Biosensors and Bioelectronics ■ (■■■■) ■■■–■■■

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Surface modification of silicon dioxide, silicon nitride and titanium oxynitride for lactate dehydrogenase immobilization

Pawasuth Saengdee [a], Woraphan Chaisriratanakul [b], Win Bunjongpru [b], Witsaroot Sripumkhai [b], Awirut Srisuwan [b], Wutthinan Jeamsaksiri [b], Charndet Hruanun [b], Amporn Poyai [b], Chamras Promptmas [a,*]

[a] Department of Clinical Chemistry, Faculty of Medical Technology, Mahidol University, 999 Phutthamonthon 4 Road, Salaya, Phutthamonthon, NakhonPathom 73170, Thailand
[b] Thai Microelectronic Center (TMEC), Chachoengsao 24000, Thailand

ARTICLE INFO

Article history:
Received 9 April 2014
Received in revised form
4 July 2014
Accepted 22 July 2014

Keywords:
Surface modification
Silanization
Immobilization
Lactate dehydrogenase
Silicon dioxide
Silicon nitride
Titanium oxynitride

ABSTRACT

Three different types of surface, silicon dioxide (SiO_2), silicon nitride (Si_3N_4), and titanium oxynitride (TiON) were modified for lactate dehydrogenase (LDH) immobilization using (3-aminopropyl)triethoxysilane (APTES) to obtain an amino layer on each surface. The APTES modified surfaces can directly react with LDH via physical attachment. LDH can be chemically immobilized on those surfaces after incorporation with glutaraldehyde (GA) to obtain aldehyde layers of APTES-GA modified surfaces. The wetting properties, chemical bonding composition, and morphology of the modified surface were determined by contact angle (CA) measurement, Fourier transform infrared (FTIR) spectroscopy, and scanning electron microscopy (SEM), respectively. In this experiment, the immobilized protein content and LDH activity on each modified surface was used as an indicator of surface modification achievement. The results revealed that both the APTES and APTES-GA treatments successfully link the LDH molecule to those surfaces while retaining its activity. All types of tested surfaces modified with APTES-GA gave better LDH immobilizing efficiency than APTES, especially the SiO_2 surface. In addition, the SiO_2 surface offered the highest LDH immobilization among tested surfaces, with both APTES and APTES-GA modification. However, TiON and Si_3N_4 surfaces could be used as alternative candidate materials in the preparation of ion-sensitive field-effect transistor (ISFET) based biosensors, including lactate sensors using immobilized LDH on the ISFET surface.

1. Introduction

The integration of biomolecules and microelectronics is being actively developed to achieve miniaturized devices (Sakata and Miyahara, 2005) for biomarker detection. Microelectronic devices using silicon dioxide (SiO_2), aluminum oxide (Al_2O_3), silicon nitride (Si_3N_4), tantalum pentoxide (Ta_2O_5), and tin oxide (SnO_2) have been investigated for their ability to incorporate protein (Lenci et al., 2011; Lue et al., 2011). Recently, titanium oxynitride (TiON) has been considered a promising candidate due to its prominent properties of high refractive index, high dielectric constant, chemical stability, and water insolubility (Bunjongpru et al., 2013). Because the permanent attachment of biomolecules onto solid substrates is a crucial factor for biosensor development, silanization has great potential as an approach to introduce reactive moieties of biomolecules onto inorganic surfaces using a surface treatment of organosilane. (3-aminopropyl)triethoxysilane (APTES) is extensively employed to functionalize surfaces with an amine layer (APTES surface) for immobilization of DNA, antibodies, and enzymes (Lenci et al., 2011; Yadav et al., 2014). There are three ethoxy ($-OCH_2CH_3$) groups in an APTES molecule. They can be hydrolyzed in aqueous environments or anhydrous organic solvents to form silanol groups (Si–OH) (Xie et al., 2010; Yadav et al., 2014). This group undergoes condensation with a hydroxylated surface via the hydrogen bond and then forms a siloxane (Si–O–Si) linkage over the treated surface. In addition, the condensation of neighbor APTES molecules also forms a polymer matrix linked by siloxane (Si–O–Si) bonds with a subsequent loss of water molecules by curing, leading to amino-terminated ($-NH_2$) surfaces (Gunda et al., 2014; Yadav et al., 2014). These amino ($-NH_2$) groups on APTES surface react with biomolecules via physical adsorption e.g., electrostatic force, hydrogen bonding, etc. (Lee et al., 2009). APTES-modified surfaces are convenient for protein immobilization on substrate surfaces; however, the immobilized protein may

* Corresponding author. Tel.: +66 2 441 4370 9x2706; fax: +66 2 441 4380.
E-mail address: chamras.pro@mahidol.ac.th (C. Promptmas).

http://dx.doi.org/10.1016/j.bios.2014.07.057
0956-5663/

denature and be easily removed from the surface during continuous processes. To overcome these problems, covalent immobilization by coupling with glutaraldehyde (GA) is preferable to produce functionalized aldehyde groups (APTES-GA-modified surface) for direct chemical interaction with biomolecules via Schiff's base (C=N) formation (Diao et al., 2005).

The SiO_2, Si_3N_4, and TiON layers were chosen as the surface materials for the ion sensitive field effect transistor (ISFET) for the development of a biomarker detection device. Each type of ISFET surface material was investigated for the proper LDH immobilization method. The achievement of LDH incorporation into the ISFET surface will ensure the further development of a lactate sensor based on the ISFET device as the immobilization method affects the ability to incorporate target biomolecules. Therefore, each surface material treated with APTES or APTES coupled with GA was chosen to obtain a reactive surface for the incorporation of LDH molecules. The surface properties and functional groups of each modified surface were characterized using CA measurement, FTIR analysis, and SEM. The efficiency of LDH incorporation on each surface was determined for both the amount of protein and LDH activity. All these experimental results will be invaluable information regarding appropriate materials and methods in lactate sensor fabrication.

2. Experimental

2.1. Chemicals and reagents

Si_3N_4, SiO_2, and TiON surfaces were obtained from Thai Microelectronic Center (TMEC). The organosilane reagent 3-aminopropyltriethoxysilane (APTES), the cross-linker glutaraldehyde (GA), lactate dehydrogenase (LDH), pyruvate, and β-nicotinamide adenine dinucleotide reduced dipotassium salt (NADH) were purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA). The Bradford reagent for protein determination was purchased from Bio-Rad Laboratories Inc. (Hercules, CA, USA). All other reagents were commercially available, analytical reagent grade.

2.2. Surface modification

All surface materials were cut into small pieces (approximately 0.5 cm × 0.5 cm) and then cleaned with piranha solution (30% H_2O_2:H_2SO_4=1:3 v/v) at room temperature for 30 min. Then, the surfaces were thoroughly rinsed with deionized water and dried before being subjected to the silanization process. This reaction was carried out in 5% (v/v) APTES in ethanol at room temperature for 2 h. The samples were then rinsed with ethanol and dried at 60 °C overnight to obtain the APTES modified surfaces. The APTES-GA modified surfaces were prepared by immersing the APTES modified surfaces into a 2.5% GA solution in phosphate buffered saline (PBS) at room temperature for 1 h and washed with PBS. This APTES-GA modified surface contains aldehyde-functionalized layer. All different modified surfaces were evaluated for their wetting property, chemical bonding composition, and surface morphology by CA measurement, FTIR analysis, and SEM, respectively.

2.3. Contact angle (CA) measurement

The CA was measured using a goniometer, Model 250 from Rame-Hart Instrument Co. (Succasunna, NJ, USA) at room temperature using the static sessile drop method and image analysis of the drop profile. A droplet of deionized water was gently placed onto each surface and measured for its CA. An average value was obtained for each surface for three different regions on the same surface.

2.4. Fourier transform infrared (FTIR) analysis

The chemical bonding on each modified surface was analyzed by the Nicolet 6700 FTIR spectrometer from Thermo Fisher Scientific Inc. (Waltham, MA, USA) operating in attenuated total reflectance (ATR) mode. Each spectrum was recorded in the range 4000–625 cm^{-1}at a resolution of 2 cm^{-1} with 64 scans. The spectra were measured in three different regions on the same surface, and the untreated surface was recorded as a sample blank.

2.5. Scanning electron microscopy (SEM) observation

The surface morphology of the modified surface was observed by Hitachi S-4700 SEM from Hitachi High-Technologies Corp. (Tokyo, Japan) with an acceleration voltage of 5 kV. All surfaces were coated with gold to increase the conductivity prior to measurement. Each surface was measured in three different regions, and the untreated surface was recorded as a sample blank.

2.6. Enzyme immobilization

LDH (1 mg/ml) was prepared in 10 mM phosphate buffer saline (PBS, pH 7.4) and applied onto each modified surface at room temperature for 1 h. It was then gently washed with PBS to remove free LDH. The immobilization efficiency was evaluated in terms of protein content and the LDH activity presented on APTES modified surfaces and APTES-GA modified surfaces.

2.7. Protein determination

The amount of LDH attached on each modified surface was indirectly quantified using the Evolution 600 UV–Vis Spectrophotometer from Thermo Fisher Scientific Inc. (Waltham, MA, USA) by measuring the difference in protein content between the original LDH solution and the remaining supernatant after immobilization. This measurement was a colorimetric method at 595 nm using Bradford protein assay reagent with bovine serum albumin as the protein standard. The amount of immobilized LDH was calculated according to the previously reported by Bradford (1976). The immobilization efficiency of each surface in terms of relative immobilized LDH (%) was calculated using the difference in protein content between the original LDH solution and the immobilized LDH on the surface.

2.8. Enzyme activity determination

The LDH activity was determined by continuously monitoring the decrease of light absorption at 340 nm resulting from the oxidation of NADH by pyruvate using the Evolution 600 UV–Vis Spectrophotometers from Thermo Fisher Scientific Inc. (Waltham, MA, USA). The reaction mixture contained 10 mM PBS, pH 7.4, 28 mM of pyruvate, and 5.58 mM of NADH. Finally, the immobilized LDH on the surfaces was added and measured for enzyme activity every 30 s for 5 min, according to the method described by Henry (1974). The immobilized LDH activity yield was calculated in terms of relative immobilized LDH activity on the surfaces (%) using the difference in LDH activity between free and immobilized forms.

Please cite this article as: Saengdee, P., et al., Biosensors and Bioelectronics (2014), http://dx.doi.org/10.1016/j.bios.2014.07.057