The Scientific Temper
Vol. 13, No. 2, July-December, 2022:pp 217-220
ISSN 0976-8653, E-ISSN 2231-6396
A Web of Science Journal
DOI: https://doi.org/10.58414/SCIENTIFICTEMPER.13.2.2022.217-220
Doc ID: https://connectjournals.com/03960.2022.13.2.217

Review- Significant Advancements in Electrochemical Detection of Neuron-Specific Enolase

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ABSTRACT

Electrochemical technique has attracted the substantial attention for the early detection of cancer biomarkers due to its imperative properties like simplicity, high sensitivity, specificity, low cost) and point of care detection. This article has reviewed the clinically relevant electrochemical immunosensors developed so far for the analysis of neuron specific enolase (NSE), a biomarker for Small cell lung cancer. Firstly, we have different Categorized the immunoassay techniques used to monitor NSE has been discussed.NSE immunosensors are particularly, divided into three main categories (a) Sandwich assay (b) Direct detection assay and (c) indirect detection assay. The Prevailing role of nano structured materials as electrode matrices and as electroactive has been discussed. Subsequently, the key performances of various immunoassays have been critically evaluated in terms of limit of detection, linear ranges and incubation time for clinical translation. Electrochemical techniques coupled with screen printed electrodes developed market level commercialization of NSE sensors have also been discussed. Finally, the review concludes the current challenges associated to available methods and provides a future outlook towards commercialization opportunities for easy detection of NSE.

Keywords: Electrochemical technique, biomarker, cancer

INTRODUCTION

Cancer, most complex and life-threatening diseases for human beings, is arising due to uncontrolled dissection and propagation of cells and causes more than 14 million new cancer cases throughout the world Bohunicky, B., and Mousa, S. A.,(2011)Yang, M., and Gong, S.,(2010). Deaths from cancer worldwide are seen 8.2 million yearly and projected to reach over 13 million in 2030Roointan, A et.al., (2019). Lung cancer is the most frequent cancer in the world, and are responsible for highest mortality and expected to continue until 2030, unless efforts for control/ detection are greatly intensified She, J et. al., (2013). Smoking, hereditary aspects and globally increased severe environmental pollution are the main causes of lung cancer. Human lung cancer, can be grouped in two major types, small-cell lung carcinoma (SCLC) and non-small-cell lungcarcinoma (NSCLC)Lung cancer Molina, Retal, (2010).Non-small cell lung cancer includes several types of lung cancers like squamous cell carcinoma, adenocarcinoma and large cell carcinoma. However, small cell lung cancer (SCLC) is one of the major fatal cancer types,accounting for about 15-20 % in various types of lung cancer and occurs almost entirely in heavy smokers Stupp, R., Monnerat, C., Turrisi III, A. T., Perry, M. C., and Leyvraz, S., (2004).Presently, a series of methods, such as ultrasound Um, S.Wetal., (2015),magnetic resonance imaging (MRI) Deng, Y etal., (2016), positron emission tomography (PET) Everitt, S etal., (2017), bone scans Faltermeier, C. M etal., (2016), computed tomography (CT) Infante, M. etal., (2015), and biopsy Sromek, M.et., (2017)have been applied for determination of SCLC. But these devices need large instruments along with highly professional operators, menace of false positives and other damages, which can restrict the early diagnosis of SCLCKarki, K et al., (2011). SCLC can be diagnosed with greatest frequency if treatment starts early and leading to an increase in patient survival rate. Quantification of tumour markers plays acrucial role in early disease diagnosis, cancer screening and therapeutic treatment efficacy. Altered levels of tumor markers from

the physiological levels in healthy human makes the foundation for developing diagnostic tests Karley, D., and Gupta, D., (2011) Swierczewska, Metal., (2012). Moreover, Tumour markers can be measured before, during and after the treatment to evaluate the progression of treatment and outcome.

This review presents the outstanding knowledge linked with electrochemical immunosensors developed for monitoring NSE.

CATEGORIES OF DETECTION METHODS

Based on reported literatures, Overall Mechanisms adopted for determination of NSE has been categorized. There are mainly three methods used for electrochemical Quantification of NSE as illustrated below in **fig(1).**

1. **Sandwich or Labelled assay: -**In sandwich assays quantify the target antigens between by sandwiching between two layers of biorecognition antibodies. The electrical signal is transduced by labelling one of the biorecognition element(antibodies) with different type of enzymes or other nanoparticles that will be discussing later in review.
2. **Direct assay or Label Free assay: -**In direct assaysanalyte is captured by surface immobilized biorecognition element and generate the electrochemical signal based on single step binding event with NSE.
3. **Indirect assay: -** This method is based on capturing the NSE antigen using other biorecognition elements like aptamers or DNA or other protein complexes. The structural changes in biorecognition element after capturing NSE antigen generates an additional detectable element resulting in the change in electrochemical signal.

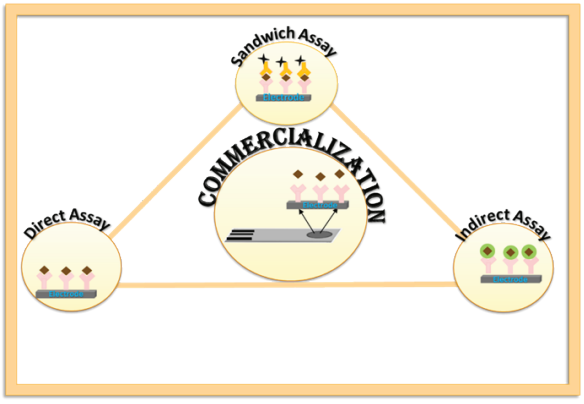

Fig1: -Schematic showing different immunoassay techniques used for electrochemical sensing of NSE and screen printed electrode showing a step forward towards commercialization.

SANDWICHOR LABELLEDELECTROCHEMICAL IMMUNOSENSORS

Commonly used sandwich technique is enzyme linked immunoassay (ELISA) typically based on the transduction of optical signal Arya, S. K., and Estrela, P., (2018). Electrochemical immunosensors are analogs to ELISA using biorecognition elements and analyte to produce signals by employing diverse electrochemical techniques. In the sandwich immunoassay secondary antibody is labelled with different enzymes such as horseradish peroxidase, alkaline phosphates (ALP) and different nanomaterials to catalyze the reduction of substrate material in the presence or absence of redox mediator for the generation of electrochemical signal. For instance,Yu, T., etal., (2012) designed an immunosensor by modifying the glassy carbon electrode with NSE covalently functionalized with SWCNT. SWCNT along with signal amplification provides numerous domains for competitive recognition of anti -NSE. Gold nano probe-Ap-Anti IgG were designed by using alkalinephosphate conjugates as labels. The AP-anti-IgG/AuNPs exhibited highly catalytic activity toward hydrolysis of alpha-naphthyl phosphate(alpha-NP), leading to a dual signal amplification of SWNTs and gold nanoprobe for detection of low-concentration target. **Scheme fig2(a).** The designed immunosensor provided a pragmatic tool for convenient detection of tumor markers in clinicaldiagnosis with the wider linear range of .**01ng/ml - 2µg/ml.** This range is the unbeatable linear range till date. Likewise, Sánchez, J. L. Aetal.,(2016)describes the introduction of disulphide linkage as anchor sites into immunoglobin structure for covalent self-assembly of antibody on to bare gold surface. Disulphide moieties were introduced via primary amines, carboxylic acid and carbohydrates present in the structure. They have compared all the strategies using SPM and concluded that carbohydrates have given the best performance in analytical response as the sugar moieties in the carbohydrates are located on the specific sites on the immunoglobin structure. Further the ability of carbohydrate strategy was investigated by EIS and DPV and of linear range and sensitivity. have shown the remarkable results. Mesoporous silica nanoparticles (MSNs) with controllable pore diameters have been used to fabricate an electrochemical immunosensor with antibodies confined to the pore channels Lin, J., Wei, Z., Zhang, H., and Shao, M., (2013). Due to poor conductivity and hydrophobicity of silica, it leads to weak electrical signals along with undesirable and poor detection limit. In this context, Wang etal., (2017)has published

DIRECT OR LABEL FREE ELECTROCHEMICAL IMMUNOSENSORS

Compared to the sandwich immunoassay, direct or label freestrategy gives pronounced results due to the direct interaction between Ab-Ag without the ease of any labelling agent as well as secondary antibody. Eradication of the secondary antibody increases the immune speed, reduces the reagent amount, decrease the false positive signals related the nonspecific bindings and simplifies the immunoassay procedure and allows in situ analysis of NSE**.** Secondly, label free technique eradicates the role of interfering agents,resolves the issue of multiple labeling, most importantly, label free technique provides the ease to measure reaction kinetics of a biological systems Tang, J., and Tang, D., (2015). Different protocols employed for label free electrochemical detection of NSE have been discussed in this section. In Zhong, Z.Y et al., (2010),fig3(a)introduced an immunosensor based on PB-SiO_2 nanocomposite using microemulsion method. Here in PB act as a redox mediator and Sio_2 provides biocompatible environment for the immobilization of antibody Also PB-SiO2 shows high catalytic activity towards H2O2. Further to improve the antibody loading APTES (3-aminopropyltriethoxy silane) was prepared by self assembling following to it Chitosan /AuNP were attached to the entire surface. The resulting **CS-nanoAu/ APTES/PB-SiO2**basedimmunosensor showed remarkable sensitivity with the linear range of **0.25–5.0 and 5.0–75 ng/mL** and limit of detection .08ng/ml. along with the longer life time of 20 days. Although the immunosensor have showed the longer life time,yet the sensitivity of purposed immunosensor was needed to be improved. In this context,Jing Han et al.(2011) investigated, a novel label free electrochemical immunosensor based on nickel hexacyanoferratenano particles (NiHCFNP) assembly over Goldnano sheets (AuNC) and in the presence of DA. And further coating of AuNP functionalized Graphene nano sheets over NiHCFNP /AuNC film increased the Anti NSE loading andenhanced the electrocatalytic activity of NiHCFNP towards the electrochemical catalysis of DA was due to large surface area and high conductivity of AuNP.The purposed immunosensor **Au–Gra/NiHCFNPs/AuNCs/GCE** exhibit the linearrange of **.001 -100ng /ml with the lowest detection limit .3pg.**Fu, X., Huang, R., Wang, J., and Feng, X., (2013)Highlighted the role of inorganic Pt nano flowers as labelsfor highly efficient enzyme free electrochemical immunoassay for NSE.Li, G.-Z., and Tian, F., (2013) reported an enzyme free strategy bio electrocatalytic reaction of most oxidizable base guanine on nanostructured graphene in the presence of $Ru(ppy)^{3+}$.They aimed at developingan insitu amplified immunoassay without the participation of enzymes. Also,this methodology has avoided the use of two working electrodes or multiple enzymes for signal amplification and made the system with an unbeatable shorter incubation time of **25 minutes.**

Summary and Future Challenges:

Various aspects of immunosensors NSE are discussed in detail. Main features and recent advances of both labeled and label free electroanalytical techniques are compared in table1.The performance of nanomaterials to enhance the analytical characteristics of immunosensor are elaborated along with the advantages and limitations of immunoassay techniques and stressing on the commercialization of Screen printed electrodes in clinical diagnosis for the future developments in this field. Although, the available electroanalytical techniques represent ideal devices for early diagnosis of NSE.There is still required to over come the challenges that restrict the immunosensing of NSE to the laboratory scale. The key issues that needs to be addressed are:

1. Many of the immunosensors attained impressive sensitivity as well as very less incubation time under optimal conditions in laboratory,but difficult to transcribe to biological samples.
2. The fabrication process is very complicated and time consuming, also the issues are with stability and reproducibility. These strategies are difficult to implement on real time analysis.
3. There is sensor to sensor variability in the properties and analytical performances of same material in different ways. which leads to the fluctuations in electrical and mechanical properties of materials and difficult to maintain the originality of the material.
4. Determining the single analyte can give false result some times because a single tumour marker is not enough to meet the strict diagnostic standard. In this context multiple antigen detection techniques should be developed to increase the diagnostic accuracy and efficiency. For NSE along with Pro GRP both give highly accurate results towards SCLCQu, Letal., (2009)

Concluding the review, we predict the bright future of NSE immunosensors based on the different immunoassay techniques. Till now, very less work has been done on NSE immunosensing in perspective commercialized purpose. By putting Constant efforts to minimize the drawbacks associated with available techniques, one can expect the extensive commercialization of immunosensor for health monitoring problems specially in remote areas.

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