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ScienceDirect
Microelectronic Engineering 84 (2007) 1698–1701
MICROELECTRONIC ENGINEERING
www.elsevier.com/locate/mee

Effect of different gold nanoparticle sizes to build an electrical detection DNA between nanogap electrodes

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Available online 15 February 2007

Abstract

An experimental study for identifying DNA strands immobilized in the different particle sizes of gold nanoparticle (AuNP) between nanogap electrodes is presented. The nanogap-based electrics chip demonstrates a significant electrical property with a target DNA (TDNA) concentration of 10 pM in the different particle sizes of AuNP to be analyzed in order to make the assay useful for an integrated lab-on-a-chip application system. The biochips with nanogap electrodes are fabricated by e-beam lithography (EBL). Different from traditional detection methods including polymerase chain reaction (PCR) based on assays which are expensive and time consuming for DNA strands research. By the method of electrical characterization, a change in measured current through the different particle sizes of AuNP can pave the way for further use in sensitive biomolecule detection study.

Keywords: Electrical detection; DNA chip; Gold Nanoparticles; Particle size; Self-assembly

1. Introduction

Some novel nanomaterials for use in bioassay applications represent a rapidly advancing field. Various nanostructures have been investigated to determine their properties and possible applications in biosensors. These nanostructures include nanotubes, nanofibers, nanorods, nanoparticles and thin films. Of these, nanoparticles have numerous possible applications in biosensors. For example, functional nanoparticles (electronic, optical and magnetic) bound to biological molecules (e.g. peptides, proteins, nucleic acids) have been developed for use in biosensors to detect and amplify various signals. Some of the nanoparticle-based sensors include the acoustic wave biosensors, optical biosensors, magnetic and electrochemical biosensors.

Electrochemical biosensors have been fabricated from mostly metallic nanoparticles. Metal nanoparticles can be used to enhance the amount of immobilized biomolecules in construction of a sensor. Because of its ultrahigh surface area, colloidal Au has been used to enhance the DNA immobilization on a gold electrode, to ultimately lower the detection limit of the fabricated electrochemical DNA biosensor.

An array-based electrical detection of DNA with nanoparticle probes was reported [1]. Some capture strands of alkylthiol-modified oligonucleotides were immobilized onto the activated surface of SiO_2 substrate between two ends of Au microelectrodes with 20 μm gaps. The binding events localized gold nanoparticles in the electrode gap. Silver deposition facilitated by the gold nanoparticles (AuNPs) bridged the gap and led to readily measurable conductivity changes. In 2002, Lu et al. [2] reported the electrical behavior of self-assembly AuNP layers using 1,6-hexanedithiol as crosslinkers.

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0167-9317/$ - see front matter © 2007 Published by Elsevier B.V.
doi:10.1016/j.mee.2007.01.266

Recently, the relationship between optical properties such as the absorption, resonance Rayleigh scattering, and resonance non-linear scattering and the characteristics of AuNPs has been discussed [3]. However, the characteristics of AuNPs using in electrochemical biosensors have not been reported. In our study, AuNPs are synthesized for the fabrication of DNA sensors. The AuNPs are self-assembled in a monolayer on the silicon dioxide film through a linker containing a mercaptan group. The thiol-modified capture DNA (CDNA) is immobilized onto the monolayer of AuNPs. Then, the target DNA (TDNA) and thiol-modified probe DNA (PDNA) are also assembled. Prior to electrical characterization, the AuNPs are again attached to the PDNA to form a multilayered gold structure. Meanwhile, gold nanoparticles with different diameters can be prepared by the sodium citrate reduction method through controlling the amounts of sodium citrate. A serious of gold nanoparticles with the mean diameters of 12, 22, 40, 60 nm can be prepared. A change in measured current through different size of AuNPs can provide an electrical detection of DNA hybridization.

2. Experiment

2.1. Synthesis of gold nanoparticles

The different particles of AuNP sizes were obtained by chemical reduction method [4] by standard procedures. Here, colloidal AuNPs can be used as assay labels, replacing standard fluorescent probes. Based on an aqueous solution 1 mM of $HAuCl_4$ (Aldrich Chem. Co.) and 3.8 mM of trisodium citrate (Aldrich Chem. Co.) mixing, the aqueous solution was stirred for 15 min. Along with reaction temperature (120, 80, 60, 25 °C) changed, the different average diameters (12, 22, 40, 60 nm) were quite easy to synthesize to fine tune the size of the particles. Owing to the higher reaction temperature, a particle size can change lightly and its average diameter also smaller than the particle size under the lower temperature [4].

2.2. Fabrication of nanogap gold electrodes

The fabrication process of a nanogap electrode is shown in Fig. 1. The silicon wafer was grown with thickness of 2000 nm silicon dioxide film by plasma enhanced chemical vapor deposition (PECVD) method. Then, the resist with 400 nm thickness was spin-coated onto the silicon dioxide and the electrodes with 300 nm gaps were patterned by EBL (Leica Weprint model-200 stepper, Jena, Germany). Prior to the 60 nm gold film was deposited, a 5 nm titanium film served the purpose of adhesion was sputtered. Finally, the acetone solvent was used to lift-off the resist for 2 h. After using acetone solvent, the silicon wafer was immersed in the piranha solution (H_2SO_4:H_2O_2 = 3:1) for 10 min. This lift-off step can clean the unexposed photoresistant and without the need of metal films.

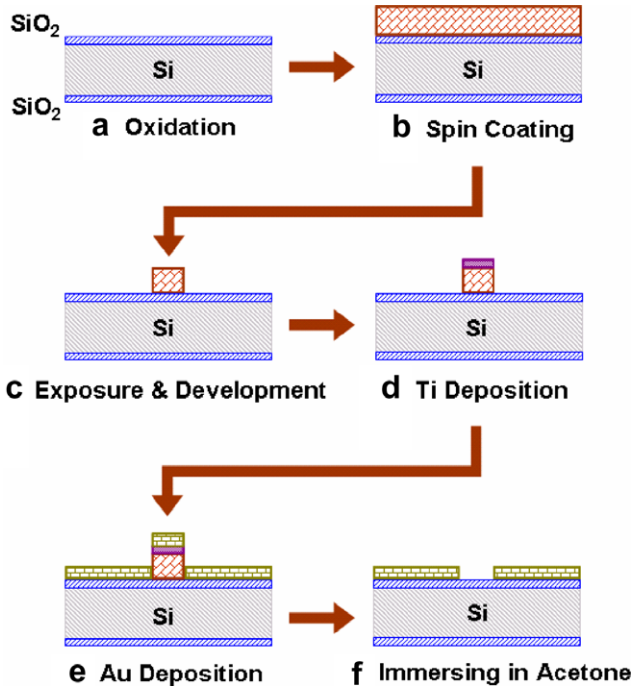

Fig. 1. Schematic illustrations of lift-off procedure based on EBL for the nanogap gold electrodes.

2.3. Procedures of DNA hybridization

In this study, four kinds of AuNP sizes were in the experimental sequence-selective DNA detection. Three

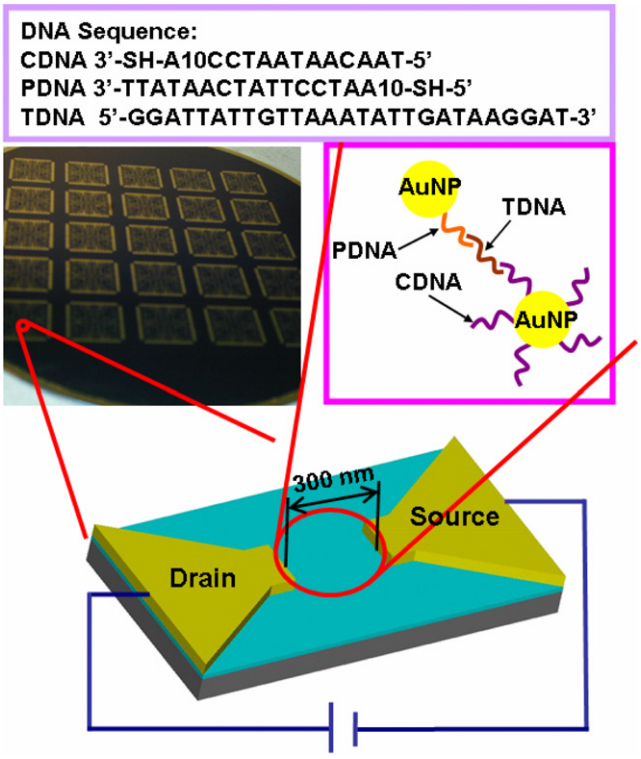

Fig. 2. Schematic illustrations of experimental setup for DNA detection with a nanogap sensing chip based on sandwich assay and electrical detection. Before detection, the APTMS has been pre-immobilized on the substrate.

DNA-bound AuNPs detected a DNA sequence that has been demonstrated in our former study [5]. Herein each monolayer and multilayer uses the same particle size of AuNPs. The analytical concept based on sandwich assay and electrical detection as shown in Fig. 2. The substrate immersed in only one kind of concentration of TDNA, CDNA, and PDNA (10 pM, 100 μL) solutions. Before the DNA chip-based detection, to begin with self-assembled monolayer AuNPs is established on SiO_2 substrate surface via 1 mM 3-aminopropyltrimethoxysilane (APTMS) (Sigma Chemical Co. St. Louis, MO) of DMSO solution for at least 2 h at room temperature, followed by rinsing with DMSO and dried under N_2. One end of the APTMS compound is to silanize the substrate surface while the thiol end of the APTMS compound is used to bind the AuNPs. Next, CDNA are immobilized on the top surface of self-assembled monolayer of AuNPs for 24 h at room temperature, followed by rinsing with SPSC buffer to remove non-covalently bound DNA, and is dried under N_2. The substrate is immersed TDNA and PDNA solutions for 2 h to hybridize, followed by immersing in a SPSC buffer to remove excess reagents. The substrate is then immersed in a solution of AuNPs in 0.3 M PBS buffer, followed by washing with the 0.3 M PBS buffer to remove excess AuNPs and is dried under N_2.

3. Results and discussion

In this study, the different AuNP sizes can affect the development of an electrical approach on sensitive detection DNA chip. Though gold is chemically more stable and is more easily conjugated to biomolecule via -SH [6], it is still concerned because of the design is nanogap electrodes. High-resolution transmission electronic micrograph (TEM, model: H-7000, Hitachi) is used to determine the size of AuNPs. The size differences and shape changes of AuNPs can be observed via TEM that different AuNP sizes with average diameters are $\sim 12 \pm 1.8$ nm, $\sim 22 \pm 2.3$ nm, $\sim 40 \pm 4.7$ nm, $\sim 60 \pm 7.1$ nm in Fig. 3a–d. The extinction spectra of the AuNPs can be measured on a Hitachi U3310 UV–vis spectrometer. A local absorption peak value in the UV–vis spectra of gold nanoparticle solution prepared by condition Fig. 4a is 523 nm, while the peak value for conditions Fig. 4b–d are 526 nm, 637 nm and

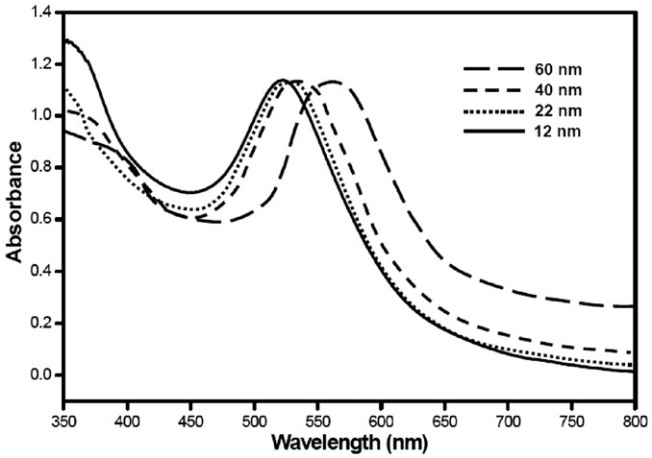

Fig. 4. Surface plasma resonance spectra of different AuNPs size using a UV–vis absorption spectrum.

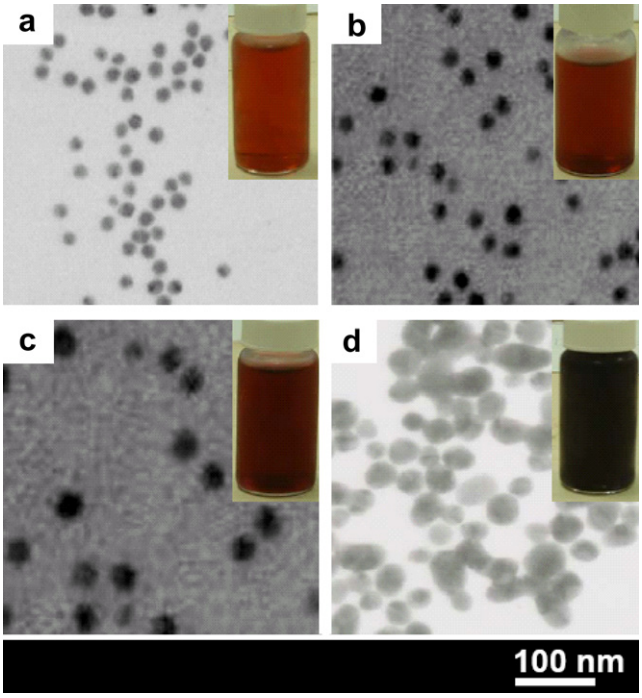

Fig. 3. TEM images of different AuNP sizes with average diameters of (a) 12 nm, (b) 22 nm, (c) 40 nm, and (d) 60 nm. Inset the different colors in small glass jars show the different gold nanoparticle sizes.

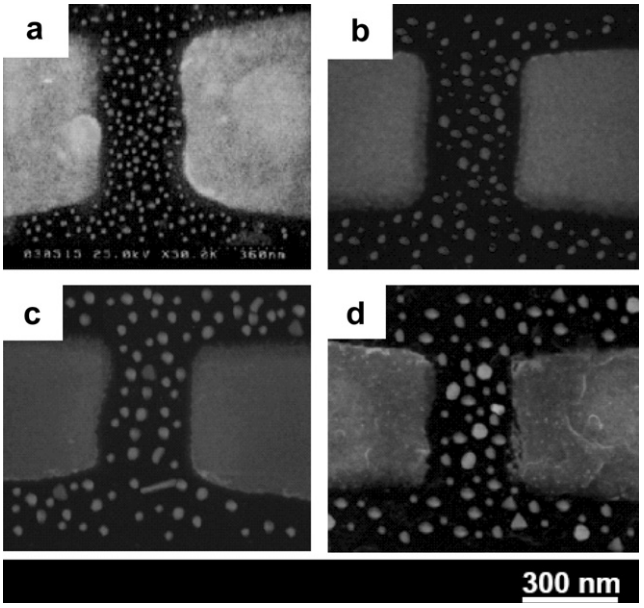

Fig. 5. SEM images of the self-assembled multilayer AuNPs with a TDNA concentration of 10 pM in the different particle sizes of AuNPs: (a) 12 nm, (b) 22 nm, (c) 40 nm, and (d) 60 nm.

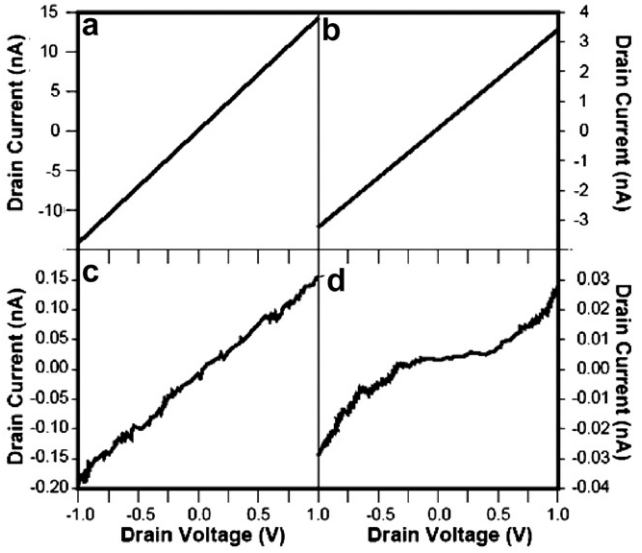

Fig. 6. *I–V* curves of the self-assembled multilayer AuNPs with a TDNA concentration of 10 pM in the different particle sizes of AuNPs: (a) 12 nm, (b) 22 nm, (c) 40 nm, and (d) 60 nm.

562 nm, respectively. According to Mie's theory and Maxwell's equation, the surface plasmon absorption and the plasmon bandwith are size-dependent with the metallic particles in the solution [7]. Consequently this peak value represents the size at which the gold nanoparticle most populates in the solution. In addition, the relationship of a peak position shift can be found between the particle size and optical absorption of UV–vis spectrum.

Fig. 5a–d shows SEM images of the self-assembled multilayer AuNPs that formed through the hybridization among TDNA, CDNA, and conjugate of the AuNPs–PDNA under the different particle sizes of AuNPs, namely, 12, 22, 40, and 60 nm. We can recognize from those images that the particle and the particle space increasing with its diameter increasing. The reason of trisodium citrate as a protecting agent is the repulsion between the particle and particle leads to the small size distribution of AuNPs will be the best in nanogap sensing system.

Next, the electrical behavior of this nanogap chip is measured using a Hewlett Packard 4156A precision semiconductor parameter analyzer in the range of −1 V to +1 V with a sweep rate of 1 mV/s. If there is no AuNPs in the nanogap, the electric current of the device would be lower than 50 fA as observed.

After the sensing step test, Fig. 6a–d shows the current–voltage (*I–V*) characteristic curves of the different particle sizes of AuNPs at a TDNA concentration of 10 pM. At the same time, the small diameter of AuNP has the evidence of larger current that our above-mentioned for well-suited particle in a nanogap is important can be seen in the result of the experiment. The different particle sizes of AuNPs can affect the signal current change that is provided a versatile and convenient factor for the design nanogap electrodes for the design of nanogap DNA sensing on-chip system.

4. Conclusion

The experimental results successfully demonstrate the feasibility of electrical detection to DNA biochip with different AuNPs sizes that give some results for the diagnostic biosensor for swift disease detection. *I–V* characteristic measurement make is clear that the electron can easily pass through the gold nanoparticle island with the smaller particle and particle space. The interrelated application for the biological samples of DNA biochip with the suitable AuNPs size to improve the commercial potential of the novel biochip platform is foreseen.

Acknowledgment

The authors deeply appreciate the facilities provide by National Nano Device Laboratories and thank the reviewers' comments which substantially improved the quality of this paper.

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