Journal of Advanced Zoology

ISSN: 0253-7214

Volume 43 Issue 01 Year 2022 Page 283:289

Biosensors for Early Cancer Biomarker Detection

Dr. Mrs. Jyoti Ramteke*

**Sevadal Mahila Mahavidhyalaya and Research Academy, Nagpur*

***Corresponding Author: -** Dr. Mrs. Jyoti Ramteke*
**Sevadal Mahila Mahavidhyalaya and Research Academy, Nagpur*

Article History:

Received: 18.04.2022
Revised: 17.05.2022
Accepted: 20.06.2022

Abstract

Cancer is a highly fatal disease, and due to contemporary lifestyle and growing, environmental pollution chances of cancer are increasing, which is a global threat. The high mortality rate in cancer is caused primarily due to its late detection, mostly in a metastatic third or fourth stage, resulting in a poor after therapy prognosis. The conventional detection methods include identification of carcinogenic features of cells such as DNA or RNA mutation, conformational changes and overexpression of some proteins, and cell morphology, which are called biomarkers or analytes. Those processes are specialist-dependent, time-consuming, and expensive.

Recently, biosensors are becoming popular as easy, quick, cheap, and highly sensitive detection tools. The biosensor technique depends on the availability of biomarkers in the sample. Thus, identifying new molecular markers for various types of cancers is a parallel issue, which is, fortunately, in rapid progress. A biosensor has a biomarker-specific layer of biorecognition elements on a transducer, which acts as an electrode. Upon binding of biomarkers with the biorecognition elements, a chemical signal gets generated. The transducer converts that signal into a measurable output for further analysis. Among several biorecognition elements, antibodies (Abs) are highly demanding, especially for the cancer diagnosis, for their unique three-dimensional structures and high specificity.

This report would focus on the complete fabrication process of biosensor for cancer biomarker detection. Results of some of the recent studies obtained in the laboratories relating to fabrication and application of nanomaterial modified biosensors for cancer biomarker detection are also mentioned.

INTRODUCTION

Cancer

Cancer is currently the leading cause of morbidity and mortality worldwide and it is estimated that more than 11 million people are diagnosed with cancer every year. Cancer is defined as abnormal and uncontrolled cell growth due to an accumulation of specific genetic and epigenetic defects, which are either environmental or hereditary in origin. The unregulated cell growth leads to the formation of a tumor mass that over time becomes independent of normal homeostatic checks and balances. As the cancer progresses, the tumor begins to spread beyond the site of origin and metastasizes to other body organs and systems, making it incurable. More than 200 distinct forms of cancer exist which include lung, prostate, breast, ovarian, hematologic, skin, and colon cancer, and leukaemia etc. Some cancers like stomach and cervical cancer are strongly associated with bacterial and viral infections, respectively. The conventional techniques for cancer detection such as centrifugation, chromatography, and fluorescence and magnetic-activated cell sorting rely on the expertise and subjective judgment of highly skilled personnel. Diagnosing a cancer via cross sectional imaging (CT scan) and biopsy is an expensive and often uncomfortable approach for patients and yield substantial false-negative rates and a limited potential for early diagnosis of disease. Therefore, technologies to recognize and understand the signatures of normal cells and how these become cancerous, promise to provide important insights into the aetiology of cancer that can be useful for early detection, diagnosis, and treatment.

Biomarker Detection

The detection of clinical biomarkers plays a crucial role in the early detection of a cancer, design of individual therapies, and to identify underlying processes involved in the disease. Biomarkers are chemical substances related with the elevation of malignant tumours which are found in blood, urine, or body tissues. They are normally produced directly by the embryonic tissue or tumour tissue. Biomarkers indicate changes in the expression of a protein that is correlated to risk or progression of a disease or its response to treatment, and that can be measured in tissues or in the blood. As a result, biomarkers can be specific cells, molecules or genes, gene products, enzymes or hormones. An ideal cancer biomarker should have high clinical sensitivity and specificity, quick release in the blood enabling early diagnosis, capability to remain elevated for longer time in the blood, and ability to be assayed quantitatively. More than 160 types of biomarkers are identified that are being used for the detection of cancer. The expression of specific biomarkers and their accurate detection can be helpful in the diagnosis, staging and effective treatment of a cancer at an early stage. Various cancer markers are being widely used for diagnosing cancer, but carcinoembryonic antigen (CEA) is known to be a tumour marker associated with colon, lung, ovarian and breast cancer that are responsible for more than half of all cancer deaths each year. CEA is a highly glycosylated cell surface glycoprotein, belonging to a group of substance known as the tumour-associated antigens. Hence, the monitoring of CEA level before and after cancer therapy facilitates early recognition of recurrences or detection of previously unremarked metastases. Determination of CEA in serum can be an interesting alternative for clinical diagnosis and monitoring of a cancer.

The quantification of biological or biochemical processes is of utmost importance for medical, biological and biotechnological applications. However, converting the biological information to an easily processed electronic signal is challenging due to the complexity of connecting an electronic device directly to a biological environment. Electrochemical biosensors provide an attractive means to analyse the content of a biological sample due to direct conversion of a biological event to an electronic signal. Over the past decades several sensing concepts and related devices have been developed. In this context, paper based electrochemical sensors are known to play an important role in on-going transition towards the POC diagnostic devices. These papers based POC can be flat, folded, simple, low cost, flexible, and lightweight. Further, intelligent use of the polymers and nanomaterials (graphene, CNT) on paper may lead to enhanced performances with increased sensitivities and lowered detection limits of several orders of magnitudes. One advantage of utilizing these nanomaterials is the high specific surface thus already enabling the immobilization of an enhanced amount of bioreceptor units.

METHODOLOGY

Electrochemical biosensors

Electrochemical sensors are powerful analytical tools used in a variety of applications due to the many benefits that they bring along. According to the state-of-the-art,40 the other analytical methods, such as spectroscopy, chromatography or electrophoresis require expensive and sophisticated equipment, complex sample pre-treatment, and skilled personnel assistance. They are also time-consuming and occasionally unsuitable. To overcome these setbacks, micro- and nanotechnologies have gained interest in the fabrication of sensing electrochemical devices as they offer unique characteristics such as miniaturization, portability, low cost, ease-of use, specificity, selectivity, and real-time on-sight monitoring capabilities. Electrochemical sensors exhibit also a much higher sensitivity towards biomolecule detection thanks to small sample volumes, reduced from ml to µl (as low as 50 µl of sample is required when using an integrated electrochemical device) and high active surface area, obtained by using nanomaterials as sensing layer, since by nano structuring the materials, the active sites are increased. Depending on the fabrication technology, the so-called "in-house" fabricated electrochemical sensors can be: 1) carbon paste-based electrodes (CPE); screen-printed electrodes, either on solid or flexible substrate (SPE) and microfabricated electrodes (MFEs). While CPEs show limitations in downsizing and integration in a miniaturized device and SPEs are disposable and easily damaged, MFEs show a better stability in time, ease of miniaturization, and they can easily be integrated in a portable device. Electrochemical sensors cover several classes of sensors according to their investigated signal during the chemical reaction: a) amperometric sensors, where a measurable current intensity is generated; b) potentiometric sensors, where a measurable potential or charge accumulation is generated; c) impedimetric sensors, where the impedance (both resistance and reactance) is recorded; d) conductometric sensors, which imply the conductive properties of a medium and lately, e) field-effect transistors (FET), which uses transistor technology to measure current as a result of a potentiometric effect at a gate electrode. The amperometric sensor is the type of electrochemical sensor that requires the three-electrode system. The current is either measured at a constant potential and it is known as amperometry, or by scanning a potential range and it is known as voltammetry.

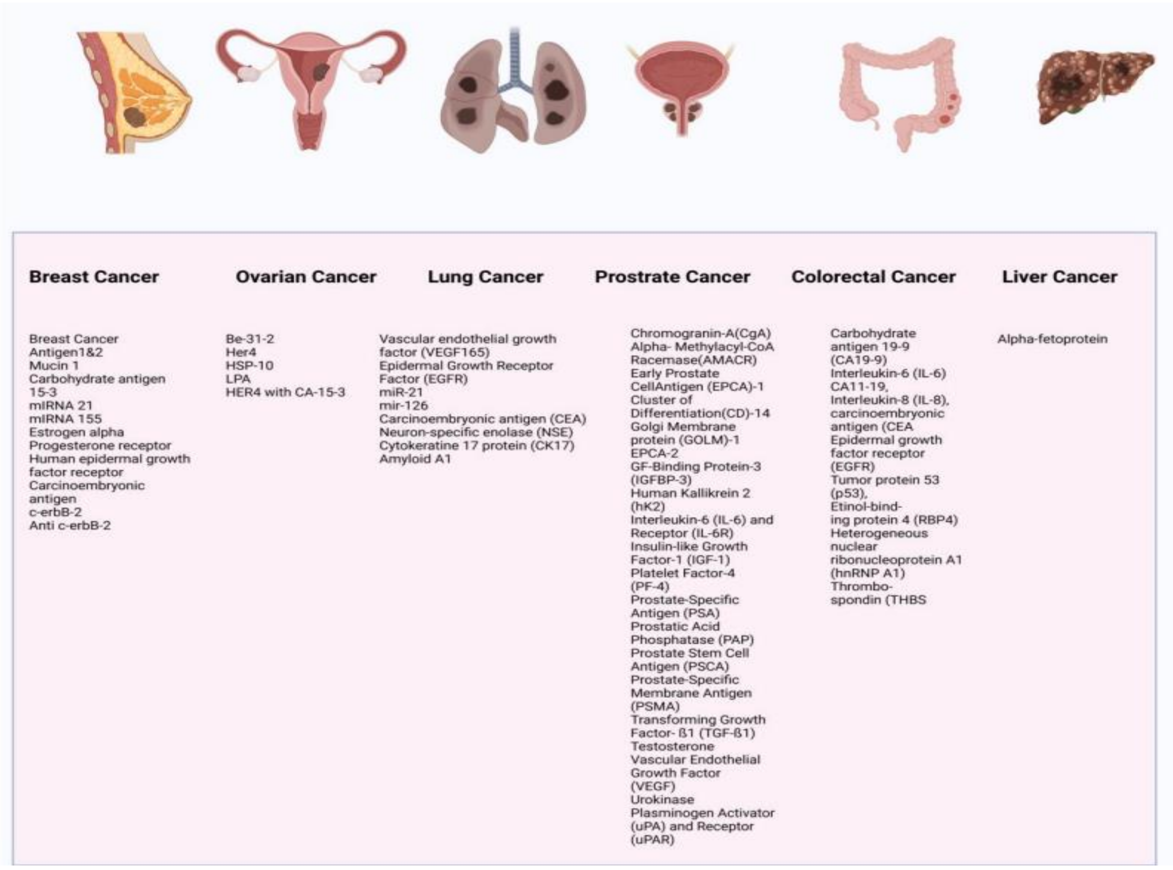
The design of microfabricated amperometric biosensors

The most common assembly of electrodes for electrochemical applications is the three-electrode system. A three-electrode structure consists in a working electrode (WE), a reference electrode (RE) and a counter electrode (CE). The working electrode serves as the transduction element in the biochemical reaction, while the counter electrode establishes a connection to the electrolyte solution so that a current can be applied to the working electrode. This system is also beneficial because it averts the RE from pushing the current which could modify its potential. The potential is applied between the WE and the RE and the CE provides the mandatory current to sustain electrolysis at the WE.
The reference electrode —A constant RE is a vital constituent of an integrated electrochemical device and is a requirement for reaching reliable analytical performance. The significant benefits of using silver/silver chloride (Ag/AgCl) as RE are the needed z electrochemical features and method compatibility. The Ag/AgCl RE principle is founded on significant exchange current density reaction, which means that at low current densities the RE is not polarized and the potential at the electrode solution interface is related just to the Cl. One known reason that limits the stability of the Ag/AgCl REs is the high solubility of AgCl. This issue can become critical for microelectrodes where RE consists only in a few hundred nanometres of AgCl. The leaking of Ag^+ into the rest of the analyte solution has been known to interfere with the measurements. In miniaturized devices it has been introduced the silver wire/ink pseudo reference electrode, which apparently wins territory due to the formation of the natural oxide layer on the silver wire/ink, eliminating the leaking and solubility issues.
The working electrode —The working electrode plays a crucial role in the design of the electrochemical sensors. The working potential window of the metal layer is limited by the potential required for oxidation of the metal, which leads to an upper positive potential limit, while on the negative potential range the limitation occurs with the onset of the hydrolysis process. The working potential window of the electrochemical sensor can be affected by the surface properties of the WE, such as the surface roughness or smoothness. The selection of an appropriate substrate is an important initial step for many studies of electrochemically active materials. The electrochemical reactivity of the substrate is a key criterion, and the behaviour of the main substrate materials were presented. The choice of sensing materials is also an important aspect that requires attention when selecting the application.
The counter electrode (CE) —The role of the CE is to complete the electrical circuit. When a potential is applied to the WE, the current is recorded as the flow of electrons between the WE and the CE. Considering the kinetics of the redox process, the surface area of the CE is usually greater than the surface area of the WE, to prevent the influence of the opposite electrochemical reaction occurring at the CE. The material chosen as substrate for the CE must be as inert as possible, such as platinum or gold materials. Even if the platinum is a better material to play the CE role, gold CEs are mainly used in the fabrication of a three-electrode system, that has also gold for WE, due to the elimination of a fabrication mask, which is a time-consuming and expensive activity.

Conventional electrodes used for cancer biomarker detection

For the fabrication of electrochemical biosensor for cancer biomarker detection various electrodes such as indium tin oxide, glassy carbon and gold electrodes have been used. The major limitation of using the electrodes is they are costly, hard, brittle, non-disposable and is difficult to functionalize. In addition to the problems with the conventional electrodes, the requirement of very high temperatures for their processing and expertise makes them more undesirable.

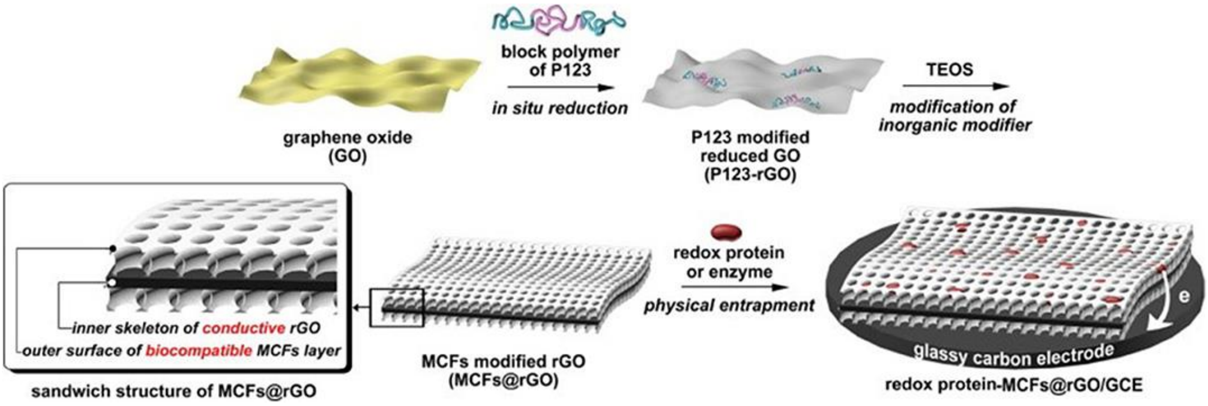
Therefore, in order to overcome these prevalent problems with the conventional electrodes, the attention has been drawn towards paper-based electrode as a potential substrate. Paper is becoming a suitable substrate for biosensing applications since it is simple, cost-effective, flexible, and are disposable. It is also beneficial in terms of cheap production, renewable raw materials and a porous structure useful for modification of paper substrate for sensing application. Conducting paper (CP) has been considered as an efficient electrochemical platform for conduction of electronic signal generate during biosensing process. For fabrication of CP both organic and inorganic materials can be used to make paper conducting. While inorganic materials often have a better electrical performance, but the drawbacks are the high material cost, processing difficulties and cracks in film during bending/sintering. However, organic materials are preferred due to the mechanical flexibility, low cost, and simple processing. In this context, carbon nanomaterial, conducting polymer and their composite is promising candidate for fabricating a CP. The advantages of paper as a sensor substrate compared to the conventional electrodes are listed.



RESULT AND DISCUSSION

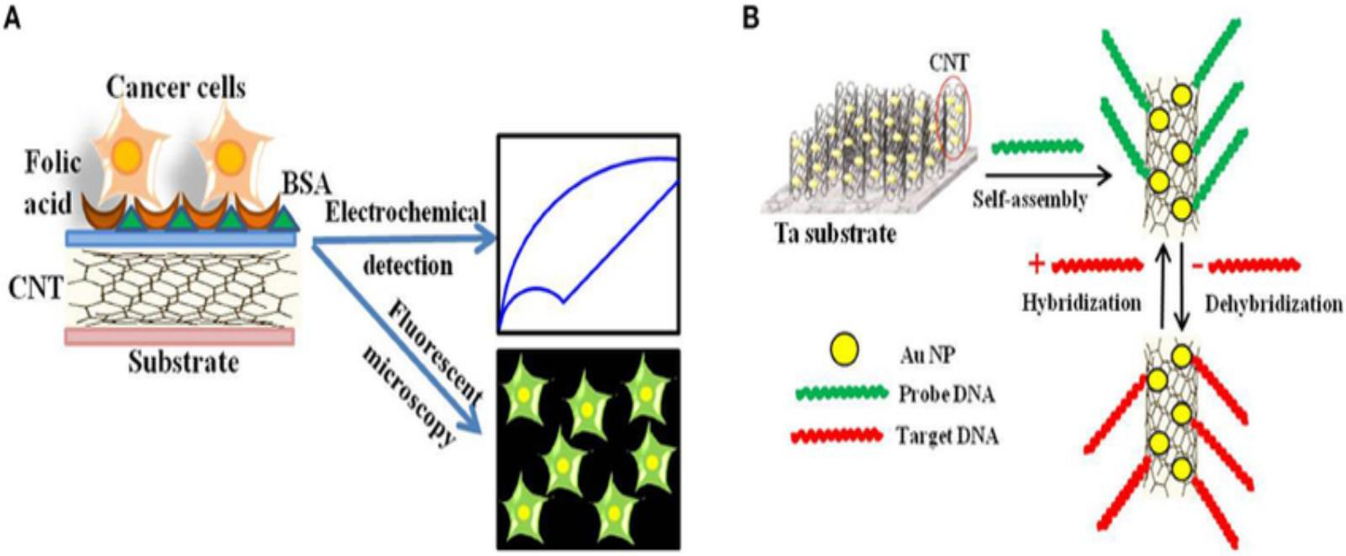
Graphene oxide integrated conducting paper for Cancer biomarker detection

Graphene is a flat monolayer of carbon atoms which are tightly packed into a two-dimensional (2D) honeycomb lattice. Graphene has received much attention due to its unique physicochemical properties such as high surface area, high mechanical strength, and ease of functionalization, excellent conductivity and mass production. Therefore, integration of graphene with conducting paper may perhaps lead to improved paper properties such as enhanced electrochemical activity, sensitivity and stability of paper sensor. Ruecha et al fabricated a paper-based cholesterol biosensor with the help of electro spraying, wax printing and screen printing using graphene/polyvinylpyrrolidone/polyaniline nanocomposite for the detection of cholesterol. The detection technique was amperometric and the fabricated electrode was found to be stable for about 2 weeks. Lu et al used graphene-gold nanoparticle for fabrication of paper based electrochemical DNA sensor by modifying paper substrate using Wax printing and screen printing. In another work Xu et al reported an electrochemiluminescence sensor by modifying paper with poly (sodium 4-styrenesulfonate) functionalized graphene composite for the detection of DNA mismatches. The electrode was fabricated with the help of photolithography and screen printing. Paper based chemiluminescence excited photoelectrochemical aptamer device for adenosine triphosphate measurement based on a gold coated paper electrode modified with a tin dioxide quantum dot/reduced graphene oxide nanocomposite has been fabricated by Wang et al. Moreover, graphene oxide (GO) has been also incorporated in paper matrix for cancer biomarker detection. The dual-signal amplification technique was used by employing graphene oxide-chitosan/gold nanoparticles(2,5-dimethoxy-1,4-phenylene)bis(ethyne-2,1-diyl) dibenzoic acid] (P-acid) functionalized nanoporous silver as signal amplification label. The immunosensor was used to detect prostate specific antigen (PSA) and carcinoembryonic antigen (CEA) using electrochemical impedance spectroscopy. The linear detection range obtained for CEA was 0.001-10ng/mL with a LOD 0.8 pg/mL. The fabricated immunosensor was also found to be stable for about 4 weeks. Li et al detect CEA on paper platform by electrochemiluminescence based method. The materials used to fabricate the electrode were gold chitosan hybrids and graphene quantum dots functionalized Au Pt. Further, Wu et al fabricated a paper based electrochemical immunodevice integrated with amplification-by-polymerization for the ultrasensitive multiplexed detection of cancer biomarkers (CEA, AFP, CA 125, CA 153). The material used in the electrode fabrication was GO and chitosan. The detection range of the fabricated sensor measured using differential pulse voltameter (DPV) was in the range, 0.01-100ng/mL [58]. The above discussed work requires wax printing, screen printing and costly conducting ink (carbon paste, silver paste etc) and hold complicated design. To overcome this problem, we have recently fabricated a paper based label free electrochemical immunosensor based on PEDOT: PSS-RGO composite for the detection of CEA by chronoamperometric technique (figure 1) [59]. PEDOT: PSS-RGO modified conducting paper electrode are prepared by simple dip coating method, wherein the conductivity is further improved from ~1.16×10-4 Scm-1 up to ~3.12×10-2 Scm-1 on treatment with ethylene glycol. The observed significant increase in electrical conductivity is due to conformational rearrangement in the polymer and is due to strong non-covalent cooperative interaction between PEDOT and the cellulose molecules. Moreover, the variation of relative conductivity of paper electrode with folding angle and folding cycle was studied. It is observed that 4% relative conductivity deviation during -180° to 180° folding angle and 20% conductivity deviation after 30 cycle of folding and unfolding (1 cycle = 360° rotation) was observed. Integration of RGO in modified paper results in improved electrochemical activity and signal stability. Further this platform is used for the immobilization of monoclonal antibody against carcinoembryonic antigen (anti-CEA). The chronoamperometric studies were performed in PBS containing 5mM [Fe (CN)6] -3/-4 at 2 V. The sensing response of PEDOT: PSS-RGO composite based paper immunoelectrode as a function of CEA concentration revealed high sensitivity of 25.8 mAng-1mLcm-2 in the physiological range of 2–8 ngmL-1 and good storage stability (21 days) for label free detection. The response of paper electrode is validated using CEA concentration of serum sample of cancer patient. This simple, low cost, flexible paper sensor can be decomposed by simple incineration and has immense potential as a smart medical diagnostic kit or point of care biosensor.



Carbon nanotube integrated conducting paper for cancer biomarker detection

Carbon nanotubes (CNTs) have been exploited for the development of electrochemical and biological sensors because of their excellent electrochemical properties, large surface area, ballistic electron transport and high mechanical strength. In addition to enhanced electrochemical reactivity, CNT-modified electrodes can be employed to immobilize biomolecules and to minimize surface fouling effects. Moreover, CNT-based electrochemical transducers offer substantial improvements in the performance of amperometric enzyme electrodes, immunosensors and nucleic-acid sensing devices. Recent studies have demonstrated that CNT can be used to enhance the electrochemical reactivity of important biomolecules and can be utilized to promote the electron-transfer reactions of proteins such as cytochrome c, ascorbic acid, xanthine oxidase, catalase, tryptophan and dopamine. Further, CNT's exceptional properties such as small size, great strength, high electrical and thermal conductivity, and large specific surface area make them excellent amplification platforms to increase the number of signal-generating molecules.



CONCLUSION
Microfabricated amperometric devices

In this report, microfabricated amperometric three-electrode system for sensors and biosensors applications were discussed both in terms of manufacturing technology and analytical performance. The main microfabrication technique used for the three-electrode configuration development was photolithography, because it provides high quality deposited films, especially in the WE area. The metal electrodes were, primarily, manufactured on glass and silicon substrates. In the development of amperometric devices, the photolithography was coupled with soft lithography or polymer moulding allowing the manufacturing of complex integrated system, containing microfluidic channels. Depending on the application fields, the analytical performances of these devices, sensitivity and selectivity were improved using strategies such as: addition of a nanostructured material, immobilization of a biological material (enzyme, antibody, DNA, etc), incorporation of a polymer or an ionic liquid, etc. Gold was the most common selected material for sensing layer or as substrate for the functionalization of the sensor, using nano- or biomaterials, due to its very low chemical reactivity. In the design of biosensors, the gold film was mainly used due to its biocompatibility and high stability. For the detection of highly electronegative heavy metals, the bismuth film was selected, displaying a more negative potential window, compared to the gold electrode. Carbon based materials have also been used as sensing layer on top of the microfabricated WE because they provide a high sensitivity due to the fast electron transfer rate. The microfabricated amperometric devices have been successfully used in several environmental applications, drug analysis and biomedical field.

Conducting paper-based biosensors

We have also discussed the prospects of CP based biosensors for cancer biomarker detection. Compared to the conventional electrode used for detection of CEA, CP based electrochemical biosensors are flexible, cost effective, light weight and can be easily disposed. The studies suggest that paper doped with MWCNT and graphene results in enhanced sensitivity, lower detection limit and wider linear detection range and have a great potential for early cancer detection. This nanomaterial modified paper-based platform should be used in the development of medical diagnostics kits, flexible electronics and energy storages devices.

FUTURE PERSPECTIVES

Although microfabricated amperometric electrodes hold much promise in the sensing and biosensing field, there are some significant technological challenges. The requirement of the clean room facilities and the use of expensive masks and custom-made optical components makes from photolithography an expensive technology for the electrode's development, more applicable for large-scale manufacturing and more complex compared to other electrodes fabrication technologies (screen printing or paste electrodes fabrication method). However, this technology provides freedom in the device design (customized design) and allows the integration of several electronic devices (nanoantenna for wireless transmission, energy-harvesting devices) in the same chip with the amperometric device for smart sensors development. In perspective, to reveal the full potential of the microfabricated integrated sensors, more attention should be paid to

i) the use of conductive polymer (such as polyaniline, polythiophene or polypyrene), as alternative to standard photolithography, enabling new designs of intelligent electrodes and insulating structures, with more controllable localized electric fields.

ii) the design of highly reliable and reproducible microfabricated electrodes which can be employed for extended periods of time; and

iii) the engineering of nanostructured materials, with high selectivity, in a resourceful way that will significantly contribute toward a clinical and environmental implementation of the biosensors.

REFERENCES

1. Review Article-Recent Progress of Biomarker Detection Sensors By Ruitao Liu, Xiongying Ye, and Tianhong Cui
2. Journal of Physics: Conference Series-Nanomaterials based biosensors for cancer biomarker detection By Bansi D Malhotra et al 2016 J. Phys.: Conf. Ser. 704 012011
3. The potential implementation of biosensors for the diagnosis of biomarkers of various cancer by Sameer Quazi
4. GenLab Biosolutions Private Limited, Bangalore, Karnataka, India. 560043
5. Department of Biomedical Sciences, School of Life Sciences, Anglia Ruskin University, United Kingdom. Corresponding Author (Colonel.quazi@gmail.com)
6. Microfabrication Techniques for Chemical/Biosensors By ANDREAS HIERLEMANN, OLIVER BRAND, CHRISTOPH HAGLEITNER, AND HENRY BALTES, FELLOW, IEEE
7. Microfabrication in Biology and Medicine By Joel Voldman, Martha L. Gray, and Martin A. Schmidt
8. Journal of The Electrochemical Society Review—Recent Advances in Microfabrication, Design and Applications of Amperometric Sensors and Biosensors

To cite this article: Angela Mihaela Baracu and Livia Alexandra Dinu Gugoasa 2021 J. Electrochem. Soc. 168 037503