A Versatile Route to Controlling Gold Nanostructures and Their Applications on Biosensing, Cytotoxicity, and Cellular Uptake Studies

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Introduction

Noble metal nanoparticles, especially of gold and silver, have attracted substantial interest recently because of their unique size-dependent properties.[1] The various applications of noble metal nanoparticles follow from their unique structural properties at nanometer dimensions. These properties can be tailored for potential applications by properly controlling the size and shape of the nanomaterials using seed-mediated growth method in the presence of a surfactant. Preferential adsorption of cetyltrimethylammonium bromide (CTAB) to the different crystal faces of gold leads to the inhibition of growth perpendicular to the long axis of the rods.[2] The growth mechanism of gold nanorods in the presence of CTAB has been widely studied to determine the influence of various experimental parameters. Among all, gold nanorods are found to be more popular and useful for potential applications such as biochemical sensing, biomedical diagnostics, and therapeutics due to possible tuning of their surface plasmon resonance in the visible and near infrared region, which is the potential window of the electromagnetic spectrum for *in vivo* applications.

This work demonstrates a versatile route for the controlled synthesis of gold nanoparticles that could alter the resulting products into desired shapes by utilizing a method similar to that described elsewhere.[3] It need to investigate fundamental aspects of synthetic conditions, such as the amount of growth solution, the introduction of foreign ions, and the reaction temperature, to propose a mechanism of the fabrication of these multishaped gold nanostructures. The other objective is to study the cytotoxicity, cellular uptake, and biosensing properties of bioconjugated gold nanorods, which will be useful for future biomedical applications. Bioconjugated nontoxic gold nanorods were utilized as molecular probes for the detection of goat IgG by the localized surface plasmon resonance (LSPR) method. The in vitro cell viability and cellular uptake studies were performed by using normal human gingival epithelioid cells (S-G) and oral cancer cells isolated from a Taiwan patient (TW 2.6).

Experimental

Materials

Hydrogen tetrachloroaurate(III) hydrate, trisodium citrate dehydrate (99%), silver nitrate (99%), ascorbic acid (AA) (99%), and cetyltrimethylammonium bromide (CTAB) (99%) were obtained from Acros Organics and used without further purification. PSS with MW 14 000 was purchased from Alfa Aesar. The peroxide-conjugated AffiniPure donkey anti-goat IgG and pure goat IgG molecules were purchased from Jackson Immuno Research Laboratories, Inc.

Aqueous 1% trisodium citrate (0.35 mL) was added to 10 mL of 0.25 mM aqueous $HAuCl_4$. After the solution had been stirred for 3 min, 0.3 mL of ice-cold, freshly prepared 0.01 M aqueous $NaBH_4$ was added, and then the solution was stirred for 5 min. The seed solution was maintained at room temperature for ~2 h before use. An aqueous solution of 0.1 M CTAB, 0~0.04 mM $AgNO_3$ and 0.25 mM $HAuCl_4$ was used as the growth solution. This solution was stored at 27 °C throughout the experiment. Gold seeds (0.1 mL) were placed in a beaker. Three volumes (1, 10, and 100 mL) of growth solution were mixed with 0.06 mL (first), 0.6 mL (second), and 6 mL (third) of freshly prepared ascorbic acid solution (10 mM), respectively. The growth solution became colorless when the ascorbic acid solution was added. These three colorless solutions were added to the quiescent gold seed solution stepwise in intervals of 30s.

Apparatus and Procedures

The UV/visible spectra of the colloidal nanoparticle solution were obtained using a SHIMADZU UV-1700 spectrophotometer. The TEM images were captured using a JEOL JEM-2010 electron microscope.. Typically, the surface was scanned at 2 Hz at a resolution of 256 lines per image and a set point of 1.4 - 4.0 V. Normal human gingival epithelioid cells (S-G) and oral cancer cells isolated from a Taiwan patient (TW 2.6) were used for cytotoxicity and cellular uptake studies. Cell cytotoxicity was evaluated by the colorimetric MTS assay. Briefly, the cells (2000 cells/90 μL) with 10 μL of gold nanorods were seeded into different wells and were incubated for 72 h followed by the addition of 20 mL of MTS in each well. The optical density (OD) of the resultant solution was determined (λ) 490 nm) by using a microplate absorbance reader (SpectraMAX 340pc, Molecular Devices, California).

Results and Discussion

Fig. 1 a-c present the products prepared in the absence of silver ions. Figure 1a indicates that the major products were spherical particles if the first addition of the growth solution was initially added to the seed solution, but a few short nanorods were obtained because a few gold ions were available. Following, the second and third growth solutions were used to grow the nanorods and the mean lengths were approximately 59 and 570 nm, respectively. When silver ions (0.004 mM) were introduced to the reaction system, the product changed from 1D nanorods to bipyramids in Fig. 1 d-f. A comparison with Fig. 1 a indicates that numerous bipyramids formed upon the first addition of growth solution, indicating that silver ions are crucial to the growth of bipyramids. Accordingly, the attempt to increase the aspect ratio of nanorods by increasing the silver ion content (0.04 mM) was unsuccessful. However,

the resulting product was dramatically altered as more silver ions were introduced into the reaction system. Following the first addition of growth solution to grow the seeds, the resulting products contained irregularly faceted particles and bipyramids (Fig. 1g). Specifically, the morphology changed with the inclusion of irregularly faceted particles and bipyramids. The surfaces of the irregularly faceted particles and bipyramids became very rough. The already nucleating small gold cluster developed on an otherwise smooth facet, and some small tips and islands were obtained upon the surfaces of irregular particles and bipyramids. (Fig. 1 h and 1 i)

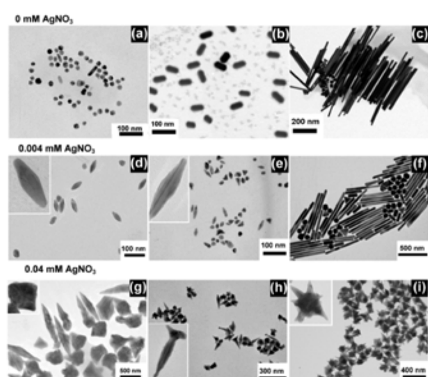

Fig. 1 TEM images of gold products synthesized under three conditions: (a-c) in the absence of silver ions; (d-f) in the presence of 0.004 mM silver ions; (g-i) in the presence of 0.04 mM silver ions.

A series of experiments was performed to investigate the effect of the temperature on the formation of gold nanocrystals. Fig. 2 shows the corresponding TEM images of the products. As the reaction temperature is increased, the yield of nanorods generally declined. Figure clearly presents triangular plates, hexagonal and spherical particles. A significant number of triangular plates with other shapes were observed at reaction temperatures of 40 ~ 60 °C. Most of the products were triangular, with an average size of 134 ± 11 nm below the reaction temperature of 60 °C.

Owing to cytotoxicity of CTAB, it is important to mask the CTAB layer for future biomedical applications. The CTAB-coated gold nanorods were further covered with PSS by electrostatic interactions. CTAB-stabilized gold nanorods showed a positive charge on the surface due to the presence of quaternary amine hydrophilic head groups from adsorbed CTAB, whereas PSS-capped gold nanorods showed a negative charge on the surface due to the presence of anionic SO_3 groups. The bioconjugation of gold nanorods and the detection of IgG are represented schematically in Figure 3.

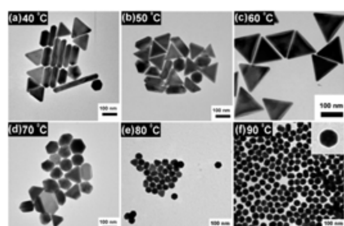

Fig. 2 (a-f) TEM images of gold nanocrystals synthesized at various temperatures as labeled in each image.

Fig. 4 shows the cell viability results of S-G and TW 2.6 cells after 72 h of exposure to CTAB-capped gold nanorods and PSS-capped gold nanorods. Similar tests were also carried out with the CTAB-capped φ-shaped (fusiform) gold nanoparticles for comparison. It was observed that CTAB-capped gold nanorods and CTAB-capped φ-shaped nanoparticles exert a higher cytotoxic effect than PSS-capped gold nanorods on both the cell lines, suggesting that PSS coating on the surface of the gold nanorods significantly decreases the cytotoxicity of the nanorods. Thus, the cellular uptake and cell viability studies suggest that the PSS-capped gold nanorods are suitable for in vivo applications.

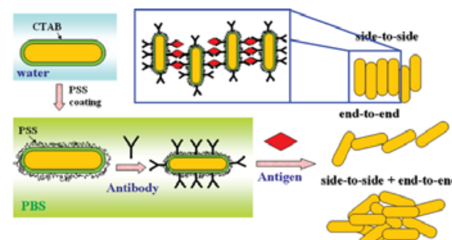

Fig. 3 Schematic representation of bioconjugation of gold nanorods and the detection of g-IgG through the aggregattion of gold nanorods.

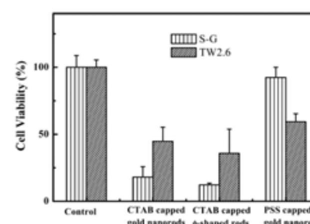

Fig. 4 Comparison of cell viability assays for CTAB-capped gold nanorods and φ-shaped gold nano particles, and PSS-capped gold nanorods after treatment with S-G and TW 2.6 cells for 72 h.

Conclusion

The morphological, structural, and spectral changes involved in the seed-mediated growth of gold nanostructures in the presence of CTAB were systematically investigated. A versatile route for the synthesis of gold nanostructures that allows the morphology of the products to be changed markedly by varying the experimental conditions was demonstrated. Gold nanorods synthesized by the seed-mediated method in the presence of CTAB have been stabilized using PSS, conjugated to the antibodies, and characterized for cytotoxicity, cellular uptake, and detection of protein, IGg.

References

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