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Influence of immobilization strategies on biosensing response characteristics: A comparative study

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ABSTRACT

The immobilization technique plays an important role in fabrication of a biosensor. NiO based cholesterol biosensor has been used to study the effect of various immobilization techniques on the biosensing response characteristics. The biosensors were fabricated by immobilizing cholesterol oxidase on NiO thin films by three different immobilization techniques viz. physisorption, cross-linking and covalent binding. The study reveals a strong dependence of biosensing response on corresponding immobilization technique. The biosensor based on immobilization by covalent bonding shows superior response characteristics as compared to others owing to its zero length. The results highlight the significance of immobilization technique for biosensor fabrication.

1. Introduction

Biosensors have gained attention of the research community over time owing to their promising applications in healthcare industry, food industry, environmental monitoringand biological analysis. Despite commercialization of various biosensors, the research is still being propelled by the need to achieve improved sensitivity and a better stability [1]. Biosensor is an analytical device that consists of a biologically active component (receptor) in contact with an appropriate transduction element (electrical, thermal, optical, etc.) for detection of analyte bio-molecules [2]. In a biosensor, the biological element responsible for sensing is the receptor biomolecule, and the sensing relies on specific bio-chemical interaction between the analyte and receptor. Matrix provides the solid support for the receptor through which it is integrated to the biosensing system and acts as a vital link between the bio-chemical interaction and external electronics by acting as a medium of electron transfer [3]. A wide range of matrices have been used for integration of the receptor to the biosensor which includes metal oxides, conducting polymers, carbon nanotubes (CNTs) and metals in the form of thin films, nanoparticles, hydrogels, monolayers, modified metals, hybrid nano-composites, all having their own advantages and disadvantages [4–7].

Though the matrix (a physical component) and the receptor (a biological component) play an important role in the sensing mechanism but the link between the two is a vital factor governing the charge transfer and stability. There are a number of ways by which the receptor is immobilized on a matrix. Immobilization techniques like, crosslinking, covalent bonding and physical adsorption are well reported in the literature [8–14]. Physical adsorption is mainly based on the electrostatic interaction between the biomolecule and the matrix surface due to difference in iso-electric points. Crosslinking is based on employing a crosslinker molecule between the matrix and the enzyme. Covalent binding involves the formation of direct covalent bond between the matrix and receptor engaging some functional groups like amino, carboxyl, thiol, sulfhydral etc. Crosslinking and covalent binding rely on immobilization via a third molecule acting as a bridge between the matrix and the receptor. However, in the later case the bulk linking molecule is subsequently removed and a zero length bond exists between the matrix and receptor [14].

Some studies have been carried out on antibody–antigen systems where various immobilization strategies and biomolecule orientation has been compared for improvement of sensing response. Binding of antibodies via different proteins have been studied by Babacan et al. [15]. Vashist et.al. have studied the

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effect of various antibody immobilization strategies on SPR sensing response [16]. Biosensing response has also been studied by carrying out antibody modification [17]. Apart from immobilization strategy the orientation of immobilized biomolecule is also known to affect the biosensing response [18]. Experimental strategies are also known to affect the sensing [19]. It is important to note that these studies are based on biosensors based antibody–antigen interactions which are affinity reaction. The immobilization strategy is expected to affect the catalytic reaction involved in enzymatic biosensors. Zhang et al. have reviewed different enzyme immobilization techniques and highlighted their effect on biosensing response [20]. Though so many reports are available but a clear inference cannot be drawn regarding the superiority of an immobilization technique from the literature. In this view, a concrete study is required where an enzyme has been immobilized using different techniques under the same working condition and a thorough sensing comparison is made.

In the present report, we attempt to study the effect of various immobilization techniques on the performance of a biosensor. Three biosensors were prepared using three different immobilization techniques keeping the matrix and the receptor fixed. NiO thin film has been chosen as the matrix owing to its inherent qualities like higher surface area, porosity, high conductivity, biocompatibility, chemical stability and electron transfer capability [21]. On the other hand, cholesterol oxidase (ChOx) has been selected as the model receptor. Three cholesterol biosensors have been designed by immobilizing ChOx on NiO thin film by physical, cross-linking and covalent immobilization techniques and their biosensing response characteristics have been studied.

2. Experiment

2.1. Chemicals and reagents

Cholesterol oxidase (ChOx) extracted from streptomyces species has been procured from SRL Pvt. Ltd., India. Triton X-100 (for the preparation of cholesterol stock solution), *N*-hydroxisuccinimide (NHS), *N*-(3-dimethylaminopropyl)-(*N*′-ethylcarbodiimide) (EDC), free cholesterol and nickel acetate tetrahydrate ($Ni(OAc)_2{\cdot}4H_2O$) were purchased from Sigma–Aldrich, USA. The chemicals were used without further purification.

ChOx solution (1 mg/ml) was freshly prepared in 50 mM Phosphate Buffer Saline (PBS) of pH 7.0 containing 0.9% NaCl. The stock solution of cholesterol was prepared in 10% Triton X-100 and stored at 4 °C.

2.2. Preparation and characterizations of NiO thin film

For the preparation of nanostructured NiO thin film, 4.35 g of $Ni(OAc)_2{\cdot}4H_2O$ was dissolved in 30 ml of de-ionised (DI) water. A separate solution was prepared by dissolving 1.5 g of NaOH in 75 ml de-ionised water containing 0.5 g PVP as a surfactant. The two solutions were mixed and spin coated onto the surface of an ITO coated corning glass substrate of area 2 cm × 1 cm of which 1 cm × 1 cm area was masked for electrical contacts. This deposition was repeated five times and the films were dried at 150 °C after each coat. Finally, the films were annealed in air at 400 °C for 2 h. All the NiO films for different immobilizations were made in the same batch.

The prepared NiO thin film has been characterized by X-Ray diffraction (XRD, Bruker D8 Discover). All electrochemical measurements were performed on Gamry Reference 600 Potentiostat/Galvanostat using a three-electrode cell in PBS (pH 7.0) with Ag/AgCl as the reference electrode and platinum as the counter electrode. Photometric studies were carried out using PerkinElmer UV–vis spectrophotometer.

2.3. Immobilization of ChOx on NiO thin film

Physical adsorption was carried out by spreading 10 μl of ChOx solution over one of the NiO thin films and incubating at 27 °C for 2 h. The bio-electrode (ph-ChOx/NiO/ITO/Glass) was kept overnight at 4 °C and washed with PBS to remove any unbound enzyme molecules. The enzyme physically adsorbs on NiO film through electrostatic interaction due to the high isoelectric point of NiO [21].

For crosslinking, 10 μl of ChOx solution was mixed with 1 μl of 1% aqueous solution of glutaraldehyde which is a widely used crosslinking agent [10,22]. It was then spread over the NiO film and incubated at 27 °C for 2 h. The bio-electrode thus obtained (cr-ChOx/NiO/ITO/Glass) was rinsed with DI water before use and stored at 4 °C when not in use.

ChOx was covalently immobilized on the NiO thin film via EDC-NHS [13]. The film was subjected to hydrolysis and silanization to activate the surface for covalent bonding. The film was immersed in a solution of $H_2O_2/NH_4OH/H_2O$ for 30 min at 80 °C for hydrolysis, followed by through rinsing with DI water and drying. The hydrolyzed film was immersed in 1% solution of 3-aminopropyl triethoxysilane (APTES) in toluene overnight at room temperature for silanization. After the coupling reaction, the electrode was rinsed with toluene and DI water to remove the unbound silanes from the surface. 10 μl of ChOx solution containing 0.4 M EDC

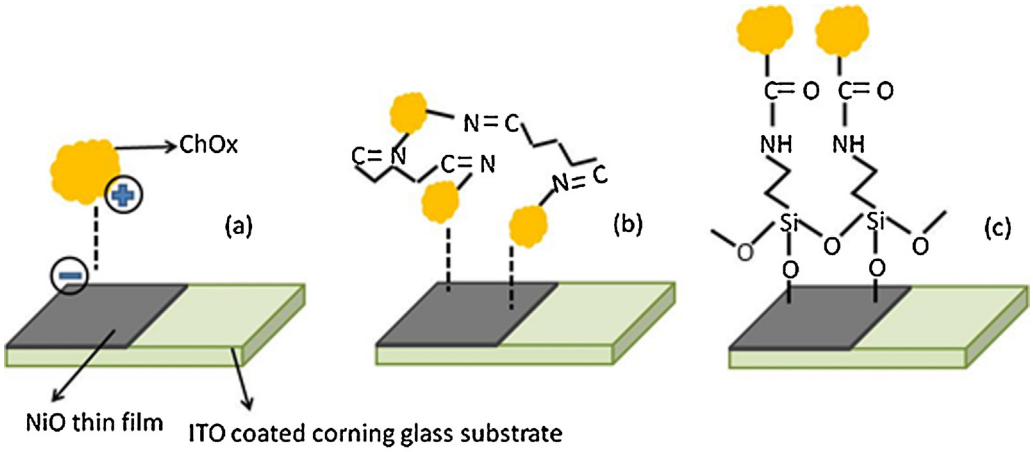

Fig. 1. Schematic diagram of enzyme immobilization by (a) physical adsorption (b) crosslinking and (c) covalent bonding.