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Electrical detection of DNA hybridization with multilayer gold nanoparticles between nanogap electrodes

C.-Y. Tsai, Y.-H. Tsai, C.-C. Pun, B. Chan, T.-Y. Luh, C.-C. Chen, F.-H. Ko, P.-J. Chen, P.-H. Chen

Abstract This study presents a DNA detection method via hybridization of a probe oligonucleotide with a target DNA and with a substrate oligonucleotide, which leads to self-assembly of gold nanoparticles and a change in the observed current. In contrast to previously reported methods, the present method can be used to detect a target DNA without a silver enhancement step. The device can be washed with 0.3 M PBS below 50 °C for a few minutes, indicating the stringency of target DNA hybridization. Target DNA concentration of as low as 10 picomolar can be detected by this method. Importantly, identification of single-base-pair mismatch in the target DNA can be accomplished with this nano-gap DNA chip.

1 Introduction

Most techniques for DNA detection have restrictive requirements on the equipment. Usually, they rely on thermal cycling of PCR, DNA hybridization, electrophoresis, fluorescent, chemiluminescent, etc. [1–4], to attain bioselectivity and immunoassay [5–9]. Recently, Mirkin has demonstrated a DNA detection methodology utilizing the optical properties of aggregated oligonucleotide-functionalized gold nanoparticles (AuNPs) [10–12]. A modification to this method was reported in 2000, which relies on the UV absorption of multilayered AuNPs on a glass substrate [13]. In another variation, the sensitivity can be further improved by the means of gold-promoted reduction of silver (1). Thus, a sensitivity of 100 times higher than conventional fluorescence imaging method in DNA base mismatch identification can be achieved [14]. A further variation of the DNA detection scheme was reported in 2002 [15]. This method involves completing of a circuit between two electrodes separated by a 20 m gap by the binding of oligonucleotide functionalized AuNPs. In this method, it is essential to use a silver enhancer solution to improve the sensitivity, since without such enhancement only a weak signal can be detected at the best. In 2002, H. L. Li and his co-workers proposed that the electrodes formed by layer-by-layer self-assembly of AuNPs using 1,6-hexanedithiol as cross-linkers have ideal microelectrode behaviors, in addition, interfacial electron transfer is promoted, consistent with a electron-transfer process [16]. In this paper, we present a DNA detection method using self-assembly multilayer AuNPs without the need of a silver enhancer, which represents an alternative method for rapid genetic disease diagnosis.

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C.-Y. Tsai, Y.-H. Tsai, C.-C. Pun
Thermal MEMS Laboratory, National Taiwan University, Taipei, Taiwan 10617, R.O.C.

B. Chan
Institute of Chemistry, Academia Sinica, Nankang, Taipei, Taiwan 115, R.O.C.

T.-Y. Luh
Department of Chemistry, National Taiwan University, Taipei, Taiwan 10617, R.O.C.

C.-C. Chen, F.-H. Ko
National Nano Device Laboratories, Hsinchu, Taiwan 30050, R.O.C.

P.-J. Chen, P.-H. Chen (✉)
Hepatitis Research Center, National Taiwan University Hospital, Taipei, Taiwan 10617, R.O.C.
e-mail: phchen@ccms.ntu.edu.tw

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2 Process development

2.1 Preparation of Au colloidal particles

Monodispersed AuNPs were prepared by the reduction of aqueous hydrogen tetrachloroaurate ($HAuCl_4$, Aldrich Chem. Co.) with trisodium citrate and tannic acid (Aldrich Chem. Co.) using a standard procedure [17]. A solution of $HAuCl_4$ (1 mg) in distilled water (80 ml), and a solution of trisodium citrate (0.05 mg) and tannic acid (0.01 mg) in distilled water (20 ml) were prepared. Both solutions were heated to 60 °C and were subsequently mixed. After development of a crimson color, the aqueous solution was heated to boiling for 10 min. The extinction spectra of the nanoparticles were measure on a Hitachi U3310 UV-vis spectrometer. High-resolution transmission electronic micrograph (HR-TEM, model: H-7000, Hitachi) was used to determine the size of AuNPs. The resulting AuNP solution has a strong surface plasma resonance at 523 nm and the size of the AuNPs was found to be in the range of 12 ± 4 nm. Particle diameter can be controlled by the amount of sodium citrate. Thus, using larger amount of sodium citrate gives smaller AuNPs, and vise versa.

2.2
The manufacturing of nano-gap gold electrode

The procedure for the manufacturing of nano-gap electrode is outlined as follow (Fig. 1): 1. Plating of a 2000 Å SiO_2 thin film using PECVD on a p-type Si (100) wafers (Phoenix, San Jose, CA). 2. Spin coating of a 7000 Å thick photoresistant on the surface. 3. Direct writing with E-beam lithography. 4. Placing the wafer in a development etchant to yield a 300–600 nm-gap on the photoresistant. 5. Thermal deposition of 5 nm titanium followed by deposition of 35 nm gold thin film. 6. Immersing the silicon wafer in acetone for 2 h.

2.3
Monolayer AuNPs

The substrates were cleaned with a 1:1 mixture of concentrated HCl and MeOH by immersing in the solution for 30 min, followed by rinsing with deionized H_2O (>18 W cm). The substrates were then immersed in concentrated H_2SO_4 for 5 min and rinsed with water, followed by boiling in deionized water for several minutes prior to chemical functionalization. 3-Mercaptopropyltrimethoxysilane was obtained from Sigma Chemical Co. (St louis, MO) and prepared as 1 mM solution in DMSO. The substrate was immersed in the silane solution for at least 2 h at room temperature, followed by rinsing with DMSO and dried under N_2. The functionalized substrate was immersed in the AuNPs solution for 8–12 h, then rinsed with distilled water and dried under N_2.

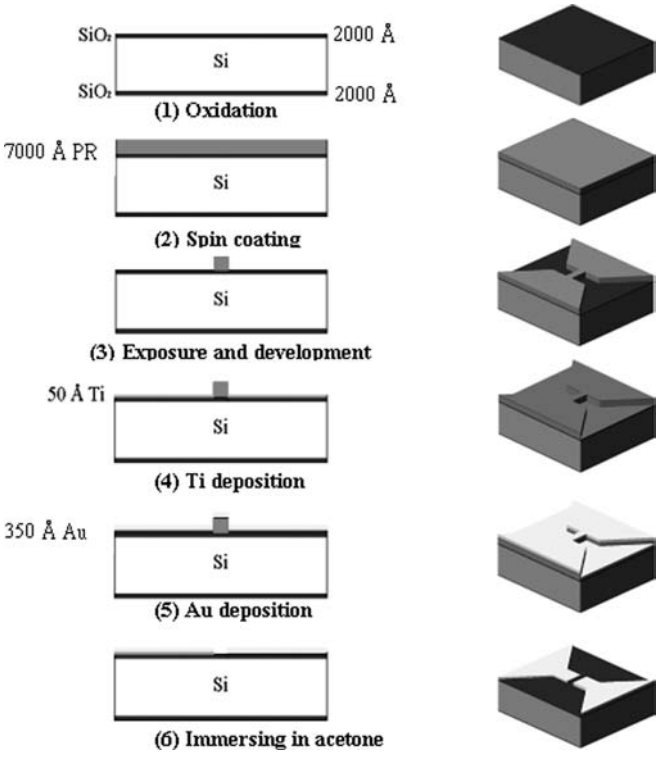

Fig. 1. Fabrication sequence of nano-gap gold electrodes using a lift-off process

2.4
Self-assembly multilayer of AuNPs by using DNA-directed immobilization

The sequences of the capture, target, probe and single base-pair mismatch of target oligonucleotides (cDNA, tDNA, pDNA and single-bp mismatch tDNA) were shown in Fig. 2, which the tDNA is complementary to the cDNA on one end and on the other to pDNA, but another single-bp mismatch tDNA is one base pair uncompletmentary to the cDNA. The oligonucleotides were prepared according to standard protocols [10–15]. The cDNA (100 μL, 1 μM) was deaerated in 10 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, J.T. Baker Chem. Co.), 5 mM EDTA buffer, pH 6.6 (HEPES buffer). The self-assembly gold-particle monolayer film was immersed in the cDNA solution for 15 hours at room temperature, followed by rinsing with 50 mM sodium phosphate, 1 M NaCl, pH 6.5 (SPSC buffer) to remove non-covalently bound DNA, and was dried under N_2. Then the monolayer film was dipped in 10 mM hexanethiol of an ethanol solution for 3 h to make a passivation surface. The chip is then washed with ethanol and DI water and is dried with N_2 gas. After this process, open space on gold nanoparticle without the immobilization of cDNA is occupied by hexanethiol for preventing thiolated pDNA from reacting with gold nanoparticle. The substrate was then immersed in four kinds of different concentration of the tDNA (0.1 μM, 1 nM, 10 pM and 1 fM, 24-mer) solution (100 μL) and the pDNA solution (12-mer, 100 μL, 0.1 μM) for 2 h to hybridize, followed by immersing in a SPSC buffer to remove excess reagents. The substrate was then immersed in a solution of AuNPs in 0.3 M PBS buffer (0.3 M NaCl, 10 mM NaH_2PO_4/Na_2HPO_4, pH 7), to remove excess AuNPs and was dried under N_2. The chip with the nano-gap electrodes and hybridized DNA was immersed in the AuNPs solution and then the AuNPs were filled the gap. Here, to make sure the target selectivity is to wash the chip with a 0.01 M PBS (0.01 M NaCl, 10 mM NaH_2PO_4/Na_2HPO_4, pH 7) buffer at room temperature to observe if differentiated with the conductivity and the single-bp mismatch tDNA dehybridized or not. This is because nanoparticles with DNA detector will show the high specificity, which washed with a stringency wash in 0.01 M PBS at room temperature and then the noncomplementary tDNA dehybridized [15]. The resulting chip was washed with 0.3 M PBS buffer to ensure the tDNA hybridization stringency. The electrical behavior of the array chip was measured by a Hewlett Packard, 4156 A

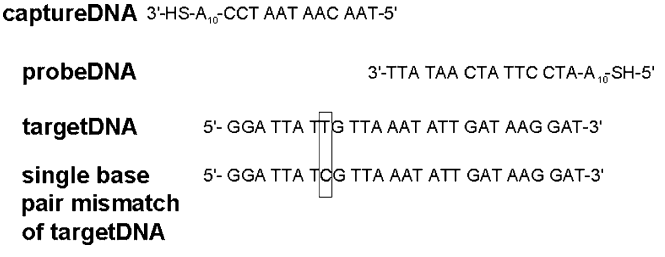

Fig. 2. The DNA sequences of the capture, target, probe and single base pair mismatch of target oligonucleotide

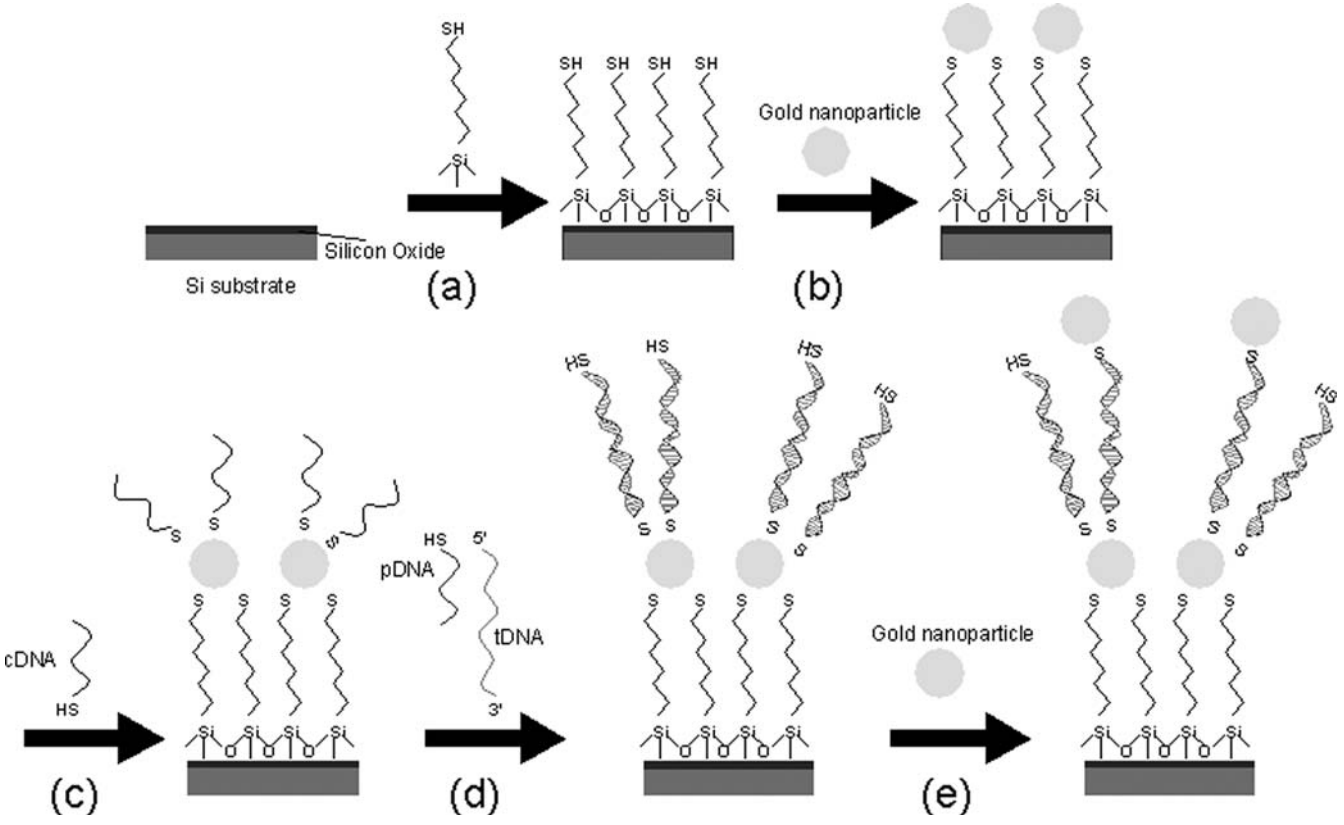

Fig. 3. The self-assembly chemistry deposition of Au-NPs monolayer onto the silicon dioxide and Chemistry used to attach thiol-modified DNA oligomers to self-assembly AuNPs monolayer. **a** Surface modification with THMS. **b** Addition of AuNPs reacts to the thiol molecule. **c** An alkanethiol-cDNA reacts with AuNPs monolayer. **d** tDNA added and hybridized with cDNA and pDNA, whose thiol reacts with multilayer AuNPs. **e** The thiol-DNA oligomer subsequently reacts with the AuNPs surface, to yield the self-assembly multilayer of the AuNPs

precision semiconductor parameter analyzer in the −1 to +1 V range with a sweep rate of 1 mV/s.

3 Results and discussion

The conductivity detection can be used to determine the quantity of the particles and tDNA that occupied the gap [15]. The signal of the chip could be increased if the density of particles was higher. Figures 1 and 3 are schematic illustrations of the chip format manufactured with electron beam lithography, and the process of gold particle immobilization and DNA hybridization. The left side of Fig. 4a and b shows field-emission scanning electron microscopic (FE-SEM: JEOL, JSM-6500F) photographs of the fabricated DNA array chip surface (a) with a monolayer of immobilized AuNPs, and (b) with multilayer of AuNPs. The current of the gap increases with increasing layer number of gold nanoparticles. Here, the distance of the nano-gap was 600 nm as obtained from the SEM images. The average particle coverage density was determined to be ∼1200 particles/μm^2 for a monolayer and ∼2900 particles/μm^2 for multilayer. Figure 5 shows a sketch of the array chip, in which the AuNPs are located in the center region. It is observed that the variation of the

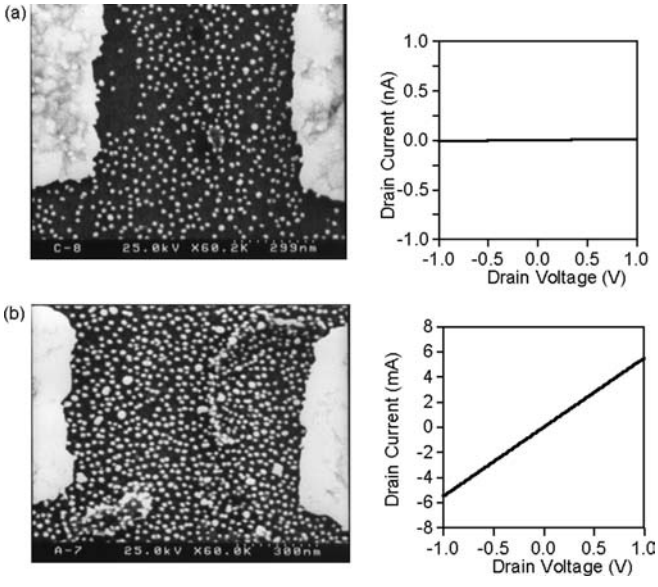

Fig. 4a, b. Measured results of self-assembly layer of gold nanoparticles. FE-SEM micrograph is shown on the left and it's the corresponding curves are shown on the right: **a** AuNPs attached with the THMS into the nano-gap electrode to become a self-assembly monolayer **b** Hybridization of AuNPs-labeled tDNA (0.1 μM) with cDNA and pDNA to become a self-assembly multiplayer

FE-SEM image is highly dependent on particle density, such that the shorter distance provided by oligonucleotide linkers significantly reduces the progressive effect of the particles.

Current-voltage (I-V) behavior of the device was measured *via* ohmic contacts as shown on the right side of

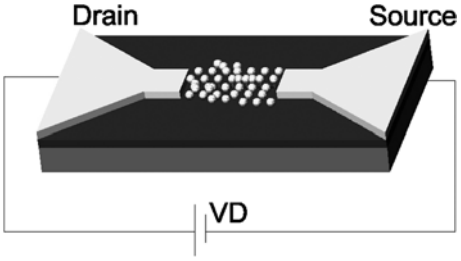

Fig. 5. The sketch map about the array chip and the center region of the AuNPs layer island

Fig. 4a and b. For an unfilled nano-gap the current of the device was lower than 50 femto amperes as observed. While the current of an AuNP monolayer was still lower than 10 pico amperes and didn't obey the ohm's law in the range −1 to 1 V at room temperature, as shown in the I-V curve of Fig. 4a.

The linear I-V curve in Fig. 4b shows that multilayer of AuNPs assembled in the nanogap *via* DNA hybridization is an ohmic device. Electrons tunnel more readily through the junction when enough energy is supplied.

FE-SEM images in Fig. 6a to d also show the self-assembly multiplayer of gold nanoparticles on SiO_2 surface treated in a manner identical to the treatment of the electrode gaps after incorporation of the second AuNPs v*ia* DNA immobilization in Fig. 4b. These FE-SEM images clearly indicate that AuNPs density increases with increasing tDNA concentration.

As shown in FE-SEM images of Fig. 6a to d, the average densities of the particles were ∼2900, ∼2600, ∼2000, and ∼1500 particles/μm^2 at a corresponding tDNA concentration of 0.1 μM, 1 nM, 10 pM, and 1 fM. The articles are complex material and when considered on an individual basis, they have slightly different levels of oligonucleotide functionalization, and hence different activities with respect to their ability to promote the conductivity. However, in the absence of tDNA and after chip was washed with a 0.3 M PBS buffer, the same signal to the monolayer of AuNPs was observed. Using this method, we have detected target oligonucleotide in 0.1 μM, 1 nM, 10 pM and 1 fM concentration ranges. The I-V curves in Fig. 6a to d show the capability of the nano-gap electrode for detecting DNA hybridization without silver enhancing.

If the chip was immersed into a buffer solution and heated to 60 °C (higher than its melting point: ∼55 °C) for 5 min to dehybridize the DNA duplexes [11], comparing the FE-SEM images of Fig. 7a and b it is clear that the AuNPs were immobilized in the nano gap by tDNA hybridization. This phenomena could be observed in the electricity differentiation between I-V curves of Fig. 7a and b.

The difference in the conductivity of multilayer with single-bp mismatch tDNA can be readily observed by washing the chip with 0.01 M PBS at room temperature. As shown in Fig. 8, before washing the DNA chip, we found the chip got higher AuNPs density in FE-SEM image and its corresponding conductivity in the Fig. 8a. But after washing with the 0.01 M PBS buffer, the density of AuNPs and conductivity of the DNA chip substantially decreases

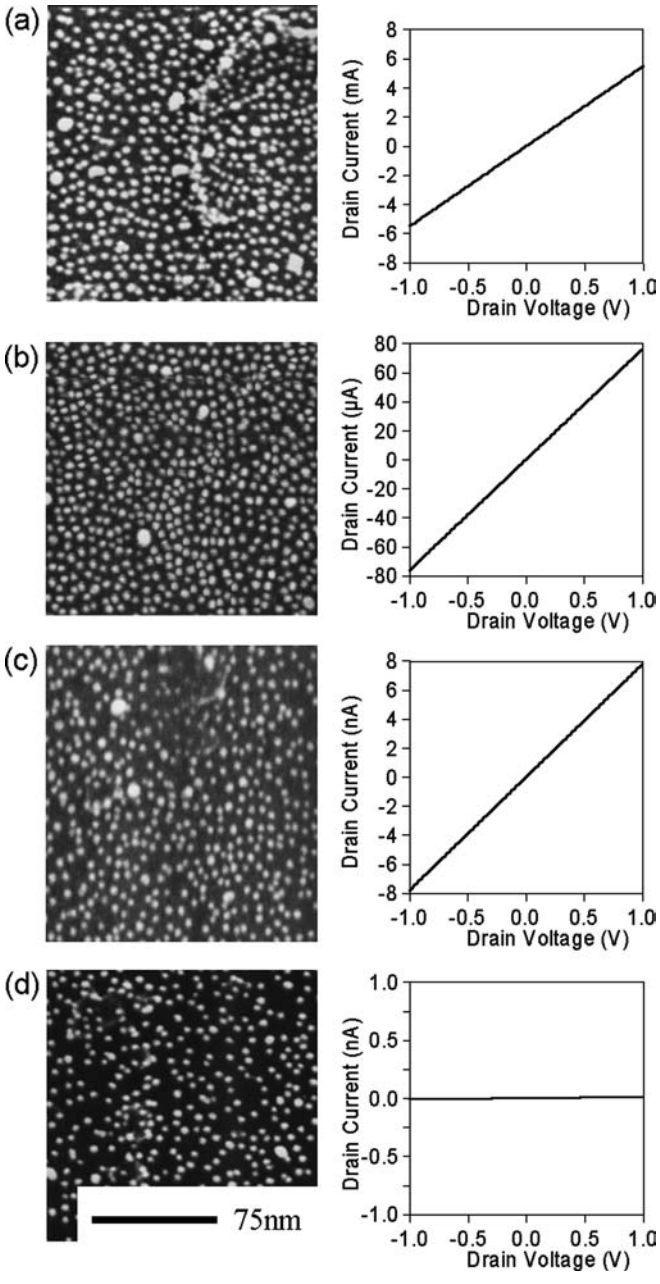

Fig. 6a–d. Measured results of self-assembly multilayer of gold nanoparticles at different tDNA concentrations: **a** 0.1 μM, **b** 1 nM, **c** 10 pM, and **d** 1 fM. Here, tDNA were hybridized to cDNA and pDNA in 0.3 M PBS for 2 h in all experiments. FE-SEM micrograph is shown on the left and its corresponding curve is shown on the right

in Fig. 8b. Here, the average particle coverage density was determined to be ∼2850 particles/μm^2 for multilayer and ∼1100 particles/μm^2 after dehybridization of noncomplementary tDNA. This observation supports that the high stringency selectivity in DNA chip detection and in differentiation of mismatched oligonucleotides. The self-assembly procedure described above provides a versatile and convenient method for DNA detection. The observation of increased AuNP density is consistent with our anticipation for DNA hybridization. Note that I-V curves in Figs. 6b, 7a, and 8a were measured for self-assembly

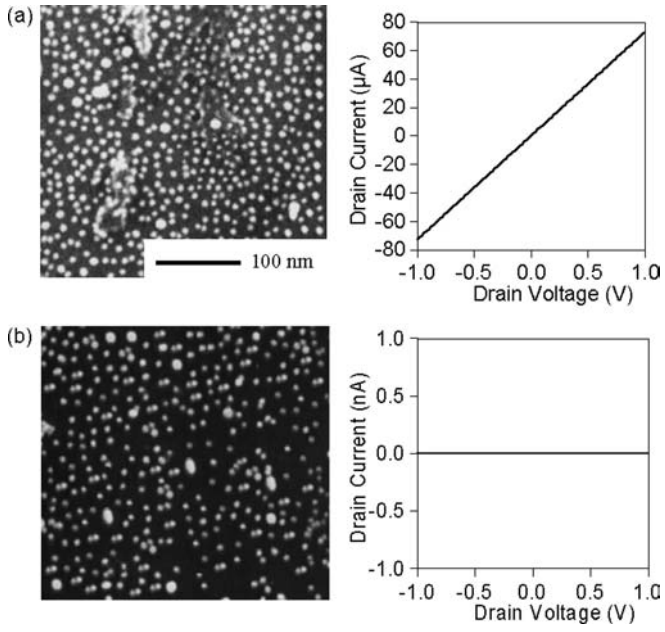

Fig. 7. **a** FE-SEM image for multilayer of AuNPs after complementary hybridization but before denaturing and its corresponding current-voltage curve on the right with a scanning rate of 10 mV/s. **b** FE-SEM image and its current-voltage curve for AuNPs after denaturing. Here, the concentration of tDNA is 1 nM for hybridization. For denaturing, the chip was immersed into the 0.3 M NaCl, PBS buffer and was heated to 60 °C for 3 min

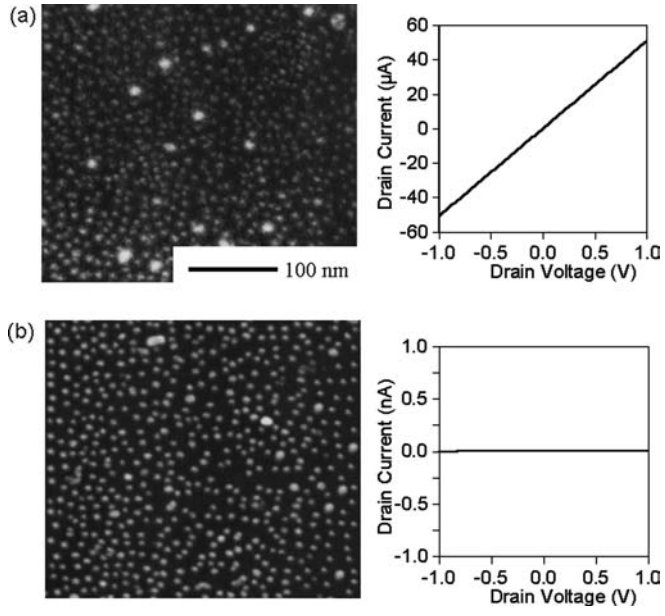

Fig. 8. **a** FE-SEM image for multilayer of AuNPs for single-bp mismatch tDNA hybridization before denaturing and its corresponding current-voltage curve on the right with a scanning rate of 10 mV/s. **b** FE-SEM image of AuNPs and its current-voltage curve on the right for Single-bp mismatch tDNA hybridization after denaturing. Here, the concentration of tDNA for hybridization is 1 nM. The chip was immersed into a salt solution of 0.01 M NaCl and PBS buffer for 2 h

multilayers of gold nanoparticles through DNA hybridization at the same tDNA concentration of 1 nM but on different sets of nano-gap electrodes. It apparently exists a measuring uncertainty in electrical conductivity of self-assembly multilayer of gold nanoparticles due to the variation in the gap distance over different set of electrodes and in the concentration of tDNA at different runs. However, the uncertainty in electrical conductivity of multilayer of gold nanoparticles is around 15% when the concentration of tDNA is 1 nM. In addition, the electronic properties of these devices might be altered by using different compounds (THMS, DNA) for the assembly of AuNPs multilayer.

4 Conclusion

Multilayer of THMS-DNA functionalized-AuNPs has been assembled on a nano-gap electrical device. This system is based on conventional nano-gap electrode for multiplexing detection through the use of electrode pair arrays. A high-impedance of the nano-gap was observed, consistent with the electrical characteristic of AuNPs in low density. Significantly, these layered structures can be assembled by conventional chemical techniques, DNA hybridization, so as to provide a genetic diseases diagnosis method. The ability to observe the spatial organization of the individual components of the molecule at the nanometer level has provided access to some of the interesting electronic properties of nanoscopic systems. This multilayer-AuNPs DNA chip and nano-gap electrode design may useful in diagnosing genetic bioselectivity and immunoassay, which contain single base-pair mismatch of oligonucleotide.

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