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REVIEW

Cardiac biomarker detection based on nanostructured biosensors

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Abstract

Early and accurate detection of plasma borne cardiac biomarkers is essential for prevention of life threatening acute myocardial infarctions. Development of low cost and point of care platforms that can ensure ultrasensitive detection of cardiac biomarkers is key to this endeavor. In this context, herein, a brief discussion is provided highlighting the contribution of nanotechnology and microfabrication towards the development of such biosensing platforms, targeting the detection of several cardiac biomarkers such as Myoglobin, Troponin T, Troponin I and creatine kinase MB. Furthermore, the challenges associated with the conventional techniques that led to the development of the ultrasensitive biosensors are described in brief. In addition to several prominent nanoscale sensors that ensure very low limit of detection of cardiac biosensors, performance analysis of a conductive nanofiber based cardiac biomarker detection platform developed by our team is presented in this paper.

Keywords: cardiac biomarker; nanostructured biosensors; myocardial infarctions

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Introduction

Providing quality healthcare to the common people, not only accentuates the need of ensuring accurate disease diagnosis but also advocates, for the development of low-cost point-of-care diagnostic platforms. In lieu of this, micro and nanofabrication assisted miniaturization of sensing platforms has emerged as one of most significant technological advancement, which would ensure that cost-effective point-of-care diagnosis is no longer a dream. Advancements in nanotechnology have not only widened the horizon of human aspirations but also have led the foundation for a better future. In general, a biosensor comprises of a sensing element (which interacts with the targeted analytes), a transducer (which converts the physio-chemical interaction between the sensing element and the targeted molecules into a recognizable electrical/mechanical/optical signal) and a data analysis unit

(which interprets the transducer output and aids in diagnosis). In recent times, miniaturized sensing elements have been extensively used to enhance the overall sensitivity and push the detection limit down into femtomolar or attomolar range. In the last decade, development of nanostructured biosensors have enabled the realization of extremely sophisticated miniaturized platforms for several important healthcare applications encompassing disease diagnosis, drug delivery and non-invasive therapeutics. Nanotechnology aids significantly in providing profound insight into the biological processes that occur at the nanoscale using accurately designed models and templates. Improved understanding of nano-sized biomolecular structures and nanoscale biological principles such as molecular self-assembly and self-organization has had an outstanding impact on fields such as medicine, genetics and point-of-care diagnosis. Nanoscale one-dimensional conductors such as nanowires and nanotubes have been used as high-gain field-effect sensors for monitoring a variety of biological events including DNA hybridization and antibody-antigen interactions. Inherent advantages of the nanoregime, namely size dependent quantum effects, macroscopic quantum tunneling effect, high surface energy, spatial confinement; reduced imperfections, tunability of material properties, enhanced electromechanical properties, high surface to volume ratio and dimensions in the order of Debye length have contributed significantly towards developing ultrasensitive biosensing platforms with highly selective functionality. In particular, nanomaterial facilitated electrochemical detection and chemiresistive sensing of DNA hybridization have greatly influenced fields such as genetics and cancerous mutation detection. Additionally, researchers have extensively worked towards the development of electrochemical sensors for detection of disease specific antibodies and other biomolecules such as proteins. This communication is focused on the detection of cardiac biomarkers using nanostructured biomarkers.

Cardiac biomarkers: Conventional methods of detection & need of the hour

Early and accurate detection of acute myocardial infarction (AMI) is of great clinical relevance given the alarming worldwide mortality associated with cardio-vascular diseases (CVD). In 2012, an estimated 17.5 million people died from CVDs, representing 31% of all global deaths. The number of deaths on account of coronary heart diseases and cardiac strokes were estimated to be 7.4 million and 6.7 million respectively. Detection of CVDs is carried out using several methods such as conventional blood tests, Electrocardiograms (ECG), Echocardiograms, Exercise stress tests in conjunction with regular ECG monitoring to follow electrical activity of the heart, thalium stress tests involving injection of radioactive isotope, CT angiograms and coronary Angiography. These widely used conventional ways of infarction detection may not provide desired information for early diagnosis of cardiac arrest, as these are not point-of-care techniques and require expert technician as well as high-end laboratory setup. In developing countries, unavailability of adequate healthcare infrastructure and state-of-the-art diagnostic platforms in rural and remote locations has been a major bottleneck in providing early diagnosis of fatal heart diseases. In addition, the cost factor associated with the conventional techniques is not convenient for most of the rural population in India. Advancements in research related to cardio vascular diseases (CVD) have led to the identification of variety of biomolecules/ proteins (Cardiac biomarkers) whose levels get elevated in blood in response to various types and stages of vascular injury. Diagnostic mechanisms based on accurate monitoring of concentration of such markers in blood can provide error-free early detection of cardiac ailments. Among these 177 odd cardiac biomarkers, creatine kinase MB, troponin I and myoglobin have been identified as vital biomarkers as they indicate early stage of vascular necrosis [1, 2]. In general, for cardiac biomarker detection, sensing materials are functionalized with antibodies corresponding to the analyte of interest. Interaction between the antibody and the respective antigen provides the desired change in certain physio-chemical properties of the sensing material, which is later transduced into a recognizable signal form. As it turns out, both labeled and label-free biosensors have been used for detection of cardiac biomarkers and each of the sensing schemes has its own advantages and disadvantages.

Biosensors for cardiac biomarker detection

Various labeled approaches proposed for the detection of these biomarkers include mass

spectrometry [3], liquid chromatography [4], luminescence [5] and colorimetric [6] detection mechanisms. Zhang et al. reported a novel gold nanoparticle (AuNP)-based optical sensing system for the detection of myoglobin (Mb). The proposed detection scheme was based on the Mb-induced aggregation of an iminodiacetic acid moiety (IDA) functionalized AuNPs resulting from the structures of Mb sandwiched between the functionalized AuNPs via Cu2+ bridges in the coordination interactions of IDA – Cu^{2+} – histidine residues available on the Mb surface. A resultant red shift in the plasmon resonance band of the AuNPs was noticed because of the induction aggregation with a change in solution color from red to purple. They also investigated the selectivity of protein assay with the functionalized AuNPs, and it is found that the optical sensing of histidine-rich proteins is closely related to number and distribution of surface histidine residues as well as the size of proteins. Liu et al. reported peptide-functionalized gold nanoparticles (AuNPs) based colorimetric assay for the detection of troponin I [7]. In their study, a 12-mer cardiac troponin I (cTnI)-specific peptide was immobilized on AuNPs of different size and concentration via the CALNN anchoring sequence and a relationship was established between the total surface area of the AuNPs (binding availability) and response (centroid shift). The colorimetric assay for cTnI operating under optimized conditions (36 nm AuNPs) yielded a limit of detection of 0.2 ng/mL, when tested in diluted serum samples with an assay time of 10 min. Wu et al. described colorimetric detection of cardiac troponin I (cTnI) using a polydimethylsiloxane (PDMS)-gold nanoparticle based composite film with silver enhancement [8]. In labeled biosensors, labels such as flourophores, gold nanoparticles are used to identify and quantify the target proteins. Attaching fluorophores or gold nanoparticles is a time taking procedure that needs to be carried out externally and hence labeled biosensors may not be ideal candidates for point-of-care diagnostics. In contrast, label-free detection mechanisms such as conductometric methods [9, 10], electrochemical detection schemes [11-14], and surface plasmon resonance (SPR) [15-18] rely on the changes in physical properties upon the binding of target proteins. Lee at al. reported single site-specific polyaniline (PANI) nanowire based conductometric biosensor for the detection of cardiac biomarkers such as Myo, cTnI, CK-MB, and BNP with ultra-high sensitivity and good specificity. The PANI nanowire was directly fabricated via electrochemical deposition growth method between pre-patterned Au electrodes and antibodies for the aforementioned cardiac markers were covalently attached to it by a surface immobilization method. Biosensing of cardiac biomarkers was performed by measuring the change in conductance of the nanowires upon adsorption of the target analytes. It has been theorized that the conductance of the nanowire can be modulated by the major carrier accumulation or depletion as the binding between immobilized mAbs and target biomarkers changes the net surface charge of the nanowire. Using single PANI nanowire-based biosensors integrated with microfluidic channels, very low concentrations of Myo (100 pg/mL), cTnI (250 fg/mL), CK-MB (150 fg/mL), and BNP (50 fg/mL) were detected. Our group [10] has also reported about a chemiresistive platform wherein multi-walled carbon nanotube doped SU8 nanofibers were used as the sensing element. These composite nanofibers were synthesized using electrospinning process. Single nanofibers were aligned between pairs of electrodes in-situ during the electrospinning process. The target proteins were detected using chemiresistive detection methodology, which involves measuring the change in conductance of the functionalized nanofibers upon the binding of the targeted antigen. The minimum detection limits of Myo, CK-MB and cTnI were experimentally found out to be as low as 6.20 and 50 fg/ml respectively. No response was observed when the nanofibers were exposed to a non-specific protein, demonstrating excellent specificity to the targeted detection. Puri et al. reported a single-walled carbon nanotube (SWNT) based ultrasensitive label-free chemiresistive biosensor for the detection of human cardiac biomarker, myoglobin [19]. In their work, poly(pyrrole-co-pyrrolepropylic acid) with pendant carboxyl groups was electrochemically deposited on electrophoretically aligned SWNT channel, as a conducting linker, facilitating the biomolecular immobilization. For the device fabrication, 2.0 µl of dispersed SWNT (in DMF) suspension were aligned across a pair of microfabricated gold electrodes (3 micron apart) using A.C. dielectrophoresis at 5 MHz frequency with a peak-to-peak amplitude of 1.5 V, followed by annealing at 300°C for 1h under a reducing environment (N_2 with 5% H_2).The device exhibited a linear response with a change in conductance in SWNT channel towards the target

analyte over the concentration range of 1 - 1000 ng ml^{-1} with a sensitivity of 118% per decade. Swati Singh et al. also reported about a label free single-walled carbon nanotubes (SWNTs) based chemiresistive genosensor, which was aimed at the early detection of Streptococcus pyogenes infection in human causing rheumatic heart disease [20]. The genosensor exhibited a linear response to S. pyogenes G-DNA from 1 to1000 ng/ml with a limit of detection of 0.16 ng/ml. Vikash Sharma and group reported a platinum nanoparticle modified single-walled carbon nanotube hybrid chemiresistive sensor for detection of antigen myoglobin (Mb) in phosphate buffer saline [21]. Change in the source–drain current of the hybrid device was correlated to the analyte concentration and the detection was established in the concentration range of 0.1 –1000 ng ml^{-1}. The hybrid device response was fitted against the Hill–Langmuir equation with a maximum response of 111.14%. Mandal et al. demonstrated rapid electrochemical detection of cardiac myoglobin using hydrothermally synthesized TiO_2nanotubes wherein denaturant induced unfolding of myoglobin facilitated efficient reversible electron transfer from protein to electrode surface. The sensitivity of the proposed platform was reported to be 18 μA mg^{-1} ml with a detection limit of 50 nM. Moreira et al. described myoglobin detection based on a smart plastic antibody material tailored on disposable screen-printed electrodes. Electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) techniques were used in their work for detecting the target analyte. Mishra et al. reported a mercaptopropionic acid capped ZnS nanocrystal based bioelectrode for the detection of the cardiac biomarker myoglobin. The devices were subjected to electrochemical impedance spectroscopy characterization in addition to atomic force microscopy, contact angle measurements and cyclic voltammetry. The EIS modeling reported in their communication revealed that the charge transfer resistance increases considerably without any significant change in the double layer capacitance after immunoreaction with protein specific antigen myoglobin. The bioelectrode exhibited a linear electrochemical impedance response in the range of 10 ng to 1 μg mL^{-1} in phosphate buffer solution (pH 7.4) with a sensitivity of 117.36 Ω cm^2 per decade. Our group [14] has also demonstrated electrochemical detection of cardiac biomarkers using electrospun nanofibers of multi-walled CNT embedded SU-8. The composite nanofibers have excellent electrical and transduction properties owing to the presence of MWCNTs in addition to ease of functionalization and biocompatibility, which can be attributed to the presence of SU-8. Glassy carbon working electrodes were surface modified with the nanofiber and antibodies specific to the targeted biomarkers were covalently attached to the surface-modified working electrode using conventional functionalization chemistry. Electrochemical Impedance Spectroscopy was used in our work for interpreting the adsorption kinetics at the electrode surface. Electrochemical studies reported therein were performed using an electrochemical analyser (CH660E, CH Instruments, USA) employing a three-electrode system comprising a 0.5 mm platinum wire as the counter-electrode and Ag/AgCl (saturated, 0.1 M KCl) as the reference electrode. EIS measurements were carried out in 0.1 mM phosphate buffer solution (pH 7.0, 5 ml volume) containing 2.5 mM each of $K_4Fe(CN)_6$ and $K_3Fe(CN)_6$, as the redox active couple. The frequency ranges used for the measurement were from 25 mHz to 1 MHz with a sine wave amplitude of 5 mV. Cyclic voltammetry measurements were carried out specifically for myoglobin detection and fresh pH 7 phosphate buffer was used as electrolyte in those experiments. The label free approaches, in addition to being highly sensitive, are devoid of any complex labelling steps, thus making them amenable for point-of-care applications. Miniaturization is the key for developing economically viable point-of-care diagnostics. Several lab-on-chip approaches and microfabrication technologies have been proposed for the detection of cardiac biomarkers and are very well reviewed by Qureshi et al. [22]. Rao's group has developed a SU-8 photoresist based peizoresistive polymer cantilever platform for detecting these biomarkers [23-25]. Lab-on-a-chip based immunosensor principles and technologies for the detection of cardiac biomarkers have been reviewed by Mohammed et al. [26]. In addition to development of nanoscale sensing materials, microfluidics also plays a critical role in developing lab-on-chip devices. Liu et al. have provided a well-compiled review of microfluidic based biosensors wherein they have summarized the recent advancements in continuous and discrete micro fluidic systems, as well as their biomedical applications [27]. Shin et al. have reported a novel microfluidic aptamer-based

electrochemical biosensing platform for monitoring damage to cardiac organoids [28]. They accentuate the need of developing novel analytical platforms to provide non-invasive, accurate information on the status of organoids at low working volumes. Zhao et al. recently reported about a paper based electrochemical platform coupled with microfluidics, which was used to demonstrate the detection of glucose, lactate and uric acid in urine [29]. Such technology can very well be extended to develop paper based portable low-cost devices for cardiac biomarkers detection. A proof of principle regarding simultaneous electrochemical detection of simultaneous detection of cTnI and C-reactive protein using a PDMS-Gold nanoparticle composite microfluidic platform was provided by Zhou et al. in 2010 [30]. CdTe and ZnSe quantum dots were bioconjugated with desired antibodies for developing a sandwich immunoassay for the targeted biomarkers and square-wave anodic stripping voltammetry was used for ensuring quantification of the biomarkers.

Conclusion

In addition to the above-mentioned research, several other prominent works have been reported in the last decade regarding the development of biosensors for detection of cardiac biomarkers. Microfluidics based lab-on-chip platforms for simultaneous detection of multiple cardiac biomarkers have also been postulated to ensure accurate diagnosis of cardiac malfunctions. However, to make these researches more beneficial to the common people, it is essential to develop low-cost devices, which can be easily operated without a great deal of technical expertise. Furthermore, making the read-out mechanism simple and effortlessly interpretable is also an important criterion.

Conflicts of interest

Author declares no conflicts of interest.

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