Rapid and Sensitive E-Coli DNA Detection by Titanium Dioxide Nanoparticles

Sh. Nadzirah, N. Zainudin, U. Hashim, M.N.B. Derman, K. F. Chong, A. Rahim Ruslinda

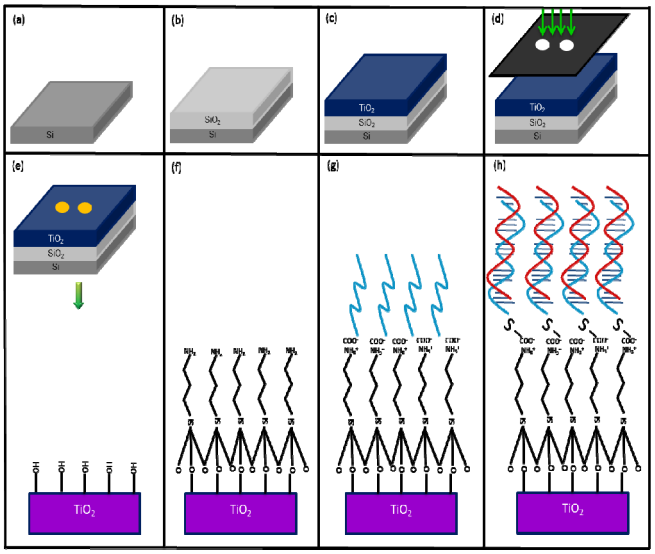
***Abstract*— Escherichia Coli (E. coli) issue has been discovered since 1985 according to its ability to cause large outbreaks of gastrointestinal illness especially O157:H7 type. A new and simple method for label free, rapid and inexpensive of titanium dioxide (TiO_2) nanoparticles biosensor based transducer on E. Coli DNA has been fabricated via sol-gel spin-coating technique. A simple quantitative approach was used to detect the existence of E. coli on the fabricated device. Extremely small steady current (picoammeters) were achieved, which indicates that this device can possibly go to very high sensitivities sensor towards DNA detection. A simple electrical signal of current-to-voltage (I-V) which provides small current was used to verify all the measurement of the device. (3-Aminopropyl)triethoxysilane (APTES) was functionalized through silanization process to modify the surface of TiO_2 nanoparticles through the covalent bond between hydroxyl groups of TiO_2 and organofunctional alkoxysilane group from APTES. The probe DNA was successfully immobilized and performed for hybridization with complementary DNA when the current is 3.5 E-10 ≤ I ≤ 4.5 E-10 A. The microchip showed reliable capture of E. coli in deionized water with an efficiency of 33.6% ± 5% at concentration of 1.0 µM.**

I. Introduction

Titanium dioxide (TiO_2) or tinania is an n-type semiconductor that can be formed in three different phases, namely, brookite, anatase, and rutile [1][2]. TiO_2 has high chemical and temperature stability [3]. The high resistance of TiO_2 towards acid and alkali makes it suitable for artificial bone or tooth fabrication. Given that TiO_2 is a biocompatible material, it is safe and non-poisonous in biosensor development. TiO_2 can generate non-poisonous CO_2 and H_2O in some inorganic products when organic pollutants are decomposed by light [5]. These multiple applications are due to its different phase formations, which have their own unique properties, causing TiO_2 to gain tremendous attention from researchers. At normal conditions, TiO_2 forms in amorphous state and changes to anatase phase once temperature is applied. Studies have reported that an increase in temperature (more than 500 °C) can transform the anatase phase to rutile phase [4][5]. TiO_2 growth has different energy gaps that depend on its phase formation. Direct and indirect band gaps exist in the rutile structure, whereas only a direct band gap exists in anatase TiO_2. The direct and indirect energy gaps of rutile are 3.02 and 3.90 eV, respectively [6][7][8]. Anatase TiO_2 has only a direct band gap of 3.20 eV [6][7]. Instead of phase transformation, the increase in annealing temperature may gradually decrease the energy gap [7][9].

Sol–gel is one of the bottom-up approaches used for the synthesis of nano-scale materials [10]. This approach comprises solutions and results in a solid phase without a precipitate [11]. The sol–gel method is used in the latest field of nanoscience because of its low cost and non-requirement of expensive equipment compared with solid-state processing routes using ball milling [12] and RF magnetron sputtering [13]. Sol–gel can be conducted under ambient temperature, namely, low-temperature processing, and can synthesize nanostructures with high homogeneity and purity [14].

Escherichia coli (*E.coli*) is a species that can be categorized as a bacterium or germ that lives in the digestive tracts of humans and animals. Most of E.coli is harmless but not the strain E. coli O157:H7 which is one of the most dangerous foodborne pathogens. Normally, this bacterium can get into raw meat, fruits, vegetables, milk and dairy products. Instead of that, people can become infected when a contaminated water supply has not been properly treated with chlorine. Thus, effective E. coli detection especially in ground beef and raw milk would have a positive impact.



Scheme 1: Schematic diagram for fabrication TiO_2 nanoparticles biosensor based transducer for E.coli DNA detection. (a) bare silicon wafer, (b) oxidation, (c) TiO_2 thin film deposition, (d) aluminium metal deposition, (f) APTES modification, (g) probe DNA immobilization and (h) complementary target DNA hybridization.

Several steps have been approach to detect this pathogen and most of the conventional methods are based on (i) culture and colony counting methods (which involve counting of

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bacteria); (ii) immunology-based methods (which involve antigen–antibody interactions); and (iii) the polymerase chain reaction (PCR). However, these methods are limited by the culturing time, high volume of sample required; false negative result also involves complicated process which needs to spend 48 to 72 hours [15].

Thus, we developed a highly sensitive sensor which able to detect E. coli DNA quantitatively using TiO_2 nanoparticles. This method is label-free and fast detection. 3-Aminopropyl triethoxy silane (APTES) was functionalized on the surface of the TiO_2 through silanization to immobilize probe DNA, furthermore performed for hybridization with complementary DNA. The aim of this study is to prove the suitability of TiO_2 nanoparticles for the detection of E. coli DNA by using simple electrical measurement current-to-voltage (I-V). The scheme for the biosensor setup used in this work is illustrated in Scheme 1.

II. Experimental

A. Chemicals and Reagents

Titanium isopropoxide ($Ti[OCH(CH_3)_2]_4$, 97%), acetic acid (AA), ammonium hydroxide [NH_4OH(27%) , hydrogen peroxide [H_2O_2 (30%)], hydrochloric acid [HCL (27%)], ethanol, aluminium, tinium isopropoxide (TIP) [16], BOE solution, hydrofluoric acid (HF) were obtained from Sigma Aldrich, USA. All the other chemicals were analytical reagent grade and purchased commercially. Distilled water (DW) was used throughout this experiment. The 30-base synthetic oligonucleotides were purchased from Bioneer, Korea. Their base sequences are as follows: 30-mer probe: 5'-AAC GCC GAT ACC ATT ACT TAT ACC GCG ACG-3' and 30-mer complementary: 5'-CGT CGC GGT ATA AGT AAT GGT ATC GGC GTT-3'. Stock solutions of all oligonucleotides were prepared with in autoclaved ultrapure water (> 18MΩ) and kept frozen (-20°C).

B. Instruments

The morphologies of TiO_2 thin film were characterized by HITACHI SU8020 Field Emission Scanning Electron Microscopy (FESEM) with 100k magnification and Atomic Force Microscopy (AFM) SII Sciko Instrument INC SPI3800N Probe station. The measurements for current to voltage were carried out by 6487 Picoammeter/Voltage Source (Keithley).

C. TiO_2 Nanoparticles Transducer Fabrication

Titanium dioxide nanoparticles were synthesized using sol-gel spin coating technique on silicon dioxide substrate. The p-type silicon substrate with <1 0 0> orientation was cleaned using RCA1, RCA2 and hydrofluoric acid (HF) to remove native oxide and small particles. RCA1 solution was prepared by mixing of DI water, ammonium hydroxide [NH_4OH(27%)], and hydrogen peroxide [H_2O_2 (30%)] by maintaining the ratio of 5 : 1 : 1. For the RCA2 preparation, hydrochloric acid [HCL (27%)] and hydrogen peroxide [H_2O_2 (30%)] were mixed in DI water by maintaining the composition at 6 : 1 : 1. The residual oxide layer was removed by dipping the substrate into a BOE solution followed by washing with DI water and drying under N_2 flow [16]. After the cleaning process, the silicon wafer was thermally oxidized to generate a 300 nm layer of SiO_2. The oxide layer is used to isolate the deposited TiO_2 film from silicon substrate in order to neglect the influence of current flow from silicon substrate.

After thermally oxidized; ethanol 96%, titanium isopropoxide ($Ti[OCH(CH_3)_2]_4$, 97%) and acetic acid (AA) were mixed with 9:1:0.1 ratios under magnetic stirrer with speed 1000 rpm at 85°C within 1 h. Ethanol as a solvent, tinium isopropoxide (TIP) as a precursor and AA as a stabilizer. Then the TiO_2 prepared solution was deposited on selected substrates at a spin speed of 3000 rpm for 30 s. The coated films then were dried on hot plate at 90°C for 15 min. After completing five times spin-coating of TiO_2, the films were annealed at 500 °C for 30 min.

Finally, aluminum (Al) metal was deposited and using a conventional lithography process, a pair of electrode of 1.3 mm^2 in size was fabricated on the SiO_2/Si substrate for electrical measurement purpose [30]. Scheme 1 shows the design of the fabricated samples. Then the electrical characterization was carried out using Kiethly6487 picoammeter interface with Labtracer2.0 software.

D. Silanization of TiO_2 Nanoparticles by APTES

TiO_2 nanoparticles were functionalized with APTES using silanization process covering the surface by self-assembly. As much as 0.5 µl APTES was dropped on the TiO_2 nanoparticles based transducer surface to perform an 'active' layer and let it dry in dry cabinet for 3h. Then, the surface was rinsed with deionized (DI) water

E. Immobilization and Hybridization DNA on TiO_2 Nanoparticles Based Transducer

After the modification of TiO_2 nanoparticles based transducer with APTES, 1.5µl of modified probe DNA with carboxyl group were immobilized on the transducer surface to form recognition layer by the covalent amide bond between the carboxyl group which already modified at DNA sequences and the amine group at APTES. After drying for 3 hours, the transducer surface was rinsed with deionized (DI) water to remove unbound probe DNA. Finally, the hybridization reaction was performed with the complementary DNA by dropping 1.5µl and left it drying at room temperature for 2 hours. Then, the surface was rinsed with deionized (DI) water before doing electrical measurement.

III. Results and Discussion

Fig. 1 shows the FESEM image of TiO_2 thin films annealed 500 °C. It was clearly observed that there were uniform particles growth with average diameter size was ~19.8 nm. When heat was applied to the TiO_2 films, the particles grew because the activation energy of TiO_2 nanoparticles was very minimal.

As shown in Fig. 2, the current-to-voltage (I-V) curves of TiO_2 nanoparticles, which at 0.5V, the real current for TiO_2 were 2.81375E-10 A. After the surface modification with silanization of APTES, the real current decreased to 2.81244E-10 A, respectively, suggesting that the APTES was

successfully modified TiO_2 particles through the silanization reaction which occur by the reaction of hydroxyl groups of TiO_2 and organofuntional alkoxysilane group from APTES. The covalent bond between the two functional groups will comprise to the 'active' layer of 'self-assembly' which covered the TiO_2 nanoparticles diameter. This behaviour determined for the reducing diameter of particles which result in decrease the effective cross-section of the area [17]. Thus, the electron mobility through the particles becomes lower which contributed to the reducing of current flow through the transducer. When the probe DNA was immobilized on APTES, the negative carboxyl groups (-COOH) which already modified at probe DNA were covalently bound with the amino groups on APTES, and neutrally amide bonds were formed on the transducer surface. After immobilized probe DNA, the current increase

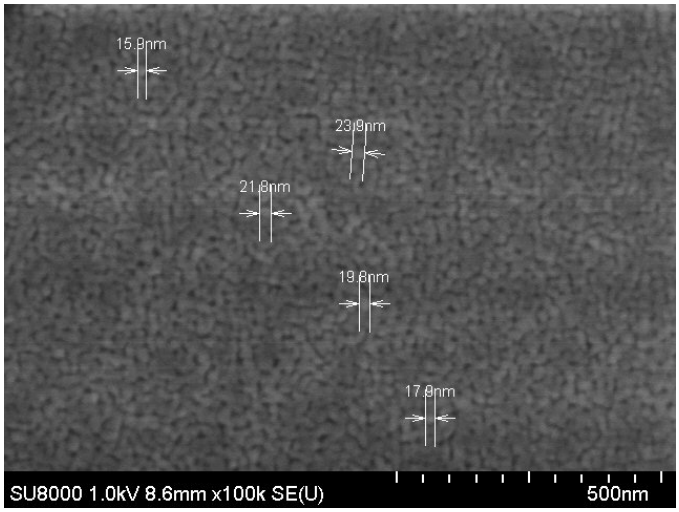

Figure 1: FESEM image of TiO_2 nanoparticles.

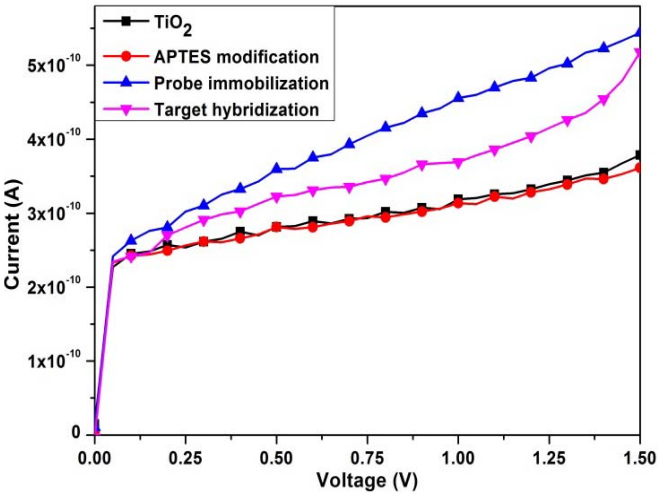

Figure 2: I-V curves of TiO_2 nanoparticles for biosensor fabrication.

greatly to 3.59488E-10 A, due to the increment in surface charge density from the negatively charged phosphate backbone of DNA. While probe DNA hybridized with its complementary DNA sequences, the lying single stranded DNA of probe become standing DNA and formed a double helix structure. The current extremely decreased to 3.22497E-10 A, indicating that complete hybridization complexes by reason of the decreased in current flow between the transducer surface and DNA as the double helix structure of DNA was abundant of negative charges. The 1.5 µL of target DNA gives an efficiency of 33.6% ± 5%.

IV. CONCLUSION

TiO_2 nanoparticles biosensor based transducer was successfully employed as a sensing platform for the E. coli through silanization of APTES. The experiments characterizations show that probe DNA was accomplished immobilized on the transducer surface through a covalent bond. For the hybridization with complementary DNA, it was shown a good electrical effect. Several works need to be improved in order to get better selectivity and sensitivity of the device.

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