Ultrasensitive Nano-structured DNA Biosensor

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Statement of Purpose: Detection of damaged and mismatched DNA is getting increasingly important for the diagnosis of genetic disease, especially the charge transport through DNA to promote oxidation and reduction in electrochemical method. Gold deposited anodic aluminum oxide (AAO) works as an innovative nano-porous template for DNA sensing, which gives a highly sensitive, specific, and rapid detection. 200 nanometer sized pores not only provide an increased surface when probing single stranded DNAs, but also make it possible to enhance DNA hybridization for higher sensitivity. Combining the simple electrochemical measurement and the nano-structure, more sensitive and quicker detection is possible with increased surface areas. Here, we report a synthesis of nano-structured DNA sensor with a porous anodic aluminum oxide (AAO) and their enhanced sensitivities in DNA detections.

Methods: Gold deposition on nano-structured anodic aluminum oxide (AAO, Anodisc 25 Whatman®) was performed by the electroless gold plating. Single stranded DNA with 19 base pairs (ATC GGC AGA CTG CGA CTTT) BT4076 from Bacteroides thetaiotaomicron was used for synthesizing probe DNA sensor. Single DNA attachment was first performed by a self assembled monolayer (SAM) of 3-mercaptopropionic acid into the Au-AAO surface which bridges between gold and single DNA molecules. Ethanol solution including 1 vol. % of 3-mercaptopropionic acid was prepared then immersed Au-AAO template in the solution for 24 hours. After bridging into Au-AAO, it stayed in 20mM phosphate buffer solution (pH 7.0) containing 5mM of EDC and 8mM of NHS for 5 minutes then moved to DNA solution including 2mM of DNA in 5mM phosphate and 50mM of NaCl (pH 7.0). Probe DNA sensors were thoroughly washed with distilled water and stored at 4°C. The hybridization with target DNA were performed by two methods; immersing probe DNA into target DNA solution, and passing target DNA solution through AAO pores. Cyclic Voltammogram (CV) were measured in a solution of 50mM Tris/20mM NaCl (pH 7.2) including redox pairs by 314 Multiplexer, Potentiostat / Galvanostat Model 273A. For the electrochemical measurement, a typical three electrode setup including a DNA probe electrode, a platinum wire counter electrode, and Ag/AgCl (sat'd KCl) as a reference was used. Potassiumferricyanide ($K_4[Fe(CN)_6]$) was used as redox molecules to transduce the electrochemical signals.

Results: DNA probe electrodes on gold coated AAO membrane templates, which have well defined 3 dimensional (3D) nanostructures, were fabricated and investigated. **Fig. 1** shows the CV curves derived by use of the corresponding ss-DNA probes and that hybridized with complementary target DNAs. Such DNA biosensors provided over 100-fold and even 1000-fold larger current values than gold macroelectrode that has a 1 dimensional (1D) smooth surface. In other worlds, the 3D structure of the AAO templates provided 100-fold to 1000-fold voltammetric signal enhancement for the resulted DNA biosensors. Based on the calculation from the peak currents (I_{pc}), an over **7 times higher signal-to-noise ratio** was also achieved with such DNA probes using AAO templates as compared to the 1D bulk gold DNA macroelectrode.

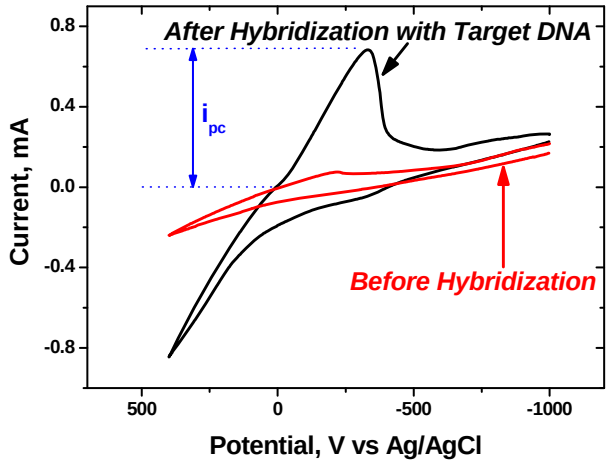

Figure1. Cyclic Voltammogram of nanostructured AAO with 50μM of $K_4[Fe(CN)_6]$ redox pair with the scan rate of 100mV/s.

Our study also demonstrated that the 3D DNA biosensor made of AAO templates can easily detect target DNA sequences with concentration of 10^{-19} M, which corresponds to ~ 60 DNA targets per ml. In comparison, the 1D DNA biosensor made of bulk gold macroelectrode can detect target DNA concentration up to only 10^{-16} M, which corresponds ~ 60,000 DNA targets per ml. Our results evidently demonstrate the 3D nanostructure effects on the improvement of DNA detection sensitivity.

Conclusions: The nano-architectural approach in designing and fabrication of DNA biosensor provided significantly improved detection sensitivity, which is believed to be due to the high surface area and the 3D structure possessed by the AAO template membrane electrodes.

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