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Nanostructured zinc oxide platform for cholesterol sensor
Third generation biosensing matrix based on Fe-implanted ZnO thin film

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Third generation biosensor based on Fe-implanted ZnO (Fe-ZnO) thin film has been demonstrated. Implantation of Fe in rf-sputtered ZnO thin film introduces redox center along with shallow donor level and thereby enhance its electron transfer property. Glucose oxidase (GOx), chosen as model enzyme, has been immobilized on the surface of the matrix. Cyclic voltammetry and photometric assay show that the prepared bioelectrode, GOx/Fe-ZnO/ITO/Glass is sensitive to the glucose concentration with enhanced response of 0.326 μA mM⁻¹ cm⁻² and low Km of 2.76 mM. The results show promising application of Fe-implanted ZnO thin film as an attractive matrix for third generation biosensing. © 2010 American Institute of Physics. [doi:10.1063/1.3496456]

Biosensors, owing to their potential applications in the field of healthcare, biological analysis, environment monitoring and food industries, have gained much attention of the research community in the last two decades. In the past few years zinc oxide, a wide band-gap semiconductor has gained much attention as a matrix for immobilization of various biomolecules due to its properties like strong adsorption ability, biocompatibility, high isoelectric point (IEP~9.5) and abundance in nature.1–5 Though the reported studies suggest biocompatibility, high isoelectric point and enhanced electron transfer property. Glucose oxidase (GOx), chosen as model enzyme, has been immobilized on the surface of the matrix. Cyclic voltammetry and photometric assay show that the prepared bioelectrode, GOx/Fe-ZnO/ITO/Glass is sensitive to the glucose concentration with enhanced response of 0.326 μA mM⁻¹ cm⁻² and low Km of 2.76 mM. The results show promising application of Fe-implanted ZnO thin film as an attractive matrix for third generation biosensing. © 2010 American Institute of Physics. [doi:10.1063/1.3496456]

Due to the absence of a redox couple in ZnO, a redox species mediator is required to provide the shuttling path for electrons, generated in biochemical reactions. ZnO-based biosensor using potassium ferricyanide as mediator has been reported earlier.6 However, attempts are continuing worldwide to remove the mediator from the electrolyte solution and proceed toward third generation biosensors based on ZnO. Though the fabrication of a matrix structure consisting of ZnO-potassium ferricyanide has been reported for possible application in third generation biosensor,5 the use of ferricyanide makes the matrix unsuitable for development of in vivo biosensors and suffers from low sensitivity. The redox peak observed in the cyclic voltammogram had been attributed to the presence of iron (Fe)-ions in the composite matrix. An alternate way to introduce the redox property in ZnO based matrix while maintaining its biocompatibility is by ion-implantation of metals having multiple oxidation states (Fe, Cu, Mn, etc.). Different properties like ferromagnetism, spin injection and electrical isolation have been obtained in ZnO using Fe implantation.10–12 However, no attempt has been made toward possible introduction of redox couple property in ZnO using ion implantation and to explore its potential application for the realization of third generation biosensors. The Fe-implanted ZnO thin film matrix combines the salient features of ZnO along with the redox behavior and enhanced electron transfer property.

In this paper, we report the fabrication of an attractive biocompatible matrix for third generation biosensor by implanting Fe into rf-sputtered ZnO thin film. Glucose oxidase (GOx) has been chosen as the model enzyme for identifying the application of the matrix toward mediator-less biosensing. The prepared bioelectrode exhibits enhanced sensing response to glucose along with long term stability without any interference from other biomolecules.

ZnO thin film (~80 nm) was deposited on ITO coated Corning glass substrate by rf-magnetron sputtering. A metallic zinc target (99.99% pure and 6 in. diameter) was sputtered in a reactive gas mixture (50% O₂+50% Ar) under a pressure of 50 mT at a power of 300 W. The as-grown ZnO film was implanted at room temperature with 15 keV Fe at a fluence of 10¹⁵ cm⁻². Since surface defects have a significant role in sensing phenomena, the low energy implantation was intentionally chosen to have the rich concentration of Fe-implant near the surface of ZnO film. A peak concentration of 8×10²⁰ Fe/cm² at a depth of 10 nm was estimated using TRIM calculations, while the ion range extend to a depth of about 25 nm.13 The as-grown films were also implanted with 20 keV Zn at a fluence of 10¹⁵ cm⁻² to create a similar damage profile for comparison. GOx enzyme was immobilized on the surface of Fe-implanted ZnO (Fe-ZnO) thin film by physical adsorption technique. The 0.5 cm² area of ZnO film was dipped in a solution of GOx (1 mg/ml) prepared in 50 mM (0.9% NaCl) phosphate buffer saline (PBS). The electrode was kept overnight for enzyme (GOx) immobilization and subsequently washed with buffer solution and dried under nitrogen flow. The prepared GOx/Fe-ZnO/ITO/Glass bioelectrode was stored at 4 °C when not in use.

X-ray diffraction (XRD) was carried out to identify the crystallographic structure of the deposited film. The activity of enzymes immobilized on the surface of Fe-ZnO films was studied by photometric assay using UV-visible spectrophotometer (Perkin Elmer Lambda 35). Scanning electron microscopy (SEM) (Zeiss Ultra plus) was employed to study the surface morphology of the thin films. Cyclic voltammetric (CV) measurement was carried out on a potentiostat/galvanostat (Gamry Inc.) using a three-electrode system in PBS solution with Ag/AgCl as the reference electrode.

Figure 1 shows the XRD spectra of as-grown and Fe-implanted ZnO thin film deposited on ITO coated glass sub-

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strate. Only reflection corresponding to (002) plane of ZnO is observed indicating growth in a preferred orientation with its c-axis normal to the substrate. The absence of ZnO peak in the Fe-ZnO film [Fig. 1] suggests amorphization or heavily defective film due to implantation. Similar observation of crystalline-to-amorphous transition in ZnO thin film by ion implantation has been reported by other workers using O+ or Si ions.

Figures 2(a) and 2(b) shows the SEM images of the as-grown ZnO and Fe-implanted films. The growth of ZnO thin film having uniformly distributed grains along with nanoporous morphology was observed [Fig. 2(a)]. The nanoporous structure provides larger surface area which is advantageous for enhanced enzyme loading on the ZnO surface. The SEM image of Fe-ZnO film shows significant changes in the surface morphology after ion implantation [Fig. 2(b)].

CV studies have been carried out in the potential window of −0.8−(−0.6 V) in PBS solution. Figure 3 shows the CV curves obtained for ZnO/ITO/Glass, Fe-ZnO/ITO/Glass and GOx/Fe-ZnO/ITO/Glass electrodes. No redox peak was observed in the CV spectra of ZnO/ITO/Glass electrode. The redox current increases with Fe-implantation into ZnO thin film in the same potential window. The redox peak is observed due to implantation of Fe having a reversible redox property. The implantation of Fe into the ZnO thin film matrix facilitates the electron transfer property due to the creation of shallow donor levels. Activation energy was measured by temperature dependant conductivity curve. A decrease in the activation energy of ZnO matrix with Fe implantation from 91 to 21 meV was observed, confirming the formation of shallow donor levels. The creation of shallow donor levels by Fe in the substitutional Zn sites due to ion implantation has been reported. To confirm that the redox couple is contributed by the implanted Fe, CV of ZnO implanted with Zn (Zn-ZnO) was also carried out. Though a slight increase in the current is seen, but no redox peak was present in the CV curves. The increase in the current may be attributed to the changes introduced by ion bombardment but no redox peak is seen as the Zn does not provide the redox couple as provided by Fe. It is interesting to note from Fig. 3 that the current increases further with immobilization of GOx on Fe-ZnO surface, despite the fact that GOx is a macromolecule of non-conducting nature. This may be due to the fact that the majority of GOx molecules exist in α-helix structure, which behave as a macro-dipole, as compared to the β-structure. The dipole structure on the surface of matrix facilitates the movement of charge carriers and increases the current [Fig. 3]. Being in a preferred α-helix structure is an implication of better catalytic efficiency of the immobilized GOx which is a possible reason for high sensitivity of the present implanted matrix.

Figure 4(a) shows the CV spectra of GOx/Fe-ZnO/ITO/Glass bioelectrode with different glucose concentrations. In the present study, no peak corresponding to oxidation of H2O2 is seen in the working potential window of the CV measurements [Fig. 4(a)]. The implantation of Fe in ZnO thin film provides the electron transfer channel and the prepared matrix acts as an efficient media for electron transfer, over bio-oxygen, resulting in a peak at a relatively lower potential (~0.06 V). The oxidation current increases with increasing concentration of glucose in the solution [Fig. 4(a)]. The variation of current, measured at a fixed potential of ~0.06 V for GOx/Fe-ZnO/ITO/Glass bioelectrode as a function of glucose concentration is shown in Fig. 4(b). A linear increase in the measured current is observed with increasing glucose concentration up to 20 mM. The sensitivity of the bioelectrode based on Fe-ZnO matrix is 0.326 μA mM−1 cm−2 with a lower detection limit of 0.31 mM. The Michaelis–Menten kinetic parameter, estimated using Hane’s plot, was 2.76 mM which is much lower than the value for free GOx (27 mM) and confirms the favorable enzymatic orientation of the immobilized GOx on the surface of Fe-ZnO matrix.

The photometric enzyme assay of immobilized enzyme was carried out by dipping the bioelectrode in 3 ml PBS solution containing 20 μl horseradish peroxidase, 20 μl o-dianisidine dye and 100 μl of biosubstrate (glucose). The difference between the initial and final absorbance value at λ=500 nm is recorded and shown in Fig. 5 as a function...
of glucose concentration. The enzyme activity increases with increase in the concentration of glucose. The amount of bound enzyme is calculated using the equation $d_{app}^{ext} = AV/ets$, where $A$ is the difference in absorbance before and after incubation, $V$ is the total volume (3.17 cm$^3$), $e$ is the millimolar extinction coefficient (7.5 for o-dianisidine at 500 nm), $t$ is the reaction time (min) and $s$ is the surface area (cm$^2$) of the electrode. The apparent enzyme activity was estimated to be $3.72 \times 10^{-2}$ Units/cm$^2$. The shelf-life study has shown that the bioelectrode retains more than 85% activity even after a period of 60 days. The selectivity study suggests that the presence of interferants, like cholesterol, urea, and ascorbic acid, have a negligible effect on the performance of the GOx/Fe-ZnO/ITO/Glass bioelectrode toward sensing of glucose.

In summary, ion-implantation technique has been exploited toward making ZnO matrix suitable for third generation biosensors. The surface of Fe-implanted ZnO matrix has been immobilized with the model enzyme, GOX, and effectively utilized for glucose sensing. In the implanted matrix the Fe provides a redox site and a good electron transfer path due to the creation of shallow donor level in ZnO. Moreover, the Fe-ZnO matrix provides a suitable environment for the enzyme to be in an orientation favorable for better enzymatic activity. The GOx/Fe-ZnO/ITO/Glass bioelectrode exhibits a good linearity with glucose up to a concentration of 20 mM and a relatively high sensitivity of 0.326 $\mu$A mM$^{-1}$ cm$^{-2}$ in a mediator-less system. The results clearly suggest that the Fe-implanted ZnO thin film provides a suitable platform for immobilization of biomolecules and proves to be a promising third generation biomatrix which could lead to development of MEMS and ISFET based miniaturized biosensors.

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