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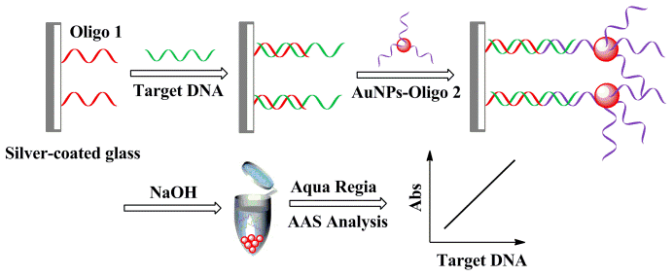

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ARTICLE TYPE

Sequence-Specific Recognition of Single-Stranded DNA with Atomic Absorption Spectroscopy

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Sequence-specific recognition of single-stranded DNA (ssDNA) with oligonucleotide modified gold nanoparticles and silver-coated glass was established by using atomic absorption spectroscopy (AAS). Oligonucleotide 5'-SH-T_{10}-TCT CTC CCA GGA CAG G-3' (Oligo 1) modified silver-coated glass was used as separation material, oligonucleotide 5'-CAC AAA CAC GCA CCT C-T_{10}-HS-3' modified gold nanoparticles (AuNPs-Oligo 2) acted as signal report component. Upon addition of target DNA (5'-GAG GTG CGT GTT TGT GCC TGT CCT GGG AGA GA-3'), it could hybridized with Oligo 1 on the surface of silver-coated glass and Oligo 2 on the surface of AuNPs. Thereby, the concentration of target DNA could be transformed into the number of AuNPs that bound to the surface of silver-coated glass, which could be detected by using atomic absorption spectroscopy. And the integrated absorbance was proportional to the concentration of target DNA over a range of 10.0 nM - 200.0 nM with a limit of detection of 0.23 nM (3σ/slope). The sensitivity of the assay could be further improved by using layer-by-layer -technique. Moreover, all processes were characterized with scanning electron microscope (SEM), contact angle (CA) and X-ray photoelectron spectroscopy (XPS) as well.

Introduction

Sequence-specific recognition of DNA is of importance and has attracted much attention because of its potential application in genetics, pathology, criminology, pharmacogenetics and food safety.[1] Therefore, a lot of methods have been established by using colorimetric method,[2,3] chemiluminescence,[4-6] fluorescence,[7,8] surface-enhanced Raman scattering spectroscopy,[9,10] electrochemistry,[11,12] dynamic light scattering[13,14] and mass spectrometry.[15,16] However, to the best of our knowledge, atomic absorption spectroscopy has not been applied to establish DNA sensor.

AAS was mainly used to detect metal and metalloid, it had good sensitivity and selectivity. Nucleic acid was composed of C, H, N, O, P and S, which was hardly to be detected with AAS, thereby it was difficult to investigate nucleic acid with AAS directly, suitable labels was inevitable to solve this problem.[17] Two main problems need to be solved during the process of DNA recognition. One is how to tag the DNA with metal element, the other is how to separate the hybridized DNA from free section.

Oligonucleotide modified glass was usually applied to separate the binding biomolecules from free ones, and a series of methods have been reported about the functionalization of glass surface. DNA could be modified on glass surface by using physical absorption, chemisorption, covalent immobilization and other methods.[18-22] The common approach was covalent binding of thiol-modified or amino-modified[23-25]oligonucleotide on the surface of organo-silane coated glass. The preparation of organo-silane coated glass must be controlled carefully, and multi-step was needed for preparation of DNA modified glass, which was complex and tedious. Thiol-modified oligonucleotide could be modified on the surface of gold film as well.[26-28] Silver-coated glass could be prepared easily by using silver mirror reaction.[29,30] Moreover, thiol-functionalized oligonucleotide could self-assembled on the surface of silver surface through Ag-S bond.[31,32] Therefore, thiol-functionalized oligonucleotide could be immobilized on the surface of silver-coated glass, which might be an ideal separation material for the assay.

Gold nanoparticles have specific photonic properties, and thiol-functionalized DNA could be assembled on its surface through Au-S bond. For that reason, a lot of DNA sensors were reported.[33-37] The concentration of gold in the solution of gold nanoparticles could be determined by using atomic absorption spectroscopy, thereby gold nanoparticle might be an ideal label for the research. Herein we explore the possibility of sequence-specific recognition of single-stranded DNA with silver-coated glass and gold nanoparticles by using atomic absorption spectroscopy.

Experimental section

Reagents

Quartz glass slides (10 mm ×10 mm ×1mm) were purchased from Guangliang High-tech Co. (Jiangsu, China). Silver nitrate, ammonia (25%) and glucose were purchased from Sinopharm chemical Reagent Co., Ltd. (Beijing, China). Tri-(2-carboxyethyl) phosphine (TCEP) and sodium citrate were purchased from Sigma-Aldrich Co. All oligonucleotides were synthesized by

Sangon Biotech Inc. (Shanghai, China). PBS buffer (pH 7.0, 10 mM) and acetate buffer (pH 5.0, 10 mM) were used in our experiments. Deionized water was used for all experiments. All the chemicals were analytical grade.

Apparatus

Centrifugation experiments were carried out on Anke GL-20G-II centrifuge (Anting Scientific Instrument Factory, Shanghai, China). UV-vis absorption spectra were obtained by a TU-1901 double-beam spectro-photometer (Beijing Purkingje General Instrument Co. Ltd, China). The surface morphology and composition were investigated by an S-4800 scanning electron microscopy (Hitachi, Japan). The surface composition and chemical state of the samples were recorded by an ESCALAB250 X-Ray photoelectron spectroscopy (Thermo-VG Scientific, USA). Water contact angle was performed by a DSA 100 drop shape analysis system (Krüss, Germany). All the samples for SEM, CA and XPS were vacuum dried at 40 ℃ for two days prior to the measurement.

Atomic absorption spectroscopy was measured with Z-2000 series polarized Zeeman atomic absorption spectrophotometer (Hitachi, Japan), equipped with a Hitachi 7J0-885 graphite tube. The measurement was performed using a gold hollow cathode lamp with a lamp current of 10 mA and a wavelength of 242.8 nm with slit width of 1.3 nm. Injection volume was 20 μL and time constant was 0.1s. The signal was measured by integrated absorbance (peak area), which had better accuracy than that of peak height. 20% NH_4NO_3 was used as the matrix modifier. The temperature program for AAS was listed in Table 1.

Table 1 Graphite furnace temperature program for the assay

Step	Start Temp (℃)	End Temp (℃)	Ramp Time (s)	Hold Time (s)	Gas Flow (mL/min)
Dry	80	140	40	0	200
Ash	400	400	20	0	200
Atomize	2400	2400	0	5	30
Clean	2600	2600	0	4	200
Cool	0	0	0	10	200

Preparation of silver-coated glass and gold nanoparticles

Silver-coated glass was prepared by using traditional silver mirror reaction. The glass slide was ultrasonically cleaned thoroughly with cleanser, hot sodium hydroxide, nitric acid and ethanol solution. Then rinsed with a large amount of ultrapure water and dried in air. To obtain $Ag(NH_3)_2OH$ solution, 1% aqueous ammonia solution was added dropwise into 5.0 ml of 3% silver nitrate solution, accompanied with gentle stirring until most precipitate was dissolved. Subsequently, the glass slide was immersed in the mixture of 400 μl $Ag(NH_3)_2OH$ solution and 800 μl glucose solution (10%) at room temperature for five minutes, then a layer of shining silver mirror was formed on the surface of glass.

Gold nanoparticles were prepared by reducing 1.0 mM $HAuCl_4$ (20 mL) with 38.8 mM sodium citrate (2.0 mL). The concentration of AuNPs was about 11.5 nM according to the result of UV-vis absorption spectrometer based on an extinction coefficient (2.6×108 $M^{-1}\bullet cm^{-1}$), average size of AuNPs was about 14 nm which was obtained from the average of 100 AuNPs in TEM image.

Preparation of Oligo 1 modified silver-coated glass

2.0 OD Oligo 1 was first dissolved into 20 μL 10 mM acetate buffer solution. Then, 8.8 μL 20 mM TCEP was added and kept at 25 ℃ in order to cleave the disulfide bond at the 5' terminal of Oligo 1. 2 hours later, the freshly unprotected Oligo 1 was added into 5.0 ml 10 mM PBS buffer (pH 7.0, with 0.1 M $NaNO_3$). Then the glass was immersed in PBS buffer for 12 h to obtain Oligo 1 functionalized silver-coated glass at room temperature. Finally, Oligo 1 functionalized silver-coated glass was rinsed by a large amount of PBS buffer.

Preparation of oligonucleotides modified gold nanoparticles

AuNPs-Oligo 2 was prepared according to the references.[38,39] 2.0 OD Oligo 2 was firstly dissolved into 20 μL 10 mM acetate buffer. Then 8.6 μL of 20 mM TCEP was added and kept at 25 ℃ for 2 h in order to cleave the disulfide bond at the 3' terminal of Oligo 2. Subsequently, 3.0 ml of 11.5 nM AuNPs was added to above mixture and incubated for about 16 h at room temperature. Consequently, 4.0 M $NaNO_3$ was added stepwise to obtain the final concentration of 0.1 M for the following 44 h. For AuNPs-Oligo 2, excess reagents were removed by centrifugation at 14000 rpm for 25 min. Following removing the suspension, the remained red oily precipitate was washed with PBS buffer. This process was repeated three times. Finally, the red oily precipitate was redispersed in PBS buffer. Oligonucleotides 5'-GAG GTG CGT GTT TGT G-T_{10}-HS-3' modified AuNPs (AuNPs-Oligo 3) was prepared with the same procedure as that for AuNPs-Oligo 2.

Detection of target DNA

In the first step, Oligo 1 functionalized silver-coated glass was immersed in 1.1 ml PBS buffer containing different concentration of target DNA and allowed to hybridize with target DNA for 4 h at room temperature. The Oligo 1 functionalized silver-coated glass was then washed with PBS buffer three times to remove free target DNA. Next, 3.7 nM AuNPs-Oligo 2 solution were reacted with the target DNA that bond on the silver-coated glass for 4.5 h at room temperature. Then the silver-coated glass was rinsed with PBS buffer for three times to remove free AuNPs-Oligo 2. Finally, the formed dsDNA that bond on the surface of silver-coated glass was dehybridized by addition 1.1 ml 50 mM NaOH solution for 1 h. Following, aqua regia was added to neutralize excess NaOH and dissolve AuNPs. Finally, 100 μL 20% NH_4NO_3 was added into the sample to act as matrix modifier and was added to 2.0 ml with water for AAS analysis.

Layer-by-layer amplification

After the first layer of AuNPs was bond on silver-coated glass, Oligo 1 functionalized silver-coated glass was further immersed into the solution of 2.0 nM AuNPs-Oligo 3 at room temperature for 45 min to form the second layer of AuNPs. AuNPs-Oligo 3 hybridized with AuNPs-Oligo 2 which was already linked on the surface of silver-coated glass. The substrate was rinsed with PBS buffer for three times to remove free AuNPs-Oligo 3. Then it was immersed into 2.0 nM AuNPs-Oligo 2 solution for 45 min at room temperature to prepare the third layer of AuNPs. The substrate was rinsed with PBS buffer for three times to remove the free AuNPs-Oligo 2. This hybridization procedure could be repeated to generate multi-layer of AuNPs on the sliver-coated

glass by immersing the silver-coated glass into the solution of AuNPs-Oligo 2 or AuNPs-Oligo 3 alternatively.

Results and discussion

Scheme of the assay

Scheme of the assay was shown in Fig. 1, silver-coated glass was prepared with silver mirror reaction, oligonucleotide 5'-SH-T_{10}-TCT CTC CCA GGA CAG G-3' (Oligo 1) was self-assembled on the surface of silver-coated glass through Ag-S bond and oligonucleotide 5'-CAC AAA CAC GCA CCT C-T_{10}-HS-3' (Oligo 2) was modified on the surface of AuNPs (AuNPs-Oligo 2). Oligonucleotide 5'-GAG GTG CGT GTT TGT GCC TGT CCT GGG AGA GA-3' was designed as target DNA, it could hybridize with Oligo 1 that functionalized on the silver-coated glass and AuNPs-Oligo 2, then AuNPs-Oligo 2 were immobilized on the surface of Oligo 1 functionalized silver-coated glass, the concentration of AuNPs could be detected by using atomic absorption spectroscopy. Therefore, the concentration of target DNA could be transferred into the absorbance of gold element.

Oligo 1 5'-HS-T_{10}-TCT CTC CCA GGA CAG G-3'

Oligo 2 5'-CAC AAA CAC GCA CCT C-T_{10}-SH-3'

Target DNA 3'-AGA GAG GGT CCT GTC CGT GTT TGT GCG TGG AG-5'

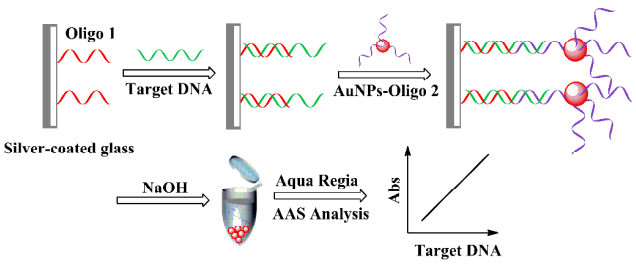

Fig. 1 Scheme for sequence specific recognition of ssDNA with silver-coated glass and AuNPs by using atomic absorption spectroscopy

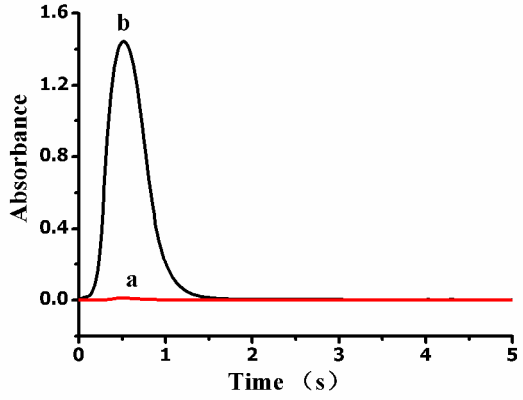

Fig. 2 Atomization curve of gold in the absence (a) and presence (b) of 273 nM target DNA.

To investigate the possibility of the assay, atomization curve of gold was studied. As shown in Fig. 2, there was almost no absorption signal of gold in the absence of target DNA, while there was strong signal of gold element in the atomization curve upon addition of 273 nM target DNA. These results revealed that AAS could be used to develop DNA sensor.

Characterization of silver-coated glass with SEM

Silver-coated glass was prepared by using silver mirror reaction. The morphology of silver-coated glass was observed by using scanning electron microscope under different conditions (Fig. 3). In the absence of target DNA, AuNPs-Oligo 2 could not adsorb on the surface of Oligo 1 modified silver-coated glass, it was almost the same with that of naked silver-coated glass. Upon addition of 0.6 μM target DNA, there were a lot of AuNPs adsorbed on the surface of Oligo 1 modified silver-coated glass, which indicated that hybridization occurred between Oligo 1, Oligo 2 and target DNA. After further addition of 50 mM NaOH solution and kept for 1 hour, most of AuNPs was removed from Oligo 1 modified silver-coated glass, which revealed the occurrence of dehybridization.

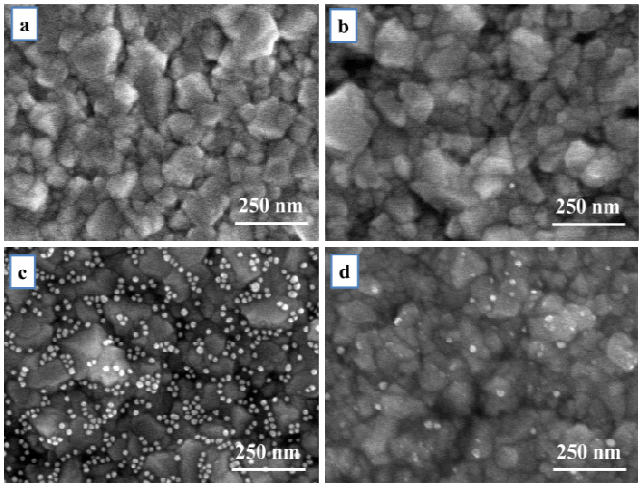

Fig. 3 SEM images of silver-coated glass under different conditions. (a) Naked silver-coated glass; (b) Oligo 1 functionalized silver-coated glass and AuNPs-Oligo 2 in the absence of target DNA; (c) Oligo 1 functionalized silver-coated glass and AuNPs-Oligo 2 in the presence of 0.6 μM target DNA; (d) c + 50 mM NaOH solution. All free AuNPs-Oligo 2 had been washed away from the surface of silver-coated glass.

Characterization of silver-coated glass with CA

Nucleic acid is hydrophilic, while metallic silver and gold are relatively hydrophobic, so surface wettability reflects the surface structure and composition, which could be determined by water contact angle (Fig. 4).[40] Silver-coated glass was prepared by using silver mirror reaction, its CA was 100.2°. After modified with Oligo 1, the CA decreased sharply to 36.9°, which indicated that hydrophilic DNA had been assembled on the surface of silver-coated glass successfully. The CA further decreased to 33.9° upon addition of target DNA, which might due to the DNA hybridization. In the presence of AuNPs-Oligo 2 and target DNA, sandwich structure would form. AuNPs-Oligo 2 were linked to the top of the surface, the linked AuNPs could change the hydrophobicity and the CA changed to 41.5°.[41]

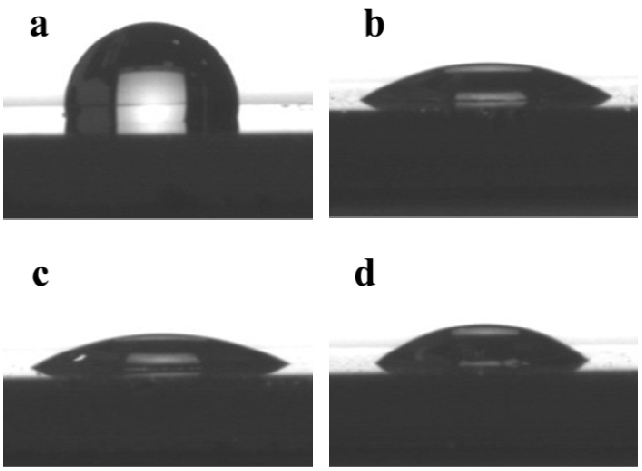

Fig. 4 Water contact angle of different surface. (a) Silver-coated glass, 100.2°; (b) Oligo 1 modified silver-coated glass, 36.9°; (c) Oligo 1 modified silver-coated glass hybridized with 0.3 μM target DNA, 33.9°; (d) Sandwich system: silver-coated glass-dsDNA-AuNPs, 41.5°.

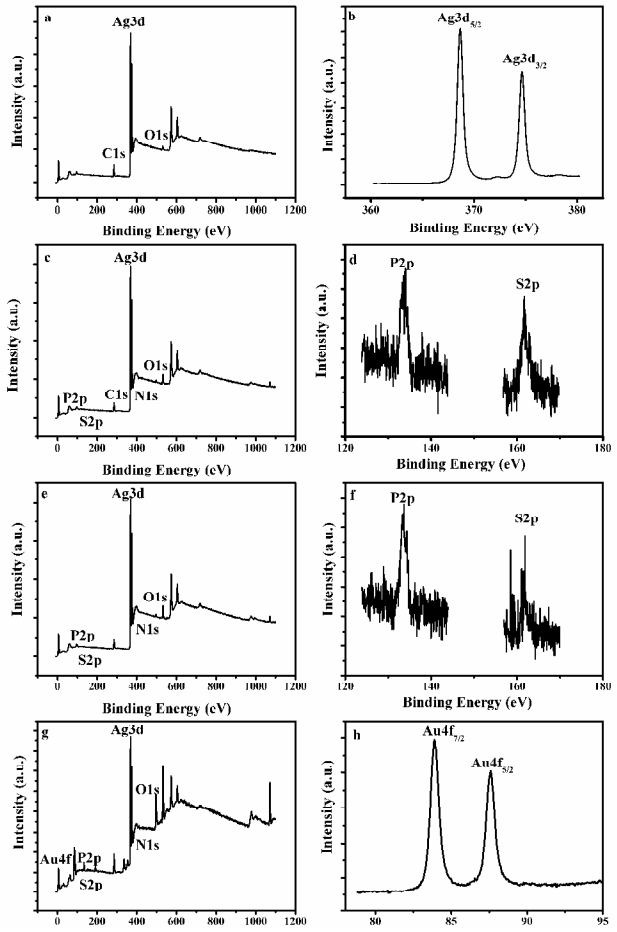

Fig. 5 XPS spectra of silver-coated glass under different conditions: (a, b) Silver-coated glass; (c, d) Oligo 1 modified silver-coated glass; (e, f) Oligo 1 modified silver-coated glass hybridization with 0.6 μM target DNA; (g, h) Sandwich system: silver-coated glass-dsDNA-AuNPs. Figure b, d, f, h were the high resolution spectra, the step size was 0.05 eV.

Characterization of silver-coated glass with XPS

To investigate the surface composition of silver-coated glass, X-ray photoelectron spectroscopy was employed. As shown in Fig. 5a and Fig. 5b, there was an evident peak of Ag3d for silver-coated glass, the high resolution spectra showed two peaks at binding energies of 368.6 eV and 374.6 eV, which could be ascribed to $Ag3d_{5/2}$ and $Ag3d_{3/2}$ respectively. They were in excellent agreement with the value of metallic silver[42] and it indicated that silver was successfully deposited on the surface of glass by using silver mirror reaction. There appeared new peaks of N1s, P2p and S2p in Fig. 5c and Fig. 5d, which revealed that DNA had been assembled on the surface of silver-coated glass successfully. After hybridized with target DNA, there was no manifest change of XPS image. After further addition of AuNPs-Oligo 2, there appeared new peaks at 83.9 eV and 87.6 eV, which could be assigned to peaks of $Au4f_{7/2}$ and $Au4f_{5/2}$, these results revealed that AuNPs-Oligo 2 had been linked to silver-coated glass through hybridization with target DNA.[43,44]

Calibration curve and detection limit for target DNA with AAS

The calibration curve of the assay was shown in Fig. 6, the integrated absorbance was proportional to the concentration of target DNA within the range of 10.0 nM-200 nM, the linear regression equation was A=0.0034C-0.052 (C in nM, R=0.9925), the detection limit was 0.23 nM, which was obtained from the equation of DL=3δ/slope. To estimate the selectivity of the assay, the absorbance of one base mismatched Oligonucleotide (5'-GAG GTT CGT GTT TGT GCC TGT CCT GGG AGA GA-3') and two base mismatched Oligonucleotide (5'-GAG GTT CGT GTA TGT GCC TGT CCT GGG AGA GA-3') were measured as well. The absorbance of mismatched DNA was lower than that of target DNA (Fig. 7), the integrated absorbance of one base mismatched DNA and two base mismatched DNA accounted for 88% and 74% of the signal of matched DNA respectively, which revealed sequence selectivity of the assay at a certain extent.[45,46]

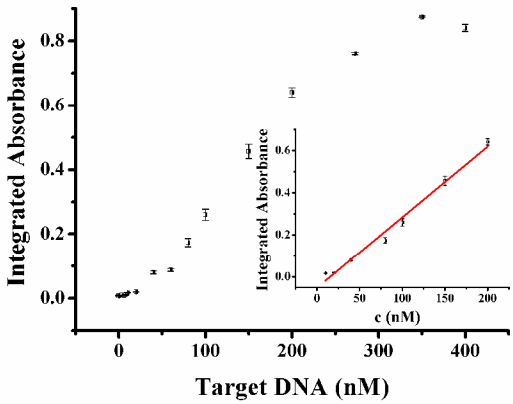

Fig. 6 Calibration curve of the assay.

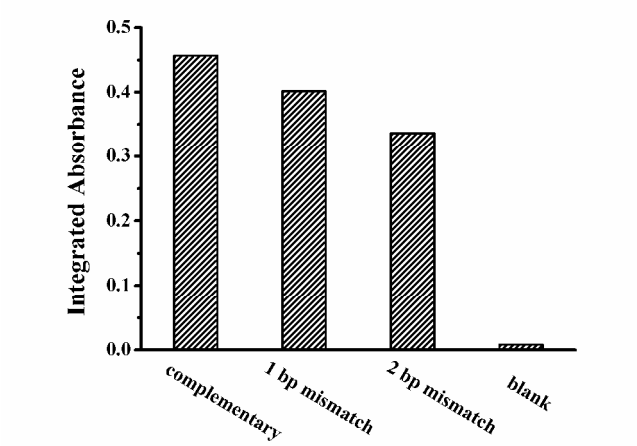

Fig. 7 Sequence selectivity of the assay, the concentration of target DNA, one-base mismatched DNA and two-base mismatched DNA was 150 nM.

Improve the sensitivity of the assay by a layer-by-layer technique

To further improve the sensitivity of the assay, the signal was amplified by a layer-by-layer technique. As demonstrated in Fig. 8, the first layer of AuNPs was formed by hybridizing target DNA with AuNPs-Oligo 2 and Oligo 1 on the silver-coated glass, AuNPs-Oligo 3 was used to hybridize with AuNPs-Oligo 2 on the surface of silver-coated glass and formed second layer of AuNPs, multi-layer of AuNPs could formed by adding AuNPs-Oligo 2 and AuNPs-Oligo 3 on the surface of Oligo 1 modified silver-coated glass alternately, which could be confirmed by the result of SEM.

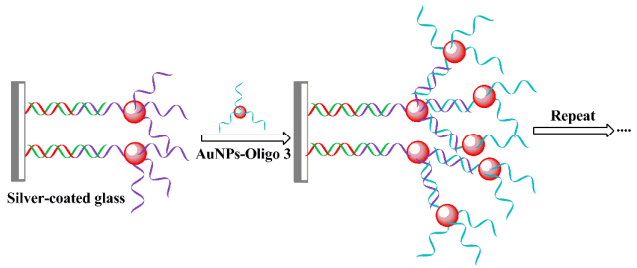

Fig. 8 Scheme for the construction of Au nanoparticle multi-layers

As shown in Fig. 9, in the absence of target DNA, there was a little change of SEM image with the increase of layer of AuNPs. However, upon addition of 5.0 nM target DNA, the SEM image changed dramatically with the change of layer of AuNPs. There was only small amount of AuNPs on the surface of silver-coated glass for one-layer sample, and the number of AuNPs increased manifest for five-layer sample and ten-layer sample. It indicated that multi-layer technique might increase the sensitivity of the assay.

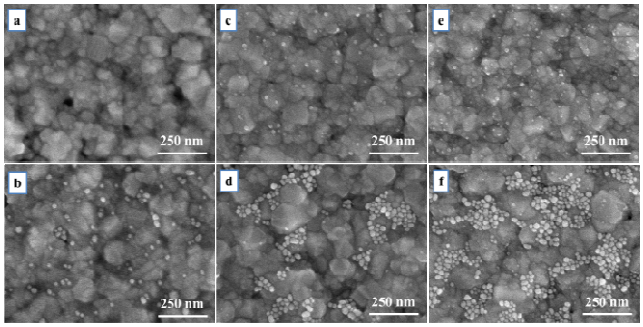

Fig. 9 SEM images of Oligo1 modified silver-coated glass in the presence and absence of 5.0 nM target DNA under different conditions. One-layer of AuNPs on the surface of silver-coated glass in the absence (a) and presence (b) of 5.0 nM target DNA; Five-layer of AuNPs on the surface of silver-coated glass in the absence (c) and presence (d) of 5.0 nM target DNA; Ten-layer of AuNPs on the surface of silver-coated glass in the absence (e) and presence (f) of 5.0 nM target DNA.

The results of absorbance coincided with the result of SEM. As shown in Fig. 10, for five-layer of AuNPs, the absorbance of blank sample was almost equivalent to that of 40.0 pM target DNA, which indicated that 40.0 pM target DNA was not detectable. For ten-layer of AuNPs, the blank signal (A_{blank}) increased to 0.1654, while that of the 40.0 pM target DNA (A) was 0.2562 and the standard deviation of blank sample (SD) was 0.0023 (n>6), which indicated that 40 pM target DNA was detectable. These results revealed that the sensitivity of the assay could be increased by using layer-by-layer technique, which was comparable to the fluorescent method.

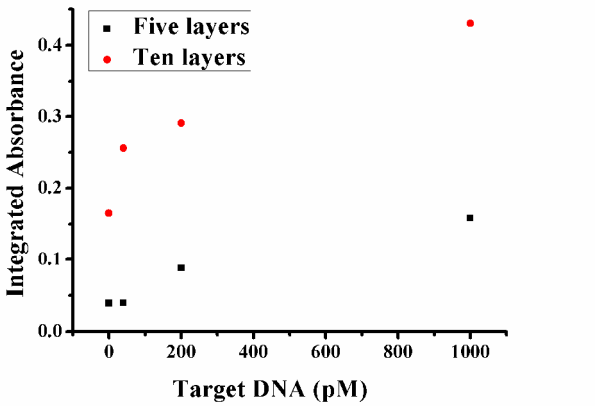

Fig. 10 Absorbance of Au in the presence of 0 pM, 40 pM, 200 pM and 1.0 nM target DNA for five-layer and ten-layer AuNPs respectively.

Conclusions

In general, atomic absorption spectroscopy was firstly and successfully applied to sequence-specific recognition of ssDNA. AuNPs-Oligo 2 acted as signal report component and Oligo 1 modified silver-coated glass was used as separation material, which could separate free AuNPs-Oligo 2 from that of hybridized. The concentration of target DNA was transformed to the signal of AuNPs, and the absorbance was proportional to the concentration of target DNA over a range of 10.0 nM-200.0 nM with a limit of detection of 0.23 nM. Moreover, the sensitivity of assay could be further improved by using layer-by-layer technique. The assay breakthrough the limit of AAS and open a

new direction for the application of AAS, enable it possible to investigate other DNA-related project in the future.

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Notes and references

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