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Opportunities in nano-structured metal oxides based biosensors

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Abstract. Nanomaterials are presently at the critical stage of the next technological revolution in solid-state electronics and are emerging as new structural materials, to serve as systems for controlled drug delivery, biomolecular electronics and are considered to have considerable impact in practically all domains of science. Among the various types of nanomaterials that have been developed, nanostructured metal oxides (NSMOs) have recently aroused much interest as immobilizing matrices for biosensors development. The unique properties of NSMOs offer excellent prospects for interfacing biological recognition events with electronic signal transduction and for designing a new generation of bioelectronics devices that may exhibit novel functions. Among the NSMOs, biocompatible zirconia (ZrO2) and its composite especially with chitosan and carbon nanotubes are technologically important exhibits high bioactivity for biomolecules with rapid and enhanced electrochemical signal. Efforts are being continuously made to explore the prospects and future challenges of NSMOs for the development of biosensing devices.

1. Introduction
Nano-structured materials can be grouped as (i) inorganic, (ii) organic and (iii) inorganic-organic nanocomposites. Inorganic nanostructures especially nano-structured metal oxides (NSMOs) e.g. iron oxide (Fe2O3), zirconium oxide (ZrO2), zinc oxide (ZnO), nickel oxide (NiO), cerium oxide (CeO2) silicon dioxide (SiO2) etc, have opened new opportunities for a plethora of applications due to their unique properties (Figure 1)7-11. Electron transport properties of NSMOs are very important for electrical and electronic applications as well as for understanding the unique one-dimensional carrier transport mechanism. Besides this, metal oxide nanoparticles have high thermal stability, chemical inertness, non-toxicity, large surface-to-volume ratio, high surface reaction activity, biocompatibility and tunable electron transport properties. However, these NSMOs are difficult to functionalize that can perhaps be addressed by combining them with organic nanostructures which are otherwise not stable alone, as they interact with the moieties present in the environment. There is, thus a synergic effect between the inorganic and organic nanostructure and these inorganic-organic nanocomposite

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offer excellent prospects for interfacing biological recognition events with electronic signal transduction and for designing new biosensor devices\textsuperscript{12-15}. According to IUPAC, a biosensor is a self-contained integral device that is capable of providing specific quantitative or semi-quantitative analytical information using a biological element. Biosensor has three major components: (i) a bio-recognition element such as DNA, enzyme, antibody etc. for recognition of an analyte also called bioreceptor (ii) an immobilization surface such as a nanomaterial/conducting polymer/sol–gel film/self-assembled monolayer, etc., used for the immobilization of a biomolecule and (iii) a transducer for conversion of a biochemical reaction product into a recognizable signal.

2. Importance of NSMOs for biosensing

Biosensor is based on the interaction of a biomolecule in conjunction with an electrode where the redox reaction could be monitored electrochemically by measurement of the loss or formation of a substrate or product, by the use of a small mediator species that shuttles between the biomolecule and the electrode, or less commonly by direct electron transfer (ET) between biomolecule redox site and the electrode. Direct ET can be difficult to achieve, since the redox site of a biomolecule is often hidden deep inside the biomolecule. Recent advances in achieving direct ET have been made by the modification of biomolecules or electrode surface through the use of novel NSMO as a mediator and the design of functional biointerface (Figure 2), which has extended throughout the field of bioelectrochemistry\textsuperscript{16-19}.

The NSMOs have recently become important as immobilization matrices for the fabrication of biosensors, as they provide excellent optical and electrical properties due to electron and phonon confinement, high surface-to-volume ratio, high surface reactivity and catalytic activity along with strong adsorption ability resulting in enhanced sensing characteristics. The adsorption of biomolecules directly onto the bare surface of bulk material may frequently result in their denaturation and loss of bioactivity. However, the adsorption of such biomolecules onto a given NSMO surface may perhaps retain the bioactivity because of the non-toxicity NSMO and their biocompatible composites with carbon nanotubes (CNT), chitosan (CHIT) etc using electrostatic and other interactive forces.

\textbf{Figure 1.} Properties of some NSMOs used for the fabrication of biosensors.
The interface formed between an NSMO and a biomolecule is of utmost importance as the surface area, surface energy, roughness, porosity etc. affect the performance of a biosensor. So, the judicious selection of NSMO is the key success for the development of an effective biosensor.

An effective NSMO biointerface helps a biomolecule, to retain its biological activity with high stability by establishing a biocompatible microenvironment, and also faster rate of electron transfer. There is thus, a considerable opportunity for the development of biosensors with improved sensitivity and detection limits, as well as lower cost and extended shelf life, through the use of NSMOs.

3. Electrochemical DNA biosensor using nano-structured biocompatible ZrO\textsubscript{2} based matrices

Fascinating electrochemistry and unique hybridization behaviour of desired nucleic acid and the excellent prospects for interfacial biological recognition event with electronic signal transduction using NSMOs, has stimulated much interest for designing new generation bioelectronic devices that may exhibit novel applications especially in the field of DNA biosensors. The high sensitivity, specificity for target analyte, faster response, ability to operate in turbid or colored solutions, easy handling, low cost, compatibility with micro-fabrication technology and portability has aroused increased interest for the fabrication of DNA biosensors.

For the fabrication of an electrochemical DNA biosensor, immobilization of DNA onto a nanocrystalline transparent metal oxide based matrices like ZrO\textsubscript{2} have drawn considerable attention due to their unique physical, chemical, and optical properties that make them promising matrices for sensing applications. Moreover, the nanostructures provide increased surface area for DNA immobilization that may lead to the improved limit of detection. Besides this, it is found to be thermally stable, chemically inert, non-toxic, and has affinity for groups containing oxygen that facilitates covalent immobilization of biomolecules without using any cross-linker that may limit sensitivity of a biosensor. The biocompatibility of ZrO\textsubscript{2} is anticipated to offer conducive environment to the fabricated biosensor which may perhaps result in enhanced stability and reusability.

Solanki \textit{et.al} have utilized sol–gel-derived nano-structured ZrO\textsubscript{2} film for immobilization of 17-base ssDNA identified from the 16sRNA coding region of \textit{Escherichia coli}. This bioelectrode exhibits high selectivity and sensitivity for hybridization, with linearity in the range of 10\textsuperscript{-6} to 10\textsuperscript{6} pM of complementary DNA. Zuo \textit{et al.} have fabricated a DNA biosensor by immobilizing ssDNA with a terminal 5’-phosphate group to a ZrO\textsubscript{2} surface utilizing the strong affinity between the oxygen atoms of the phosphoric group and zirconium. This biosensor has been found to have high sensitivity and selectivity for hybridization detection (≤20 nM complementary DNA). Yang \textit{et al.} have fabricated a DNA biosensor using a composite comprising of Nano ZrO\textsubscript{2}-CNT-CHIT and observed
that the bioelectrode can effectively detect the complementary DNA $1.49 \times 10^{-10} - 9.32 \times 10^{-8}$ using differential pulse voltammetry.

Inspired by the characteristics of nano ZrO$_2$, Das et al. have fabricated electrochemical DNA biosensor for *M. tuberculosis* detection using nano-structured ZrO$_2$, from an aqueous solution of zirconium oxychloride (ZrOCl$_2$) onto gold coated glass plate (Au) (Figure 3). They have observed that ssDNA-NanoZrO$_2$/Au electrode can detect target analyte from $640 - 0.065$ ng.$\mu$L$^{-1}$, with lower detection limit of $0.065$ ng.$\mu$L$^{-1}$ (20nM) and 1 ng.$\mu$L$^{-1}$ for genomic DNA indicating that it can be used for rapid and early detection of *M. tuberculosis*.

In spite of being an interesting matrix for biomolecule immobilization, nano-structured ZrO$_2$ based electrode suffers from the problems of cracking and aggregation leading to limited application of ZrO$_2$ nanoparticles to biosensing. This problem can perhaps be addressed by modifying ZrO$_2$ nanoparticles with CHIT. CHIT has been found to be an interesting biopolymer for immobilization of biomolecules because of its excellent film-forming ability, high permeability, biocompatibility, low cost etc. Moreover, chemical modification of amino groups of CHIT provides hydrophilic environment for the biomolecules. Feng et al. have reported nanoporous CeO$_2$/CHIT composite film as the immobilization matrix for fabrication of electrochemical DNA biosensor for colo-rectal cancer detection. This biocomposite matrix can be used for increased loading of probe DNA to obtain enhanced biosensor response. Kaushik et al. have developed a nucleic acid sensor based on Fe$_2$O$_3$/CHIT hybrid matrix for pyrethroid detection.

We have proposed chitosan-zirconium-oxide (CHIT-NanoZrO$_2$) nanocomposite, deposited via electrophoretic deposition of onto indium-tin-oxide(ITO) coated glass plate from a colloidal suspension of CHIT-NanoZrO$_2$ in acetonitrile, to reveal its application in DNA biosensor for *M. tuberculosis* detection. Efforts have been directed to propose a mechanism for the electrophoretic deposition of CHIT-NanoZrO$_2$ colloidal particles onto given ITO plate in presence of DC electric field.

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reduced at the cathode surface, a localized pH gradient is generated, resulting in cathodic electrodeposition of thin CHIT-NanoZrO₂ film (180 nm) with ZrO₂ molecules entrapped within CHIT chains due to strong electrostatic interactions between these oppositely charged moieties.

The morphological studies carried out using SEM and TEM techniques clearly reveal incorporation of the hexagonal grains of ZrO₂ (30-50 nm) in the amorphous network of CHIT (Figure 4). Interestingly, the nano grained ZrO₂ have been found to be inter-linked and well-dispersed into the CHIT matrix, corroborating the homogeneous nanobiocomposite formation. This has been attributed to electrostatic interactions between cationic CHIT and surface charged ZrO₂ nanoparticles. The DNA/CHIT-NanoZrO₂/ITO bioelectrode, fabricated by covalent immobilization of biotinylated probe DNA specific to *M. tuberculosis* onto this nanobiocomposite matrix, has been found to be selective and can detect complementary target up to 0.78 nM with sensitivity of 6.38×10⁻⁶ A μM⁻¹.

Contemporary interest in metal and metal oxide/CNT composites stems from their utilization as an emitter in field emission displays, a thermal interface material, a super capacitor, interconnects in large scale integrated circuits etc. Keeping in view, the advantages of CNT-metal oxide composites in various emerging fields, there is an augmented interest in exploring the synergy of electroactive CNT and biocompatible metal oxides in the potential field of biomolecular electronics. In this context, bio-functional devices based on CNT/ZrO₂ nanocomposites have recently gained much attention. In the fabrication of a biosensing device composed of CNT/ZrO₂, the thickness and the morphology of the ZrO₂ coating is considered to play a critical role. The uniform, dense pin-hole free thin coating provides large surface area that can be useful for biosensor applications. In this context, electrophoretic technique has achieved much interest for deposition of the uniform, dense and electrochemically reversible films from colloidal suspension. In addition, this technique provides a unique strategy to prepare nano-patterned composite film with nanoporosity and densely packed structure with controlled thickness and morphology.

Electrophoretic deposition of ZrO₂ grafted CNT, has been obtained by Das *et al.* via isothermal hydrolysis of zirconium oxychloride (ZrOCl₂) in presence of CNT, onto ITO coated glass plate (Figure 5). The structural characterization of this nanocomposite (Figure 6) divulge that there is transition of crystal structure of ZrO₂ from its pristine monoclinic to deviated monoclinic arrangement with increased unit cell volume of 163 Å³. The high resolution TEM and SEM studies reveal that the ZrO₂ is present in and around the nanotubes resulting in deviated monoclinic crystalline arrangement having crystallite size of 28.63 nm. The change in the crystalline structure provides increased surface area to immobilize the biomolecules required for the fabrication of an effective biosensor.

Based on this idea, an impedimetric genosensor (ssDNA-NanoZrO₂-CNT/ITO) is fabricated by *in-situ* entrapment of 21-mer DNA probe specific to *Mycobacterium tuberculosis*.
**Figure 5.** Electrophoretic fabrication of CNT/ZrO₂ nanocomposite and the interaction between DNA and the nanocomposite.

**Figure 6.** (A) XRD; (B) TEM; (C) SEM and (D) EDX pattern of NanoZrO₂-CNT composite.
The ssDNA-NanoZrO$_2$-CNT/ITO electrode is capable of detecting DNA hybridization process by showing the variation in charge transfer resistance ($R_{CT}$) obtained from the diameter of the semicircle of the Nyquist plot. The results of DNA hybridization with the complementary target DNA strands reveal wider detection range of target DNA concentration ($1\times10^{-2}$ mM to $1\times10^{-8}$ mM) and exhibit detection limit as low as $1\times10^{-9}$ mM or 0.01 nM with storage stability of 24 weeks.

Table 1 exhibits the various biosensing characteristics obtained for the fabrication of DNA biosensors for $M.$ tuberculosis detection using ZrO$_2$ and its composite by Das et al.

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Film fabrication method</th>
<th>Detection limit</th>
<th>Reusability</th>
<th>Stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>NanoZrO$_2$</td>
<td>Electrochemical</td>
<td>20 nM</td>
<td>10-12 times</td>
<td>16 weeks</td>
</tr>
<tr>
<td>NanoZrO$_2$-CHIT</td>
<td>Electrophoretic</td>
<td>0.78 nM</td>
<td>10-12 times</td>
<td>18 weeks</td>
</tr>
<tr>
<td>NanoZrO$_2$-CNT</td>
<td>Electrophoretic</td>
<td>0.01 nM</td>
<td>~ 15 times</td>
<td>24 weeks</td>
</tr>
</tbody>
</table>

4. Conclusions
With the rapid emergence of nanotechnology, hybrid bio/nanostructures with well-defined morphologies have been popularly employed to modern materials science. Capitalizing the properties of NSMOs, novel composite nano-scale materials have opened up a new horizon in materials science with a synergistic effect of the individual components. The highly sensitive detection protocol for the fabrication of biosensor using NSMOs especially with ZrO$_2$, suggest that future interdisciplinary research is likely to lead to a new generation of electrochemical biosensors for the detection of infectious agent in an early stage.

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