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Research Article

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Controllable microstructure of Au nanoparticle-DNA oligonucleotide conjugates

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ABSTRACT

The Au nanoparticle (AuNPs) and DNA oligonucleotide conjugates will have complicated microstructures because one AuNP can be conjugated to more than one single-stranded DNA (ssDNA) on its surface. In this paper, the AuNPs-DNA conjugates with controllable microstructure were prepared on SiO_2 microsphere carriers. The reactions between AuNPs with ssDNA on SiO_2 carriers were sterically restricted because only out part surface of AuNPs on carriers could react with ssDNA. When two kinds of AuNPs with different diameters, 15 nm and 50 nm, were conjugated with two complementary ssDNA, cleaved from carries and hybridized each other, the AuNPs-DNA conjugates were prepared. The TEM results indicated that when the particle size of AuNPs was about 50 nm, 1 AuNP could be conjugated to 2 ssDNA on its surface, while 1 AuNP (15 nm) particle was only conjugated to 1 ssDNA on its surface.

Keywords: Au nanoparticles, DNA, Conjugates, Microstructure

INTRODUCTION

Colloidal nanoparticles of metals and semiconductors have potentially useful optical, optoelectronic and material properties because of their small (nanoscopic) size [1, 2]. These properties might lead to applications including chemical sensors, spectroscopic enhancers, quantum dots and nanostructure fabrication, and micro-imaging methods [1]. A great deal of control can now be exercised over the chemical composition, size and polydispersity of colloidal particles, and many methods have been developed for assembling them into useful aggregates and materials [2]. Gold nanoparticles (AuNPs) are widely used in gene delivery [3], vivo cell labeling [4] and imaging [5] because they are bioinert, nontoxic and easily synthesized and functionalized [6, 7]. And they also can be as a multifunctional platform for both therapeutic and diagnostic purposes [8, 9]. Finally, AuNPs can be accumulated preferentially at tumor sites by proper functionalization, which provide a powerful tool for cancer gene therapy [10].

The concept of DNA-directed self-assembly was proposed in 1996 [11, 12] and gained extremely high popularity [13]. DNA is an ideal template for the formation of nanoparticle clusters due to its ability to form well-defined secondary and tertiary structures and its similarity in size to nanoparticle clusters. When the single-stranded DNA (ssDNA) has thiol group at the terminus, it can react directly with the surfaces of the AuNPs [14]. The conjugates of DNA with AuNPs have already used as building blocks for analytical and bioanalytical detection strategies, including schemes based on their high density, their ability to bind large numbers of probe molecules, and their optical properties [15]. Although the DNA-directed assembly of building blocks has been investigated for over a decade, the control of nanobuilding blocks into controllable discrete microstructure still remains a key challenge [16]. Alivisatos *et al*. developed an anisotropic approach to allow one to functionalize nanoparticles with as few as one oligonucleotide per particle [14, 17]. Although this was an important step forward in anisotropic functionalization of nanoparticle, it has been limited to very small particles and typically leads to mixtures of products that must be separated by electrophoretic means [18]. Mirkin *et al*. functionalized AuNPs with two different types of oligonucleotides in a site-specific manner by using a magnetic sphere as a geometric restriction

template and obtained the DNA-AuNPs nanoparticle conjugates with cat paw, satellite, and dendrimer-like microstructures [18].

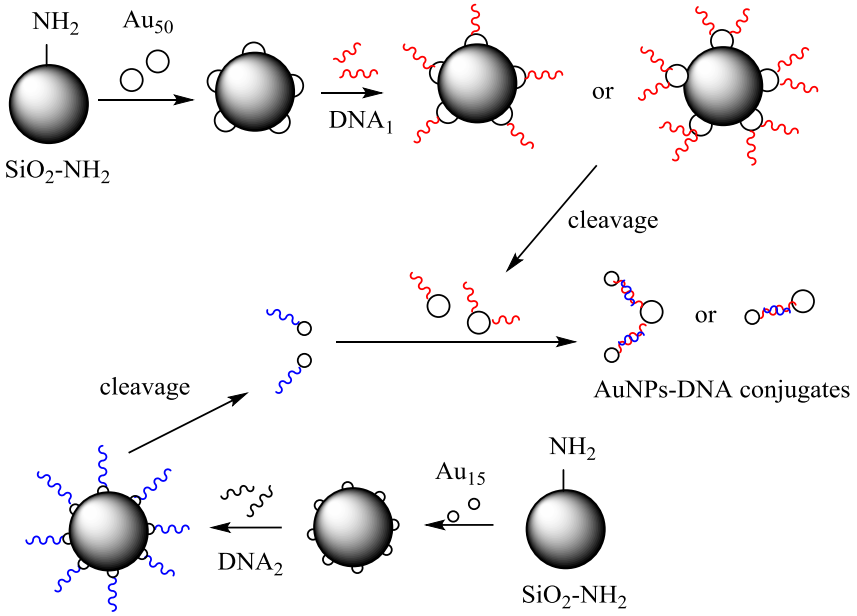

Fig. 1 Scheme of AuNPs assembly method and microstructure of AuNPs-DNA conjugates

In this paper, a method of controlled microstructures by solid-phase organic synthesis on SiO_2 microspheres is presented. Microspheres are sterically stable by the solvent-swollen gel surface layer in good solvents [19], therefore, AuNPs could be partly embedded into the solvent-swollen surface gel layer of carriers, which would hinder and control the consequent self-assembly of DNA on AuNPs. Therefore, when AuNPs with different particle sizes, 15 nm (Au_{15}) and 50 nm (Au_{50}), were conjugated with complementary ssDNA respectively and hybridized each other, the microstructure of DNA-AuNPs nanoparticle conjugates can be controllable (Fig. 1).

EXPERIMENTAL SECTION

2.1. Materials

Tetraethyl orthosilicate ($Si(OEt)_4$, TEOS) was purchased from Tokyo Kasei Kogyo Co., Ltd. Ammonium (25%) was obtained from Tianjin Chemical Reagent Ⅲ (Tianjin, China). (3-Aminopropyl) diethoxymethylsilane (APDEMS) (97%) was availabled from Alfa Aescar Co.. All Other reagents were of analytical grade and used as received without any further purification. Double distilled water was used in all of the experimental processes. Single-stranded DNA (ssDNA) was synthesized by Shanghai invitrogen Biotechnology Co., Ltd, and the sequences were listed as follows:

DNA_1: 5'-HS-TTATAAACTCGTTGGATGCAGGTACG-3'
DNA_2: 5'-TGCATCCAACGAGTTTATAA-HS-3'

2.2. Synthesis and modification of Silica Microspheres

Silica microspheres were prepared according to the classical Stöber method as follows [20]: 12 mL of TEOS was added to the mixture of 200 mL ethanol, 20 mL double distilled water and 16 mL aqueous solution of 25% ammonium with vigorous stirring at room temperature and the reaction was continued further for 24 h with stirring. The silica microspheres modified by APDEMS were afforded by adding silane coupling agent [21, 22]. 0.4 g of APDEMS was added into 19.6 g of ethanol solution containing 0.5 g of silica microspheres and the resulting mixture was incubated for 24 h at room temperature. The solution was purified by repeating centrifugation, decantation, and resuspension in ethanol for three times.

2.3. Preparation of DNA conjugated AuNPs on microspheres

AuNPs (15 nm or 50 nm) were prepared by reducing $HAuCl_4$ with trisodium citrate [23]. The AuNPs (2 mL, 225 μM aqueous solution) were self-assembled on the modified SiO_2 microspheres (0.02 g in 10 mL of ethanol-water solution (4:1 V/V) for 24 h at room temperature under shake. DNA-functionalized AuNPs on microspheres were prepared according to the previously reported procedures [24]. 8 μM of thiolated DNA was incubated with microspheres linkage 15 nm AuNPs (or 50 nm AuNPs) solution at room temperature for 16 h. The solution was

allowed to stand for 40 h after adding 400 μL of 0.1 M NaCl/PBS in the solution. The microspheres with DNA conjugated AuNPs were centrifuged and washed with 0.1 M NaCl/PBS to remove the excess DNA1(or DNA2), and kept in the dark at 4 °C.

RESULTS AND DISCUSSION

3.1. Preparation and modification of SiO_2 microspheres

SiO_2 microspheres prepared by Stöber method [20] have plenty of active hydroxyl groups on the surfaces and can be modified by silane coupling agent, such as (3-aminopropyl) diethoxymethylsilane (APDEMS), to get different surface functional groups easily. Therefore, they are an excellent template for assemblies.

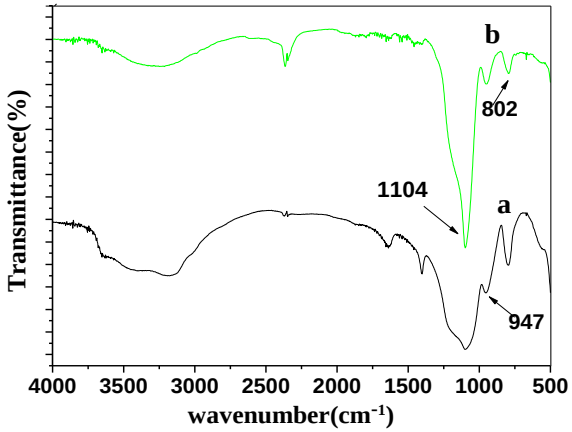

Fig. 2 FT-IR spectra of polymer microspheres
a) silica microspheres; b) APDEMS-modified silica microspheres

The FT-IR spectra of SiO_2 microspheres and NH_2-modified silica microspheres were measured and shown in Fig. 2. The absorption peak at 1104 cm^{-1} and 802 cm^{-1} were respectively attributed to the symmetrical stretching vibration and asymmetrical stretching vibration absorption peak of Si-O-Si in silica microspheres. The absorption at 947 cm^{-1} was the stretching vibration of Si-OH, which indicated that the silica microspheres were modified by silane coupling agent.

3.2. Preparation of DNA conjugated AuNPs (Au_{50}-DNA_1 and Au_{15}-DNA_2)

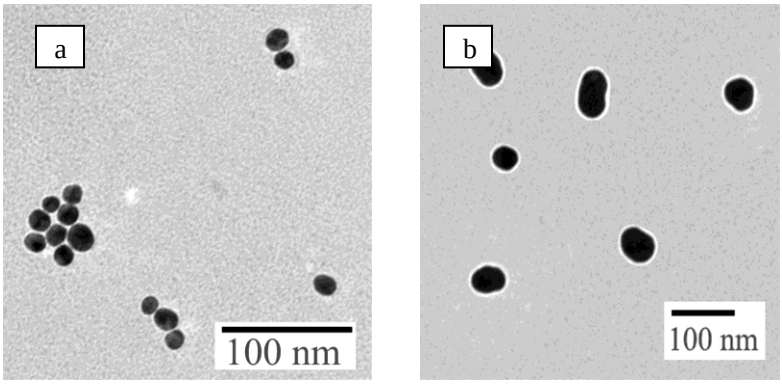

Fig. 3 TEM image of AuNPs
a) 15 nm; b) 50 nm

AuNPs were prepared using trisodium citrate as the reducing reagent. The average particle size was about 15 and 50 nm in diameters measured by TEM (Fig. 3). To link with the AuNPs, silica microspheres need to modification with silane coupling reagents (3-aminopropyl) diethoxymethylsilane (APDEMS) to get active amino groups on their surface. Because of the reaction between the Au atoms and the amino groups on the silica microspheres, the AuNPs were easily self-assembled onto the surface of the SiO_2 microspheres (Fig. 4).

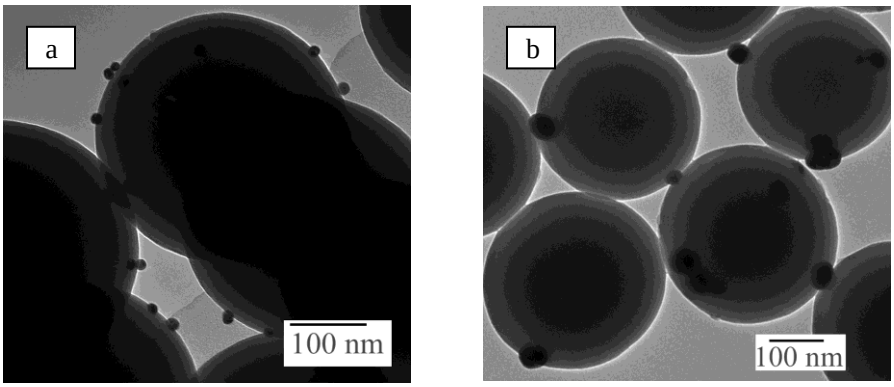

Fig. 4 TEM micrographs of different AuNPs on silica microspheres
a) 15 nm AuNPs; b) 50 nm AuNPs

The results indicated that SiO_2 microspheres were about 370 nm in diameters and had smooth surfaces and AuNPs self-assembling SiO_2 microspheres had a strawberry-like structure. The self-assembly affinity was from the reaction between amino groups and Au atoms, when the lone pair electrons of N atoms inserted into the empty orbital of the Au atoms. The outside part of AuNPs on carriers still had active Au atoms which could be reacted with thiol-terminated ssDNA because of the Au-S bond formation [12]. The deconstruction characteristics of Au_{50}-DNA_1 and Au_{15}-DNA_2 from carriers were explored under different pH conditions by measuring the UV-vis absorbance of the supernatant after ultracentrifugation at 260 nm. The results indicated that both Au_{50}-DNA_1 and Au_{15}-DNA_2 were mostly released from the SiO_2 microsphere surface when pH of solution was about 14.

3.3. Microstructures of DNA-AuNPs conjugates

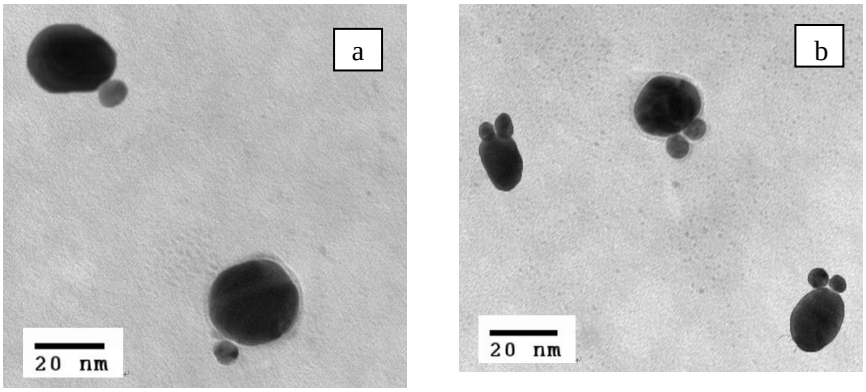

Fig. 5 TEM micrographs of DNA-AuNPs conjugates
a) Au_{50}-DNA_1:Au_{15}-DNA_2=1:1 (mol:mol); b) Au_{50}-DNA_1:Au_{15}-DNA_2=1:5 (mol:mol)

Mirkin and co-workers highlight particle size as an additional key variable: as the curvature of the Au surface increases, it is possible to pack considerably more oligonucleotides into a given area [25]. In this work, the amount of ssDNA on AuNPs could be under control because the active surface area of AuNPs on carriers was restricted. Therefore, when the Au_{50}-DNA_1 and Au_{15}-DNA_2 were released from SiO_2 microspheres and hybridized each other, the microstructures of DNA-AuNPs conjugates could be controllable. The TEM micrographs of DNA-AuNPs conjugates were shown in Fig. 5 when the ratio of Au_{50}-DNA_1 and Au_{15}-DNA_2 was 1:1 and 1:5 (mol/mol, according to the concentration of ssDNA). The results showed that the DNA-AuNPs conjugates could had two microstructures (A_{50}:A_{15}=1:1 or A_{50}:A_{15}=1:2), which indicated that each Au_{50} particle was conjugated to 2 ssDNA on surface because of a large surface area, while each Au_{15} particle was conjugated to only 1 ssDNA on its surface.

CONCLUSION

A controlled method of Au Nanoparticles-DNA (AuNPs-DNA) conjugates was presented in this paper, when the AuNPs-DNA conjugates were prepared on SiO_2 microspheres. The results indicated that when the diameter of AuNPs was about 50 nm, each AuNP could conjugated to two single-stranded DNA (ssDNA), while the diameter of AuNPs was about 10 nm, each AuNP could only conjugated to 1 ssDNA.

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