Biosensors and Bioelectronics 34 (2012) 12–24

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

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

Recent trends in antibody based sensors

Timothy R.J. Holford, Frank Davis, Séamus P.J. Higson*

Cranfield Health, Cranfield University, Cranfield, Bedfordshire MK43 0AL, UK

ARTICLE INFO

Article history:
Received 22 August 2011
Received in revised form 6 October 2011
Accepted 13 October 2011
Available online 20 October 2011

Keywords:
Immunosensor
Point of care
Lab on a chip
Reusable
Continuous monitoring

ABSTRACT

This review details recent advances in the fields of immunosensors and closely related immunoassays in the past decade, together with a discussion of possible future trends.

Immunosensors can be classified by the way in which they transduce the signal produced upon the formation of an antibody antigen complex. Recent advancements to these methods of detection and transduction are discussed in detail, with particular focus on electrochemical, optical, piezoelectric and magnetic based sensors. The varying applications of these sensors are also discussed.

Some of the most significant advances include development of immunosensors for the continuous monitoring of analytes, point of care (PoC) devices, with lower unit costs, automation, reusability and ease of use. Immunosensor technology has advanced at a prolific rate since its conception and has grown into a diverse area of ongoing research.

1. Introduction to immunosensors

The concept of using immunological components as sensing agents was first described within an immunoassay for plasma insulin in human subjects (Yalow and Berson, 1959). The high dissociation constants (Kd) with which antibodies bind to their target antigen has continued to be exploited, with the most well-known immunoassay being the enzyme linked immunosorbent assay (ELISA) (Engvall and Perlmann, 1971). The ELISA test has since been seen as a 'gold-standard' for immunoassays for comparison against all newly developed immunoassays and immunosensors. Briefly, ELISAs involve the immobilisation of a reactant (an antibody or antigen) onto a solid surface – with enzymes being used as markers for the presence and abundance of a specific antibody-antigen (Ab/Ag) interaction (Butler, 2000).

Immunoassays make specific and sensitive measurements of target analytes by harnessing the high specificity of the Ab/Ag interaction – and this phenomena was used to develop what led to the first commercially available immunoassay – the home pregnancy test (measuring human chorionic gonadotrophin (hCG). Immunoassays for detecting hCG were first described in the 1960s, with radioimmunoassays following in the 1970s, however these lacked specificity towards hCG until the first lateral-flow immunoassay measuring the hCG-β subunit was developed which could distinguish between hCG and luteinising hormone (LH) (Vaitukaitis et al., 1972).

In the late 1980s the first pregnancy tests were made available to the public for home use, and ever since, the technology has been applied to a diverse range of uses. Other workers (Clark and Lyons, 1962) described the development of the first biosensor, which coupled the biological specificity of enzymes with an electrode and transducer. Immunosensors, by definition, also incorporate this transduction stage to link the specific Ab/Ag interaction with the signal generation. Early work on immunosensors has been reviewed (Hock, 1997) and there are more recent reviews (Cosnier, 2005; Diaz-Gonzalez et al., 2005; Rodriguez-Mozaz et al., 2006; Centi et al., 2009).

Several methods exist to transduce a signal created by the binding of antibody and analyte, each with associated advantages and disadvantages, leading to a wealth of research into fabrication of working immunosensors and immunoassays. Recent research is focussed towards point of care (PoC) systems, reusable and portable devices, miniaturisation, fabrication of more reliable platforms and the use of aptamers, molecularly imprinted polymers, nanoparticles and other relevant species. It is now possible to produce antibody fragments with a high specificity for their target analyte and for a much wider range of uses than available with naturally formed antibodies (Nassef et al., 2009; Lu et al., 2007).

The future of this area of research seems set towards developing more sensors with the characteristics described above, with particular emphasis towards PoC applications and greater capabilities for simultaneous multiple analyte analysis (multiplexing) and high-throughput screening.

1.1. Immunosensors: Present day status

The home pregnancy test was first established as a lateral flow-cell immunoassay to measure levels of hCG in urine (Vaitukaitis

* Corresponding author. Tel.: +44 1234 758300.
E-mail address: s.p.j.higson@cranfield.ac.uk (S.P.J. Higson).

0956-5663/$ – see front matter © 2012 Published by Elsevier B.V.
doi:10.1016/j.bios.2011.10.023

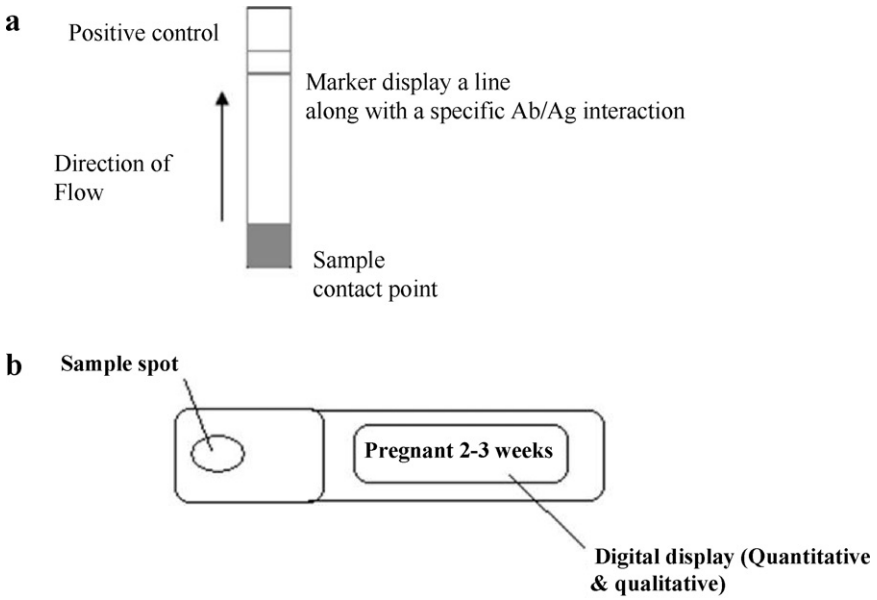

Fig. 1. (a) The lateral flow immunoassay format (marker shows if positive or negative for hCG) and (b) the present day digital readout which displays in written format whether the user is pregnant or not, and if pregnant then for how long.

et al., 1972). Lateral-flow immunoassays generally consist of an absorbent strip along which sample flows on a single axis from the sampling pad until the analyte reaches its specific antibody which is conjugated to a coloured marker (Marino et al., 2009). The target (hCG), bound to its coloured antibody continues to a capture site where further anti-hCG immobilised along a line binds the Ab/Ag complex. If hCG is present in the urine, the Ab/Ag complex will bind at the capture site, forming a visible line to indicate the presence of hCG. Urine continues to flow up the strip to a pH sensitive control area in which a second line becomes visible to indicate that the sample has progressed all the way along the test strip. The appearance therefore of two solid lines on the strip indicates a positive result, one line a negative result. Today many lateral-flow tests determining the intensity of the coloured Ab/Ag complex to generate a digital readout of binding (Johnson et al., 2011) (Fig. 1).

PoC testing is an 'on site' test, detailed within a large proportion of contemporary front-line research, with numerous devices, diverse in terms of analytical targets. However, none offer a complete set of the necessary characteristics for a good PoC sensor including full automation, portability, precision, accuracy and sensitivity, low cost and ease of use (Von Lode, 2005; Warsinke, 2009). In recent years some PoC devices have become available for the detection and monitoring of cancer development (Rusling et al., 2009; Mani et al., 2009; Yu et al., 2006). There has been a continued, sustained effort towards lowering the limits of detection on presently existing immunosensors and developing new immunoassays for other targets of clinical significance.

Non-invasiveness is another important characteristic when considering PoC sensor design, preferably samples such as urine, sweat or saliva are used. Minimising the pain associated with blood sampling, as in blood glucose detection, is possible by reducing sample size; with devices utilising samples of 0.3 μl blood now on the market (Warsinke, 2009) although when sampling for proteins, larger sample sizes of 1 μl are typically needed. Miniaturisation of devices could help produce lowered sample sizes, they operate on a smaller scale, can minimise pain upon sample collection and reduce unit costs.

2. Principles of immunosensors

2.1. Definition of immunosensors

The journal Biosensors and Bioelectronics in the abstracts of the Fifth World Confernce on Biosensors defines a biosensor as an: '*...analytical device incorporating a biological material, a biologically derived material, or a biomimic, intimately associated with or integrated within a physicochemical transducer or transducing microsystem...*'. Biosensors can incorporate many different biological sensing agents e.g. enzymes, cell receptors, nucleic acids, microorganisms and, in the case of immunosensors, antibodies or antibody fragments. Immunosensors employ the high Ab/Ag specificity to detect the presence of its analyte as shown schematically (Fig. 2).

Immunosensors can be either direct or indirect, meaning that the detection mechanism operates either directly via the Ab/Ag interaction, or a further label, such as an enzyme or fluorescent molecule, is used in order to detect whether a binding event has occurred.

2.2. Electrochemical immunosensors

Electrochemical sensors can be based on potentiometric, amperometric or impedimetric transduction principles. Inherent benefits of electrochemical sensors include selectivity, ease of use, low limits of detection and scope for miniaturisation.

2.2.1. Potentiometric immunosensors

Devices based on this principle use potential changes which are logarithmically proportional to a particular ion activity and reactants are neither destroyed nor consumed during the measurement process. Therefore no concentration gradients are formed and stir independent responses are observed, facilitating ease of use. A sensor design which transduces the potential across a membrane into a digitised signal – or field effect transistors (FETs) whose semiconductive surface potential changes upon a change in analyte concentration (Luppa et al., 2001) are examples of potentiometric sensors.