

Immunosensors for Biomarker Detection in Autoimmune Diseases

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Received: 30 March 2016 / Accepted: 4 July 2016 / Published online: 3 September 2016
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Abstract Autoimmune diseases occur when the immune system generates proinflammatory molecules and autoantibodies that mistakenly attack their own body. Traditional diagnosis of autoimmune disease is primarily based on physician assessment combined with core laboratory tests. However, these tests are not sensitive enough to detect early molecular events, and quite often, it is too late to control these autoimmune diseases and reverse tissue damage when conventional tests show positivity for disease. It is fortunate that during the past decade, research in nanotechnology has provided enormous opportunities for the development of ultrasensitive biosensors in detecting early biomarkers with high sensitivity. Biosensors consist of a biorecognition element and a transducer which are able to facilitate an accurate detection of proinflammatory molecules, autoantibodies and other disease-causing molecules. Apparently, novel biosensors could be superior to traditional metrics in assessing the drug efficacy in clinical trials, especially when specific biomarkers are indicative of the pathogenesis of disease. Furthermore, the portability of a biosensor enables the development of point-of-care devices. In this review, various types of biomolecule sensing systems, including electrochemical, optical and mechanical sensors, and their applications and future potentials in autoimmune disease treatment were discussed.

Keywords Biosensor · Biomarker · Autoimmune diseases · Nanotechnology

Abbreviations

APS	Antiphospholipid syndrome
RA	Rheumatoid arthritis
SLE	Systemic lupus erythematosus
MS	Multiple sclerosis
SPR	Surface plasmon resonance
SS	Systemic sclerosis
IBD	Inflammatory bowel disease
ACPA	Anti-citrullinated protein antibody
MWCNT-PS	Multi-walled carbon nanotube–polystyrene
CFFSP1	Chimeric fibrin–filaggrin synthetic peptide 1
HRP	Horseradish peroxidase
ANCA	Anti-neutrophil cytoplasmic antibodies
SPCE	Screen-printed carbon electrodes
EIS	Electrochemical impedance spectroscopy
SERS	Surface-enhanced Raman spectroscopy
CV	Cyclic voltammetry
QCM	Quartz crystal microbalance

Introduction

Autoimmune diseases are caused by the loss of immunologic tolerance to self-antigens, causing the formation of autoantibodies which mistakenly attack the body's own organs, tissues and cells (Davidson and Diamond 2001). Autoimmune diseases are a significant cause of morbidity and mortality and may affect 5–10 % of the world population (Shoenfeld et al. 2012). As chronic and debilitating illnesses, autoimmune diseases have become a massive burden on patients, their families, and society with high medical costs and reduced quality of life. Autoimmune diseases are heterogeneous in that

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they may afflict specific organs or multiple organ systems in different patients.

The analysis of biomarkers, such as specific autoantibodies in clinical samples, is one of the methods applied in the detection of the diseases. Biomarkers can be objectively evaluated as indicators for early diagnosis, disease prediction, and prognosis (Maecker et al. 2012). Current methods to detect these biomarkers are not sufficiently rapid and convenient, especially for point-of-care monitoring (Kobeissy et al. 2014). To achieve the goal of point-of-care monitoring, novel technology platforms are needed for the detection of autoimmune diseases, and one of the most promising ways is the elaboration of biosensors (Mascini and Tombelli 2008). Biosensors are analytical devices harnessing the exquisite sensitivity and specificity of biology in conjunction with physicochemical transducers to deliver complex bioanalytical measurements with simple, easy-to-use formats (Turner 2013). Biosensors can be used to detect biomarkers that indicate the early stages of a disease to intervene as early as possible. For clinical trials of new drugs, authorities employ traditional methods to determine their efficacy. However, traditional tests are not specific enough to pinpoint the drug responses that are critical to treatment. The use of biosensors may provide opportunities to monitor and assess useful drug responses to facilitate governmental approval of novel drugs during clinical trials. Biosensors have great potential to be used as point-of-care devices to monitor the status of a disease, which could give physicians the ability to determine if a patient's disease is progressing into a more severe phase or help them decide if a medication needs to be modified (e.g., dosage). Besides the widely used blood glucose biosensor, other types of biosensors have also been developed to detect biomarkers in the clinical management of certain diseases, notably cancer and cardiovascular disease (Qureshi et al. 2012; Soper et al. 2006). Point-of-care biosensors for cardiac biomarkers such as troponin, myoglobin, and C-reactive protein have been commercially available (Mohammed and Desmulliez 2011). In the field of autoimmune disease, various biomarkers have been discovered during the development of biotechnologies in the past several decades. However, the studies on novel platforms for sensing autoimmune biomarkers are inadequate. On the other hand, standardized guidelines for biomarker discovery and validation are particularly important in terms of experimental design, biobanking protocols, sample size, methodology accuracy and data analysis (Gnanapavan et al. 2014; Teunissen et al. 2009). These are crucial in determining whether a biomarker has a predictive value and worth investing for further development of respective biosensors in clinical diagnostics.

In this review, various types of biosensors used in autoimmune disease detection and treatment are

summarized, and the future of the development and application of biosensors for autoimmune diseases is discussed.

Biomarkers in Autoimmune Diseases

Autoimmune diseases include various disorders due to an abnormal immune system and the generation of autoantibodies that attack one or more tissues/organs. (Suurmond and Diamond 2015). Common autoimmune diseases include systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), multiple sclerosis (MS), systemic sclerosis (SS), type 1 diabetes, inflammatory bowel disease (IBD), and antiphospholipid syndrome (APS). The cause of autoimmune disease is complicated and involves genetic and environmental factors (Costenbader et al. 2012). The heterogeneity of autoimmune diseases makes the physician assessment and key laboratory tests difficult and inaccurate. For example, it is difficult to diagnose an autoimmune disease at an early stage and to determine the risk of the disease developing into a more severe stage. However, the use of biosensors allows the detection of small concentrations of biomarkers which are present in various quantities as the disease progresses. Biomarkers can be used to diagnose, classify, and guide therapeutic decision-making for patients with autoimmune disease. An ideal biomarker should have high selectivity and sensitivity and allow for easy acquisition and analysis.

Multiple autoantibodies have been studied and found to have different specificities in individual autoimmune diseases: some are associated with multiple diseases and others are disease-specific, which allows them to serve as diagnostic biomarkers (Tan 2012). SLE is a prototype of autoimmune disease, as various autoantibodies can be detected in lupus and multi-organ systems are involved. The diagnostic criteria of SLE include a list of autoantibodies: anti-dsDNA antibodies, anti-Sm antibodies, lupus anticoagulant, and antiphospholipid, among others (Petri et al. 2012). Other autoimmune diseases are marked by different specific autoantibodies, such as anti-citrullinated protein antibody (ACPA) in RA, anti-neutrophil cytoplasmic antibodies (ANCA) in IBD and ANCA-associated vasculitides (Iskandar and Ciorba 2012; Seo and Stone 2004), anti-annexin II and V antibodies in APS and SS (Iaccarino et al. 2011), and autoantibodies against one or more pancreatic islet antigens in type 1 diabetes (Bottazzo et al. 1974). A more detailed spectrum of autoantibodies is now widely used as a diagnostic tool, and has been reviewed by others (Conrad et al. 2002, 2011).

Innate immune responses, such as the production of proinflammatory mediators, are the most common features of autoimmune diseases involved in triggering the disease

process (Mills 2011). It has been reported that first-degree relatives of SLE patients present with increased production of interferon- α , and that an increased production of proinflammatory cytokines presented in IBD patients is associated with genetic alterations in the inflammasome pathway (Hamilton et al. 2012; Niewold 2011). Several cytokines, chemokines, and adhesion molecules were found to be promising markers of autoimmune diseases, including IL-1, IL-6, IL-8, IL-12, VCAM-1, CXCL16, and angiostatin, and some of them have demonstrated superior sensitivity and specificity in preliminary studies (Hughes-Austin et al. 2013; Kunz and Ibrahim 2009; Rovin et al. 2005; Wu et al. 2007, 2013). Therefore, a sensitive and accurate detection system for these biomarkers could not only significantly aid in the early diagnosis and clinical management of autoimmune diseases, but may also provide a potential therapeutic strategy.

Biosensors as Diagnostic Tools

A biosensor is a bioanalytical device designed to convert biorecognition responses into quantifiable physicochemical signals (Mascini and Tombelli 2008). It generally integrates a biorecognition element (e.g., antigen–antibody, DNA–DNA, enzyme–substrate, receptor–ligand) with a suitable transducer. Biosensors can be categorized into either biocatalytic sensors or affinity biosensors based on the nature of the biological recognition process. The first-generation biosensors are catalytic systems that incorporate enzymes which recognize the target analyte and generate electronic signals. The most successful commercial application of a biocatalytic sensor to date is the personal blood glucose detector that utilizes glucose oxidase to selectively detect glucose in blood samples, as reviewed by others (Wang 2001). Glucose oxidase is a readily available, inexpensive, and stable enzyme from *Aspergillus niger* (Ronkainen et al. 2010). However, the number of biological analytes of interest with available corresponding enzymes that are both sufficiently selective and stable is limited. As an alternative method, affinity biosensors emerged, which take advantage of the interactions between different biorecognition elements. These interactions are mainly determined by the complementary size and shape of the binding site to the analyte of interest, such as antigen–antibody, DNA–DNA, or protein–nucleic acid binding. Affinity biosensors are very sensitive and selective due to the high affinity and specificity of the binding between a biomolecule and its ligand, which are the target analyte and the immobilized biomolecule on the transducer. Because autoantibodies are among the most important and common diagnostic biomarkers in the field of autoimmune disease, the

biorecognition processes employed in biosensors are mostly based on antigen–antibody interactions. The transducers used in biosensors can be optical, electrochemical, mechanical or magnetic. So far, the most effective biosensors are electrochemistry-based or surface plasmon resonance (SPR)-based, as illustrated in Fig. 1. A summary of collated biosensors associated with different autoimmune diseases is shown in Table 1.

Electrochemical Biosensors

The electrochemical transducer is one of the most widely used sensor technologies due to the low cost, ease of use, portability, and simplicity of fabrication (Ronkainen et al. 2010). The detection of the target analyte on electrochemical biosensors generally produces a measurable current (amperometry), a measurable charge accumulation or potential (potentiometry), or change in conductivity (conductometry/impedimetry). In such biosensors, the concentration of detecting agents, such as the secondary antibody labeled with an enzyme or other reporter that can help in electroactive species generation, does not influence the electrochemical signal that is produced. Therefore, removal of unbound detecting agent is not necessary, which significantly reduces the time of operation. A typical electrochemical biosensor is either a three-electrode (a working electrode, a reference electrode and a counter electrode) or a two-electrode (only a working electrode and a reference electrode) system. The three-electrode system has the advantage of protecting the reference electrode from changing its half-cell potential, because the charge from electrolysis passes through the counter electrode (Ronkainen et al. 2010). On the other hand, the two-electrode system is simpler and has a lower cost as a disposable sensor. The electrodes of biosensors are easily miniaturized, and various nanomaterials such as nanowires, nanoparticles, and carbon nanotubes can be incorporated into them to increase their sensitivity (Grieshaber et al. 2008). A significant advantage of using a smaller electrode, especially when working with limited clinical samples, is that the amount of sample needed for testing is reduced significantly.

Amperometry

Amperometry is a technique used to measure changes in a current generated by electrochemical oxidation or reduction while maintaining a constant potential between the working electrode and a reference electrode. In a biosensor that employs amperometry, current is proportional to the concentrations of the electroactive species in the sample, which are specific to the biorecognition element. The

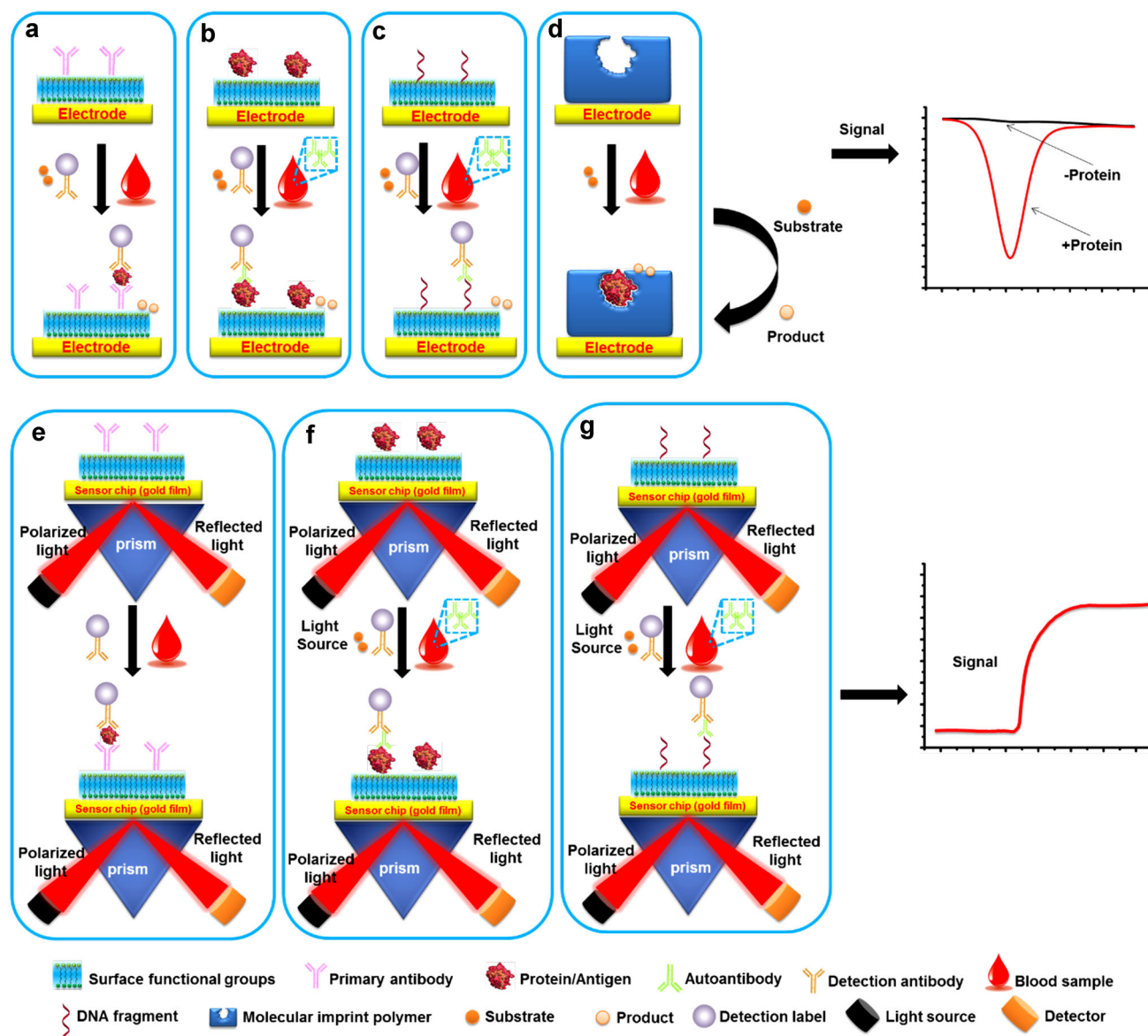


Fig. 1 Schematic representation of the platforms and principles of major biosensors including electrochemical biosensors (a–d) and surface plasmon resonance-based sensors (e–g) for biomarker detection in autoimmune diseases. These versatile biosensors are capable of detecting proteins/antigens (a, d, e) or autoantibodies including anti-protein/peptide antibodies (b, f) or anti-DNA (c, g) antibodies in the blood samples from patients with autoimmune diseases. These applicable biosensors are mostly based on antigen–antibody

interactions (a–c, e–g); however, the rapid growth of nanotechnologies has also provided enormous opportunities for the development of novel biosensors which could be based on protein–polymer interactions, such as molecular imprint which is antibody-free and label-free (d). Among these, electrochemical biosensors (a–d) have the advantages of high sensitivity and portability, whereas surface plasmon resonance-based immunosensors (e–g) have great potentials in ultrasensitivity

advantage of amperometric detection is the fixed potential leading to a minimized background signal and improved limit of detection. It has been reported that a multi-walled carbon nanotube–polystyrene (MWCNT–PS) transducer with modified electrodes has been employed for the development of an electrochemical amperometric biosensor for the diagnosis of RA (Villa et al. 2011). ACPAs are autoantibodies in the sera of RA patients specific for different citrullinated peptides and proteins such as fibrin or

filaggrin. Chimeric fibrin–filaggrin synthetic peptide 1 (CFFSP1) was used as a biorecognition element immobilized on the electrode, and amperometry was performed to detect ACPAs in human sera (Villa et al. 2011). The electrode used in this sensor was fabricated by depositing COOH-functionalized MWCNT–PS on thin gold film or platinum electrodes using standard Si/SiO₂/metal micro-electronic technology. The electrochemical properties of only MWCNT or only PS-modified electrodes were

Table 1 Biomarkers for autoimmune diseases detected by biosensors of different transduction platforms

Disease	Biomarker	Biosensor/transducer	LOD	References
Rheumatoid arthritis	ACPA	SPR immunosensor	31.25 nM	(Real-Fernández et al. 2015; Rossi et al. 2014)
	Glycosylated antibody	Electrochemical impedance sensor	3.2 nM	(Bertok et al. 2013, 2015)
	hnRNP A2/B1	SPR imagery	ND	(Beleoken et al. 2013)
	CXCL12	SPR immunosensor	5 nM	(Vega et al. 2013)
	Anti-chimeric fibrin–flaggrin citrullinated peptide	Electrochemical immunosensor	ND	(Villa et al. 2011)
Celiac disease	ACPA	QCM	ND	(Drouvalakis et al. 2008)
	Autoantibody against glucose-6-phosphate isomerase	SPR immunosensor	0.84 AU	(Cha et al. 2004; Kim et al. 2003)
	Anti-transglutaminase antibodies	Electrochemical sensor	2.7 AU	(Giannetto et al. 2014; Martín-Yerga et al. 2014; Martín-Yerga and Costa-García 2015)
	Anti-transglutaminase antibodies	Electrochemical magneto immunosensor	16.95 AU	(Kergaravat et al. 2013)
	Antibodies against deamidated gliadin peptides	Electrochemical immunosensor	46 ng/ml	(Neves et al. 2013; Rosales-Rivera et al. 2011)
Multiple sclerosis	Toxic gliadin	Electrochemical immunosensor	5.5 ng/ml	(Nassef et al. 2008, 2009)
	Genotyping HLA-DQ2/DQ8	Electrochemical sensor	ND	(Joda et al. 2014)
	Autoantibodies to tissue transglutaminase, gliadin, IgA	Electrochemical immunosensor	ND	(Joda et al. 2014; Ortiz et al. 2015)
	MMP-9	Electrochemical impedance sensor	50 ng/ml	(Biela et al. 2015)
	MBP	Electrochemical immunosensor	0.15 ng/ml	(Derkus et al. 2013)
Systemic lupus erythematosus	Glycopeptide CSF114(Glc) antibody	Electrochemical immunosensor, SPR immunosensor	ND	(Real-Fernandez et al. 2008, 2012)
	Serum IL-12	Electrochemical immunosensor	3.5 pg/ml	(Bhavsar et al. 2009; La Belle et al. 2007)
	hnRNP A2/B1	SPR imagery	ND	(Beleoken et al. 2013)
	Anti-DNA antibody	Electrochemical biosensor	10 µg/ml	(Rubin et al. 2014)
	Serum IL-6	Combination tapered fiber-optic biosensor	5 pM	(Wang et al. 2010)
Type 1 diabetes	Anti-dsDNA	SPR immunosensor	ND	(Buhl et al. 2007; Fiegel et al. 2010)
	Anti-chromatin autoantibodies	Electrochemical biosensor	ND	(Konstantinov et al. 2009)
	β2-glycoprotein I-reactive autoantibodies	SPR immunosensor	ND	(Metzger et al. 2007)
	Anti-dsDNA	QCM immunosensor	ND	(Fakhrullin et al. 2007)
	Continuous glucose monitoring	Chip-based near infrared sensor	4 mg/dl	(Ben Mohammadi et al. 2014, 2015)
Autoimmune hepatitis	Serum insulin	Amperometric electrochemical sensor	2 mM	(Castle et al. 2012; Liu et al. 2012)
	Insulin antibodies	Voltammetric immunosensor	15 pM	(Singh and Krishnan 2015)
	Non-invasive glucose monitoring	SPR immunosensor	ND	(Trabucchi et al. 2013)
	Anti-glutamic acid decarboxylase antibody	Electrochemical impedance sensor	ND	(Caduff et al. 2006; Pickup 2015)
	hnRNP A2/B1	SPR immunosensor	ND	(Choi et al. 2005)
Antiphospholipid syndrome	Antibodies to β2-glycoprotein I, prothrombin, cardiolipin	SPR imaging sensor	ND	(Beleoken et al. 2013)
	Antibodies to β2-glycoprotein I	Polarized imaging reflectometric interference spectroscopy	ND	(Bleher et al. 2014; Schindler et al. 2015)
	Antibodies to β2-glycoprotein I	SPR immunosensor	62.5 nM	(Schlichtiger et al. 2013)

ND not determined, AU Arbitrary unit, LOD limit of detection, ACPA anti-citrullinated protein/peptide antibodies, SPR surface plasmon resonance, hnRNP heterogeneous nuclear ribonucleoprotein, QCM quartz crystal microbalance, MMP-9 matrix metalloproteinase-9, MBP myelin basic protein, ds double-stranded

compared with the composite electrode, and the latter was recorded as having a well-defined reversible faradaic signal with a higher faradaic to capacitive current ratio. A sandwich immunoassay format was applied by first anchoring CFFSP1 peptide to the surface of a MWCNT–PS-modified electrode, incubating the electrodes in the sera, and finally adding secondary antibodies labeled with horseradish peroxidase (HRP) and tetramethylbenzidine as a redox mediator to generate electroactive species. A comparative study between a control serum from a blood donor and the sera from two RA patients was conducted to confirm the selectivity of the biosensor. Although the report mentioned that the detection limits were comparable to those achieved by commercial ELISA test, the limit of detection was not published (Villa et al. 2011). Also, the repeatability and reproducibility of the experiment were limited due to a small sample size in the study.

Voltammetry

In the voltammetry method, the potential is scanned over a potential range. In a voltammetry-based biosensor, current is proportional to the concentration of the electroactive species in the sample as a signal format of a peak or a plateau. Various voltammetry methods include cyclic voltammetry (CV), linear sweep voltammetry, differential pulse voltammetry, square-wave voltammetry, AC voltammetry, polarography and stripping voltammetry, among which CV is the most common. Neves et al. (2012) fabricated a disposable electrochemical immunosensor for the detection of celiac disease using CV method. Celiac disease is a gluten-induced autoimmune enteropathy with the specific presentation of tissue transglutaminase (tTG) autoantibodies in patients' serum. In this work, tTG was used as a biorecognition element and was immobilized on the transducer surface of screen-printed carbon electrodes (SPCE) nanostructured with carbon nanotubes and gold nanoparticles. The carbon–metal nanoparticle hybrid conjugation on the transducer surface was excellent for the amplification of immunological interactions. The immunoassay was performed by exposing the immobilized tTG on the surface of the transducer to the sample, followed by CV detection after the addition of alkaline phosphatase-labeled anti-human IgA or IgG antibody. The electrochemical signal was generated by the anodic redissolution of enzymatically generated silver. The results from the electrochemical immunosensor as compared to a commercial ELISA test were not quantitatively matched, but qualitatively fit (i.e., positive or negative) (Neves et al. 2012). Because diagnosis often relies more upon qualitative results, this SPCE-based disposable biosensor may be a good point-of-care diagnostic device.

Electrochemical Impedance Spectroscopy

Electrochemical impedance spectroscopy (EIS) measures both the resistive and capacitive properties of materials by applying a small sinusoidal AC potential excitation with a wide range of frequencies to an electrochemical cell, which are represented as the real (Z_{real}) and imaginary (Z_{imag}) components in a complex impedance (Z), respectively. The impedance method is powerful and capable of measuring electron transfer at high frequency and mass transfer at low frequency, and could simulate a biochemical reaction as an equivalent electrical circuit. The impedance is commonly presented in either a Bode plot or a Nyquist plot. In a Nyquist plot, the semi-circle part represents the capacitance and resistance, and the linear part represents the diffusive effect. The changes in impedance as antibodies bind to antigens are proportional to the concentration of the target analyte, which makes EIS perfect for label-free biosensors (Daniels and Pourmand 2007).

A novel, sensitive, label-free impedimetric biosensor was developed to detect anti-myelin basic protein autoantibodies in human cerebrospinal fluid and serum samples from MS patients (Derkus et al. 2013). MS is a neuro-inflammatory and neurodegenerative disease that involves the damage of myelin sheaths in the central nervous system by the immune system (such as by autoantibodies against myelin basic protein—MBP). MBP was chosen as the biorecognition element, and the transducer was fabricated by the immobilization of MBP on gelatin and gelatin–titanium dioxide (TiO_2)-modified platinum electrodes. Nanoparticles on the electrode surface increase the surface area and enhance the electron transfer rate. It turned out that these nanomaterial-modified immunosensors exhibited superior sensitivity compared to the ELISA method (Derkus et al. 2013).

The advantages of electrochemical biosensors include the simplicity of sensor setup, low cost of microelectronic circuits, easy interface for electronic readout and data processing, robustness, small volume requirement for samples/analytes and good sensitivity as exemplified in Table 1. Also, it is easy to miniaturize and multiplex for high-throughput purpose. The disadvantage of electrochemical biosensors lies in the lack of surface architectures which limits the signal-to-noise ratio, but this could potentially be addressed by utilizing nanotechnologies for favorable surface modifications.

Optical Biosensors

Optical transducers are based on the changes in the phase, amplitude, polarization, or frequency of the input light in response to the biorecognition process. Common types of optical biosensors include colorimetric, fluorescence,

luminescence, surface plasma resonance and fiber-optics-based biosensors. The biorecognition element is either labeled with chromogenic or fluorescent dyes, or is label-free. Although optical biosensors are considered to be one of the most sensitive techniques—allowing detection limit down to pg/ml (Justino et al. 2010)—the instrumentation is bulky, expensive, time-consuming and requires sophisticated personnel to conduct the tests.

Fluorescence-Based Biosensors

Fluorescence-based biosensors are widely used in the detection of biomarkers by using fluorescent probes in the form of an antibody–antigen–antibody sandwich structure. This requires that the primary antibody be immobilized onto the sensor surface to capture the target analyte in the sample, after which the secondary antibody tagged with a fluorescent probe is added to detect the analyte. Any unbound detector antibody needs to be removed from the sensor area by a washing step, which increases the operational demands and time for a single assay. Then, fluorescence is emitted by the excitation of the fluorescent probe; the detected fluorescent signal is proportional to the concentration of captured biomarkers. The selection of fluorescent probes is based on spectral output, quantum efficiency, cost, and particular requirements such as toxicity and functional linking group. The readout of fluorescence intensity comes from a fluorescence microscope or photodetector. In the work by Yoo et al. (2014), a microarray-based fluorescence immunoassay was developed using *Escherichia coli* immobilized on microplates with auto-displayed Ro proteins [Ro(+)-*E. coli*] to diagnose SLE. Ro(+)-*E. coli* was first incubated in rabbit serum to block nonspecific binding of the outer membrane by proteins other than Ro proteins, after which the F_c region from those nonspecific binding antibodies were cleaved by papain. Modified Ro(+)-*E. coli* was applied to SLE patients' sera and detected using F_c -specific anti-human IgG antibodies labeled with HRP and determined by chromogenic reaction with TMB solution. The results showed a sensitivity of 82.6 % and a selectivity of 87.5 % (Yoo et al. 2014).

SPR-Based Biosensors

SPR-based biosensors utilize changes in the refractive index or thickness of the sensor surface with the excitation of light to detect the biorecognition element. The immobilized antibodies on the metallic surface bind the target antigen, which causes a shift in the resonance curve of the reflected light; this is the basic principle behind SPR immunoassay. The characterization of insulin autoantibodies for type 1 diabetes in terms of concentration and affinity was described using an SPR biosensor (Trabucchi

et al. 2012). The commercial SPR sensor was used for protein immobilization and assays. Analyzing the affinity and concentration results from the comparative tests between childhood-onset and adult-onset diabetic patients indicated a distinct autoimmune process involved in childhood-onset and adult-onset diabetic patients. The report shows that SPR is a powerful technique for a fine description of the thermodynamic parameters of antigen–antibody interaction, although it is not eligible for routine marker screening (Trabucchi et al. 2012).

Surface-Enhanced Raman Spectroscopy-Based Biosensor

Surface-enhanced Raman spectroscopy (SERS)-based biosensors involve surface-sensitive techniques that use Raman scattering enhanced by molecules adsorbed on a rough gold or silver metal surface. With the enhancement of the Raman signal, SERS biosensors have been proven to have a detection limit of 1 pg/ml, which is more sensitive than fluorescence-based immunosensors by several orders of magnitude (Lin et al. 2008). The most commonly used sandwich format consists of primary antibodies immobilized on a sensor substrate and secondary antibodies labeled with a metallic Raman reporter molecule, which is similar to fluorescence biosensors labeled with a fluorescent probe. Unlike fluorescent probes, the labels for Raman sensors have less background signal and could enhance the signal-to-noise ratio. Macromolecular single-walled carbon nanotubes have been used as multi-color Raman labels to detect human autoantibodies against proteinase 3, a biomarker for the autoimmune disease Wegener's granulomatosis (Chen et al. 2008).

The advantages of optical biosensors include the capability of detection via real-time binding reaction for kinetic evaluation of affinity interactions and the speed of detection. The most significant disadvantages of optical biosensors include the relatively limited sensitivity, especially for label-free analytes, and the high cost of instrumentation, e.g., the price ranges \$100,000–\$300,000 for SPR instruments in the market.

Mechanical Biosensors

Transducers based on mechanical techniques are capable of detecting changes in mechanical properties, such as mass, surface stress, effective Young's modulus, and viscoelasticity in biorecognition interactions (Tamayo et al. 2013). Mechanical biosensors are not as popular as optical or electrochemical sensors due to their complexity. Among mechanical biosensors, quartz crystal microbalance (QCM) sensors are the most established ones, which are based on

quartz crystal resonators (Länge et al. 2008). The electrical signal is generated when the crystal is deformed using the piezoelectric method. There is a mass change when the analyte binds to the biorecognition element immobilized on the crystal surface. Peptide-coated single-walled carbon nanotube (SWCNT)-based QCM biosensors have been attempted in RA (Drouvalakis et al. 2008). RA-specific (cyclic citrulline-containing) peptide was used to detect corresponding autoantibodies in patient serum by fixing it to the functionalized SWCNT and deposited on a QCM sensing crystal. The results show that 34.4 % more RA patients with anti-citrullinated peptide antibodies were found by this QCM sensor than those found by ELISA.

The advantages of mechanical biosensors such as QCM include the high sensitivity and wide dynamic range of detection spanning nanomolar to femtomolar range and the flexibility to use virtually any surface coating for assays. The major disadvantage is the relatively cumbersome procedures in handling samples (converting from liquid phase to solid phase) which are susceptible to cause measurement artifacts (Arlett et al. 2011).

Future Directions

Highly sensitive assay systems for the detection of biomolecules are critical in basic research and clinical applications. Large instruments, such as sophisticated mass spectrometry, are capable of detecting various biomolecules, including proteins/peptides and the status of their post-translational modifications as well as other small molecules such as metabolites which could indicate a disease state at molecular levels. However, these large instruments are expensive and usually need specifically trained personnel to operate. Also, significant variations could easily be generated from different experimenters, different operation dates and different models of instrumentation. Obviously, it is impractical to adopt these sophisticated instruments for general clinical diagnostics, especially for the point-of-care purposes; more importantly, the feasibility and affordability of using these big platforms in the remote areas of low-resource countries could be a significant issue.

Biosensors, particularly electrical and electrochemical biosensors, could provide a new hope in the development of novel point-of-care devices and next-generation technologies for clinical diagnostics, due to the following potentials: (1) Capability of attaining ultra-sensitivity driven by the discovery and application of novel materials: the integration of novel materials such as carbon nanotubes, graphene, TiO₂, and MoS₂, among others, could significantly improve the sensitivity of the biosensors (Zhang et al. 2013; Zhou et al. 2013; Zhu et al. 2013). This is of great importance in

the detection of low or even ultralow concentrations of biomarkers at early stages in diseases such as lupus, before progression to end-organ damage occurs. (2) Versatility in the detection of various novel biomarkers: biosensors are able to detect various biomolecules including peptides/proteins and their post-translational modifications, autoantibodies, nucleic acids such as microRNAs, and even metabolites. This will tremendously expand the capability of biosensors in detecting biomarkers specific for a certain biological/physiological process at the molecular level. Traditional antibody-based assays, such as ELISA and Western blot, are totally dependent on the availability of antibodies, and the success of these assays relies largely on the quality of the antibodies, i.e., their specificity and affinity. However, the availability of high-quality antibodies has apparently become a major bottleneck for the development of antibody-based assays. In fact, commercially available antibodies may only cover fewer than 5 % of proteins in the entire proteome, which means it is impossible to detect the majority of human proteins using antibody-based assays due to the lack of antibodies. Therefore, there is a great need to find alternative technologies to tackle this challenge. Fortunately, the emerging aptamer technology shows great promise in binding and recognizing target proteins with high specificity (Skerra 2007); further development and expansion of a high-quality aptamer library will enable the development of a new generation of highly specific aptamer biosensors to detect various important biomarkers in autoimmune diseases. Recently, a novel capacitive biosensor technology that uses an electropolymerized molecular imprint (Aghaei et al. 2010; Cai et al. 2010) (Fig. 1d) is emerging, where a target molecule could be used to generate a template-specific molecular imprint in a polymer. This will allow for the detection of template molecules in body fluids obtained from patients. This structure-based technology eliminates the use of antibodies or aptamers and will give more flexibility and increased capability in recognizing various types of biomarkers in autoimmune diseases.

Collectively, biosensors are versatile and will provide enormous opportunities in the detection of various important disease biomarkers in the diagnostics and prognostics of autoimmune disease.

Acknowledgments This work is partly supported by the Lupus Research Institute and the startup fund from the University of Houston (to T. Wu).

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