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Electrochemical methods for detection of biomarkers of Chronic Obstructive Pulmonary Disease in serum and saliva

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ABSTRACT

Chronic obstructive pulmonary disease (COPD) is the fourth leading cause of death nowadays, and its underdiagnosis is still a great challenge. More effective diagnosis method is in urgent need since the traditional spirometry has many limitations in the practical application. The electrochemical (EC) detection methods have their unique advantages of high accuracy, short response time and easy integration of the system. In this review, recent works on the EC methods for COPD biomarkers including interleukin 6 (IL-6), interleukin 8 (IL-8) and C-reactive protein (CRP) are summarized. Five types of EC methods are highlighted in this study, as enzyme-labelled immunosensors, nanoparticle-labelled immunosensors, capacitive or impedimetric immunosensors, magnetoimmunosensors, and field effect transistor (FET) immunosensors. To date, EC immunosensors have been exhibiting high analytical performance with a detection limit that can achieve several pg/mL or even lower. The simplicity of EC immunosensors makes them a perfect solution for a future point-of-care device to use in settings for COPD diagnosis and follow-up. Nevertheless, more efforts need to be paid on the simultaneous detection of multiple biomarkers, a demand for the clinical diagnosis, and processes of assay simplification towards achieving one-step detection.

1. Introduction

Chronic obstructive pulmonary disease is a disease affecting millions of patients worldwide, and it is reported as the fourth leading cause of death at present, and it is estimated to be the first cause by 2030 (López-Campos et al., 2016; Mannino and Martinez, 2011; The US Burden of Disease Collaborators et al., 2018). Although COPD is mainly caused by tobacco smoking, other causes such as secondary and tertiary smoking, occupational exposure to dust and ambient air pollution are also proved to be related to COPD (Halbert et al., 2006; Postma et al., 2015). Studies showed that maternal smoking during pregnancy would increase as much risk of COPD for children as smoke when they grow up (Silva et al., 2015). Besides, biomass smoke exposure is also proved to be associated with COPD. It was found that a higher risk of developing COPD will occur if biomass exposure index is over 60 h-year for women (Silva et al., 2015).

Since COPD is a heterogeneous disease, the underdiagnosis is one of the greatest challenges for now, especially for non-smoker patients (Bednarek et al., 2008; Lamprecht et al., 2015; Murphy and Panos, 2013). It is usually not diagnosed until it is clinically apparent and moderately advanced (Fromer and Cooper, 2008). In the Spanish EPI-SCAN study, 73% of patients with non-reversible airflow obstruction were not diagnosed as COPD before the study (López-Camposet al., 2016). As for now, the most commonly used diagnosis method is spirometry, measuring the obstruction of the incompletely reversible airway which is a ratio of the forced expiratory volume in 1 s (FEV1) to forced vital capacity (FVC) (Price et al., 2009). According to the Global Initiative for Chronic Obstructive Lung Disease (GOLD), the patient is

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diagnosed as COPD if the FEV1/FVC is less than 0.7. The value of FEV1 is also used to predict the exacerbation of COPD. However, this method still has many limitations, since patients need to go to the medical center regularly, and the results need to be evaluated by health care professionals. Though spirometry in primary care becomes more readily available nowadays, only 31–37% of COPD patients have clinically documented spirometry data as reported (Damarla et al., 2006; Lee et al., 2006). What's more, the spirometry method is doubted whether it is effective for making really good decisions for COPD patients (Arne et al., 2010; Enright, 2008; O'Donnell, 2000). The normal value of FEV1/FVC changes with age. For women around 30 years old, it is abnormal already when FEV1/FVC ratio is 0.75; while for people older than 70 years old whose FEV1/FVC ratio is less than 0.7, over 30% of them are healthy (Postma et al., 2015). Many over-diagnosis cases that patients with a diagnosis of COPD have normal lung cancer were reported (Enright, 2006; Wilt et al., 2005). Thus, more accurate and convenient COPD diagnosis method is in urgent need.

COPD is a disease related to the changes in lung airway caused by the inflammatory response of macrophages (Mapel et al., 2000). The number of macrophages will significantly increase in the lung of COPD patients. Macrophages activated by smoking, for example, will secrete many inflammatory proteins, including certain cytokines, chemokines, reactive oxygen species and elastolytic enzymes (Barnes, 2004). COPD patients' levels of inflammatory proteins, as CRP (Bhavsar et al., 2015; Patel et al., 2016; Torres et al., 2006) and proinflammatory cytokines, as IL-6 and IL-8 were found to be increased (Bathoorn et al., 2009; Blicharz et al., 2009; Casaburi et al., 2013; Karadag et al., 2008). Besides, fibrinogen, matrix metalloproteinase (MMP-8 and 9), tumour necrosis factor alpha (TNF-α) and neutrophil elastase (NE) are also the biomarkers considered to be related to the state of COPD (Cao et al., 2012; Gao et al., 2013; Koutsokera et al., 2013). By detecting the change of inflammatory proteins in body fluids such as sweat, saliva, sputum and blood, an accurate diagnosis can be conducted, and the state of the COPD patients can be monitored.

IL-6 and IL-8 are two cytokines associated with the inflammation in the lungs. IL-6 is a pleiotropic cytokine which is related to growth and differentiation of cells and is an important mediator of inflammatory (Gauldie et al., 1987). It is demonstrated to be relevant to the pathogenesis of many diseases even cancers, such as rheumatoid arthritis (Hirano and Kishimoto, 1989), head and neck carcinomas (Malhotra et al., 2010), gastrointestinal (De Vita et al., 2001) and COPD (Aaron et al., 2010; Bhowmik et al., 2000; Donaldson et al., 2005; Eickmeier et al., 2010; Ji et al., 2014; Seemungal et al., 2000; Wilkinson et al., 2006). The IL-6 concentration in serum of healthy individuals is about 6 pg/mL (Hong et al., 2007). As reported, the average concentrations of IL-8 in oral cancer patients are about several thousand pg/mL but less than several hundred pg/mL in healthy subjects. CRP is an acute phase plasma protein produced in the liver, which will significantly increase in the inflammatory process. The normal level of CRP in serum can increase by several orders of magnitude (Pepys and Hirschfield, 2003). It is reported that the level of CRP can help to predict several diseases such as the coronary heart disease (Danesh, 2000), ischemic stroke (Cao et al., 2003), acute myocardial infarction (Suleiman et al., 2006) and COPD (Broekhuizen et al., 2006; de Torres et al., 2008; Dev et al., 1998; Stolz et al., 2007). It is reported that immunonephelometric or immunoturbidimetric assays are used to determine the level of CRP in the lab, which is quite expensive and time-consuming (Şişman et al., 2007).

Due to the complexity of the human body, using single biomarker cannot predict disease accurately. Diagnosis decisions based on one single biomarker usually has a high rate of misdiagnosis. The typical accuracy for a single salivary biomarker are from 0.65 to 0.85, as reported in (Li et al., 2004). However, it is still far from meeting the requirements for clinical diagnosis. Researches show that a combination of multiple biomarkers can improve the diagnosis accuracy instead of single biomarker (Agusti et al., 2016; Kozak et al., 2005; Oikonomopoulou et al., 2008). In this paper we focus on the EC detection method of CRP, IL-6 and IL-8 in serum and saliva, verifying the possibility of an accurate and portable device for COPD diagnosis which utilizes the changes of several relevant biomarkers simultaneously.

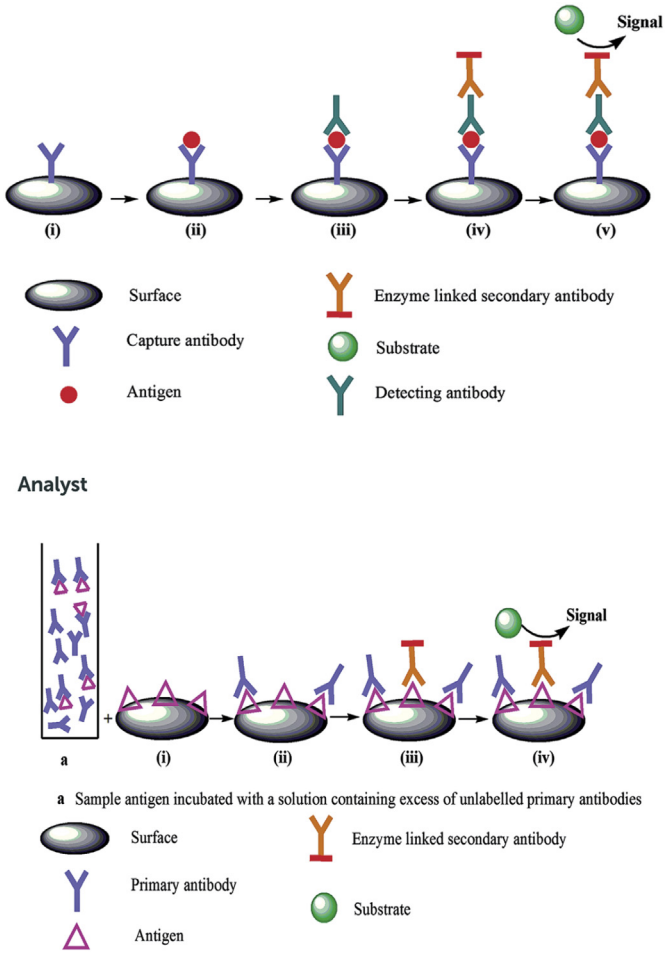

Fig. 1. Schematic representation of different ELISA formats: sandwich format (upper one), and competitive format (below one). Reprinted with permission from (Mistry et al., 2014).

2. Gold standard of biomarker detection

At present, the most commonly used method for biomarker detection is the enzyme-linked immunosorbent assay (ELISA). It is considered as a gold standard of biomarker detection and is widely used as the control group for EC detection, which can reach a detection limit of 1–3 pg/mL (Kingsmore, 2006; Lilja et al., 2008). ELISA is an approach established on the highly specific antigen-antibody reaction (usually the non-covalent binding reaction) and the efficient catalysis of the enzyme. It has two different types, the sandwich type and the competitive type, as shown in Fig. 1. For the sandwich type, the measured antigen binds to the capture antibody and the reporting antibody. It, therefore, requires that the antigens have at least two binding sites for the certain kind of antibody. The binding reaction between the immobilized capture antibodies and the target antigens cannot be monitored directly. Thus an extra labelling step is necessary, and the enzyme-labelled reporting antibodies are introduced in. The binding reaction between the enzyme-labelled reporting antibodies and the target antigens will produce a detectable response such as current and fluorescence, which is the key to show the number of target antigens. The signal detected is proportional to the concentration of the target antigen. For the competitive type, the measured target antigens

compete with the enzyme-labelled reporting antigens to bind with immobilized antibodies. Thus the signal detected is inversely proportional to the concentration of the target antigen (Kokkinos et al., 2016; Mistry et al., 2014; D. Tang et al., 2009). Sandwich ELISA is highly specific since a single antigen is captured and detected by two antibodies. This type of ELISA is more suitable for complex samples since the antigen does not require any purification before analysis. The vast majority of EC immunosensors using enzymatic labelling is based on a "sandwich" mode of operation. Appropriate labels should be chosen to amplify the detecting signal, which is usually detected by the amperometric or voltammetric method (Li et al., 2011). However, the high cost of test kits and the complexity of the signal reading system limit the popularization of this method in clinical diagnosis (Arugula and Simonian, 2014; Elshal and McCoy, 2006).

3. Electrochemical method

Lots of works focused on the immunosensors of COPD biomarkers in recent years, utilizing different methods such as surface-enhanced Raman scattering (SERS) (Kamińska et al., 2017; Rong et al., 2018; Zamora-Mendoza et al., 2019), fluorescence (Huang et al., 2018) and micro-cantilever (Ku et al., 2018). Electrochemical (EC) detecting method is a method with relatively simple structure, has its unique advantages in detecting accuracy, fabrication simplicity and rapid response time, and could be easily integrated with electronics and highly amenable to mass production (Chikkaveeraiah et al., 2012; Dong and Barbosa, 2015; Gau et al., 2001; Xiao et al., 2007), compared to electrochemiluminescence (ECL) method, which needs precise alignment of optical components and bulky and power-intensive laser sources and detectors (Honrado and Dong, 2014). Further, since the sensitivity of the ECL method follows the Lambert-Beer law, the sample volume and path length should be minimized to achieve higher sensitivity (Dong and Pires, 2017; Ricci et al., 2012).

The signal amplification method and the efficient bio-interface are two critical problems for EC detecting. An efficient bio-interface needs to be able to immobilize enough molecules on the probe surface and keep the biological activity of them at the same time (Liang et al., 2008; Nuno M M Pires et al., 2011). Graphene is used to be considered as an ideal material for the bio-interface due to its high electrocatalytic activities and unique electronic transport properties (Kang et al., 2009; L. Tang et al., 2009). However, since the graphene sheet is easy to fall off from the electrode and the loading amount of protein will be limited due to the surface coverage decrease of the electrode, the application of graphene sheet as bio-interface still have many problems (Hong et al., 2010; Tang et al., 2010). As for signal amplification, increasing detection sensitivity is the key aim. Searching for the proper label or enzyme has been the focus of many research works for labelled immunosensors. Many researches have focused on label-free EC immunosensors as well. Therefore, in this review, we divide the EC immunosensors for COPD biomarkers into enzyme-labelled immunosensors, nanoparticle-labelled immunosensors, capacitive or impedimetric immunosensors, magnetoimmunosensors (Kokkinos et al., 2016) and field effect transistor (FET) immunosensors (Farka et al., 2017). A summary of the developments in these EC sensors will be discussed below.

3.1. Enzyme-labelled immunosensors

Most of the enzyme-labelled immunosensors utilize sandwich structures for now. Binding of the antigen and antibody is turned into an electrochemical signal, which can be monitored by amperometric or voltammetric detection. The key point of enzyme-labelled immunosensors is the realization method of this conversion and signal amplification to achieve higher sensitivity. The horseradish peroxidase (HRP) enzyme is usually employed as a label to catalyze the oxidation of a mediator, with hydrogen peroxide usually. After that, the mediator is electrochemically reduced at the surface of electrodes. Different kinds of nanostructures like carbon nanotube forest are designed to close the distance between the enzyme label to the electrode.

As mentioned above, signal amplification is a critical problem for EC immunosensors. Multiple enzymes method is usually adopted to solve this problem. The multiple enzymes solution is to make the reporting antibody combine with carries (like metal nanoparticles, Au hollow microspheres, carbon nanotubes and magnetic beads) which can carry multiple enzymes. The more enzymes one carry can support, the better the signal amplification effect is, and the higher sensitivity can be achieved (Yang, 2012).

Many researchers have been working on the enzyme-labelled immunosensors for COPD related biomarkers including IL-6, IL-8 and CRP, which will be listed and summarised as follows.

3.1.1. Enzyme-labelled immunosensors for IL-6

Multiple enzymes strategy is quite commonly used to amplify the signal of IL-6 immunosensor. In (Chikkaveeraih et al., 2009), an EC sandwich immunosensor using single-wall carbon nanotube (SWNT) forests was proposed by researchers in University of Connecticut, which could detect four biomarkers in a serum sample simultaneously, including prostate specific antigen (PSA), prostate specific membrane antigen (PSMA), platelet factor-4 (PF-4), and IL-6. HRP was used as a label combined with the secondary antibodies. In order to obtain the necessary sensitivity for the detection of IL-6, the secondary antibodies bind to streptavidin-HRP conjugates provided 14–16 labels per antibody. The detection limit was 30 pg/mL as reported. The same group also compared two strategies of multi-label immunosensor for IL-6 in serum in another paper (Malhotra et al., 2010). The first one was the same strategy as described above. While the second strategy employed HRP-multiwall carbon nanotube (MWNT)-HPR as label, having 106 active HRPs per 100 nm of carboxylated carbon nanotubes as shown in Fig. 2. A 60-fold lower detection limit (0.5 pg/mL) for IL-6 was obtained with this strategy. These immunosensors prove that SWNT immunosensors utilizing multi enzymes strategy have excellent promise for IL-6 detection in clinical applications.

Besides the signal amplification problem, the stability and activity of the immobilized biomolecules is also a key factor of the immunosensor. The interface between the sensor surface and the fluid medium determines the levels of signal and noise in the electrochemical detection process. The sandwich immunoassay format and multi-enzymes strategy were adopted by Wang et al. to detect IL-6 (Wang et al., 2011). They used Au nanoparticles (AuNP)-Poly-dopamine (PDOP) as the sensor platform for immobilization of biomolecules and AuNP-PDOP@carbon nanotubes (CNT) as enzyme carrier. At pH 6.8, every milligram of AuNP-PDOP@CNT could hold about 120 μg of HRP. The linear range of this immunosensor was 4.0–800 pg/mL, and the detection limit could reach 1.0 pg/mL by the amperometry determination.

Wang et al. fabricated an IL-6 electrochemical immunosensor based on Au nanoparticles (AuNP)-graphene-silica sol-gel as bio-interface immobilized on the ITO electrode (Wang et al., 2014). HRP-AuNP-polydopamine coated carbon nanotubes (PDA@CNT) were used as the label, which is shown in Fig. 3. Since the sol-gel structure can provide a three-dimensional network, proteins entrapped in have a better activity and functionality and are much easier to store and operate than those adsorbed on a traditional two-dimensional substrate.The chip was put into an electrochemical cell to measure the current signal with hydrogen peroxide which could reach a dynamic range of 1–40 pg/mL and a detection limit of 0.3 pg/mL (at 3s).

Bernard et al. compared the performances of single-wall nanotube (SWNT) forests and glutathione-protected gold nanoparticles (GSH-AuNP) when they were applied to detect IL-6 (Munge et al., 2009). Both SWNT forests and AuNPs were fabricated on the surface of pyrolytic graphite (PG) disks. Sandwich structures were employed for both immunosensors. Multiple (Damarla et al., 2006; Enright, 2008; O'Donnell, 2000) HRP were bound to a secondary antibody used as labels. The results showed that the GSH-AuNP immunosensor could reach a

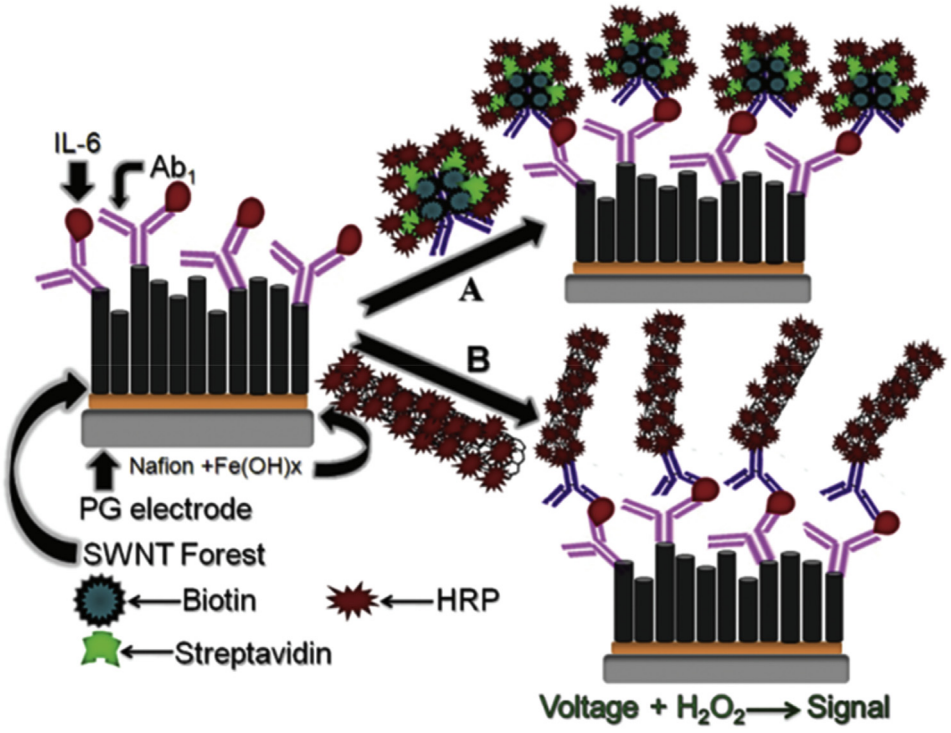

Fig. 2. Two strategies for multilabel detection in the amperometric immunosensor. Method A employed biotinstreptavidin-HRP as a label, and method B employed HRP-multiwall carbon nanotube-HPR as a label. Reprinted with permission from (Malhotra R et al. Ultrasensitive electrochemical immunosensor for oral cancer biomarker IL-6 using carbon nanotube forest electrodes and multilabel amplification[J]. Analytical chemistry, 2010, 82(8): 3118–3123.). Copyright (2010) American Chemical Society.

detection limit of 10 pg/mL in 10 μL calf serum which was three times better than the SWNT forests immunosensor. The GSH-AuNPs immunosensor also had a larger dynamic range (20–4000 pg/mL) than the SWNT forests one (40–150 pg/mL). However, the SWNT forests one shows a better sensitivity in low concentration range than the GSH-AuNP.

Jensen et al. reported a multi-enzyme sandwich EC immunosensor for IL-6 using low-cost inkjet printing Au nanoparticle electrodes arrays, which were printed on the polyimide substrate (Jensen et al., 2011). Each electrode array costed only 0.2 euro according to this paper, making it possible for clinical application. A biotinylated reporting antibody with 16–18 HRP labels was used. The detection limit was 20 pg/mL in calf serum, and the linear dynamic range was 20–400 pg/mL.

3.1.2. *Enzyme-labelled immunosensors for IL-8*

Wan et al. developed an ultrasensitive EC immunosensor for PSA and IL-8 based on sandwich format and HRP label, which is shown in Fig. 4. (Wan et al., 2011). They proposed a multi-labelled nanoprobe by loading HRP and reporting antibody onto MWNT. A 16 channel disposable screen-printed carbon electrode (SPCE) array was used as the sensor platform with three electrodes including a carbon working

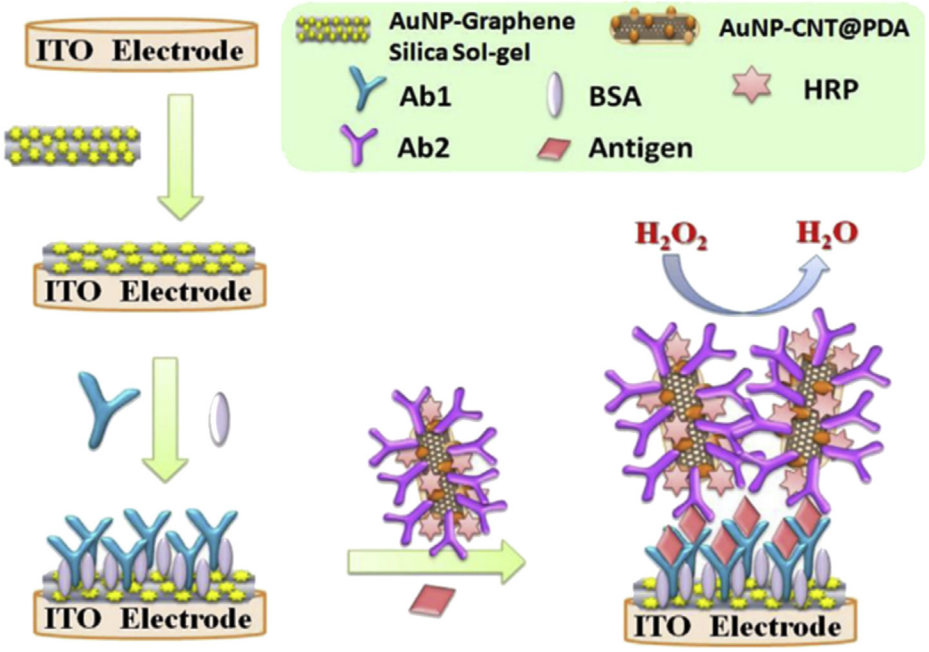

Fig. 3. Schematic view of sandwich-type electrochemical detection of IL-6 based on HRP-AuNP-polydopamine coated carbon nanotubes label. Reprinted with permission from (Wang et al., 2014).

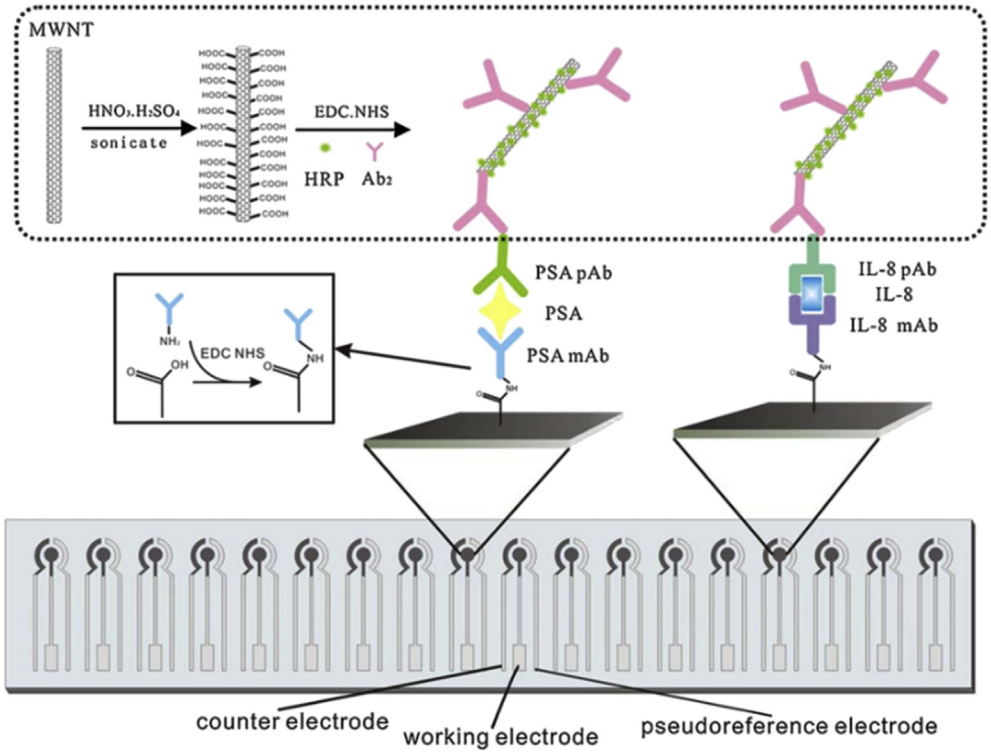

Fig. 4. Schematic demonstration of the PSA and IL-8 immunosensor. A disposable 16 channel SPCE array was employed as the sensor platform. Reprinted with permission from (Wan et al., 2011).

electrode, a carbon counter electrode and a silver pseudoreference electrode, which showed potentials in applying for the point-of-care test in clinical diagnostics. The dynamic range was 8–1000 pg/mL and the detection limit of IL-8 was 8 pg/mL.

3.1.3. Enzyme-labelled immunosensors for CRP

A disposable EC immunosensor for CRP was developed by Buch et al. (Buch and Rishpon, 2008). Screen-printed carbon electrodes were modified with MWNTs and protein A to enhance the immobilization of anti-CRP antibodies. HRP was used as label and 3,5,3',5'- tetramethylbenzidin (TMB) was added to generate electrochemical signal which was measured by amperometric detection. The detection limit of CRP in this paper is 0.5 ng/mL.

While Sakda et al. proposed an EC immunosensor for CRP employing L-cysteine/gold modified screen-printed graphene electrodes and a novel redox label (Jampasa et al., 2018). Anthraquinone (AQ) was the redox label used in their research for its stable redox-active property and small size, which was beneficial for reducing the interference between the biomolecules as reported. In the previous researches of their group, AQ was also proved to be an effective redox marker in an EC E-DNA immunosensor, in which AQ showed high sensitivity, and a detection limit in nM range was obtained (Jampasa et al., 2014). Differential pulse voltammetry (DPV) was used to detect the EC signal, with which the detection limit could reach 1.5 ng/mL.

3.1.4. Brief discussion

Enzyme-labelled immunosensors for IL-6 and IL-8 could reach a detection limit from 0.3 pg/mL to tens pg/mL, and for CRP could reach around 1 ng/mL, which are within the physiologically relevant concentration range. Also, disposable substrate like Polyimide was reported to be used. The detection limit of this type of immunosensors was improved by employing different kinds of enzymes carriers such as MWNT and AuNP-CNT. However, the higher sensitivity is obtained by using a more sophisticated enzyme carrier, which will increase the cost of each test. Besides that, the sandwich structure of the enzyme-labelled immunosensors not only increase the cost but also make the procedure more complex.

3.2. Nanoparticle-labelled immunosensors

Recently, metal NPs like Au and Ag, metal sulfide like CdS and PbS, and quantum dots (QDs) are widely used in EC immunosensors due to their unique properties including chemical stability and high surface area-to-volume ratio. Metal NPs used in EC immunosensors have several different functions. The first one is to modify the surface of the electrode, serving as the catalyst or electron wire to enhance the electron-transfer. The second one is to serve as the carry of enzyme labels. The third one is to serve as electrochemical labels (Kokkinos et al., 2016).

QDs are also commonly utilized as labels for sandwich type EC immunosensor in recent years. In QDs-based EC immunosensors, QDs labels are usually oxidized by HCl or HNO3 and then release cations which will be transferred to an electrochemical cell. Anodic stripping voltammetry (ASV) is often used for the detection of released metallic cations, which is proved to be an ultrasensitive detecting method with short response time and low cost.

3.2.1. Nanoparticle-labelled immunosensors for IL-6

Peng et al. synthesized a silver nanoparticle–hollow titanium phosphate sphere (AgNP–TiP) hybrid for a label for EC detection of IL-6, which is shown in Fig. 5. (Peng et al., 2011, p. 6). AgNPs are widely used for recognition, transportation, and catalysis of biomolecules such as proteins in these years due to their unique electronic properties (Kim and Lee, 2010). The diameter of hollow TiP spheres used in this paper was 30 nm and the average shell thickness was 40 nm. The hybrid attached to the reporting antibody showed high silver loading properties and excellent biocompatibility. DPV signal of the immunoassay was observed to show the concentration of IL-6. A magnetic sensing array combined with this novel label could reach a detection limit of 0.1 pg/mL in a linear range of 0.005–10 ng/mL.

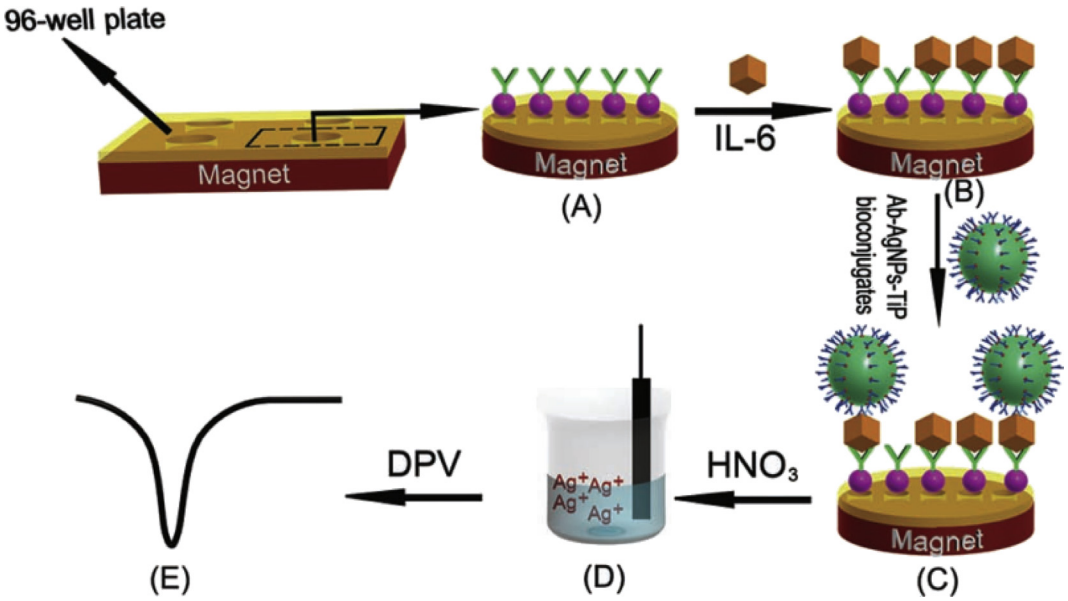

Fig. 5. Schematic illustration of electrochemical immunoassay of IL-6. A) the Magnetic array; B) IL-6 antigens captured by antibodies; C) secondary antibodies with labels captured; D) silver ions release by reaction with acid; E) DPV signal of the immunosensor. Reprinted with permission from (Peng et al., 2011, p. 6).

A competitive EC immunosensor for IL-6 with dual signal amplification was proposed by Lou et al. (2014). Electrochemically reduced graphene oxide (ERGO) and gold-palladium bimetallic nanoparticles (AuPdNPs) were used as the platform of the sensor. AgNPs were adopted as labels in this immunosensor and electrically heated carbon electrode (HCPE) technique was used to enhance the electrochemical signal. In the HCPE technique, the electrode was heated to improve mass transportation without changing the temperature of the solution. The immunosensor showed a wide linear range from 0.1 to 100000 pg/mL, and the detection limit was 0.059 pg/mL.

3.2.2. Nanoparticle-labelled immunosensors for CRP

Kokkinos et al. proposed a PbS quantum dot labelled EC immunosensor for the detection of CRP (Kokkinos et al., 2015) with bismuth citrate-modified graphite screen-printed electrodes. Screen-printed electrodes were fabricated on a polyester sheet. The CRP capture antibody was immobilized on the surface of the sensor first, binding with CRP, reporting antibody and streptavidin-conjugated PbS QDs. After the acidic dissolution of the QDs, the concentration of the target biomarker was detected by ASV detection of the released Pb(II) at the bismuth precursor-modified transducer. The linear range of this CRP immunosensor was reported to be 0.2–100 ng/mL, and the limit of detection was 0.05 ng/mL.

3.2.3. Brief discussion

Lower detection limit could be obtained by **nanoparticle-labelled immunosensor** for all three biomarkers. However, metal NPs-labelled electrochemical immunosensors have difficulties in detecting several biomarkers simultaneously due to the close formal potential values of the commonly used NPs. The main advantage of QDs immunosensor is that it can be used for detection for multi biomarkers simultaneously since the oxidation potentials vary from different QDs labels. It is worth mentioning that the metal QDs are toxic, limiting their real application in point-of-care diagnosis device.

3.3. Capacitive and impedimetric immunosensors

Unlike the EC immunosensors mentioned above, capacitive and impedimetric immunosensors are label-free immunosensors, which attract the attention of more researchers in recent years, since they are more low-cost and simple in structure. The capacitive immunosensor is actually a biochemical capacitor, usually with two parallel conductive plates and a dielectric layer. The capacitance can be described as follow:

$$C = \varepsilon\varepsilon_0 A/d \quad (1)$$

Where A is the surface area of the plates, ε is the dielectric constant of the dielectric layer, ε_0 is the permittivity of free space (8.85419 pF = m), and d is the thickness of the insulating layer (Kokkinos et al., 2016).

In a two-electrode EC capacitive immunosensors, the working electrode is considered as one of the plates of the capacitor and the electrolyte solution is considered as the other plate. When antibodies are immobilized on the surface of the electrode, the distance between the plates will increase. After the interactions between the immobilized antibodies and the target biomarker, the distance will increase more while the capacity will change correspondingly, which is the principle of the capacitive immunosensors. The capacitive immunosensors will show higher sensitivity when the target biomarker is bigger due to the larger distance between the surface of the electrodes.

For impedimetric immunosensors, due to the interaction between the target biomarker and the capture antibody, the flux of the redox probe to the surface of the electrode will decrease. Thus the impedance will increase, which is utilized as the parameter to show the concentration of the target biomarker. Electrochemical impedance spectroscopy (EIS) is a usual method to monitor the changes in impedance or capacity.

3.3.1. Capacitive and impedimetric immunosensors for IL-6

An impedimetric immunosensor constructed by depositing Au nanoparticles on single-walled carbon nanotube array was proposed by Ting et al., which is shown in Fig. 6. (Yang et al., 2013). The minimum concentration of IL-6 in serum they could detect is 0.01 fg/mL, which was much lower than the detection limit of those immunosensor utilizing Au nanoparticles or carbon nanotube alone, and was also lower than the concentration of IL-6 in a healthy individual, making this immunosensor qualified for the diagnosis of a certain disease. In their study, the single-walled carbon nanotubes (SWCNTs) array was fabricated on a wafer substrate made of SiO2/Si using EtOH-CVD method. The charge transfer resistance was measured to ensure those arrays fabricated have similar properties. The combination between the antigen and antibody was measured by EIS. When the concentration of IL-

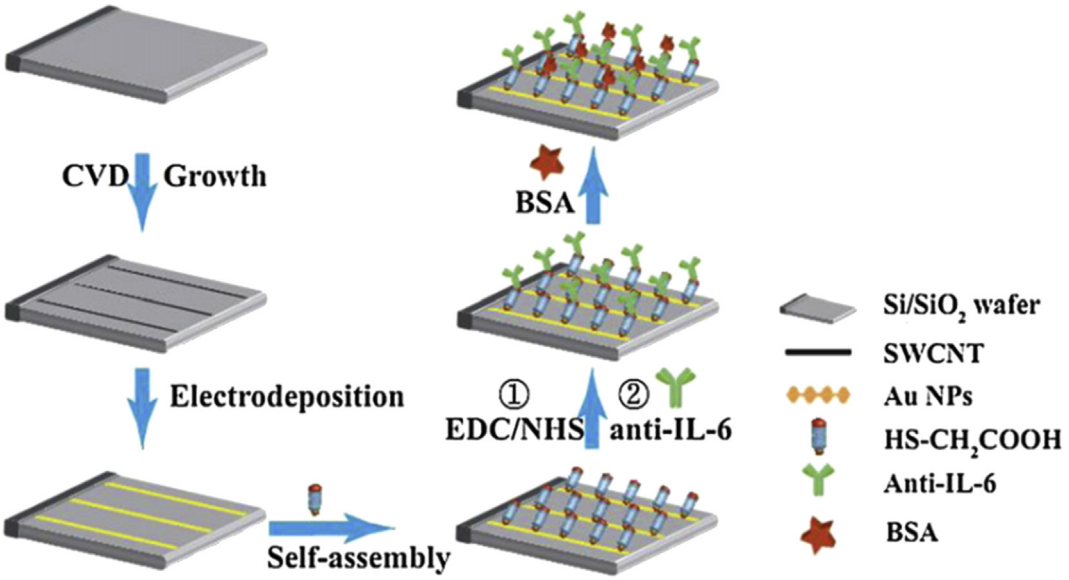

Fig. 6. Schematic illustration of the impedimetric immunosensor for IL-6 by depositing Au nanoparticles on single-walled carbon nanotube array. Reprinted with permission from (Yang et al., 2013).

6 antigen in the sample increases, the measured charge transfer resistance increases as well.

A label-free and fast response (2.5min) impedimetric immunosensor (Fig. 7.) utilizing gold multi-microelectrodes for the detection of IL-6 in serum was developed by Russell et al. (Russell et al., 2019, p. 6). Needle-shaped microelectrodes array had eight 2.5 μm-diameter microelectrodes, as shown in Fig. 1, the surface of which was functionalized by IL-6 antibodies. A decrease in impedance and an increase in DPV peak current would happen when the concentration of IL-6 increased. The detection limit of this immunosensor was reported as 20 pg/mL, and the linear range of which was 20–60 pg/mL. The sensor showed a non-linear increase from 80 to 100 pg/mL, so it might be applicable from 20–100 pg/mL.

An aptamer-based label-free impedimetric immunosensor for IL-6 detection in serum was proposed in (Tertis et al., 2019), as shown in Fig. 8. Thio-terminated IL-6 aptamers were immobilized on p-aminobenzoic acid, p-aminothiophenol and gold nanoparticles modified glassy carbon electrode. The aptamer-based immunosensor could reach a linear range from 5 pg/mL to 100 ng/mL by EIS test, and the detection limit was 1.6 pg/mL.

3.3.2. *Capacitive and impedimetric immunosensors for IL-8*

Sharma et al. reported an ultrasensitive EC impedance-based immunosensor for the detection of IL-8 in serum, as shown in Fig. 9. (Sharma et al., 2016). The non-antibody-based binding strategy was employed in this work by using small scaffold proteins, which showed excellent selectivity of the target biomarker. The Au electrode was coated with a monothiol-alkane-PEG acid SAM to enhance the selectivity. The carboxylic acid groups of the SAM were then activated with carbodiimide hydrochloride (EDC)/N-hydroxysuccinimide (NHS), binding with capture proteins. The change in the phase of the impedance from the sample baseline was measured to detect the concentration of IL-8. Short response time of 15 min was reached, which was qualified for the application for clinical diagnosis. The linear range was from 900 fg/mL to 900 ng/mL, and the detection limit was around 90 fg/mL.

An impedimetric immunosensor for the detection of IL-8 using disposable ITO electrodes was fabricated by Aydın et al. (2018). A new kind of conductive composite slurry was spin coated on the surface of the ITO electrode to achieve a better electron transfer and immobilization of antibodies, which was a mixture of star shape polymer,

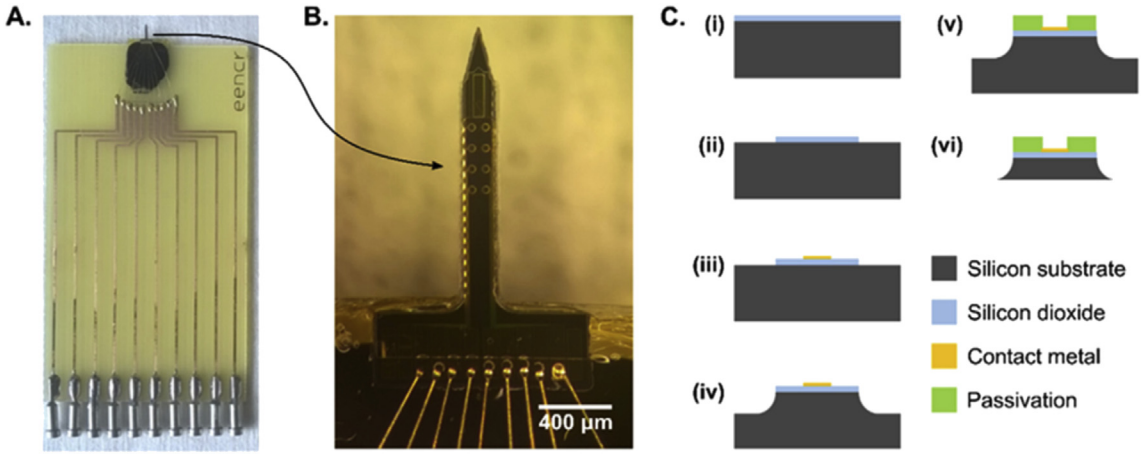

Fig. 7. (A) Image of the whole impedimetric immunosensor (B) Image of the needle-shaped electrode with eight gold electrodes on it. (C) Schematic illustration of the fabrication process of the device. Reprinted with permission from (Russell et al., 2019, p. 6). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

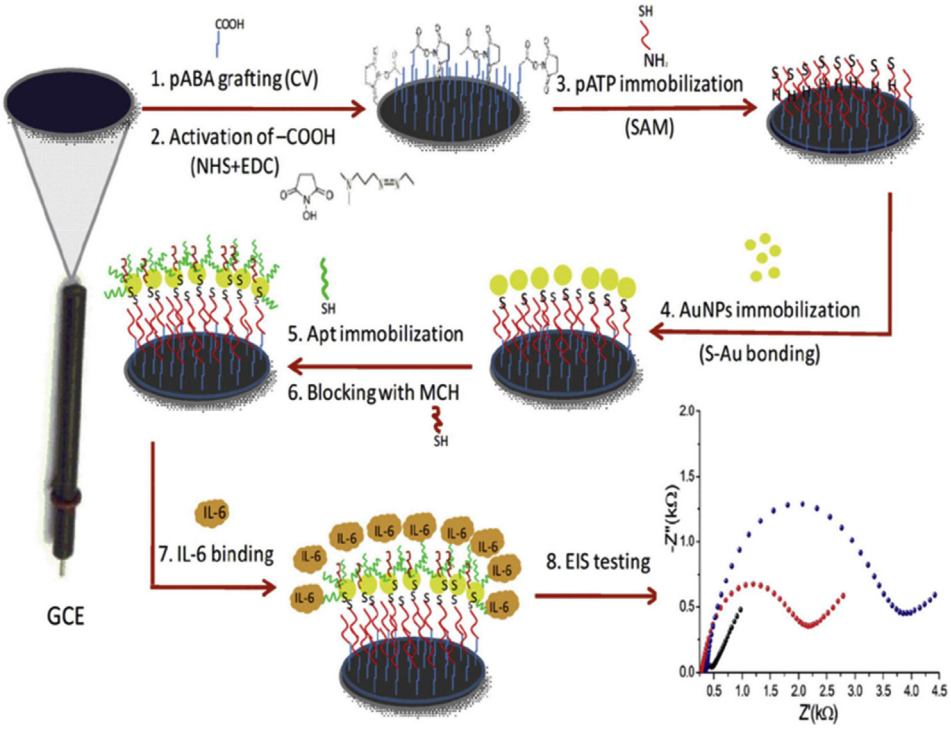

Fig. 8. Schematic illustration of the aptamer-based label-free impedimetric immunosensor. Reprinted with permission from (Tertis et al., 2019).

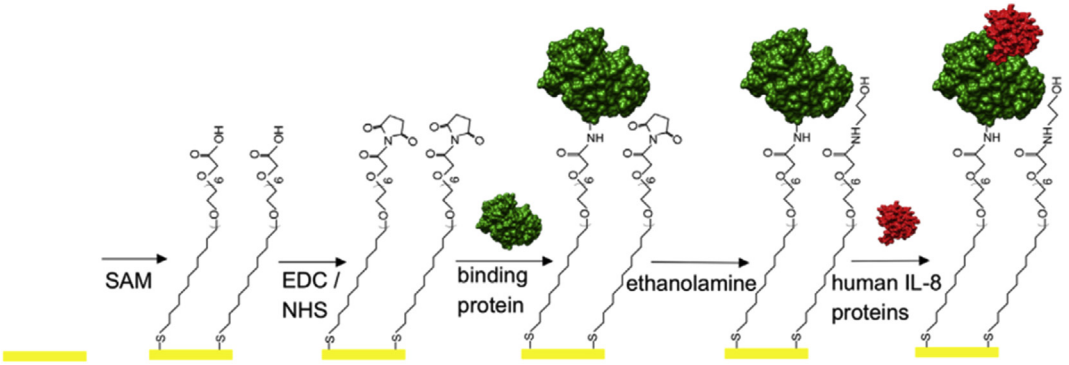

Fig. 9. Schematic illustration of the immunosensor for IL-8. Reprinted with permission from [98].

Super P carbon black conductivity agent and polyvinyldenefluoride. IL-8 antibodies were combined with star shape polymer covalently to capture the antigen. A linear range of 0.01–3 pg/mL and a detection limit of 3.3 fg/mL were reached by EIS test. Furthermore, the accuracy of this immunosensor was tested by real serum and saliva samples, which showed a compatible result with ELISA kits. The same group also reported a similar impedimetric immunosensor for IL-8 based on 6-phosphonohexanoic acid (PHA) modified ITO electrode with a linear range from 0.02 pg/mL to 3 pg/mL and a detection limit of 6 fg/mL in (Aydın and Sezgintürk, 2018).

Verma et al. reported an impedimetric label-free immunosensor for IL-8 detection in saliva based on gold nanoparticles reduced graphene oxide (AuNPs-rGO) composite film and ITO coated glass electrodes with a linear range of 500 fg/mL to 4 ng/mL and a detection limit of 72.73 ± 0.18 pg/mL (Verma et al., 2017). The IL-8 antibodies were covalenty combined with the rGO on the electrode surface. DPV was utilized to see the different concentration of IL-8 in their immunosensor.

3.3.3. Capacitive and impedimetric immunosensors for CRP

Thangamuthu et al. developed a label-free impedimetric immunosensor for CRP detection in serum by using screen-printed carbon electrode (SPE) which was modified with AuNPs and CRP antibodies and an external redox mediator Fe(CN)64- (Thangamuthu et al., 2018). A linear range of 0.047–23.6 μg/mL and a detection limit of 17 ng/mL was achieved by detecting the oxidation current of the redox indicator which was Fe3 + /Fe2 + in their immunosensor.

Also, a simple folding electrochemical impedimetric immunosensor with disposable paper-based electrode was developed by Boonyasit et al. (2019). The phosphocholine-modified screen-printed carbon electrode was proved highly sensitive to the concentration of CRP, which could achieve a linear range of 0.005–500 μg/mL with a detection limit of 0.001 μg/mL.

A capacitive immunosensor for the detection of CRP based on nanosized nickel patterned electrodes with higher durability and lower cost was proposed in (Kallempudi and Gurbuz, 2011). Image reversal techniques were employed in this work to fabricate nickel electrodes instead of etching. This work showed an excellent potential of nickel-based electrodes to replace gold based electrodes, which were commonly used in previous researches. The detection limit of CRP was reported to be 1 ng/mL.

Ha-Wook et al. also proposed a label-free capacitive immunosensor

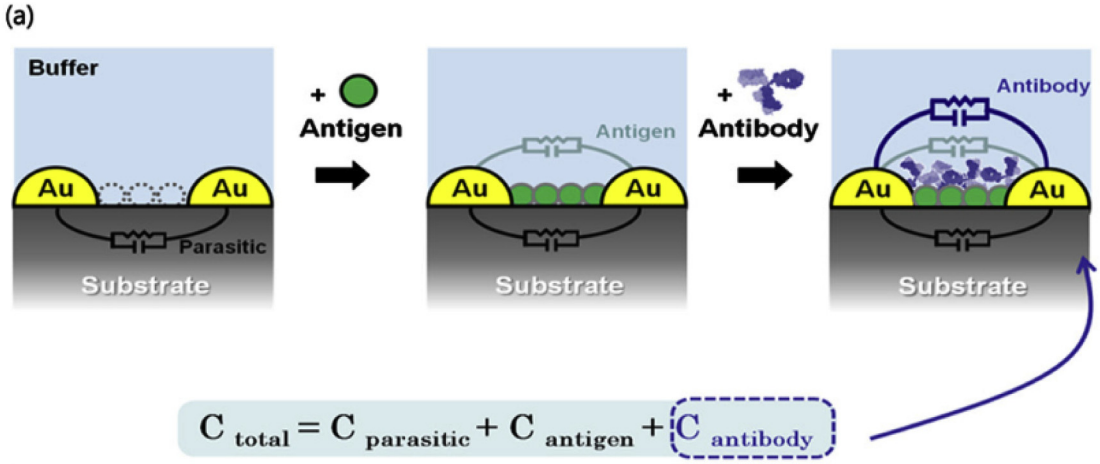

Fig. 10. The principle of the immunosensor for CRP based on the IDE with nanoislands. Reprinted with permission from (Jung et al., 2014).

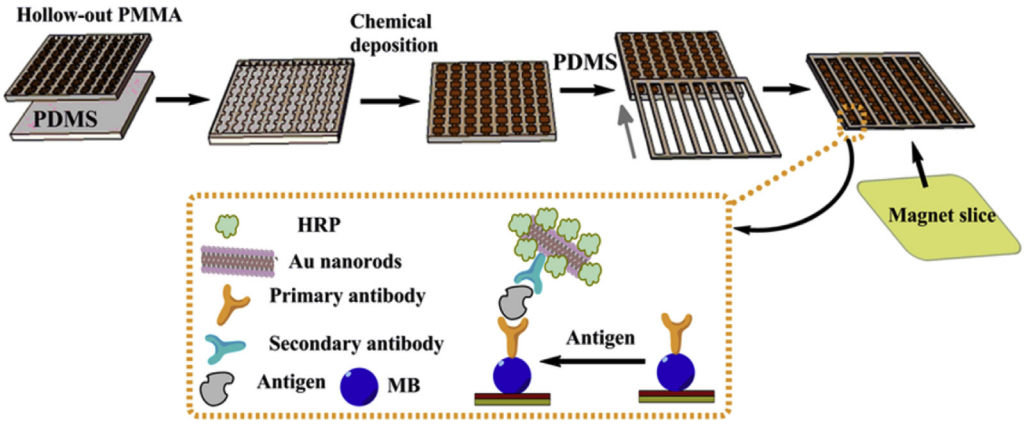

Fig. 11. The fabrication process of the PDMS microchip and the MB on the electrode holding capture antibody-target antigen-reporting antibody-multilabels. Reprinted with permission from (Liu J et al. Flexible gold electrode array for multiplexed immunoelectrochemical measurement of three protein biomarkers for prostate cancer[J]. ACS applied materials & interfaces, 2014, 6(22): 20137–20143.). Copyright (2014) American Chemical Society. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

for the detection of CRP based on an interdigitated electrode (IDE) with nanoislands, which was the guarantee of the sensitive detection of protein in this work (shown in Fig. 10.) (Jung et al., 2014). A parylene-A film on the IDE was used to ensure the stability of protein immobilization. The principle of this biosensor was the utilization of the different relative permittivity values between protein and water. The capacitance would change when the protein was adsorpted on the surface of electrodes. A more significant change would occur if the distance between the electrodes was shortened, which could be concluded from Equation (1). IDE with nanoislands was fabricated in this research to shorten the distance between the electrodes of IDE, which was proved to improve the sensitivity of the detection of capacitance.

A capacitive immunosensor based on gold interdigitated electrodes for simultaneous detection of TNFα, IL-6 and CRP was developed by Anjum et al. in (Qureshi et al., 2010). Both pure and mixture of antibodies immobilization were conducted in this paper. In pure antibodies immobilization experiment, three capacitor arrays were used with TNFα, IL-6 and CRP antibodies respectively. CRP and IL6 could be detected from 25 pg/mL to 25 ng/mL, and TNFα could be detected from 25 pg/mL to 1 ng/mL. While in the mixture of antibodies immobilization experiment, all three biomarkers could be detected from 25 pg/mL to 25 ng/mL, which showed great potential for the application of capacitive immunosensor for clinical diagnosis.

3.3.4. Brief discussion

The capacitive and impedimetric immunosensors provide a promising alternative for point-f-care diagnosis since they are label-free, low cost compared to sandwich type immunosensors and fast in detection. Since their sensitivity is related to the size of the target biomarker, more efforts are needed to increase the sensitivity of the detection of small biomarkers.

3.4. Magnetoimmunosensors

Magnetoimmunosensor draws great attention in recent years since it can simplify the experiment procedure by magnetical separation. The magnetic beads (MBs) are developed as label holders and have been proved to be an effective strategy to improve the detecting sensitivity and minimize matrix effects of the EC immunosensor (Bettazzi et al., 2012). Due to the magnetical separation procedure, magnetoimmunosensors can be applied for the detection of target biomarkers in raw samples without any purification steps, showing great potential in the application of clinical diagnosis.

3.4.1. Magnetoimmunosensors for IL-6 detection

Liu et al. (2014) proposed an EC multi-biomarker magnetoimmunosensor for PSA, PSMA, and IL-6 (shown in Fig. 11.). The magnetoimmunosensor was fabricated on a polydimethylsiloxane (PDMS) slice with 8*8 nano-Au electrodes deposited on it. Different capture antibodies linked with MBs were immobilized on the electrodes by magnet. HRP was used as labels and the Au nanorods served as enzyme carrier bound with reporting antibodies. The linear range between current signal and the logarithm of antigens concentration was from 5 pg/mL to 1000 pg/mL and the limit of detection of IL-6 in serum samples was 5 pg/mL.

An EC magnetoimmunosensor for IL-6 was proposed in (Ojeda et al., 2014), using poly-HRP-strept conjugates as labels, which could obtain enhanced signal-to-blank ratios. The reporting antibodies were

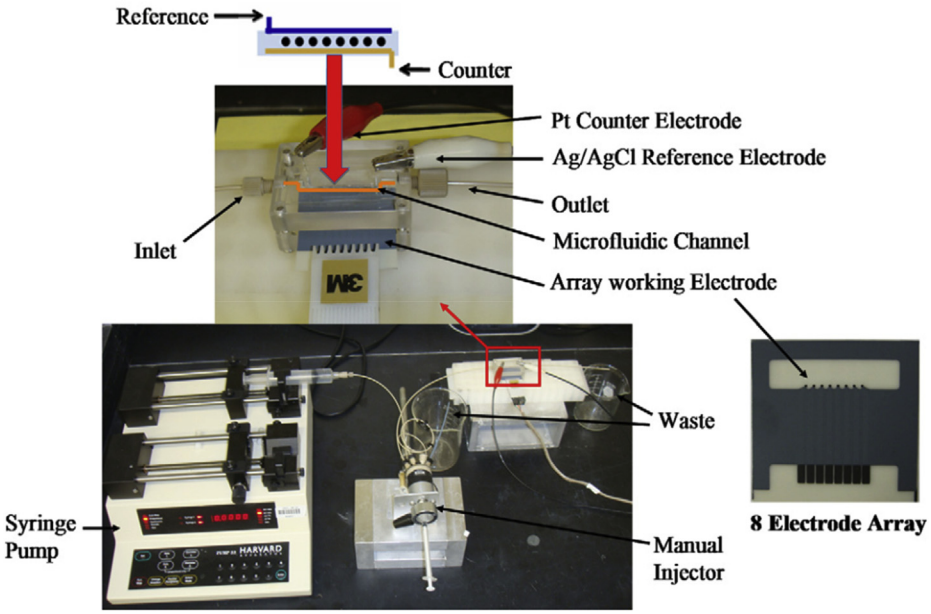

Fig. 12. Schematic illustration of the microfluidic EC immunosensor system. Reprinted with permission from (Chikkaveeraiah et al., 2011).

immobilized onto carboxyl-functionalized magnetic microparticles. Screen-printed carbon electrodes were used as working electrodes, and a neodymium magnet was placed on the bottom of the electrode to immobilize the MBs onto the surface of electrodes. Amperometric measurements were conducted after adding hydrogen peroxide solution onto the electrode. A linear relationship could be achieved between the measured current and log of IL-6 concentration in the range of 1.75–500 pg/mL, and the limit of detection was 0.39 pg/mL.

A microfluidic EC immunosensor system, including a pump and a sample injector connected to a 1.5 mm polydimethylsiloxane (PDMS) channel, was developed by Chikkaveeraiah et al. (2011) for the simultaneous detection of PSA and IL-6 in serum (shown in Fig. 12). The capture antibodies were immobilized on a disposable 8-electrode chip, coated with a dense layer of 5 nm glutathione-decorated gold nanoparticles. Superparamagnetic particles (MPs) were used as carriers for over 200000 HRP labels, which was the key to improve the sensitivity for this immunosensor. Target biomarkers were captured by a heavily HRP-labelled antibody-magnetic particle bioconjugate and could be separated from the sample magnetically. Amperometric signals were developed by injecting a solution of mediator and hydrogen peroxide. The limit of detection was 0.30 pg/mL for IL-6 obtained from diluted serum mixtures.

3.4.2. Magnetoimmunosensors for IL-8 detection

Munge et al. reported an ultrasensitive sandwich magnetoimmunosensor (shown in Fig. 13) for IL-8 in serum based on glutathione-protected Au nanoparticle (GSH-AuNP) (Munge et al., 2011). The limit of detection could reach 1 fg/mL by utilizing novel massively labelled paramagnetic particles. A dense film of GSH-AuNP was coated on the electrode of sensor, and the capture antibodies were attached on it. Superparamagnetic beads loaded with about 500000 HRP labels were used. Hydrogen peroxide and hydroquinone were added as well to activate the labels.

An EC magnetoimmunosensor for the simultaneous determination of IL-8 protein and its messenger RNA, IL-8 mRNA in undiluted human raw saliva was successfully developed in (shown in Fig. 14) (Torrente-Rodríguez et al., 2016). It used functionalized magnetic beads and a specific hairpin DNA sequence for IL-8 mRNA. Disposable screen-printed carbon electrodes was adopted as working electrodes. Carboxylic-modified magnetic beads (HOOC-MBs) were used for immobilization of IL-8 antibodies, and labelled with HRP. Amperometric detection was conducted to after the HRP-labelled sandwich immune-complexes hold by MBs was transferred to the working electrode. The limits of detection of IL-8 was 72.4 pg/mL and the linear range was reported to be 87.9–5000 pg/mL.

3.4.3. Magnetoimmunosensors for CRP detection

An ultrasensitive magnetoimmunosensor for the detection of CRP was proposed in (Esteban-Fernández de Ávila et al., 2013), which is similar to the magnetoimmunosensors for IL-6 and IL-8, with disposable Au screen-printed electrodes (Au/SPEs) as working electrodes and HOOC-MBs holding the sandwich antiCRP-CRP-biotin-antiCRP structure. Strep-KRP was used as labels and the 3,3,5,5- tetramethylbenzidine (TMB) was used as electron transfer mediator. The linear range of this CRP magnetoimmunosensor was between 0.07 and 1000 ng/mL, and the detection limit could reach 0.021 ± 0.005 ng/mL.

A rapid magnetoimmunosensor for the simultaneous detection of two biomarkers, including aminoterminal pro-B-type natriuretic peptide (NT-proBNP) and CRP was reported by Berta et al. in (Ávila et al., 2014). Carboxylic acid-modified magnetic beads were covalently bonded with capture antibodies. Dual screen-printed carbon electrodes and HRP label were employed in this immunosensor. A detection limit of 0.47 ng/mL was reached in this paper.

3.4.4. Brief discussion

The great strength of magnetoimmunosensor is it can separate the target antigen to get a preconcentration of the sample, so that it can prevent the influence of matrix and increase the selectivity of the sensor. Thus, magnetoimmunosensor has its potential to be used in raw sample test.

3.5. FET immunosensors

FET immunosensors attracted wide interest in recent years. The principle FET immunosensor is to control the current flowing between the drain and source terminals by the voltage on the gate terminal (Farka et al., 2017).

3.5.1. FET immunosensors for IL-6 detection

Hu et al. propose a liquid-gated field-effect transistor (FET) immunosensor for IL-6 based on SWCNT (shown in Fig. 15.) (Chen et al.,

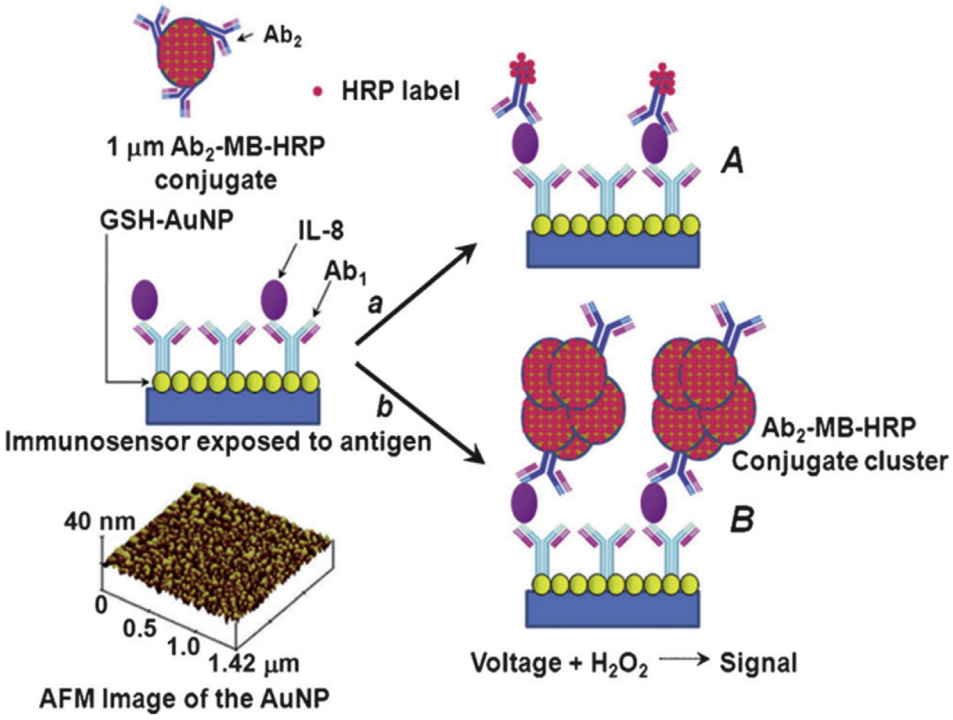

Fig. 13. Schematic illustration of AuNP magnetoimmunosensor for IL-8. (A) The immunosensor with 14–16 HRP labels per reporting antibody (B) The immunosensor with massively labelled Ab2–MB–HRP particles to obtain amplification. Reprinted with permission from (Munge et al., 2011).

2016). The drain current of the transistor was measured to predict the interactions of IL-6 and IL-6 antibody on the SWCNT. Interactions of the target analyte and antibody will lead to a change of the transducing layer conductance, which can be measured by the drain current. The horizontally aligned CNT employed in the sensor were grown on quartz substrate, approximately 3–5 tubes/μm, and IL-6 antibodies were immobilized on nanotubes linked by 1-pyrenebutanoic acid succinimidyl ester. The voltage bias is applied between the Au source and drain electrode and the gate potential is applied through a reference electrode. The proposed sensor can reach the sensitivity of 1.37 pg/mL by reducing the contact resistance between each tube, which is achieved by good horizontal alignment of carbon nanotubes.

In (Gentili et al., 2018), a combination of organic electrochemical transistors (OECTs) and immune-affinity regenerated cellulose (RC) membranes was employed to detect IL-6 (Fig. 16.). The immune-affinity membranes were functionalized with protein G and immobilized with antibodies, whose binding affinity towards IL-6 would change with PH variation, which could be used for a preconcentration of IL-6. The IL-6

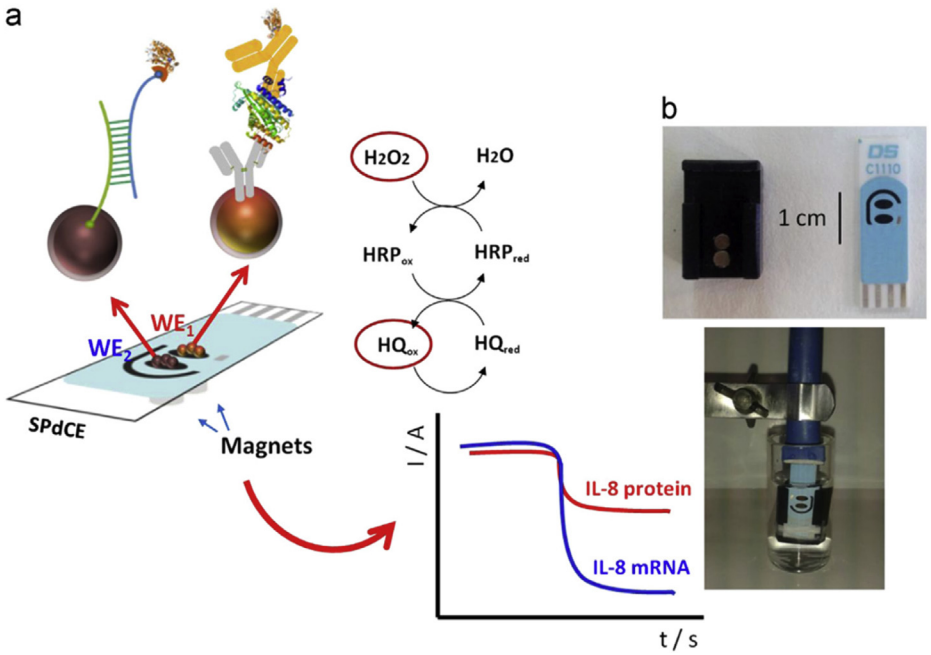

Fig. 14. Schematic of the disposable dual magnetoimmunosensor for the simultaneous determination of IL-8 protein and IL-8 mRNA (a). The working electrodes and the modified MBs (b). The real scale of magnetoimmunosensor. Reprinted with permission from (Torrente-Rodríguez et al., 2016).

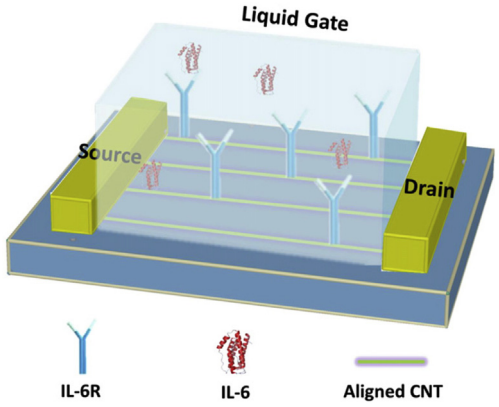

Fig. 15. Schematic illustration of the liquid-gated field-effect transistor sensor based on horizontally aligned single-walled carbon nanotubes for IL-6 detections. IL-6 antibodies were immobilized on carbon nanotubes to captured interleukin-6 molecules. Reprinted with permission from (Chen et al., 2016).

antibodies were immobilized on the surface of the gate electrodes which were 1 mm diameter gold wires to capture the antigen. They also used oligo(ethylene glycol) (OEG)-terminated self-assembled alkanethiolate monolayers (SAMs) to functionalize the gate electrode helping to prevent non-specific protein binding. The detection limit of this immunosensor was 220 pg/mL.

An integrated portable immunosensor for the detection of IL-6 in saliva was reported by Hao et al., which is shown in Fig. 17. (Hao et al., 2019). Graphene-based field effect transistor (GFET) with the buried-gate geometry was immobilized with 1- Pyrenebutanoic acid succinimidyl ester (PASE) through π-π stacking and then combined with aptamers, which could achieve a linear range of 50–800 pM and a detection limit of 12.2 pM in saliva. The immunosensor was integrated on printed circuit boards for data process and transmission. This device can also transmit data wirelessly to a smart-phone, showing a potential to be used for point-of-care diagnosis.

3.5.2. FET immunosensors for CRP detection

Magliulo et al. deveploed a label-free immunosensor based on the electrolyte-gated field-effect transistor (EGOFET) (Magliulo et al., 2016). Antibodies were immobilized on the poly-3-hexyl thiophene substrate. Nonspecific binding was prevented by a hydrophobic blocking polymer layer. A wide linear range from 4 pM to 2 μM and a detection limit of 220 ng/L could be achieved in this paper.

3.5.3. Brief discussion

An obvious advantage of FET immunosensors is the simplicity of operation, which is suitable for application in clinical diagnosis. Besides, this kind of immunosensors is low-cost for it's label free and use less antibodies than sandwich type. However, the sensitivity of FET immunosensors might not be as high as the impedimetric possibly due to the nonspecific binding and other ions present in the sample (Farka et al., 2017).

4. Remarks

This review focuses on the researches of EC immunosensors for the COPD biomarkers including IL-6, IL-8 and CRP. EC immunosensors have been showing superior results to ELISA, including faster response time and more simple operation. The summary of the EC immunosensors for COPD biomarkers is listed in Table 1. Different EC immunosensors have their advantages and disadvantages, which are also discussed above. The limit of detection of EC immunosensors can reach several pg/mL or even lower, showing great potentials in the application for clinical diagnosis.

4.1. Electrodes

The choice of electrodes is crucial for the sensitivity of EC immunosensors. Inert materials are commonly used for electrodes such as ITO (Wang et al., 2014), disposable screen-printed carbon (Torrente-Rodríguez et al., 2016, p. 8), graphite (Kokkinos et al., 2015), and Au (Sharma et al., 2016). Although Au electrodes are widely utilized in lab experiments, it still has difficulties in application due to the cost and washing procedure. Instead, carbon has many outstanding properties such as good conductivity and electrochemically stability. Thus, disposable screen-printed carbon electrodes which have mature fabrication process already and much lower cost than precious metal electrodes as Au are more appropriate for mass production and commercial application.

4.2. Receptors

Both antibody-based and aptamer-based immunosensors are listed in this review. Antibodies are widely used in the development of all kinds of immunosensors. It was reported that due to the large size of antibodies, it may be a challenge to get lower detection limit of biomarkers (Tertis et al., 2019). Besides, antibodies have some more limitations in storage condition and high cost. However, Aptamers have many advantages such as smaller size, higher stability, longer shelf life and lower in cost compared to antibodies, making them more promising

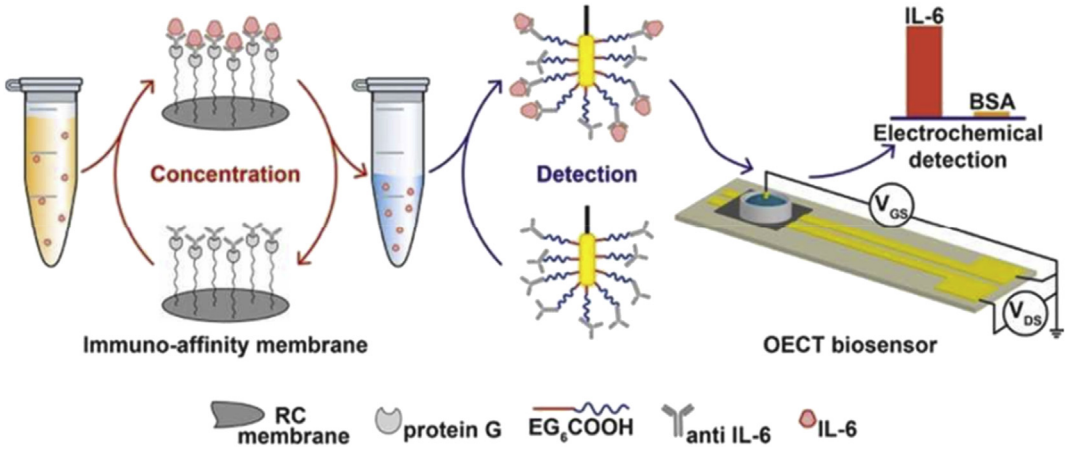

Fig. 16. Schematic illustration of the preconcentration of IL-6 using RC membrane and subsequent electrochemical detection of IL-6. Reprinted with permission from (Gentili et al., 2018).

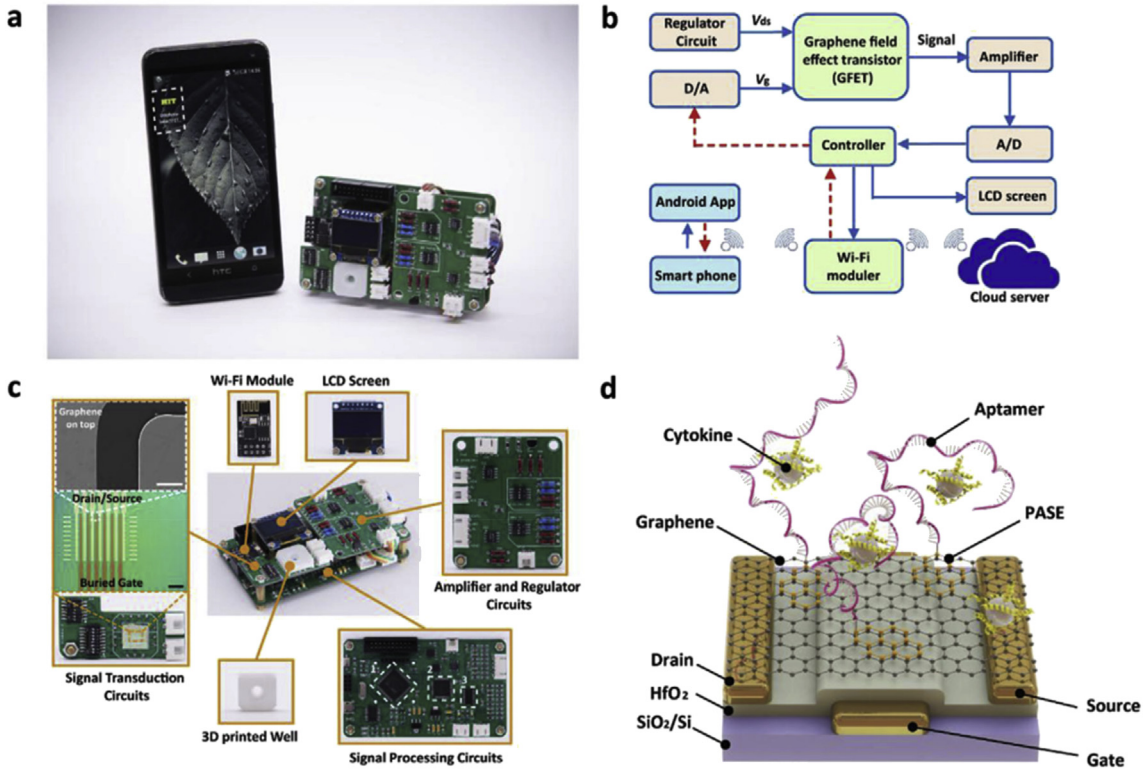

Fig. 17. (a) Schematic illustration of the integrated portable immunosensor for IL-6 detection. (b) System-level block diagram of the whole system. (c) Image of the GFET nanosensing system on printed circuit boards. (d) Schematic illustration of the GFET and aptamers for IL-6 detection. Reprinted with permission from (Hao et al., 2019).

for point-of-care devices (Hao et al., 2019).

4.3. Sample type

As a non-invasive sample, saliva is considered as a promising alternative for point-of-care diagnosis (Eftekhari et al., 2019). It can be easily collected by patients themselves without external stimulus or skin-piercing (Hao et al., 2019). In recent years, many researchers began to focus on the detection of relevant biomarkers in saliva, such as cardiac troponin I (Hao et al., 2019), cortisol (Chekin et al., 2018; Kämäräinen et al., 2018), fetuin (Dhull et al., 2019) and TNF-α (Sánchez-Tirado et al., 2018). However, there are not many literatures on electrochemical immunosensors for COPD biomarkers in saliva, especially raw saliva. Difficulties including high viscosity, the background interference of raw saliva and the extremely low concentration of biomarkers need to be solved before saliva can be used for samples for point-of-care diagnosis.

4.4. Labels

According to the research works listed above, HRP is the most commonly used label for the labelled type of EC immunosensor to catalyze the oxidation of a mediator and often used with hydrogen peroxide since it has fewer requirements for the pretreatment of samples, thus can be used for most of the circumstances. Metal nano-particles and QDs are other alternatives of labels which are aforementioned discussed. However, in recent years, more and more attention has been paid on label-free immunosensors, which are more simple in principle and lower in cost as well. For label-free immunosensors, there is still difficulty in achieving the same detection limit with the labelled immunosensors, which we believe, will be the focus of future research.

4.5. Nanostructured materials

Nanostructured materials are commonly used in immunosensors, including nanoparticles and nanostructures on electrodes. Different functions of nanostructured materials can be summarized as follows. Firstly, nanostructured materials are often employed as labels. Secondly, nanostructured materials can serve as electron wires to transfer the electrons. Thirdly, nanostructured materials often serve as carriers of multi-enzyme labels. Moreover, the last, they are often employed as catalysts to amplify the measured EC signals. The use of nanostructured materials makes it possible to integrate the immunosensor system by reducing volume, which is an important factor for the application of the EC method.

4.6. Future research directions

From the listed literature it can be concluded that more attention has been paid on impedimetric immunosensors and FET immunosensors in recent years since they are label-free, low cost compared to sandwich type immunosensors and fast in response, which is more suitable for the application in portable diagnosis device. Besides, aptamer-based immunosensors become a new trend as well, which we believe will replace antibody-based immunosensors.

5. Conclusions and perspectives

EC immunosensors for single or multi biomarkers (which is relatively rare), can be achieved in the lab, with excellent sensitivity and relatively shorter response time comparing to ELISA method. However, the EC immunosensors are still hindered for clinical diagnosis due to several limitations, which can be the focus of future research works. First of all, due to the complicated fabrication process and high cost of nanostructures, the patients cannot afford it, especially for COPD

Table 1
Summary of the EC immunosensors for COPD biomarkers.

Sensor type	Bio-marker	Label	Comments	Linear range	Limit of detection	Ref
Enzyme-labelled	IL-6	HRP	SWNT forests as carries for multiple enzymes	/	3×10^{-2} ng/mL	Chikkaveeraih et al. (2009)
Enzyme-labelled	IL-6	HRP	HRP-MWNT-Ab2 bioconjugate having 106 active HRPs per 100 nm of carboxylated carbon nanotubes	/	0.5 pg/mL	Malhotra et al. (2010)
Enzyme-labelled	IL-6	HRP	AuNP-PDOP as the sensor platform and AuNP-PDOP@ CNT as enzyme carrier	4–800 pg/mL	1.0 pg/mL	Wang et al. (2011)
Enzyme-labelled	IL-6	HRP	a new biointerface based on grapheneAuNP sol–gel and using AuNP-PDA@CNT as carrier	1–40 pg/mL	0.3 pg/mL	Wang et al. (2014)
Enzyme-labelled	IL-6	HRP	compared the performances of SWNT forests and GSH-AuNP	GSH-AuNPs:20–4 x 10^3 pg/mL SWNT: 40–150 pg/mL	GSH-AuNPs:10 pg/mL SWNT: 30 pg/mL	Munge et al. (2009)
Enzyme-labelled	IL-6	HRP	Au nanoparticle electrodes arrays costed only 0.2 euro	20–400 pg/mL	20 pg/mL	Jensen et al. (2011)
Enzyme-labelled	IL-8	HRP	a 16 channel disposable SPCE array as sensor platform	/	8 pg/mL	Wan et al. (2011)
Enzyme-labelled	CRP	HRP	disposable screen-printed carbon electrodes modified with MWNTs and protein A	/	0.5 ng/mL	Buch and Rishpon (2008)
Enzyme-labelled	CRP	AQ	L-cysteine/gold modified screen-printed graphene electrodes was adopted	/	1.5 ng/mL	Jampasa et al. (2018)
Metal NPs-labelled	IL-6	AgNP–TiP hybrid	a magnetic sensing array combined with this novel label	5×10^{-3}-10 ng/mL	0.1 pg/mL	Peng et al. (2011)
Metal NPs-labelled	IL-6	AgNPs	ERGO and AuPdNPs as the platform	0.1–10^5 pg/mL	5.9×10^{-2} pg/mL	(Lou et al., 2014, p. 6, p. 6)
Quantum dot-labelled	CRP	PbS quantum-dot	bismuth citrate-modified graphite screen-printed electrodes were used	0.2–100 ng/mL	5×10^{-2} ng/mL	Kokkinos et al. (2015)
Impedimetric immunosensors	IL-6	None	electrochemically depositing Au NPS on horizontally aligned SWNT array	0.01–100 fg/mL	10^{-2} fg/mL	Yang et al. (2013)
Impedimetric immunosensor	IL-6	None	Needle shaped microelectrodes array with eight disk microelectrodes on it	20–60 pg/mL	20 pg/mL	Russell et al. (2019)
Impedimetric immunosensor	IL-6	None	A lable-free impedimetric immunosensor using aptamers instead of antibodies	5 pg/mL-100 ng/mL	1.6 pg/mL	Tertis et al. (2019)
Impedimetric immunosensors	IL-8	None	Non-antibody-based binding molecules employ small scaffold proteins	900 fg/mL to 900 ng/mL	90 fg/mL	Sharma et al. (2016)
Impedimetric immunosensor	IL-8	None	A new kind of conductive composite slurry was coated on ITO electrodes	0.01–3 pg/mL	3.3 fg/mL	Aydın et al. (2018)
Impedimetric immunosensor	IL-8	None	An impedimetric immunosensor based on PHA modified ITO electrode	0.02–3 pg/mL	6 fg/mL	Aydın and Sezgintürk (2018)
Impedimetric immunosensor	IL-8	None	AuNPs-rGO) composite film and ITO coated glass electrodes were used	500 fg/mL to 4 ng/mL	72.73 ± 0.18 pg/mL	Verma et al. (2017)
Impedimetric immunosensor	CRP	None	SPE modified with AuNPs and CRP antibodies and an external redox mediator Fe(CN)64- were used	0.047–23.6 μg/mL	17 ng/mL	Thangamuthu et al. (2018)
Impedimetric immunosensor	CRP	None	A simple folding electrochemical impedimetric immunosensor with disposable paper-based electrode	0.005–500 μg/mL	0.001 μg/mL	Boonyasit et al. (2019)
Capacitive immunosensor	CRP	None	nanosized nickel patterned electrodes were reported to replace gold electrodes	/	1 ng/mL	Kallempudi and Gurbuz (2011)
Capacitive immunosensor	CRP	None	An interdigitated electrode (IDE) with nanoislands	/	/	Jung et al. (2014)
Capacitive immunosensor	IL-6 CRP	None	simultaneous detection of TNFα, IL-6 and CRP	/	25 pg/mL	Qureshi et al. (2010)
Magnetoimmunosensor	IL-6	HRP	fabricated on a PDMS slice with 8*8 nano-Au electrodes deposited on	5 pg/mL-10^3 pg/mL	5 pg/mL	Liu et al. (2014)
Magnetoimmunosensor	IL-6	poly-HRP	carboxyl-functionalized magnetic microparticles as label carriers	1.75–500 pg/mL	0.39 pg/mL	Ojeda et al. (2014)
Magnetoimmunosensor	IL-6	HRP	MPs used as carriers for over 2×10^5 HRP labels	/	0.30 pg/mL	Chikkaveeraiah et al. (2011)
Magnetoimmunosensor	IL-8	HRP	GSH-AuNP coated on the electrode and superparamagnetic beads loaded with about 5×10^5 HRP labels	/	1 fg/mL	Munge et al. (2011)
Magnetoimmunosensors	IL-8	HRP	Disposable dual screen printed carbon electrodes.	87.9–5 x 10^3 pg/mL	26.4 pg/mL	(Torrente-Rodríguez et al., 2016, p. 8, p. 8)
Magnetoimmunosensors	CRP	HRP	anti-CRP immobilized onto HOOC-MBs	0.07–10^3 ng/mL	0.021 ± 5×10^{-3} ng/mL	Esteban-Fernández de Ávila et al. (2013)

(*continued on next page*)

Table 1 (*continued*)

Sensor type	Bio-marker	Label	Comments	Linear range	Limit of detection	Ref
Magnetoimmunosensors	CRP	HRP	Carboxylic acid-modified magnetic beads were covalently bonded with capture antibodies	/	0.47 ng/mL	Ávila et al. (2014)
FET immunosensors	IL-6	None	a liquid-gated field-effect transistor (FET) immuosensor for IL-6 based on SWCNT	/	1.37 pg/mL	Chen et al. (2016)
FET immunosensors	IL-6	None	A combination of OECTs and immune-affinity RC membranes was employed	/	200 pg/mL	Gentili et al. (2018)
FET immunosensors	IL-6	None	GFET with the buried-gate geometry was immobilized with PASE	50–800 pM	12.2 pM	Hao et al. (2019)
FET immunosensors	CRP	None	EGOFET with a hydrophobic blocking polymer layer to prevent nonspecific binding	4 pM-2 μM	220 ng/L	Magliulo et al. (2016)

patients, since several tests need to be conducted per month to monitoring the exacerbation of the disease. Secondly, although the EC detection method can reach less response time than the ELISA method, it still costs usually several hours, which is considered too long for clinical equipment. Thirdly, simultaneous detection of multiple biomarkers still incur difficulties in non-specific binding.

Overall, the identified challenges in COPD biomarker detection demands methods with both the low-cost and fast response time, while detecting multiple biomarkers with sufficient specificity. Disposable sensor platforms would offer a friendlier solution to the patients, which can be further explored in future work. We believe that with joint efforts of global researchers, the application of EC immunosensor in clinical diagnosis is in the near future.

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this article.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Xuan Chen: Writing - original draft, Writing - review & editing, Investigation. **Tao Dong:** Conceptualization, Methodology, Writing - review & editing, Supervision, Project administration, Funding acquisition. **Xueyong Wei:** Conceptualization, Methodology, Investigation, Writing - review & editing, Supervision. **Zhaochu Yang:** Conceptualization, Methodology. **Nuno Miguel Matos Pires:** Investigation, Writing - review & editing. **Juan Ren:** Investigation. **Zhuangde Jiang:** Conceptualization, Supervision.

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