Synthesis of Gold Microstructures with Surface Nanoroughness Using a Deep Eutectic Solvent for Catalytic and Diagnostic Applications

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We synthesized highly monodisperse gold microparticles (AuMPs) using a deep eutectic solvent (DES) which composed of choline chloride and malonic acid as both a reaction medium and structure-directing agent. These microparticles exhibit distinctive surface nanoroughness and highly defined diameters that can be precisely controlled over a range of a few micrometers under different reductive conditions. The internal and external structures of the particles are thoroughly investigated by electron microscopy, which is further analyzed in association with their optical properties. We also investigate the gold microparticle concentration-dependent catalytic property employing a reductive reaction of 4-nitrophenol to 4-aminopenol as a model system. Importantly, the gold microparticles are densely functionalized with DNA and reversibly assemble with DNA-gold nanoparticle conjugate probes for the colorimetric detection of target DNA sequences, demonstrating that these novel structures can be utilized as platforms that quickly regulate the optical properties of plasmonic nanoparticles for diagnostic applications.

Keywords: Gold Microstructures, Deep Eutectic Solvent, DNA, Catalysis, Sensing.

1. INTRODUCTION
Syntheses of spherical gold microstructures, or gold microparticles (AuMPs), whose diameter spans a range of a few micrometers have attracted researchers’ interest in particular areas. For example, these particles play important roles in biolistic delivery of nucleic acids into cells,1 and in coating for larger electrode surface area.2 These usages are based on the unique properties of the AuMPs such as negligible cellular toxicity and electrochemical activity, which could find further practical applications under various conditions. In contrast to gold nanoparticles (AuNPs), however, the AuMPs have been far less investigated, primarily because of the synthetic difficulties lying in the way of controlling the size, shapes, and their distributions of AuMPs. To date, a number of materials and methods have been reported for the synthesis of AuMPs, such as polyvinylpyrrolidone (PVP),3 photoreduction with halide ions,4 and slow reduction in organic5 or aqueous6 solutions. Unfortunately, however, they have to suffer from poor shape- or size-dispersity, slow kinetics, or incapability of controlling the particle size. Therefore, the development of a new synthetic method for monodisperse AuMPs with controllable sizes would be significant progress in material chemistry.

Deep eutectic solvent (DES) is a form of ionic liquid composed of eutectic mixtures between organic halide salt and complexing chemicals which form hydrogen bonds with the anion.7,8 Compared to the conventional ionic liquids, DES is non-reactive with water, biodegradable, and relatively non-toxic, all of which contribute to its potential excellence in various applications, particularly associated with materials synthesis. In fact, it has been used in the synthesis of zeolite analogues,7 polymers,10 metal oxide nanostructures,11 nanoporous silver films,12 and AuNPs.13,14 DES is also ideal anhydrous media for the structural investigation of oligonucleotides.15 Based on these previous studies on DES as a medium for materials synthesis, the investigation of DES for the efficient synthesis of AuMPs would be of substantial importance.

Herein, we present a novel synthesis of AuMPs using the DES composed of choline chloride and malonic acid. This method takes advantage of the DES as reaction
media for the formation of spherical microparticles with controlled sizes and surface roughness. Importantly, this synthesis does not rely on any surfactants or polymers, suggesting the essential role of DES as a structure-directing reagent and particle stabilizer. Importantly, to the best of our knowledge, this work is the first synthesis of the AuMPs based on the chemical properties of DES, which exhibit advantages over the conventional methods.

2. EXPERIMENTAL METHOD

2.1. Materials
Malonic acid (99%; cat. #M1296), choline chloride (98%; cat. #C1879), tetrachloroauric acid trihydrate (HAuCl₄; 99.9%; cat. #520918), ascorbic acid (cat. #A5960), 4-nitrophenol (cat. #241326), sodium borohydride (NaBH₄; 99.99%; cat. #480886) and sodium dodecyl sulfate solution (SDS; 10%; cat. #L4522) were purchased from Sigma-Aldrich (USA) and used as received. Ultrapure water (18.2 MΩ-cm, Direct-Q3 system) was used for the reactions. The HPLC-purified DNA probe sequences (TAACAATAATCCCTCA10-SH 3′) were purchased from Genotech (Daejeon, Republic of Korea).

2.2. Preparation of the Deep Eutectic Solvent (DES)
The deep eutectic solvent (DES) was prepared following the literature method using malonic acid and choline chloride. Malonic acid and choline chloride in powder forms were combined at the same molar ratio, and mixed with a spatula at 80 °C for a couple of hours. Eventually the powder appeared to melt down, forming a clear, colorless, and viscous liquid. The resultant DES was allowed to cool down at room temperature.

2.3. Synthesis of Gold Microparticles (AuMPs)
For the synthesis of gold microparticles (AuMPs), two hundred microliters of aqueous HAuCl₄ solution (0.15 M) was combined with 20 mL of DES and homogeneously mixed at 50 °C, to which 200 μL of aqueous ascorbic acid solution (0.1 M) was injected. After the mixture was stirred for 3 hours at 50 °C, it turned colorless from yellow, indicating the reduction of Au³⁺ into Au⁰ and further metallic Au. The mixture was further stirred for 12 hours at 25 °C to complete the reaction and then undisturbed overnight. The precipitates were obtained and washed three times with pure water, and finally dispersed in pure water. The final [AuMP] was adjusted to the 40 times of its original concentration, and was considered as ‘1×’.

2.4. Reductive Reaction of 4-Nitrophenol to 4-Aminophenol with AuMPs
Various concentrations of the AuMP solutions (1×, 0.8×, 0.6×, 0.4×, 0.2×, 0×) were combined with the solutions containing 4-nitrophenol (4.5 mM) and freshly prepared sodium borohydride (88 mM). As the reaction proceeded, the color of solutions turned colorless from yellow, which was monitored by UV-vis spectroscopy.

2.5. Synthesis of DNA-Functionalized AuMPs (DNA-AuMPs)
The DNA-functionalization of the AuMPs was conducted following a literature published by our group.16 In brief, the monothiol DNA sequences were deprotected in a 0.10 M DTT solution (0.17 M phosphate buffer, pH 8.0), and purified using a NAP-5 column. The purified DNA solution (67.5 nmol) was combined with the AuMP solution (20 mL, 1×) and the mixture was buffered to 0.15 M NaCl in a phosphate buffer (0.01% SDS, pH 7.4, 10 mM phosphate). The final mixture was incubated overnight and washed by centrifugation (13000 RPM for 20 min) three times with a buffer (0.01% Tween 20, pH 7.4, 10 mM phosphate).

2.6. DNA Detection Using DNA-AuMPs
The DNA-functionalization of the AuMPs was conducted following a literature published by our group.17 A solution containing both the DNA-AuMPs and DNA-AuNPs was combined with Target at various concentrations from 0.1 to 100 nM (1.0 M NaCl, 0.01% Tween 20, pH 7.4, 10 mM phosphate). The hybridization was allowed to proceed for 15 min, during which the color change of the reaction mixtures occurred in turn from the one containing the highest [Target]. The assembly of the DNA-AuMPs and DNA-AuNPs was observed by SEM (Hitachi S-4800).

3. RESULTS AND DISCUSSION
The synthesis of the AuMPs typically begins with the preparation of DES. In our work, choline chloride and malonic acid were employed at the same molecular ratio, whose melting temperature is supposed to be 10 °C, thus existing as liquid at room temperature. Because the solubility of gold salt and ascorbic acid in the DES is not sufficient, their concentrated aqueous solutions (a few hundreds microliters to a few milliliters) were prepared first and injected to the larger amount of DES (20 mL), to form a homogeneous DES solution. Once the AuMPs were synthesized, we first analyzed their morphologies using scanning electron microscopy (SEM). The AuMPs synthesized with 200 μL of ascorbic acid solution (0.1 M) were highly monodisperse, whose diameter is ~3 μm (Fig. 1(A)). Interestingly, the surface of these AuMPs was noticeably rough. As the amount of the ascorbic acid solution increased to 2 mL and 4 mL, the diameter of the AuMPs systematically decreased to 2 μm and further 1 μm, respectively (Figs. 1(B) and (C)). The smaller AuMPs obtained with the higher concentration of ascorbic acid indicate that the synthesis of the AuMPs is based on the typical ‘nucleation-growth’ mechanism, where more
nuclei are generated by higher concentration of reductant to grow into smaller particles. These AuMPs were also observed by transmission electron microscopy (TEM) for the investigation of the internal structures, which could suggest the fully packed, solid spherical structures without the internal vacancy (Fig. 1(D)). The UV-vis spectra of the AuMPs also demonstrate an obvious size-dependence, which exhibits the red-shift of the wavelength where the maximum extinction takes place (Fig. 2(A)), which was further confirmed by slicing the AuMP at the center using focused ion beam, and directly observing the cross-section using SEM (Fig. 2(B)). The AuMPs were also proven to be composed of only gold without any other metallic element by energy-dispersive X-ray spectroscopy (Fig. 2(C)).

To further investigate the effect of the reaction temperature on the synthesis of AuMPs in DES, we synthesized the AuMPs ($D = 1 \mu m$) in DES with the appropriate amount of ascorbic acid (4 mL) at elevated temperatures such as 70 and 90 °C (Figs. 3(A) and (B), respectively), and observed the morphologies of the resultant materials. At 70 °C, we obtained isotropic, smaller particles whose diameter is almost 100 nm, indicating that elevated temperature could accelerate the initial nucleation process to result in a higher number of the seeds and thus smaller particles based on the seed-growth mechanism. On the other hand, 3-dimensionally networked gold nanostructures were obtained at 90 °C. This result could be explained by the highly temperature-dependent chemical and physical properties of DES, which plays a key role as a shape-directing reagent. In addition, we investigated how the order of the chemical addition into the reaction mixture affected the resultant structures. Unlike our standard synthetic conditions in which the ascorbic acid is combined with the gold salt-DES solution, gold salt was subsequently added to the ascorbic acid-DES solution, and resulted in planar structures whose diameter is about 2 to 5 μm (Fig. 3(C)). Additionally, the effect of the increased amount of water up to 50% on the particle shape formation was also investigated, which leads to giant polyhedral with various shapes (Fig. 3(D)).
Considering the surface roughness the AuMPs and their consequent large surface area, we could expect the catalytic activity of the AuMPs. To evaluate the catalytic properties of the AuMPs, we prepared six mixtures containing 4-nitrophenol (4.5 mM), sodium borohydride (88 mM) and the AuMPs at different concentrations (0×, 0.2×, 0.4×, 0.6×, 0.8×, and 1×). At the beginning, all the mixtures were yellow owing to the strong absorption of 4-nitrophenol at ~400 nm, as observed by UV-vis spectroscopy (Fig. 4(A), inset). As the reaction went on, however, the color of the 1× turned colorless first in half an hour (Fig. 4(A)). This color change implies that 4-nitrophenol was completely reduced to 4-aminophenol in the presence of AuMPs, suggesting that AuMPs have similar catalytic properties as AuNPs do. Subsequently, the 0.8×, 0.6×, 0.4×, and 0.2× also sequentially turned colorless in turn at different reaction rates, respectively (Fig. 4(B)). The strong dependence of the catalytic activity of the AuMPs on their concentration evidently demonstrates that the AuMPs possess high potential for their catalytic applications.

We further investigate the diagnostic applications of the AuMPs for the detection of DNA targets. Importantly, we took advantage of the plasmon-regulating capability of the AuMPs, which is based on the distance-dependent optical properties of the plasmonic nano- and micromaterials. For the given sequence of the DNA target, we designed two probe sequences: one is complementary to the half of the target and for the AuMPs, and the other to the other half of the target for the AuNPs. In the presence of the target DNA, therefore, the AuNP probes are hybridized onto the surface of the AuMPs via DNA–DNA interconnect formation with a concomitant extinction decrease, whose [target]-dependence in association with the hybridization time is demonstrated in Figures 5(A) and (B). We further observed the hybridized AuNPs on an AuMP using SEM (Fig. 5(C)).

4. CONCLUSIONS

We developed a facile synthetic method for the size-controlled spherical AuMPs in a DES. Significantly, these AuMPs exhibit distinctive structural, optical, and catalytic properties, which would be of potential importance for other chemical and biological applications. As an example, a diagnostic application of the AuMPs for the colorimetric detection of DNA targets was demonstrated. The synthetic strategy in this work will be further investigated for the synthesis of anisotropic gold structures in a DES with assistance of other surfactants or polymers.

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References and Notes

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