Carbon Nanotubes Based Electrochemical Biosensors: A Review

[1]Jasmeen Saini, [1]Rupesh Kumar, [1]Vishnu Prabhakar
[1]*Nanotechnology Research Center, DAVIET, Jalandhar, Punjab, India*

Abstract- Carbon nanotubes (CNTs) can be define as a smart material having unique combination of excellent mechanical, electrical and electrochemical properties, which has stimulated increasing interest in the application of CNTs as components in (bio) sensors. CNTs large length-to diameter aspect ratios provide for high surface-to-volume ratios. Moreover, CNTs have an outstanding ability to mediate fast electron-transfer kinetics for a wide range of electroactive species. Electrochemical biosensors are currently among the most popular variant of biosensors and various materials has been used for design architectures of CNT-based electrochemical biosensors is successfully demonstrated. This is followed by a collection of fabrication electrochemical biosensors that have proven valuable for the detection of specific biomolecules. This review highlights various design methodologies for CNT-based electrochemical biosensors and their employment for the detection of a number of biomolecules. The next section describes single-wall carbon nanotube (SWCNT)-based electrochemical biosensors that have been used for biosensing detection. After a critical discussion of the factors that currently limit the practical use of CNT-based biosensors with an outline of potential future applications for CNTs in biology and medicine.

Keywords- *Biosensors, CNTs, Electroactive species, biomolecules.*

I. INTRODUCTION

Electrochemical sensors have been diversely used in industries and other analytical instruments used in environmental, food, pharmaceutical, or clinical laboratories. Glucose biosensors used widely in glucometers and pH electrodes are the important and known examples of the electrochemical sensors. Day by day, the numbers of sensors or biosensors are increasing. Next generation of sensor or biosensors will require considerable improvements in sensitivity, selectivity, and accuracy to meet the future needs in diversity of fields. The nanoparticles have different effects on response of the sensor or biosensor besides improving their thermal, electrical and mechanical properties (F. Faridbod *et al.*, 2011). Electrochemical biosensors have been studied for a long time. These provide an attractive means to analyze the content of a biological sample due to the direct conversion of a biological event to an electronic signal (F.A. Armstrong *et al.*, 1997). An electrochemical sensor is a device that transforms electrochemical information into an analytically useful signal. Electrochemical sensors usually composed of two basic components, a chemical (molecular) recognition system which is the most important part of a sensor and a physicochemical transducer which is a device that converts the chemical response into a signal that can be detected by modern electrical instrumentations (F. Faridbod *et al.,* 2011).

Electrochemical biosensors combine the sensitivity of electroanalytical methods with the inherent bioselectivity of the biological component. The biological component in the sensor recognizes its analyte resulting in a catalytic or binding event that ultimately produces an electrical signal monitored by a transducer that is proportional to analyte concentration. Some of these sensor devices have reached the commercial stage and are routinely used in clinical, environmental, industrial, and agricultural applications (N.J. Ronkainen *et al.,* 2010). The recognition process or the reaction mechanism that these biosensors employ include ZnO based enzymatic reaction, carbon nano-tube enzymatic-gelatin reaction and whole cell system. The fabrication techniques used in these developments include micro-fabrication and printing technologies. Nano-tube and nano-particles are incorporated in these advancements. Electrochemical based sensors provide the detection mechanism for the biosensor. This detection mechanism can be incorporated by a recognition process with the analyte through an enzymatic reaction, antibody and antigen interaction, whole cell recognition process or otherwise. A very successful example of an electrochemical based biosensor is the blood glucose strip (single-use, disposable biosensor) currently used for diabetic patient management. In this biosensor, the enzymatic reaction with blood glucose generates H_2O_2, which is then electrochemically oxidized producing a current to quantify blood glucose (C.C Liu, 2012).

The combination of knowledge in bio- and electrochemistry, solid-state and surface physics, bioengineering, integrated circuit silicon technology and data processing offers the possibility of a new generation of highly specific, sensitive, selective and reliable micro (bio-) chemical sensors and sensor arrays addressing these remaining issues. Emerging devices for electrochemical biosensors inspired by advances in microelectronics and nanotechnology will be introduced.

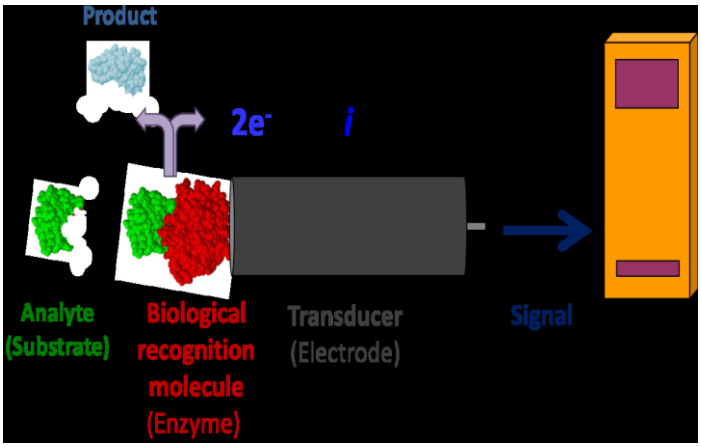

Figure 1. A typical design of an enzyme modified electrochemical biosensor (Putzbach *et al.* 2013)

II. MATERIALS USED IN CNT BASED ELECTROCHEMICAL BIOSENSOR

Materials used in electrochemical biosensors are classified as: (1) materials for the electrode and supporting substrate, (2) materials for the immobilisation of biological recognition elements (3), materials for the fabrication of the outer membrane and (4), biological elements, such as enzymes, antibodies, antigens, mediators and cofactors (S. Zhang *et al.*, 2000).

A. Electrodes and supporting substrates

Metals (platinum, gold, silver etc.) and carbon (graphite, carbon black etc.) are commonly used to prepare solid electrode systems and supporting substrates. Metals have excellent electrical and mechanical properties whereas carbon holds high chemical inertness, providing a wide range of anode working potentials with low electrical resistivity and have a very pure crystal structure that provides low residual currents and a high signal-to-noise ratio (Ce´spedes *et al.*, 1996). More recently, a number of new mixed materials have appeared for the preparation of electrodes. A novel electrode by the use of a flexible conductive polymer film of polypyrrole doped with polyanions and a microporous layer of platinum black was also fabricated (G.F. Khan *et al.*, 1996). Glucose sensors produced with this material provided a H_2O_2 oxidation current at a lower applied potential than conventional sensors.

B. Materials used for the immobilisation of biological elements

Materials being traditionally used for immobilization purpose are multifunctional agents such as glutaraldehyde and hexamethyl diisocyanate, which form crosslinks between biocatalytic species, or proteins. Organic conductive polymers provide advantages, including the formation of an appropriate environment for enzyme immobilisation at the electrode and for its interaction with metallic and carbon conductors (P.N. Bartlett *et al.*, 1993; M. Trojanowicz *et al.*, 1995). Alternatively, non-conductive polymers, such as polyacrylamide and polyphenol, can be used to entrap elements physically.

C. Membrane materials and biocompatibility

Biosensors are usually covered with a thin membrane that has several functions, including diffusion control, reduction of interference and mechanical protection of the sensing probe. Commercially available polymers, such as polyvinyl chloride (PVC), polyethylene, polymethacrylate and polyurethane are commonly used for the preparation of these membranes due to their suitable physical and chemical properties (L.E. Donald *et al.*, 1996). Materials with hydrogel-like properties are generally considered to favour biocompatibility. Water associates with the water-soluble polymers and the presence of water around the polymer hinders protein adsorption due to the energetically unfavourable displacement of water by protein and compression of the polymer upon the approach of protein (S.I. Jeon *et al.*, 1991). Surfaces grafted with water-soluble polymers have been developed using a number of techniques, including end-grafting (M.S. Shoichet *et al.*, 1994) and in situ polymerization by photo- or wet- chemistry, and by radio frequency glow discharge deposition (G.P. Lopez *et al.*, 1992). Polyethylene glycol (PEG) has been used extensively to modify surfaces, so that protein adsorption and platelet interactions with the foreign surface are reduced (M.M. Amiji *et al.*, 1994). Surface modification of polymers has led to modest improvements in biocompatibility, but it is still not satisfactory for long-term in vivo applications, so there is an urgent need to design and develop new biocompatible materials.

D. Biological elements

Improvements have frequently been directed at the incorporation of active molecules, including enzymes such as glucose oxidase (L.C. Clark *et al.*, 1962) and lactate oxidase (P. Vadgama *et al.*, 1986). Deoxyribonucleic acid (DNA) has recently been suggested as a biological recognition element for such biosensors (P. Vadgama *et al.*, 1992; A. Mulchandani *et al.*, 1995). The unique nucleotide base structure of DNA provides the basis of the technique which allows singlestranded DNA (ssDNA) to be used to identify other ssDNA molecules with the complementary bases (J.H. Zhai *et al.*, 1997). In electrochemical DNA biosensor an ssDNA strand is covalently bound to the surface of an electrode. Hybridisation of the immobilised sequence with its dissolved complement forms the double strand that can be detected using a DNA-specific redox-active metal:polypyridine complex. Damaged segments of DNA can also be detected by measuring changes in the redox signals of base residues in DNA immobilised on carbon electrodes (E. Palecek *et al.*, 1998). Peptide nucleic acids (PNAs) have been found to exhibit unique and efficient hybridisation properties that may offer significant advantages for sequence-specific

recognition compared to their DNA counterparts (S. Zhang *et al.*, 2000).

III. FABRICATION PROCESS

A. Dispersion and Stabilization by Oxidative Acids

Although well-ordered, all-carbon hollow CNTs are excellent candidates for biosensors, but they have two major limitations imparted by their hydrophobic nature. These include spontaneous coagulation and lack of solubility in aqueous media (J. Wang *et al.*, 2007). Using the –COOH groups, the functionalized CNTs can be chemically adsorbed onto an electrode surface. A dark stable suspension can be achieved after immobilization via removal of the excess carboxylic acid groups. Kovtyukhova *et al.*, (2003) developed a novel method for immobilization of SWCNTs using an oxidative technique previously developed for transformation of graphite to graphite oxide. This process involved treatment with a H_2SO_4 containing $(NH_4)_2S_2O_8$ and P_2O_5 solution, followed by H_2SO_4 and $KMnO_4$. The oxidized CNTs slowly formed hydrogels at low concentration. The authors attributed this to the formation of a hydrogen-bonded nanotube network. The oxidized tubes bonded readily to amine-coated surfaces, on which they adsorbed as a single-layer film.

B. CNT Adsorption on the Transducer Substrate

To prevent the coagulation that occurs when CNTs are placed in aqueous media, dissolving them in non-polar organic solvents such as N,N-dimethylformamide (DMF) or chloroform followed by sonication allows the formation of homogeneous CNT dispersions that can be used to drop-cast or spin coat transducer surfaces (C. Baj-Rossi *et al.*, 2012). The solvent quickly evaporates leaving behind a porous, 3-D structure of CNTs on the electrode surface to which the biomolecules can be immobilized. These methods are very popular for CNT immobilization due to their ease and simplicity. The major limitation of adsorption immobilization is the resulting random distribution of nanomaterials that is not reproducible on the transducer surface. The most common subtrates are gold, platinum, glassy carbon, carbon fiber, and glass (C.S.S.R. Kumar, 2007). Baj-Rossi *et al.*, prepared a biosensor for electrochemical detection of anti-cancer drugs in human serum using chloroform solubilization followed by sonication and drop-casting of MWCNTs with diameter of 10 nm, length of 1–2 μm, and 5% –COOH groups content. The CNTs were directly immobilized onto screen printed graphite working electrodes. The authors demonstrated that simultaneous detection of two drugs can be achieved with a careful selection of the isoform as enzyme probe according to the drug to be detected.

C. Dispersion by Surfactant Interaction

Multiple groups have explored noncovalent immobilization methods which preserve the intact CNT structures after their dispersion. The nanostructures were first centrifuged, filtered, distilled, and sonicated followed by a simple noncovalent immobilization by spin coating, evaporation or casting onto the sensor surface (P.J. Britto *et al.*, 1996; M. Ouyang *et al.*, 2002). However, dispersing and anchoring the CNTs onto the sensor surface in a controlled manner can be challenging due to the hydrophobic properties of the nanostructures. Noncovalent surfactant and polymer assisted aqueous dispersion which utilize the hydrophilic caps of CNTs have helped overcome some of the limitations seen with simple physical stabilization (W. Putzbach *et al.*, 2013)

D. Surface Functionalization

This method requires covalent modification of the CNT and/or electrode surface with functional groups that will bind to the electrode or substrate surface. The modification of CNTs usually involves the ends, sidewalls, or defects which result from the oxidative acid pretreatment of CNTs and are rich in CNT-bound carboxylic groups (C.S.S.R. Kumar, 2007). The linkages between the functional components and CNTs, which may or may not involve coupling agents, are typically based on carboxylate chemistry via esterification therefore involving covalent bonding or alternatively ionic interactions that are noncovalent in nature.

Moreover, activating CNT surfaces is important in order to improve the performance of the prepared biosensors. CNT solubilization in aqueous media is important for use of CNTs as supporting matrix for the immobilization of proteins. This can be achieved by the surface functionalization of CNTs with ionic, hydrophilic groups, or with water-soluble polymers. Soluble CNTs have been shown to have electronic properties similar to CNTs that were not functionalized (M. Melle-Franco *et al.*, 2004).

E. Incorporation into a Composite

Perhaps, the easiest and most popular method of CNT immobilization is the incorporation of the nanomaterial into a composite. A composite mixture of CNTs and pi-conjugated polymers such as graphite can be viewed as an extreme form of a conducting polymer, offering a high surface area-volume ratio and enhanced electronic properties. Needle type biosensors were prepared and optimized for glucose using composite of MWCNTs, graphite powder, and freeze-dried powder of GOx inside a glass capillary (J. Jia *et al.*, 2008). MWCNTs were treated with strong acid and then agitated. MWCNTs were then filtered, rinsed with water, and dried in an oven. The acid treated MWCNTs, GOx and graphite powder were mixed into a paste and pressed into the cavity at the end of a glass capillary containing a copper wire. Finally, the end surface of the electrode was soaked in paraffin, oven dried, and polished to a smooth surface with weighing paper (W. Putzbach *et al.*, 2013).

F. Carbon Nanotube Array Biosensors

CNT arrays consist of vertically aligned bundles of relatively short CNTs. CNT arrays have many of the same desirable properties that were observed for individual CNTs such as good electrical conductivity and efficient electron transfer reactions (J. Wang, 2008). Direct electron transfer between redox active enzymes such as Glucose oxidase and CNT arrays has been reported (J.J. Gooding *et al.*, 2003). However, they may not be robust requiring the use of a polymer or a glass casing as a protective outside support. Carbon nanotube needle biosensors can be prepared in a cost effective manner by welding a bundle of MWCNTs in an inert atmosphere onto the tip of a tungsten needle under a bright field microscope. The needle can later be encased in glass and a UV curing polymer coating to electrically insulate the tugsten needle leaving only the tip exposed to the analyte (Y. Yun *et al.*, 2007). The bundle of nanotubes at the tip of the transducer may be sharpened using acid etching or electrical discharge to further lower the sensor detection limits.

IV. CURRENT AND FUTURE APPLICATIONS

CNTs are extremely attractive for fabricating electrochemical biosensors due to their outstanding properties, especially the excellent conductive, adsorptive and biocompatibility. The practical application of CNTs in electrochemical biosensors can be found in a very limited area only.

A. Current Perspective

1. Real Sample Analysis

CNTs-based sensors can be applied in real sample analysis in different areas such as biomedical, food, agriculture, and fishing industries. CNTs-based sensors can be used in commercial food samples to detect undesired chemical residues resulting from animal drugs, food additives, pesticides, and other environmental contaminants in raw and processed foods (N. Liu *et al.*, 2007). . CNTs based electrochemical sensors are also widely used in real blood and urine samples analyses.

2. *Amperometric biosensing of Organo Phosphorus compounds*

The CNT-based transducer leads to a highly sensitive and stable detection of the enzymatically (OPH) liberated *p*-nitrophenol. Such coupling of OPH-based biorecognition and amperometric transduction on CNT transducers is advantageous over AChE-based CNT biosensors that lack specificity towards OP compounds and require addition of the substrate and an incubation period. Detection of cysteine on Pt/CNT electrodes by cyclic voltammetry has been carried out. The electrochemical detection of NADH was also demonstrated using scattered-CNT electrodes (K. Maehashi *et al.*, 2009). The use of OPH amperometric biosensors for direct measurements in untreated natural water samples has been demonstrated. Such on-site applications would greatly benefit from the use of disposable OPH/CNT-coated screen printed electrodes (K. Maehashi *et al.*, 2009). Potential interferences from easily oxidizable species should be considered in such real-life applications; the Nafion coating should alleviate such electroactive interferences.

3. As Molecular Wires

Aligned CNT "forests" can act as molecular wires to allow efficient electron transfer between the detecting electrode and the redox centers of enzymes to fabricate reagentless biosensors. Electrochemical sensing for DNA can greatly benefit from the use of CNT based platforms since guanine, one of the four bases, can be detected with significantly enhanced sensitivity. CNTs fluoresce, or emit light after absorbing light, in the near infrared region and retain their ability to fluoresce over time. This feature will allow CNT-based sensors to transmit information from inside the body (J. Wang, 2007). The combination of micro/nanofabrication and chemical functionalization, particularly nanoelectrode assembly interfaced with biomolecules, is expected to pave the way to fabricate improved biosensors for proteins, chemicals, and pathogens. However, several technical challenges need to be overcome to tightly integrate CNT-based platforms with sampling, fluidic handling, separation, and other detection principles. Carbon nanotube patents look more controversial in electronics but are less problematical in energy, health care, and cosmetics. The use of CNTs in biosensing looks very promising as reflected by some significant patents in this area and other research and development endeavors.

4. Drug Analysis

The development of sensitive analytical techniques of drugs has drawn much attention due to the promising applications in environment protection, pharmacology and biomedical studies. To improve the detection sensitivity, various nanomaterials are employed to fabricate modified electrodes with high performance. Among these materials, CNTs have also been widely to improve the sensitivity of some drugs also CNTs have some advantages, such as good conductivity and strong electrocatalytic activity (C. Hu *et al*., 2009). The enhanced adsorption of some water insoluble or conjugated drugs on the hydrophobic and π-conjugated sidewalls of CNTs contributes to the sensitive determination of these species at CNT electrodes.

5. OP pesticides determination in food and in the environment

Various nanomaterials used as acetylcholinesterase immobilization matrices in electrochemical biosensors for organophosphorus pesticides determination; along with biosensors performance characteristics such as sensitivity, linear dynamic range, and detection limit are evaluated. The

alternative route leading to biosensors sensitivity, selectivity and stability increase involves the incorporation in the biosensing platform of biorecognition elements with tailor designed properties (FQPA, 1996). Two main strategies to improve electrochemical biosensors performances emerged during the recent years: nanomaterials transducer modification and genetic engineering of the biological recognition element. The nanotechnological approach in electrochemical biosensors development, takes advantage of the electrocatalytical properties of the nanostructures, their action as electron transfer mediators or electrical wires, large surface to volume ratio, structural robustness, and biocompatibility (K. Balasubramanian *et al.*, 2006). Therefore, it yielded the following chief issues: electrode potential lowering, enhancement of the electron transfer rate with no electrode surface fouling, sensitivity increase, stability improvement, and interface functionalization.

B. Future Perspectives

Beyond the applications outlined above, the field of CNT based (bio)chemical sensors is currently experiencing a wealth of future developments. One promising research direction is the large-scale fabrication of nanoelectrode arrays made of CNTs (Y. Tu *et al.*, 2005). For this purpose, CNTs are vertically grown at a low density using a plasma-enhanced chemical vapor deposition method, and then the interstitial spaces between the CNTs are filled with an electrically insulating epoxy resin, whereupon only the tips of the CNTs are exposed for the electrochemical detection. Such arrays can provide information about the ambient environment via specificity in the pattern of collected responses (H.E. Katz *et al.*, 2004).

Significant progress has also been made in identifying the electrocatalytically active sites of MWCNTs. Specifically, the oxidation of NADH has been found to be preferred at the open tube ends and defects ("edge plane sites") along the tube axis (C.E. Banks *at al.*, 2005). Further to this, immuno-CNTs, which have recently been shown to be capable of recognizing pathogen cells via specific antibody–antigen interactions (T. Elkin *et al.*, 2005), may contribute to expanding the detection scope of CNT sensors. Another intriguing development is the integration of biological cell membranes and CNT transistors, which opens the door to obtaining important information like the distribution of charges within the membrane (K. Bradley *et al.*, 2005). Finally, recent advances in the implantation of CNT biosensors into living biological tissue (S. Mancuso *et al.*, 2005), as well as the development of novel, fluorescence-based CNT nanosenors are worth mentioning (P.W. Barone *et al.*, 2005).

Every important issue related to the integration of CNTs into biological cells and tissues is the need to study their cytotoxicity towards biological species. SWCNTs have been reported to be toxic to mammalian cells beyond 10 μmol/L, while in another study they have been found to be nontoxic up to a concentration of 0.05 mg/Ml. On the other hand, most recent results suggest that chemically modifying CNTs can reduce their cytotoxicity to a certain extent (C.M. Sayes *et al.*, 2006). However, in-depth systematic studies of the effect of CNTs on human cells and tissues as well as information related to safety issues are still lacking, and future work must concentrate on addressing these aspects.

V. CONCLUSION

Electrochemical nanobiosensors offer without doubts an important step toward development of selective, down to few target molecules sensitive biorecognition device for medical and security applications. Electrochemical nanobiosensors consisting from single carbon nanotube are another future path of biosensor development. There is high expectation that such devices will develop toward reliable point-of-care diagnostics of cancer and other diseases, and as tools for intra-operation pathological testing, proteomics and systems biology.

In the future, efforts will need to be directed toward preventing nonspecific adsorption of biomolecules onto the tube walls, although promising advances have already been made in this direction. Further improvements may be expected from extending the range of modifying molecules that can be attached to the tubes; enzymes, nucleic acids and metal nanocrystals have been mostly employed for this purpose so far. Particularly promising in this respect are electropolymerized coatings, which can be prepared with a broad range of compositions and with precisely controlled thicknesses.

VI. REFERENCES

[1] A. Mulchandani and A.S. Bassi, "Principles and applications of biosensors for bioprocess monitoring and control", Crit. Rev. Biotechnol., (1995), 15, 105–124.

[2] C.C. Liu, "Electrochemical Based Biosensors", Biosensors, (2012), 2, 269-272.

[3] Baj-Rossi, G. de Micheli and S. Carrara, "Electrochemical detection of anti-breast-cancer agents in human serum by cytochrome P450-Coated Carbon nanotubes", Sensors, (2012), 12, 6520–6537.

[4] C.E. Banks and R.J. Compton, "Exploring the electrocatalytic sites of carbon nanotubes for NADH detection: an edge plane pyrolytic graphite electrode study", Analyst, (2005), 130, 1232–1239.

[5] Hu and S. Hu, "Carbon Nanotube-Based Electrochemical Sensors: Principles and Applications in Biomedical Systems", Journal of Sensors, (2009), 40 pages.

[6] C.S.S.R. Kumar, "Carbon Nanotube-Based Sensor. In Nanomaterials for Biosensors", Wiley-VCH: Weinheim, Germany, (2007), pp. 27–89.

[7] C.M. Sayes, F. Liang, J.L. Hudson, J. Mendez, W. Guo, J.M. Beach, V.C. Moore, C.D. Doyle, J.L. West, W.E. Billups, K.D. Ausman and V.L. Colvin, "Functionalization density dependence of single-walled carbon nanotubes cytotoxicity in vitro", Toxicol Lett, (2006), 161(2), 135–42.

[8] Palecek, M. Fojta, M. Tomschik and J. Wang, "Electrochemical biosensors for DNA hybridisation and DNA damage", Biosens. Bioelectron., (1998), 13, 621–628.

[9] F. Faridbod, K. Gupta and H. Zamani, "Electrochemical Sensors and Biosensors", International Journal of Electrochemical Science, (2011), ISSN 1452-3981 2011, Article Id 352546.

[10] F.A. Armstrong, H.A. Heering and J. Hirst, "Reactions Of Complex Metalloproteins Studies By Protein Film Voltammetry", J. Chem. Soc., Rev., (1997), 26, 169-179.

[11] Ce´spedes and S. Alegret, "New materials for electrochemical sensing: glucose biosensors based on rigid carbon-polymer biocomposites", Food Technol. Biotechnol., (1996), 34, 143–146.

[12] FQPA, H.Rept. 104-669, part 2, 104th Congress, 2nd sess., p. 6, 1996.

[13] G.F. Khan and W. Wernet, "Platinization of shapable electroconductive polymer film for an improved glucose sensor", J. Electrochem. Soc., (1996), 143, 3336–3342.

[14] G.P. Lopez, B.D. Ratner, C.D. Tidwell, C.L. Haycox, R.J. Rapoza, T.A. Hobett, "Glow-discharge plasma deposition of tetraethylene glycol dimethyl ether for fouling-resistant biomaterial surfaces", J. Biomed Water Lenz. Res., (1992), 26, 415–439.

[15] H.E. Katz, "Chemically Sensitive Field-Effect Transistors and Chemiresistors: New Materials and Device Structures", Electroanalysis, (2004), 16, 1837–1842.

[16] J.J. Gooding, R. Wibowo, J. Liu, W. Yang, D. Losic, S. Orbons, F.J. Mearns, J.G. Shapter and D.B. Hibbert, "Protein electrochemistry using aligned carbon nanotube arrays", J. Am. Chem. Soc., (2003), 125, 9006–9007.

[17] J. Jia, W. Guan, M. Sim, Y. Li and H. Li, "Carbon nanotubes based glucose needle-type biosensor", Sensors, (2008), 8, 1712–1718.

[18] J. Wang, "Electrochemical glucose biosensors", Chem. Rev., (2008), 108, 814–825. J.H. Zhai, H. Cui and R.F. Yang, "DNA based biosensors", Biotechnol. Adv., (1997), 15, 43–58.

[19] J. Wang, "Carbon-Nanotube Based Electrochemical Biosensors: A Review", Electroanalysis, (2005), 17, 1.

[20] K. Maehashi and K. Matsumoto, "Label-Free Electrical Detection Using Carbon Nanotube- Based Biosensors-Review", Sensors, (2009), 9, 5368-5378.

[21] K. Balasubramanian and M. Burghard, "Biosensors based on carbon nanotubes", Anal Bioanal. Chem., (2006), 385, 3, 452-468.

[22] K. Bradley, A. Davis, J.C.P. Gabriel and G. Gruner, "Integration of cell membranes and nanotube transistors", Nano Lett, (2005), 5, 841–845.

[23] L.C. Clark and C. Lyons, "Electrode systems for continuous monitoring in cardiovascular surgery", Ann. NY Acad. Sci., (1962), 102, 29–45.

[24] L.E. Donald and A.H. Jeffrey, "Surface treatment of polymers for biocompatibility", Ann. Rev. Mater. Sci., (1996), 26, 365–394.

[25] M. Trojanowicz and T.K.V. Krawczyk, "Electrochemical biosensors based on enzymes immobilised in electropolymerised films", Mikrochim. Acta, (1995), 121, 167–181.

[26] M. Melle-Franco, M. Marcaccio, D. Paolucci, F. Paolucci, V.D. Georgakilas, M. Guldi, M. Prato and F. Zerbetto, "Cyclic voltammetry and bulk electronic properties of soluble carbon nanotubes", J. Am. Chem. Soc., (2004), 126, 1646–1647.

[27] M. Ouyang, J. Huang and C.M. Lieber, "Fundamental electronic properties and applications of single-walled carbon nanotubes", Acc. Chem. Res., (2002), 35, 1018–1025.

[28] M.M. Amiji and K. Park, "Analysis on the surface-adsorption of peo ppo peo triblock copolymers by radiolabelling and fluorescence techniques", J. Appl. Polym. Sci., (1994), 52, 539–544.

[29] M.S. Shoichet, S.R. Winn, S. Athavale, J.M. Harris, F.T. Gentile, "Poly(ethylene oxide) grafted thermoplastic membranes for use as cellular hybrid bioartificial organs in the central-nervous system", Biotechnol. Bioeng., (1994), 43, 563–572.

[30] N.J. Ronkainen, H.B. Halsall and W.R. Heineman, "Electrochemical Biosensors", Chem. Soc. Rev., (2010), 39, 1747-1763.

[31] N.A. Monteiro-Riviere, R.J. Nemanich, A.O. Inman, Y.Y. Wang and J.E. Riviere, "Near-infrared optical sensors based on single-walled carbon nanotubes", Toxicol Lett., (2005), 155, 377–384.

[32] N.I. Kovtyukhova, T.E. Mallouk, L. Pan and E.C. Dickey, "Individual single-walled nanotubes and hydrogels made by oxidative exfoliation of carbon nanotube ropes", J. Am. Chem. Soc., (2003), 125, 9761–9769.

[33] N. Liu, X. Cai, Y. Lei, Q. Zhang, M.B. Chan-Park, C. Li, W. Chen and A. Mulchandani, "Single-Walled Carbon Nanotube Based Real-Time Organophosphate Detector", Electroanalysis, (2007), 19, 616 – 619.

[34] P.W. Barone, S. Baik, D.A. Heller and M.S. Strano, "Near-infrared optical sensors based on single-walled carbon nanotubes", Nature Mater, (2005), 4, 86–92.

[35] P.N. Bartlett and J.M. Cooper, "A review of the immobilisation of enzymes in electropolymerized films", J. Electroanal. Chem, (1993), 362, 1–12.

[36] P.J. Britto, K.S.V. Sunthanam and P.M. Ayajan, "Carbon nanotube electrode for oxidation of dopamine", Bioelectrochem. Bioenerg., (1996), 41, 121–125.

[37] P. Vadgama, A.K. Covington and K.G.M.M. Alberti, "Amperometric enzyme electrode system for extracorporeal lactate monitoring based on lactate dehydrogenase", Analyst, (1986), 111, 803–807.

[38] P. Vadgama and P.W. Crump, "Biosensors – Recent trends – A review", Analyst, (1992), 117, 1657–1670.

[39] S. Zhang, G. Wright and Y. Yang, "Materials and techniques for electrochemical biosensor design and construction", Biosensors & Bioelectronics, (2000), 15, 273–282.

[40] S.I. Jeon and J.D. Andrade, "Protein surface interactions in the presence of poyethylene oxide. 1. Effect of protein size", J. Colloid Interface Sci., (1991), 142, 159–166.

[41] S. Mancuso, A.M. Marras, V. Magnus and F. Baluska, "Non-invasive and continuous recordings of auxin fluxes in intact root apex with a carbon nanotube-modified and self-referencing micro electrode", Anal Biochem., (2005), 341, 344–351.

[42] T. Elkin, X.P. Jiang, S. Taylor, Y. Lin, L.R. Gu, H. Yang, J. Brown, S. Collins and Y.P. Sun, "Immuno-carbon nanotubes and recognition of pathogens", ChemBioChem., (2005), 6, 640–643.

[43] W. Putzbach and N.J. Ronkainen, "Immobilization Techniques in the Fabrication of Nanomaterial-Based Electrochemical Biosensors: A Review", Sensors (2013), 13, 4811-4840.

[44] Y. Yun, A. Bange, V.N. Shanov, W. Heineman, H.B. Halsall, Z. Dong, A. Jazieh, Y. Tu, D. Wong and S. Pixley, "A carbon nanotube needle biosensor", J. Nanosci. Nanotechnol., (2007), 7, 2293–2300.

[45] Y. Tu, Y. Lin, W. Yantasee and Z. Ren, "Carbon Nanotubes Based Nanoelectrode Arrays: Fabrication, Evaluation, and Application in Voltammetric Analysis", Electroanalysis, (2005), 17, 79–84.