Biosensors and Bioelectronics 47 (2013) 1–11

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Development of electrochemical immunosensors towards point of care diagnostics

Ying Wan [a], Yan Su [a,*], Xinhua Zhu [a,**], Gang Liu [b], Chunhai Fan [c]

[a] School of Mechanical Engineering, Nanjing University of Science and Technology, Nanjing 210094, China
[b] Shanghai Institute of Measurement and Testing Technology, Shanghai 201203, China
[c] Laboratory of Physical Biology, Shanghai Institute of Applied Physics, Chinese Academy of Sciences, Shanghai 201800, China

ARTICLE INFO

Article history:
Received 17 January 2013
Received in revised form
27 February 2013
Accepted 28 February 2013
Available online 14 March 2013

Keywords:
Electrochemical immunosensor (EI)
Point of care (POC)
Oriented immobilization
Nanotechnology
Multiplexing analysis
Integration

ABSTRACT

Electrochemical immunosensors (EI) has attracted numerous interests due to its inherent benefits over the other transduction schemes, such as a high sensitivity, ease of use, a possible automation and integration in compact analytical devices, mostly cheap and relatively simple technology of its production. Thus, EIs have great potential in point of care (POC) diagnostics for early detection of diseases. During last decades, numerous efforts have been put into EIs development. Firstly, different fabrication methods and amplification strategies have been employed to achieve high sensitivity. To be pointed, nanotechnology has been involved in the fabrication and signal amplification of EIs, which present great superiority. Secondly, EI arrays have been used for multiparametric analysis. Thirdly, several attempts have been made to construct integrated systems, which showed promising applications for POC test. Several of them are commercially available for POC use. Herein, we will review briefly the recent achievements and progress in developing EIs towards POC diagnostics.

Contents

* Corresponding author. Tel.: +86 258 430 3258; fax: +86 258 431 3250.
** Corresponding author. Fax: +86 25 84313250.
E-mail addresses: suyan@njust.edu.cn, suyannjust@gmail.com (Y. Su), zhuxinhua@mail.njust.edu.cn (X. Zhu).

0956-5663/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bios.2013.02.045

1. Introduction

Rapid, sensitive and selective detection of certain protein biomarkers in early diagnosis is critical for the cancer therapy. Recently, considerable attention has been paid to point of care (POC) diagnostic which can be performed at the bedside or in the clinic. The realization of POC requires not only fast, sensitive and selective detection, but also small, cheap and integrated device. Electrochemical immunosensor (EI), with the inherent advantages of high sensitivity, low cost, low power requirement and potential of automation, has been a promising approach for POC diagnosis (Centi et al., 2009; Tothill, 2009; Vestergaard et al., 2007; Von Lode, 2005; Warsinke, 2009; Warsinke et al., 2000).

To realize POC diagnostic, there are several demands to be satisfied such as high sensitivity, multitarget detection and integration. There are two key points for the improvement of sensitivity: (1) the way to immobilize the recognition layer onto electrodes; (2) the way to amplify the electrochemical signal of the binding event between the antibody and its antigen. Considerable efforts have also been devoted towards the following two key points: (3) multiplexing analysis; (4) integration of EI devices with greater capabilities for home-health care.

Although there has been substantial progress in the development of EIs, the way to POC diagnostic is still hard and long. This review tried to trace the development of EIs and summarize the challenges for them being applied to POC diagnostics.

2. The way to immobilize the recognition layer onto electrodes

The recognition layer immobilization is a potentially important prerequisite for the fabrication of EIs. For most of the current EIs, the recognition layers are proteins (antibodies or antigens), and the transducers are made from metals (e.g., gold, platinum), semiconductor materials (e.g., indium tin oxide, Iridium oxide) or carbon materials (e.g., carbon paste, glassy carbon, graphite). Based on the physical and chemical properties of both the electrode and the protein, a number of immobilization methods have been proposed. The key problem during the immobilization is how to fully maintain the protein's conformation and activity.

2.1. Physical immobilization

Physical immobilization is based on the adsorption of proteins onto electrode surfaces via noncovalent interactions, mainly electrostatic force, ionic bonds and hydrophobic interactions. However, physical immobilization often results in random orientation and weak attachment.

To enhance the attachment of proteins with electrode surface, several strategies were developed by entrapping proteins into a polymer matrix (Dai et al., 2003). As reported by Wilson and Rauh, antibodies were immobilized in electrochemically grown iridium oxide (IrO_x) thin film matrices (Wilson and Rauh, 2004). During the electrochemical growth of iridium oxide (IrO_x) films, antibodies were physically entrapped and available for immunological binding. Though physical entrapping of the proteins can enhance the attachment, the recognition efficiency is limit due to the burying of the active sites. Thus other non-physical immobilization strategies have been developed.

2.2. Covalent immobilization

Covalent bonds are mostly formed between side-chain-exposed functional groups of proteins with suitably modified electrodes, resulting in an irreversible binding and producing a high surface coverage.

The modification of carbon electrodes can be achieved by electrochemically activating the electrode surface, resulting in carboxyl groups for the binding with amines of proteins. We have

Table 1
Comparison of different approaches for covalent immobilization.

Electrode		Functional groups in capture probe	Cross linker	References
Metal	Gold	Amine	11-Mercaptoundecanoic acid	Ahmad and Moore (2012)
	Gold	Amine	22-(3,5-bis((6-mercaptohexyl)oxy)phenyl)-3,6,9,12,15,18,21-heptaoxadocosanoic acid; 1,2-dithiolane-3-pentanoic acid	Nassef et al. (2008)
	Gold	Amine	Thiourea; thioctic acid; 3-mercaptopropionic acid	Limbut et al. (2006)
	Gold	Amine	Glutaraldehyde	Jiang et al. (2003)
	Gold	Amine	Carboxymethyldextran	Darain et al. (2005)
Semiconductor	Iridium oxide	Amine	(3-aminopropyl)triethoxysi-lane and glutaraldehyde	Salam and Tothill (2009)
	ITO	Amine	(3-glycidoxypropyl)-trimet-hoxysilane	Wei et al. (2009)
Carbon	Graphite		4-carboxymethylaniline	Wilson (2005)
	Graphite	Amine	Poly-terthiophene carboxylic acid	Corgier et al. (2005)
	Glassy carbon	Amine	Pyrrolepropylic acid	Dong et al. (2006)
	Boro-doped diamondn	Amine	o-Aminobenzoic acid	Preechaworapun et al. (2008)
	Carbon paste	Carboxylic	4-Carboxyphenyl diazonium	Hayat et al. (2011)

ITO: Indium–tin oxide.