Applied Surface Science 258 (2012) 6056–6063

Contents lists available at SciVerse ScienceDirect

Applied Surface Science

journal homepage: www.elsevier.com/locate/apsusc

Immobilization of streptavidin on 4H–SiC for biosensor development

Elissa H. Williams[a,b,c,*], Albert V. Davydov[b], Abhishek Motayed[b], Siddarth G. Sundaresan[b,d], Peter Bocchini[b,e], Lee J. Richter[b], Gheorghe Stan[b], Kristen Steffens[b], Rebecca Zangmeister[b], John A. Schreifels[a], Mulpuri V. Rao[c]

[a] Department of Chemistry and Biochemistry, George Mason University, Fairfax, VA 22030, USA
[b] Material Measurement Laboratory, NIST, Gaithersburg, MD 20899, USA
[c] Department of Electrical and Computer Engineering, George Mason University, Fairfax, VA 22030, USA
[d] GeneSiC Semiconductor Inc., Dulles, VA 20166, USA
[e] Department of Mechanical Engineering, University of Delaware, Newark, DE 19716, USA

ARTICLE INFO

Article history:
Received 1 October 2011
Received in revised form 1 February 2012
Accepted 25 February 2012
Available online 5 March 2012

Keywords:
4H–SiC
3-Aminopropyltriethoxysilane
Biotin
Streptavidin
Bioconjugation
Surface functionalization

ABSTRACT

A sequential layer formation chemistry is demonstrated for the functionalization of silicon carbide (SiC) appropriate to biosensing applications. (0 0 0 1) 4H–SiC was functionalized with 3-aminopropyltriethoxysilane (APTES) and subsequently biotinylated for the selective immobilization of streptavidin. Atomic force microscopy, X-ray photoelectron spectroscopy, ellipsometry, fluorescence microscopy, and contact angle measurements were utilized to determine the structure, thickness, wettability, and reactivity of the resulting surface after each functionalization step. Optimization of the APTES layer was found to be critical to the success of the subsequent steps; multilayer, polymeric films resulted in irreproducible behavior. It was shown that there was significant non-specific (electrostatic) binding of streptavidin to APTES functionalized SiC, thus revealing the importance of a uniform biotinylation step prior to streptavidin attachment. The experimental results demonstrate that the APTES functionalized and biotinylated SiC surface has the potential to be employed as a biosensing platform for the selective detection of streptavidin molecules.

1. Introduction

Over the past few years, there has been an increased interest in the fabrication of semiconductor biosensors that have the ability to electrically detect adsorbed biomolecules with great selectivity and sensitivity [1–5]. The efficiency of such sensors is enabled by chemi-resistive transduction mechanisms, where the change in surface potential due to selective molecular absorption affects the device conductance [6]. Of the readily available semiconductor materials, SiC is an attractive substrate for biosensing applications due to its unique electronic properties, mechanical robustness, chemical inertness, thermal stability, non-toxicity, and biocompatibility [7–11]. In order to enable SiC-based devices that utilize direct, electronic sensing of biomolecules, one must first develop an analyte-specific functionalization of SiC and deduce mechanisms by which the functional and analyte molecules bind to the SiC surface. Numerous conjugation techniques have been developed for the attachment of specific biomolecules to surfaces through the use of various linking molecules [12,13]. The covalent attachment of APTES on oxidized surfaces is widely used as the first step of surface functionalization for the optical and electrical detection of proteins and DNA [1,3,14–16]. APTES functionalized semiconductor surfaces result in great versatility and adaptability since amine groups can react with any biomolecule containing or modified with an ester group to form a stable amide bond. To date, only one research group, Yakimova et al. [7], has studied the immobilization of APTES on SiC surfaces. Yakimova et al. reported the successful covalent attachment of APTES to (0 0 0 1) 4H–SiC using a vapor phase deposition.

The biotin–streptavidin system, in which four biotin molecules bind to one streptavidin molecule, is one of the most stable and selective noncovalent biological binding couples known and hence it is often employed as a model system to study bio-recognition events [15,16]. Additionally, the multi-functionality of streptavidin makes it a useful coupling agent for nano-engineering applications [17,18]. A commercially available sulfonated and esterified form of biotin (sulfo-NHS-biotin) enables simple and efficient

Abbreviations: SiC, silicon carbide; APTES, 3-aminopropyltriethoxysilane; AFM, atomic force microscopy; XPS, X-ray photoelectron spectroscopy; cy3, cyanine 3; FITC, fluorescein isothiocyanate; BSA, bovine serum albumin; FBS, fully biotinylated-streptavidin; rms, root-mean-square.

* Corresponding author at: Department of Chemistry and Biochemistry, George Mason University, Science and Technology 1, Room 343, 4400 University Drive, Fairfax, VA 22030-4444, USA. Tel.: +1 703 993 1070; fax: +1 703 993 1055.
E-mail address: ehwill@nist.gov (E.H. Williams).

0169-4332/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.apsusc.2012.02.137

biotin labeling of any primary amine containing molecule [3]. Thus, biotinylation of an APTES functionalized SiC surface and subsequent streptavidin immobilization is achievable and presents a protein–ligand interaction that is ideal for the development of a biosensor [19].

This paper presents results of the first detailed and comprehensive study of APTES attachment to the (0 0 0 1) Si-face of 4H–SiC. AFM, XPS, ellipsometry, and contact angle measurements were utilized to determine the optimum surface conditions for the covalent attachment of APTES. The effects of APTES deposition duration on the structure, thickness, reactivity, and wettability of the resulting APTES layer on SiC were investigated. The immobilization of biotin and streptavidin on the APTES functionalized SiC surface and the effect of APTES deposition conditions on the attachment of biotin and streptavidin were studied. Selective streptavidin immobilization on biotinylated SiC was monitored using fluorescence microscopy. In addition, the non-specific binding of streptavidin as well as BSA to bare, APTES functionalized, and biotinylated SiC surfaces was examined experimentally.

2. Material and methods

Silane functionalization, biotinylation, and streptavidin immobilization were performed on SiC substrates using Scheme 1. A commercial 76.2 mm (3 in.) diameter wafer of (n-doped) 4H–SiC (0 0 0 1)[1] was cut into 5 mm × 5 mm squares. The samples were immersed for 5 min in trichloroethylene, followed by acetone, and then in isopropanol. They were further cleaned using a standard RCA cleaning procedure [20].

To improve the reactivity of the SiC surface and to remove any remaining organic contaminants, the SiC samples were treated in an oxygen plasma [21] (Fischione Instruments, Model 1020 Plasma Cleaner[2]) in a 20% oxygen/80% argon gas mixture for 1 min. Oxygen plasma treatment grows a thin oxide that facilitates the creation of surface silanol groups (see step A in Scheme 1) which are necessary for APTES condensation on the SiC surface [22]. The SiC samples were exposed to air for 2–3 h following oxygen plasma cleaning to ensure surface chemisorption of water molecules. It has been proposed that APTES hydrolysis can occur most efficiently if there is sufficient surface water available for the reaction [23]. The moist silanol-terminated SiC samples were immersed in a 1:49 volume fraction (v/v) solution of APTES (99% APTES, Sigma–Aldrich) in toluene (99.8% anhydrous, Sigma–Aldrich) for a duration of 5 min, 1 h, or 16 h followed by ultrasonication to minimize the non-specific attachment of APTES to the SiC surface. A glove bag with a nitrogen environment at room temperature was utilized for APTES attachment. Following the controlled deposition of APTES (see Scheme 1, step B) on the SiC surface, the samples were ultrasonicated for 10 min in toluene and for 1 min in isopropanol (99% anhydrous, Oriole Brand, Warner–Graham Co.) to remove any loosely adsorbed APTES molecules [22]. The functionalized SiC samples were dried under a stream of nitrogen gas. Biotinylation of the SiC surface (see Scheme 1, step C) was achieved by placing the APTES functionalized samples in a 5 mg/mL solution of sulfo-NHS-biotin (sulfo-*N*-hydroxysuccinimide biotin ester sodium salt, Thermo Scientific) in 0.01 M phosphate buffer (pH = 7.4, Sigma–Aldrich) for 2 h at room temperature. The terminal amino group of the APTES functionalized samples reacts with the ester linkage in sulfo-NHS-biotin thus leading to the formation of a stable amide bond and a biotinylated surface. The biotinylated SiC samples were ultrasonicated in phosphate buffer for 15 min to remove physisorbed biotin molecules, rinsed with deionized water, and dried with a stream of nitrogen gas [15]. Streptavidin immobilization on the biotinylated surface (Scheme 1, step D) was achieved by exposing the samples to a 0.058 mg/mL solution of streptavidin-cy3 conjugate protein (1 mg/mL, pH 7.4, Sigma–Aldrich) in 0.01 M phosphate buffer (pH = 7.4, Sigma–Aldrich) containing 0.05% Tween20 (polyethylene glycol sorbitan monolaurate, Sigma–Aldrich) for 2 h at room temperature. A 2 h protein exposure time is typical for functionalization protocols described in the literature [3,4,15], and was thus utilized in the present study. However, it is important to point out that since biotin–streptavidin binding occurs on the second time-scale, a much shorter exposure time can be used in device sensing experiments [3]. Tween20 was used to minimize non-specific binding of streptavidin to the biotinylated SiC surface. After the immobilization of streptavidin, the samples were rinsed in a phosphate buffer containing 0.05% Tween20 solution for 15 min to remove loosely adsorbed streptavidin molecules, rinsed in phosphate buffer for 30 s to wash off the Tween20 solution and physisorbed streptavidin molecules, and dried under a stream of nitrogen gas.

The binding of FBS to 5 min APTES functionalized SiC and to biotinylated SiC was studied as follows: 10 mL of 5 mg/mL biotin (in 0.01 M phosphate buffer, pH = 7.4) was mixed with 12 mL of 0.058 mg/mL streptavidin-cy3 (in 0.01 M phosphate buffer, pH = 7.4) and 11 μL of Tween20 resulting in a solution of FBS. This solution was allowed to incubate at room temperature for 10 min. APTES and biotinylated SiC samples were subsequently immersed in the FBS solution for 2 h at room temperature, rinsed in a phosphate buffer containing 0.05% Tween20 solution for 15 min, rinsed in phosphate buffer for 30 s, and dried under a stream of nitrogen gas.

To test the selectivity of biotinylated SiC, the 5 min APTES functionalized and biotinylated samples were immersed in a solution of 0.058 mg/mL FITC labeled BSA (Sigma–Aldrich) in 0.01 M tris buffer (tris(hydroxymethyl)aminomethane, pH = 7.4, Sigma–Aldrich) containing 0.05% Tween20 for 2 h at room temperature. BSA is a serum protein that has no affinity for biotin and thus there should be no conjugation of BSA to the biotinylated SiC samples. Following sample immersion in the BSA solution, the samples were rinsed in a tris buffer containing 0.05% Tween20 solution for 15 min, rinsed in tris buffer for 30 s, and dried under a stream of nitrogen gas.

SiC samples were characterized using ellipsometry, fluorescence microscopy, AFM, XPS, and contact angle measurements.

2.1. Ellipsometry

Optical thickness measurements were performed on the SiC samples with a multichannel, spectroscopic ellipsometer (M2000-DI, J.A. Woollam Co., Inc.) at an incident angle of 70°. A weakly focused, ≈0.3 mm diameter beam was used. To avoid coherent artifacts due to reflection from the back of the double sided polished wafers, only the spectral region from 190 nm to 350 nm, where SiC is absorbing, was included in the analysis. The layer thickness was determined with vendor supplied software using up to five layers (SiC, SiO_2, organic, organic, and protein). The dielectric function for SiC was derived from a multi-angle, multi-sample (freshly etched, plasma treated) analysis. The analysis treated the SiC as isotropic [24] and the results were similar to those in the literature [25]. The dielectric function for SiO_2 was taken from the literature [26]. It was necessary to account for the UV absorption of both the organic (APTES/biotin) and protein layers. A simple model with a single

[1] Hereafter, all functionalization steps with the corresponding characterization data are reported for the Si-terminated (0 0 0 1) face of SiC; both Si- and C-terminated faces exhibited practically identical bio-functionalization behavior based on preliminary results.

[2] Commercial equipment and material suppliers are identified in this paper to adequately describe experimental procedures. This does not imply endorsement by NIST.