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Electrochemical biosensors based on nanofibres for cardiac biomarker detection: A comprehensive review

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ABSTRACT

The vital importance of early and accurate diagnosis of cardiovascular diseases (CVDs) to prevent the irreversible damage or even death of patients has driven the development of biosensor devices for detection and quantification of cardiac biomarkers. Electrochemical biosensors offer rapid sensing, low cost, portability and ease of use. Over the past few years, nanotechnology has contributed to a tremendous improvement in the sensitivity of biosensors. In this review, the authors summarise the state-of-the-art of the application of one particular type of nanostructured material, *i.e.* nanofibres, for use in electrochemical biosensors for the ultrasensitive detection of cardiac biomarkers. A new way of classifying the nanofibre-based electrochemical biosensors according to the electrical conductance and the type of nanofibres is presented. Some key data from each article reviewed are highlighted, including the mechanism of detection, experimental conditions and the response range of the biosensor. The primary aim of this review is to emphasise the prospects for nanofibres for the future development of biosensors in diagnosis of CVDs as well as considering how to improve their characteristics for application in medicine.
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Contents

1. Introduction

Biomarkers are proteins found in body fluids or tissues, that reflect the normal or abnormal activity of the organism (Bohunicky and Mousa, 2011). Nowadays, it is well known that the appearance of biomarkers or variation of their concentrations are symptoms of various diseases. Cardiovascular disease (CVD) is one of the most pervasive non-communicable disorders which majorly threats human health (WHO, 2010). CVD is in directly linked with the heart and blood vessels. The decreasing in human mobility and increase in stress, which are the consequences of industrialisation

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and urbanisation during the recent centuries, are the main causes of CVD (Alwan, 2011). There are also other factors which can lead to CVD such as high blood pressure, being overweight, smoking, genetic transmission and *etc.* (Bahadır and Sezgintürk, 2015; Martín-Ventura et al., 2009).

Early treatment of myocardial defects are really beneficial, preventing irreversible damage to the heart and acute myocardial infarction (AMI). However, such interventions require early and accurate diagnosis of CVD (Kakoti and Goswami, 2013; Mohammed and Desmulliez, 2011). The World Health Organization (WHO) has identified some medical inspections which are routinely performed in hospitals for diagnosis of CVD: characterisation of a patient's chest pain (*Effort Angina* or *Spontaneous Angina*) (Rapaport, 1979), examining changes in the electrocardiogram (ECG), and measurement of specific cardiac biomarkers in the blood samples (Bahadır and Sezgintürk, 2015; Tunstall-Pedoe et al., 1994). Since the results from the first two (chest pain and ECG) are not entirely reliable for CVD diagnosis (Qureshi et al., 2012; Stubbs and Collinson, 2001), biochemical identification of cardiac biomarker concentration is required for confirmation for most of the patients suspected of CVD. Moreover, measurement of cardiac biomarkers concentration can provide prognostic information about the disease, which then aids clinicians in deciding how aggressively they need to treat the disease. Biochemical markers have played a critical role in facilitating accurate diagnosis of the cardiac damage for over half a century (Aldous, 2013; Tang and Casas, 2014). Cardiac biomarkers are defined as biological analytes that are released into circulation during the continuum of CVD or immediately after myocardial injury (Kakoti and Goswami, 2013; Thygesen et al., 2012). There are various type of biomarkers within the blood serum or plasma such as C-reactive proteins (CRP), creatine kinase-MB (CK-MB), creatine kinase MM (CK-MM), myoglobin (Myo), cardiac troponins (cTnI & cTnT), Heart-type fatty-acid-binding protein (H-FABP) and etc. (Kehl et al., 2012; Singh et al., 2010). A good biomarker is something that is easily measured and can be used as a surrogate marker for disease and its severity (Chan and Ng, 2010). Therefore, among the mentioned cardiac biomarkers, cardiac troponins are the preferred biomarkers as a CVD diagnostic and prognostic indicator because of their high sensitivity and specificity (Adams et al., 1993; de Lemos, 2013). *Ergo*, these biomarkers are deemed to be the gold standard for detecting myocardial damages (Chan and Ng, 2010; Falahati et al., 1999).

The concentration of cardiac biomarkers can be identified in the range of pM to nM through clinical analytical procedures (Hasanzadeh et al., 2013). For instance, Enzyme-Linked Immunosorbent Assay (ELISA) is one of the most widely applicable biochemical laboratory analyses (Lequin, 2005) and has been adopted as a standard approach because of its high sensitivity (Chua et al., 2009). Despite ongoing clinical diagnostic techniques enjoying accurate and trustworthy results for specific cardiac biomarker recognition, these techniques can be time-consuming and expensive (Yang and Zhou, 2006). It is desirable for a clinician to make a quick and absolute measurement of cardiac biomarker concentration at the time of hospital admission (Kost and Tran, 2005). Point of care (POC) tests are rapid and relatively inexpensive diagnostic instruments which can improve patient safety and efficacy of treatment (Justino et al., 2013; McDonnell et al., 2009). In this regard, biosensors can make a major contribution to the development of point-of-care diagnostic instruments (Wang, 2006).

In general, classification of biosensors is proposed in terms of the type of biological element immobilised on the surface of transducer or the mechanism of detection (Scheller et al., 1989). Enzyme sensors are a kind of biosensor in which an enzyme catalyses the conversion of analyte to product. In order to facilitate the shuttling of electrons between the active site of the enzymes and the electrode surface, and subsequently amplify the analytical response as well as minimise the interference, chemical mediators with reversible redox capability have been widely used in enzyme electrodes. Affinity sensors or "immunosensors" are another type of biosensor in which, for example, the detection of antigen as a target analyte is a result of the specific binding of the antigen to the particular region of an antibody attached the surface of the transducer (Bahadır and Sezgintürk, 2015; Moina and Ybarra, 2012). In respect that the binding or affinity constant between antibody and antigen is usually very large, such systems are almost always irreversible or single-use biosensors (Thévenot et al., 2001).

On the other hand, the mechanisms of detection can be considered as a starting point for another type of classification. The largest number of studies have been conducted on optical and electrochemical biosensors (Perumal and Hashim, 2014). Most quantitative immunoassays also use optical sensing schemes (like fluorescence or luminescence detection methods). They yield reliable results and high molecular sensitivity, but expensive and bulky optical imaging equipment is required for observing the optical variations by labelled immunological detection (Warsinke et al., 2000). On the contrary, since electron-transfer process in the electrochemical reactions directly generates an electronic signal, electrochemical immunosensors greatly simplify signal transduction, potentially avoiding the requirement for expensive equipment (Belluzo et al., 2008). Common types of electrochemical detection are potentiometric, capacitive and amperometric modes. Among them, amperometric biosensors are the most common type because they are potentially more convenient and easy-to-use (Claussen et al., 2007; Hahm, 2011). A detailed list of this classification as well as schematics of their detection mechanisms are shown in Fig. 1.

A crucial aspect to consider in the fabrication of electrochemical biosensors is how to immobilise the recognition elements on the surface of transducer (Védrine et al., 2003). Recognition elements may be immobilised using gels, polymeric membranes, conductive salts, carbon-based pastes and *etc.* (Manesh et al., 2008). Biosensors have been hugely influenced by recent progress in nanotechnology, which has provided a means to increase the surface area for immobilisation and sensing, thus enhancing the catalytic properties of electrodes and providing nanoscale sensors (Cash and Clark, 2010).

Most reviews in the field of electrochemical nano-biosensors have discussed the application of some special nanomaterials such as carbon nanotubes (CNTs), gold nanoparticles (GNPs) and graphene nanolayers (Alwarappan et al., 2009). However, there has been no comprehensive survey focusing on nanofibre-based electrochemical biosensors. In this review nanofibre structures are introduced and the advances and importance of utilising nanofibres in biosensors explored. Research in this field are reviewed according to two main categories: conductive and nonconductive nanofibres. Moreover, we emphasise the opportunities for further improvement of nanofibre-based electrochemical biosensors.

2. Nanomaterials

Nanostructured materials (Table 1) are commonly defined as materials with at least one dimensions below 100 nm. At the nanometre-scale, mechanical properties differ from those of the bulk material, and surface properties are dominant (Chopra et al., 2007). In biotechnology applications, use of nanomaterials can provide an attractive route for fabrication of highly sensitive and miniaturised nano-biosensors which require small amounts of sample for detection (Tang and Casas, 2014; Tiwari and Turner,

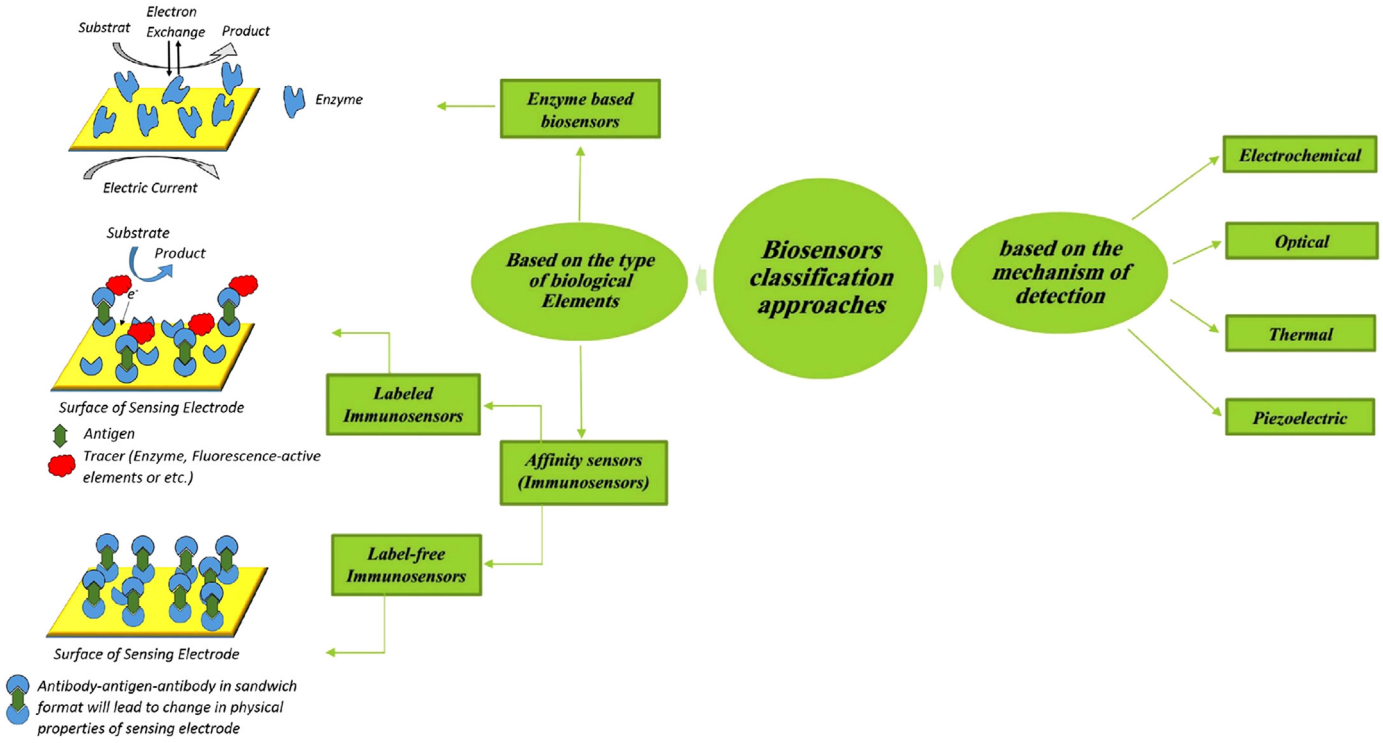

Fig. 1. Cascade diagram of different categories of biosensors with schematics of their detection mechanisms.

2014).

Nanoscale materials have inherently large surface-area-to-volume ratios, which can provide excellent places for biological molecule attachment and improve the loading capacities of these molecules over conventional materials such as polymeric membranes and fibrous structures (Wang et al., 2008). In addition, immobilisation of biomolecules on the surface of nanostructured materials can maximise the activity and lifetime of these molecules due to comparable sizes of the nanomaterials and biological molecules (Fathil et al., 2015; Noor and Krull, 2014). Altogether, the mentioned salient properties of nanostructured materials make them ideal candidates for use in biosensing devices. Nanostructured substrates such as nanoparticles, nanotubes, nanowires and nano-patterned surfaces have been employed as electrodes in biosensing devices. Recent developments in nanotechnology open the possibility of utilisation of nanofibre structures as ultrasensitive biosensors for proteins detection. High specific surface area, unique and tailorable chemical surfaces and large pore volume per unit mass make nanofibres a superb foundation for biosensor applications (Sadir et al., 2014).

Table 1
Classification of nanostructured materials based on the International Organization for Standardization (ISO, 2008).

Dimensions in nanoscale	Direction of confinement (X,Y,Z) in Cartesian coordinates	Type of class	Schematic view	
0D	X,Y,Z	Nanoparticles	Nanospheres	< 100 nm
			Nanoclusters	< 100 nm
1D	X,Y	Nanofibres family	Nanofibres	
			Nanowires and nanorods	
			Nanotubes	
2D	X	Nanolayers	Thin films and nanoplates	

3. Why nanofibre structures?

The family of nanofibres are defined as one-dimensional (1D) nanostructure materials, which are nano-objects with two similar dimensions at the nanoscale (< 100 nm) and the third dimension notably larger than the other two dimensions (ISO, 2008; Matlock-Colangelo and Baeumner, 2014). The family of nanofibres are further divided into typical nanofibres (organic and inorganic nanofibres), nanotubes (hollow nanofibre), nanorods (solid or non-flexible nanofibre), nanowires (electrically conducting or semiconducting nanofibre) and nanowhiskers (ISO, 2008; Ravichandran et al., 2015; Reiners, 2013). In the recent years, nanofibre structures have become more and more alluring on account of their numerous advantages, namely increase in the reaction rate and sensitivity of the biosensor due to the high surface area of nanofibre structures (Luo et al., 2012; Wang et al., 2002), the ability to produce nanofibre structures from a variety of materials according to the application (Wang et al., 2009b), low barrier to diffusion of materials due to their high porosity and interconnectivity (Macagnano et al., 2015), high activity of biological elements immobilised on the surface of nanofibres (Sadir et al., 2014; Tran and Balkus, 2012) and excellent structural mechanical properties (Ding et al., 2010). Besides, it has been found that for electrochemical biosensors, the nanofibre structures can provide faster electron transfer compared with a nanoparticle-based films of the same material (Mondal et al., 2014; Wanekaya et al., 2006).

In spite of the overwhelming advantages of nanofibre structures, research on nanofibre-based electrochemical biosensors are mostly confined to the detection of a few analytes, namely glucose, urea and cholesterol (Ding et al., 2010; Manesh et al., 2007; Ren et al., 2006). There are a few informative articles which consider the role of nanofibre structures for the detection of cardiac biomarkers in electrochemical biosensors. Nevertheless, this area of research is in its infancy. This particular review will, consequently, focus on developments nanofibre-based electrochemical biosensors for detection of cardiac biomarkers in the last years.

4. Nanofibre-based electrochemical biosensors

According to the electrical properties of the nanofibrous mat employed as a substrate for immobilising the biological elements, articles in this area can be classified into two major categories: conductive nanofibres and non-conductive nanofibres. This classification clearly illustrates the mechanism of detection in nanofibre-based biosensors. The surface of transducer in biosensors with conductive nanofibres is shifted to the surface of nanofibres. Thus, the exchanged electrons in the biochemical reactions can rapidly transfer to the electronic circuit by these nanofibres. On the other hand, when the biomarker/antigen–antibody interaction depends on their inherent affinity to each other and takes place without any electron exchange, it is helpful to engage enzyme labels coupled to the antibodies or antigens, to deliver higher sensitivity (Thévenot et al., 2001). In case of nonconductive nanofibre-based biosensors, nanofibre structures are just employed as 3D nanostructures with special porosities for immobilising the biological elements. In this system, for instance in electrochemical immunosensors, attachment of antibody to antigen/biomarker will lead to changing the porosity of nanofires mat and formation of a hindrance in front of the diffusion of probe through the 3D nanofibrous structure (Mertz et al., 2011).

5. Conductive nanofibres

Conductive nanofibres are attached to the surface of sensing electrode to enhance the electron-transfer activities and heighten the sensitivity of electrochemical detection (Ekabutr et al., 2013). There are various procedures to fabricate conductive nanofibres from the different materials, such as plasma enhanced chemical vapour deposition (PECVD), self-assembling polymerisation (Ekabutr et al., 2013), oil/water emulsion (He, 2005), sol–gel electrospinning (Ahmad et al., 2010; Wang et al., 2009a) and solution electrospinning (Veisi et al., 2013). Among them, electrospinning is one of the most appealing methods which has gained widespread attention in that it is known to be an effective fabrication tool for preparing nanofibres with various special structures (Wang et al., 2006) (we will discuss the mechanism of electrospinning process in more detail in the nonconductive nanofibres section). For instance, conductive nanocomposite nanofibres are frequently fabricated in the electrospinning process by doping polymer solutions with different nanostructured materials such as metal nanoparticles, graphene, or carbon nanotubes (CNTs) (Ekabutr et al., 2013). Most of the research on the nanofibre-based electrochemical biosensor for cardiac biomarkers detection has utilised nanofibres with inherent conductivity. There are, however, several studies on the electrochemical biosensors with nanocomposite nanofibres including some aforementioned conductive additives (Gomathi et al., 2011; Manesh et al., 2008). Research performed on conductive nanofibre-based electrochemical biosensors for cardiac biomarker detection can be categorised into three groups: CNTs and Carbon nanofibres (CNFs), nanofibres based on metals, metalloids and their oxides and conductive polymer-based nanofibres. So, in the following, the emphasis is on an in-depth investigation of these categories.

6. CNTs and CNFs

Carbon is one of the most compelling elements of the Periodic Table in view of its vast variety of allotropic forms in the micro and nanoscale (Huang et al., 2010). Specifically, CNTs and CNFs are carbon-based nanostructured materials which have commanded particular attention in electroanalysis and biosensing on account of their mechanical, electrical, thermal, optical and structural properties (Al-Saleh and Sundararaj, 2009; Matlock-Colangelo and Baeumner, 2012). The key factor resulting in ample mechanical strength and high electrical and thermal conductivity of CNTs is the sp^2 hybridisation of carbon atoms in the structure, which can bring about stronger bonds between the carbon atoms than those of sp and sp^3 hybridisations (Li et al., 2003; Shao et al., 2009). The internal structure of CNFs and CNTs varies and is composed of different arrangements of modified graphene sheets. CNTs can be considered as graphene sheets rolled into cylindrical form that is particularly extended throughout the symmetric axis of the material with a hollow cavity in the centre (Melechko et al., 2005).

Although CNTs have a simple chemical composition and periodic atomic bonding arrangements, these materials include a wide variety of structures, according to the number of rolled graphene sheets along the tube axis (single-walled CNTs (SWCNTs) or multi-walled (MWCNT)), and the direction of the rolling vector along the graphene sheet (armchair, zigzag or chiral tubes) (Dai, 2002; Kuzmany et al., 2004). In addition to the aforementioned properties for CNTs, they have some other distinctive characteristics such as large edge plane/basal plane ratio, the capability to vary the surface chemistry by functionalisation process, low cost and chemical inertness in most electrolyte solutions (Yang et al., 2013; Zhang et al., 2015). All these fascinating characteristics support CNTs as one of the prime candidates for developing a new generation of biosensor with higher sensitivity and lower detection limits, able to recognise a myriad of clinically important analytes and biomarkers (Wang, 2005; Yang et al., 2013). The application of CNTs as an immobilisation matrix in electrochemical biosensors

not only enhances the electron transfer rate between the active sites of biological elements and sensing electrode, but can also lead to sustaining their bioactivity on the sensing electrode; this may be explained by the mutual biocompatibility of CNTs with biological elements (Justino et al., 2010; Tran et al., 2009). Moreover, CNTs are extensively employed in field effect transistors (FETs) and serve as a gate for electron transfer (Kim et al., 2008). In spite of the above-mentioned spectacular properties of CNTs, poor dispersibility in polar solvents culminates in aggregation and agglomeration of CNTs, which restricts the direct utilisation of these nanostructured materials (Bagheri et al., 2010). Therefore, some primary processes should be performed to improve the dispersibility of CNTs such as functionalisation of CNTs with polar functional groups or ultrasonication and introduction of the proper surfactant for wrapping and stabilising the individual CNTs (Jacobs et al., 2010; Rastogi et al., 2008).

Here, we take account of the articles that utilised CNT-based electrochemical biosensors to determine the concentration of cardiac biomarkers within the blood serum. Buch and coworkers (Buch and Rishpon, 2008) took advantage of MWCNTs/screen-printed carbon electrodes (MWCNTs/SPE), modified with polyethylenimine and glutaraldehyde, as a sensing electrode for labelled electrochemical measurements of CRP biomarker concentrations. The presence of CNT layer on the surface of SPE can contribute significantly to the promotion of electrochemical activity of the sensing electrode. Another modification was performed by grafting protein A on the surface of the electrode to achieve a proper orientation of the bound biological element. To this end, the Anti-CRPs were crosslinked to the protein A using dimethyl pimelimidate dihydrochloride (DMP) to form a molecular recognition surface. The fabricated electrodes were immersed in human serum solutions containing various concentrations of CRP, and finally in a solution including goat anti-CRP antibody conjugated with horse radish peroxidase (HRP) as the tracer. The enzymatic reactions of tetramethylbenzidin (TMB) with the entrapped HRP in the sandwich format were measured by amperometry. The calibration curve of the assay, constructed from the measurement of the reduction currents of different CRP concentrations, covers very wide range of CRP concentrations from 0.5 ng/mL to 200 ng/mL. In other relevant work, Gomes-Filho and coworkers (Gomes-Filho et al., 2013) set up an electrochemical immunosensor based on carboxylated CNTs supported by a conductive polymer film of Polyethyleneimine (PEI) on a gold electrode to detect cardiac Troponin T (cTnT). EDC/NHS were utilised to activate the COOH-CNTs and form successful attachment of anti-cTnT on the functionalised CNTs. The electrode was incubated in glycine solution to block remaining active sites and avoid non-specific binding. Then, the fabricated electrodes were firstly immersed in solutions with different concentrations of cTnT in PBS, and were subsequently incubated in a solution containing specified amount of anti-cTnT-HRP. The amperometric signal was generated by reaction of hydrogen peroxide (H_2O_2) with HRP conjugated to anti-cTnT. The designed immunosensor showed a low detection limit of 0.033 ng/mL and a linear range between 0.1 and 10 ng/mL for the cTnT concentration, which is comparable with the ones described by conventional laboratory tests for cTnT. In other similar work, Freitas and coworkers (Freitas et al., 2014) produced electrochemical immunosensors based on amino-functionalised MWCNTs/SPE to detect cTnT. The amino-functionalisation of MWCNTs was performed to improve the stability and orientation of antibodies on the surface of sensing electrode and consequently enhance the efficiency of the immunosensor. The amino-functionalised MWCNTs/SPE were placed in a sandwich-type immunoassay for measuring the cTnT, in which the electrochemical measurements were obtained through H_2O_2 reaction with HRP conjugated to the secondary antibody. This electrochemical immunosensor prototype exhibited broad linear range of detection from 0.02 to 0.32 ng/mL with low detection limit of 0.016 ng/mL.

Despite the utmost precision and accuracy of the labelled biosensor results, operation of these types of biosensors is somewhat time-consuming due to the several incubation steps. Hence, recent research has focused on label-free recognition techniques (Jacobs et al., 2010). For instance, Silva and coworkers (Silva et al., 2013) presented a label-free electrochemical immunosensor involving amino-functionalised CNTs/SPE for detection of the cTnT. Amine-functionalised CNTs were incorporated into a printing ink and formed a thin film as sensing electrode. Immobilisation of anti-cTnT on the surface of sensing electrode was performed and after incubation in cTnT solution, amperometric responses were obtained by differential pulse voltammetry (DPV) in the presence of $[Fe(CN)_6]^{3-/4-}$ as probe. DVP measures the current facilitated by the probe by generating successive and regular voltage pulses superimposed on the potential linear sweep or stair steps (Bard and Faulkner, 1980). The main advantage claimed for this technique is that no labels are necessary for tracing the antigen–antibody interactions. However, an electrochemical probe is still required. The analytical response to antigen–antibody attachment was obtained taking into account the difference between the peak current (ΔI) before and after cTnT attachment. The calibration curve of the designed immunosensor indicated a linear response from 0.0025 ng/mL to 0.5 ng/mL with low detection limit of 0.0035 ng/mL, which is lower than the other reported immunosensors results for this biomarker and is on a par with those of conventional analytical methods.

In comparison with CNTs, CNFs do not have perfect arrangement of atoms and the hollow cavity in the centre (Serp et al., 2003; Tran et al., 2009). In general, CNFs, are cylindrical and consist of graphene layers arranged as stacked cones, cups or plates, and typically have lengths on the order of micrometres (Matlock-Colangelo and Baeumner, 2012; Wang and Lin, 2008). There are two approaches for the manufacture of CNFs, analogous to the fabrication procedures of the other nanostructured materials: top-down and bottom-up.

Laser-ablation (De Jong and Geus, 2000) and electrospinning are in the realm of top-down approach for CNFs synthesis (Feng et al., 2014; Zhang et al., 2014). The bottom-up approaches for synthesising carbon nanostructures include arc-discharge method, catalytic chemical-vapour deposition (C-CVD) and catalytic plasma enhanced chemical-vapour deposition (C-PECVD). From an application point of view, the C-PECVD process is really noteworthy for achieving favourable features of the produced CNFs thanks to a number of operational advantages, namely control over such adjustable parameters as position, alignment, diameter, length, chemical composition, or other characteristics of individual nanostructures (De Jong and Geus, 2000; Melechko et al., 2005). The CNFs synthesised by this method have special characteristics; they are vertically aligned (which called VACNFs) on the substrate and the disposition of the graphene sheets in the internal structure differs according to the type of catalyst and the manufacturing conditions. Ribbon-like CNFs (R-CNFs), platelet CNFs (P-CNFs) and herringbone or fishbone CNFs (H-CNFs) are three more known structures of CNFs with the variations in the stacking of graphene sheets along the nanofibre axis (as shown in Fig. 2).

Recently, nanoelectrode arrays (NEAs) constructed based on VACNFs grown by the C-PECVD process have become more and more attractive for use as highly sensitive electrodes in a wide range of electrochemical biosensors, taking into account their superb properties such as unique electrical and thermal conductivities, high signal to noise ratio, wide electrochemical window, ease of surface modification and biocompatibility (Baker et al., 2006; Gupta et al., 2014a; Wang and Lin, 2008). In order to

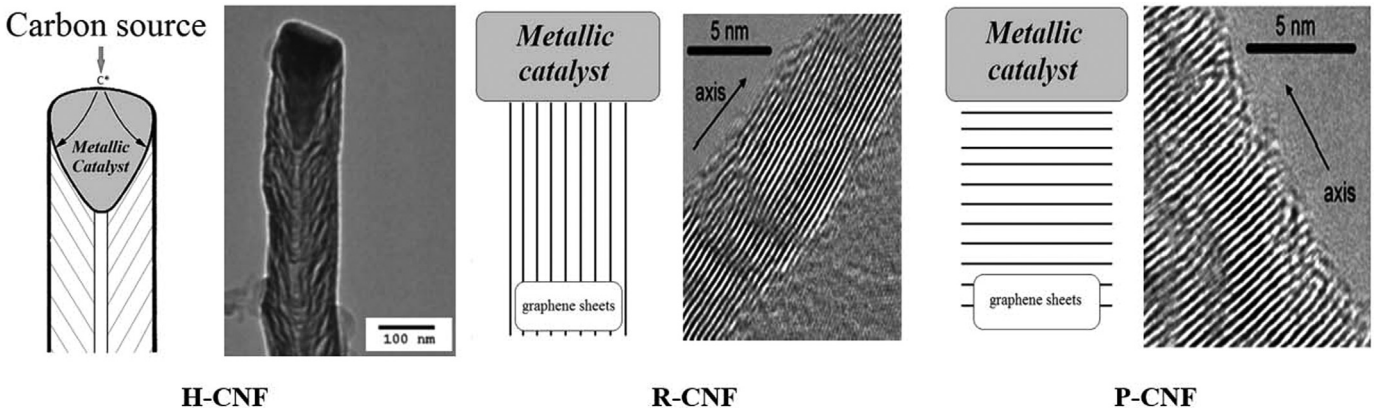

Fig. 2. Different types of CNFs according to the disposition of the graphene sheets in the internal structure (Shao et al., 2009).

Immobilise the biological elements on the edge of graphene sheets of CNFs, modification of CNFs must be carried out to introduce functional groups on the surface of the nanofibres (Lee et al., 2004). There are various strategies for the surface modification of CNFs, such as photochemical reaction, electrochemical reduction (Baker et al., 2005), soaking in acidic solutions (Periyakaruppan et al., 2011), oxidative methods, using of covalently coupling biomolecules and *etc.* (Fletcher et al., 2006; Huang et al., 2010). There follows two of the most interesting electrochemical biosensor studies applying CNFs to recognise cardiac biomarkers to have been investigated.

Periyakaruppan and coworkers (Periyakaruppan et al., 2013) produced a label-free VACNFs-based electrochemical immunosensor for cardiac cTnI detection. Chemical modification of the VACNFs was carried out by soaking the NEAs in HNO_3 solution to introduce COOH groups on the top of the CNFs for subsequent immobilisation of the probe antibody (anti-cTnI). The resultant electrodes were utilised for electrochemical detection of human-

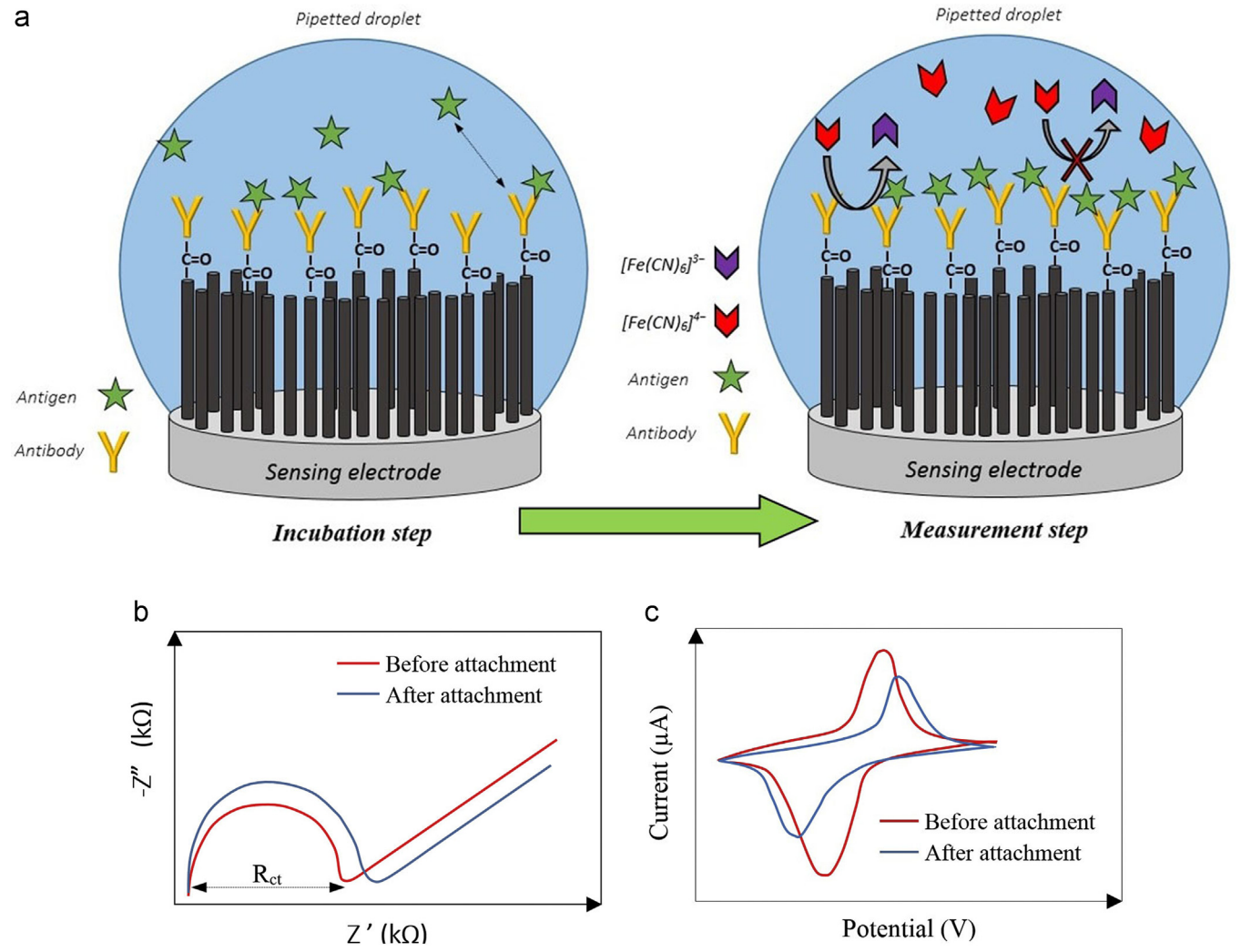

Fig. 3. Schematic view of label-free VACNFs-based electrochemical biosensor for biomarkers detection, (a) incubation and measurement steps, (b) EIS and (c) CV results.

cTnI at different concentrations. Electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) techniques were deployed to evaluate the immunoreaction between the moiety anti-cTnI (on the electrode surface) and target human-cTnI, after incubation of NEAs in different cTnI solutions for 1 h. The antibody-biomarker attachment on the top of CNFs will act as a hindrance for mediators movement and significantly confine their diffusion and electron exchange with CNFs. Therefore, this confinement will lead to a less measured current and a more charge transfer resistance (R_{ct}) in CV and EIS results, respectively. A schematic view of the mechanism of biomarker detection by CNFs-based NEAs is given in Fig. 3.

Their outcomes showed that the immunosensor was capable of detecting cTnI at concentrations as low as ~0.2 ng/mL with linearity, high sensitivity and good specificity. In addition, the detection limit of the produced immunosensor was 25 times lower than that achieved by conventional methods, which confirms the unique capability of VACNFs for use in point-of-care diagnostic instruments.

Gupta and coworkers (Gupta et al., 2014b) utilised the same NEAs for detection and quantification of another cardiac biomarker (CRP), implementing both CV and EIS techniques. CRP presence at an anti-CRP-modified VACNF electrode was identified by investigating the changes in reduction current in CV plots and R_{ct} in EIS plots. After antibody-CRP attachment on the surface of NEAs, the magnitude of current in CV plots was found to decrease and the R_{ct} in EIS plots was found to increase, proportional to the concentration of the CRP target; this behaviour stems from the insulating nature of the CRP which acts as a barrier for electron transport. The detection limit of the biosensor was found to be ~90 pM or ~11 ng/mL, which corresponds to the clinically relevant range.

7. Nanofibres based on conductive polymers

Intrinsically conducting polymers (CPs) or electroactive conjugated polymers are a special class of organic materials with a capability of overlapping of the polymer molecular orbitals (caused by extended Pi-conjugation structures along the polymer backbone) (Ahuja et al., 2007; Gupta et al., 2006). CPs are present in the form of cationic salts with adjustable electrical properties, according to their redox or doping/dedoping states (Li et al., 2015). Actually, the accepted mechanism behind electrical variations of CPs is justified by the unique reversible switching capability of these materials between conducting oxidised state (doped) and insulating reduced state (undoped or neutral) (Takashima and Kaneto, 2004). These unique materials have received considerable attention for use in various advanced technologies. As an example, CPs have been recently regarded as one of the most promising materials for development of advanced sensors by virtue of their relative stability, low ionisation potential, capability of being directly synthesised on the sensor electrode by electrochemical oxidation of monomers, adjustable thickness by controlling the deposition charge, redox conductivity, poly-electrolyte characteristics and optical features (Sadik et al., 2003; Takashima and Kaneto, 2004). Furthermore, in contrast to inorganic metals and semiconductors, CPs possess mechanical flexibility, compatibility with organic biomolecules and good processability, which boost their popularity for biosensing applications (Li et al., 2015; Xia et al., 2010).

It should be noted that for an electrochemical biosensor involving CPs, the detection is related to detectable changes in the electrical properties of these materials, which is proportional to the concentration of a specific biological element or set of them. In the recent years, nanostructured CPs have piqued more scientific interest in the area of biosensing applications, because of their special capability for biomolecule stabilisation, high electron transfer efficiency, great sensitivity and selectivity, which are due to the extreme surface to volume ratio, high surface free energy and some special quantum phenomena (Li et al., 2015; Xia et al., 2010). Concerning this, nanofibre based-CPs have been frequently utilized in the biosensors for recognition of various analytes thanks to their significantly faster response time, good features for the incorporation of biological elements and high porous and permeable matrices leading to faster both doping and dedoping processes (Ekabutr et al., 2013; Xia et al., 2010). Continuing this theme, some of the biosensors that employed CPs-based nanofibres to detect the cardiac biomarkers have been investigated.

Lee and coworkers (Lee et al., 2012) presented a label-free conductometric biosensor whose single PANi-NWs were integrated with microfluidic channels to detect very low concentrations of Myo (100 pg/mL), cTnI (250 fg/mL), CK-MB (150 fg/mL), and b-type natriuretic peptide (BNP, 50 fg/mL). An electrode of pre-patterned Au was deployed as substrate on which single PANi-NWs was formed by electrochemical deposition method. The patterned Au electrode was used to assist the controlled growth of PANi-NWs in the pattern direction. Then, the biosensor was manufactured by covalent functionalisation of PANi-NWs with myocardial antibodies (mAbs). The detection of cardiac biomarkers was carried out applying the conductometric sensing method in which the conductance changes of the nanowire were measured. During a slow flow of phosphate buffer solution (pH 7.4) containing target biomarker into the microfluidic cannel, binding between the immobilised mAbs and the target biomarkers led to a change in conductance of single PANi-NWs. The selectivity of the produced biosensor was investigated by the injection of bovine serum albumin (BSA) (acting as a non-specific molecule) into the microfluidic channels. The fabricated biosensor showed 106 times greater specificity for the cardiac biomarkers than that of BSA. In an analogous study, these researchers made use of a conductometric biosensor with a single PANi-NWs, which was formed between a pair of patterned Au electrodes, as a sensing element to identify immunoglobulin G (IgG) and Myo biomarkers. The detection limits were 3 ng/mL and 1.4 ng/mL for IgG and Myo, respectively (Lee et al., 2011).

Anwar and coworkers (Anwar et al., 2013) took advantages of electrospun polystyrene/PANi blend nanofibres as a sensing element to set up a label-free electrochemical biosensor for CRP detection. In order to attain the conductive nanofibres, doping the PANi was implemented during the solid state protonation reaction between the PANi and camphorsulfonic acid (PANi-HCSA). PS was added into the electrospinning solution to promote the electrospinnability of PANi and help immobilise the biological element. The electrospun nanofibre mat was placed on a comb-shaped gold microelectrode platform as a chip and was encapsulated in a microfluidic manifold with a 150 μL capacity. Functionalisation of nanofibres with anti-CRP was conducted with dithiobis (succinimidyl propionate) (DSP)/NHS-ester. EIS results demonstrated that the fabricated nanotextured electrochemical biosensor had a low detection limit of 100 fg/mL and a wide dynamic linear range of 100 fg/mL–1 μg/mL for detection of CRP concentration.

8. Nanofibres based on metals, metalloids and their oxides

1D nanostructured materials based on metals and metal oxides are another promising candidate for use in electrochemical biosensors involving the transformation of the analytical signals in solution to electrical signals (Shalev et al., 2013). Nanowires and nanofibres based on metals, semiconductors and metal oxides, which can be synthesised by the aforementioned and some other

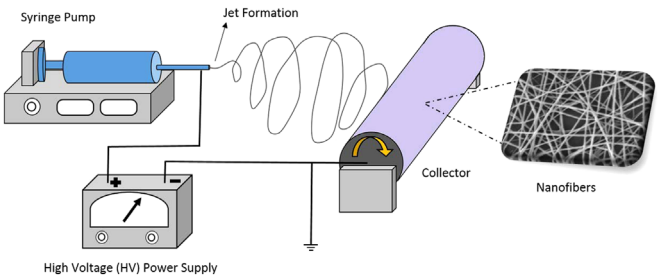

Fig. 4. Schematic representation of conventional electrospinning process and nanofibres formation.

techniques, have recently aroused much interest as FETs for creating ultrasensitive biosensor devices. In addition, they enjoy some remarkable features including their comparable sizes with biological molecules and their extraordinary mechanical, electrical, thermal and multifunctional properties (Chen et al., 2008; Hahn et al., 2012; Solanki et al., 2011). Considering these unique features, any interaction between the specific biological molecules and these 1D nanostructured materials will induce significant changes in the electrical characteristics. In recent years, a large variety of nanowires and nanofibres of metals, metalloids and their oxides such as ZnO nanofibres (Ahmad et al., 2010), Si-NWs (Chee et al., 2009), Pd (IV)-doped CuO composite nanofibres (Wang et al., 2009a), Electrospun gold nanofibres (Marx et al., 2011), TiO_2 nanofibres (Mondal et al., 2014) *etc.* have been utilised to design biosensors with different mechanism of sensing from surface plasmon resonance (SRP) to electrochemical approaches (Solanki et al., 2011). Among them, biosensors involving Si-NWs have drawn much interest due to their fast, efficient and inexpensive detection of proteins, unlike the other time-consuming methodologies such as ELISA or western blot (Chee et al., 2009; Shen et al., 2015). Zhang and coworkers (Zhang et al., 2011) took advantage of an integrated chip consisting of a filtration part for plasma separation from fingerprick blood and Si-NWs (with ~80 nm widths) array part for detection of three cardiac biomarkers (cTnI, CK-MM and CK-MB) simultaneously. A steady-state measurement of Si-NWs resistance changes before and after incubation in the buffer solutions was performed to verify the interactions of biomarkers with immobilised antibodies on the surface of nanowires. Their results indicated that the fabricated biosensor is able to attain a low detection limit of 1 pg/ml for the three cardiac biomarkers from 2 μl blood sample in a shorter time (45 min) compared with clinical methods.

In another relevant study, Chua and coworkers (Chua et al., 2009) presented a new label-free biosensor which served the complementary metal-oxide semiconductor (CMOS)-compatible Si-NWs array platform as a FET immunosensor to detect cTnT in the assay buffer as well as in undiluted serum samples. In order to attach the biological probe molecules to the surface of Si-NWs, the chemical modification process of the Si-NWs array chips took place through immersing the chips in a solution of ethanol/H_2O containing 2–3% aminopropyl triethoxy silane (APTES), which can lead to the formation of amine groups on the surface of NWs. Their experimental outcomes showed that the low detection limit of cTnT in an assay buffer solution is lower than 1 fg/mL, and likewise, in an undiluted human serum environment is about 30 fg/mL.

Most of the published reports on metal and metal oxides nanofibre-based electrochemical biosensors for cardiac biomarkers detection revolve around Si-NWs. There are, however, some studies which deployed another metal oxide nanofibres family for detection of these biomarkers. Ibupoto and coworkers (Ibupoto et al., 2012) set up an electrochemical biosensor based on ZnO nanotubes to detect CRP within a phosphate buffer solution. The authors presented two major reasons for choosing the ZnO nanotubes as sensing electrode; the first one is the good piezoelectric properties of this material and other is the high isoelectric point (IEP) about 9.5. The piezoelectricity can help generate the voltage along with the charged environment provided by the CRP. Besides, since high IEP of ZnO is the evidence for the strong electrostatic bonding with the immobilised antibody, the immobilisation mechanism of anti-CRP on the surface of ZnO nanotubes may be justified by physical adsorption. The electrochemical response of the presented biosensor was measured by the potentiometric technique and the results revealed a detection range of 1.0×10^{-5}–1.0 mg/L of CRP concentration with the time response of less than 10 s.

9. Nonconductive nanofibres

In addition to the mentioned conductive nanostructures, it is beneficial to use several nonconductive polymeric nanofibres for fabrication of nanofibre-based biosensors in light of their relatively easy methods to produce, controllable porous structure and surface chemistry, which are particularly appropriate for immobilizing the biological molecules (Hahm, 2011; Macagnano et al., 2015). In fact, polymeric nanofibres are often applied as a three dimensional mesoporous media to immobilize the biological elements on the surface of electrodes and increase the sensitivity of the biosensors, especially those that work through optical, electrochemical, and mass-sensitive mechanisms of detection (Arecchi et al., 2010; Scampicchio et al., 2010). Considering the various fabrication techniques of polymeric nanofibres, electrospinning process has gained widespread attention since it is known to be an effective approach for preparing relatively large quantities of continuous and uniform nanofibres at a reasonably low cost (Rezaei et al., 2014). In this process, usually a continuous strand of a polymeric flow (*i.e.* solution or melt) is drawn through a needle by high electrostatic forces to deposit randomly on a grounded collector as a nonwoven mat (Luo et al., 2012; Rezaei et al., 2014). A schematic representation of the conventional electrospinning process is presented in Fig. 4.

Hitherto, the main emphasis has been on biosensors employing nonconductive electrospun nanofibres to detect glucose (Ding et al., 2010). Apart from the single component nonconductive electrospun nanofibres, some researchers take advantage of the electrical properties of nanocomposite electrospun nanofibres to boost the sensitivity and low detection limit of the electrochemical biosensors. For instance, MWCNTs/polymethylmethacrylate (PMMA) nanofibres (Manesh et al., 2008), GNPs/chitosan nanofibres (Gomathi et al., 2011) and MWCNTs/poly(acrylonitrile-co-acrylic acid) (PANCAA) (Wang et al., 2009c) served as benign matrices to detect glucose, cholesterol and glucose, respectively. In the last few years, however, some efforts have been reported in the literature, aiming to exploit nonconductive nanofibres for detection of cardiac biomarkers and some other proteins. Sadir and coworkers (Sadir et al., 2014) deployed different nanofibres (poly-L-lactic acid (PLLA) and cellulose acetate (CA) nanofibres) as immobilisation platforms for anti-CRP to fabricate an optical colorimetric ELISA. The optical colour intensity parameter was the key criterion to estimate the level of CRP.

Detection of the antibody–antigen/biomarker attachment in nonconductive nanofibre-based electrochemical biosensors can be measured in terms of changes in dielectric/charge transfer resistance (R_{ct}) constants. Kunduru and coworkers (Kunduru et al., 2010) assessed the effect of scaling down of textured polystyrene (PS) polymer (from micro- to nanoscale) on the sensitivity of electrochemical biosensor. For this purpose, micro beads and

Table 2
A summary of the electrochemical biosensors based on the family of nanofibres for detection of cardiac biomarkers.

Cardiac biomarker detection	Type of nanofibres family	Assay format	detection range	Low detection limit	Detection time (incubation times+ evaluation time)	Ref.
CRP	MWCNTs	Sandwich form using HRP as tracer	0.5–200 (ng/mL)	0.5 ng/mL	180 min+600 s	Buch and Rishpon (2008)
cTnT	Carboxylated CNTs	Sandwich form using HRP as tracer	0.1–10 ng/mL	0.033 ng/mL	60 min+ –	Gomes-Filho et al. (2013)
cTnT	Amino-functionalized MWCNTs	Sandwich form using HRP as tracer	0.02–0.32 ng/mL	0.016 ng/mL	90 min+120 S	Freitas et al. (2014)
cTnT	Amino-functionalized CNTs	Label-free immunosensor	0.0025–0.5 ng/mL	0.0035 ng/mL	40 min+ –	Silva et al. (2013)
cTnI	VACNFs	Label-free immunosensor	0.25–1.0 and 5.0–100 ng/mL	~0.2 ng/mL	70 min+ –	Periyakaruppan et al. (2013)
CRP	VACNFs	Label-free immunosensor	0.05–0.5 and 2.5–5 mg/ml	~11 ng/mL	60 min+ –	Gupta et al. (2014a,b)
Myo	Single PANi NWs	Label-free conductometric biosensor	–	100 pg/mL	0+300 s	Lee et al. (2012)
cTnI			300 fg/mL–3 ng/mL	250 fg/mL	0+300 s	
CK-MB			–	150 fg/mL	0+300 s	
BNP			50 fg/mL–3 ng/mL	50 fg/mL	0+300 s	
IgG	Single PANi NW	Label-free conductometric biosensor	3 ng/mL–3 μg/mL	3 ng/mL	–	Lee et al. (2011)
Myo			1.4 ng/mL–2.5 μg/mL	1.4 ng/mL	–	
CRP	Electrospun polystyrene/PANi nanofibres	Label-free immunosensor	100 fg/mL–1 μg/mL	100 fg/mL	15 min+ –	Anwar et al. (2013)
cTnI	Si-NWs	Label-free electrical detection	–	1 pg/ml	30 min+ –	Zhang et al. (2011)
CK-MM			–		30 min+ –	
CK-MB			–		30 min+ –	
cTnT	CMOS-compatible Si-NW	Label-free electrical detection	–	1 fg/mL in buffer solution 30 fg/mL human serum	100–200 s 100–200 s	Chua et al. (2009)
CRP	ZnO nanotubes	Label-free electrochemical biosensor	1.0×10^{-5} mg/L–1.0 mg/L	1.0×10^{-6} mg/L	0+ < 10 s	Ibupoto et al. (2012)
CRP	PS nanofibres	Label-free electrochemical biosensor	1 pg/mL–1 μg/mL	1 pg/mL	15 min+ –	Kunduru et al. (2010)

nanofibres of PS were fabricated as solid supports for anti-CRP immobilisation. The authors inferred that the immobilisation of antibody occurred through van der Waals attraction between the hydrophobic areas of the adsorbed antibodies and the polymer surface. The amount of the CRP biomolecules bound to the immobilised anti-CRP was estimated by applying EIS to measure the double-layer capacitance fluctuations, which stem from the modulation of the surface charge. Their results indicated that scaling down to the nanoscale leds to much higher surface-to-volume ratio, thereby increase the immobilisation locations for the antibodies and finally heightening the chemoelectronic transduction along the nanotextured surface. Plus they deduced that not only is the performance of the nanotextured immunosensor platform on a par with that of ELISA assay, but also this nanofibre-based biosensor will lead to lower volume of serum and lower cost per assay. Some of the more important analytical characteristics of the reviewed research on electrochemical biosensors based on the family of nanofibres are listed in Table 2.

10. Outlook

In recent years, the emergence of nanotechnology has sparked a revolution in the design and fabrication of the biosensors and opened new horizons for the production of biosensors with desirable capabilities and miniaturised dimensions. Specifically, the use of nanomaterials in electrochemical biosensing is considered a promising strategy to accelerate the reaction rate and intensify the sensitivity of the biosensors. There are various types of nanostructured materials, such as nanoparticles, nanofibres and nanolayers, employed in electrochemical biosensor design to detect a wide range of analytes ranging from glucose to nucleic acids. Among them, biosensors based on the nanofibre family have demonstrated exceptional ability to boost the sensitivity, specificity and throughput of the electrochemical biosensors. Moreover, these biosensors offer the long-term potential to develop towards single-molecule biosensing. In the present review we have attempted to reflect the state of the art of nanofibre-based electrochemical biosensors for correct and rapid diagnosis of CVDs. In this regard, most of the recent relevant articles were reviewed and the mechanisms of detection for each sensor was evaluated. By and large, it is expected that the biosensors exploiting various types of the nanofibres will occupy a pivotal role in future applications for health-care testing in general, and CVDs diagnosis in particular.

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References

Adams, Jr, Bodor, G.S., Davila-Roman, V.G., Delmez, J., Apple, F., Ladenson, J., Jaffe, A., 1993. Circulation 88 (1), 101–106.
Ahmad, M., Pan, C., Luo, Z., Zhu, J., 2010. J. Phys. Chem. C 114 (20), 9308–9313.
Ahuja, T., Mir, I.A., Kumar, D., 2007. Biomaterials 28 (5), 791–805.
Al-Saleh, M.H., Sundararaj, U., 2009. Carbon 47 (1), 2–22.
Aldous, S.J., 2013. Int. J. Cardiol. 164 (3), 282–294.
Alwan, A., 2011. World Health Organization.
Alwarappan, S., Erdem, A., Liu, C., Li, C.-Z., 2009. J. Phys. Chem. C 113 (20), 8853–8857.
Anwar, R.M., Vattipalli, K., Myrah, E., Asmatulu, R., Prasad, S., 2013. Adv. Sci. Eng. Med. 5 (7), 633–640.
Arecchi, A., Scampicchio, M., Drusch, S., Mannino, S., 2010. Anal. Chim. Acta 659 (1), 133–136.
Bagheri, B., Abdouss, M., Shoushtari, A., 2010. Materialwiss. Werkst. 41 (4), 234–240.
Bahadır, E.B., Sezgintürk, M.K., 2015. Talanta 132, 162–174.
Baker, S.E., Colavita, P.E., Tse, K.-Y., Hamers, R.J., 2006. Chem. Mater. 18 (18), 4415–4422.
Baker, S.E., Tse, K.-Y., Hindin, E., Nichols, B.M., Lasseter Clare, T., Hamers, R.J., 2005. Chem. Mater. 17 (20), 4971–4978.
Bard, A.J., Faulkner, L.R., 1980. Electrochemical Methods – Fundamentals and Applications. Wiley, New York.
Belluzo, M.S., Ribone, M.É., Lagier, C.M., 2008. Sensors 8 (3), 1366–1399.
Bohunicky, B., Mousa, S.A., 2011. Nanotechnol. Sci. Appl. 4, 1.
Buch, M., Rishpon, J., 2008. Electroanalysis 20 (23), 2592–2594.
Cash, K.J., Clark, H.A., 2010. Trends Mol. Med. 16 (12), 584–593.
Chan, D., Ng, L.L., 2010. BMC Med. 8 (1), 34.
Chee, R., Chua, J., Agarwal, A., Wong, S., Zhang, G., 2009. Label-free detection of proteins with surface-functionalized silicon nanowires. In: Proceedings of the 13th International Conference on Biomedical Engineering. Springer, pp. 838–841.
Chen, P.-C., Shen, G., Zhou, C., 2008. Nanotechnol. IEEE Trans. 7 (6), 668–682.
Chopra, N., Gavalas, V.G., Bachas, L.G., Hinds, B.J., Bachas, L.G., 2007. Anal. Lett. 40 (11), 2067–2096.
Chua, J.H., Chee, R.-E., Agarwal, A., Wong, S.M., Zhang, G.-J., 2009. Anal. Chem. 81 (15), 6266–6271.
Claussen, J.C., Shi, J., Diggs, A.R., Porterfield, D.M., Fisher, T.S., 2007. Nanotechnologies for the Life Sciences. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany.
Dai, H., 2002. Acc. Chem. Res. 35 (12), 1035–1044.
De Jong, K.P., Geus, J.W., 2000. Catal. Rev. 42 (4), 481–510.
Ding, B., Wang, M., Wang, X., Yu, J., Sun, G., 2010. Mater. Today 13 (11), 16–27.
Ekabutr, P., Chailapakul, O., Supaphol, P., 2013. J. Appl. Polym. Sci. 130 (6), 3885–3893.
Falahati, A., Sharkey, S.W., Christensen, D., McCoy, M., Miller, E.A., Murakami, M.A., Apple, F.S., 1999. Am. Heart J. 137 (2), 332–337.
Fathil, M.F.M., Md Arshad, M.K., Gopinath, S.C.B., Hashim, U., Adzhri, R., Ayub, R.M., Ruslinda, A.R., Nuzaihan, M.N., Azman, M., Zaki, A.H., Tang, T.-H., M., 2015. Biosens. Bioelectron. 70 (0), 209–220.
Feng, L., Xie, N., Zhong, J., 2014. Materials 7 (5), 3919–3945.
Fletcher, B.L., McKnight, T.E., Melechko, A.V., Simpson, M.L., Doktycz, M.J., 2006. Nanotechnology 17 (8), 2032.
Freitas, T.A., Mattos, A.B., Silva, B., #xe1, M., rV., Dutra, R.F., 2014. Biomed. Res. Int. 2014, 9.
Gomathi, P., Ragupathy, D., Choi, J.H., Yeum, J.H., Lee, S.C., Kim, J.C., Lee, S.H., Do Ghim, H., 2011. Sens. Actuators B: Chem. 153 (1), 44–49.
Gomes-Filho, S.L.R., Dias, A.C.M.S., Silva, M.M.S., Silva, B.V.M., Dutra, R.F., 2013. Microchem. J. 109 (0), 10–15.
Gupta, N., Sharma, S., Mir, I.A., Kumar, D., 2006. J. Sci. Ind. Res. 65, 549–557.
Gupta, R.K., Meyyappan, M., Koehne, J.E., 2014a. RSC Adv. 4 (43), 22642–22650.
Gupta, R.K., Periyakaruppan, A., Meyyappan, M., Koehne, J.E., 2014b. Biosens. Bioelectron. 59, 112–119.
Hahm, J.-i, 2011. Sensors 11 (3), 3327–3355.
Hahn, Y.-B., Ahmad, R., Tripathy, N., 2012. Chem. Commun. 48 (84), 10369–10385.
Hasanzadeh, M., Shadjou, N., Eskandani, M., de la Guardia, M., Omidinia, E., 2013. TrAC Trends Anal. Chem. 49, 20–30.
He, Y., 2005. Mater. Sci. Eng.: B 122 (1), 76–79.
Huang, J., Liu, Y., You, T., 2010. Anal. Methods 2 (3), 202–211.
Ibupoto, Z.H., Jamal, N., Khun, K., Willander, M., 2012. Sens. Actuators B: Chem. 166, 809–814.
ISO, N., 2008. International Organisation for Standardisation (ISO). Genève, Switserland.
Jacobs, C.B., Peairs, M.J., Venton, B.J., 2010. Anal. Chim. Acta 662 (2), 105–127.
Justino, C.I., Rocha-Santos, T.A., Duarte, A.C., 2013. TrAC Trends Anal. Chem. 45, 24–36.
Justino, C.I.L., Rocha-Santos, T.A., Duarte, A.C., Rocha-Santos, T.A., 2010. TrAC Trends Anal. Chem. 29 (10), 1172–1183.
Kakoti, A., Goswami, P., 2013. Biosens. Bioelectron. 43 (0), 400–411.
Kehl, D.W., Iqbal, N., Fard, A., Kipper, B.A., Landa, A.Dl.P., Maisel, A.S., 2012. Transl. Res. 159 (4), 252–264.
Kim, J.P., Lee, B.Y., Hong, S., Sim, S.J., 2008. Anal. Biochem. 381 (2), 193–198.
Kost, G.J., Tran, N.K., 2005. Cardiol. Clin. 23 (4), 467–490.
Kunduru, V., Bothara, M., Grosch, J., Sengupta, S., Patra, P.K., Prasad, S., 2010. Nanomed.: Nanotechnol. Biol. Med. 6 (5), 642–650.
Kuzmany, H., Kukovecz, A., Simon, F., Holzweber, M., Kramberger, C., Pichler, T., 2004. Synth. Met. 141 (1), 113–122.
Lee, C.-S., Baker, S.E., Marcus, M.S., Yang, W., Eriksson, M.A., Hamers, R.J., 2004. Nano Lett. 4 (9), 1713–1716.
Lee, I., Luo, X., Cui, X.T., Yun, M., 2011. Biosens. Bioelectron. 26 (7), 3297–3302.
Lee, I., Luo, X., Huang, J., Cui, X.T., Yun, M., 2012. Biosensors 2 (2), 205–220.
Lequin, R.M., 2005. Clin. Chem. 51 (12), 2415–2418.
Li, L., Shi, Y., Pan, L., Shi, Y., Yu, G., 2015. J. Mater. Chem. B 3 (15), 2920–2930.
Li, W., Liang, C., Zhou, W., Qiu, J., Zhou, Z., Sun, G., Xin, Q., 2003. J. Phys. Chem. B 107 (26), 6292–6299.
Luo, Y., Nartker, S., Wiederoder, M., Miller, H., Hochhalter, D., Drzal, L.T., Alocilja, E. C., 2012. Nanotechnol. IEEE Trans. 11 (4), 676–681.
Macagnano, A., Zampetti, E., Kny, E., 2015. NanoScience and Technology.
Manesh, K., Kim, H.T., Santhosh, P., Gopalan, A., Lee, K.-P., 2008. Biosens.

Bioelectron. 23 (6), 771–779.
Manesh, K., Santhosh, P., Gopalan, A., Lee, K.-P., 2007. Anal. Biochem. 360 (2), 189–195.
Martín-Ventura, J.L., Blanco-Colio, L.M., Tuñón, J., Muñoz-García, B., Madrigal-Matute, J., Moreno, J.A., Vega de Céniga, M., Egido, J., 2009. Rev. Española Cardiol. 62 (6), 677–688.
Marx, S., Jose, M.V., Andersen, J.D., Russell, A.J., 2011. Biosens. Bioelectron. 26 (6), 2981–2986.
Matlock-Colangelo, L., Baeumner, A.J., 2012. Lab Chip 12 (15), 2612–2620.
Matlock-Colangelo, L., Baeumner, A.J., 2014. Annu. Rev. Anal. Chem. 7, 23–42.
McDonnell, B., Hearty, S., Leonard, P., O'Kennedy, R., 2009. Clin. Biochem. 42 (7–8), 549–561.
Melechko, A.V., Merkulov, V.I., McKnight, T.E., Guillorn, M., Klein, K.L., Lowndes, D. H., Simpson, M.L., 2005. J. Appl. Phys. 97 (4), 041301.
Mertz, T.O., Kunduru, V., Patra, P.K., Vattipalli, K., Prasad, S., 2011. Patterned polymer nanofibers based biosensors. MRS Proc. 1358, 1303–1307.
Mohammed, M.-I., Desmulliez, M.P., 2011. Lab Chip 11 (4), 569–595.
Moina, C., Ybarra, G., 2012. Adv. Immunoass. Technol.
Mondal, K., Ali, M.A., Agrawal, V.V., Malhotra, B.D., Sharma, A., 2014. ACS Appl. Mater. Interfaces 6 (4), 2516–2527.
Noor, M.O., Krull, U.J., 2014. Anal. Chim. Acta 825 (0), 1–25.
Periyakaruppan, A., Arumugam, P.U., Meyyappan, M., Koehne, J.E., 2011. Biosens. Bioelectron. 28 (1), 428–433.
Periyakaruppan, A., Gandhiraman, R.P., Meyyappan, M., Koehne, J.E., 2013. Anal. Chem. 85 (8), 3858–3863.
Perumal, V., Hashim, U., 2014. J. Appl. Biomed. 12 (1), 1–15.
Qureshi, A., Gurbuz, Y., Niazi, J.H., 2012. Sens. Actuators B: Chem. 171, 62–76.
Rapaport, E. 1979. "Nomenclature and Criteria for Diagnosis of Ischemic Heart-Disease-Report of the Joint-International-Society-and-Federation-of-Cardiology-World-Health-Organization Task-Force on Standardization of Clinical Nomenclature." 607-609.
Rastogi, R., Kaushal, R., Tripathi, S., Sharma, A.L., Kaur, I., Bharadwaj, L.M., 2008. J. Colloid Interface Sci. 328 (2), 421–428.
Ravichandran, M., Oza, G., Velumani, S., Ramirez, J.T., Garcia-Sierra, F., Andrade, N.B., Garza-Navarro, M.A., Garcia-Gutierrez, D.I., Lara-Estrada, R., Sacristán-Rock, E., 2015. RSC Adv. 5 (22), 17223–17227.
Reiners, G., 2013. Safety Aspects of Engineered Nanomaterials p. 1.
Ren, G., Xu, X., Liu, Q., Cheng, J., Yuan, X., Wu, L., Wan, Y., 2006. React. Funct. Polym. 66 (12), 1559–1564.
Rezaei, B., Askari, M., Shoushtari, A.M., Malek, R.A.M., 2014. J. Therm. Anal. Calorim. 118 (3), 1619–1629.
Sadik, O.A., Ngundi, M., Wanekaya, A., 2003. Microchim. Acta 143 (2-3), 187–194.
Sadir, S., Prabhakaran, M.P., Wicaksono, D.H., Ramakrishna, S., 2014. Sens. Actuators B: Chem. 205, 50–60.
Scampicchio, M., Arecchi, A., Lawrence, N.S., Mannino, S., 2010. Sens. Actuators B: Chem. 145 (1), 394–397.
Scheller, F., Schubert, F., Pfeiffer, D., Hintsche, R., Dransfeld, I., Renneberg, R., Wollenberger, U., Riedel, K., Pavlova, M., Kühn, M., 1989. Analyst 114 (6), 653–662.
Serp, P., Corrias, M., Kalck, P., 2003. Appl. Catal. A: Gen. 253 (2), 337–358.
Shalev, G., Landman, G., Amit, I., Rosenwaks, Y., Levy, I., 2013. NPG Asia Mater. 5 (3), e41.
Shao, Y., Liu, J., Wang, Y., Lin, Y., 2009. J. Mater. Chem. 19 (1), 46–59.
Shen, S.-H., Wang, I.-S., Cheng, H., Lin, C.-T., 2015. Sens. Actuators B: Chem.
Silva, B.V., Cavalcanti, I.T., Silva, M.M., Dutra, R.F., 2013. Talanta 117, 431–437.
Singh, V., Martinezclark, P., Pascual, M., Shaw, E.S., O'Neill, W.W., 2010. Coron. Artery Dis. 21 (4), 244–256.
Solanki, P.R., Kaushik, A., Agrawal, V.V., Malhotra, B.D., 2011. NPG Asia Mater. 3 (1), 17–24.
Stubbs, P., Collinson, P.O., 2001. Clin. Chim. Acta 311 (1), 57–61.
Takashima, W., Kaneto, K., 2004. Sens. Actuators B: Chem. 102 (2), 271–277.
Tang, L., Casas, J., 2014. Biosens. Bioelectron. 61, 70–75.
Thévenot, D.R., Toth, K., Durst, R.A., Wilson, G.S., 2001. Biosens. Bioelectron. 16 (1), 121–131.
Thygesen, K., Alpert, J.S., Jaffe, A.S., White, H.D., Simoons, M.L., Chaitman, B.R., Katus, H.A., Apple, F.S., Lindahl, B., Morrow, D.A., 2012. J. Am. Coll. Cardiol. 60 (16), 1581–1598.
Tiwari, A., Turner, A.P., *Biosensors Nanotechnology*, 2014, John Wiley & Sons, New Jersey, USA.
Tran, D.N., Balkus Jr, K.J., 2012. Top. Catal. 55 (16–18), 1057–1069.
Tran, P.A., Zhang, L., Webster, T.J., 2009. Adv. Drug Deliv. Rev. 61 (12), 1097–1114.
Tunstall-Pedoe, H., Kuulasmaa, K., Amouyel, P., Arveiler, D., Rajakangas, A.-M., Pajak, A., 1994. Circulation 90 (1), 583–612.
Védrine, C., Fabiano, S., Tran-Minh, C., 2003. Talanta 59 (3), 535–544.
Veisi, Z., Ceylan, M., Mahapatro, A., Asmatulu, R., 2013. An electrospun polyaniline nanofiber as a novel platform for real-time COX-2 biomarker detection. In: Proceedings of the ASME 2013 International Mechanical Engineering Congress and Exposition. American Society of Mechanical Engineers, pp. V03BT03A028–V003BT003A028.
World Health Organization. *World health statistics 2010*. World Health Organization, 2010.
Wanekaya, A.K., Chen, W., Myung, N.V., Mulchandani, A., 2006. Electroanalysis 18 (6), 533.
Wang, D., Sun, G., Xiang, B., Chiou, B.-S., 2008. Eur. Polym. J. 44 (7), 2032–2039.
Wang, J., 2005. Analyst 130 (4), 421–426.
Wang, J., 2006. Biosens. Bioelectron. 21 (10), 1887–1892.
Wang, J., Lin, Y., 2008. TrAC Trends Anal. Chem. 27 (7), 619–626.
Wang, W., Li, Z., Zheng, W., Yang, J., Zhang, H., Wang, C., 2009a. Electrochem. Commun. 11 (9), 1811–1814.
Wang, X., Drew, C., Lee, S.-H., Senecal, K.J., Kumar, J., Samuelson, L.A., 2002. J. Macromol. Sci. Part A 39 (10), 1251–1258.
Wang, Z.-G., Wan, L.-S., Liu, Z.-M., Huang, X.-J., Xu, Z.-K., 2009b. J. Mol. Catal. B: Enzym. 56 (4), 189–195.
Wang, Z.-G., Wang, Y., Xu, H., Li, G., Xu, Z.-K., 2009c. J. Phys. Chem. C 113 (7), 2955–2960.
Wang, Z.G., Xu, Z.K., Wan, L.S., Wu, J., Innocent, C., Seta, P., 2006. Macromol. Rapid Commun. 27 (7), 516–521.
Warsinke, A., Benkert, A., Scheller, F.W., 2000. Fresenius' J. Anal. Chem. 366 (6–7), 622–634.
Xia, L., Wei, Z., Wan, M., 2010. J. Colloid Interface Sci. 341 (1), 1–11.
Yang, T., Wang, S., Jin, H., Bao, W., Huang, S., Wang, J., 2013. Sens. Actuators B: Chem. 178, 310–315.
Yang, Z., Zhou, D.M., 2006. Clin. Biochem. 39 (8), 771–780.
Zhang, G.-J., Luo, Z.H.H., Huang, M.J., Kang, T.G., Ji, H., 2011. Biosens. Bioelectron. 28 (1), 459–463.
Zhang, H., Zhang, J., Zheng, J., 2015. Measurement 59, 177–183.
Zhang, L., Aboagye, A., Kelkar, A., Lai, C., Fong, H., 2014. J. Mater. Sci. 49 (2), 463–480.
de Lemos, J.A., 2013. JAMA 309 (21), 2262–2269.