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Review

Biosensors for cardiac biomarkers detection: A review

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ABSTRACT

The cardiovascular disease (CVD) is considered as a major threat to global health. Therefore, there is a growing demand for a range of portable, rapid and low cost biosensing devices for the detection of CVD. Biosensors can play an important role in the early diagnosis of CVD without having to rely on hospital visits where expensive and time-consuming laboratory tests are recommended. Over the last decade, many biosensors have been developed to detect a wide range of cardiac marker to reduce the costs for healthcare. One of the major challenges is to find a way of predicting the risk that an individual can suffer from CVD. There has been considerable interest in finding diagnostic and prognostic biomarkers that can be detected in blood and predict CVD risk. Of these, C-reactive protein (CRP) is the best known biomarker followed by cardiac troponin I or T (cTnI/T), myoglobin, lipoprotein-associated phospholipase A(2), interlukin-6 (IL-6), interlukin-1 (IL-1), low-density lipoprotein (LDL), myeloperoxidase (MPO) and tumor necrosis factor alpha (TNF-α) has been used to predict cardiovascular events. This review provides an overview of the available biosensor platforms for the detection of various CVD markers and considerations of future prospects for the technology are addressed.

Contents

1. Introduction

Cardiovascular disease (CVD) is a major cause of human death in both developing and developed countries. According to the World

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Health Organization (WHO), an estimated 17.5 million (30%) of all global deaths in 2005 are associated with CVD and it is estimated that by 2015, CVD can be the leading cause of death in the developing countries [1]. Recently, according to the new European cardiovascular disease statistics 2008, a staggering figure of over 4.3 million deaths in Europe alone and 2 million deaths in European Union are caused by CVD, and it is overall estimated to cost the EU economy €192 billion a year [2]. The early and quick diagnosis of cardiovascular disease is extremely important and crucial not for only patient survival but also saving cost and great deal of time in successful prognosis of the diseases. Existing methods of diagnosis for CVD rely heavily on classical methods which are based on tests conducted in central laboratories that may take several hours or even days from when tests are ordered to when results are received [3]. The diagnosis of CVD has been based on the WHO criteria, whereby patients must meet at least two of three conditions: characteristic chest pain, diagnostic electrocardiogram (ECG) changes, and elevation of the biochemical markers in their blood samples [3]. Although, ECG is an important management tool for guiding therapy [4,5], but it is a poor diagnostic test for CVD, because about half of the CVD patients who present to the Emergency Department show normal or no diagnostic electrocardiograms, which makes early diagnosis of CVD more difficult [4–7]. Therefore, measurement of cardiac markers is critical in assisting the diagnosis of CVD. A more sensitive and rapid technology platform is therefore needed to fulfill the rapid diagnosis requirements in CVD detection. The elaboration of biosensors is probably one of the most promising ways to solve some of the problems concerning sensitive, fast and cost effective measurements [8]. Biosensor can help in rapid diagnosis, providing better health care and reducing the waiting time for results dissemination which is highly stressful to the patients. Recently, lab-on-a chip and microfluidics based biosensor technology is reviewed for the detection of cardiac markers [9]. This review provided information on commercially available a few point-of-care immunosensing instruments and chip based technology for the detection of different cardiac biomarkers. In the present paper, we reviewed the developments in application of biosensors over the past 10 years for the detection of cardiovascular risk assessment. This review also summarized the frequently targeted CVD biomarkers in various biosensor platforms and highlighted the major clinically relevant parameters, such as their detection limit/range and designing of bioassay.

2. Cardiac biomarkers

CVD is not a single disease, but it is a group of different disorders that affect heart and blood vessels. CVD includes atherosclerosis condition that develops when a plaque builds up in the walls of the arteries. This plaque narrows the arteries and makes it difficult for blood to flow through and causes a heart attack or stroke. CVD can be caused by a range of factors and disorders that include genetic, gender, age, high blood pressure and cholesterol, diabetes, obesity and overweight, smoking and stress. The causes of CVD are more diverse that clinical testing becomes increasingly complex. There are a number of diseases associated with CVD that affect different parts of the body. Although, great progress has been made in the treatment of this disease, current medical knowledge is unable to effectively predict its risk. With regards to predicting CVD risk, one of the active research areas recently is the use of diagnostic and prognostic biomarkers that can be identified in blood [10]. On the basis of diagnostic and prognostic standpoint, CVD biomarkers can be categorized into pathogenetic and therapeutic types. The diagnostic and prognostic biomarkers also provide therapeutic value in medical applications. The vascular wall releases molecules into the bloodstream that can reflect the pathological processes taking

Fig. 1. Most frequently studied biomarkers in relation to the different mechanism involved in CVD risk [10].

place. In theory, the concentrations of the molecules involved in different pathological processes could be the biomarkers. However, not all of these molecules are suited to this aim but should fulfill certain conditions [10].

There are several important characteristics that an ideal cardiac biomarker should exhibit. These include: (a) high clinical sensitivity and specificity, (b) quick release of biomarker in the blood enabling early diagnosis, (c) capability to remain elevated for longer time in the blood, and (d) ability to be assayed quantitatively [10]. It is difficult to select a specific marker for the diagnosis of CVD. Therefore, a range of biomarkers can potentially be analyzed simultaneously for the accurate disease diagnosis [6,7,10–13]. Anderson et al. reported a set of 177 candidate biomarkers that are potential plasma markers for CVD and stroke [13]. Recently, the most frequently studied biomarkers are summarized in relation to the different mechanisms involved in development and rupture of atherosclerotic plaque, such as endothelial dysfunction, inflammation, oxidative stress, proteolysis, and thrombosis (Fig. 1) [10].

Several other cardiac-specific biomarkers have emerged as strong and reliable risk predictors for coronary heart disease, as listed in Table 1. Of which, CRP has been the most frequently used single biomarker for cardiovascular risk (CVR). The CVR defined by the American Heart Association (AHA) and the Center for Disease Control and Prevention (CDC) is regarded as low risk for a CRP concentration below 1.0 mg L^{-1}, moderate for 1.0–3.0 mg L^{-1}, and high risk for concentrations over 3.0 mg L^{-1} [14]. CRP can rise as high as 1000-fold because of inflammation induced by infection or injury, often leading to CVR [15]. Recent research suggests that patients with elevated basal levels of CRP are at an increased risk of diabetes and hypertension as well as CVD [15].

Myoglobin, although not a very specific marker, but it is the first marker released after the damage occurred to myocardial muscle cells. B-type natriuretic peptide (BNP), cardiac troponin I (cTnI), and CRP are released after myoglobin, but they are specific markers for coronary events. BNP is useful for the emergency diagnosis of heart failure and for the prognosis in patients with acute coronary syndromes (ACS) [16]. CRP is an important prognostic indicator of CVR and ACS. cTnI has become a standard marker for the detection

Table 1
A summary of primary clinically utilized cardiac biomarkers, highlighting their respective cut-off values.

Cardiac biomarker	Type of cardiovascular diseases involved	Cut-off levels	Specificity (low, medium, high)	MW (kDa)	Initial elevation	Time to peak	Return to normal	POC test available
Troponin I (cTnI)	Detection of acute myocardial infarction (AMI)	0.01–0.1 ng mL^{-1}	High	23.5	4–6 h	12–24 h	6–8 days	Yes
Troponin T (cTnT)	Detection of AMI	0.05–0.1 ng mL^{-1}	High	37	4–6 h	12–24 h	7–10 days	Yes
Myoglobin	Early detection of AMI	70–200 ng mL^{-1}	Low	18	1–3 h	6–12 h	24–48 days	Yes
C-reactive protein (CRP)	Early detection of inflammation/cardiac risk factor	<10^3 ng mL^{-1} low risk 1–3 × 10^3 ng mL^{-1} intermediate risk >3–15 × 10^3 ng mL^{-1} high risk (no definitive)	High	125	ND	ND	ND	Yes
Creatine kinase MB subform (CK-MB)	Early detection of AMI	10 ng mL^{-1}	Medium	85	4–6 h	12–24 h	3–4 days	Yes
B-type natriuretic peptide (BNP)	Acute coronary syndromes/diagnosis of heart failure/ventricular overload		High	3.4	ND	ND	ND	Yes
N-terminal pro-B-type natriuretic peptide (NT-proBNP)	Acute coronary syndromes/diagnosis of heart failure/ventricular overload	0.25–2 ng mL^{-1}	High	8.5	ND	ND	ND	Yes
Myeloperoxidase (MPO)	Detection of inflammation	Patients with elevated MPO levels >350 ng mL^{-1} stratification risk	Medium	150	ND	ND	ND	Yes
Heart fatty acid binding protein (H-FABP)	Myocardial necrosis	Patients with elevated H-FABP levels elevated ≥6 ng mL^{-1} stratification risk	Low	15	2–3 h	8–10 h	18–30 h	Yes
TNF-α	Inflammation/cardiac risk factor	<0.0036 ng mL^{-1} low risk ≥0.0036 ng mL^{-1} high risk	ND	ND	ND	ND	ND	ND
Interlukin-6 (IL-6)	Inflammation/cardiac risk factor	Low < 0.0013 ng mL^{-1} Mid 0.00138–0.002 ng mL^{-1} High > 0.002 ng mL^{-1}	ND	ND	ND	ND	ND	ND
Fibrinogen		Low < 3.58 × 10^6 ng mL^{-1} Mid 3.58–4.20 × 10^6 ng mL^{-1} High > 4.20 ×10^6 ng mL^{-1}	ND	ND	ND	ND	ND	ND

Source: Adapted from Refs. [9,11,54,88–92].

Table 2
Cardiac markers detection on different transduction platforms and their detection range reported in the literature. The time required for the detection of cardiac markers using the methods listed in table is estimated to be within ~20 min to 1 h.

Target/biomarker	Transduction platform	Detection range	Assay type	Reference
Optical biosensors				
CRP Myoglobin cTnI	Fluorescence	CRP-LOD – 30 ng mL^{-1}	Microfluidic with multianalyte	[22]
NF-α, IL-8, IL-6, IL-4, IL-2γ, IL-2, IL-1b, IL-12, IL-10, interferon-gamma (IFN-γ)	Fluorescence	All cytokine 0.01–10 ng mL^{-1} LOD – 0.01 ng mL^{-1}	Multianalyte	[23]
IL-6	Fluorescence using labeled nanoparticles	0.02–1.25 ng mL^{-1} LOD – 0.007 ng mL^{-1}	Single analyte	[24]
TNF-α	Fluorescence	1 ng mL^{-1}	Single analyte	[25]
TNF-α	Fluorescence	1–0.0016 ng mL^{-1}	Single analyte	[93]
CRP	Fluorescence	0.1 ng mL^{-1}	Single analyte	[26]
CRP	Fluorescence	10–10^5 ng mL^{-1}	Single analyte	[27]
CRP	Fluorescence	20 ng mL^{-1}	Single analyte	[28]
cTnI	Fluorescence	0.1–100 ng mL^{-1}	Single analyte	[29]
Heart-type fatty-acid binding protein (H-FABP) CRP	ELISA	H-FABP-LOD < 6×10^3 mL^{-1} CRP-LOD < 10^3 ng mL^{-1}	Multianalyte	[30]
cTnT	ELISA	0.1–100 ng mL^{-1} LOD – 0.027 ng mL^{-1}	Single analyte	[31]
Myoglobin	ELISA	20–230 ng mL^{-1} LOD – 16 ng mL^{-1}	Single analyte	[32]
cTnI	ELISA	Rat troponin I protein (5.3×10^4 to 5.3×10^6 ng mL^{-1}) or human troponin I protein (4.3×10^4 to 4.3×10^6 ng mL^{-1})	Single analyte	[33]
CRP and IL-6	Fluorescence/colorimetric	1–1000 ng mL^{-1} LOD-CRP – 1 ng mL^{-1}	Microfluidic with multianalyte	[35]
Myoglobin, creatine kinase mb (CKmb), cTnI, and fatty acid-binding protein (FABP)	Chemiluminescence	Myoglobin – 1.2 ng mL^{-1} CKmb – 0.6 ng mL^{-1} TnI – 5.6 ng mL^{-1} and FABP – 4 ng mL^{-1}	Multianalyte	[37]
CRP	Chemiluminescence	50–5×10^4 ng mL^{-1} LOD – 12.5 ng mL^{-1}	Multianalyte	[34]
CRP	Chemiluminescence	10 to 10^4 ng mL^{-1}	Microfluidic with Single analyte	[14]
cTnI	Colorimetric; PDMS–gold nanoparticle composite film	10^4 to 5×10^3 ng mL^{-1}	Single analyte	[36]
Low-density lipoprotein (LDL)	Electrochemiluminescence	0.025–16 ng mL^{-1} LOD – 0.006 ng mL^{-1}	Single analyte	[38]
cTnI	Electrochemiluminescence	0.002 ng mL^{-1}	Single analyte	[39]
Metalloproteinase (MMP)-2	SPR	LOD – 0.036 ng mL^{-1}	Single	[42]
CRP	SPR	LOD – 10^3 ng mL^{-1}	Single marker	[43]
B-type natriuretic peptide (BNP)	SPR	5 pg mL^{-1} to 100 ng mL^{-1} LOD – 0.005 ng mL^{-1}	Microfluidic with single analyte	[48]
CRP	SPR	2–5×10^3 ng mL^{-1} LOD – 10^3 ng mL^{-1}	single analyte	[44]
CRP	SPR	1 ng mL^{-1} to 5×10^4 ng mL^{-1} LOD – 1 ng mL^{-1}	Single analyte	[45]
cTnT	SPR	0.03 up to 6.5 ng mL^{-1} LOD – 0.01 ng mL^{-1}	Single analyte	[49]
cTnT	SPR	0.05 and 4.5 ng mL^{-1} LOD – 0.05 ng mL^{-1}	Single analyte	[50]

Table 2 (*Continued*)

Target/biomarker	Transduction platform	Detection range	Assay type	Reference
Myoglobin and cTnTI	SPR	LOD – below 1 ng mL^{-1}	Multianalyte	[52]
CRP	SPR	10^4 ng L^{-1}	Single analyte	[46]
TNF-α	SPR	1–2 × 10^3 ng mL^{-1}	Single analyte	[53]
CRP	SPR	0.1–200 ng mL^{-1}	Single analyte	[47]
cTnT	SPR	100 ng mL^{-1}	Single analyte	[51]
cTnT	SPR	0.068 ng mL^{-1}	Single analyte	[18]
Myeloperoxidase (MPO)	SPR	50 ng mL^{-1}	Single analyte	[54]
BNP, cTnI, myoglobin, and CRP	Optical fiber	BNP – 0.1 ng mL^{-1} I cTnI – 7 × 10^{-3} ng mL^{-1} MG – 70 ng mL^{-1} CRP – 700 ng mL^{-1}	Multianalyte	[16]
CRP	Optical fiber	5–12.5 × 10^3 ng mL^{-1}	Single analyte	[55]
Nerve growth factor (NGF)	Optical fiber	1–200 ng mL^{-1}	Single analyte	[57]
IL-6	Optical fiber	LOD – 0.12 ng mL^{-1}	Single analyte	[58]
CRP	Optical fiber/fluorescence/evanescent	4.4 × 10^{-11} to 2.9 × 10^{-9} ng mL^{-1} and 1.3 × 10^{-10} to 2.29 × 10^{-8} ng mL^{-1}	Single analyte	[56]
CRP	Photonic microring resonator	3 × 10^{-6} ng mL^{-1}	Single analyte	[59]
IL-6	Photonic crystal resonant	0.001–0.01 ng mL^{-1}	Multianalyte	[60]
cTnT	Optomagnetic	0.0039–3.9 ng mL^{-1} (1–1000 pM) LOD – 0.0117 ng mL^{-1}	Multianalyte	[61]
cTnT	Optomagnetic	0.03 ng mL^{-1}	Single analyte	[62]
IL-6	Surface acoustic wave	20 ng mL^{-1} to 2 × 10^3 ng mL^{-1}	Single analyte	[19,20]
CRP	Resonant acoustic profiling	0–231 ng mL^{-1} Direct assay – LOD 20 ng mL^{-1} Sandwich assay – 3 ng mL^{-1}	Single analyte	[63]
Electrochemical biosensors				
Myoglobin	Faradaic, Impedance/interdigitated electrodes	100 ng mL^{-1}	Single analyte	[64]
CRP	Faradaic, CNT modified carbon electrodes	0.5–500 ng mL^{-1} LOD – 0.5 ng mL^{-1}	Single analyte	[65]
CRP	Faradaic, impedance/gold electrodes	to 20 ng mL^{-1} LOD – 0.1 ng mL^{-1}	Single analyte	[66]
Lipoprotein-associated phospholipase A(2)	Faradaic, iridium-modified carbon electrodes	0–150 U mL^{-1}	Single analyte	[72]
IL-6	Faradaic, gold electrodes	LOD – 4.1 × 10^{-3} ng mL^{-1}	Microfluidic with single analyte	[94]
Low-density lipoprotein (LDL)	Faradaic, impedance/AuNPs-AgCl@PANI-modified glassy carbon electrode	LOD – 3.4 × 10^{-3} ng mL^{-1}	Single analyte	[73]
CRP	Faradaic, magnetic beads with carbon electrodes	LOD – 5.4 × 10^{-11} ng mL^{-1}	Single analyte	[67]
cTnT	Non-Faradaic, impedance/Al interdigitated electrodes	0.07–6.83 ng mL^{-1}	Multianalyte	[81]
CRP	Faradaic, impedance/gold electrodes	1.15 × 10^{-13} to 1.15 ng mL^{-1} LOD – 6 × 10^{-14} ng mL^{-1}	Single analyte	[68]
CRP	Faradaic, diamond like carbon electrodes	LOD – 10^{-4} ng mL^{-1}	Single analyte	[69]
IL-6	Faradaic, gold interdigitated electrodes	LOD – 0.005 ng mL^{-1}	Single analyte	[95]
CRP	Non-Faradaic, gold interdigitated electrodes	25–800 ng mL^{-1}	Single analyte	[70]
CRP and MPO	Faradaic, Impedance/iridium oxide modified electrodes	LOD-CRP – 1 ng mL^{-1} and LOD-MPO – 0.5 ng mL^{-1}	Multianalyte	[71]
cTnI and CRP	Faradaic, poly(dimethylsiloxane)–gold nanoparticle composite microreactors	cTnI – 0.01 ng mL^{-1} and CRP – 0.5 ng mL^{-1}	Microfluidic with single analyte	[74]
Cardiac biomarker N-terminal pro-B-type natriuretic peptide (NT-proBNP)	Faradaic, nanostructural gold and carbon nanotubes composite	0.006 ng mL^{-1}	Single analyte	[96]
CRP	Non-Faradaic, impedance/Al interdigitated electrodes	0.1 ng mL^{-1}	Single analyte	[82]
cTnT	Faradaic, streptavidin-microsphere modified screen printed electrodes	0.2 ng mL^{-1}	Single analyte	[97]

Table 2 (*Continued*)

Target/biomarker	Transduction platform	Detection range	Assay type	Reference
CRP	Faradaic, nanotextured polystyrene (PS) electrode	0.001–10^3 ng mL^{-1}	Single analyte	[75]
Myoglobin	Faradaic, nanoparticles modified electrodes	17.8–1780 ng mL^{-1}	Single analyte	[98]
cTnI	Faradaic, gold nanoparticles modified ITO electrodes	1–100 ng mL^{-1}	Single analyte	[76]
Myoglobin	Faradaic, nanoparticles modified Fe graphite electrodes	5 ng mL^{-1} in human plasma	Single analyte	[77]
cTnT	Conductance, metal-oxide semiconductor-compatible silicon nanowire	In buffer solution – 10^{-6} ng mL^{-1} Undiluted human serum – 3×10^{-6} ng mL^{-1}	Single analyte	[78]
Myoglobin	Polyaniline nanowires/conductance	1.4 ng mL^{-1}	Single analyte	[79]
cTnI	SnO_2 nanobelt FET sensor/IV curve	~2 ng mL^{-1}	Single analyte	[80]
Magnetic biosensors				
CRP and creatine kinase isoenzyme MB fraction (CKMB)	Magnetometer sensing coil/coated paramagnetic particles	CKMB – 1–15 ng mL^{-1} CRP – 1–40 ng mL^{-1} LOD-CKMB – 2 ng mL^{-1} LOD-CRP – 3 ng mL^{-1}	Multianalyte	[86]
CRP	Streptavidin-coated magnetic beads and avidin-coated polystyrene microspheres/beads	10^{-5} ng mL^{-1}	Single analyte	[87]
CRP	Solid phase (polyclonal anti-CRP conjugated silica microparticles), labeling agent	2×10^{-10} ng mL^{-1}	Single analyte	[88]
CRP	Antibody conjugated dextran iron oxide nanoparticles (70 nm) as superparamagnetic labels	3×10^3 ng mL^{-1}	Single analyte	[89]
CRP	Magnetometer sensing coil/coated magnetic beads	25 ng mL^{-1} to 2.5×10^3 ng mL^{-1} LOD – 25 ng mL^{-1}	Single analyte	[90]
cTnI	Magnetic tweezer/magnetic beads	0.368 ng mL^{-1}	Single analyte	[20]

of acute myocardial infarction (AMI). During the heart infarction, the troponin T (TnT) is immediately released to the bloodstream, a biosensor able to monitor this biomarker in a short time could improve patient care by allowing a definite diagnosis of myocardial infarction in real time.

Elevated concentrations of these cardiac markers in serum are associated with recurrent CVD events and higher death rates. Simultaneous quantification of these biomarkers allows clinicians to diagnose CVD quickly and/or to accurately design a patient care strategy. A fast and reliable detection of these proteins will also help medical professionals differentiate diseases among those showing similar symptoms. The clinically significant sensing ranges of myoglobin, BNP, cTnI, and CRP are extremely low (pM to nM), and therefore, assay methods for these biomarkers need to be highly sensitive. In recent years, different biosensor platforms have been designed for detection of available cardiac disease biomarkers. Here, we summarized the most prominently used cardiac biomarkers and their detection by different biosensor platforms.

3. Biosensors for CVDs

A biosensor is a device designed to detect and quantify target molecules that is widely used as a powerful analytical tool in medical diagnostics [8]. It includes proteins detection, nucleic acids or monitoring antigen–antibody interaction. In principle, it is generally fabricated by immobilizing a biological receptor material, for instance, antibody, DNA, or RNA on the surface of a suitable transducer that converts the biochemical signal into quantifiable electronic signals. A range of sensors has been developed for CVD markers detection. Here, a summary of available different sensor platforms for the detection of CVD and most prominently used CVD markers is presented and summarized in Tables 1 and 2). This signal can be electrochemical, optical, mass change (piezoelectric/acoustic wave) or magnetic in nature [17–20].

3.1. Optical biosensors

Optical biosensors are considered as the most sensitive techniques that are based on the change in the phase, amplitude, polarization, or frequency of the input light in response to the biorecognition processes. Optical biosensor can be classified in to following categories, such as colorimetric, fluorescence, luminescence, surface plasma resonance (SPR) and fiber optics/bio-optrode based biosensors. In colorimetric and fluorescence-based detection, either target or biorecognition molecule is labeled with chromogenic/fluorescent tag, such as dyes. The change in the intensity of the color/fluorescence signal indicates the presence of the target molecules, which is extremely sensitive, with the detection limit down to a single molecule [21]. In label-free detection methods, the target molecules are not labeled, and are detected in their native forms. This type of detection is relatively easy and cheap to perform that also allows quantitative/kinetic measurement of molecular interactions by surface plasma resonance or optical fiber biosensors [reviewed in 21]. Although, optical biosensors are highly sensitive, they are bulky, expensive and require dedicated personnel to perform the tests. Additionally, colorimetric, fluorescence and luminometric type of sensors require difficult labeling procedures that depend on indirect indicator based signal schemes.

3.1.1. Fluorescence based biosensors for cardiac markers detection

A range of different cardiac biomarkers has been detected on different optical based biosensor platforms. Current research has more focused on the methodologies for multi-analyte detection and the integration of immunosensors into chip based microfluidic platforms. For example, a self-regulating microfluidic networks

based immunosensor is designed for the multianalyte cardiac marker detection that includes CRP, Mb and cTnI [22]. Fluorescence microsphere immunoarray based platform was employed using antibody sandwich assay for the detection of cytokines such as, TNF-α, IL-1, IL-6, and IL-8 [23]. In such type of multi-analyte detection platform, simultaneous detection of multiple markers, the rapid availability of results and the simplicity of the experimental setup makes this approach an option for point-of-care testing devices. A novel sandwich fluoroimmunoassay for IL-6 detection with functionalized Rubpy-encapsulated fluorescent core–shell silica nanoparticles labels has been developed [24]. Here, IL-6 was measured based on the specific interaction between captured IL-6 antigens and functionalized fluorescent core–shell nanoparticles-labeled anti-IL-6 antibodies. The reported method offered potential advantages of sensitivity, simplicity and reproducibility for the determination of IL-6 in serum samples. In another approach, a 20-fold enhancement of emitted light by fluorescent molecules was achieved by employing photonic crystals coupled with colloidal quantum dot emitters that resulted in high signal-to-noise ratio for cardiac marker (TNF-α) detection [25]. Improvements in photonic crystal performance can be made to further lower the detection limits that is useful in detecting new biomarkers associated with the disease. While Jung et al. developed a competition-based tagged internal standard assay to sensitively measure the sub-nanogram levels of CRP in human serum [26].

In another approach, onchip sandwich assay for the detection of CRP, which is similar to microarrays using fluorescent tags is reported [27]. This method was superior in terms of CRP measuring range, but lower in data reproducibility compared to other methods. A relatively new method without employing antibody for the estimation of CRP in serum was developed [28]. This method centered on the variation of fluorescence intensity of copolymers, containing a fluorophore (Fluoreseinamine isomer 1) and a CRP ligand, O-phosphorylethanolamine (PEA). Here, the copolymer containing fluorophore quenches the fluorescence in the presence of PEA that eventually determines the CRP levels. Further, an antibody-tagged fluoro-microbead guiding chip integrated with microchannels for simultaneously five identical tests was developed for the detection of cTnI in human plasma [29]. Design, picture and schematic diagram of the sandwich assay on fluoro-microbeads guiding chip is shown in Fig. 2. This chip contained five gold functional surfaces to perform five identical tests simultaneously through cTnI antibody-linked fluoro-microbeads as the detection component (Fig. 2c: System 1). To amplify the antigen–antibody binding signal, the avidin–biotin affinity interaction was employed. After immobilization of the capture antibody and binding of the target antigen cTnI, biotin-conjugated cTnI detection antibody was loaded into the chip and reacted for 30 min. After washing the micro-channels with PBS, 0.005% avidin-conjugated fluoro-microbeads were injected (Fig. 2c: System 2). Then the amplified signal from the avidin–biotin reaction was detected under a fluorescence microscope. Human plasma samples were spiked with varying concentration of cTnI for testing in the Systems 1 and 2 for sensitively detecting cTnI in complex mixtures (Fig. 2c). However, addition of microfluidic components can minimize procedural steps such as sample preparations.

3.1.2. Luminescence and colorimetric methods for cardiac marker detection

Recently, luminescence based biosensing platforms and methods were examined for the early cardiac biomarker detection [14,30–35]. The luminescence methods are broadly classified into two types, namely chemiluminescence and electroluminescence. In chemiluminescence, luminescent signal is generated by the action of an enzymatic antibody label in the presence of luminogenic substrate while in the case of electroluminescence; the luminescent signal is induced following an electron transfer reaction of a luminescent compound immobilized near the proximity of an electrode surface.

A cardiac chip was developed by exploiting a geometry that allows for isolation and entrapment of single polymeric spheres in micro-machined pits, while providing to each bead the rapid introduction of a series of reagents/washes through microfluidic structures. Optical signals derived from single beads are used to complete immunological tests for the simultaneous detection of the cardiac risk factors, such as C-reactive protein and interleukin-6 (IL6) in human serum samples [30]. In another approach, PDMS surface was directly modified with a randomly oriented protein antibody spots during the polymerization [31] relies on the ability of the PDMS polymer to entrap macromolecules at its surface while the polymerization process occurs. This method was employed to develop a sensitive sandwich immunoassay for CRP detection. The main advantage of this concept is to be able to combine both the macromolecule immobilization, without the need of additional chemicals, and the easy 3D structured platform for sandwich assays. CRP measurements were also performed by using an integrated microfluidic chip incorporated with magnetic beads [14]. The magnetic beads coated with CRP-specific DNA aptamers recognize, purify and enrich the target CRP from the sample that was sandwiched by binding with acridinium ester-labeled CRP-antibody for chemiluminescence signal. The development of this microfluidic system is promising for fast, accurate, and sensitive detection of CRP.

Currently, electroluminescence (ECL) and colorimetric biosensing platforms have been less extensively examined by researchers for cardiac markers detections [34,35]. Based on the ECL of CdS nanocrystals, a novel label-free ECL biosensor for the detection of low-density lipoprotein (LDL) has been developed by using self-assembly and gold nanoparticle amplification techniques [34]. The LDL concentration was measured through the decrease in ECL intensity resulting from the specific binding of LDL to apoB-100 (ligand of LDL receptor). The ECL peak intensity of the biosensor decreased linearly with LDL concentration in the range of 0.025–16 ng mL^{-1} with a detection limit of 0.006 ng mL^{-1}.

Colorimetric method using poly(dimethylsiloxane) (PDMS)–gold nanoparticles (AuNPs) composite film as basis with silver enhancement has been reported for the detection of cardiac troponin I (cTnI) [30,32]. In this study PDMS material was used because its advantages. Firstly, the polymer matrix of PDMS could protect AuNPs from aggregation, so this PDMS–AuNPs composite film could be well stored at 4 °C for several months which enhanced the stability and prolong shelf-life of the device. Experimental procedure for silver enhancement colorimetric detection of cardiac troponin I, is shown in Fig. 3. For cTnI detection, the monoclonal antibody against cTnI was firstly immobilized on the PDMS–AuNPs composite film, followed by blocking solution and cTnI under the procedure as shown in Fig. 3. The mechanism of gold basis silver enhancement method is that AuNPs play a role of catalyst during reactions of silver reduction, and this catalytic ability could be inhibited when there were proteins covering the surface of AuNPs, which influence the amount of reduction silver metal, which led to the color difference in the reaction mixture. This inhibition effect could be distinct due to different species, quality and/or quantity of covering proteins. The results were consistent with that from Enzyme-linked immunosorbent assay (ELISA), which is considered to be one of the most sensitive and standard techniques [30].

3.1.3. ELISA based methods for cardiac marker detection

ELISA typically relies on optical properties of chromogenic reporters to give rise to a sensitive signal into either colorimetric, fluorogenic or luminescent forms, respectively. ELISA has been

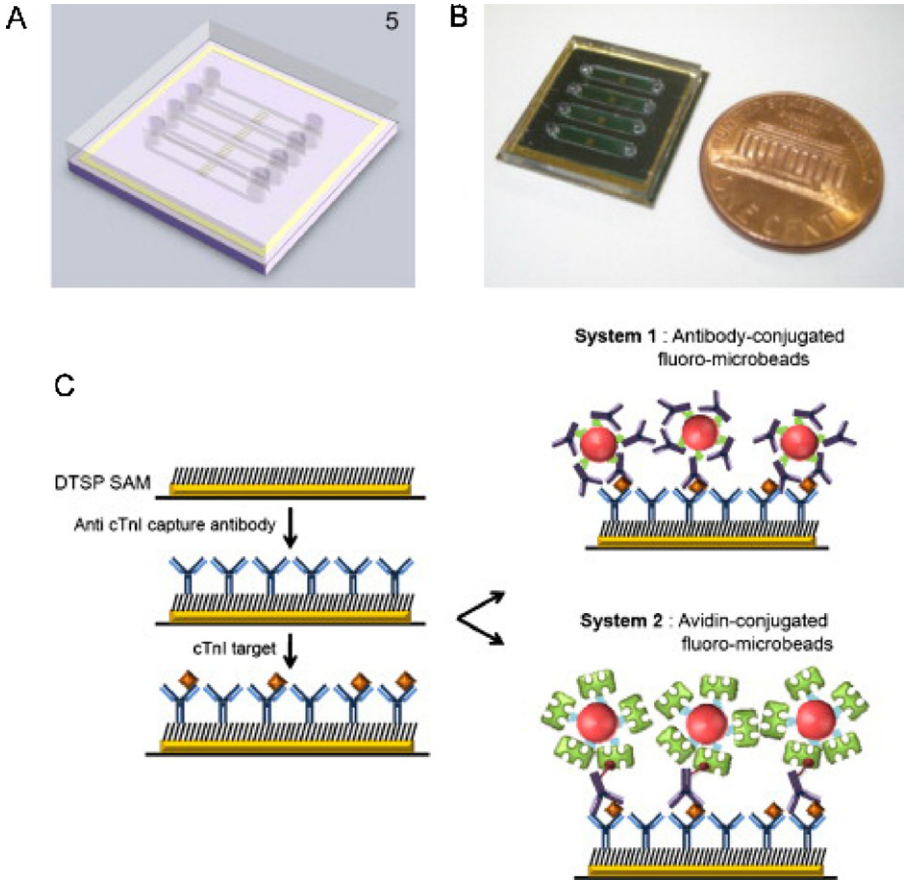

Fig. 2. (a) Design, (b) photograph and (c) schematic diagram of the sandwich immunoassay on the fluoro-microbeads guiding chip [29].

widely utilized for the detection of cardiac markers [36–39]. Darain et al. reported a simple and highly efficient sandwich ELISA in which in-channel polystyrene was immobilized with antibodies, and fabricated plastic based microfluidic chip for the detection of myoglobin [38]. Cardiac multianalytes, such as heart-type fatty-acid binding protein (H-FABP) and C-reactive protein (CRP) were detected in a simple, rapid and "digital-style" semi quantitative lateral flow assay using an in-house ELISA [36]. This assay was able to simultaneously detect cardiac markers and enables predicting early CVD risk, simply by counting the number of red lines in the test without any expensive reading instruments. Cho et al. reported a chemiluminometric biosensor system for point-of-care testing using an immuno-chromatographic assay combined with an enzyme (e.g., horseradish peroxidase) tracer, which produces a light signal measurable on a simple detector [37]. In this work, to enhance the sensitivity, biotin–streptavidin capture technology was employed in preparing an immuno-strip that was then integrated onto the chip in order to generate the ELISA-on-a-chip (EOC) biosensor, and sensitively detected cTnI as low as 0.027 ng mL^{-1} detection limit. However, further miniaturization of sensor platform has to be provided without sacrificing the detection capability.

A relatively different method involving specific peptide sequence of cardiac marker detection has been reported. This method utilizes polyvalent phage display to isolate unique linear peptide motifs which recognize both the human and rat homologs of troponin I [39]. The peptide specific for human troponin I has a sequence of FYSHSFHENWPS, while FHSSWPVNGSTI for the rat troponin I that later detected by ELISA. It was shown that the binding affinities of the phage displayed peptides were decreased by the presence of complex tissue culture media, and the addition of 10% calf serum further interfered with the binding of the target proteins. In this study, kinetic indirect phage ELISAs revealed that both troponin I binding peptides were found to have nanomolar affinities for the troponin proteins while attached to the phage particles [31]. These new peptides may have potential utility in the development of new clinical assays for cardiac injury as well as in monitoring of cardiac cells grown in culture.

3.1.4. SPR based biosensor for cardiac marker detection

Surface plasmon resonance (SPR) is a unique optical transduction method, which has been commercially employed for optical biosensors [40]. SPR biosensors exploit special electromagnetic waves – surface plasmon to probe changes in the refractive index (RI) at surfaces of metals. SPR biosensors can therefore be used to monitor the interaction between an analyte and its biospecific element immobilized on the metal surface without the use of labels. The basic SPR apparatus is referred to as the Kretschman prism arrangement [40].

In the SPR, a thin film of metal (usually a 400–500 Å thick gold or silver film) is coated on the prism onto which a biosensing layer is coated by immobilization of biorecognition elements (proteins/antibodies/DNA/RNA). When an incident light of an appropriate wavelength strikes on the dielectric–metal interface at a particular angle, it induces SPR phenomenon at the metal surface. Depending upon the thickness of a molecular layer at the metal surface, SPR results in a graded reduction in the intensity of the reflected light [41]. Thus, one can observe a sharp minimum of light reflectance when the angle of incidence is at this proper resonant angle. Since the resonance angle depends on several factors (e.g., the wavelength of the incident light, the metal, and the nature of the media in contact with the surface) the nature of the media can be sensed by measuring this resonance angle. Further,

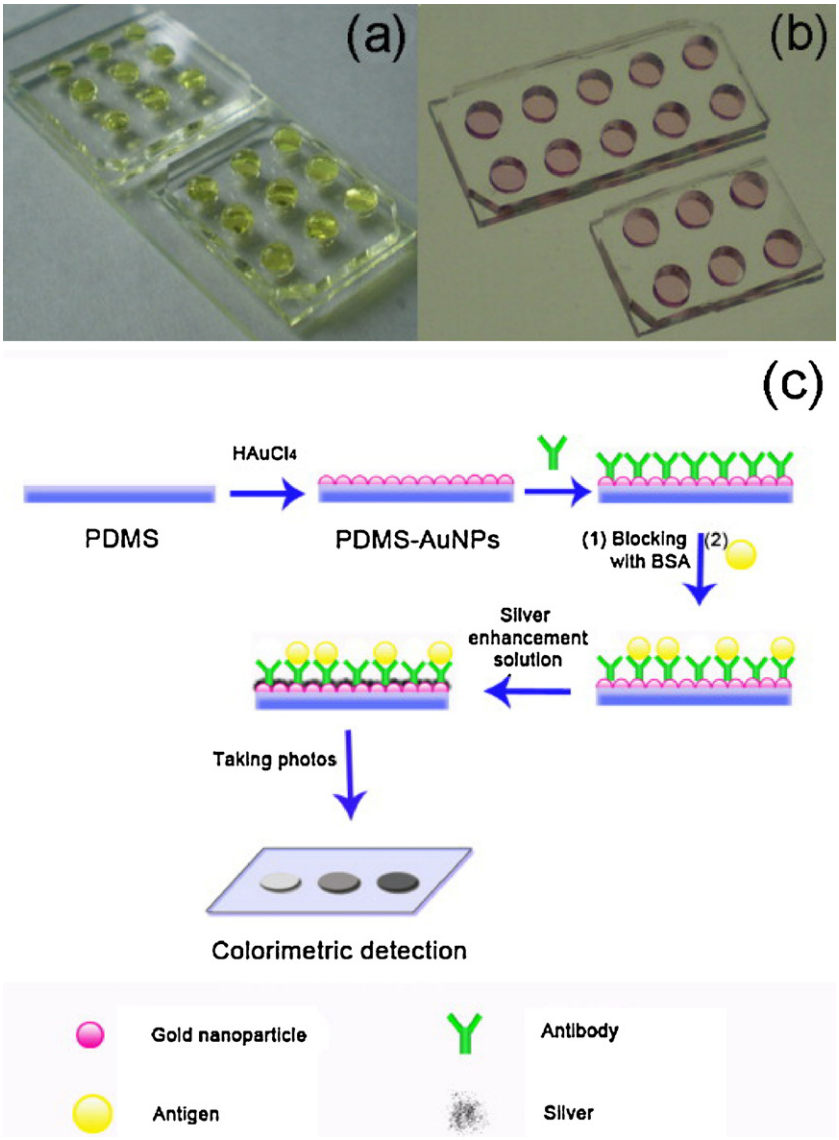

Fig. 3. Experimental procedure for silver enhancement colorimetric detection of cardiac troponin I: (a) PDMS chip with $HAuCl_4$ solution, (b) photo of PDMS–AuNPs composite film and (c) schematic diagram for colorimetric detection [32].

in addition to the prism coupling, SPR sensors can also be based on optical fibers or integrated optical waveguides. Biomedical applications take advantage of the exquisite sensitivity of SPR to the RI of the medium next to the metal surface, which make it possible to measure accurately the capturing of target biomarkers on the metal surface [41].

SPR based sensing platforms have been extensively studied and applied for the detection of cardiac makers that include metalloproteinase (MMP)-2 [42], CRP [42–47], B-type natriuretic peptide (BNP) [48], cTnT [49–51], myoglobin [52], cTnI [18,52], TNF-α [53], and MPO [54]. A one-step SPR based sandwich assay format for the quantification of MMP-2 has been reported with Biacore 2000, using colloidal gold with a particle diameter of about 20 nm for signal amplification [42]. The reported assay has a detection limit below the level documented by established methods for MMP-2 detection (Table 2). A modified CRP (mCRP) was detected using SPR based sensor because mCRP is regarded as a powerful inducer than pentamer CRP (pCRP), therefore, mCRP is considered to be important indicator for assessing the risk of developing CVD. Three monoclonal antibodies (Mabs), C8, 8D8, and 9C9, were immobilized on a protein G layer for subsequent CRP detection. It is shown that detecting pCRP using Mab C8, the SPR bioassay provided sufficient sensitivity to evaluate whether or not a patient is at risk of developing CVD [43]. A miniaturized immunosensor is designed to determine trace levels of cardiac marker, B-type natriuretic peptide, using a microfluidic device combined with a portable SPR sensor system that showed a detectable concentration range 5 pg mL^{-1} to 100 ng mL^{-1} by monitoring the SPR angle shift, which covers the required detection range for the B-type natriuretic peptide concentrations found in blood [48].

A carboxymethyldextran hydrogel planar sensor chip was employed for the detection of cTnT [49]. This sensor was coupled to the SPR AutoLab Spirit® system (Eco Chemie, Netherlands) attached at the bottom of the prism with cedar oil and a flow cell was installed on the chip. In this work, the well-established biotin–streptavidin chemistry was used to increase the amount of immobilized antibodies by highly specific interaction between the biomolecules. Further, two myocardial infarction biomarkers, myoglobin and cardiac troponin I, were quantified at biological levels and in undiluted serum without sample pretreatment using SPR sensors and the detection limits for both markers were below 1 ng mL^{-1} [52]. A modified surface chemistry on SPR

sensor surface for CRP detection is developed in which poly(3-(2-((N-succinimidyl)succinyloxy)ethyl)thiophene) (P3SET), a poly(thiophene) with pendant NHS ester groups was prepared and used for the formation of a self-assembled monolayer (SAM) on a gold surface for CRP biosensor fabrication [46]. Improvements in the fabrication of SPR sensors have been attempted and demonstrated the enhancement of sensitivity for an SPR interface using Kretschmann's configuration, through nano-gratings combined with nano-patterned immobilization of surface bioreceptors [53]. This configuration resulted in high electromagnetic field intensity for the detection of TNF-α. In an interesting approach *Escherichia coli* outer membrane with auto displayed Z-domain was used as a molecular recognition layer for the detection of CRP using SPR biosensor [47]. From this study, the LOD of SPR biosensor was estimated to improve more than 100-fold compared to the SPR biosensor with the antibody-layer by physical adsorption. The main problem of SPR sensor could be mainly because of its fouling ability, low affinity and specificity that affect sensitivity of biosensing transducers. One study attempted to improve these factor by utilizing a modified method of SAM formation using a homogeneous mixture of oligo(ethylene glycol) (OEG)-terminated alkanethiolate and mercaptohexadecanoic acid (MHDA) on gold surface for detecting cardiac troponin T [51].

3.1.5. SPR based fiber optic biosensors for cardiac marker detection

Recently, SPR based on optical fibers have been utilized for the detection of cardiac specific biomarkers BNP [55], cTnI, Mg [55], CRP [16,55,56], nerve growth factor (NGF) [57], and IL-6 [58]. The detection principle of a large class of fiber optic biosensors (FOBs) is based on the principle of total internal reflection fluorescence (TIRF) [55,56]. An evanescent wave forms when the light propagates in the fiber core at an incident angle greater than the critical angle, which results in total reflection of incident light. This is due to the change in high to low refractive indices of the media, respectively. The evanescent wave can be used to excite fluorophores of the immobilized fluorescently labeled species, resulting in the evanescent wave excitation of fluorescence. The intensity of the evanescent wave decays exponentially along the direction perpendicular to the interface (typical penetration depth of 100–200 nm range). This characteristic enables real-time monitoring of the kinetics behavior between receptors and target analytes during the measurement. Moreover, the detected signal intensity of an FOB depends on the amount of fluorophores in the captured target molecules, and the relationship between the signal response and the concentration of target molecules can be determined accurately. Studies involving use of novel FOB has been reported to determine the mCRP-anti-CRP binding kinetics [55]. In this FOB platform the fluorescence signal excited by evanescent wave in the near field region of fiber core surface and a photomultiplier tube (PMT) facing the fiber wall, and perpendicularly to the fiber axis was fixed that enabled to collect the fluorescence signal more efficiently in the FOB. The reported FOB was capable of detecting CRP at physiological concentration. Sandwich immunoassay has also been performed using FOB in which fiber optic probe was used as a dip probe for the sensitive detection of IL6 (5 pM or 0.12 ng mL^{-1}) [58].

3.1.6. Other optical based biosensors

Refractive index based other optical biosensors have been reported. These include, resonant biosensor to detect CRP markers and silicon photonic mircroring resonators using optofluidic platform to detect IL-6 [59,60]. Optomagnetic biosensors have been applied for the detection of cardiac troponin cTnT and I [61,62]. While a prototype hand held device was developed actuated magnetic nanoparticle labels (500 nm) bound to the sensor surface via a sandwich immunoassay for the optomagnetic detection of cTnT [61]. Optomagnetic detection of cardiac biomarkers using functionalized magnetic particles has high potential in analytical performance and ease-of-use that is suitable for applications in point-of-care diagnosis [61,62].

3.2. Acoustic biosensors for detection of cardiac markers

Acoustic biosensors allow label-free detection of biomolecules and analysis of binding events mainly for CRP and interleukin family of proteins. The detection of these markers is based on the mass of captured analyte by an immobilized receptor molecule on the quartz crystal resonator surface, which is proportional to the resonant frequency [63]. Their detection has also been demonstrated by using surface acoustic wave based biosensor platform [19,63]. It was found that $ZnO/SiO_2/Si$ Love mode surface acoustic wave (SAW) biosensor for the detection of interleukin-6 (IL-6) has been reported with high sensitivity. This sensor consisted of fully integrated CMOS Si chips for portable real time detection of interleukin family of proteins in human serum [19]. However, performances of acoustic biosensors depend on factors, such as its temperature dependence, attenuation and the Raleigh waves are surface-normal waves, deposition of non-specific molecules, dust or other contaminants on sensor crystals may contribute to large background signal noises that may affect sensitivity and specificity of the sensors.

3.3. Electrochemical biosensors

Electrochemical biosensors are affinity based biosensors where they use an immobilized recognition element (probe) that binds the target/analyte molecule selectively. The detection of binding a target to probe in solution is governed by detecting change at a localized surface in terms of change in currents and/or voltages. Based on their operating principle, the electrochemical biosensors can employ potentiometric, amperometric and impedimetric transducers converting the chemical information into a measurable amperometric signal. Thus, this category excludes sensors which require light (e.g., surface plasmon resonance or fluorescence), mechanical motion (e.g., quartz crystal microbalance or resonant cantilever), or use of magnetic particles. Due to their low cost, low power and ease of miniaturization, electrochemical biosensors hold great promise for applications where minimizing size and cost is crucial, such as point-of-care diagnostics and bio-warfare agent detection.

The first scientifically proposed and successfully commercialized biosensors were those based on electrochemical sensors for multiple analytes. Currently, transducers based on semiconductors and screen printed electrodes represent a typical platform in electrochemical biosensor for detection of CVR markers. Electrochemical biosensors for the detection of cardiac markers, including cardiac troponin I or T (cTnI/T), myoglobin, CRP, Lipoprotein-associated phospholipase A, IL-6, LDL and MPO have been published in the last few years. Most notably, the detection of myoglobin was performed by a miniaturized point-of-care sensor, which was based on impedimetric sensing of cardiac enzyme captured by an antibody layer immobilized on a planar gold electrode sensor [64]. Gold/Ti-on-glass substrate has been used on which the working electrode was immobilized with antibodies that are in turn integrated with a microfluidic system. The presented biosensor was able to detect the myoglobin antigen concentration of 100 ng mL^{-1}. A number of studies on developing electrochemical biosensors for the detection of CRP have been published in the last few years [65–71].

A three-dimensional ordered macroporous (3DOM) gold film modified electrodes have been employed for the development of electrochemical impedance biosensor for the detection of CRP [66].

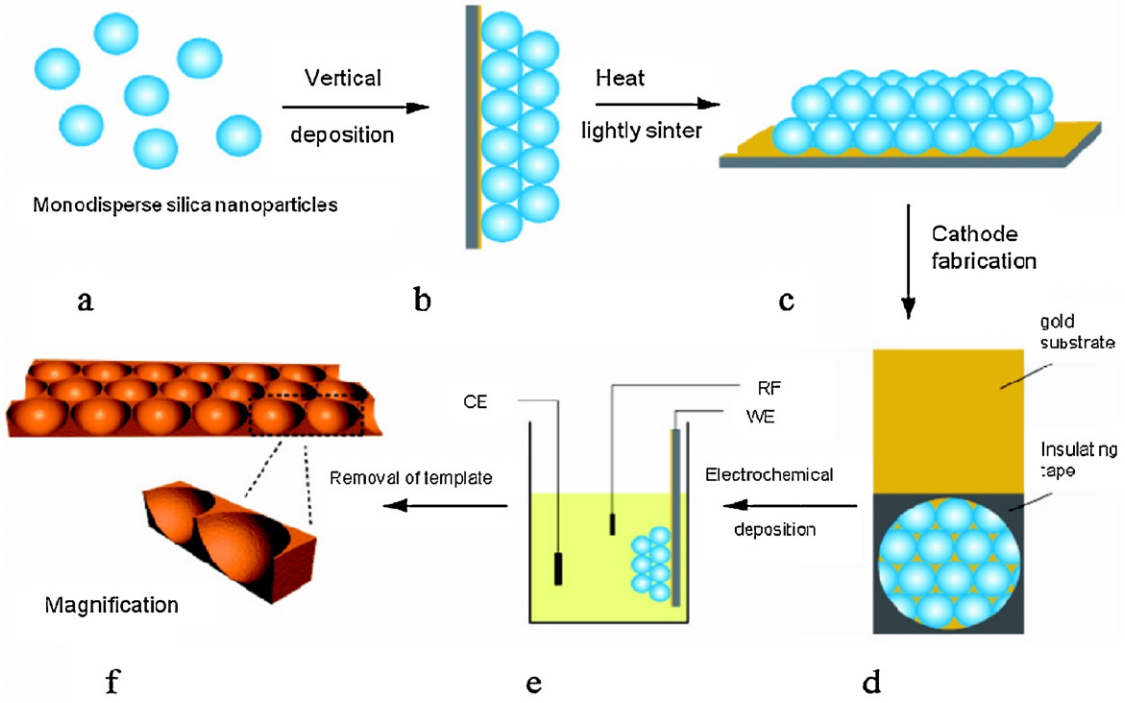

Fig. 4. Procedure for preparation of 3DOM gold film [66].

The procedure for preparation of 3DOM gold film electrodes is shown in Fig. 4.

The electrode used in this sensor was electrochemically fabricated with an inverted opal template that enabled enhanced surface area of the 3DOM gold film up to 14.4 times higher compared to classical bare flat electrodes. The presented CRP immunosensor was developed by covalently conjugating CRP antibodies with 3-mercaptopropionic acid (MPA) on the 3DOM gold film electrode and schematic is presented in Fig. 5. The CRP concentration was measured through the increase of impedance values in the corresponding specific binding of CRP antigen and CRP antibody. This enhanced sensor surface area of 3DOM gold film after immobilizing CRP antibodies increased the electron-transfer resistance (Ret) values that were proportional to the CRP concentrations in the range of 0.1–20 ng mL^{-1}. The lowest detection limit here for CRP antigen was as low as 100 pg mL^{-1} [66].

A possibility of using a range of dc and ac electrochemical techniques to probe associative interactions of CRP with CRP antibody immobilized on a gold electrode surface was investigated by Hennessey et al. [68]. It was demonstrated that the investigated electrochemical techniques can be used efficiently to probe these interactions over a wide CRP concentration range, from 1.15×10^{-5} to 1.15 mg L^{-1}. The measured sensitivity of the techniques was in the following decreasing order: differential pulse voltammetry, charge-transfer resistance obtained from electrochemical impedance spectroscopy (EIS), cyclic voltammetry, chronoamperometry, and double-layer capacitance deduced from EIS measurements which gave the poorest sensitivity. The lowest detection limit of CRP antigen concentration was 6×10^{-6} mg L^{-1}. A capacitive biosensor based on chronoamperometry was developed by using diamond-like carbon (DLC) film as an electric isolating layer and applicability of the biosensor was demonstrated by detecting CRP [69]. The lowest detection limit of CRP antigen concentration in this method was 0.01 pg mL^{-1}. Centi et al. developed electrochemical sandwich assay for the detection of CRP [67]. The screen-printed carbon electrodes and magnetic beads were employed in this assay to develop electrochemical assay that had a limit of detection of 0.054 mg L^{-1} CRP in serum.

Electrochemical biosensors based detection offer sensitivity, selectivity and reliability, making them very attractive tools for biomarker protein detection. However, these biosensors suffer from perturbations on the sensor surface that are influenced by different pH, ionic strength and co-existing molecules in biological fluids. The biological recognition elements used in a majority of electrochemical biosensors are antibodies. The fact that these antibodies are generated, isolated and purified after the administration of a desired antigen in to the animal models making them inherently trained to function in normal physiological solutions. This makes antibodies difficult to contain specific binding ability outside of the physiological environments or synthetic buffers. The functionality of antibodies may further decline in presence of external redox mediators that are essentially mixed with the sample solution for electrochemical sensor platforms.

3.3.1. *Use of nanomaterials in electrochemical biosensors*

Use of nanomaterials in electrochemical biosensors has received considerable attention by the research groups over the past decade. The demonstration of novel and unique detection platform for cardiac marker detection by utilizing nanoparticles, nanotubes, nanowires and nanocomposites has been reported. There is much versatility in the selection of nanomaterials to measure the detectable signal by which analyte concentrations can be determined. Recently, a sensitive, disposable, and easy to operate sensor based on heterogeneous sandwich immunoassay for high sensitivity CRP (hs-CRP) measurement was developed [65]. This biosensor utilized screen-printed electrodes modified with multi-walled carbon nanotubes (SPE/CNTs) and protein A to ensure the oriented immobilization of anti-CRP antibodies and sensitively detected CRP at 500 pg mL^{-1} levels. In another study, detection of lipoprotein-associated phospholipase A2 (Lp-PLA2) is reported in which carbon paste doped with iridium nano-particles as working electrodes was used as biosensor [72]. Here, the detection of Lp-PLA2 was in the range 0–150 U mL^{-1} with a sensitivity of 1.45 nA/U. The detection of low-density lipoprotein (LDL) using silver chloride@polyaniline (PANI) core–shell nanocomposites (AgCl@PANI) combined with Au nanoparticles (AuNPs) hybrid material has been reported [73]. The

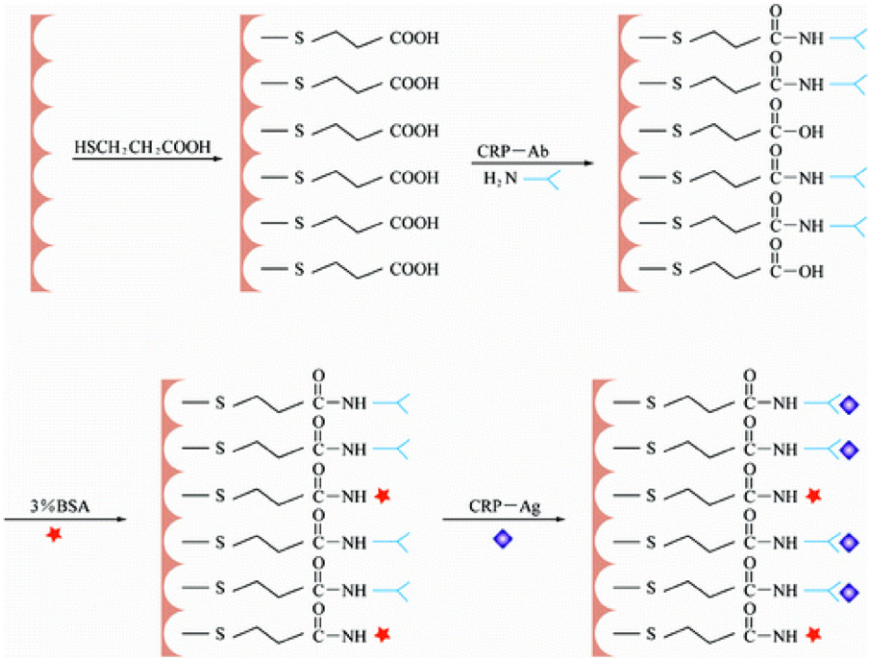

Fig. 5. Schematic of the immunosensor fabrication process [67].

hybrid material was used to provide surface for high antibody loading due to its large surface-to-volume ratio. The method was based on adsorption of antibody to apolipoprotein B-100 (aopB-100) on an AuNPs-AgCl@PANI-modified glassy carbon (GC) electrode. The biosensor exhibited a highly sensitive response to LDL with a detection limit of 0.34 pg mL^{-1}.

In a study by Zhou et al. developed an electrochemical immunoassay for the simultaneous detection of cardiac troponin I (*c*TnI) and CRP in which CdTe and ZnSe quantum dots were utilized for sandwich immunoassay. Here, Cd^{2+} and Zn^{2+} were detected by square-wave anodic stripping voltammetry to enable the quantification of the 2 biomarkers in 20 human serum samples [74]. Similarly, Kunduru et al. designed nanotechnology-based electrochemical biosensor using micro- and nano textured polystyrene polymer structures. The results demonstrated that scaling down the surface texturing from the micro- to the nano scale enhances the sensitivity of this detection method for CRP [75]. In the work by Ahammad et al., gold nanoparticles have been electrodeposited on indium tin oxide (ITO) and applied to detect molecular interaction between human cardiac *c*TnI and specific antibody by measuring open circuit potential (OCP). In this work, a new strategy has been adapted to obtain an electrical signal due to the enzyme-based immune catalytic reaction and detected 1–100 ng mL^{-1} I *c*TnI [76]. Electrochemical immunosensor based on metal nanoparticles for cardiac myoglobin detection in human blood plasma has been reported [77]. In this work, cardiac myoglobin detection was based on direct electron transfer between the Fe(III)-heme and the electrode surface that was modified with metal nanoparticles (gold, silver, and copper) stabilized by didodecyldimethylammonium bromide and antibodies. The method proposed does not require labeled secondary antibodies that resulted in a detection limit of 5 ng mL^{-1} with a broad range of working concentrations.

The iridium oxide (IrOx) nanowires based electrochemical biosensor was presented for the detection CRP and MPO cardiovascular disease biomarkers [71]. This biosensor was based on electrical detection of protein biomarkers wherein an immunoassay was built onto the iridium oxide nanowires that in turn undergoes specific electrical parameter perturbations during each binding event associated with the immunoassay. The study demonstrated that the iridium oxide nanowires has an ability to detect very small changes to the surface charge and this capability is utilized for achieving the performance metrics and forms the basis of the key innovations of this technology to improve selectivity and sensitivity of detection.

3.3.2. *Electrochemical biosensors without electron mediators*

Another class of electrochemical biosensors that do not utilize redox mediators known as non-Faradaic electrochemical sensor has been employed for the cardiac marker detection in buffer and complex serum greatly varies because of the interference of the non-specific molecules and ions. Capacitive electrochemical (non-Faradaic) immunosensor has recently been presented [78] for the detection of TnT based on the use of immobilized antibodies specific for TnT. The device was able to detect TnT levels in the range 0.07–6.83 ng mL^{-1} in human serum from patients with cardiac diseases and in the range 0.01–5 ng mL^{-1} for TnT in phosphate buffer saline. In the work by Qureshi et al. [79], gold interdigitated capacitor arrays were employed for the development of reagent-less, label-free capacitive electrochemical biosensors for the detection of CRP that is based on charge distribution on the surface of capacitors. The sensitive detection of CRP capturing on capacitors was observed in a dynamic range 100–500 pg mL^{-1} under standard conditions that includes the defined geometry of electrodes and specified biochemical assay conditions.

Electrochemical capacitive immunoassays are promising alternatives to existing immunochemical tests for the development of hand-held devices for point-of-care applications. The attraction of affinity-based capacitive sensors is that they are able to determine the analyte directly in a sample with no or very little sample preparation. The sensing principle of these sensors is based on changes in dielectric properties, charge distribution, and/or conductivity change brought on by bioreceptor–target analyte complexes formed on the surface of the electrodes. Capacitive affinity biosensors can be constructed by immobilizing recognition elements on the electrodes, and measuring changes in the dielectric/surface properties when an analyte binds. It can then be correlated to the bound target protein molecules and the amount captured by bioreceptors immobilized on the surface. For providing

larger sensor surface area, conductors can be made into a pattern of micro to nano-interdigitated fingers. However, non-specific and large background signals affect these types of sensors and reduce signal-to-noise ratio and therefore, further improvements is required for improving these label-free biosensors.

3.4. Magnetic biosensors

Magnetic particles/beads have been established in life sciences and used for many years in biological assays. A wide variety of biological species, such as cells, proteins, antibodies, pathogens, toxins, and DNA can be labeled by attaching them to superparamagnetic microbeads. These particles range in size from a few nanometers to a few microns, and the normal magnetic bead structure consists of many iron oxide (magnetite) crystallites, which provide the paramagnetic attraction of the particles to a magnet and they are encapsulated in plastic or ceramic spheres. The beads are coated with a chemical or biological species (e.g., DNA or antibodies) that selectively binds to the target analyte. To date, several authors described the interaction of magnetic beads with a magnetic field and the ensuing visualization of binding effects [80], or use the beads as a separation and magnetic immobilization platform [81,82] although magnetic beads/particles based biosensing platforms have been less extensively examined by researchers for cardiac markers detections.

A rapid immunoassay system based on antibody-coated micrometer-sized paramagnetic particle was reported by Luxton et al. [83] for the detection of cardiac markers including CRP and creatine kinase isoenzyme MB fraction (CKMB). This assay do not require sample preparation or washing step, and results can be obtained in less than 3 min after introducing the sample into the vessel with sensitivities in the normal clinical range.

The use of magnetic beads in CRP detection has also been described by other research group [84] where magnetic bead was used only for separation of bound and unbound aggregates of sandwich assay. The quantification of magnetic beads for the CRP detection has also been described by Kriz et al. [85]. In this publication, the authors describe the sedimentation of magnetic beads complexed with silica microparticles upon appearance of CRP in the sample. The content of magnetic beads in the sedimented phase of the measurement is then analyzed by a single magnetic resonant coil. The measurement needs only 11.5 min, but shows detection limits of 0.2 mg L^{-1} and a coefficient of variation (CV) of 11%. The same research group published a rapid detection system based on the same technique [86] which provides results after 5.5 min and has a detection limit of 3 mg L^{-1} and a CV of 10.5%. In another approach, CRP detection was reported by another research group [87] where the most significant difference between previously methods is the measurement head. This reported system uses two different excitement coils in a frequency mixing mode. This enables the very low detection limit of 25 μg L^{-1}, accompanied by the low CV of 4.2%.

A new strategy for the detection of cardiac troponin I that relies on competitive exchange interactions of an analyte was reported [20]. In this method, use of superparamagnetic beads and of magnetic tweezers apparatus allows a small force to be applied to the tethers, providing a driving force for the competitive exchange reaction and selectively detected protein in a sensitive reagentless fashion. Although, to obtain the reproducible results from the device, further optimization in the fabrication of sensor would be necessary for better sensitivity.

4. Conclusions and perspectives

It is critically important to diagnose CVD at early stages of its progression, which allows successful treatment and recovery of patients. Therefore, it is essential to develop simple and sensitive CVD diagnostic methods that can detect multiple cardiac biomarkers at very low concentrations in biological fluids. Current biosensing platforms can fulfill these demands but require sophisticated laboratory equipment and training. In this review, available biosensing platforms for CVD biomarkers detection are summarized. Most frequently used CVD biomarker/s for biosensing are in the order of CRP > cTnI > myoglobin > IL-6 > TNFα on optical, electrochemical and magnetic transduction formats. A majority of cardiac biomarkers are also markers of common inflammation, making it difficult to distinguish the cardiovascular risk. An inflammatory process in patients can occur without the existence of cardiovascular disease, which may incur a high rate of false-positives. Therefore, a multiple marker detection strategy needs to be adapted using a combination of established and new cardiac markers, which helps in making correct clinical decisions. Future innovation in biosensor technology can be directed toward (a) identification of new and non-inflammatory cardiac biomarkers and their validation, (b) development of novel biorecognition elements in place of classical antibodies, (c) direct detection of biomarkers in biological fluids (blood) without sample preparations, (d) miniaturization of electronic transducers for portability, and (e) patterning arrays and microfluidics suitable for multiple biomarker detection. Combinations of all the above features are essential to making devices of high potential for early CVD diagnosis with precision. Improving sensitivity, specificity, and making biosensors a low-cost and point-of-care capability is another challenge.

In recent years, nanomaterials have shown wide applications in biosensing. One of the most striking advantages of such nanomaterials is to use surface activated magnetic nanoparticles (SAMN). These SAMN enable functionalization and concentration of target protein molecules directly from the complex mixtures, such as blood/serum. Concentration of target protein from the complex serum can be achieved by magnetic nanoparticles functionalized with specific affinity ligands (antibodies/aptamers). The magnetic properties of nanoparticles provide magnetic separation simply by placing a magnet in close proximity of the suspension, making them concentrate with captured target proteins. This methodology holds great promise in electrochemical chip based biosensing, mainly for drawing the target captured magnetic nanoparticles on the sensor surface immobilized with specific affinity ligands. This method enables washing away the magnetic nanoparticles with no target while withdrawing the magnet away, and leaving behind only those that bound to the target proteins for specific detection and quantification. The concept of using nanomaterials as one of the candidates to further improve the sensitivity in developing highly sensitive devices for early diagnosis and point-of-care applications. Early diagnosis will aid in increase in the human survival rate that requires precise diagnosis, which is only possible with multiple biomarker detection for CVD risk. However, appropriate investment and funding support mechanisms are needed to facilitate moving this technology from research to the commercial applications.

Acknowledgements

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