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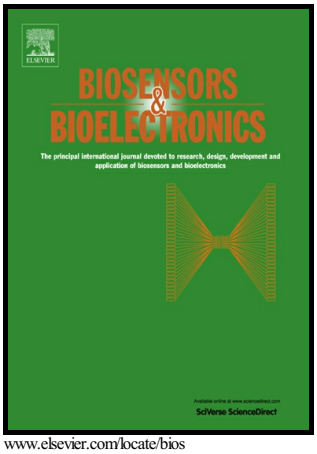

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Electrically nanowired-enzymes for probe modification and sensor fabrication

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Abstract

Enzymes are highly specific and selective due to their precise, intricate three-dimensional catalytic- structure. Electron transfer in enzymes normally occurs through an active-metal centers or tunneling events that are highly insulated by the surrounding globular protein structure. In case of electrochemically active enzymes/proteins, the distance between the redox-active cofactor and the electrode surface plays key role during direct communication. Therefore, the long electron-tunneling distance can be overcome by introducing mobile redox mediators such as nanostructures specially nanowires which can diffuse into and out of the enzyme active site, ferrying reducing or oxidizing equivalents with them. Therefore, nanowire-conjugated enzymes have gained great interest in the development of biosensor devices and other electrocatalytic-biological applications. Herein we present a comprehensive review about the electrochemical enzyme-based sensor using nanowires. Over the past decade, nanowires were investigated as a versatile platform for various applications including sensors and biosensors because of their high aspect ratio and a high surface-to-volume ratio. This review aimed to summarize

some of the recent developments in the enzyme based sensor research that have been achieved with various metallic and non-metallic one-dimensional nanostructure i.e. nanowires. Due to low or no toxicity and biocompatibility, enzymes conjugated with nanowires are still highly specific, sensitive and biologically active. This review demonstrates the potential usability of nanowired-enzymes for the bioanalytical applications. The review includes various types of nanowires, mode of the enzyme integration or immobilization methodologies, probe modification, biosensor fabrication and real or spiked sample testing. Biosensor parameters such as linear range and sensitivity, selectivity and detection limit of reported sensors were also considered herein. We also introduce some of the new nanowire materials which have not yet been used for biosensing or biosensor application. The limitations, challenges and prospects for the use of nanowired-enzymes in electrochemical and other real-time sensing systems as well as fabrication technologies are also discussed in this review.

Keywords: Enzyme electrode, nanowire, immobilization, linear range, lower detection limit, electrochemical biosensor.

1. Introduction

Enzymes have raised enormous interest in sensing area because of their remarkable specificity and catalytic mechanism. Although the inherent architecture with active center allows electron shuttle in three dimensional enzyme structure, globular structure based bioelectrocatalysis may be shielded by the surrounding structure and long electron tunneling distance. However, conjugation of mediator or nanomaterials with enzymes can reduce or overcome this insulating effect. They allow an efficient bio-electrical communication between active site of enzymes and electrodes which is of great interest in developing advanced devices for various applications. Enzyme-based detection and quantification of different analytical species are important in environment, biomedicine and many other areas (Patolsky et al., 2006a). Biosensors in particular, represent a powerful detection platform for a broad range of biological and chemical target species. Many technologies

have been either developed or modified for enzyme sensor applications, such as spectrometric bioassays, enzyme-linked immunosorbent assay (ELISA), surface plasmon resonance (SPR), microcantilevers, conductometry and poentiometry. However, quest for rapid and sensitive detection with ease of miniaturization and operation remain challenge for bioanalytical and medical diagnostic communities. Optical based sensing systems are widely used due to their high sensitivity, but it has major drawbacks such as precise alignment of light source, the needs of large and traditional equipment, requirement of skilled personnel, tedious sample preparation, and difficulties in miniaturization. While electrochemical sensing is somewhat simpler, more flexible with advanced portable instruments, highly specific and selective, rapid on field detection, and more feasible for point-of-care applications.

The nanostructures, particularly nanowires, can be integrated and/or immobilized onto a biological/chemical entity and meritoriously affect the electrical performance of the sensing devices due to their one dimensionalities and exceptional properties. Among an enormous number of nanostructures, nanowires have their unique properties. Nanowires are one-dimensional nanostructure, with a diameter of the order of 10^{-9} m (Xia et al., 2003; Wang et al., 2008; Davami et al., 2015; Rahong et al., 2016), and the length to width ratio greater than 1000. Unlike nanotubes, nanowires are non-hollow and in the form of wire. They can be synthesized by diverse techniques for various applications (Laocharoensuk et al., 2013). Nanowires are confining electrical conductors with ideal mechanical and thermal properties and large surface-to-volume ratio (Chern et al., 2010; Bagal-Kestwal et al., 2013). The cross-section of a nanowire can be round, hexagonal, or polyhydron. The length of the nanowires varies from a few hundred meters to millimeters or even micrometers (Davami et al., 2015). Analytical and biochemical applications of nanowires are increasing due to their close proximity/biocompatibility to the biological molecule, surface binding ability, and size similarity to the biomolecules such as DNA, RNA, PNA, and enzyme/proteins. (Gao et al., 2007; Rahong et al., 2016). Nanowires are made from a variety of conducting and semiconducting materials like copper, gold, iron, silver, silicon, zinc oxide, germanium, carbon material, etc. Native enzymes are fragile, temperature and pH

sensitive, and can be affected by microenvironment. However, nanowired-enzymes with versatile electrical, biocompatible, optical and magnetic properties can perform superiorly for real-time and label-free sensing, imaging and commercial device fabrication (Wu et al., 2009; Vu et al., 2010; Zhang et al., 2010a; 2010b).

In this review, we focus on nanowire-based enzyme sensors. By reviewing their applications in various areas they currently encounter and their future prospects, we hope that this review will help the researchers to find future research directions. The discussion for reported nanowired sensors are categorized into two main sections: sensors with organic nanowires and sensors with inorganic nanowires. Further, the sub-sections are divided into conducting, non-conducting and semiconducting metal, non-metal and their oxides for easy understanding.

2. Electrochemical nanowired enzyme sensors

In enzyme-based electrochemical/amperometric biosensors, the production of a current is monitored when a fixed potential is applied between standard and working electrodes. This type of transducer platform is extensively used due to its special features such as easy to miniaturize, quantifiable signal, and can be operated with small sample volumes (Rocchitta et al., 2016). Enzyme-based electrochemical sensors become more attractive and complementary analytical tools due to their easy modification with various nanostructures. Nanomaterial-based enzyme sensors not only show wide range of analyte detection but also overcome issues like stability, matrix and species interference, low signal output, lagged response time, etc. Different kinds of nanomaterials including carbon and metal, and nanostructures including nanoparticles, nanotubes, nanowires, nanodiamonds and nanostars have been successfully applied to develop enzyme-based electrochemical biosensors (Bagal-Kestwal, 2018). Enzyme based electronic sensors coupled with nanostructures, in particular nanowires, are able to detect biological and chemical species including cancer markers (Zheng et al., 2005), nucleic acids (Hahm and Lieber, 2003; Li et al., 2004; 2005), proteins (Cui et al., 2001; Stern et al., 2007; Kim et al., 2007; Tain et al., 2010), whole cells (Tain et al., 2011; Patolsky et al.,

2006a; Park et al., 2007) and viruses (Patolsky et al., 2004) with high sensitivity and precision. In this section we will discuss about various nanowire materials and new approaches for constructing nanowired-enzyme sensors and their unique features.

2.1. Strategies for electrode modification using nanowired enzymes

Sensor/biosensor's features are highly influenced by many factors including type of signal transduction, amount of recognizing element/mediators, signal generated during interaction between analyte and integrated biomolecule and system. Figure 1 illustrates the various strategies used for the modification of electrodes using nanowired-enzymes. An innovative immobilization of biomolecules on nanowire/polymer/inert probe not only improves turn over but also provides prolonged active biomolecule. Therefore, proper immobilization technique is an important prerequisite for the integration and fabrication of nanowired sensor devices. Different immobilization methodologies are available including adsorption or absorption, encapsulation, cross-linking or covalent bonding, electrodeposition, etc. Nanowired woven films or membrane can be produced using techniques such as monolayer, sandwich, multilayer, layer by layer technique and co-deposition. However, many parameters need to be considered before nanocomposite matrix is prepared such as nature of the enzyme (oxidative or non-oxidative), mediators, chemicals, geometric area of the probe, surface area of the nanowires, adsorption and functionalization capacity, and tunable porosity of the polymer/matrix.

In most of the cases, nanowired-enzymes are used to replace mediators which efficiently shuttles electrons between enzyme and electrode. These conjugated nanowires not only establish an electrical connection but also provide shield to the biologically active enzyme. Therefore, a thorough understanding of binding events of enzyme and nanowire and their link to probe will certainly influence the response output and device fabrication with respect to its application. The mechanism of enzyme-nanowire conjugation greatly affects the accuracy of the device, limit of detection, reusability and sensing capability. For example, use of conducting polymer matrix during nanowire-enzyme immobilization also accelerates the current or

electron flow in the system while natural hydrogels will provide excellent micro-environment for confined proteins/enzymes. However, use of harsh chemicals, like cyanogen bromide, formaldehyde, glutaraldehyde, ethyl choloroformate may denature enzyme and also affect nanostructures morphology during cross-linking or covalent bonding. On other hand, carrier matrices used for encapsulation and entrapment can provide protection for the integrated enzyme-nanowire and help to increase functional efficiency of the modified biological entity. Such film/membrane confined nanowired-enzymes also showed improved resistance towards extreme physical and chemical conditions.

2.2. Methods for the characterization of nanowired enzymes on probes

The structural orientation of the immobilized nanowired-enzymes when used for electrode modification, can be studied and understood by various techniques such as X-ray photoelectron spectroscopy (XPS), circular dichroism (CD) spectroscopy, surface plasmon resonance (SPR), electron microscopy (field emission scanning electron microscopy (FESEM), scanning electron microscopy (SEM), transmission electron microscopy (TEM), atomic force microscopy (AFM), Fourier transform infra-red spectroscopy (FTIR), etc. (Bagal et al., 2013). These techniques are very important for understanding the effect of different nanowires deposition/ integration methods, film/membrane composition/configuration, topology of the probe and film, morphological changes of different casting methods, film hydrophilicity/hydrophobicity and their direct effects on sensing performance.

3. Organic nanowires for sensor fabrication

Like organic matter, the organic nanowires (ONWs) are defined as nanowires derived from carbon-based compounds found in natural environment. ONWs are advantageous for their facile and large-scale synthesis using bottom-up approaches, solution processability, predefined chemical properties and molecular-electronic tunability (Vasseur, et al., 2016). ONWs are flexible, stretchable, and have good electrical properties, and therefore have great potential for use in next-generation smart hybrid materials, biomedical devices, textile and wearable electronics (Min et al., 2015). 1-D organic nanostructures/nanowires from π-

conjugated small molecules or polymers possess many advantages making them highly suitable for practical device applications. However, limitations in bulk production, lack of reliable techniques to control the desired number of nanowire produced, etc. are main hurdles for the practical and industrial applications of ONWs.

3.1. Protein and biopolymer nanowires

Primary structure of protein (presence of the repeated peptide groups and unique radical groups) plays vital role in electron shuttling in the living entity. Recently, Suprun and Shmeleva (2015) reported that the protein acts as a typical semiconductor with nanowire manifests due to radicals of amino acids for electronic transport in the metabolism (redox process) in homogeneity systems. This intrinsic property of protein can be directly give new directions in biologics. Omichi et al. demonstrated a versatile platform for functionalizing the surface of protein molecules with an extremely large surface area. They used Human serum albumin (HSA) to prepare biotinylated HSA nanowires (HSANWs) and successfully demonstrated their biological function for avidin–biotinyl interactions and peroxidase activity (Omichi et al., 2014). Figure 2 shows the atomic force microscopic images of fabrication of protein nanowires and their applications in biosensing.

Special biological nanowires (chitosan nanowires CSNWs) using multiwalled carbon nanotubes (MWNTs) grafting was prepared by phase separation method (Gomathi et al., 2011). This new nanobiocomposite with synergistic contributions from chitosan and carbon nanomaterial was further exploited for the development of amperometric glucose biosensor and the biosensor had an excellent performance for glucose detection with high sensitivity, fast response time in a wide concentration range with good reproducibility and storage stability.

3.2. Indium phosphide nanowired enzymes

Indium phosphide nanowires (InpNWs), the one-dimensional organic binary semiconducting nanowires mainly formed by chain polymerization reaction, have potential for nanoelectronics applications (Janissen et al., 2017). InP and its nanoform exhibit longer carrier lifetimes due to low surface recombination rates, and

this behavior supports a strong argument for applying them as biosensor conduction channels mainly electrically active field-effect transistors (FET). The developed InP nanowire biosensor has proved its qualification for ultrasensitive and highly selective disease biomarker detection. The amperometric biochemical sensors based on InP porous nanostructures functionalized by glucose oxidase (GOx) have been fabricated as amperometric glucose sensors (Sato et al., 2010). In the future, InPNWs need to be explored for its compatibility with many important enzymes for immobilization and subsequently for sensor fabrication.

3.3. Polyaniline nanowired enzymes

Chemical synthesis such as polymerization of aniline-monomer in acidic solutions and/or by chemical template-based synthesis as well as electrochemical polymerization/deposition are the most common techniques used for synthesizing one-dimensional polyaniline nanowires (PANINWs). PANINWs are formed by chemical oxidation of aniline monomers, while aniline monomers are electrochemically oxidized and polymerized on the surface of the anode of the electrochemical cell (Song and Choi, 2013, Zhai et al., 2013). A single PANINWs-based biosensor integrated with microfluidic channels was developed to detect cardiac biomarkers such as myoglobin (100 pg mL^{-1}), cardiac troponin I (250 fg mL^{-1}), creatine kinase-MB (150 fg mL^{-1}), and b-type natriuretic peptide (50 fg mL^{-1}) at extremely low concentrations. The fabricated PANINWs-based devices showed a fast response satisfying respective reference conditions for cardio markers for diagnosis of heart failure and possible determination of the stage of the disease. Superior sensing reliability and biocompatibility with the feasibility of label-free detection and cost-effective processing are accountable features of this system which can be applied to other biosensors for cancer or other diseases (Lee et al., 2012).

GOx based glucose sensors/biosensors showed wide range of applications including medical diagnosis, bioprocess monitoring, beverage industry and environmental monitoring (Zhai et al., 2013; Bagal-Kestwal, 2018). The GOx can be incorporated on PANINWs which are directly grown on carbon cloth supported electrode via electrochemical polymerization. This PANINWs system can be readily extended to other biocatalytic sensing, based on the ample selection of enzymes, thus they possess enormous potential for

biosensors, bioenergy and bioelectronics applications (Horng et al., 2009). A highly ordered PANINWs nanowire array was produced via a one-step template-free electrochemical approach on reduced graphene oxide modified graphite electrode. The as-fabricated GOx based sensor also acted as biofuel cell which used PANINWs based enzyme electrode as a bioanode and an electrochemically controlled cathode and showed a maximum output of 115.4 mW cm^{-2}. Thus, PANINWs can play excellent role in biosensor as well as biofuel cell performance (Xia et al., 2015). In addition, a PANINWs microelectrode junction platform was prepared by GOx cross-linking via glutaraldehyde for glucose sensing without the need of redox mediators. This enzyme-nanowired electrode junction exhibited good performance in terms of dynamic range of detection, short response time and linearity (1.0 mM to 20 mM) with excellent sensitivity of 12 µA/mM (Koinkar et al., 2015). Though, PANINWs were utilized for versatile sensing, their applications are limited by a few factors. For example, PANINWs suffer from loss of conductivity and natural degradation when exposed to medium with pH higher than 5.0 (Song and Choi, 2013). The structural deformation may lead to relatively low response time and practical constraint for commercialization of device.

4. Inorganic nanowires for sensor fabrication

Materials derived from non-living sources fall into this category including metals, non-metals and metalloids. In this section we try to give an in-depth overview of different nanowired enzymes which are directly or indirectly bonded with conducting, semiconducting, metallic, non-metallic and oxide materials.

4.1. Titanium nanowired enzymes

Silver colored titanium, a biocompatible transition metal, is corrosion resistant and has the highest strength-to-density ratio among the metallic elements. It has many medical uses including dental and surgical implements due to its low elastic modulus, non-toxicity and bio-adhesivability (Zakabunin et al., 2008; Ai et al., 2014). Titanate nanostructures have attractive properties such as large specific area and pore volume, high mechanical strength, thermal and chemical stability, optical and electrical properties, biocompatibility, low-cost and ease of manufacturing (Curulli et al., 2005; Zhao et al., 2008; Liu et al., 2010). Titanium with

different salt oxides in nano-form (nanotubes, nanowires, film, etc.) have been employed as the supporter for pursuing the efficient immobilization/binding of biomolecules including DNA (Lo et al., 2008), trypsin (Zakabunin et al., 2008), anatase (Ding et al., 2013), horseradish peroxidase (HRP) (Xu et al., 2002; Sovic et al., 2011), alkaline protease (Farhadyar and Sadjadi, 2011) etc., due to its interactive functional groups and non-toxic nature. During polymer composite manufacturing, TiNWs act as fillers and significantly alter the properties of the matrix. They also form core-shell like structure easily. Gold-TiO_2 core–shell nanowires were first prepared by the sol-gel method and alkaline protease was immobilized on the surface of the nanowires (Farhadyar and Sadjadi, 2011; Sadjadi et al., 2011). Another core/shell nanonanocomposite porous structures based on nickel oxide nanoflakes and titanate nanowires (TiNWs) was fabricated and evaluated for photo-degradation application. Experimental results indicated significant increase in catalytic stability and surface area of nanowired based heterostructure formed and proved its possible application for the wastewater treatment (Chen et al., 2016). Titanate electrical wires were also used for covalent immobilization of HRP for biosensor with fast and sensitive response (Nicolini et al., 2015; 2016). TiNWs have been modified with (3-aminopropyl) trimethoxysilane and glutaraldehyde to immobilize peroxidase covalently. The HRP-TiNWs-based screen-printed carbon electrode showed low reduction potential, detection limit and dynamic range in micromolar scale with good enzyme stability (Ferraz et al., 2017).

A potential photo-electrochemical biosensor for convenient, efficient and low-cost biomarker detection and disease diagnosis was developed using hydrothermally-grown single-crystalline TiO_2NWs with surface-functionalized GOx (Tang et al., 2014). TiO_2NW-GOx-based anodic probe was operated in the range of 0–1.0 mM with high sensitivity for glucose. These versatile and tunable TiNWs served as an efficient electron shuttling mediator between the catalytic center of enzyme and the transducer surface to amplify the signal-to-noise ratio and the sensitivity of the device. An amperometric lactate oxygenase (LOx) biosensors using TiNWs as electron mediators were developed for the H_2O_2 detection (Yang et al., 2017). TiNWs-based sensor was constructed using gold electrode as the working electrode which can work in presence of

interfaces such as ascorbic acid and uric acid. The three-dimensional architecture formed from TiNWs and Nafion was helpful for preventing LOx from denaturation, hence facilitating their extensive applications in environmental monitoring, food processing, clinical diagnostics, and medical research (Yang et al., 2017). TiNWs can be further tested for immobilization of various analytical enzymes whereas immobilization is critical for amperometric transducers and diagnostic purpose.

4.2. Manganese oxide nanowired enzymes

Electrochemical properties of the Manganese oxide (MnO_2) as supercapacitor material attract them to be used in cyclic voltammetry, galvanostatic charge–discharge studies and their application in batteries (Tang et al., 2009; Bai et al., 2008). MnO_2 nanostructures with different specific surface area showed direct electrocatalytic ability toward the reduction/oxidation reaction. This interference-free synergistic effect is due to the inherent physical and chemical properties of MnO_2 and its nano forms. The MnO_2 one-dimensional nanostructures including nanowires (MnO_2NWs) are efficient electron transporters. They facilitate fast electron communication between the enzyme's active sites and the screen printed electrode and can be used directly for versatile sensing and bio-sensing. Bai et al. (2008) synthesized Nano-MnO_2 with various crystalline structures and dimensions, including amorphous MnO_2 nanoparticles, α-MnO_2 nanoparticles, and β-MnO_2 nanowires via an electrochemical deposition method, and tested them for detection of H_2O_2. Furthermore, co-deposition of chitosan hydrogel, choline oxidase (ChOx), and different MnO_2 nanomaterials onto GC electrodes was carried out to fabricate choline biosensors. The α-MnO_2NPs and β-MnO_2NWs based choline chloride biosensors showed a wide linear detection range with micromolar detection limit. Zhang et al., (2013) modified GCE with composite mixture of GOx-MnO_2NWs. GOx was immobilized via simple solvent casting processes on the β-MnO_2NWs surface in Nafion solution. Prior to probe preparation, β-MnO_2NWs (diameter 40–100 nm and length 2.0- 4.0 μm) were prepared by a hydrothermal method with high purity and crystallinity. The biosensor with β-MnO_2NWs as a mediator, GOx as sensing material and Nafion as a polymer backbone enabled amperometric glucose detection with high sensitivity and short response

time. MnO_2NWs also showed their potential to be used as labels for immunoassay where they act as enzyme mimetic. Enzyme-linked immunosorbent assay (ELISA) with integrated nanomaterials has become a useful analytical tool for the detection of toxicologically important analytes especially in complicated biological systems (Wan et al., 2012). The authors designed a sandwich assay format using MnO_2NWs-ELISA for quantitative detection of the sulfate-reducing bacteria/pathogen. This hybrid ELISA using 3,3',5,5'-tetramethylbenzidine as a substrate was allowed to react with the MnO_2NWs without H_2O_2 in the reaction system. The kinetic parameters analysis showed the effectiveness of the catalytic nanowire-based biosensor for its sensitive detection of the pathogen by mimicking enzyme.

Recently, the novel negatively charged tetraglutamate-fused phage (M13-E4)-template with MnO_2NWs (20−30 nm in diameter and 1.0 μm in length) were synthesized through a bio-mineralization strategy (Figure 3). The designed biosensor showed good inter-assay and intra-assay reproducibility, and satisfactory storage stability with immobilized GOx (Han et al., 2016). This sensor was operated in the linear range of 5.0 μM to 2.0 mM with 1.8 μM limit of detection for glucose based on the electro-oxidation of H_2O_2 at neutral pH. Overall, the facile synthesized MnO_2 one-dimensional nanowires showed an attractive future in biosensor platform development and analytical research.

4.3. Copper nanowired enzymes

Copper nanowires (CuNWs) have potential application in fabrication of sensing systems because of their remarkable electro-activity, thermal conductivity, low cost and mechanical properties (Hassan et al., 2015; Chu et al., 2016). Porous CuNWs generated by facile one-step electrodeposition using hydrogen bubbles can provide 1.4 times larger surface area than a film. This corrosion-free, stable CuNWs as a working electrode showed stable response (95 injections of 0.20 μmol L^{-1} of glyphosate) for glyphosate in fresh fruits and vegetables when used in custom-built flow cell sensor. High-quality ultra-long CuNWs-MWCNTs hybrid was proven to have great potential application in the development of biosensors for extremely low glucose concentration in cell samples (Huang et al., 2013). CuNWs also showed novel avenues for monitoring of

organophosphate pesticides (OPs) in vegetables and fruits. Acetylcholinesterase (AChE) immobilized on the palladium-copper nanowires (Pd-CuNWs) modified GCE electrode can catalyze the hydrolysis of acetylthiocholine chloride (ATCl) and generate an irreversible oxidation peak during Cyclic voltammetry quantification (Song et al., 2017).

4.4. Zinc oxide nanowired enzymes

Zinc oxide nanowires (ZnONWs) have advantages for use in solar cells, light emitting diodes, chemical sensors and nano-size biosensor (Zang et al., 2007; Umar et al., 2009; Rodrigues et al., 2017). Specifically, nanowires or arrayed nanorods in a highly oriented and ordered array are of crucial significance for the development of novel devices for realistic and practical applications (Vayssieres, 2003). Researchers have suggested their additional applications for the immobilization of enzymes and nano-size biosensor electrodes because of the large surface area, direct electron pathway in the *c*-axis direction, biocompatibility and chemical stability (Zhou et al., 2006; Kumar and Chen, 2008). The feasibility of realizing light-weight, flexible, high-performance sensing devices using ZnONWs was illustrated by Pradhan et al. (2010). In this case, the nanoconjugate of enzyme-ZnONWs deposited on gold-coated polyester bioelectrode was evaluated for the amperometric glucose detection. Direct and one-step electrodeposition procedure offered a simple, methodology to produce uniformly distributed and vertically oriented ZnONWs for fast target-sensing performance. ZnONWs with their high specific surface area and suitable isoelectric point were used for efficient immobilization of high amount of acidic enzyme, especially GOx. An amperometric glucose sensor was developed by the immobilization of GOx with hydrothermally grown ZnONWs on Si wafers. Various coupling agents, namely (3-aminopropyl) trimethoxysilane (APTMS), (3-aminopropyl) triethoxysilane (APTES), and (3-aminopropyl) methyldiethoxysilane (APS), were used to bind GOx to ZnONWs. The experimental data showed that the APS-treated biosensor had a fast response time of less than 5 s, three-fold higher sensitivity as compared to other glucose sensors, and the lowest Michaelis–Menten constant with maximum amount of enzyme loading and low electron transfer resistance (Jung and Lim, 2013). In another research, HRP sensor based on carbon-decorated

ZnONWs array was fabricated for fast and sensitive potentiometric response for H_2O_2 biosensing (Liu et al., 2009). This sensor was not only a cost-effective but also high-performance alternative for other expensive metal nanostructures. Another attempt involved the construction of a glucose platinum electrode biosensor based on self-sustained films of ZnONWs grown on compacted graphite flakes by the vapor transport method (Gallay et al., 2016). The fabricated enzyme sensor was workable with the detection limit ~9.0 μM and limit of quantification (LOQ) ~30 μM. Thus, ZnONWs have been proven that they will play important role in the establishment of promising clinical biosensors in the near future.

4.5. Silver nanowired enzymes

Silver nanowires (AgNWs) are one-dimensional silver nanostructures with diameters in the range of 10–200 nm, and lengths in the range of 5–100 μm with aspect ratio greater than 10 (Zhang et al., 2017). AgNWs were mainly prepared via electrochemical method, template method, liquid polyol method, self-assembly method, ultrasonic reduction method and wet chemical method (Schuette et al., 2014; Ma and Zhan, 2014; Ma et al., 2014; Ahmad et al., 2015; Xiang et al., 2016). However, low uniformity and low yield were main limitations of these methods. The hard-template and soft-template methods are used to synthesize AgNWs under controlled conditions for large scale manufacturing of high quality product (Zhang et al., 2017) (See Figure 4). AgNWs are versatile materials due to their excellent optical, electrical, mechanical and chemical properties. They are used for many applications including transparent conductors, films, hybrid and adhesive materials, as electro-actuators (Mi et al., 2014), electronic devices (He and Ye, 2015; Xiang et al., 2016), etc.

A biocompatabile film composed of AgNWs was used for sensor application owing to its electrocatalytic activity and flexibility (Patolsky et al., 2006b). AgNW-chitosan (CS)-GOx film based sensor has enhanced electron transfer between the immobilized GOD and the surface of electrode, which is attributed to the large surface-to-volume ratio and high conductivity of AgNWs (Wang et al., 2013). In another report, AgNWs with controlled properties were synthesized via a polyol process and used to modify GCE for its catalytic performance toward H_2O_2 reduction. This polyvinylpyrrolidone (PVP) based GOD-

AgNWs/GCE sensor showed fast response (less than 2 s) and current linearity from 20 μM to 3.62 mM (R = 0.998) with a detection limit of 2.3 μM for detecting glucose in human blood serum (Yang et al., 2012). The same sensor without enzyme was also highly sensitive for H_2O_2, proving the high electrocatalytic and pseudo-enzymatic properties of AgNWs.

Recently, Kumar-Krishnan claimed that chitosan supported AgNWs are not only sensitive but also low-cost material for their diverse applications in medical diagnosis, healthcare, and environmental monitoring when used in glucose sensor (Kumar-Krishnan et al., 2016). The fabrication of an amperometric cholesterol biosensor based on AgNWs and cholesterol oxidase (ChOx)–graphene oxide (GO)–chitosan film was studied using cyclic voltammetry by Xu et al. (2016). The developed biosensor possessed high sensitivity (13.628 $\mu A\ mM^{-1}\ cm^{-2}$) and low detection limit of 0.427 mg dL^{-1} in physiological conditions. These results revealed the possibility of ChOx-wired-AgNWs as a promising tool for the clinical determination of cholesterol. Similar cholesterol biosensor with high sensitivity and selectivity was also developed using AgNWs, ZnO, chitosan and an indium tin oxide (ITO) electrode (Wu et al., 2016).

4.6. Platinum nanowired enzymes

Platinum (Pt) is one of the noble metals, its potential applications have been widely demonstrated in various areas such as fuel cells, catalysts and sensor fabrications (Yang et al., 2006; Bagal et al., 2008). The Pt nanostructures such as ultrathin nanowires have photonic, electronic, magnetic, and catalytic properties and can provide large surface area for protein/enzyme loading (Xia et al., 2013). When conjugated with electrochemically inactive enzyme, Pt nanowire (PtNWs) serve as mediator to improve electronic communication between transducer probe and imprisoned biomolecules (Bagal-Kestwal, 2013; 2017). Scientists have used various microfabrication techniques such as microlithography, thin-film deposition and wafer-scale ion beam etching to prepare PtNWs based arrays for multiplex detection (Xuan et al., 2010). Yang et al. reported a PtNW based nanoelectrode array and its possible applications in the detection of low

levels of analytes. Such sensors can be of great importance for modern medicine, environmental control, and food industry due to their high sensitivity and interference-free during determination of analyte(s). Researchers demonstrated the facile method to synthesize PtNWs (250 nm in diameter and 2.0 μm in length) by electrodeposition on polycarbonate membrane for sensor array building. The PtNWs showed direct response to H_2O_2 at zero potential with wide linear detection range of 1×10^{-7} to 6×10^{-2} M. The PtNWs array sensor showed sensitivity 50 times higher than that of the conventional platinum electrode for H_2O_2. When GOx was spatially patterned on array, the PtNWs nanoelectrode sensor showed 10^{-6}–3×10^{-2} M wide linear detection range for glucose in blood samples (Yang et al., 2006). The PtNWs prepared by electrodeposition strategy using nanopore alumina template and dispersed in chitosan were also tested for H_2O_2 and GOx cross- linking via glutaraldehyde. The short response time (< 8 s) with a linear range of 10^{-5}–10^{-2} M and detection limit of 5×10^{-6} M features were reported for this PtNW electrochemical glucose biosensor (Lu et al., 2007b). Different surface modification techniques using gelatin gel with SiO_2, polyvinyl alcohol with Prussian blue mediator and cysteamine to achieve long term stability of GOx on PtNWs and sensitive detection of glucose were also investigated (Le et al., 2010). An excellent amperometric sensors with and without GOx were prepared using PtNWs and CNTs in chitosan for glucose and H_2O_2 respectively. The synergic action of PtNWs and CNTs helped to facilitate electron-transfer in electrochemical sensor with response time less than 10 s (Qu et al., 2007b). In another study, Phan et al. (2013) also fabricated a similar GOx sensor based on electrochemical platform for glucose using cyclic voltammetry. The GOx-PtNW chips functionalized with cysteamine in PBS (1.0 M, 7.0 pH) successfully determined glucose level in diabetic blood samples from 125 μM to 16.5 mM with deviation of ± 3%, indicating the possible use of a next–generation GOx-PtNW chip in application diabetic diagnosing. From the above discussion, we can also conclude that the enzyme adsorbed on NWs surface does not hamper the conductance property of the wire and its superior performance.

4.7. Gold nanowired enzymes

Most of the noble metals are conductance and enhancing agents for effective acceleration of direct or indirect electron transfer between electrode and detection molecules, which can lead to more rapid current response for targeted molecules (Birenbaum et al., 2003). Gold nanoparticles (AuNPs) have also shown their ability to form a powerful transduction platform (Mbindyo et al., 2001). Like gold nanoparticles, gold nanowires (AuNWs) also have tremendous potentials in many fields including biomedical and imaging. They are the best choice for the analyses of chemical and biological molecules where high sensitivity and accurate quantitation are needed. A crucial problem in the commercial development of biosensors is the ability to immobilize sufficient amount of enzyme on an electrode while maintaining its biological activity. The use of AuNWs can provide a huge surface area per unit volume for the immobilization of a large amount of enzyme (Cusma et al., 2007). Vastarella et al. synthesized AuNWs by electroless deposition within the pores of polycarbonate particle track-etched membranes for the immobilization of GOx. Cyclic voltammetric electrochemical investigation of nanostructured gold wired array showed potential applications for glucose detection (Vastarella et al., 2006, 2010). While in another attempt, the AuNWs (diameter 250 nm and length about 10 μm) were prepared by an electrodeposition strategy using nanopore polycarbonate membrane and further dispersed into chitosan solution to modify GCE surface. The sensor showed glucose detection in the linear range of 1.0 $x10^{-5}$–2×10^{-2} M and detection limit of 5.0 x 10^{-6} M at -0.2 V in a short response time (Lu et al., 2007a). In addition, sensing of localized enzymatic peroxides activity for imaging biosensor application was explored by Vatsyayan et al. (2016). They covalently linked the AuNWs to HRP and immobilized on platinum disk ultramicroelectrode (UME). Positive feedback and localized HRP activity over individual AuNWs were imaged by switching the probe in various potential ranges with the topographic details (Figure 5). Qin et al. (2016) reported the glucose biosensor in combination with AuNWs arrays, carbon nanotubes (CNTs) and reduced graphene oxide nanosheets. The low working potential, high sensitivity, good anti-interference ability and high throughput of 45 h^{-1} are some of the analytical features of the developed glucose microfluidics biosensors. Excellent sensitivity, unparalleled selectivity against

interfering agents and high stability with repeated uses was achieved with a highly ordered gold nanowire array (AuNWA) by several research groups (Aravamudhan, et al., 2007; Cui et al., 2014; Xia et al., 2015; Ogabiela et al., 2015). Successful integration of highly ordered AuNWA with pyruvate oxidase (PyOx) and co-factors was carried out to fabricate a novel phosphate amperometric nanobiosensor. While GOx was integrated on AuNWA with the aid of Nafion membrane to form glucose sensor (Ogabiela et al., 2015).

4.8. Silicon nanowired enzymes

Silicon nanowire (SiNW) is a promising material in bioanalytical field. Some of the major challenges in diagnostics are easily overcome by SiNW's unique mechanical, electronical, and optical properties (Talin et al., 2006; Chen et al., 2011; Rashid et al., 2013; Choi et al., 2013). SiNWs can be produced by different approaches, such as laser ablation, thermal evaporation, chemical vapor deposition, molecular beam epitaxy, chemical etching, solution growth (Zhang et al., 2008). Recent studies also proved that silicon nanostructures are the best matrices for enzyme immobilization and sensor fabrication with low mass transfer resistance. Silica nanostructures including nanowires were phyto-fabricated on different surfaces by using pomegranate (*Punica granatum*) leaf. The SiNWs developed on Zn surfaces were further functionalized with amine groups and used for the covalent immobilization of *Candida rugosa* lipase.

The lipase immobilized on SiNWs showed improved pH and temperature stability and retained 80% activity after 20 cycles of testing (Rao et al., 2012). SiNW-based sensors using different detection methods such as surface-enhanced Raman scattering (SERS), fluorescence, electrochemical methods, and field-effect transistors (FET) have been fabricated (Rashid et al., 2013; Bagal-Kestwal et al., 2018). The small dimension, good electron transfer, accumulation of charge, high aspect ratio, and stable architecture make SiNWs the best candidate in the development of these ultrasensitive sensors (Zhang et al., 2010a). Su et al., incorporated SiNWs into nano-complex to increase the electrical conductivity of modified electrode and facilitate electron transfer of AChE for detection of organophosphate pesticide. The SiNWs-AuNPs modified

AChE-nanowired probe showed rapid response in the detection of acetylcholine in the range of 1.0 μM–1.0 mM and was highly sensitive down to 8.0 ng L^{-1} (Su et al., 2008).

4.8.1. SiNWs-GOx based sensor

Glucose oxidase (GOx/GOD) is one of the most extensively used enzyme for glucose and hydrogen peroxide (H_2O_2) sensor (Bagal and Chiang, 2017). SiNWs have shown good biocompatibility with the GOx by providing microenvironment around the enzyme. In a work by Chen et al., SiNWs were prepared by thermal evaporation of silicon monoxide powder, which resulted in a crystalline Si core (~15–20 nm) with a Si oxide shell of ~3–5 nm. GOx was then covalently linked to SiNWs via the carboxyl groups of the nanowires. In this work, the real-time detection of glucose of the SiNWs-GOx biosensor had a linear response in the range of 0.1–15 mM (Chen et al., 2006). Another electrochemical glucose biosensor was prepared by immobilizing GOx on glassy carbon electrode (GCE) decorated with SiNWs and AuNPs grown in situ. This glucose sensor had high biological affinity with the apparent Michaelis–Menten constant of 0.902 mM, which is much smaller than other reported values for GOx of nanomaterials-incorporated electrodes (Su et al., 2010). Moreover, GCE was modified with GOx and SiNWs for monitoring blood-glucose levels with the detection range of 4.4–6.6 mM (Su et al., 2010). While in another investigation, cyclic voltammetry-based amperometric Pd-Ni/SiNWs electrode was tested with different concentrations of glucose without GOx and achieved sensitivity of 190.7 μA mM^{-1} with detection limit of 2.88 μM (Hui et al., 2011). These impressive results are attributed to the successful integration of biological molecules with high specificity and biocompatibility of silicon nanowires.

4.8.2. SiNWs-based field effect transistors

Among various potentiometric techniques, semiconductor nanowire-based sensing using field-effect transistors (FETs) has attracted considerable attention in diagnostics. Semiconductor nanowire-based sensors also compile features such as miniaturization, fast response time, label-free and parallel sensing of analytes in real time using electronic device (Poghossian et al., 2014; Kaisti, 2017). SiNW-FET-based biosensors have

been studied extensively for the detection of various targets, as they are highly sensitive, label-free and cost-effective electrical tools. They also hold great promise due to simplified transistor structures and biological modulation for point-of-care (POC) devices in critical disease management (Zhang and Ning, 2012). A typical SiNW-based FET device is composed of source, drain and gate electrodes and allows to detect electrical signals related to chemical or biological actions (Bunimovich et al., 2006; Zhang et al., 2009; Stern et al., 2010). The sensing mechanism of the enzyme-based field effect transistors (ENFET) involves the change in charge density induced at SiNW gate surface due to enzyme-substrate reaction and a change in the electric field. One of the challenges in ENFET design is the method of generating the enzyme layer, i.e., immobilization, near the gate surface. A negatively charged biomolecule integrated to the outer surface of an n-type SiNW could increase the resistivity of the device and vice versa if using p-type SiNWs (Zhang and Ning, 2012). This technique ultimately determines the sensitivity, response time and reuse of the ENFET. A polymer coat on the gate can lead to slower response times due to diffusion barrier of the analyte (Vijayalakshmi et al., 2008). Therefore, a variety of methods were used, such as cladding, multilayer technique, covalent attachment, polymer entrapment, and layer by layer technique to modify the gate surface using nanomaterials (Zheng et al., 2005). The SiNWs not only guarantee high-quality catalytic activity and conductivity but also ultrahigh sensitivity over carbon nanotubes. An electrochemical approach has played crucial role in biosensing by applying SiNWs based array electrodes in an integrated architecture of work, counter and reference electrodes. The fabricated biosensors were able to detect the electrochemical response using cyclic voltammetry, double plus voltammetry, FERT, etc. The complementary metal-oxide semiconductor (CMOS) field effect transistor has become more popular after successful detection of cardiac biomarker in real time using these array sensors, and the ultrahigh detection of human cardiac troponin-T (cTnT) has been demonstrated (Chua et al., 2009). In another study, CMOS-based integrated SiNW-chip sensor was designed to detect three cardiac biomarkers, creatine kinase MM (CK-MM), creatine kinase MB (CK-MB), and cTnT in a short span of time (Zhang et al., 2011). Third-generation electrode array with high

conductivity, excellent electron transfer capability and good biocompatibility is a promising platform for mediator-free enzyme-based sensors mainly for H_2O_2 based on SiNWs (Vu, et al., 2010). A nanoscale sensor device based on urease was fabricated with a top-down lithography approach. This sensor showed a linear relationship between surface potential change and urea concentration in the range of 0.1-0.68 mM (Chen et al., 2017). Hsu et al. reported a poly-SiNWs sensor fabricated by a top-down method with immobilized GOx on the nanowires for determining glucose concentration. The proposed reusable sensor with SiNWs (3.0 µm in length) exhibited a theoretical resolution of 0.003 mg dL^{-1} and the highest sensitivity of 0.03 µA / (mg dL^{-1}) (Hsu et al., 2015). Another SiNW-FET with uniformly distributed and size-controlled SiNWs and GOx was also fabricated by "top-down" approach. This SiNW-FET showed further improved sensitivity of 0.988 ± 0.030 nA / (mg dl^{-1}) upon illumination at 480 nm light as compared to that without illumination as 0.486 ± 0.014 nA / (mg dl^{-1}). These finding showed that the nanowired-enzyme device was fast, stable and sensitive to light and can be used as sensitive biological sensing platform for bio-analyte(s) (Ahn et al., 2014). Another possible use of SiNWs and a new wafer-scale nanofabrication technology is for ultrasensitive detection of biological species/biomarkers for diagnosing of several diseases (Pham et al., 2011). Deposition and etching under angle technique was used for the fabrication of tunable nanowire conductance and low contact resistance SiNWs to build up the biosensor to detect analyte with concentrations down to about pM level (Pham et al., 2011). Thus, all studies revealed that the SiNWs are unique building blocks for future nanoscale biosensor devices merging electronic sensing and nanofluidics (Cui et al., 2001). And SiNWs configuration can be applied in direct, real-time detection systems and also label-free unambiguous detections.

4.9. Tin oxide nanowired enzymes

Semiconducting and amphoteric tin oxide (SnO_2) is a key functional material that has been used extensively for optoelectronic devices, gas sensors and other electronics (Dai et al., 2002). The one-dimensional SnO_2 nanostructures have been synthesized by various processes, such as wet-chemical approach, high temperature thermal oxidation, hydrothermal, carbothermal reduction, plasma-enhanced chemical vapor deposition, etc.

(Garcia et al., 2012). Novel tin oxide field-effect-transistors for pH and protein detection in the healthcare sector have been reported. These SnO_2 nanowires (SnO_2NWs) possess an intrinsic n-type doping and thus avoid the need for intentional doping and make them suitable for the biosensing systems (Jackob et al., 2017). SnO_2 nanostructures especially one-dimensional un-doped nanowire have shown excellent capability for fabricating real-time, label-free and high sensitive sensors used in the detection of molecules such as DNA, hydrogen peroxide (H_2O_2) and glucose (Wan et al., 2007; Le et al., 2015; Jakob et al., 2017). These nanowires play important role in signal enhancement when used in electrochemical impedance spectroscopy (EIS), optical and other amperometric transducers.

The Sb-doped SnO_2NWs were used as immobilization matrix for the enzymes and further integrated to fabricate a mediator-free, HRP-based biosensor. In comparison with the un-doped SnO_2NWs, the Sb-doped SnO_2NWs exhibited excellent electron transfer properties for the enzymes and high electroactivity toward H_2O_2 analyte. The fabricated sensors showed good performance along with high sensitivity, wide linear range, and long-term stability. This improved performance of the SnO_2NWs based HRP-sensor was attributed to the enhanced carrier density arising from Sb doping and biocompatible microenvironment provided by the Sb-doped SnO_2NWs (Li et al., 2010). Further, the inherent properties of SnO_2NWs such as aspect ratio, size scale, tunable electron transport, prolongs conductivity lifetime are also playing important role to retain the enzymatic activity during immobilization (Haider et al., 2017).

4.10. Carbon nanowired enzymes

Carbon and most of its derivatives are organic. However, a few types of carbon-containing compounds, such as carbides, carbonates, oxides of carbon such as CO and CO_2 and cyanides are considered as inorganic. Allotropes of carbon, such as diamond, graphite, graphdiyne, fullerenes, and carbon nanotubes are also inorganic because they are simple substances composed of only a single element and are not considered to be chemical compounds (Wu et al., 2002; Wang, 2009; Shen et al., 2010). Similarly, a single-crystalline anisotropic nano-microarchitectures of carbon nanowires falls into inorganic category. Therefore, we classify

them under inorganic semiconducting nanowires section (Wu et al., 2002; Wang, 2009; Shen et al., 2010; Volder et al., 2011; Xu et al., 2018). Nano-scale carbon and its allotropes (graphene, nanotubes, carbon nanowires (CNWs), etc.) offer superior electrical, thermal and mechanical properties and they are now poised to become the energy efficient building blocks of next-generation electronics. (Shulaker et al., 2013, Peng et al., 2014, Thiha et al., 2018). CNWs have extremely high surface to volume ratio and large surface area which can be used for modification so that specific chemical or biological molecular binding is possible and results in electric change. The electrically generated signal can be further transmitted along the nanowire to the detector, thus yields a highly sensitive, fast and advanced system. Recently, Tiha et al., reported the sensitive and specific *Salmonella Typhimurium* whole cell detection with a detection limit of 10 CFU mL^{-1} using highly suspended carbon nanowires (Thiha et al., 2018). The sensor platform was integrated with a microfluidic chip to form a lab-on-chip device for label-free chemiresistive biosensing. However, CNWs are very expensive and slow for mass-manufacturing and also difficult to fabricate into any desired shapes as compare to carbon nanotubes (CNTs). This may be one of the reason, CNWs are relatively less used in enzyme immobilization and sensor fabrication as compared to CNTs. On other hand, diamond nanowires (DNWs) exhibits numerous advantages, such as chemical inertness, high mechanical strength, high thermal and electrical conductivity, etc. DNWs also showed their biocompatibility when tested for various strategies to functionalize their surface (Szunerits et al., 2015). An enhanced electrochemical performance of electrode comprising of 3D-networked CNT/diamond core-shell nanowires showed its use in biosensor applications especially the analytes at very low concentrations (Lee et al., 2014). Wang et al., explored the possibility of photoactivable DNWs interfaces for covalent modification for small molecules and enzymes. Reusability of DNWs-lysozyme or DNWs-HRP electrodes made them attractive for the controlled release of biomolecules from a substrate surface which is a highly challenging task (Wang et al., 2015). However, application of DNWs and other carbon-based nanowires are still limited in the area of enzyme immobilization, electrochemical or optical sensing (Szunerits et al., 2017).

4.11. Other nanowired enzymes for sensor fabrication

Controlled morphologies of nonstoichiometric cerium (IV) oxide, also known as ceric oxide (CeO_2) nanomaterials have raised special attention because of their tunable and favorable shape-dependent physico-chemical properties (Muthukumaran et al., 2016; Ibrahim et al, 2017). The metal-doped ceria nanomaterials are highly promising due to their high catalytic activity to enhance the electrocatalytic oxidization. The CeO_2 nanowire arrays were synthesized by hydrothermal approach (calcinations and chemical vapor deposition). Subsequently, CNTs coating on the surface of CeO_2 nanowires (CeO_2NWs) was done by chemical vapor deposition at high temperature under H_2/Ar atmosphere. These nonstoichiometric CeO_2@CNT core/shell-nanowire arrays were used as substrate to fabricate cholesterol flow injection based biosensors. Amperometric cholesterol sensor showed high sensitivity with excellent selectivity due to the synergistic effect of CeO_2 and CNTs nanostructures (Luo et al., 2017). In another attempt, nanowires from lead (II) nitrate (PbNWs) with ~ Φ60–80 nm were fabricated by a simple and effective way using L-cysteine-assisted self-assembly route and AuNPs were decorated through –SH–Au specific interaction onto the PbNW surface. This biosensor exhibits good reproducibility, long-term stability and relative good anti-interference with sensitivity of 135.5 μA mM^{-1} cm^{-2}, LOD of 2.0 μM (S/N = 3), and the response time less than 5 s in a linear range of 5.0–2200 μM (Wang et al., 2009).

On the other hand, Karyakin and his research group have demonstrated that the Prussian blue nanowire (PBNW) array is a superior transducer for electrochemical detection of a hydrogen peroxide. They prepared PBNW array through lyotropic liquid crystalline templates and used in flow injection analysis (Karyakin et al., 2004). Later Qu et al. used these PBNWs arrays with GOx for the electrochemical reduction of H_2O_2 in neutral media. The as prepared sensor showed its capability for rapid, selective and sensitive determination of glucose with 3 s response time and 1.0 μM detection limit, with an excellent electrocatalytic activity for H_2O_2 (Qu et al., 2007a).

Similarly, poly-thionine nanowires (PTHNWs) were synthesized by template electrodeposition and used to fabricate the H_2O_2 biosensor. The biosensor based on encapsulated HRP exhibited long-term stability, good reproducibility and response time less than 5 s when operated in pH 6.98 phosphate buffer. The PTHNWs can also form composite with other enzymes for various applications (Shi et al., 2008).

A good bioanode for glucose based biofuel cell was fabricated by combining GOx and vertically aligned Palladium nanowires (PdNWs) (5.57 µm in length and 64.28 nm in diameter) array electrode. GOx was entrapped within a composite UV cross-linked HEMA-hydrogel composite and deposited on PdNWs array electrode. This study revealed the possibility of the PdNWs immobilized redox enzyme/GOx not only for glucose based biofuel cells, but also biosensor development (Slaughter and Amoah, 2012).

5. Application of nanowire sensors

5.1. General applications of nanowires

In this review, we have already discussed different sensing and biosensing systems and their respective applications using nanowired enzymes (Table 1). Apart from biological sensors, nanowired architecture can be effectively used for photovoltaic devices, smart electronics, renewable energy storage devices (batteries)/ solar cells, chemical sensors, pH and gas sensors, etc. Thus, from application point of view, nanowires can be fabricated and reframed for the state-of-the-art. Due to versatile and tunable structural compositions, nanowires can be used for customized applications such as electrochemical and optical fluidic platforms, probe modification, chemo resistive sensor devices, etc. (Wu et al., 2009; Vu et al., 2010; Zhang et al., 2010a; 2010b; Laocharoensuk et al., 2013; Bagal et al., 2018). Still there is a great need of low-cost, large-scale, highly efficient, and flexible optoelectronic applications in various fields. An enzyme-nanowires can be the best solution for such increasing demand for portable and wearable electronic devices.

5.2. Nanowire-sensing in living cells

Nanowire's geometries make them ideal for interfacing with cells and measuring intracellular potentials of neurons with minimal invasiveness. Such pre-patterned tool is gaining attention due to its usefulness for addressing fundamental questions in medicinal science particularly neurobiology. An artificial, pre-patterned mammalian neuronal network opens opportunity to develop nanoelectronic-array based devices (neurochips and biosensors) for detecting their cellular responses. Sub-micrometrical scale 3D SiNWs-FET devices were successfully fabricated and used as unique probes for electrical characterization of intrinsic behavior of neurons and its dynamic network function (Kwait et al., 2012). In another attempt, NW-array was used to record intracellular activity from primary rodent and human stem cell derived neurons. This study showed the potential of cell–electrode interface for independent and scalable electrical addressability in intracellular and extracellular configurations to understand the basic functionality of neurons/brain (Liu et al., 2017). The neuron-nanowire array technology can serve as a platform for the modulation of individual neural circuits or even at single cell-level (Casanova et al., 2018), while minimally invasive method for probing enzyme activity in situ to understand the role and function of enzyme in the context of an intracellular signal transduction pathway requires. Activity detection of enzymes like protease, phosphatase and protein kinase has potential in uncovering the physiological roles of various enzymes. These enzymes are important biomarkers in disease diagnostics. Vertical silicon nanowires (SiNWs), when used as a culture substrate, have been demonstrated to provide direct physical access to the cells' interior without affecting their viability or function. The endogenous activity of protein tyrosine phosphatases in HeLa cells even in the absence of external stimuli was tracked without affecting cell viability and membrane integrity (Na et al., 2012). In another study, low density nanowire array was used for Chinese hamster ovary (CHO) cells to understand the time-resolved nanowired-cargo material delivery in cell cytoplasm with minimal perturbation (Xu et al., 2013). The effect of nanowires on cells in terms of viability, cell–nanowire interface morphology, cell's activity, changes in gene expression, cellular stress markers and unexplored issues were well discussed by Prinz et al. (2015). Nanowired enzyme based arrays, for example GOx-NW array based electrodes, can be

implanted in living organisms, like rats, insects and snails. These nanowire adapted bioelectrodes composed of oxidizing enzyme anodes (GOx, glucose dehydrogenase or cellobiose dehydrogenase, laccase, bilirubin oxidase), would allow applications in sugar-driven portable electronics, blood power-driven wireless sensors and implantable medical devices (Zhang et al., 2016; Cadet et al., 2016; Shoji et al., 2016; Song et al., 2017; Kumar et al., 2018).

6. Challenges

Although the 1-D nanowires have made countless progress in various areas especially in sensing and bio-sensing, there are still many limitations and these challenges need to be overcome in order to make these systems into commercial and affordable realistic technology. Lack of single nanowire fabricated biosensors due to technical difficulties in handling of a single nanowire precisely from bulk is one limitation, and poor reproducibility is another problem. In addition, many conducting nanowires face practical problems such as fragility, structural degradation when exposed to harsh chemicals or environment. Some of nanowires lose their conductivity as well as sensitivity in physiological pH or electrolyte solution due to nanoscale morphological entities. There are various nanowires which are explored for sensor application but not yet employed for enzyme immobilization. In depth research is needed for the application of such ideal nanowires for designing, fabrication and miniaturization of detecting devices. Selenium is one of the required elements of the human body and it boosts the activity of the seleno-enzyme glutathione peroxidase. However, application of Se nanowires in biosensing is not yet explored. Nickel oxide (NiO) is a material with high electron transfer capability and good biological compatibility. However, NiO nanowire and NiO hybrid nanowires have not been much studied for sensing applications.

There is also strong need of facile, convenient mass-production of uniform and pure nanowires with controlled dimensions and characteristics. In addition, nanowire's toxicity, electrode fouling, and device miniaturization related issues need more critical evaluation before invasive and in vivo applications in

biomedical area. Specific application oriented challenges and limitations such as nanowires orientation, size limitation, biocompatibility, reliability, reproducibility, stability, background noise, resistance, etc. need careful examinations at the device level. Further, biodegradability and disposal are issues need to be considered when nanowires combined with non-biodegradable materials for sensor devices. Thus, more work is needed in this direction.

7. Future prospective

Nanowired enzymes can be used for constructing more flexible and cost-effective sensors with superior and novel functionalities as compared with the conventional sensors. The robust biofunctionalized nanowires will surely offer modulation and adaptation with a unitary host material in sensing technology. So that nanowired enzyme-hierarchical assemblies will be more useful for biological-chemical imaging and sensing with great selectivity, specificity, and superior sensitivity. Longevity and repeated use are important for practical applications of all sensors, therefore, more attention is needed on these aspects. The customized method with robust and simple mechanism will definitely broaden the nanowire's application in various fields including detectors for various sensing targets. In conclusion, nanowire- interfaces based research should emphasize not only on new innovative technologies but also to cut down the distance from laboratory to market. The significant improvement in device and nanodevice design and ease of handling of enzyme-based biosensors incorporated with nanowires will open new horizons in interdisciplinary technology research and will definitely take biosensors directly from laboratory to person for improving life quality.

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Notes

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Fig 1: Various strategies for electrode modification using nanowired-enzymes for electrochemical sensing. (A) Unmodified disc electrode, (B) Unmodified screen printed electrode, (C) Array template before modification; immobilization methods using NWs and enzyme for electrode surface modification (D) adsorption or absorption, (E) encapsulation, (F) cross-linking or covalent bonding, (H) electrodeposition, film or membrane techniques (I) monolayer, (J) sandwich, (K) multilayer, (L) layer by layer technique and (M) co-deposition.

Fig 2: Schematic representation of fabrication of various protein nanowires for sensing application (Omichi et al., 2014). (Reproduction with permission).

Fig 3: Schematic diagram of engineered M13-mediated synthesis of MnO_2NWs (Not to the Scale) (Han et al., 2016).

Fig 4: (a to d) The SEM images of AgNWs synthesized with PVP molecules of different chain length and (a1 to d1) SEM images of silver nanowires synthesized with different concentrations of $AgNO_3$ (reprinted from Zhang et al., 2017 with permission).

Fig 5: Schematic representation of HRP-linked AuNWs for biosensor fabrication (Vatsyayan et al., 2016) (Reproduction with permission).

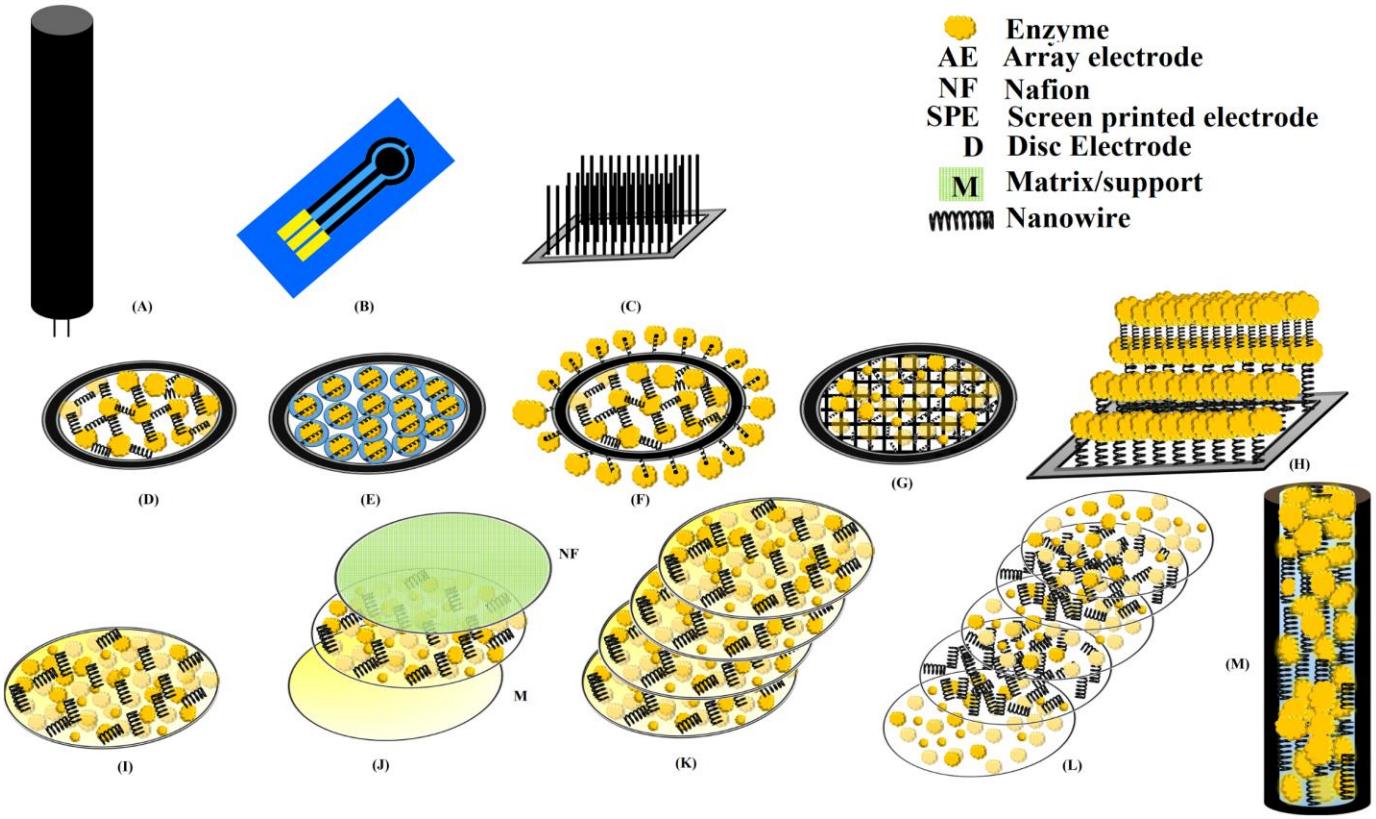

Figure1:

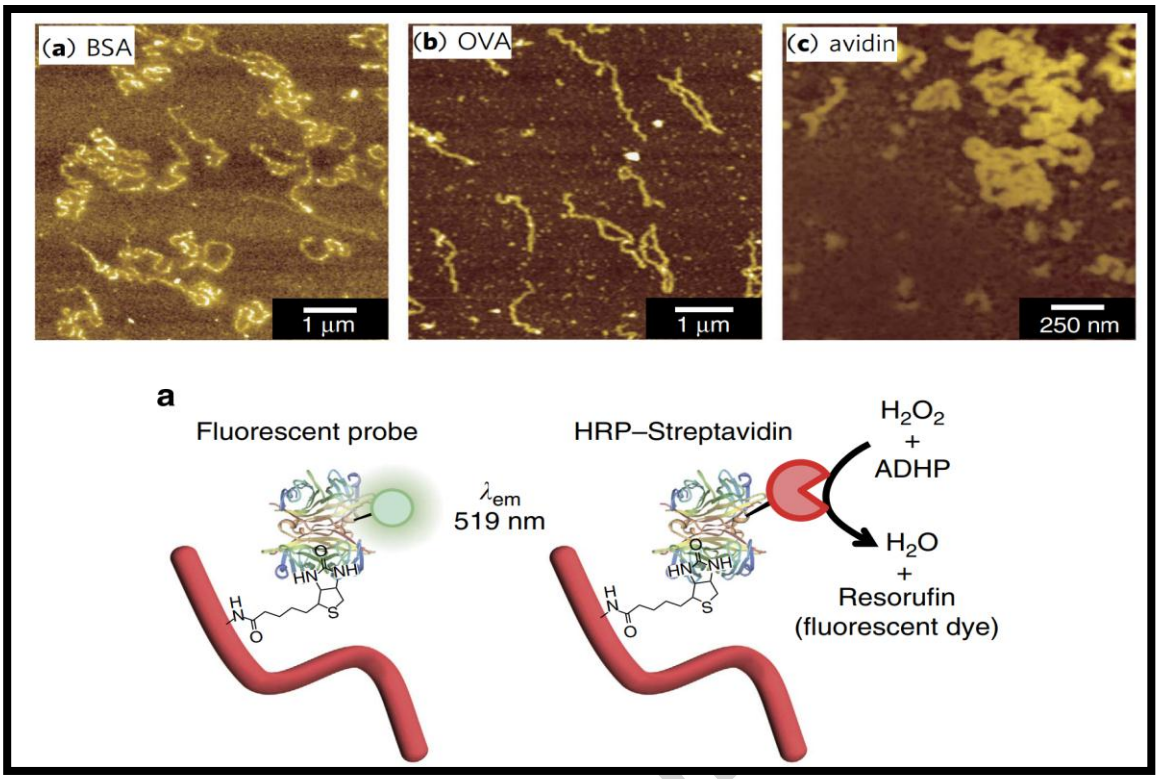

Figure 2:

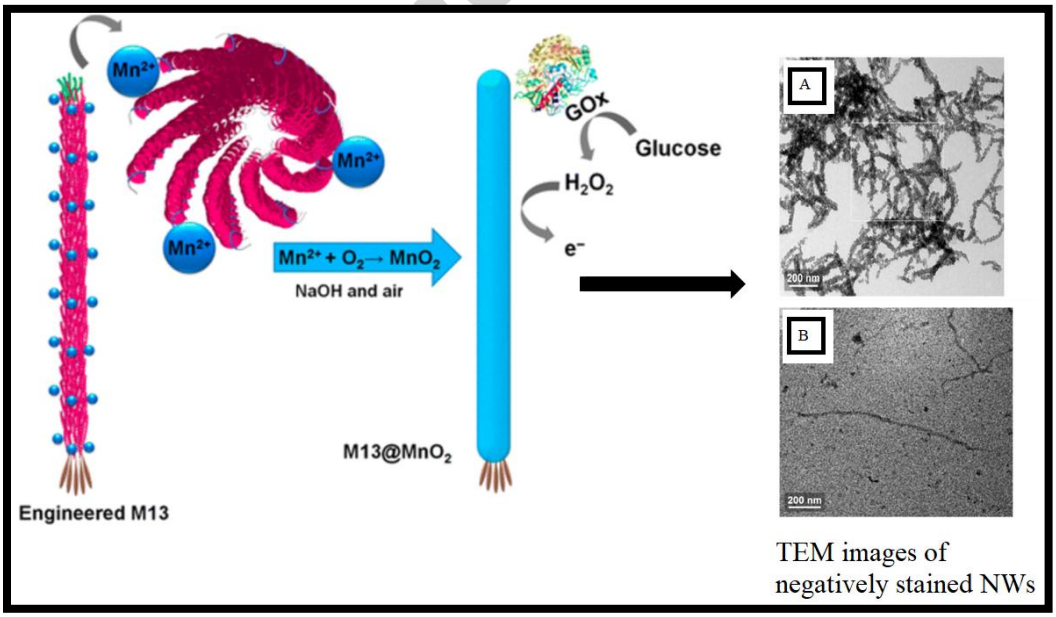

Figure 3:

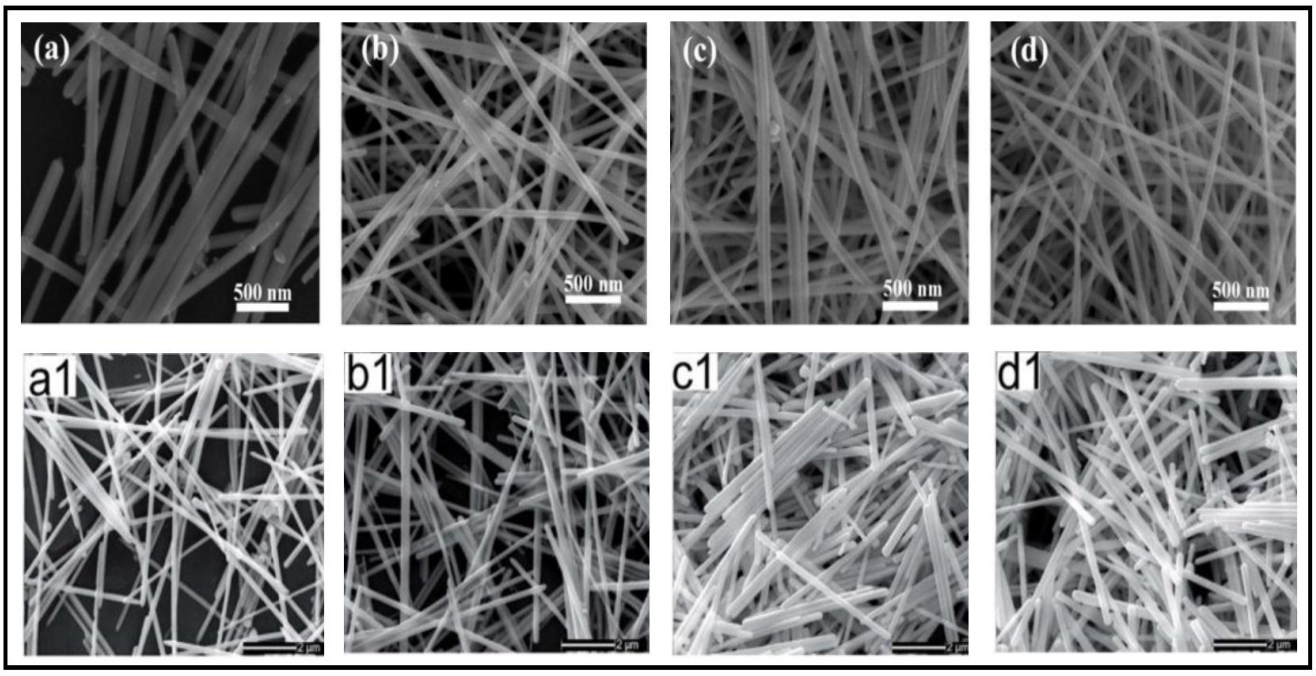

Figure 4:

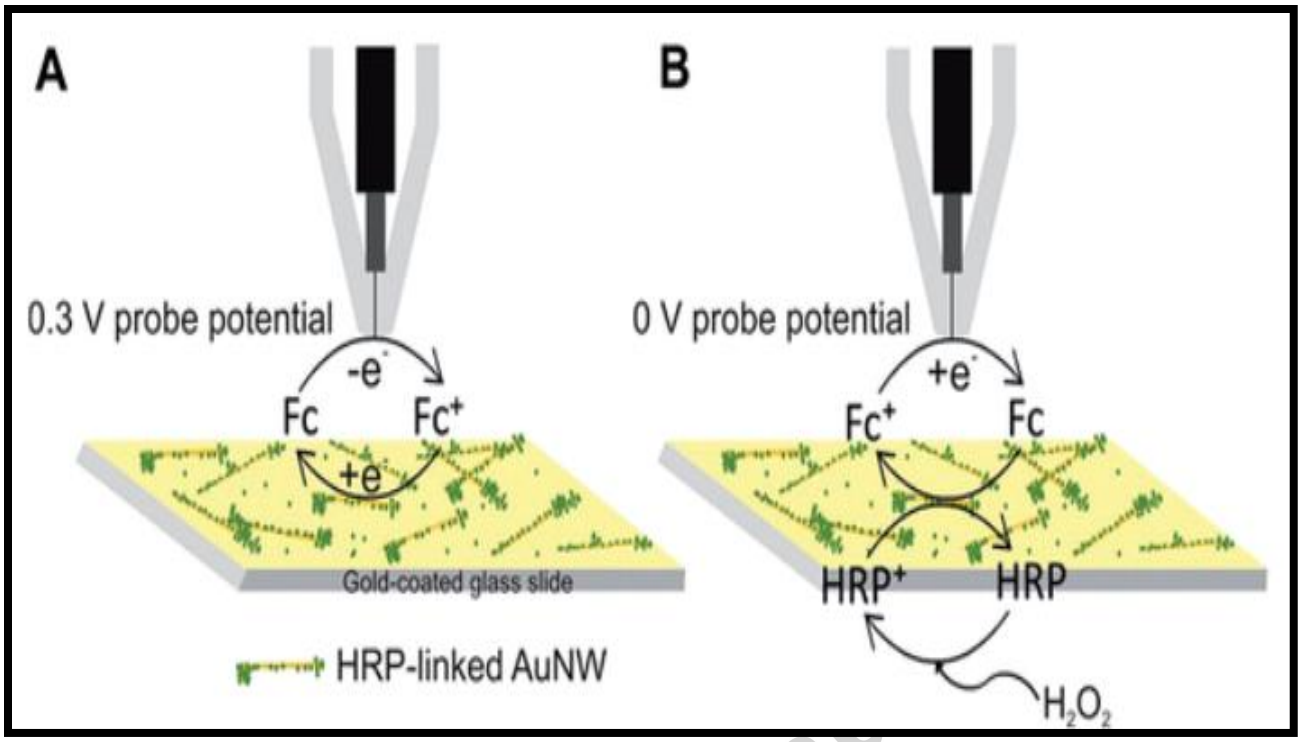

Figure 5:

Table 1 –An account of nanowired-enzyme sensors and their applications

Electrode/Sensor	Enzyme	Nanowire	Linear range	Detection limit	Application	References
Organic nanowires						
GOx/MWNT-CSNWs/ITO-ME	GOx	CSNWs	1.0–10 mM	1.0 mM	Glucose sensor	Gomathi et al., 2011
GOx/AuNPs/PANINWs	GOx	PANINWs	0.001−20 mM	0.001 mM	Glucose sensor	Chowdhury et al., 2014
GOx/PANINWs/RGO/GE	GOx	PANINWs	0.1– 8.5 μM	0.7 μM	Glucose sensor	Xia et al., 2015
Inorganic nanowires						
GOx/TiO_2NWs	GOx	TiO_2NWs	0.9 nM –100 mM	~0.9 nM	Glucose sensor	Tang et al., 2014
HRP-TiNWs/SPCE	HRP	TiNWs	40 − 560 μmol L^{-1}	10.7 μmol L^{-1}	H_2O_2 sensor	Ferraz et al., 2017
MnO_2NWs-ELISA	HRP	MnO_2NWs	$1.8x10^4$−$1.6x10^7$ CFU mL^{-1}	NA	Bacterial sensor	Wan et al., 2012
GOx/Naf/MnO_2NWs/GCE	GOx	MnO_2NWs	0.2 − 3.8 mM	25.56 μM	Glucose sensor	Zhang et al., 2013
GOx/MnO_2 NWs/GCE	GOx	MnO_2NWs	5.0 μM – 2.0 mM	1.8 μM	Glucose sensor	Han et al., 2016
Uricase/ZnONWs/Au	Uricase	ZnONWs	100~590 μM	25.6 μM	Uric acid sensor	Zhao et al., 2013
GOx-ZnONWs-APTMS GOx-ZnONWs-APTES GOx-ZnONWs-APS	GOx	ZnONWs	0 – 1.6 mM 0 − 1.6 mM 0 − 1.2 mM	NA	Glucose sensor	Jung and Lim, 2013
GOx/ZnO-NWs/graphite	GOx	ZnONWs	0.03 – 1.52 mM	~9.0 μM	Glucose sensor	Gallay et al., 2016
GOx/AgNWs/AuE	HRP	AgNWs	4.8 nM–0.31 μM	1.2 nM	H_2O_2 sensor	Song et al., 2010
GOx-PVP-AgNWs/GCE	GOx	AgNWs	20 μM − 3.62 mM	2.3 μM	Glucose/H_2O_2 sensor	Yang et al., 2012
GOx-CS/AgNWs	GOx	AgNWs	0.010 − 0.8 mM	2.83 mM	Glucose sensor	Wang et al., 2013
GOD/Nafion-AgNWs/GCE	GOx	AgNWS	0.005 − 10 mM	50 μM	Glucose sensor	Ahmad, et al., 2015
ChOx/AgNWs/GO/CS/ITO	ChOx	AgNWs	0.5–400 mg dL^{-1}	0.427 mg dL^{-1}	Cholesterol sensor	Xu et al., 2016
ChOx/ZnO/Ag/GO–CS/ITO	ChOx	AgNWs	0.25–400 mg dL^{-1} (6.5 μM − 10 mM)	0.287 μM	Cholesterol sensor	Wu et al., 2016
CS/AgNWs/GOx-GCE	GOx	AgNWs	1.0−15 mM	2.1 mM	Glucose sensor	Kumar-Krishnan et

						al., 2016
GOx/PtNWs/NEA	GOx	PtNWs	$1.0x10^{-6}$–$3.0x10^{-2}$ M	$5.0x10^{-7}$ M	H_2O_2 sensor	Yang et al., 2006
GOx/PtNWs	GOx	PtNWs	$1.0x10^{-5}$–$1.0x10^{-2}$ M	5×10^{-6} M	Glucose sensor	Lu et al., 2007
GOx/PtNWs/CHIT/GCE	GOx	PtNWs	$5.0x10^{-6}$ – $5.0x10^{-2}$ M	3 μM	Glucose sensor	Qu et al., 2007b
GOx/PVA/PBPtNWsE	GOx	PtNWs	0–12 mM	60μM	Glucose sensor	Le et al., 2010
GOx/PtNWs	GOx	PtNWs	$1.0x10^{-5}$–$1.0x10^{-2}$ M	5×10^{-6} M	Glucose sensor	Lu et al., 2012
GOX/PtNWs-Cys	GOx	PtNWs	125 μM − 16.5 mM	125 μM	Glucose sensor	Phan et al., 2013
GOx/SAM/NEE/SPS	GOx	AuNWs	$1.0x10^{-5}$ −$4.0x10^{-2}$ M	$1.5x10^{-5}$ M	Glucose sensor	Vastarella et al., 2006
HxD/AuNWsE	HxD	AuNWs	10 – 80 μM 5.0 – 30 μM	10 μM 5.0 μM	Cortisol sensor Hydrocortisone sensor	Kumar et al., 2007
ChOx/CE/AuNWs/PtE	ChOx/CE	AuNWs	10–60 μM	10 μM	Cholesterol sensor	Aravamudhan et al., 2007
GOx/AuNWs-PC/CHIT/GCE	GOx	AuNWs	$1.0x10^{-5}$–2.0×10^{-2} M	$5.0x10^{-6}$ M	H_2O_2 sensor	Lu et al., 2007
ALP/AuNWs	ALP	AuNWs	10 – 100 ng ml^{-1}	10 ng ml^{-1}	Cancer biomarker sensor (Cytokeratin-7)	Patil et al., 2008
HRP/AuNWs/nano-TiO_2/AuE	HRP	AuNWs	$2.3x10^{-6}$–$2.4x10^{-3}$ M	$7.0x10^{-7}$ M	H_2O_2 sensor	Zhong et al., 2011
GOx-BSA-GLA-AuNWA	GOx	AuNWs	5.0–6000 μM	0.1 μM	Glucose sensor	Cui et al., 2014
GOx–CNT/AuNWA GOx–rGO/AuNWA	GOx	AuNWs	100 – 3000 μmol L^{-1} 50 – 4000 μmol L^{-1}	20 μmol L^{-1} 10 μmol L^{-1}	Glucose sensor	Qin et al., 2016
PyOx/AuNWA-GLA-BSA-FAD-TPP	PyOx	AuNWs	12.5–1000 μM	100 nM	Phosphate sensor	Ogabiela et al., 2015
AChE/AuNPs/SiNWs/GCE	AChE	SiNWs	1.0 μM–1.0 mM	8.0 ng L^{-1}	Organophosphate pesticide sensor	Su et al., 2008
ISFET/SiNWs	Urease	SiNWs	0.1−0.68 mM	0.1 mM	Urea sensor	Chen et al., 2017
NF-GOx-SiNWs@AuNPs/GC	GOx	SiNWs	1.0–16 mM	50 μM	Glucose sensor	Su et al., 2010
HF/SiNWs/GOxE	GOx	SiNWs	0.1–15 mM	0.01 mM	Glucose sensor	Chen et al., 2006
SiNWs-GOx	GOx	SiNWs	10 − 300 mg dL^{-1}	10 mg dL^{-1}	Glucose sensor	Hsu et al., 2015
PBNWs/GCE	GOx	PBNWs	$1.0x10^{-7}$–$5.0x10^{-2}$ M	$5x10^{-8}$ M	H_2O_2 sensor	Qu et al., 2007a
GOx/PBNWs/GCE			$2.0x10^{-6}$ −$1.0x10^{-2}$ M	1.0 μM	Glucose	

					sensor	
GOx-PdNWs-bioanode	GOx	PdNWs	1.0 –13 mM	1.0 mM	Biofuel and glucose sensor	Slaughter and Amoah, 2012
AChE-Cs/Pd-Cu NWs/GCE	AChE	Pd-CuNWs	5–1000 ppt 500–3000 ppb	1.5 ppt (4.5 pM)	Organophosphate pesticide sensor	Song et al., 2017
HRP/PNRNWs/GCE	HRP	PNRNWs	1.0 μM − 8 mM	1.0 μM	H_2O_2 sensor	Qu et al., 2007c
HRP/PTHNW/GNP	HRP	PTHNW	0.5 μM–13.0 mM	0.3 μM	H_2O_2 sensor	Shi et al., 2008
HRP/SnO_2NWs/GCE	HRP	SnO_2NWs	10 – 450 μM	0.8 μM	H_2O_2 sensor	Li et al., 2010
GOx/CPANINWEJ	GOx	CPNWEJ	1.0 − 20 mM	1.0 mM	Glucose sensor	Konikar et al., 2015

Note- ALP-alkaline phosphatase, APS-(3- aminopropyl)methyldiethoxysilane, APTES- (3-aminopropyl)triethoxysilane, APTMS- (3-aminopropyl)trimethoxysilane, AuNWA-gold nanowires array, AuNWAs- gold nanowire arrays, BSA-bovine serum albumin, CE-cholesterol esterase, CHIT-chitosan, ChOx-cholesterol oxidase, CPANINWEJ-conducting polyaniline nanowire electrode junction, CSNWs-Chitosan nanowires, Cys- cysteamine, ELISA-enzyme-linked immunosorbent assay, FAD-flavin adenine dinucleotide, GCE-glassy carbon electrode, GLA-glutaraldehyde, GOD/GOx-glucose oxidase, GO-graphene oxide, CS-chitosan, H_2O_2-hydrogen peroxide,
HxD-3α-hydroxysteroid dehydrogenase, ISFET -ion sensitive field-effect transistor, ME-modified electrode, NA-not available, NEA-nanoelectrode array, PBNWA-Prussian blue nanowire array, PC-polycarbonate, PNRNWs- poly (neutral red) nanowires, PTHNW-Polythionine nanowire, PVP- polyvinylpyrrolidone, PyOx-pyruvate oxidase, TPP-thiamine pyrophosphate.

Highlights

- Review includes selection, synthesis and immobilization strategies for nanowired enzymes
- Comments and discussion on modification and characterization of nanowired enzyme probes
- Their potential usability for the bioanalytical applications with high accuracy and reliability.
- Summary of reported nanowired sensors with respect to detection limit, reproducibility, linear range, long-term enzyme stability.