

A Comprehensive Review of ZnO nanostructures and thin films for biosensor applications

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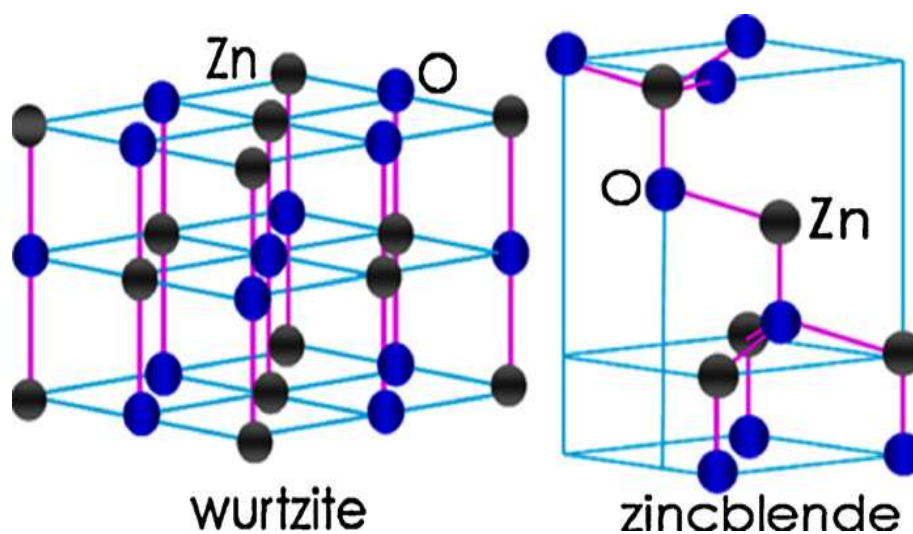
Abstract: ZnO nanostructured materials, such as films and nanoparticles, could provide a suitable platform for development of high performance biosensors due to their unique fundamental material properties. The performance of biosensors depends on their components, among which the matrix material, i.e., the layer between the recognition layer of bio molecule and transducer, plays a crucial role in defining the stability, sensitivity and shelf-life of a biosensor. This paper reviews different preparation techniques of ZnO nanocrystals. Efforts are made to summarize and analyze existing results regarding surface modification for successful biofunctionalization and understanding of the mechanisms involved.

I. Introduction:

Recent advances in interdisciplinary research and molecular diagnostics have led to the rapid development of different classes of biosensors for a wide range of bioanalyte detection with improved sensing characteristics. In addition, continuous developments in the fields of engineering and nanotechnology have contributed to miniaturization and multifunctionality in biosensor technology.

In a biosensor sensing biomolecule need to be integrated into the system on a solid support. The type of solid support that holds the sensing biomolecule (receptor) is known as a matrix. A suitable matrix enhances signal transduction and helps to immobilize biomolecule with retained or enhanced activity. The physico-chemical properties of the matrix dictate the method of immobilization and the operational stability of the biosensor [1]. Moreover, the matrix alters the resistance of the biomolecule to various physical and chemical changes, such as pH, temperature and chemical composition changes. Recently, ZnO thin films and nanostructures have gained much attention as a suitable matrix for biomolecule binding. This review is intended to comprehensively report on recent advances in the synthesis protocols of various ZnO nanostructures and thin films in addition to the applications of ZnO structures in biosensors.

The present review highlights potentials of ZnO nanocrystals, addressing mainly nanoparticles, for bio sensing by modifying the surface properties. For comparison, results obtained on nanostructured ZnO films or/and crystals are, where suitable, brought into consideration. Related issues such as biocompatibility are also discussed.



Schematic representation of ZnO crystal structures: wurtzite and zinc blende [2].

Zinc oxide:

Although known and utilized for a very long time, it is only during the last decade that zinc oxide (ZnO) has been the focus of research in relation to promising photonic and electronic applications of this material. As a direct wide band gap (3.37 eV) semiconductor ZnO is attractive for short-wavelength light emitting devices while, as an oxide semiconductor, it is highly interesting for a range of sensors – from gas sensors to biological sensors. The latter applications are highly promising because ZnO nanostructures exhibit relevant properties including high catalytic efficiency and strong adsorption ability. Recently, interest has been focused towards applications of ZnO in biosensors due to its high isoelectric point (IEP) of ~9.5, biocompatibility, and abundance in nature (3). The high isoelectric point of ZnO results in a unique ability to immobilize an enzyme with a lowisoelectric point through electrostatic interaction. Furthermore, nontoxicity, high chemical stability and high electron transfer capability make ZnO a promising material for immobilization of bio molecules without an electron mediator and can be employedfor developing implantable biosensors (4, 5).

Physical properties of wurtizeZnO:

Properties	Value
Lattice constants (T = 300 K)	
a_0	0.32469 nm
c_0	0.52069 nm
Density	5.606 g/cm ³
Melting point	2248 K
Relative dielectric constant	8.66
Gap Energy	3.4 eV, direct
Intrinsic carrier concentration	< 10 ⁶ cm ⁻³
Exciton binding Energy	60 meV
Electron mobility (T = 300 K)	200 cm ² /V s
Hole mobility (T = 300 K)	5-50 cm ² /V s

The renewed interest in ZnO has been driven by the success in growth of single crystals, epitaxial layers and nanostructures with controlled properties. Concomitantly, novel device concepts and implementations emerge, e.g. nanopiezotronics and nanogenerators (6), excitonic solar cells (7 - 9), and probably many more will appear owing to the multifunctional features of ZnO. The nanowire dye-sensitized solar cell (DSC) is an exciting variant of the most successful of the excitonic photovoltaic devices. As an ordered topology that increases the rate of electron transport, a nanowire electrode may provide a means to improve the quantum efficiency of DSCs in the red region of the spectrum, where their efficiency is currently limited (8). In a single crystal state ZnO can be prepared as two inch diameter bulk crystals, e.g. (10), thin films (11, 12) and distinct nanostructures of large morphological varieties, such as nanobelts (13), nanorods (14, 15), distinct nanowires (16), etc. Structural quality and stoichiometry control are key characteristics of materials intended for modern device applications. The availability of single crystal substrates and epitaxial layers of ZnO promises to realize reliable transducers for high performance bioelectronic devices. The application of nanomaterials to the design of biosensors is nowadays one of the most active research fields due to their high activity, good selectivity, and outstanding specific surfaces (17). One-dimensional single-crystalline ZnO nanostructures have been synthesized successfully in several groups (16, 18 - 21). Such structures exhibit high surface to volume ratios and superior mechanical stability making them ideal candidates for sensors. Gas sensors based on ZnO nanorods and thinfilms have been reviewed in (22). In a recent review S.J. Pearton and co-authors (23) have illustrated the significance of wide band gap semiconductors, including ZnO, in sensor applications. It is stated for example, that the use of enzymes or adsorbed antibody layers on the semiconductor surface leads to highly specific detection of a broad range of antigens of interest in the medical and homeland security fields.

Zh.L. Wang (6) has recently brought to the forefront the significance of ZnO nanowires and nanobelts in the field of nanotechnology. Furthermore, ZnO nanowires have been shown to be bio-safe and biocompatible (24) which make them attractive for applications as implantable biosensors. This opens unexplored possibilities to novel biosensor platforms. Nanoparticles(NPs) and nanostructured films of ZnO have, through the years, maintained vast research interests and realization in the field of gas sensors (25 - 28) and biomedical sensors (29, 30). Among the advantages of these materials is the ease of preparation while possessing high sensing performance and quantum properties. A comparison of the gas sensing capability of nanoparticles and nanostructured films has been conducted by J. Eriksson and co-authors (31). The investigation was made for oxygen detection in a carrier gas of nitrogen where it was shown that nanoparticles had higher sensitivity.

Keeping in mind that the sensing mechanism of semiconducting oxide gas sensors is based on the surface reaction of the semiconducting oxides (32), it is expected that their microstructure is one of the most important factors for high sensitivity, i.e. nanoparticles have an advantage because of their larger surface area. However, in other studies it has been revealed that the aggregation of ZnO nanoparticles limits the properties of the sensors (33).

Another issue which should be considered when using nanostructured ZnO materials with a textured structure, i.e. containing grains, is the difficulty in maintaining stoichiometric composition with the concentration of oxygen vacancies (VO) typically increasing. This mainly has an impact on optical properties but, it has also been demonstrated to affect sensor performance (34). In particular, a resistive sensor prototype under oxygen exposure showed a gradual decrease of the conductivity due to oxygen diffusion into the bulk of the films and a subsequent elimination of the oxygen vacancies (as the source of intrinsic charge carriers). The results suggested a suitable pretreatment procedure for improvement of the stoichiometry of the ZnO films, which is related to the material stability and oxygen sensitivity (34). Nanoparticles and nanostructured thin films have been studied in terms of their gas sensing abilities, where it was shown that a rough surface with a larger surface area had a positive influence on the sensor response (31). As such, ZnO thin films and ZnO nanoparticles are both very interesting for electrically based bio sensing (1). A review of the state of the art of ZnO nanocrystals utilization for enzyme immobilization in electrochemical biosensors has been published recently covering key issues in ZnO synthesis methods and related features such as biosensor performance and biosensor construction e.g., modified electrodes and enzyme immobilization. The content of the review is oriented toward bio sensing of glucose, hydro peroxide, phenol, cholesterol, uric acid and urea, respectively (35). In bioscience, the special properties of ZnO nanoparticles have gradually gained increasing attention. The biocompatibility and fast electron transfer ability allow the nanoparticles to function as a bio mimic membrane material with the ability to fix and modify proteins. The advantages of ZnO nanoparticles may also be applied to develop enzymatic detection devices. (36). A biosensor often consists of biological recognition elements covalently attached to the transducer. Therefore, the functionalization of the ZnO surface with selected molecular species is of major importance. Self-assembled monolayers (SAMs) of organosilanes are widely used as a first step for the immobilization of bio molecules on oxidized silicon, e.g., for fabrication of on-chip bio devices (37, 38). Systematic investigation on functionalization of diamond surfaces has been reported (39 - 41). Only a few studies have been performed for other wide band gap semiconductor surfaces, including ZnO. Recently, a survey on the concepts and possible applications of direct biofunctionalization of various semiconductors has been reported (38).

II. ZnO nano structure in biosensors:

In recent years compared to bulk ZnO films the nanostructured ZnO has attracted great interest. [42-52] Nanostructures give numerous unique features and show great promise for faster responses and higher sensitivity than planar sensor configuration. Nanoscale ZnO structures can achieve single molecule detection because of their small dimensions, increased sensing surface and strong binding properties. ZnO can be grown to form highly anisotropic nanostructures on various substrates including glass, silicon, sapphire and conductive surfaces with different morphologies [53,46]. ZnO nanomaterials work as an excellent fluorescence enhancing substrate for the detection of bio molecules and ZnO quantum dots (QDs) are excellent quantitative labels for biological assays based on their high aspect ratio and substantial optical and electronic signal amplification [47,48]. ZnO nanostructures can be synthesized by various physical and chemical routes which are found suitable for various biosensor applications. These nanostructures include wires (ZnONW) [54,45,46,49,50], rods (ZnONR) [47,51,52], combs [55,56], forks [57], fibers [42], flakes [43], waxberries [58], bundles [59], spheres [44], composites [60], tetrapods [61,62], particles (ZnONP) [63-65], nanorods spheres [66], tubes (ZnONT) [67,68], belts [69,70], flowers [71-73] and sheets/disks [74-76]. In addition to their great potential for fundamental studies on the roles of dimensionality Fig. 4. SEM images of ZnO nanobelts [69]. Nonporous and nanostructured ZnO films have also been used for biosensor applications [77-79]. The ease of fabrication using low cost processes, which can yield a wide range of nanostructures, makes ZnO-based matrices a promising platform for low cost biosensors. Further characterization of these nanostructured materials is essential to advance the field of electrochemical biosensors and reach the goal of a sensitive, fast and inexpensive point-of-care diagnostic device. The following sections describe the details of various synthesis processes used for the preparation of these nanostructures.

III. Synthesis of ZnO nano structure:

The various physical and chemical properties of the nanomaterials vary as a function of size, shape and surface chemistry. The synthesis of the ZnO nanostructures mainly by two methods 1) wet-chemical/solution based method 2) physical technique based method [57]. Physical technique such as vapour-liquid-solid (VLS), vapour solid and chemical vapour deposition (CVD) in addition to thermal evaporation typically require high temperature and pressures as well as particular substrates and result in low product yield. This method produce high quality ZnO nanostructures; however the methods are energy and costly.

I. wet chemical/ solution based approaches:

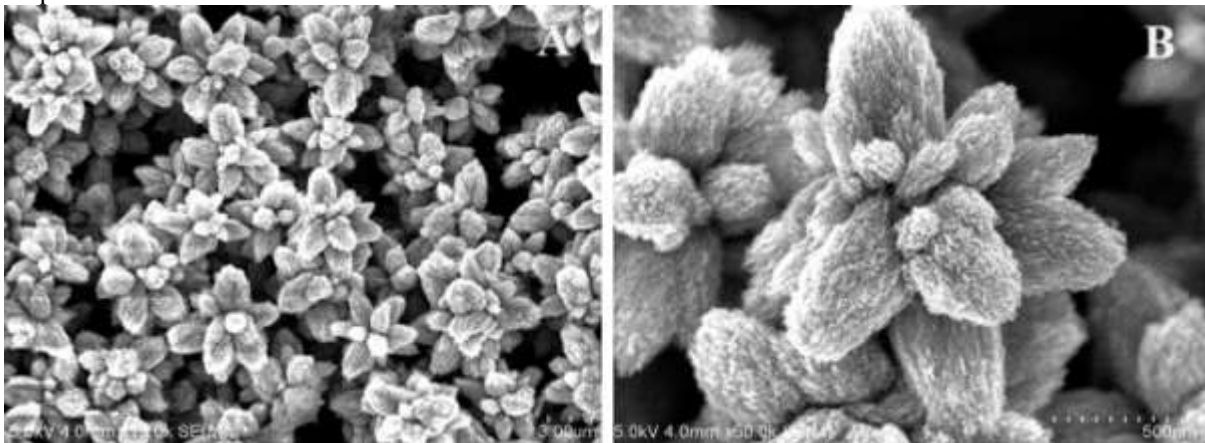
The Wet-chemical/solution based methods include hydrothermal/Solvothermal processes, solution-liquid-solid (SLS) and capping agents/surfactant-assisted synthesis. Zinc readily forms hydroxyl and ammonia complexes and these methods are based on the hydrolysis of such complexes at elevated or room temperatures. Thus, wet-chemical/solution based methods provide convenient, facile manipulation, potential for scale-up and a lower temperature pathway for the fabrication of the desired ZnO nanostructures [50, 57, 80–83]. Among these methods, the solution based method via seed layer deposition followed by ZnO growth is the most common procedure for growing nanostructures.

II. Physical techniques:

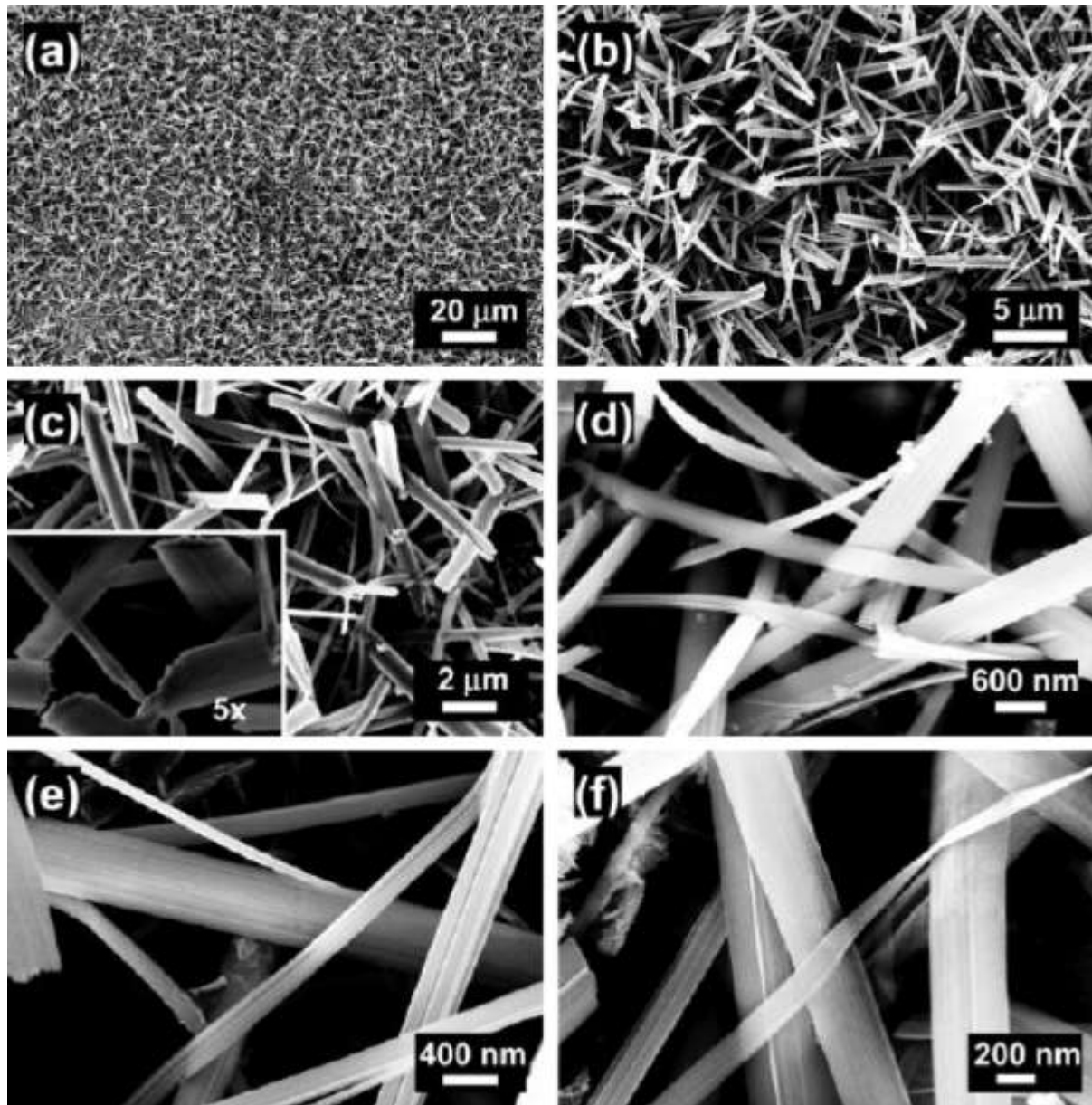
Physical techniques are the dry methods to obtain ZnO nanostructures. In these methods, one can obtain ZnO nanostructures with adequate control; however, these techniques typically require harsh temperature conditions. Most commonly employed physical techniques to grow ZnO nanostructures involve the carbothermal reduction process [84], thermal evaporation [46,49,85] vaporphase transport method [56,61,86], etc

III. Electrochemical methods:

Recently, electrochemical techniques have gained much interest for growing ZnO nanostructures [44,68,70,75,87–90]. Electrochemical method allows the enhancement of nucleation density and the simple synthesis of doped ZnO nanostructures. Furthermore, in this method, many substrates do not require pre-treatment to generate a seed layer. The most common method for the electro-deposition of ZnO involves the reduction of oxygen or nitrate on the substrate in the presence of a Zn salt at neutral pH and elevated temperature.



SEM images of the prepared ZnO products. The scale bars of (A) and (B) represent 300nm and 500 nm [71].



SEM images of ZnO nanobelt-like structures electro-deposited onto conducting PET substrate at 0 °C in 0.1 M $Zn(NO_3)_2 \cdot 6H_2O$ mixed with 0.1 M KCl electrolyte for 180 min [70].

Summary of different morphologies, results and methods reported for ZnO with different precursors and temperature:

Deposition Method	Precursor Solution	Synthesis Temperature	Resulting Morphology	Ref.
Hydrothermal	Zinc acetate dihydrate and Urea	90–180 ^o C	2-D petal-like ZnO nanostructure	[91]
Hydrothermal	Zinc nitrate hexahydrate, Sodium hydroxide and ethylenediamine	180 ^o C	Nanorods	[92]
Wet chemical route	Metallic zinc powder and Sodium hydroxide	200 ^o C	Urchin-like multidimensional	[93]
Hydrothermal	Zinc nitrate hexahydrate and Hexamethylenetetramine	70 ^o C	Tubular ZnO	[94]
Chemical solution Route	Zinc nitrate hexahydrate, PVA and diethylenetriamine	95 ^o C	Dendrite-like ZnO nanostructures	[95]
Pyrolysis	Zinc (II) oleate, oleic acid and noctadecene solvent	317 ^o C	2-D ZnOnanopellets	[96]
Facile solution method under mild conditions (Refluxing)	Zinc acetate dihydrate and Hexamethylenetetramine	90 ^o C	Dumbbell-like ZnO microcrystals	[97]
Hydrothermal	Zinc nitrate hexahydrate and Hexamethylenetetramine	90 ^o C	Nanorods and nanotube	[98]
Electrodeposition	Zinc nitrate hexahydrate & Potassium Chloride	70 ^o C	Nanospikes & Nanopillars	[99]
Ultrasonic spray	Zinc acetate dihydrate	300 ^o C	Islands with different sizes	[100]
Simple aqueous solution route	Zinc chloride and Sodium hydroxide	85 ^o C	Needle- and flowerlike ZnO	[101]
Sol-gel route	Zinc acetate dihydrate and Oleic acid	Zinc acetate dihydrate and Oleic Acid	Hexagonal faceted ZnO quantum dots	[102]
Chemical bath deposition (CBD)	Zinc acetate dihydrate, ammonia and triethanolamine	80 ^o C	Nanorods and nanospines	[103]

IV. Conclusion:

The widespread interest in nanostructured materials over the past decade has resulted from the development of novel synthesis methods and better characterization techniques that allowed the creation of new functionalities. These nanostructures, due to their remarkable variations in fundamental electrical, optical and physico-chemical properties, showed great promise for faster responses and higher sensitivity compared to planar configurations in biosensor applications.

Nanostructured ZnO had proven its potential as a biomaterial for bio sensing applications. This extensive review on ZnO nanostructures as a biosensor matrix material highlights the various procedures that can be employed for the formation and the applicability of ZnO structures in bio molecule mobilizations. The ease of fabrication using low-cost processes, which can yield a myriad of nanostructures, makes ZnO based matrices a promising platform for low-cost biosensors. Moreover, the biocompatible nature of ZnO and compatibility with MEMS technology will play a major role in the use of this material in designing miniaturized, wireless and implantable biosensors. Furthermore, doping of ZnO with noble metals offers an

effective approach to the enhance physicochemical properties of nanostructures, which is crucial for their practical application. It is not possible to address all of the recent works in this review. However, we have attempted to cover most ZnO nanostructure and thin film based biosensors, considering the morphological aspects along with various transduction techniques including piezoelectric,electrochemical,optical and field effect transistor based detection.

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