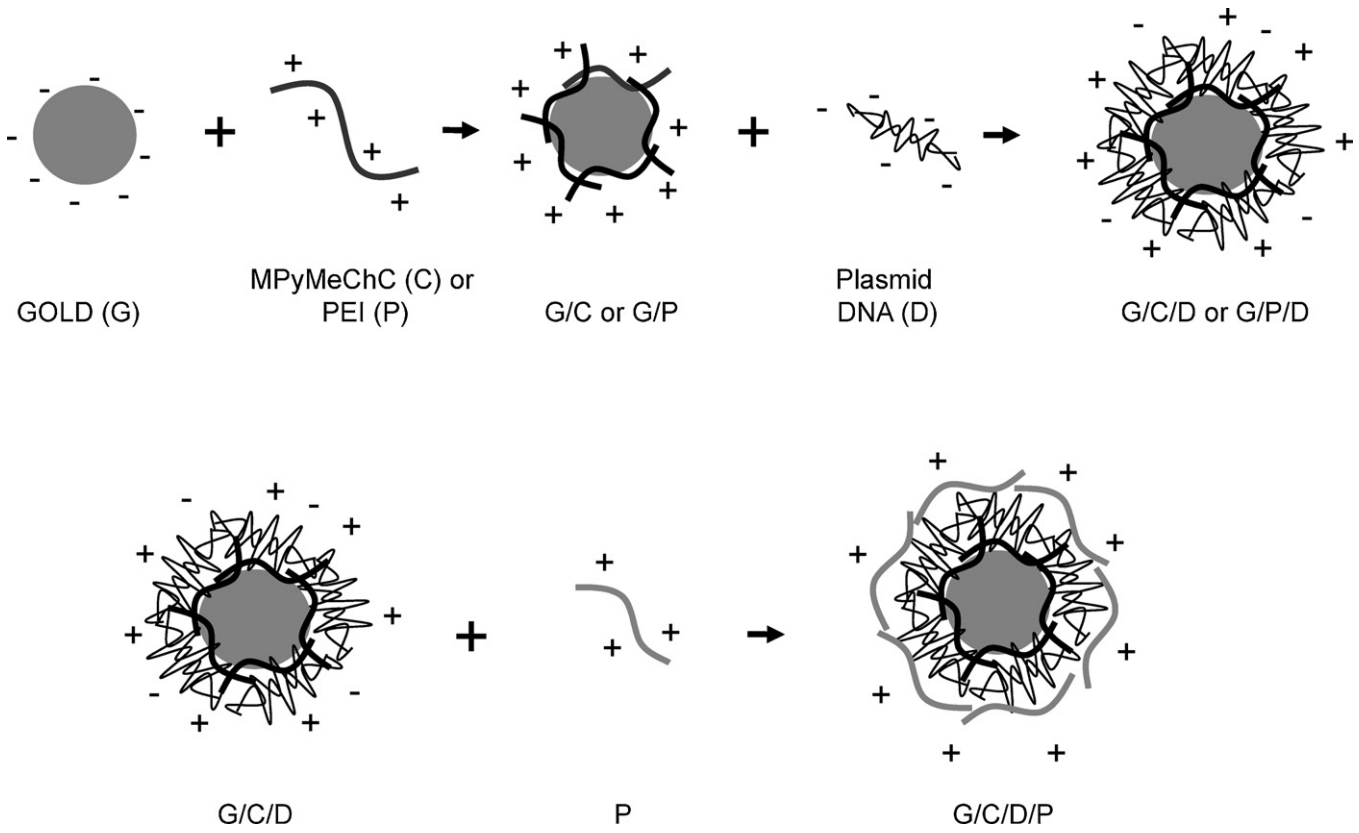

Fig. 2. Layer-by-layer formation of gold/cationic polymer nano-scaffold condensed with DNA; G, gold; C, MPyMeChC; D, DNA and P, PEI. The surface charges of polymer and complex are indicated.

and DNA. Thus, G/D complex formation could not occur resulting in free migration of DNA in agarose gel. Our result revealed that the complex formation of C/D and P/D was completely formed at the weight ratios of 5/1 and 1/1, respectively. It implied that either chitosan or PEI can directly bind with DNA to form nano-polyplexes at tested ratios; therefore, the movement of DNA was retarded on the agarose gel. Correspondingly, the high binding affinities of scaffold G/C, G/P and G/C/P with 1 μg of DNA were detected since DNA bands were shifted. This result demonstrated the capability of gold/polymer based nano-scaffold as a vehicle for DNA delivery.

3.4. Size and zeta potential analysis

The particle size and zeta-potential were determined at pH 7.4. The DNA at 1 μg was constantly applied to form complexes with gold/cationic polymers. All complexes of G/D, C/D, G/C/D, P/D, G/P/D and G/C/D/P were prepared as described in Section 2. The C/D, G/C/D, P/D, G/P/D and G/C/D/P complex sizes analyzed by DLS were in the range of 310–380 nm (Fig. 5). Their sizes were significantly larger than the colloidal gold nanoparticles. For non-scaffold polyplexes (C/D and P/D), zeta potential measurement showed that both types of polyplexes possessed strong positive charge surfaces at

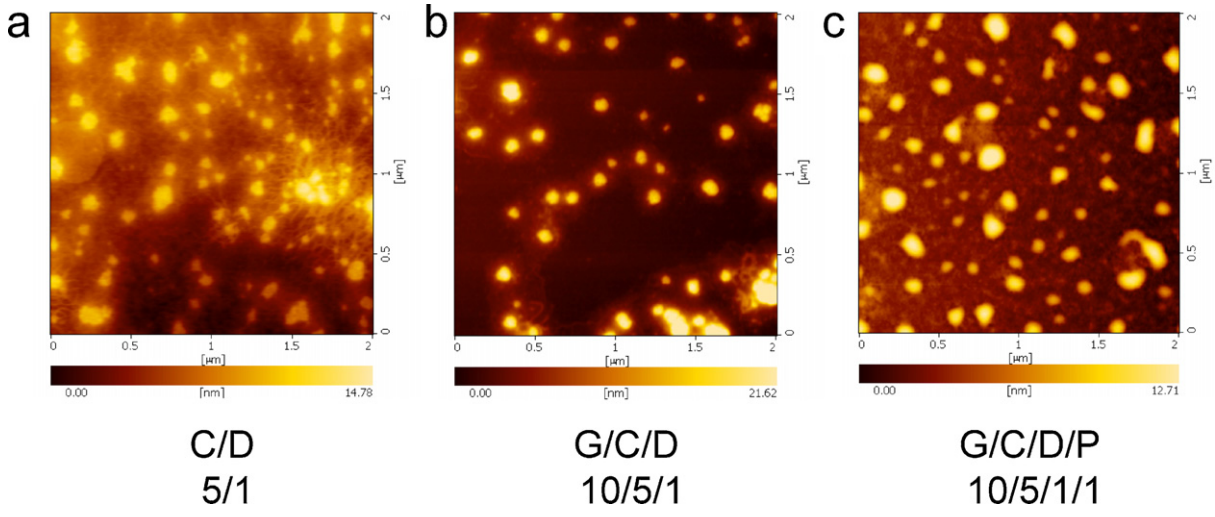

Fig. 3. Atomic force microscope images topology of polyplex MPyMeChC/DNA at the weight ratio 5/1: (a) gold/MPyMeChC/DNA (G/C/D) complex at the weight ratio 10/5/1 (b) and gold/MPyMeChC/PEI/DNA (G/C/P/D) at the weight ratio 10/5/1/1 (c).

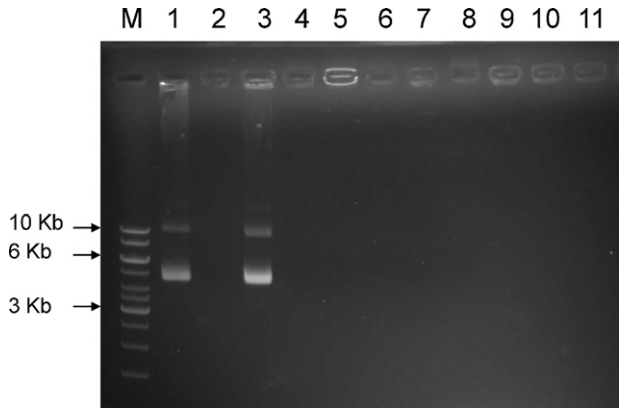

Fig. 4. Gel retardation assay. One microgram of DNA was applied to observe complex formation with gold, MPyMeChC and PEI. M is 1 kb ladder marker (fermentus life science). Lane 1 is the free plasmid DNA; Lanes 2 and 4 are the controls without DNA of G and C at the weight of 10 and 5, respectively. Lanes 6, 8 and 10 are the controls without DNA of G/C, G/P and G/C/P at the weight ratios of 10/5, 10/1 and 10/5/1, respectively. Lanes 3, 5, 7, 9 and 11 are the gold/polymer-based nano-scaffolds of G/D, C/D, G/C/D, G/P/D and G/C/D/P at the weight ratios of 10/1, 5/1, 10/5/1, 10/1/1 and 10/5/1/1, respectively.

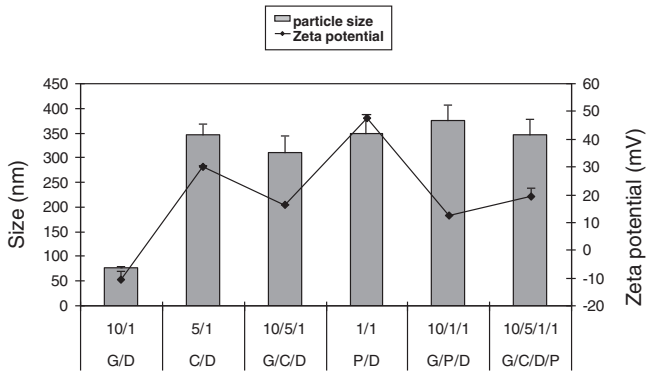

Fig. 5. The size and zeta-potential of complexes of G/D, G/C/D, G/P/D and G/C/D/P at the weight ratios 10/1, 10/5/1, 10/1/1 and 10/5/1/1, respectively. The bars correspond to size and the line corresponds to zeta-potential.

30 and 48 mV, respectively. Combination of colloidal gold in the system slightly decreased the charges of complexes because zeta potential of G/C/D, G/P/D and G/C/D/P showed moderately positive surface charge at 12–19 mV. However, all complexes were in nano-sizes carrying positive charges on their surfaces. This is additional evidence that shows the C/D, P/D, G/C/D, G/P/D and G/C/D/P are applicable for gene transfection.

3.5. 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, G/P/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 florescence microscope together control free cells (Fig. 6). The image revealed that the complexes were found to be successfully delivered into HeLa cells. At post transfection for 4 h, the complexes have agglomerated around the cell surfaces. Meanwhile the distribution of complexes in the cytoplasm and nucleus compartment remained observed at post transfection 24 h which is the time after luciferase has been expressed. This data suggested that gold/polymer nano-scaffold is practical to be use as a DNA delivery vehicle.

3.6. Transfection efficiency

To determine the ability of cationic polymer coated gold nano-scaffold as a vehicle for a gene carrier in mammalian cell lines, two types of cell lines, a human lung adenocarcinoma epithelial cell line (A549) and a human cervical cancer cell line (HeLa),

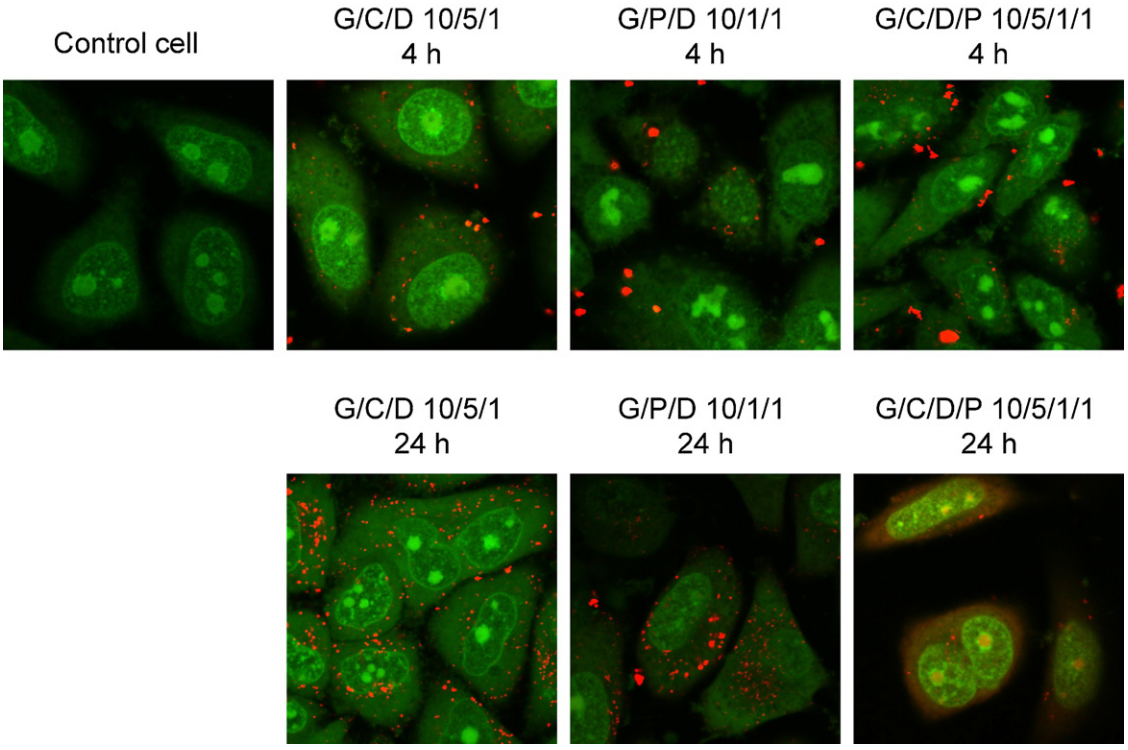

Fig. 6. Confocal microscopy images show DNA internalization into the HeLa cell at 4 and 24 h after transfection.

were employed for gene transfection study. The experiment was performed by using 1 μg of pGL3-basic vector containing CMV enhancer/promoter region for preparation of complexes as described in Section 2. Free cells, cells incubated with naked plasmid and Lipofectamine 2000™, were included to serve as controls. Generally, transfection profile in both A549 and HeLa cells appeared in the same tendency (Fig. 7a and b). In both cell lines, low expression level of 10^4 RLU/mg protein was found when using C/D complex as a gene carrier. However, addition of gold to generate scaffold-based carrier G/C/D improved gene transfection in both cell types, especially as high as 10^5 RLU/mg protein in A549 cells. It is possible that colloidal gold could condense DNA on the complex in the nano-sizes and promote dispersity property. For the transfection via P/D complexes at the weight ratio of 1/1 in both A549 and HeLa, gene expression signal was approximately 10^6 and 10^7 RLU/mg protein in A549 and HeLa, respectively. Similarly, addition of colloidal gold enhanced gene expression level approximately 10 times in both cell lines. Furthermore, the result indicated that PEI-based polyplex provided higher transfection efficiency more than chitosan-based polyplex in HeLa and A549 cell lines. Therefore, we continue to apply colloidal gold in the system to improve transfection efficiency. The result indicated that the combination of gold and PEI (G/P/D) enhanced transfection efficiency 10 times more than transfection via P/D complex. Meanwhile, addition of both PEI and MPyMeChC into the system (G/C/D/P) also slightly enhanced transfection efficiency (Fig. 7b), but it was likely that amount of added PEI would limit transfection result. In summary, 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™ in both tested cell lines. 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/P/D complexes obviously enhanced transfection efficiency comparing with C/D and P/D polyplexes. Using colloidal gold as scaffold would improve the mono-dispersity of the gene carrier and finally facilitate gene internalization into the cells. In addition, the transfection result indicated that transfection efficiencies varied depending upon the types of cationic polymer used. Once the complexes enter into the cells, lysosome will digest the complexes causing the DNA disassociation. Then, non-digested DNA escaped from lysosome will move into the nucleus for further transcription. However, the mechanism of PEI is slightly special because PEI posses the proton sponge effect inside the lysosome. PEI containing complexes are able to buffer the endolysosomal pH, and lead to endosome disruption via osmotic swelling, and eventually DNA can be released (Boussif et al., 1995). Therefore, the PEI containing gold nanoparticles (G/P/D and G/C/D/P) provided higher transfection efficiency than that of MPyMeChC (G/C/D). Based on transfection result using the same type and amount of polymers, the advantage of gold/polymer nano-scaffold (G/C/D, G/P/D) over the polyplex system (C/D and P/D) in gene delivery is that DNA adsorbed on scaffold surfaces are easily released after cell internalization. In the polyplex system, DNA is tightly condensed for packaging and protection, therefore, the disassociation of DNA from nanoparticle in the cell is more difficult (Wong et al., 2007).

3.7. 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. Cells were treated with different DNA carriers under the identical conditions to the transfection process. The results tested in HeLa and A549 are shown in Fig. 8a and b, respectively. The control was free cells which

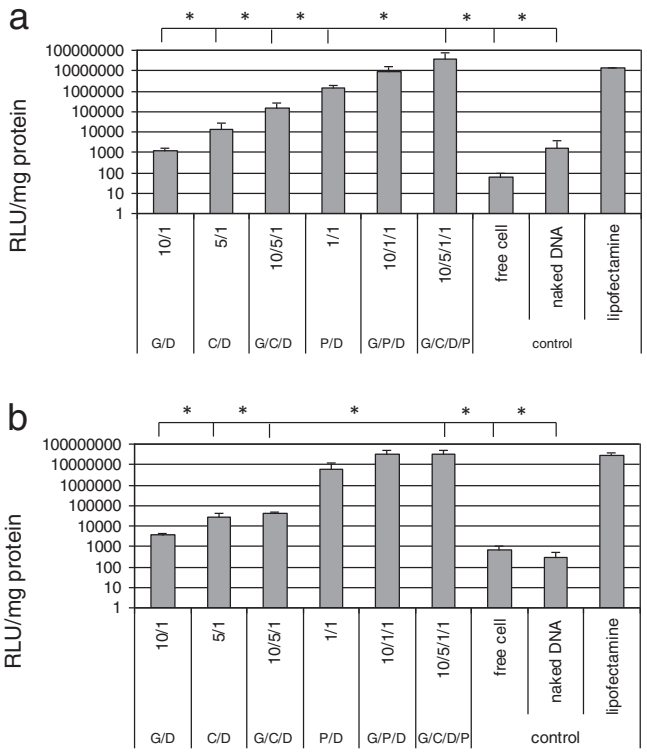

Fig. 7. Transfection efficiencies of complexes in HeLa cells (a) and A549 (b) at all tested ratios. The transfection efficiency was compared to non-transfected free cells, cell transfected with positive control Lipofectamine 2000™ (Invitrogen, USA) and negative control (naked DNA; plasmid pGL-3-basic containing CMV promoter/enhancer). Experiments were carried out with triplicates. One-way ANOVA with Turkey's post hoc test was applied for statistical analysis. Asterisks show significant differences between G/C/D/P and other treatments.

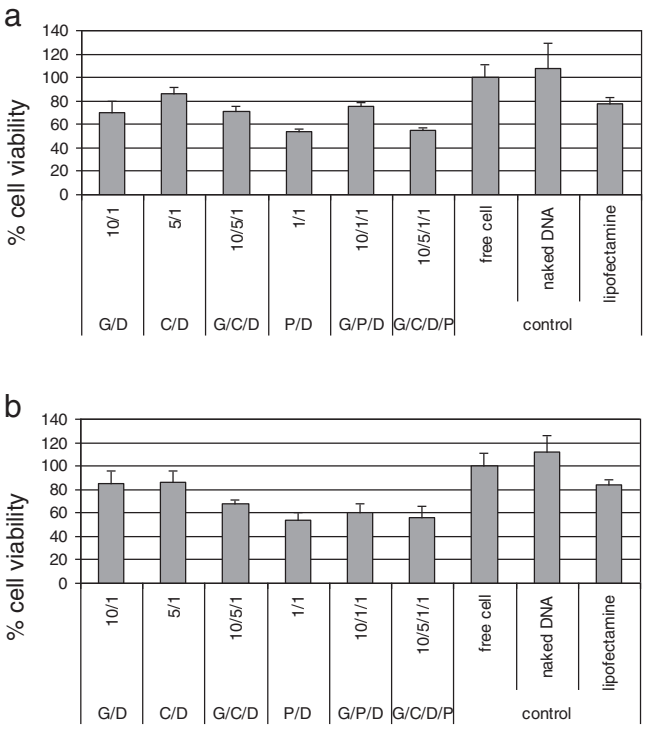

Fig. 8. Cell viability assay in HeLa cells (a) and A549. (b) Cells were treated with the complex at the weight ratios as indicated. Cytotoxicity was determined by MTT assay. Percentage viability of cells was expressed relative to free cell.

retained cell viability of 100%. Meanwhile, C/D polyplex and Lipofectamine 2000™ were relatively non-toxic since cell survival at 80% was evident. In both cell lines, the viability of cells transfected with G/P/D and G/C/D/P was approximately 60–80% 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 (Kraljevic & Pavelic, 2005; Shea, Smiley, Bonadio, & Mooney, 1999). Therefore, the use of gold as nano-scaffold should be a compromise between transfection efficiency and cell cytotoxicity.

4. Conclusions

In this study, we developed an alternative approach for simple preparation of the vehicles for gene delivery. 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. Two human cell lines, A549 and HeLa, were selected to evaluate transfection efficiency of our developed system. The obtained result demonstrated the potential of gold/cationic polymer–DNA (G/P/D and 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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