Nano-crystalline 3C-SiC Electrode for Biosensing Applications

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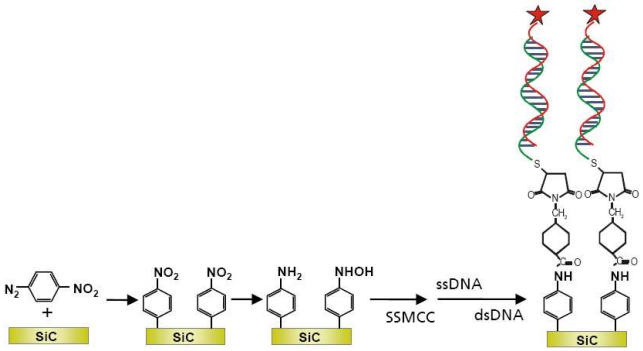
Experimental Section

Nano-crystalline 3C-SiC films were deposited on single-crystalline p-type Si (100) wafers in an ASTeX 1.5kW reactor.[18] The deposition was carried out at a constant gas pressure of 25 Torr, a substrate temperature of 700 °C, and a microwave power of 700 W. Tetramethylsilane (TMS, 1% TMS diluted in H_2) was the source gas for the SiC growth. The flow rates of H_2 and TMS were maintained at 400 and 20 sccm (cubic centimeter per minute at STP), respectively. The films were grown for a period of 6 h. To remove any inorganic and organic contaminations on the surface, the film was washed firstly by using methanol for 3 min in ultrasonic bath after deposition. To form a dense surface SiO_2 layer, the sample was further washed using mixed solutions of HCl-H_2O_2-H_2O (V:V:V=1:1:6) at 80 °C for 5min and NH_4OH-H_2O_2-H_2O (V:V:V=1:1:5) at 80 °C for 5 min. Afterwards, the sample was cleaned in the ultrasonic bath in distilled water for 10 min. For electrochemical experiments, the film was treated by 2.5% HF solution for 3 min followed by washing in distilled water for 6 min in ultrasonic bath to remove the surface SiO_2 layer.

All electrochemical experiments were conducted on a Biological Multi-Channel Potentiostat with a three-electrode configuration with a platinum counter electrode and an Ag/Ag^+ (0.01 M) reference electrode in organic solvents or an Ag/AgCl (3M) reference electrode in aqueous solutions. The working electrode was a 3C-SiC electrode. For XPS measurements, a monochromated Al *Kα* beam was used as X-ray source. The samples were examined under 45° degrees with respect to the surface normal. The fluorescent images were recorded on an AXIO Imager (ZEISS) fluorescent microscope.

Electrochemical attachment of nitrophenyl molecules to SiC electrodes using cyclic voltammetry was performed in 1.0 mM 4-nitrobenzene diazonium in dehydrated acetonitrile (H_2O < 50 ppm) containing 0.1 M tetrabutylammonium tetrafluoroborate. The scan rate was 200 mV s^{-1}. The electrochemical reduction of 4-nitrophenyl groups ($–C_6H_5NO_2$) to aminophenyl groups ($–C_6H_5NH_2$) was performed in 0.1 M KCl solution of EtOH-H_2O (V/V=1:9) solvent. The scan rate was 100 mV s^{-1}.

For probe DNA attachment, the amino-phenyl layer is then reacted with 14 nM solution of the heterobifunctional crosslinker sulphosuccinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate in 0.1 M pH 7 triethanolamine (TEA) buffer for 20 minutes at room temperature in a humid chamber. The NHS-ester group in this molecule reacts specifically with the -NH_2 groups of the linker molecules to form amide bonds. The maleimide moiety was then reacted with (2 - 4) µl thiol-modified DNA (300 µM thiol DNA in 0.1 M pH 7 TEA buffer) by placing the DNA directly onto the surface in a humid chamber and allow to react for 1h at room temperature. As probe DNA we used the 23-mere cancer marker cytokeratin 20 (CK20: 5'-HS-C_6H_{12}-T_9-CTG TTT TAT GTA GGG TTA GGT CA-3') and as target the complementary sequence 5'Cy5-TGA CCT AAC CCT ACA TAA AAC AG-3'), where Cy5 indicates the presence of a red fluorescence marker. For hybridization of DNA, 5 µl of SSPE buffer containing complementary DNA was place on the sensor surface for 1 h at 20 °C in a humid cell. The density of complementary DNA has been varied from 10 µM to 1 pM to investigate sensitivity properties of the sensor. The sensitivity limits have detected in a container using 100 µl of SSPE. After hybridization the samples have been washed in de-ionized water for 1 h at 37 °C to remove non-intentional bonded DNA molecules. Stability measurements have been performed using 1 µM solutions over extended cycles of hybridization /denaturation treatments. Denaturation was performed in 8.3 M urea-solution for 30 minutes at 37 °C, followed by rinsing in de-ionized water.



Scheme 1. Schematic plot of bio-functionalization of SiC with DNA. The term of SSMCC stands for the cross-linker of sulphosuccinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate.