

높은 안정성을 갖는 초미립 폴리에틸렌이민-금 나노입자

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Ultrasmall Polyethyleneimine-Gold Nanoparticles with High Stability

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초록: 본 연구는 수계에서 오랜 시간 동안 안정한 생체적합 금 나노입자의 제조에 관한 것으로, 환원제와 안정제의 역할을 동시에 수행하는 폴리에틸렌이민을 이용하여 응집성이 낮은 초미립 폴리에틸렌이민-금 나노입자를 상온의 수용액 상에서 합성하였다. 폴리에틸렌이민-금 나노입자의 평균 입자크기는 8~12 nm이었고, 수계의 나노콜로이드 상에서 50 nm 내외의 클러스터를 형성하였으며, 매우 뛰어난 안정성을 보였다. 상대적으로 낮은 금속 전구체의 농도에서 나노입자를 제조하였을 때, 입자의 크기가 두드러지게 감소하는 경향을 나타내었다. 폴리에틸렌이민-금 나노입자의 X-선 회절분석 결과, 금에서 나타나는 전형적인 결정 피크가 발견되었다. 또한 세포배양실험 결과, 폴리에틸렌이민과는 달리 폴리에틸렌이민-금 나노입자는 98%의 세포 생존율을 보여 세포독성은 거의 없는 것으로 나타났다. 이상의 결과로부터 본 연구에서 합성된 폴리에틸렌이민-금 나노입자는 CT 조영제 등으로의 활용이 기대된다.

Abstract: This study is related to the preparation of biocompatible gold nanoparticles (AuNPs) which are stable in aqueous solutions for a long time. Ultrasmall polyethyleneimine (PEI)-capped AuNPs (PEI-AuNPs) with limited agglomeration were prepared in aqueous solutions at room temperature, which were based on the roles of PEI as a reductant and a stabilizer. PEI-AuNPs with an average size of 8~12 nm formed highly stable nanocolloids with an average hydrodynamic cluster size of around 50 nm in aqueous media. At a low concentration of metal precursor hydrogen tetrachloroaurate(III), the particle size was reduced noticeably. The typical peaks of gold were observed in the X-ray diffraction pattern of AuNPs. The cell viability of 98% was obtained in the case of PEI-AuNPs, while PEI was cytotoxic. The PEI-AuNP is considered to be a potential candidate as a contrast agent for computed tomography.

Keywords: gold, nanoparticles, polyethyleneimine, reductant, stabilizer.

Introduction

The development of synthesis protocols for nanostructured materials is an important goal in nanotechnology.^{1,2} Recently, the preparation of ultrafine metal particles has received much attention since they can offer highly promising and novel options for a wide range of technical applications.^{3,4}

For example, silver and gold nanoparticles (AuNPs), which are able to form stable nanocolloids in aqueous solutions, have been synthesized and applied extensively owing to their unique physical, chemical, and biological properties.^{5,6} There has been much of interest in the nanoparticles as imaging probes for *in vivo* biomedical applications.^{7,8} The preparation of nanoparticles is the one of the core technologies in molecular imaging as magnetic resonance (MR)^{9,10} and optical¹¹⁻¹⁴ imaging probes.

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X-ray computed tomography (CT) one of the most useful diagnostic tools. Different tissues can be distinguished by the method of CT since they provide different degree of X-ray attenuation. Current contrast agents for CT are predominantly based on iodinated small molecules because among nonmetal atoms, iodine has a high X-ray absorption coefficient.^{15,16} However, current CT contrast agents, iodine containing molecules, show several disadvantage. At first, iodinated compounds allow very short times for imaging due to rapid clearance by the kidney. Accordingly, it is difficult to use such iodine contrast agents for a patient having a kidney disease since they cause renal toxicity.^{17,18} Besides, iodine may lead to various symptoms such as a pain, a hot flash, and an allergy.¹⁹ Secondly, this material is hard to be conjugated to most biological components or cancer markers, so it is nonspecifically targeted. The feasibility of biocompatible polymer coated AuNPs were reported as a potential CT contrast agent, based on the fact that gold has a higher X-ray absorption coefficient than iodine.²⁰⁻²² The AuNPs are easy to control the size and shape, and highly flexible in terms of functional groups for coating and targeting.²³⁻²⁵ Furthermore, AuNPs are stable, nontoxic, and biocompatible.^{26,27}

In the present study, the ultrasmall polyethyleneimine (PEI)-capped AuNPs (PEI-AuNPs) with limited agglomeration for a long time were prepared in aqueous solutions at room temperature, which was based on the roles of PEI as a reductant and a stabilizer. The characteristics of the PEI-AuNPs were studied by transmission electron microscopy (TEM), field emission scanning electron microscopy (FE-SEM), X-ray diffractometry (XRD), and ultraviolet-visible (UV-VIS) spectroscopy. Cytocompatibility of the PEI-AuNPs was examined by checking cell viability.

Experimental

Preparation of PEI-AuNPs. 0.01 g of branched PEI ($M_w \sim 25000$ by light scattering, Aldrich, USA) was dissolved completely in 100 mL of distilled water for an hour. Then, the metal precursor hydrogen tetrachloroaurate(III) (HAuCl_4) (Aldrich, USA) was dissolved in the aqueous solution of PEI under magnetic stirring for another hour. The mixture was stirred vigorously at room temperature without adding reductant such as sodium borohydride (NaBH_4). In this case, PEI played a role of mild reductant so that a slow color change from yellow to dark purple or red wine was observed, indicating the formation of AuNPs. The mixture was stirred for 24 hrs in order to lead entire reduction. Then, the reacted mixture was then separated by a super speed vacuum

centrifuge (VS-30000i, Vision Scientific, Korea) at 15000 rpm for 30 min. The supernatant of the suspension was dialyzed using a dialysis tube (Membrane Filtration Products Inc., USA) with a molecular weight cut-off of 1000 Da with repeated water changes for more than 2 days to eliminate the unreacted chemical remains and the salts formed while synthesis. After dialysis, the PEI-AuNPs were collected by ultracentrifuge at 25000 rpm for an hour. The collected PEI-AuNPs were dispersed finely in water with ultra-sonicator (UP 200s, Hielscher Ultrasonics GmbH, Germany) to prepare aqueous solutions of PEI-Au nanocolloids.

Analysis. The morphology and the particle sizes of PEI-AuNPs were investigated by TEM (H-7600, Hitachi, Japan) and FE-SEM (S-4300, Hitachi, Japan). For the TEM, a tiny quantity of PEI-Au nanocolloid was placed onto a carbon coated copper grid and dried at room temperature for 1 day. Colloidal suspensions of AuNPs were freeze-dried and sputter-coated with platinum for the FE-SEM. The mean size of nanocluster was measured by dynamic light scattering (DLS) (ELS-800, Photal Otsuka Electronics, Japan) equipped with vertically polarized light supplied by a He-Ne laser, operated at 10 mW at room temperature. UV-VIS spectroscopy was carried out with UV-1700 spectrophotometer (Simadzu, Japan) to investigate the color change of the nanocolloids prepared at different synthesis conditions of AuNPs. The XRD data of dried AuNPs were collected using $\text{Cu-K}\alpha$ radiation with X'pert APD X-ray diffractometer (X'pert PRO MRD, Philips, Netherland).

Cell Viability. For sterilization, the freeze-dried AuNPs were irradiated by UV in a clean bench for 2 hrs. Then, they were moved onto 96 well cell culture plates. The 5×10^3 primary human fibroblast cells were seeded into the culture plates with 300 μL of growth medium and then the culture was performed in a humidified atmosphere of 5% CO_2 at 37 °C for 48 hrs. After replacing a new medium, the cell viability was checked by a colorimetric tetrazolium the CellTiter 96 aqueous one solution assay (Promega, USA) according to the manufacturer's procedure. The absorbance at 490 nm was measured by the microplate reader. Cell viability (%) was expressed as the relative number of vital cells to that of control.

Results and Discussion

In this study, different amounts of HAuCl_4 (0.02, 0.04, 0.06, 0.08 wt%) were added in an aqueous solution of PEI (0.01 wt%). Without PEI, gold synthesis could not be achieved because there was no reducing agent. In this case, an addition of reductant like NaBH_4 would lead to the

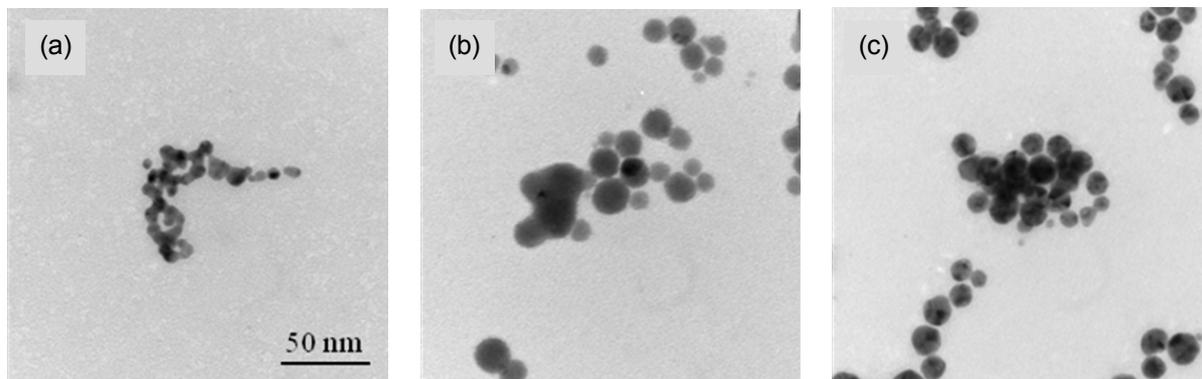


Figure 1. TEM images of PEI–AuNPs prepared at different concentrations of HAuCl₄: (a) 0.02 wt%; (b) 0.04 wt%; (c) 0.06 wt% of HAuCl₄.

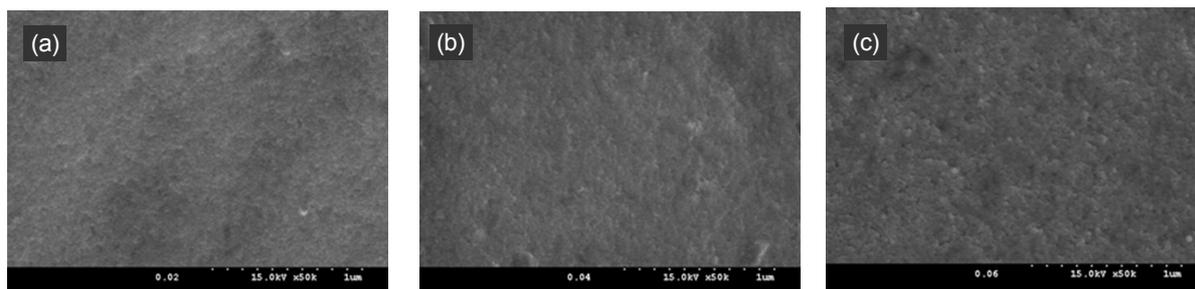


Figure 2. FE–SEM images of PEI–AuNPs prepared at different concentrations of HAuCl₄: (a) 0.02 wt%; (b) 0.04 wt%; (c) 0.06 wt%.

synthesis of gold but AuNPs could not be prepared due to agglomeration followed by precipitation. This implies that PEI played a role of a reductant and a stabilizer. At the HAuCl₄ concentration of 0.08 wt% or higher, it was impossible to perform analysis because there were serious agglomerations in the solution. Thus, the AuNPs were not able to form a stable nanocolloid in this case, although gold metal precipitates were observed on the wall and the bottom of a glass vial.

Figure 1 depicts the morphology of PEI–AuNPs observed by TEM. The AuNPs synthesized in the presence of PEI exhibited a spherical shape with a cluster size of 20~100 nm. PEI molecules, which contain a large number of amino groups in the long molecular chain, are capable of forming complexes with metal ions via coordination.²⁸ In an aqueous solution, positively charged PEI molecules have strong repulsion each other. For this reason, the PEI–AuNPs formed a stable nanocolloid in water against agglomeration. It is notable that the AuNPs had different size according to the concentration of HAuCl₄. The FE–SEM images of freeze-dried PEI–AuNPs are shown in Figure 2. The clusters of AuNPs were well demonstrated and appeared to be closely packed.

Hydrodynamic size of nanoclusters, which was measured by DLS, is a useful quantity to control the steps of the nanoparticle preparation and to develop structural models for

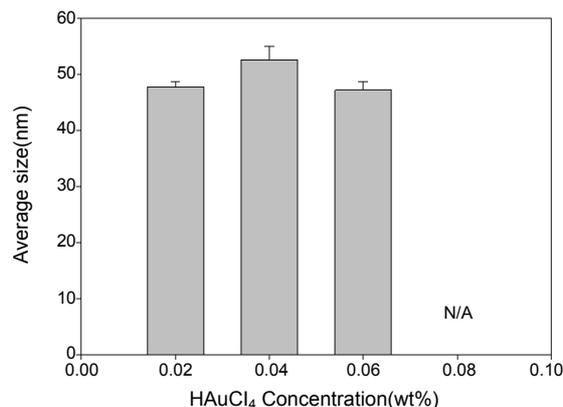


Figure 3. Average hydrodynamic cluster sizes of PEI–AuNPs measured by DLS.

the particles. It was impossible to measure the cluster size of PEI–AuNPs prepared at the HAuCl₄ concentration over 0.08 wt% due to serious agglomeration. The nanoclusters of PEI–AuNPs prepared at the HAuCl₄ concentration of 0.02~0.06 wt% had the average size around 50 nm (Figure 3) to form stable nanocolloids in aqueous media. There was little significant relationship between the metal precursor concentration and the average hydrodynamic size of nanoclusters when the concentration of HAuCl₄ was controlled to 0.02~0.06 wt%, although the hydrodynamic cluster size of the AuNPs prepared at the HAuCl₄ concentration of

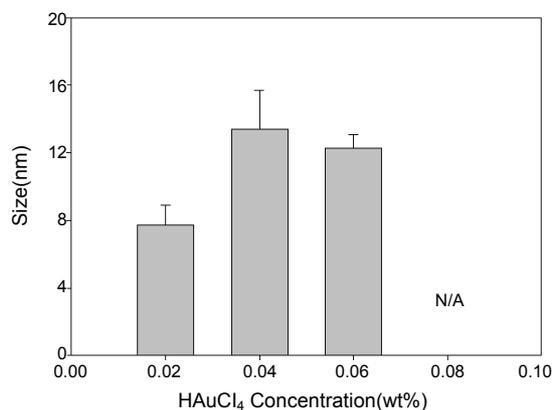


Figure 4. Average particle sizes of PEI-AuNPs measured from TEM images. Each value is the mean diameter of at least 30 nanoparticles.

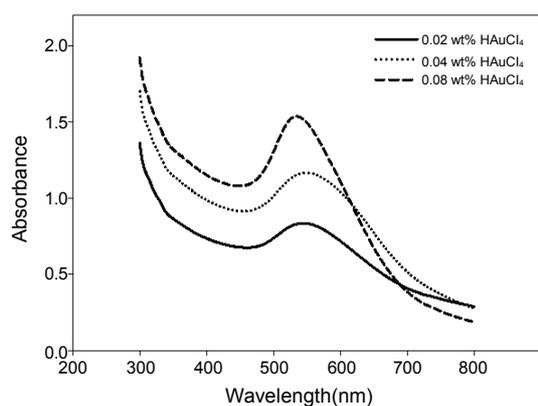


Figure 5. UV-VIS spectra of PEI-AuNPs prepared at the various concentrations of HAuCl₄.

0.04 wt% increased slightly as compared with the others.

On the other hand, the average size of nanoparticles, which was measured directly from the TEM images, was dependent upon the HAuCl₄ concentration as shown in Figure 4. At the low concentration (0.02 wt%) of metal precursor, the particle size was reduced to 8 nm. When the concentration of HAuCl₄ was high, Au particles formed by the reductive action of PEI were placed close so that it was easy to aggregate in the solution before they were stabilized by capping with PEI, resulting in the larger particles. Altogether, the ultrasmall PEI-AuNPs with the average size of 8~12 nm formed the highly stable nanocolloids with the average cluster size around 50 nm.

The red or purple color of each solution gives one piece of evidence for the formation of AuNPs.²⁹ Thus, the AuNPs formation and the particles size were checked by the peaks of the plasmon resonance band located at 520~540 nm in the UV-VIS spectrum (Figure 5). Moreover, a red-shift of the plasmon absorption band is attributed to increasing

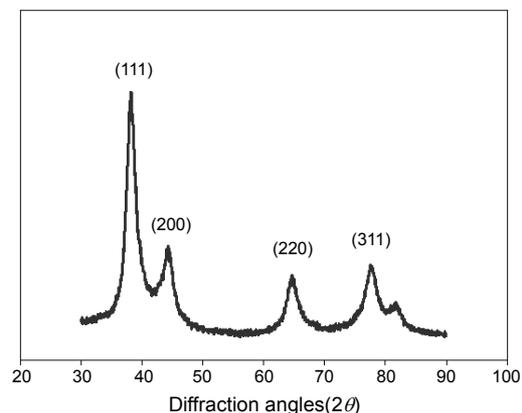


Figure 6. X-ray diffraction pattern of PEI-AuNPs prepared at HAuCl₄ concentration of 0.06 wt%.

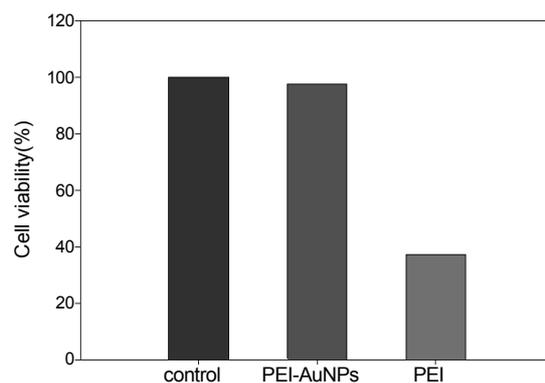


Figure 7. Cell viability for PEI and PEI-AuNPs prepared at the HAuCl₄ concentration of 0.06 wt%.

particle size.³⁰ In the case of PEI-Au nanocolloid prepared at the HAuCl₄ concentration of 0.04 wt%, the red-shift of the plasmon absorption band was conspicuous as compared with the others. This suggests that the particle and the cluster size of PEI-AuNPs prepared at the HAuCl₄ concentration of 0.04 wt% should be increased, which is well coincident with the results in Figures 3 and 4.

The X-ray diffraction pattern of PEI-AuNPs is represented in Figure 6. Several distinct X-ray diffraction peaks are assigned to the reflections from the 111, 200, 220, and 311 planes of the face-centered-cubic gold particles.³¹ Four peaks corresponding to those of gold appeared at the X-ray diffraction pattern of PEI-AuNPs.

PEI has been reported to be toxic to cells. However, the cytotoxic effects of PEI can be greatly lessened when it is in a particulate form. Figure 7 denotes the cytotoxicity of PEI-AuNPs, as well as PEI itself. The cell viability of 98% was obtained in the case of PEI-AuNPs, while PEI was cytotoxic. The detrimental property of PEI was negligible when it was incorporated with AuNPs. This is supposed to be related with two things. First, lots of PEI molecules were

removed by centrifuge and a tiny amount of PEI molecules was incorporated into AuNPs. Second, the amino groups in the long molecular chains of PEI were involved in metal ion complexes via coordination. For this reason, the cytotoxic effects due to the amino groups in PEI were possibly diminished. Accordingly, the PEI–AuNPs are considered to be a cytocompatible candidate as a molecular imaging probe such as a contrast agent for CT.

Conclusions

Hydrophilic polymers are efficient agents for the preparation of stabilized AuNPs at room temperature without aggregation. However, an addition of strong reducing agent like NaBH₄ often leads to large particles with a broad size distribution. In this study, the PEI–capped AuNPs were prepared in an aqueous solution, which was based on the roles of PEI as a reductant and a stabilizer. The ultrasmall PEI–AuNPs with the average size of 8–12 nm formed the highly stable nanocolloids with the average cluster size of around 50 nm in aqueous media, which satisfies the basic condition of AuNPs as a contrast agent for CT. At the low concentration of HAuCl₄, the particle size was reduced noticeably. The red–shift of the plasmon absorption band in the UV–VIS spectra provided an information of increase in the size of PEI–AuNPs and the nanoclusters. The XRD peaks corresponding to those of the gold particles appeared at the X–ray diffraction pattern of PEI–AuNPs. The cell viability of 98% was observed in the case of PEI–AuNPs, while PEI was cytotoxic. The results obtained from this study suggest that the PEI–AuNPs are feasible as a contrast agent for CT. In addition, the PEI–AuNPs are expected to extend the utility in the biological labeling and the cell recognition since PEI has a number of reactive amino groups which is available for attachment of the luminescent materials such as quantum–dots and dyes for optical molecular imaging.

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