

Branched PEI Capped Gold Nanoparticles in Water for siRNA Delivery to Cancer Cells

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ABSTRACT

Herein we describe a simple method for the synthesis of different sizes of polyethylenimine-capped gold nanoparticles (AuNPs-PEI) in water and assess their potential to deliver siRNA to cancer cells. AuNP-PEI with diameters ranging between 25-150 nm have been synthesised in aqueous solutions using PEI (25 KD and 2KD) as capping ligands, and hydroxylamine-O-sulfonic acid or ascorbic acid as reducing agents. Different parameters were found to affect the final size of nanoparticles core (*i.e.* gold salt concentrations, PEI molecular weight/concentrations, and temperature). The obtained AuNP-PEIs were characterized using UV-visible spectroscopy, Scanning Electron Microscopy (SEM), and Dynamic Light Scattering (DLS). The ability of AuNP-PEIs to complex siRNA was analysed by gel electrophoresis, while the potential application of AuNP-PEIs in siRNA delivery was investigated using PC-3 prostate cancer cells.

Keywords: gold nanoparticles, polymers, stabilization, siRNA delivery, cancer.

1 INTRODUCTION

Cancer is one of the leading cause of death worldwide, therefore emerging nanotechnology innovations in cancer research diagnosis for early detection and treatment are a high value to society [1]. One of the methods for cancer therapy is based on RNA interference (RNAi) effectors such as siRNA and miRNA that can selectively down-regulate any gene implicated in the pathology of a disease, implying the potential of siRNA-based therapeutics in cancer. siRNA-based therapeutics can achieve downregulation of a target gene expression in the tumor cells, thus inhibiting proliferation [2]. To date, studies have shown that naked siRNA suffer from poor bioactivity mainly due to short plasma half-life caused by enzymatic degradation, renal clearance, inability to cross the

negatively charged phospholipidic cell membrane and hepatic metabolism [3]. Non-viral delivery using nanoparticles for small interfering RNAs (siRNAs) may overcome these barriers, facilitating the clinical application of siRNA-based therapeutics for cancer treatment [4, 5]. In fact, nanoparticles not only protect naked siRNA, but also their surface can be functionalized with a specific targeting ligand to recognize a tumor cell. Several nanoparticulate siRNA delivery system have been introduced recently and are well reviewed in the literature [3,6-8]. Positively charged polymers are known to increase cellular interactions and to facilitate endocytosis of positively charged nanoparticles by cells [9, 10]. Furthermore, polyethyleneimine cationic polymers have been shown to facilitate endosomal escape upon acidification, leading to the absorption of the protons and consequently the destabilization of the endosomes membranes. Therefore the use of such cationic polymers in the delivery of siRNA in major cancer types is of high interest [7].

Moreover, metal nanoparticles especially gold (AuNPs) have recently gained considerable attention for a wide range of applications in diagnostics, thermal therapy, and drug/gene delivery [9, 11]. AuNPs offer size and shape dependent optical properties due to surface plasmon resonance, a low cytotoxicity and an ease of bioconjugation through a high affinity to thiols and amino groups containing biomolecules. Therefore, they provides opportunities to interact with biomolecules (*i.e.* antibodies, nucleotides, peptides, proteins, and targeted ligands) [12]. One of the challenge for successful application of AuNPs in drug delivery is to maintain their physicochemical properties in biological fluid. Although attachment of polymers and surfactants can increase the stability of NPs, it may also increase toxicity and decrease cellular uptake. Recently we have shown that positively charged Au-L-cysteine-cysteine nanoparticles were less cytotoxic to a range of different cell types (human and murine), compared to corresponding AuNPs produced using the commonly employed surfactant, cetyl trimethyl ammonium bromide

(CTAB), under similar conditions [13]. The use of Polyethyleneimine (PEI) in the synthesis of nanoparticles and delivery of RNA have attracted further attention, it should be noted here that most of the studies found in the literature use PEI as both a reducing agent and stabilising agent. Wang et al. used PEI (25 KD) as both the reductant and stabilizer of AuNPs, the later were used to bind siRNA by electrostatic interaction at an appropriate weight ratio. Moreover, PEI-capped AuNPs/siRNA targeting endogenous cell-cycle kinase, an oncogene polo-like kinase 1 (PLK1), were found to display significant gene expression knockdown and to induce enhanced cell apoptosis, when compared to the cells treated with PLK1 siRNA using PEI as the carrier [4]. Similarly Xia et al. demonstrated that PEI coating enhances the cellular uptake of Mesoporous Silica Nanoparticles and allows safe delivery of siRNA and DNA constructs [14].

Herein we describe a simple method for the synthesis of different sizes of PEI-capped gold nanoparticles (AuNPs-PEI) in water using ascorbic acid as a reducing agent (hydroxylamine-O-sulfonic acid was also tested). The effects of different parameters (*i.e.* gold salt concentrations, PEI molecular weight/concentrations, and temperature) on the final size of the nanoparticle core were evaluated as detailed below. Furthermore, the resulting AuNPs-PEI were tested for potential siRNA delivery to PC-3 prostate cancer cells.

2 PREPARATION AND CHARACTERIZATION BRANCHED PEI CAPPED GOLD NANOPARTICLES IN WATER

AuNP-PEI with diameters ranging between 25-150 nm were obtained by chemical reduction of $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ with ascorbic acid or hydroxylamine-O-sulfonic. The resulting AuNP-PEIs were fully characterized using UV-visible spectroscopy, Electron Microscopy (EM), and Dynamic Light Scattering (DLS). Figure 1 (up left) represent the UV-visible spectra of selected Au NPs synthesised in this study at room temperature, the effect of different concentrations of PEI 2KD which fixed concentrations of Ascorbic acid (0.388 mM) and $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ (0.25 mM) on the final AuNPs is evaluated. It is clearly seen from UV-vis that variations in the concentration of PEI 2KD, affect the position of the plasmon resonance band of the resulting AuNPs, which a band shift of about 40 nm from 532 nm to 572 nm when the concentration of PEI 2KD was decreased from 40 μM to 2.5 μM . This plasmon shift also translated into a change in the colour of the colloidal AuNPs solution. Therefore, the AuNPs-PEI offer size dependent optical properties with a plasmon band shift to longer wavelengths related to an increase in the size of the AuNPs with decreasing PEI concentrations as demonstrated from SEM micrographs analysis (Figure 1) and DLS measurements (Figure 2). Figure 1 (up right) shows that the Au NPs-PEI

obtained with a high concentration of PEI (40 μM) are well dispersed with a diameter of $\sim 26 \pm 5$ nm as estimated from *Image J* software analysis; while the size was found to increase to 70 ± 15 nm (down left) and to $\sim 90 \pm 10$ nm (down right) when the PEI concentration is decreased to 5 μM and 2.5 μM respectively.

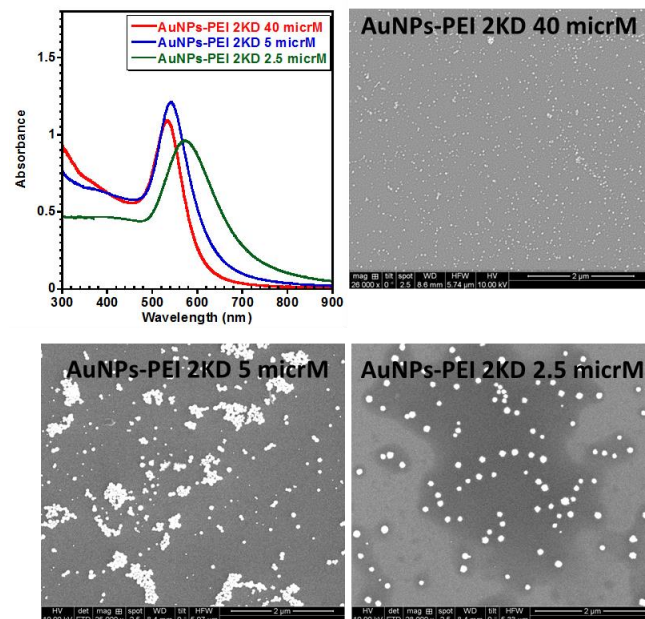


Figure 1: UV-visible spectra (up right) of selected Au NPs obtained with different PEI 2KD concentrations, 40, 5 and 2.5 μM . SEM micrographs of the corresponding AuNPs-PEI (scale bar 2 μm) showing that the AuNPs are well dispersed and nearly spherical in shape with diameter ~ 25 nm (up right); ~ 70 nm (down left) and ~ 90 nm (down right)

In addition, DLS analysis was used to determine the hydrodynamic diameter (D_h) of AuNPs-PEI hybrids particles in colloidal solution [15]. DLS measurements shown in Figure 2a represent the size distribution by intensity of the same samples imaged using SEM in Figure 1. DLS indicated that all samples were nearly monodisperse with one size distribution. It is clearly seen that the hydrodynamic diameter increases from around 55 nm for 40 μM PEI to 97 nm with 5 μM PEI and 123 nm with 2.5 μM PEI, further confirming that the size of the AuNPs-PEI increase when the concentration of the PEI decreases. It was also noticed here that the polydispersity index (PDI) decreased with the cocentration of PEI (*i.e.* from 0.266 to 0.109 when the concentration of PEI decreased from 40 μM to 2.5 μM). Furthermore, the size distribution by intensity from DLS was also found to be much larger than the size measured by SEM, indicating the AuNPs were successfully coated with a layer of branched PEI. To further confirm that the nanoparticles are capped with branched PEI, the zeta potential (ζ) was measured and the results are shown in Figure 2b. Zeta potential measurements showed that all AuNPs-PEI samples were positively charged with a ζ -

potential in the range of 37 ± 3 mV, leading to a very high stability of the colloidal solution for several months when stored at 4 °C.

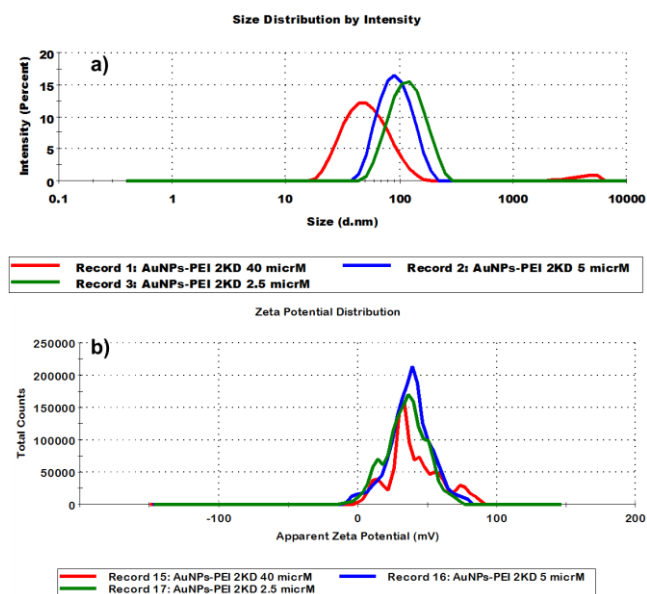


Figure 2: a) size distribution by intensity of AuNP-PEI 2KD from DLS measurements, showing that the size increase from 55 nm to 123 nm when the concentration of PEI 2KD was decrease from 40 µM to 2.5 µM. (b) zeta potential for the corresponding AuNPs-PEI 2KD showing that all AuNPs-PEI samples were positively charged with a ζ -potential in the range of 37 ± 3 mV.

In contrast, PEI 25KD was also used as a capping ligand in the synthesis of AuNPs using a similar method, the concentration of PEI 25KD was also found to affect the final size of AuNPs similarly to PEI 2KD. However, it should be noted that the resulting AuNPs-PEI 25KD were found to be less spherical in shape especially at large AuNPs diameters (results not shown). Moreover, beside PEI concentrations, different parameters such as the $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ concentration, temperature and type of reducing agent were also found to affect the final size of nanoparticles core, for example the AuNPs-PEI core size were found to increase when the concentration of $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ increases and to decrease as the temperature increases (results not shown).

3 APPLICATION OF AUNPS-PEI IN SIRNA DELIVERY

The potential application of AuNPs-PEIs in siRNA delivery to PC-3 prostate cancer cells was also investigated in this study. The ability of AuNPs-PEIs to complex siRNA was analysed by gel electrophoresis. Results indicated that AuNP-PEI 2KD and AuNP-PEI 25KD could complex siRNA from MR0.5 and MR0.25 onwards, respectively

(Figure 3a and 3b), suggesting that AuNP-PEI 25KD has a better siRNA binding capacity than AuNP-PEI 2KD.

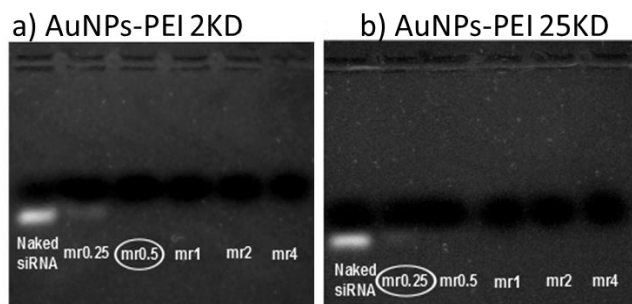


Figure 3: Complexation of siRNA (0.25 µg) with ~ 150 nm (a) AuNP-PEI 2KD and (b) AuNP-PEI 25 KD at different mass ratios (MRs) using gel retardation (1% agarose gel at 120 mV for 30 min).

Cellular uptake was also performed on PC-3 cancer cells following 24 h incubation, Figure 4. Uptakes studies indicated that AuNP-PEI 25KD achieved significantly higher fluorescein-positive cells (~ 98%) relative to that of AuNP-PEI 2KD (~ 5%), suggesting that AuNP-PEI 25KD, but not AuNP-PEI 2KD, could deliver siRNA into cells.

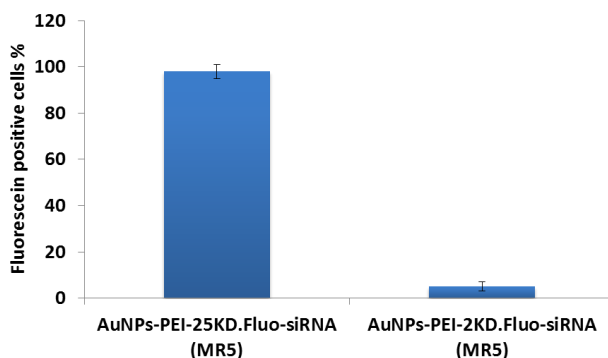


Figure 4: Fluorescein-positive PC-3 cells (% , mean \pm SD), Cell were transfected by 50 nM FAM siRNA complexed with AuNP-PEI 25 KD and AuNPs-PEI 2K (MR5) and incubated for 24 h in normal growth medium, after 24 h transfection ten thousands cells per sample were measured following the procedure outlined in Becton Dickinson FACScalibur manual.

4 MATERIALS AND METHODS

4.1 Chemicals and Materials:

Purified H_2O (resistivity ≈ 18.2 M Ω cm) was used as a solvent for AuNPs synthesis. All glassware was cleaned with aqua regia (concentrated HCl/concentrated HNO_3 3/1), rinsed with distilled water, ethanol and acetone, and oven-dried before use. Tetrachloroauric acid trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$), L-ascorbic acid ($\text{C}_6\text{H}_8\text{O}_6$), hydroxylamine-o-sulfonic acid $\text{H}_2\text{NOSO}_3\text{H}$, Branched PEI 2KD and 25 KD

solutions 50 % w/v were purchased from Sigma Aldrich.

4.2 Preparation of Au NPs-PEI

Diameter of ~25 nm AuNPs-PEI 2KD: To 64 mL of an aqueous solution of $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ (0.25 mmol L^{-1}) at room temperature. 1.115 mL of a 2.3 mmol L^{-1} PEI 2KD aqueous solution was rapidly added. The colour of the solution changed from pale yellow to deep yellow upon addition of PEI, afterward 0.145 mL of 109 mmol L^{-1} was quickly added the solution turn deep red within about 1 minute. Stirring was maintained overnight after addition of Ascorbic acid. The Au NPs obtained with this procedure were $\sim 26 \pm 5 \text{ nm}$.

Preparation of larger AuNPs-PEI 2KD and AuNPs-PEI 25KD. A similar protocol was used for the synthesis of larger AuNPs-PEI 2KD and AuNPs-PEI 25KD while decreasing the PEI concentration.

4.3 Preparation of AuNP.siRNA complexes

AuNPs-PEI ($500 \mu\text{g mL}^{-1}$) were added to the siRNA solutions at different mass ratios (MRs) of AuNPs to siRNA, followed by 1 h incubation with slightly shaking at RT. The ability of AuNPs to complex siRNA was assessed by gel retardation. Complexes of AuNPs and siRNA (containing $0.25 \mu\text{g}$ siRNA) at different MRs were loaded onto 1 % (w/v) agarose gels in Tris/Borate/EDTA buffer containing SafeView™ (NBS Biologicals, UK). Electrophoresis was performed at 120 V for 30 min and the resulting gels were analysed under UV

4.4 Fluorescence activated cell sorting

PC-3 (100,000 per well) cells were seeded in 24-well plates and incubated for 24 h under normal growth conditions. Cells were transfected by 25 and 50 nM FAM siRNA complexed with AuNPs-PEI (MR5) and incubated for 24 h in normal growth medium. Following incubation cells were washed twice with PBS and trypsinised. Cells were subsequently centrifuged (1,000 rpm for 5 min) and re-suspended in 1000 μL ice-cold PBS in Polystyrene Round-Bottom Tubes (Becton Dickinson). Ten thousands cells per sample were measured following the procedure outlined in Becton Dickinson FACScalibur manual. Fluorescein-positive cells (%) were displayed by Dot Plot [16].

4 INSTRUMENTATION

Optical spectra were obtained on a CARY Uv-vis spectrophotometer with a Xenon lamp (300–900-nm range, 0.5 nm resolution).

Dynamic Light Scattering (DLS) and Zeta Potential: Measurements were carried out with the Malvern instrument (Zeta sizer Nano series) at 25°C . Measurements on each sample were performed in triplicate.

Scanning Electron Microscopy (SEM) AuNPs were deposited from solution onto a Silicon wafer and air-dried prior to analysis using a FEI 630 NanoSEM equipped with an Oxford INCA energy dispersive X-ray (EDX) detector operated at 5 kV.

6 CONCLUSION

Different sizes of AuNPs-PEI were synthesized in water and characterized, using UV-vis, DLS and SEM. The PEI concentration used in the synthesis of AuNPs was found to affect their final size by preventing their extensive growth. AuNPs-PEI were shown to be stable and successfully complex siRNA, AuNP-PEI 25KD, but not AuNPs-PEI2KD was found to deliver siRNA into PC3 cancer cells. The surface of AuNPs-PEI can be further conjugated with thiolated polyethylene glycol (SH-PEG) (results not shown). Moreover, we have previously shown that covalently bonded targeting ligand (such as Anisic Acid or Folic acid) could be also chemically grafted on AuNPs-PEI, leading therefore to a multifunctional nanoparticle that may be promising in the field of Nanobiotechnology and Nanomedicine [17,18].

Acknowledgments: We acknowledge financial support from the National Council for Scientific Research Lebanon (CNRS-L-GRP2015-3538), and Science Foundation Ireland and AMBER (Grant 12/RC/2278).

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