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Mini Review

Signal Transducing Methods for Immuno-sensing Devices

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Abstract

Immunosensors, which are basically integrated and miniaturized immunoassay systems, are advantageous in several ways. In addition to their high sensitivity, selectivity, simplicity, multi-analysis capability, and on line and real time detection abilities, they are both very small and relatively inexpensive instruments. The signal transducing system, a core technology in immunosensor manufacture, has been extensively studied, especially with regard to three primary categories; namely, electrochemical, optical, and mass sensitive transducers. This article addresses signal transducing technologies that have been developed in the past few years, and also discusses possible future major trends in signal transducers.

Keywords: Transducer, Electrochemical, Optical, Mass sensitive, Immunosensor

Introduction

Biosensors are analytical devices based on sensing biomolecules (microbes, enzymes, antibodies, nucleotide, and artificial receptors). The reaction of a sensing biomolecule with a target analyte results in a conversion to a detectable signal. The principal advantages of biosensing devices include their specificity, sensitivity, simplicity, short detection time, and compact integration[1]. Following the production of the first biosensor by Clark and Lyons in the 1960s[2], the technology has been increasingly employed in a variety of applications in which measurements of biological information are required. Biosensing devices have been utilized in clinical, environmental, pharmaceutical, and food safety applications, because they are capable of detecting trace amounts of target analytes, via the use of highly sensitive and selective bioreceptors.

The immunoassay method, in particular, has attracted a great deal of attention in this regard, since antibodies are capable of recognizing target analytes among structurally relevant molecules, and can be developed to operate against any specific molecule. Immunoassay methods including enzyme-linked immunosorbent assay (ELISA), Western blotting, fluoroimmunoassay, chemiluminescent immunoassay, and radioimmunoassay (RIA) techniques have been developed for the detection of target analytes. Among these methods, competitive and sandwich binding immunoassay (e.g., ELISA) are the most extensively utilized for quantitative measurements (Figure 1). Sandwich ELISA, as compared to competitive ELISA, evidences both high specificity and sensitivity, due to the use of two paired antibodies. Despite the high degree of sensitivity, selectivity, and precision possible with these immunoassay techniques, the methods also have attendant disadvantages. Firstly, analysis time with these techniques is relatively long due to the multi-step nature of the procedure. Secondly, the techniques tend to be somewhat expensive, and require qualified experts and expensive apparatus for proper application[3,4]. Therefore, the development of a novel immuno-sensing system with high sensitivity, high selectivity, low cost, multi-analysis capability, and high throughput is considered to be an attractive objective, and the production of such a tool would probably result in the supplantation of conventional immunoassay techniques.

Immunosensors, which are integrated and miniaturized immunoassay systems, have already been developed as alternatives to the conventional techniques. They combine the high specificity of immunoassays and the convenience of an integrated detection system. Immunosensors have many advantages over conventional immunoassay methods, most notably their simplicity, convenience, multi-analysis capability, on line and real time detection abilities, economy, and size. Due to these advantages, immunosensors are currently regarded as a promising candidate for point-of-care-tests (POCT) in clinical applications and on site monitoring in the environmental and food processing fields.

According to the signal sensing principle, immunosensors can be classified into three principal categories; electrochemical, optical, and mass sensitive. In this study, immunosensors are described in detail, with an emphasis on the signal transduction technique.

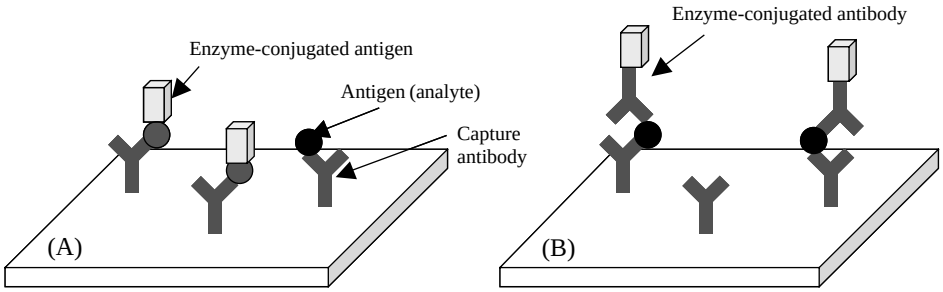

Figure 1. Schematic procedures of (A) competitive ELISA and (B) sandwich ELISA.

Electrochemical Immunosensor

Electrochemical immunosensors are capable of detecting immuno-affinity binding on electrochemical transducers, including potentiometric, amperometric, impedance, capacitive, and conductometric transducers. Potentiometric immunosensors are predicated on the measurement of surface charges or potential changes on the interface of transducers, via immunoreaction. Many researchers have developed potentiometric immunosensors for the measurement of clinical or environmental analytes[5]. However, potentiometric immunosensors evidence two serious disadvantages, in terms of non-specific binding with heterogeneous antigens, and high background signal.

Impedance immunosensors can measure directly the electrical properties of an immunoassay. The formation of an antigen-antibody complex on a conductive support alters the impedance characteristics of the interface, and impedance spectroscopy has been extensively utilized to measure the resistive or capacitive change attending this reaction[6]. Recently, Miao and Guan developed an impedance based immunosensor for α-fetoprotein by immobilizing an anti-human monoclonal α-fetoprotein IgG onto a polyaniline modified carbon electrode. Electrochemical impedance spectroscopy (EIS) confirmed that the electron transfer resistance increased with α-fetoprotein levels in the range of 200-800 ng/mL[7].

The principle of capacitive immunosensors involves measurements of changes in electrical conductivity at constant voltage, caused by ion generation or consumption upon immuno-reaction. A non-competitive capacitive immunosensor which could detect PSA was previously developed by Fernandez Sanchez *et al*.[8]. Their lateral-flow immunosensor was fabricated on an electrochemical transducer, which was coated with a pH-sensitive polymer. This immunosensor format is useful in the fabrication of a disposable immunosensor. They also described an impedance immunosensor which operated via a similar principle[9].

Amperometric immunosensors can measure the current generated via electrochemical reactions. Frequently, electrochemically active labeling is required in order to elicit the electrochemical reaction of the analyte, as the analytes usually cannot function as redox partners. The most common electrochemical labels are enzymes, most notably horseradish peroxidase (HRP) or alkaline phosphatase (AP), as they are able to catalyze the electrochemical reaction. A variety of studies have involved the development of amperometric immunosensors[10-12]. Amperometric techniques have also been employed to monitor analyte binding in the absence of a labeled compound. An antibody immobilized on a polymer-modified electrode has been also utilized with pulsed amperometric detection (PAD). The current acquired at the immunochemical/polypyrrole based electrodes occurs via the following steps: the diffusion of ions to the electrode; charge transfer at the porous polypyrrole membrane interface; migration through the polymer membrane; and finally the adsorption-desorption of the analyte at the immunochemical/polypyrrole interface with the solution. The slow rate limiting step of the adsorption -desorption process can be controlled by the selection of electrical potential[13]. PAD immunoassay techniques are utilized for analyte detection in either static or flow injection mode via the application of pulsed potentials between sensor surfaces[14].

Recently, Kerman *et al*. described a label-free electrochemical immunosensor for clinical diagnosis, which was predicated on the direct electrochemical response (current signal) of the antibody-antigen binding[15]. This work established an extraordinarily useful platform for the development of simple and effective amperometric immunosensors, without the need for labeling. In recent years, screen-printed electrodes have come to the forefront as an alternative material for the development of disposable electrochemical immunosensors. Figure 2 shows the principles of competitive electrochemical immunosensors constructed using screen-printed electrodes.

Conductometric transducers have also been utilized in the fabrication of immunosensors. Kim *et al*. investigated an immuno-chromatographic assay system

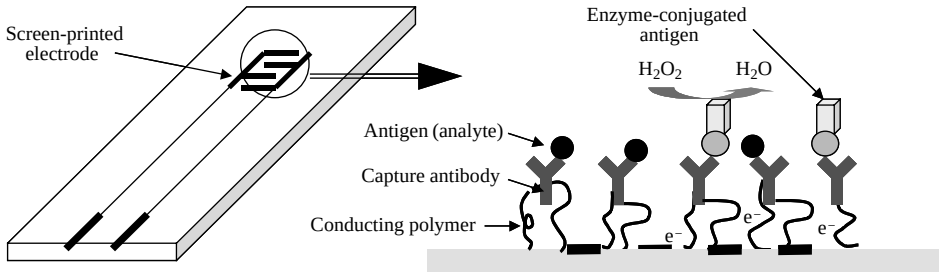

Figure 2. Principles of competitive electrochemical immunosensor using screen-printed electrode.

predicated on conductometric detection by using colloidal gold with polyaniline, as a signal generator, bound to the metal surface[16]. In order to overcome the electron transfer barrier resulting from the presence of protein, they introduced a conducting polymer (polyaniline) as a conductivity modulatory agent on the gold surface after immobilizing an antibody specific to human albumin, which was employed as the target analyte. The signal was then amplified via the conductometric technique, and the results were compared with those generated via conventional analytical methods.

Optical Immunosensor

Optical detection methods, including colorimetric, fluorescent, chemiluminescent, and surface plasmon resonance (SPR), have been applied to immunoassay systems as well, largely due to their ability to provide visible, quick, and electric noise free signal generation. The colorimetric detection technique is extensively utilized for rapid test kits in clinical diagnoses. Conventionally, color dye or gold particle tagged immunoassays have been commercially utilized for the detection of clinical analytes. Our group previously described the fabrication of a colorimetric immuno sensor based on sandwich ELISA technology and lateral flow immuno-chromatography, which was used to detect *Escherichia coli* O157 : H7. The performance of the proposed immuno sensor was evaluated in water samples. The results indicated that the lower limit of *E. coli* O157 : H7 detection was 10^3 colony forming units (CFU) per mL[17]. In clinical applications of immunosensors, a fluorescent detection system has also been widely applied in the design of immunosensors[18,19].

Chemiluminescent immunosensors are predicated on the measurement of a sensitive chemiluminescent signal generated via immunoreaction. This form of immunosensor may prove to be an effective optical immunosensing system, as it does not require a light source, making it a fairly simple instrument, and also evidences highly sensitive measurement capacity. The most common chemiluminescent analytical techniques include direct chemiluminescent tags, chemiluminescent substrate labels, and enzyme labels. In the direct chemiluminescent tag method, chemiluminescent tags (e.g., acridinium ester and acridinium sulfonamide ester) are labeled on an antibody or antigen. Folllowing immunoreaction, the labeled tag reacts with peroxide to emit light. In both methods using chemiluminescent substrates, including luminol and isoluminol, and enzymes (e.g., HRP and AP), a chemiluminescent enzyme reaction occurs, resulting in the generation of chemiluminescent products after immunoreaction. Chemiluminescent signals are proportional to the concentration of the antigen-antibody complex. The use of enzymes can amplify the signal generated in a chemiluminescent immunosensing system. Early researchers developed a chemiluminescent immunosensor using phenacyl phosphate as a chemiluminescent substrate and microtube[20]. However, this immunosensor was both time consuming and irreducibly complex. Therefore, many researchers have attempted to fabricate a more simple and better automated sensing system. Lin *et al*. described a non-competitive enzyme immunoassay which utilized flow-injection chemiluminescence[21]. Additionally, our group reported that a chemiluminescent immunosensor predicated on the sandwich ELISA technology and the lateral flow immuno-chromatography technique was fabricated in order to detect *E. coli* O157 : H7[22].

SPR-based immunosensors utilize an evanescent wave phenomenon to detect changes in the refractive index of the surface medium, as is shown in Figure 3. Over the past few years, immunosensors utilizing surface plasmon resonance (SPR) have been developed for the measurement of antigens bound to antibodies immobilized on the SPR sensor surface, which can detect analytes (antigen) in complex biological media. These immunosensors provide sensitive, label-free, real-time detection[23-26]. However, due to the fact that the concentrations of analytes in a biological system tend to be extremely low, the sensitivity of such sys-

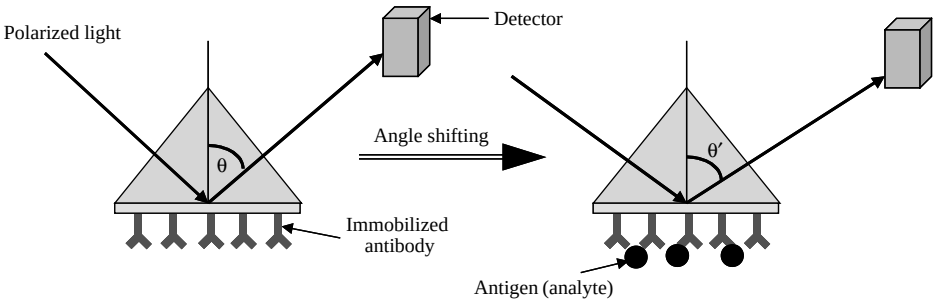

Figure 3. Label-free detection principle of surface plasmon resonance (SPR) based immunosensor.

tems must be significantly enhanced in order to reliably detect biological materials. The sensitivity of a SPR immunosensor for the detection of very low-concentration antigens can be augmented by controlling the orientation of antibodies immobilized on the SPR sensor surface. When antibodies are immobilized on a solid surface, their binding activity is generally less profound than that of free antibodies. Therefore, the immobilization method for antibodies in such systems requires also that the antibodies be immobilized in a highly oriented manner. Several techniques have been assessed for the fabrication of oriented antibody molecules on solid matrix surfaces. For instance, the Langmuir Blodgett (LB) technique and the self-assembly technique have been applied to the fabrication of protein thin films[27-29].

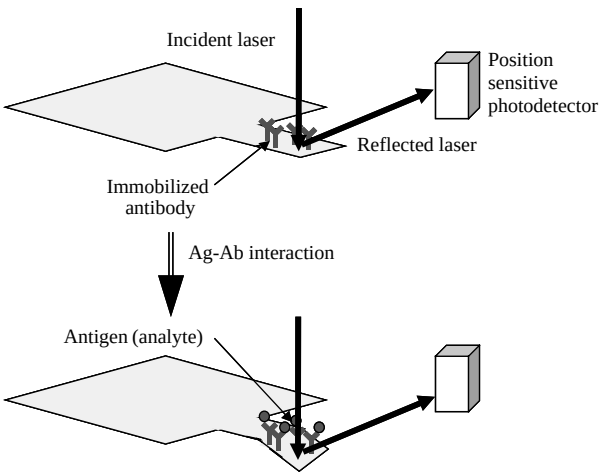

Figure 4. Principles of microcantilever immunosensor; cantilever bending caused by antigen-antibody interaction and optical read-out method for a cantilever bending.

Mass based Immunosensor

The study of mass based immunosensors has focused principally on quartz crystal microbalance (QCM) immunosensors. The QCM device is composed of a quartz crystal wafer, the thickness of which can vary, sandwiched between two metal electrodes[30-32]. Due to its simplicity, convenience, low cost, and real time detection capability, this method has proven important for biomolecule detection.

In recent years, microcantilever sensors have been introduced as a type of mass-based sensor[33,34]. Microcantilever sensors can extremely sensitively measure the molecular adsorption on a microcantilever, which results in a change in its resonant frequency, as is shown in Figure 4. When the specific binding of biomolecules occurs on the surface of the microcantilever, intermolecular nano-mechanical force induces the cantilever to bend, which allows for the measurement of changes in cantilever deflection. Microcantilever sensors are capable of detecting signals caused by the presence of a molecule on a biosensor surface, without the need for labels and reporter molecules[35-37]. Microcantilevers are typically constructed of silicon/silicon nitride or polymer materials, and their dimensions range from tens to hundreds of μm long, tens of μm wide, and hundreds of nm in thickness. This type of immunosensor can be fabricated in arrays comprising many microcantilevers, and they are therefore useful as an alternative to conventional protein chips, without the need for labeling[37].

Conclusion and Prospectus

The immunoassay method has been recognized as an important assay system, because antibodies are able to recognize the target analyte among structurally relevant molecules, and can be developed against any specific molecules. Despite the many advantages of the immunoassay technique (high sensitivity and selectivity), it has disadvantages as well including relatively long analysis time due to the multi-step procedure, relatively high cost, and the need for qualified experts and expensive apparatus for proper operation.

To overcome these disadvantages, a novel immunosensing system has been developed, which is highly sensitive, highly selective, inexpensive, high throughput, and has multi-analysis capability. Immunosensors, which are essentially integrated and miniaturized immunoassay systems, have many advantages compared to conventional immunoassay systems, namely; simplicity, convenience, multi-analysis capability, online and real time detection, miniaturization, and economy. The signal transducing system, a core immunosensor technology, has been extensively studied, specifically with regard to three major categories; electrochemical, optical, and mass sensitive transducers. In particular, label free sensing systems (e.g., SPR and QCM) have been under intensive development over the past few years. These signal transducing technologies allow for the direct measurement of biomolecular interactions, without the need for labeling.

Additionally, major trends in immunosensors include tendencies toward higher throughput, miniaturization, lower detection limits, higher integration, and more user-friendly and prosaic equipment. Nanotechnology and micro-electro mechanical systems (MEMS) technology are currently being widely applied to immunosensor research, and will eventually lead to the development of novel immunosensors with low detection limits, miniaturized and integrated instrumentation, high throughput, and simultaneous multianalyte detection capability. These trends should apply in parallel in the future development of immunosensors for clinical, environmental, and food safety monitoring applications.

Acknowledgements

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