Applied Surface Science 316 (2014) 46–53

Contents lists available at ScienceDirect

Applied Surface Science

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

Stable functionalization of germanium surface and its application in biomolecules immobilization

Qi Cai[a,c], Baojian Xu[a], Lin Ye[b,c], Teng Tang[a,c], Shanluo Huang[a,c], Xiaowei Du[a,c], Xiaojun Bian[a], Jishen Zhang[a], Zengfeng Di[b,*], Qinghui Jin[a], Jianlong Zhao[a,**]

[a] State Key Laboratory of Transducer Technology, Shanghai Institute of Microsystem and Information Technology, Chinese Academy of Sciences, No.865, Changning Road, Shanghai 200050, China
[b] Sate Key Laboratory of Functional Materials for Informatics, Shanghai Institute of Microsystem and Information Technology, Chinese Academy of Sciences, No.865, Changning Road, Shanghai 200050, China
[c] University of Chinese Academy of Sciences, No.19A, Yuquan Road, Beijing 100049, China

ARTICLE INFO

Article history:
Received 20 June 2014
Received in revised form 19 July 2014
Accepted 21 July 2014
Available online 30 July 2014

Keywords:
Germanium
Functionalization
11-Mercaptoundecanoic acid
Self-assembled monolayers (SAMs)
Protein immobilization

ABSTRACT

As a typical semiconductor material, germanium (Ge) has the potential to be utilized in microelectronics and bioelectronics. Herein, we present a simple and effective method to immobilize biomolecules on the surface of functionalized Ge. The surface oxide of Ge was removed with the pretreatment of hydrochloric acid and the Cl-terminated Ge reacted with 11-Mercaptoundecanoic acid (11-MUA). The surface of Ge was coated with 11-MUA self-assembled monolayers (SAMs) due to the bonding reaction between the sulfhydryl group of 11-MUA and Cl-terminated Ge. Furthermore, typical biomolecule, a green fluorescent protein was chosen to be immobilized on the surface of the functionalized Ge. Contact angle analysis, atomic force microscopy and X-ray photoelectron spectroscopy were used to study the characteristics including wettability, stability, roughness and component of the functionalized Ge, respectively. Fluorescence microscopy was utilized to indicate the efficiency of protein immobilization on the surface of the functionalized Ge. With these studies, stable and uniform functionalized monolayer was obtained on the surface of Ge after 11-MUA treatment and the functionalized Ge was effectively applied in protein immobilization. Furthermore, this study may pave the way for further applications such as the integration of bioelectronics and biosensors with the attractive semiconductor material-Ge in future work.

1. Introduction

Over the past few years, there has been an increasing interest in the semiconductor biosensors that have the ability to electrically detect different targets with great selectivity and sensitivity [1–3]. In this field, the effective immobilization of organic or biological molecules on traditional semiconductor materials is a primary and important area of research for the development of hybrid bioelectronics devices [4,5]. Such hybrid semiconductor substrates functionalized with biological or organic molecules can be used in a variety of sensing applications [3,6].

As a traditional and typical semiconductor material, silicon (Si) has been very well studied more than any other element in the periodic table because of its critical importance in the microelectronics industry. Silicon forms a very stable oxide (SiO_2) and can be chemically passivated with a variety of organic species. The reaction mechanisms have been extensively investigated for solution-phase and vapor-phase oxidation, metallization, nitridation, and organic monolayer passivation of both the well characterized Si surfaces of monolithic single-crystal substrates and the poorly characterized surfaces of porous Si [7]. On the other hand, germanium (Ge), as a congener of Si, it is well known that the water solubility of GeO_2 and the complex oxidation behavior of Ge is strongly dependent on the oxidative environment and influenced by illumination conditions and crystal orientation [7–9]. The lack of a stable passivation layer on its surface has retarded Ge from being utilized as an electronic material in the past. However, compared with Si, the mobilities of holes and electrons in Ge are more than twice of Si and Ge has gained renewed interest as a candidate of choice for future electronics. These excellent electrical properties of Ge may suggest its potential application in biosensors and the increased interest in Ge has prompted the development of robust chemistries to passivate and protect against chemical oxidation.

* Corresponding author. Tel.: +86 21 62511070x8605; fax: +86 21 62511070x8714.
** Corresponding author. Tel.: +86 21 62511070x5801; fax: +86 21 62511070x8714.
E-mail addresses: zfdi@mail.sim.ac.cn (Z. Di), jlzhao@mail.sim.ac.cn (J. Zhao).

http://dx.doi.org/10.1016/j.apsusc.2014.07.127
0169-4332/

Up to now, a number of routes to nominally stable termination/passivation layers of Ge have been reported [7–25]. Generally, these passivation schemes commonly rely on an initial chemical priming of the semiconductor surface with an etchant including HF [13], NH_4F [9,26], HCl [16,18], or HBr [17], to remove surface oxide and provide either a hydrogen-terminated surface that can react via Lewis-acid-mediated, photochemical [7], arenediazonium salts [27,28], or thermally activated hydrogermylation reactions [17], or a halogen-terminated surface that reacts with alkyl Grignard reagents [14]. Notably, the ambient stability of H/Cl-terminations of Ge just can last several minutes and less than 2 h [16], respectively. And thermally initiated hydrogermylation reactions and thiol passivation are suitable monolayer precursors and the passivated layer can protect the Ge from oxidation for more than 12 h [17]. However, detailed understanding of how various species on Ge surfaces affect the electrical properties of Ge is lacking. In addition, only a few published literatures [6,29–31] mentioned the feasibility in biological molecules immobilization on the functionalized Ge substrate. Korgel and coworkers [30] studied the PEGylation of carboxylic acid-functionalized Ge nanowires and Toone et al. [6,31,32] successfully patterned protein on the NHS-terminated self-assembled monolayers (SAMs) on Ge, which provide guidance for our work.

Here, we developed a simple and effective method to immobilize the biomolecules on the functionalized Ge surface. This method could result in the feasibility in protein molecules immobilization on the surface of 11-MUA functionalized Ge. The schematic of green fluorescence protein effective immobilization on the surface of Ge was shown in Fig. 1. Firstly, the surface oxide of Ge was removed and Cl-termination was realized with HCl aqueous solution immersion. Then the functionalized SAMs were obtained through immersing the Cl-terminated Ge in 11-Mercaptoundecanoic acid ($HS(CH_2)_{10}COOH$, 11-MUA) isopropyl alcohol solution. Finally, facilitated by zero-length cross linker EDC/NHS, the carboxyl groups on the free end of SAMs were activated and a green fluorescent protein (GFP, FTIC-tagged goat anti-mouse IgG antibodies) were immobilized on functionalized Ge surface. Contact angle analysis, atomic force microscopy (AFM) and X-ray photoelectron spectroscopy (XPS) were utilized to monitor the detail process of functionalization on Ge samples and fluorescence microscopy was performed to analyze the efficiency of GFP immobilization.

2. Material and methods

2.1. Materials and reagents

All reagents were analytical grade (AR). Isopropyl alcohol ($(CH_3)_2CHOH$, IPA), acetone, ethanol absolute and hydrochloric acid were purchased from Shanghai Lingfeng Chemical Reagent Co., Ltd. And *n*-dodecyl mercaptan ($HS(CH_2)_{11}CH_3$, NDM) was purchased from Sinopharm Chemical Reagent Co., Ltd. 11-Mercaptoundecanoic acid ($HS(CH_2)_{10}COOH$, 11-MUA), 1-ethyl-3-[3-dimethy- laminopropyl] carbodiimide hydrochloride (EDC) and N-hydroxysuccinamide (NHS) were obtained from Sigma–Aldrich. The green fluorescent protein Alexa Fluor*488 goat anti-mouse IgG antibody was supplied by Molecular Probes. All solutions including phosphate-buffered saline (PBS, pH7.4, 10 mM) and 2-(N-Morpholino) ethanesulfonic acid hydrate (MES, pH6.0, 25 mM) were prepared by deionized (DI) water (18.2 MΩ cm) obtained from a MilliQ filtration system.

2.2. Sample preparation and monolayer formation

Ge functionalization and biomolecules immobilization were performed on Ge substrates following Scheme 1 (shown in Fig. 1). Commercial p-type Ge(1 1 1) wafer (Ga-doped, WSKtech) was cut into $5 \times 5\ mm^2$ squares, then cleaned by 10 min sonication in acetone and ethanol absolute in turn and dried with nitrogen. The cleaned samples were then rinsed in DI water for 10 min to preliminary dissolve the natural oxide on the surface of Ge substrate. After water rinse, the Ge samples were dried with nitrogen and immersed into 10% HCl (w/w) aqueous solution for 30 min to remove the residual oxide entirely and realize the Cl-termination [6,16]. The Cl-terminated Ge samples were subsequently washed with DI water and IPA, and then immediately immersed in 11-MUA solution (25 mM in IPA) for 24 h to obtain high quality monolayer film [15,30]. To avoid the substantial evaporation of IPA during exposure, the sample container was sealed with Parafilm. After the reaction was accomplished, the Ge samples were cleaned by sonication in neat IPA for 1 min to remove the physical absorption of 11-MUA molecules on the surface of Ge samples, and dried with nitrogen. All these steps were carried out in ambient conditions.

For comparison, several control groups were prepared in this section. Firstly, to understand the different surface behaviors of SAMs on Ge, the 11-MUA was replaced with NDM (25 mM in IPA), and NDM coated Ge samples were prepared [15]. In term of molecular structure, the major difference between 11-MUA and NDM was carboxyl group in the free end of 11-MUA molecular while alkyl group in NDM. In addition, in order to explore the characteristic of SAMs on Ge samples further, 11-MUA coated and NDM coated Au samples were also prepared in this section. The detail process was described in the supporting information.

2.3. Immobilization of fluorescent protein

After the 11-MUA-Ge samples were obtained, the chosen green fluorescent proteins were immobilized on the sample surface to illustrate the feasibility of biomolecules immobilization on the surface of Ge.

In order to immobilize the fluorescent protein, the 11-MUA-Ge samples were immersed in the MES buffer containing coupling agents: 50 mM NHS and 20 mM EDC at room temperature for 30 min [30,33]. Water-soluble EDC and NHS were used to activate the carboxyl group at the free end of 11-MUA SAMs. The activated Ge samples were washed with MES and PBS buffer twice respectively, then the EDC/NHS solution was replaced by PBS buffer, containing various concentrations of GFP. After 3 h reaction, samples were flushed with PBS and DI water, then dried with nitrogen.

Meanwhile, to understand the real effect of EDC and NHS in the immobilization process and the physical adsorption of protein on the surface of 11-MUA-Ge samples, two controls were performed in this section. The EDC and NHS treatment was bypassed and the functionalized Ge samples were incubated in the GFP solution directly. Moreover, the cleaned Ge samples without 11-MUA coated were incubated in GFP solution directly to have a further understanding of physical adsorption of 11-MUA film.

2.4. Characterization

Contact angle analysis, atomic force microscopy and X-ray photoelectron spectroscopy were performed to characterize the surface property of the functionalized Ge samples. Furthermore, fluorescence microscopy was utilized to evaluate the chosen GFP immobilization efficiency on the surface of functionalized Ge.

2.4.1. Contact angle analysis

The contact angles (θ) were measured in air using a goniometer (KRÜSS Easydrop Model FM40). DI water was used to contact with the sampling dimension by the sessile drop method and the volume of the drops was ~2 μL. At least five contact angles values