**Procedure 28**

**Atomic force microscopy characterization of a DNA electrochemical biosensor**

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28.1 OBJECTIVES

Determination of the optimal experimental conditions for the atomic force microscopy (AFM) characterization of the surface morphology of a DNA electrochemical biosensor obtained using different immobilization procedures of calf-thymus double-stranded DNA (dsDNA) on a highly oriented pyrolytic graphite (HOPG) electrode surface.

28.2 MATERIALS AND INSTRUMENTS

Calf thymus dsDNA, sodium salt, type I (Sigma-Aldrich Química, Madrid, Spain) used without further purification. Electrolyte pH 4.5, 0.1 M acetate buffer solution prepared using analytical grade reagents and purified water from a Millipore Milli-Q system (conductivity < 0.1 μS/cm). DNA solutions obtained by direct dilution of the appropriate volume in acetate buffer.

HOPG, grade ZYH, of rectangular shape with 15 × 15 × 2 mm dimensions (Advanced Ceramics Co., UK/Germany) used as a substrate.

PicoSPM controlled by a MAC Mode module and interfaced with a PicoScan controller (Molecular Imaging Corp., Tempe, AZ, USA); CS AFM S scanner with the scan range 6 μm in x–y and 2 μm in z (Molecular Imaging Corp.); silicon type II MAClevers of 225 μm length, tip radius of curvature less than 10 nm, 2.8 N/m spring constant, and 60–90 kHz resonant frequencies in air (Molecular Imaging Corp.) used in MAC Mode AFM.
28.3 DNA ELECTROCHEMICAL BIOSENSORS PREPARATION

Freshly cleave the HOPG with adhesive tape prior to each experiment, image it by AFM in order to establish its cleanliness, and then modify it with a thin or thick dsDNA film.

- Prepare the thin-film dsDNA electrochemical biosensor by free adsorption from 100 μL of 60 μg/mL dsDNA solution in pH 4.5 0.1M acetate buffer onto the HOPG surface and incubate it to 3 min. Stop the adsorption process by gently rinsing the sample with a jet of Milli-Q water, dry the HOPG with adsorbed DNA with nitrogen and image it in air [1].
- Prepare the multilayer-film dsDNA electrochemical biosensor by evaporation on the HOPG surface of three consecutive drops, each containing 5 μL of 50 μg/mL dsDNA in pH 4.5 0.1M acetate buffer electrolyte solution and leave the electrode in sterile atmosphere to dry [2].
- Prepare the thick-film dsDNA electrochemical biosensor by covering the HOPG electrode surface area with 100 μL solution, prepared by dissolving 3 mg of dsDNA in 80 μL pH 4.5 0.1M acetate buffer electrolyte solution, and leave the electrode in sterile atmosphere to dry overnight [1].
- Image the sample surface by MAC Mode AFM in air [1–5].

28.4 ATOMIC FORCE MICROSCOPY EXPERIMENTAL CONDITIONS

- Take all AFM images (256 samples per line × 256 lines) at room temperature, with scan rates of 1.0–2.5 lines/s. Process the images by flattening in order to remove the background slope, and adjust the contrast and brightness.
- Perform section analysis over ODN molecules and films with PicoScan software version 5.3.1 (Molecular Imaging Co.) and with Origin version 7.5 (OriginLab Corporation, USA).
- Calculate the mean values of the heights using 50 measurements over different scanned AFM images. Use Origin version 7.5 (OriginLab Corporation) to calculate standard deviation and all the experimental height/thickness distribution graphs.
28.5 DISCUSSION

Electrode surface characteristics represent an important aspect in the construction of sensitive DNA electrochemical biosensors for the rapid detection of DNA interaction and damage. MAC Mode AFM images in air are used to characterize three different immobilization procedures for immobilizing nanoscale dsDNA surface films on carbon electrodes. A thin dsDNA adsorbed film forming a network structure with holes exposing the electrode surface, a multilayer and a thick dsDNA film completely covering the electrode surface, with a much rougher structure, are investigated.

Knowledge of the electrochemical DNA biosensor surface morphology and of the electrochemical behaviour of the drug at a bare electrode is most important to avoid possible misinterpretations during utilization of DNA electrochemical biosensors. Uniform coverage of the electrode surface by DNA is a must, since non-uniform coverage allows the adsorption of the hazard compound on the electrode surface, leading to contributions from both simple adsorbed analyte and from products of damage to immobilized DNA, which need to be carefully distinguished.

SELECTED LITERATURE