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Diagnostic biosensors in medicine – A review

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ABSTRACT

Biosensors consist of a biocatalyst that can detect a biological element and a transducer which can convert the combination event of the biocatalyst and the biological element into a detectable parameter. The biocatalyst can be biomolecules like, enzyme, DNA, RNA, metabolites, cells, oligonucleotides etc., and the transducers can be electrochemical, optical, piezoelectric, acoustics, calorimetric etc. In disease diagnostics biosensors utilizing immobilized cells, enzymes, nucleic acids have come into the field in recent years. Nanobiosensors exploiting the ultrasmall size and unique properties have also been used for engineering disease diagnostic biosensors. The use of biosensors can rapidly assess the health status, onset of the disease and its progression and can help to plan treatment for many diseases with the aid of multidisciplinary combination of chemistry, medical science and nanotechnology. The devices are cost effective, highly sensitive, rapid, user friendly and can be produced in bulk for human use. This review focuses on the different biosensors for the diagnosis of three major diseases like diabetes, cardiovascular disease and cancer.

1. Introduction

Biosensors, coined by Cammann, are analytical devices that convert a biological response into an electrical signal. Typically, biosensors should be extremely specific and irrespective of physical limitations like pH, temperature and may be recyclable (Mehrotra, 2016). Practical approach to design a biosensor needs fabrication, immobilization, transducing devices which altogether offers engineering of multidisciplinary research in chemistry as well as in biology (Bhalla, 2016). The material needed in biosensors are divided into four groups based on its working mechanism like; 1. Biocatalytic i.e. enzymes based biosensors. 2. Bioaffinity group, i.e. involvement of antibodies, antigen and nucleic acid. 3. Microbes i.e. biosesnsors containing microorganisms. 4. Nanosensors i.e. sensors with active nanoparticle that usually increase sensitivity and specificity towards early detection of disease (Byrne, 2009; Cash and Clark, 2010; Holford, 2012; Omidfar, 2013; Rocchitta, 2016). These various types of biosensors allow to sense levels of hormones, drugs, toxins, pollutants, heavy metals, pesticide etc. with considerable precision. In the present review an attempt was made to highlight various types of biosensors which have indispensable application in medicines, for instance enzyme specific biosensors, immunosensors, nano specific biosensors and DNA biosensors.

Biosensors are devices that usually estimate the levels of biological markers or any chemical reaction by producing the signals that are mainly associated with the concentration of an analyte being in the chemical reaction (Mehrotra, 2016). Such biosensor usually help to monitor diseases, drug discovery, detection of pollutants, detection of disease causing bacteria and markers that typically indicate the diseased conditions, like body fluids (saliva, blood, urine, sweat etc.) (Ngoepe, 2013). A common biosensor depicted in Fig. 1.

A typical biosensor consists of;

1. **Analyte**: A substance of interest which is needed to be detected, for example glucose for diabetes.
2. **Bioreceptor:** A molecule which recognizes the analyte can be a bioreceptor for example enzymes.
3. **Transducer:** It usually converts a bio recognition event into measurable signal, known as signalization.
4. **Electronics:** It generally processes the transduced signal in display form.
5. **Display:** Usually its liquid crystal display in combination with hardware and software for generation of biosensor result into user friendly manner (Kazemi-Darsanaki, 2012).

The first biosensor was commercialized in the year of 1975 by Yellow Spring Instruments which were designed to detect glucose level in blood (Yoo and Lee, 2010). Table 1 shows the historical survey of the biosensor in the duration of 1970–1992 (Bhalinge, 2016).

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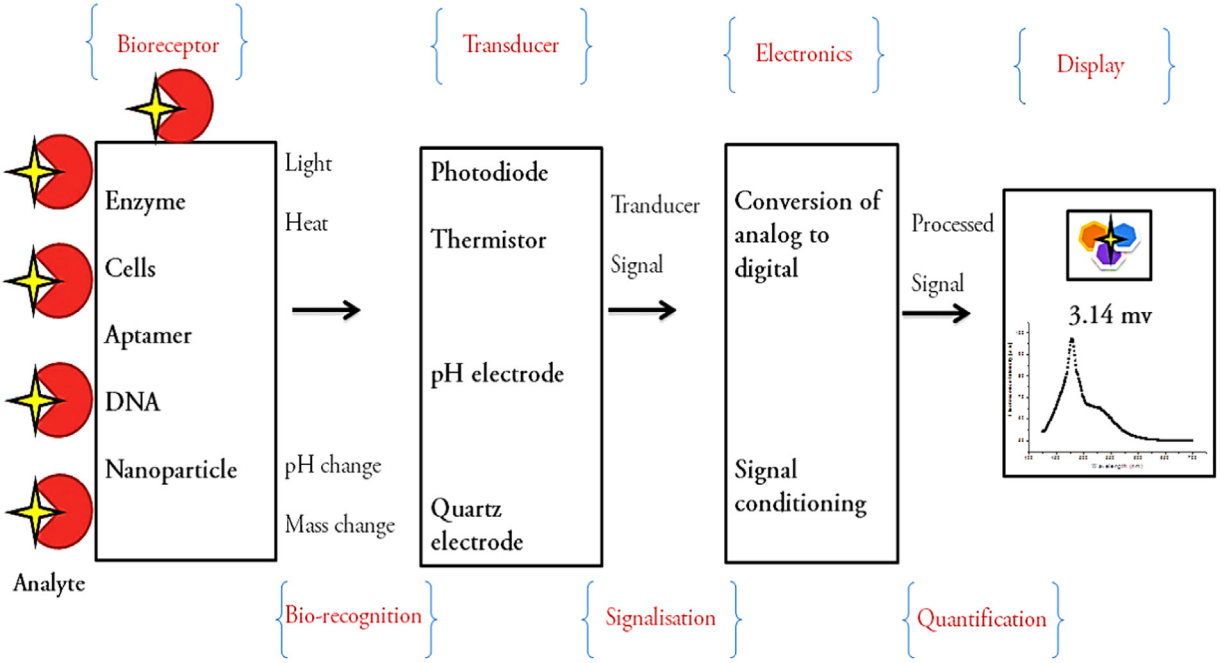

Fig. 1. Schematic depiction of biosensor.

Table 1
Historical overview of biosensor development.

Year	Event
1970	Discovery of ion-sensitive field-effect transistor (ISFET) by Bergveld (Bergveld, 1970)
1975	Fibre-optic biosensor being carbon dioxide and oxygen detection by Lubbers and Opitz (Vestergaard et al., 2015)
1975	First commercial biosensor for glucose detection by YSI (Yoo and Lee, 2010)
1975	First microbe-based immunosensor by Suzuki et al. (Suzuki et al., 1975)
1982	Fibre-optic biosensor for glucose detection by (Schultz, 1982)
1983	Surface plasmon resonance (SPR) immunosensor by Liedberg et al. (Liedberg et al., 1983)
1984	First mediated amperometric biosensor: ferrocene used with glucose oxidase for glucose detection (Cass et al., 1984a)
1990	SPR-based biosensor by Pharmacia Biacore (Vestergaard et al., 2015)
1992	Handheld blood biosensor by i-STAT (Vestergaard et al., 2015)

The typical characteristics of biosensors are;

1. **Selectivity:** This feature is most important in biosensor because this depends on the potential of bioreceptor to detect the specific analyte in a mixture of sample and contaminants. For example; specific interaction of antibody with particular antigen (Bhalla, 2016).
2. **Reproducibility:** It is just reproducibility of biosensor to program identical output after repetition of the experimental setup (Bhalla, 2016).
3. **Stability:** It is the ability of biosensor to be non-susceptible in ambient conditions in and around the biosensing system. Any disturbance can modulate the output signals of biosensor for its measurement which may lead to error and can affect the efficiency of the biosensor. The transducer and electronics can be temperature sensitive, which may create disturbance in the performance biosensor. Therefore, precision tuning is must to ensure stable output of a biosensor. In addition to these, another factor can influence the biosensor which is the binding affinity of analyte towards the bioreceptor. Thus, the biosensor needs to be highly stable to overcome these issues (Bhalla, 2016).
4. **Sensitivity:** Sensitivity can be defined based on its limit of detection (LOD). In many medical application, biosensors have to detect analyte as small as in the range of concentration of ng/mL or fg/mL, to affirm the existence of analyte in the sample. For example, in Prostate cancer, the prostate specific antigen (PSA) concentration will have a value of nearly 4 ng/mL in blood, which needs to be detected with high precision (Bhalla, 2016).
5. **Linearity:** It attributes to the accuracy of the measured response. It can be depicted mathematically as $y = mc$, where c is the concentration of the analyte, y is the output signal and m is the affectability of the biosensor. A little change in concentration of an analyte makes a difference in the yield of the biosensor. Another term that is taken into consideration associated with is the linearity in a linear range, which can be defined by the small amount of change in concentration, giving a considerable change in the signal (Bhalla, 2016).

There are several applications of biosensor which has been implemented in various fields like medical science, marine sector, food industry, etc., and also these biosensors are programmed for better sensitivity and linearity in comparison with traditional methods (Mehrotra, 2016). In the present review, we are attempting to cover biosensors which are related to the medical science. In the field of medical science, the application of the biosensor is growing rapidly. The glucose biosensor is most widely used in the medical application for the diagnosis of diabetes mellitus.

2. Glucose biosensors in diabetic management

Blood glucose monitoring has become a valued tool in diabetes management because maintaining a regular blood glucose level is usually done by consulting the clinicians that made a series of

Table 2
Historical overview of glucose biosensors.

Year	Event
1962	First description of a biosensor by Clark and Lyons (Clark and Lyons, 1962)
1967	First practical enzyme electrode by Updike and Hicks (Updike and Hicks, 1967)
1973	Glucose enzyme electrode based on detection of hydrogen peroxide (Guilbault and Lubrano, 1973)
1975	Relaunch of first commercial biosensor, i.e., YSI analyzer (Newman and Turner, 2005a)
1976	First bedside artificial pancreas (Miles) (Bruttomesso and Grassi, 2015)
1982	First needle-type enzyme electrode for subcutaneous implantation by Shichiri (Newman and Setford, 2006)
1984	First ferrocene mediated amperometric glucose biosensor by Cass (Cass et al., 1984b)
1987	Launch of the MediSense ExacTech blood glucose biosensor (Newman and Setford, 2006)
1999	Launch of a commercial *in vivo* glucose sensor (MiniMed)
2000	Introduction of a wearable noninvasive glucose monitor (GlucoWatch)

development of blood glucose sensors. Diabetes mellitus is highest prevailing endocrine disorder of carbohydrate metabolism (Hameed, 2015) having more number of morbidity and mortality in the developed countries (Tabish, 2007). Multiple tests are common for investigating and monitoring of diabetic markers in the diabetic patients. The major diagnostic criteria for diabetes are blood glucose level which involves self-monitoring of glucose level by diabetic patients. Studies have shown that the control of blood glucose level in the normal range can help amelioration of microvascular (nephropathy, neuropathy, and retinopathy) and macrovascular (coronary artery disease and stroke) complications (Martín-Timón, 2014). Blood glucose is commonly found in the range of 4.9–6.9 mM in healthy individuals that can increase up to 40 mM in diabetic patients after glucose intake (Bruen, 2017). Although various types of glucose sensors are available commercially, advancements in glucose biosensors have changed over the years which summarized in Table 2 (Yoo and Lee, 2010).

2.1. First generation of glucose biosensor

Clark and Lyons proposed the first glucose biosensor in 1962 which was dependent on electrochemical approach with the use of glucose oxidase (GOx). Due to the electrochemical approach, this biosensor offered high level of sensitivity and its fabrication involved relatively low cost. GOx was employed in this enzyme based sensor which have high sensitivity towards glucose and also it could tolerate changes in pH and temperature, unlike other enzymes such as hexokinase and glucose 1-dehydrogenease (Wang, 2001).

GOx usually oxidizes glucose into gluconolactone in the presence of oxygen with production of hydrogen peroxide (H_2O_2) and water as by product (Milton, 2013). Further, it undergoes reaction which produces the carboxylic acid product named gluconic acid. GOx needs redox cofactor to execute the oxidation process with the input of flavin adenine dinucleotide (FAD^+). FAD^+ is an electron acceptor which is reduced to $FADH_2$ by redox reactions. Subsequent reaction with oxygen that produces H_2O_2 regenerates the FAD^+ at anode, which can sense the number of transferring electrons that is correlated with the amount of H_2O_2 production and hence, the amount of glucose present (Milton, 2013; Turale and Agrawal, 2018).

The glucose biosensor has a thin layer of GOx enmeshed over an oxygen anode (by means of semipermeable dialysis film), and the oxygen devoured by the enzyme-catalyzed response can be observed, as shown in Fig. 2. The Clarks original patent covered with use of one or more enzyme which usually converted the electroinactive substrate to electroactive products. The first glucose sensor was launched by the Yellow Spring Instrument company in 1975 (the Model 23 YSI analyzer) for the forthright assessment of glucose in 25 μL samples of whole blood (Wang, 2008). Further, Updike and Hicks developed the glucose biosensor by the use of two oxygen electrodes in which one was secured with enzyme and was used to estimate the difference in current for correcting the background variation in the samples (Yoo and Lee, 2010).

In 1973, Guilbault and Lubrano developed an enzyme electrode for the determination of glucose, which was based on amperometric transducer i.e. anodic by monitoring the discharge of H_2O_2 (Wang, 2008). During the 1980s biosensors became a trending topic which made them to develop second generation glucose biosensor i.e., commercial strips forself-monitoring of glucose, modified form of the electrode, which will enhance the sensitivity of the sensor (Yoo and Lee, 2010; Wang, 2001). The first generation was usually based on natural oxygen substrate and depended on the detection of hydrogen peroxide (Rocchitta, 2016). The major problem with first generation which was based on amperometric measurement of hydrogen peroxide that demanded high operation potential with high selectivity. Another drawback was the confined dissolvability of oxygen in biological fluids, which delivered changes in the oxygen tension, known as the "oxygen deficit" (El-Laboudi, 2014).

2.2. Second generation of glucose biosensor

To overcome the above discussed limitations, the second generation glucose biosensor has come into the picture. The enhancements were accomplished by substituting oxygen with non-physiological electron acceptors, called redox mediators that could convey electrons from the enzyme to the surface of the working electrode (Bakker and Qin, 2006). A reduced mediator is formed rather than hydrogen peroxide and after that reoxidized at the electrode, giving an amperometric signal and recovering the oxidized form of the mediator (Rahman, 2010). Variations in mediators, for example, ferrocene, ferricyanide, quinines, tetrathialfulvalene (TTF), tetracyanoquinodimethane (TCNQ), thionine, methylene blue, and methyl viologen were utilized to enhance the sensor execution. The unique properties of ferrocene as mediator, like, it does not react with oxygen in oxidized and reduced forms, independent of pH, shows the reversible electron transfer kinetics and reacts fast with enzyme, makes it a suitable mediator in glucose biosensor. There were extensive studies, which was the shuttling between electrodes and the mediators like GOx and glucose dehydrogenase pyrroloquinolinequinone (GDH-PPQ) (Harper and Anderson, 2010).

In 1987, the first electrochemical self-monitoring blood glucose (pen sized biosensor) implemented commercially in to the market named ExaTech by the company Mediasense Inc., which used GDH-PPQ and a ferrocene derivative. This implementation of biosensor was the revolution among the diabetic patients and many of today's biosensors are somewhat based on the use of ferrocene or ferricyanide mediators (Newman and Turner, 2005b).

2.3. Third generation of glucose biosensor

This type of generation is usually reagent less and there is a direct exchange of electrons among the enzyme and electrode without the use of mediators (Fig. 3). Mediators have high toxicity, so here electrode acted as a direct electron transfer mode by the use of organic conducting material which is based on charge- transfer complexes, like,

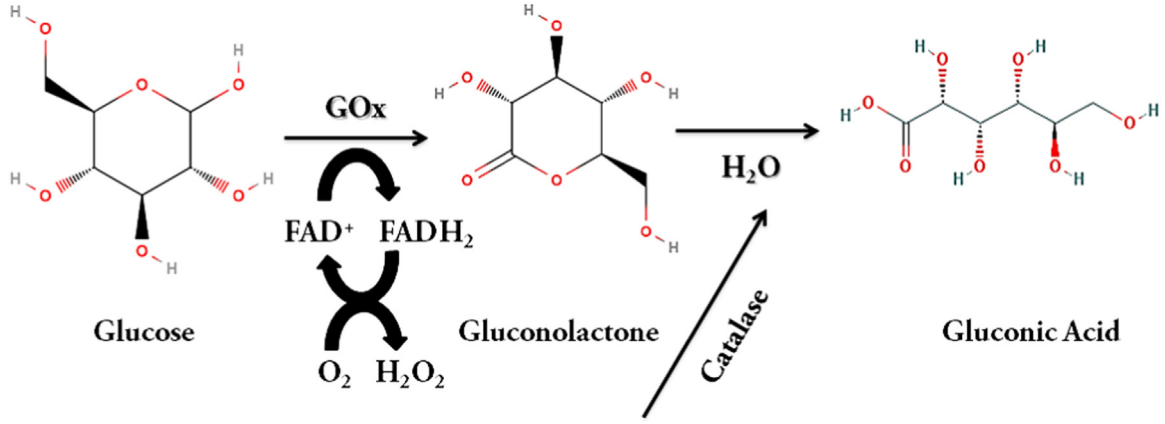

Fig. 2. The transition of glucose to gluconic acid using glucose oxidase.

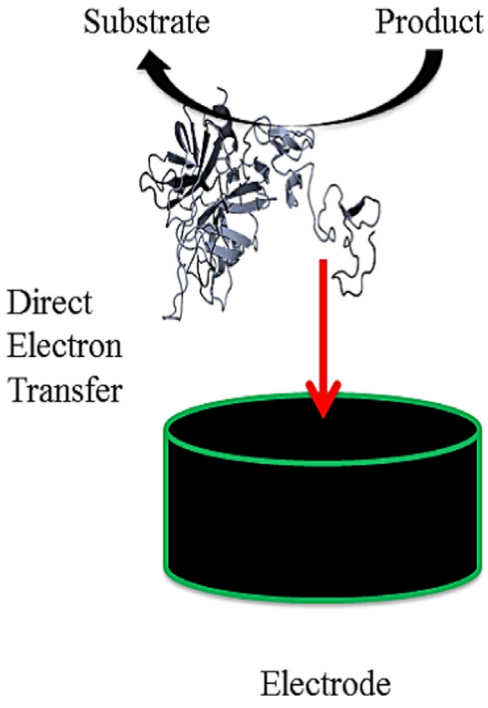

Fig. 3. Third generation of glucose biosensor.

conducting organic salt, tetracyanoquinodimethane (TTF-TCNQ). This TTF-TCNQ mediates the electrochemistry between the GOx and GDH-PPQ. This absence of mediators provided super selectivity to the glucose biosensors (Zhang and Li, 2004).

2.4. Non enzymatic glucose biosensor

Continuous glucose monitoring (CGM), with respect to discontinuous self-checked blood glucose (SMBG) values, has managed critical changes in the administration of diabetes mellitus (Rodbard, 2017). Nonenzymatic glucose sensors can be believed as the fourth generation of glucose biosensors. The fabrication of catalytic electrode for oxidation of glucose was the main moto for the non enzymatic glucose biosensors. Usually, in this type of biosensor, the electrodes are modified by using the dealloying, etching, electrodepostion, and electrochemical anodization (Hwang et al., 2018). This can be carbon based materials, metal-metal oxides and layers of the hydroxides (Rahman, 2010). Some of the fourth generation example are discussed below.

The study described non enzymatic novel glucose biosensor fabricated by the lithographic process of gold on sensing electrode. The biosensor was developed in multi-step like; 1. AZ-1518 spin-coating photoresist onto a reclaimed silicon wafer, 2. To expose and develop a hexagonal close-packed column array of photoresist AZ-1518, masked with a hexagonal close-packed circle array, 3. To convert each photoresist column into a photoresist hemisphere, thermal melting was employed and, 4. A gold thin film and GNPs over the hemisphere array sputtered on AZ-1518 to form the sensing electrode. The effective sensing space of the projected electrode was found to be 10.2-fold bigger compared with the plain gold electrode. Existing glucose measurements disclosed that the projected biosensing theme might operate in an exceedingly linear manner with detection range varying from 55.56 μM–13.89 mM with a sensitivity of 749.2 μA mM^{-1} cm^{-2} and a detection range up to 9 μM. The steadiness of the projected nonenzymatic glucose biosensor was proved through twenty cycles of Cyclic voltammetry and a chain investigation for a couple of months. The projected non-enzymatic novel glucose biosensor has the benefits of being enzyme free, easy to fabricate, less cost, and simple to preserve for a long-run basis and may be employed in clinical applications in future (Hsu, 2016).

Another step towards the fabrication of highly-efficient non-enzymatic glucose device electrode was achieved by directly growing ZnO nanorods (ZnO NRs) on fluorine doped tin oxide (FTO) conductor followed by CuO modification. The distinctiveness of CuO-ZnO NRs/FTO electrode is that, the directly-grown ZnO NRs on electrode surface provided straight forward substrate with penetrable structure, and huge expanse for CuO modification that successively enhanced electrochemical activity for glucose detection. The sensing electrode exhibited outstanding high performance in terms of sensitivity, wide response, variation in time interval, property, reliability, repeatability, and stability. In addition, the glucose detection in real human blood showed the electrode's quality for sensible detection (Ahmad, 2017; Chung, 2017).

This improved sensing performance is especially attributed to the matured nanostructures that offered a wonderful contact between the nanostructure and electrode with high expance for catalytic sites, facilitating appropriate path for electron transport thoughout the electrochemical activity. Overall, the fabricated electrodes by chemical vapor deposition and lithographic process may be visualized as promising technique for utilization of non-enzymatic glucose activity in real clinical samples which can acquire extended edges for various biomolecule detection (Ahmad, 2017; Chung, 2017).

3. Cardiovascular disease detection using biosensors

Cardiovascular disease (CVD) is the number one cause of death globally and more people die because of CVD than any other cause (Yusuf, 2001). An estimated 17.7 million people died from CVD upto 2015 that represents overall 31% of all global deaths. Among these 7.4

million were due to coronary heart diseases and 6.7 million due to the stroke (Al-Mawali, 2015). A person with CVD needs more early detection and management by the way of medicine and counseling. The existing technique to detect CVD depends on classical method which is generally based on tests that may take several hours or sometimes days. These diagnosis criteria are set by WHO, where patient should meet at least one of conditions like; diagnostic electrocardiogram (ECG) changes, elevation of the biochemical markers in their blood samples and, acteristic chest pain. ECG is an important parameter for management of therapy, but in case of CVD, the ECG is a poor diagnostic test because half of the CVD patients show normal cardiogram which makes it more difficult to diagnose CVD (Kost and Tran, 2005). Biosensor may assist in quick diagnosis, giving excellent health care and decreasing the delay time for results dissemination which is immense stressful for the patients.

3.1. Biomarkers of CVD

CVD is a class of various types of disorders that involves the heart and blood vessels. CVD includes the condition that gives appearance of plaque which construct the walls of the arteries known as atherosclerosis (Wilson, 1998). This plaque usually reduces the lumen size of the arteries and makes more effort for the blood to flow, which eventually causes stroke and heart attack. CVD can be provoked by various factors like genetic, gender, age, elevated blood pressure, diabetes, overweight, stress, cholesterol and other lifestyle changes (Martín-Timón, 2014). On the basis of diagnostic and sensing point of view, CVD biomarker can be classified into pathogenic and therapeutic types. A release of a molecule from vascular walls into the blood stream that can imitate the pathological process taking place is the biomarker and can be detected using the biosensors. In theory, the biomarkers could be a molecule that is involved in different pathological processes, but not all these molecules suits for CVD biomarker and certain conditions has to be fulfilled to be a suitable biomarker for CVD.

The ideal characteristicts of CVD biomarker should possess; 1. High clinical specificity and sensitivity. 2. Quick release of biomolecule into the blood stream for early detection. 3. Should have capability to stay a longer time in blood 4. To be able to assayed quantitavely. It is dificult to have a specific selective marker for CVD (King, 2016). The most studied biomarkers are shown in Fig. 4.

3.2. Biosensors for CVD

3.2.1. Fluorescence based cardiac biosensors

Various types of biosensors have been fabricated using optical biosensors. The recent research focused on the detection for the multi-analyte and the addition of immunosensors for microfluidic platforms.

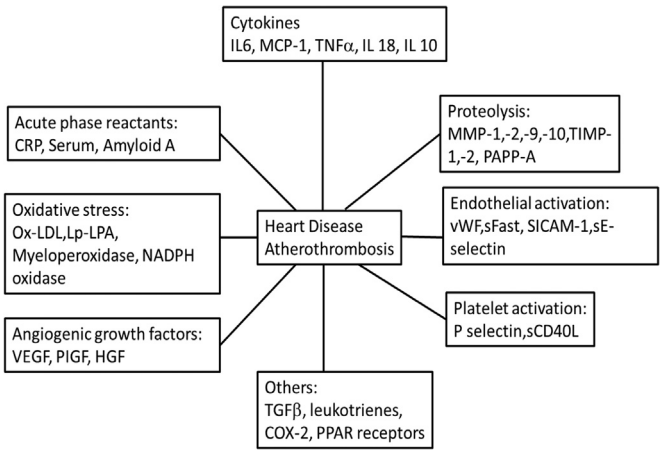

Fig. 4. Most studied biomarkers for CVD.

For example, a proof of concept in which a combination of micromosaic immunoassays (μMIAs) with self regulating microfluidic networks (μFNs) to detect C reactive protein (CRP) and other cardiac markers such as myoglobin (Mb) and cardiac Troponin (cTnI) was engineered. With this method, CRP can be quantitavely detected within 10 min in 1 μL of human plasma down to concentration up to 30 ng mL^{-1}. Such multiple analyte detection, rapid availability of results with a simple experimental approach has made point-of- care testing devices (Wolf, 2004). A novel sandwich of Rubpy-encapsulated fluroscent core shell silica nanoparticles in combination with a fluoroimmunoassay for recombinant human interleukin-6 (IL-6) has been developed for the determination of IL-6 in human serum samples and also it was enabled by fluroscence microscopy imaging for detection of IL-6. This sandwich gave a detection limit up to 7 pg/mL (Hun and Zhang, 2007). In another approach, a biosensor has 20 fold improvement of the fluorescence signal in the detection of the cytokine TNF-α in a sandwich immunoassay. A competition-based tagged-internal standard (TIS), assay was developed to estimate the elevation of blood proteins in the human serum. In this biosensor, a target protein in the serum compete to bind with TIS to an antibody array which was fabricated by immbolization of a target protein with specific antibody on carboxylate-modified latex bead, surface well type-arrays. This approach yielded high-throughput, quantitative, and label-free TNF-α detection and may be useful in the rapid serodiagnosis of human disease (Jung, 2009).

In another approach aptamer-antibody on chip sandwich immunoassay was employed to detect CRP in spiked serum. Use of RNA aptamer-based biochip with high affinity and having specificity for CRP (exist in concentration in the range of 1–3 mg/L) is showed in Fig. 5.

To detect CRP using an RNA aptamer method was superior, but lower in data reproducibility. So another approach in which biosensor have been developed without involving antibody to detect CRP selectively with the use of specific fluorophores, like fluoreseinamine isomer 1 and O-Phosphorylethanolamine (PEA). This fluorophore gives high emission in the existence of CRP in solution upto a concentration of 20 ng/mL. This approach is simple, cost effective and sensitive in detection at low level of CRP (Pultar, 2009). Further a fluoro-microbead guiding chip (FMGC) was developed to detect the level of cardiac troponin I (cTnI). cTnI is preferred marker for myocardial infarction. This

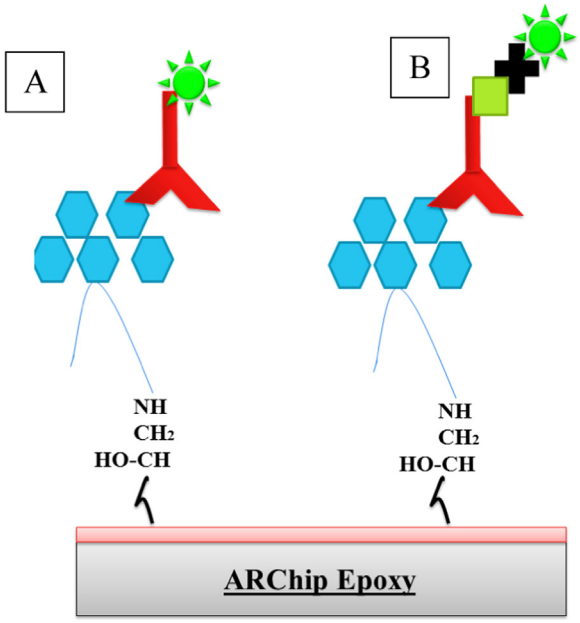

Fig. 5. Biosensor which is based on sandwich immunoassay by using the RNA aptamer for the detection level of CRP A) Dy647-labeled anti-CRP was used for single step detection; B) biotinylated anti-CRP and Dy647-Streptavidin for two step detection.

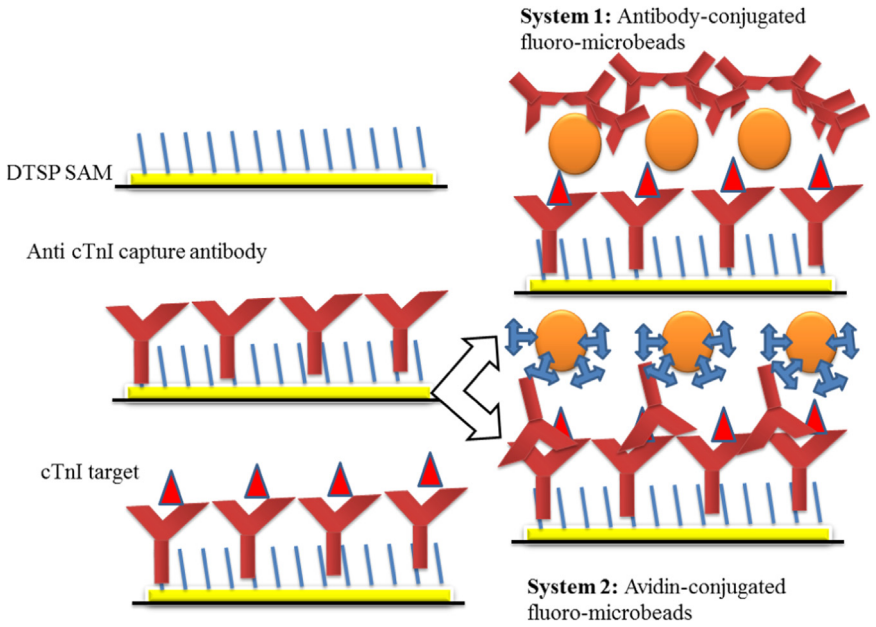

Fig. 6. Schematic diagram sandwich immunoassay using antigen/antibody binding system 1 and avidin/biotin affinity binding (system 2) on the FMGC.

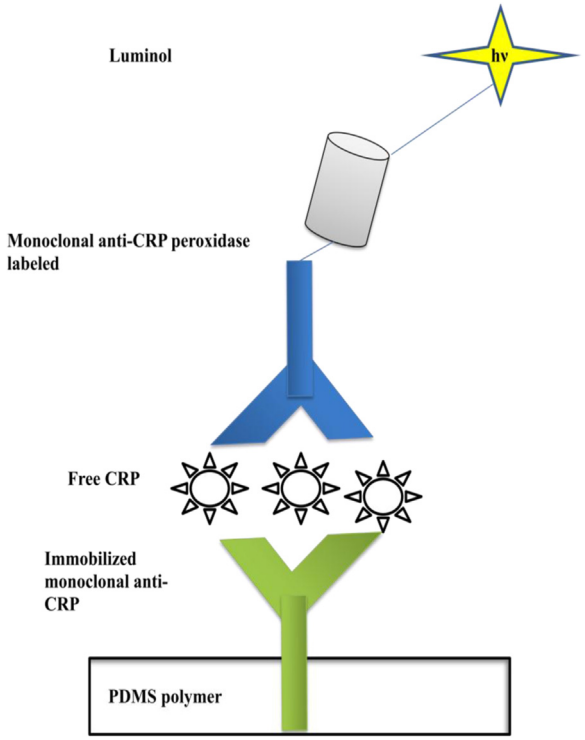

Fig. 7. Schematic representation of sandwich CRP assay.

FMGC biosensor has four immune reaction sites on a silicon substrate, with five gold patterns imprinted on each region for multiple assay (Han, 2016). This assay can detect the immunospecific binding from the non specific binding through optical signals from outside and inside pattern. To capture cTnI specific antibody, this biosensor used conjugated FMGC surface with cTnI specific antibody by reaction with 3-3-dithiobis-propionic acid N-hydroxysuccinimide ester to make a self-assembling antigen-sensing monolayer (DTSP SAM) on the chip as shown in Fig. 6.

This FMGC based sandwich immune assay has accomplished high accuracy and minimizes non-specific binding.

3.2.2. Luminescence based cardiac biosensors

The luminescence based methods are used for the detection of early CVD biomarkers. This method is categorized in two types, namely chemiluminescence (luminescent signal by the action of an enzyme labeled antibody) and electroluminescence (luminescent signal generated by electron transfer reaction of luminescent compound) (Qureshi, 2012; Yang, 2009).

The cardiac chip was developed to detect CRP and IL-6 by designing newly fashioned chip which admits the entrapment and isolation of a single polymeric sphere in micromachined pits. The support of each bead can quickly establish a series of reagents/washes through microfluidic structures. The merging of these miniaturized components, developed the integration of multiplex assays with brief analysis times by employing the small sample volumes (Zhao, 2017).

Another technique involved the relying of macromolecules to PDMS transfer in which direct entrapment of macromolecules spots, during PDMS polymerization was done. Before being masked with liquid PDMS, macromolecules spots were patterned using piezo arrayer on 3D glass or Teflon. Then after healing at high temperature it produced an elastomeric polymer which can be easily separated from the mold. Once PDMS was peeled off, the polymer incorporated randomly grafted protein, spots at the PDMS air interface (Heyries, 2009). The idea was to combine the macromolecule immobilization, without the need of extra chemicals, and easy 3D structure achievement according to the initial mold conformation. This biosensor used sandwich immunoassay for CRP detection using PDMS air interface. CRP assay is usually clinically accepted and offers a low limit of detection to high limit of detection in order to fit in a wide range of healthy as well as an affected person (0.05–50 mg/L). Such sensitivity can be achieved by using two monoclonal antibodies; one was used for the capture and the other to detect target protein. So here anti-CRP monoclonal antibodies were immobilized on PDMS air interface and chemiluminescence was used to detect free CRP as shown in Fig. 7.

Currently electrolumiscence, are very less studied for CVD. Cadmium Sulfide (CdS) nanocrystal based electroluminescence biosensor was developed for the detection of low density lipoprotein with the high sensitivity achieved by gold nanoparticle (Allijn, 2013). In aqueous solution, electrochemical reduction of Mercaptoacetic acid (RSH)-capped CdS nanocrystals (NCs) takes place which further reacts with the co-reactant $S_2O_8^{2-}$ to produce strong electrochemiluminescence. This biosensor was developed as follows; gold

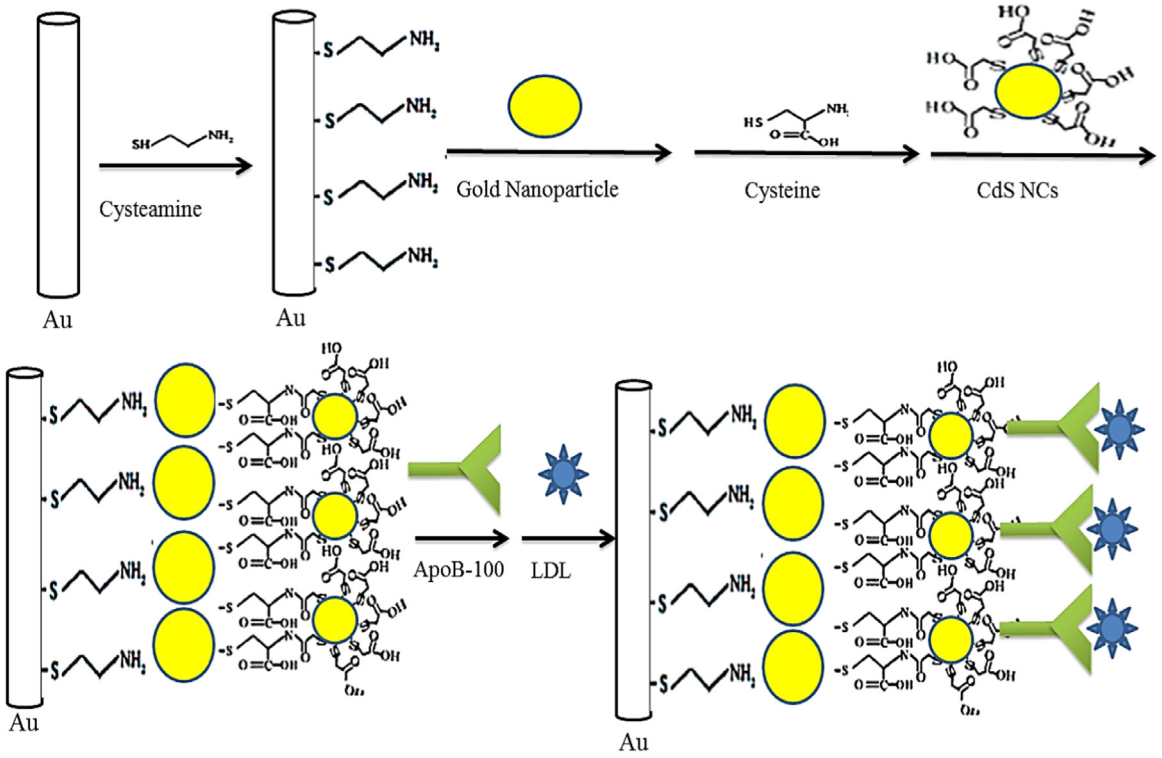

Fig. 8. Schematic diagram of the biosensor fabrication and LDL binding.

nanoparticles were first massed onto an acysteamine monolayer on the gold electrode surface as shown in Fig. 8. Cysteine treated gold nanoparticle-covered the electrode and reacted with CdS NCs to afford a CdS NC-electrode. Finally, apoB100 (ligand of LDL receptor) was covalently combined to the CdS NC-electrode. This modification allowed for LDL detection with a high sensitivity, with values as low as 0.006 ng mL^{-1} (Jie, 2007).

3.2.3. SPR based cardiac biosensors

In the last two decades, surface plasmon resonance (SPR) and its improvement with imaging (SPRi) made them convenient and reliable platform in the clinical analysis for label free and real time interaction of biomolecules (Nguyen, 2015). SPR is defined as a charge-density oscillation at the interface among two media, with dielectric constants of opposite signs (e.g., metal and a dielectric), which produces a surface plasmon wave (SPW) with a propagation constant β, expressed by the following equation;

$$\beta = \frac{\omega}{c}\sqrt{\frac{\varepsilon M \varepsilon D}{\varepsilon M + \varepsilon D}}$$

where ω is the angular frequency, c is speed of light in vacuum, and εD εM are the dielectric constants of dielectric and metal, respectively (Mariani and Minunni, 2014).

Ultrasensitive magnetic field assisted SPR was used recently for the detection of cardiac troponin I. In this approach a thin film gold was evaporated on glass state which acted as a sensing SPR film. Further it was modified with hollow gold nanoparticle (HGNPs) and polydopamine (PDA) sequentially and then immobilized with antibodies which can detect specific antigen (Mouse anti-human cTnI). The SPR electron coupling originated from HGNPs and the gold film led to the amplification of SPR response (SPR). The PDA film altered the gold film via self-polymerization of dopamine (DA) which made straight immobilization of capture antibodies (cAb). To isolate and upgrade the target analyte, PDA-wrapped magnetic multi-walled carbon nanotubes (MMWCNTs-PDA) were mixed with detection antibodies (dAb) and used as the extracting agent for the magnetic extraction of cTnI in sample. The combination of the nanoparticle assisted drug binding brought in the substantial sensitivity and improvement of the SPR immunoassay. The concentration of cTnI with least detectable SPR response as accomplished by the current assay was 1.25 ng mL^{-1}, which was 1000-fold less than that accomplished by the conventional established SPR immunoassay based on PDA-modified gold film. In another approach the SPR based immunosensor was designed for rapid and sensitive estimation of cTnI. This device was made with crosslinking of monoclonal antibody P-II-13, which was produced in contradiction of a loop region (aa 84–94) of cTnI protein as an epitope peptide, on top of a chemically improved thin gold film. The output was studied by SPR signal strength vs cTnI concentration. The least revealing limit of the sensor was 68 ng/L of cTnI, which was comparable to ELISA-based commercial cTnI concealment systems (Kwon, 2011).

Detection of target analyte concentration using multistep processes for signal amplification was also developed further. B-type (or brain) natriuretic peptide (BNP) which is associated with cardiac heart failure can be detected using nanoparticle-improved surface plasmon resonance (SPR). In this approach, a DNA aptamer was immobilized on a chemically modified gold surface in combination of the specific adsorption of antiBNP layered gold nanocubes as the biomarker target. This set up of biosensor can detect attomolar concentrations of the target in undiluted human serum (Jang, 2013).

3.2.4. ELISA based cardiac biosensors

ELISA typically works on sensitive signal of colorimetric, fluorogenic or luminescent forms usually generated from optical properties of chromogen reporter. It has wide application in detection of cardiac biomarkers. The immunoassay is one of the most valuable analytical procedures for detection of specific analyte (Jang, 2013; Leung, 2005; Cho, 2009).

A novel digital style approach was used to detect an early cardiac marker like heart-type fatty-acid binding protein (H-FABP) and C-reactive protein (CRP). This type of biosensors was very quick and produced result within 15 min by simple calculating the quantity of red lines on test zone without the involvement of expensive device. The

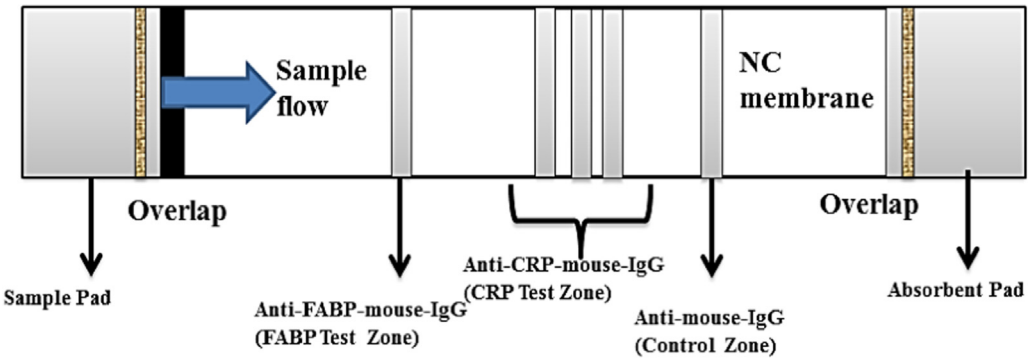

Fig. 9. Schematic diagram of digital style rapid test.

present biosensor involved conjugation of the of H-FABP (i.e.anti-FABP-mouse-IgG) and anti-mouse-IgG for CRP as depicted in Fig. 9 (Leung, 2005).

Further, dispensable immunosensor cartridge was refined that included immobilized antibodies on the exterior of myoglobin, which is early diagnostic marker of acute myocardial infarction (AMI) using fluorescence technique. The anti-myoglobin antibody was immobilized on a polystyrene substrate build on covalent bonding via silanization. The immunosensor chip layers were formulated from sheets by $CO(_2)$-laser excision. The antigen-antibody reaction as a sandwich ELISA included horse radish peroxidase conjugated with secondary antibody (HRP-anti-myoglobin), addition of fluorogenic substrate that produced a fluorescent dye which was quantified on-chip using fluorescent technique. The detection limit achieved with this biosensor was up to 16 ng mL^{-1} which is the least cut off value for myoglobin in healthy patients (Darain, 2009).

4. Biosensor for detection of cancer

Cancer is the one of the most deadly disease and recently many of the researchers have developed biosensor for early detection of cancer. Usually most of the cancer is detected by MRI, ultrasound or biopsy methods which rely on physical properties of tumor and its appearance and makes the detection involving either sophisticated instruments or invasive (Bohunicky and Mousa, 2011). Mainly cancer is caused by the changes in gene sequences, i.e. mutation and therefore it demands for early detection before the disease progresses. Early detection of cancer makes treatment easier and effective which opens a platform for biosensor for sensing early stages of cancer. Many researchers believed that early detection can be possible in case of cancer because abnormalities in chemical and genetic composition can be sensed well before the onset of the disease. Uncontrolled and abnormal growth of cells, known to be a cancer in general, happens because of accumulation of specific genetic mutation and epigenetic defects (Sharma, 2010). These tumor cells show resistant for apoptosis and anti-growth defence mechanism of the body (Elmore, 2007). The cancer becomes incurable when it progresses and starts to expand to other body organs and systems i.e. metastasize stage (Valastyan and Weinberg, 2011). The two most important tumorigenesis mechanisms are stimulation of oncogenes and reducing the activity of tumor suppressor genes (TSGs). Due to mutation or duplication of normal gene (proto-oncogene), activation of oncogene takes place, which plays key roles like, regulation of cell growth, proliferation, and/or differentiation, for the disease. Such type of mutations directs the gene to produce excess amount of its gene product that leads to disregulation of cell division, cell growth, and tumor establishment (Lee and Muller, 2010). Many oncogenes, growth factor receptors have been considered as promising cancer biomarkers. The human epidermal growth factor receptor Her-2, is intensified in ~ 33% of all breast cancers, and cancers with enhanced Her-2 tend to develop and increase more aggressively. Thus, knowledge of Her-2 status is important in concluding the possible course of medication (Loo, 2005). Trastuzumab, a recombinant humanized monoclonal antibody aimed across Her-2 as straight therapy for breast cancer, is now a typical adjuvant treatment for patients with this type of amplified gene expression (Vu and Claret, 2012). TSGs are associated in the regulation of inappropriate cell growth and proliferation by reducing or halting the cell division (Lee and Muller, 2010). Three of the well-studied TSGs in cancer are retinoblastoma protein (Rb), BRCA1/2, and p53 (Sharma, 2010). The Rb is a master regulator of cell division, and mutation of Rb plays a major role in various cancers (Thomas, 2015; Giacinti and Giordano, 2006). Point mutations and deletions are the most ordinary causes of inactivation of the Rb1 gene (Ayari-Jeridi, 2015). BRCA1 is a DNA repair enzyme, which is associated with 'proofreading' of newly replicated DNA for fidelity and to check any mutations. DNA repair enzymes usually function to excise replication errors before the cell divides. BRCA1 gene mutations narrates 50% of hereditary breast cancers and 80–90% of hereditary breast and ovarian cancers. Finally, the p53 protein is a key regulator of apoptosis or programmed cell death. Mutations in p53 are found in brain, breast, colon, lung, hepatocellular carcinomas, and leukemia. Another major involvement with the loss of p53 is that it contributes in the mechanism of chemotherapeutic drug resistance (Yoshida and Miki, 2004). The improvement of biosensors that can identify the presence of mutations in p53, Rb, and BRCA1 is highly warranted and can enable us to determine early cancer susceptibility with accurate prognosis and treatment regimes.

4.1. Cancer biomarkers

The National Cancer Institute (NCI) defines a biomarker as "a biological molecule found in blood, other body fluids, or tissues that are a sign of a normal or abnormal process or of a condition or disease" (Henry and Hayes, 2012). A biomarker can be used to see how the body responds to cancer therapy. Biomarkers can be of various molecular origins, including RNA, or protein (i.e., hormone, antibody, oncogene, or tumor suppressor) and DNA (i.e., specific mutation, translocation, amplification, and loss of heterozygosity) (Bohunicky and Mousa, 2011). These biomarkers are typically detected in body fluids (blood, urine, and cerebral spinal fluid) and also can be present in and on cancer cells. The list of commonly used biomarkers for cancers are shown in Table 3.

4.2. Biosensors for prostate specific antigen

Prostate-specific antigen was one of the initial tumor biomarkers to be known and was in clinical use of screening and diagnosing of prostate cancer. Studies have shown that elevated PSA levels compared to healthy individuals (4.0 ng/mL) correlate directly with prostatic cancer. A study conducted by Smith et al., found that roughly 30% of men with a PSA level between 4.1 and 9.9 ng/mL had prostatic cancer (Smith, 1997). The elevated level of PSA may also be associated with benign

Table 3
Common biomarkers for cancer detection.

Type of cancer	Biomarker
Breast	BRCA1, BRCA2, CA 15–3, CA 125, CA 27.29, CEA, NY-BR-1, ING-1, HER2/NEU, ER/PR (Bohunicky and Mousa, 2011)
Esophageal	SCC (Tan, 2016)
Liver	AFP, CEA (Bohunicky and Mousa, 2011)
Ovarian	CA 125, HCG, p53, CEA, CA 549, CASA, CA 19–9, CA 15–3, MCA, MOV-1, TAG72 (Coticchia et al., 2008)
Lung	CEA, CA 19–9, SCC, NSE, NY-ESO-1 (Zamay, 2017)
Melanoma	Tyrosinase, NY-ESO-1 (Aung et al., 2014)
Colon	CEA, EGF, p53 (Lech et al., 2016)
Prostate	PSA (Smith, 1997)

prostatic hyperplasia, prostatitis (inflammation of the prostate), or little neoplasm that is not fatal. Many men with elevated PSA don't show the prostate cancer at all and in such cases biosensors can be employed to remove the uncertainity surrounding the ordinary screening methods. Some of the latest development of biosesnors for detection of prostate cancer is described below.

The ultrasensitive detection of PSA becomes crucial in case of prostate cancer at an early stage. The capacitive biosensor was developed to detect the PSA in real time with high and selective detection of PSA. The biosensor was developed by the use of microcontact imprinting method in which PSA was imprinted on capacitive sensor. Microcontact imprinting is a surface coating for the formation of recognition cavities for macromolecular templates. In the normal process, the target protein was immobilized onto a glass support to form the protein stamp. Then, it was brought in viscinity of the sensor surface. By this way, a thin polymer film was formed on the sensor surface via UV polymerization. When the template was removed from the surface, specific protein recognition sites were produced at the bottom of the imprinted support. This technique helped to reduce activity loss of biorecognition which enhanced long term stability of the biosensor. In this biosensor, after an alteration of the gold surface with poly-tyramine and acryloyl chloride, the protein stamp was influenced to contact with the capacitive electrode, and PSA imprints were preceded onto the sensor surface. At the same time, electrodes with immobilized anti-PSA antibodies (anti-PSA electrodes) were developed via glutaraldehyde activation, to corelate the detection performances and selectivities of two methods as depicted in Fig. 10. The detection limits were found as 8.0×10^{-5} ng mL^{-1} (16×10^{-17} M) and 6.0×10^{-4} ng mL^{-1} (12×10^{-16} M) for PSA-MIP and anti-PSA electrodes, respectively. The outcomes were promising and demonstrated that when the affectability of the capacitive system was mixed with the selectivity and reproducibility of the microcontact-imprinting methodology, the subsequent framework may be utilized effectively for different analytes even in low concentrations (Ertürk, 2015).

Further to detect PSA, an innovative and competitive electrochemiluminescence (ECL) aptasensor for PSA assay was build using gold nanorods functionalized graphene oxide (GO@AuNRs) multilabeled with glucose oxidase (GOD) and streptavidin (SA) toward luminol-based ECL system. A powerful signal was accomplished by the use of electrodeposited gold (DpAu) on the electrode in which gold nanoparticle (AuNps) enhanced the luminol signal. The multiple signal amplification was achieved by prepared use of GOD and SA-biotin-DNA on GO@AuNRs. Such integration of amplifying effect improved the sensitivity and selectivity for PSA detection (Cao, 2018).

To detect PSA, for the first time aggregation induced emission (AIE)-active molecules with silica nanospores (AIE-SiO_2 NP) based label free fluorescent aptasensor for the sensitive "turn-on" detection was reported. AIE usually gives an intelligent fluorescence activation mechanism and high sensitivity. The positively charged amino-functionalized SiO_2 NPs were used as efficient nanocapturer to electrostatically adsorb single stranded PSA aptamer (PA) to form SiO_2 NP-PA nanocomposite as well as adsorb negatively charged tetraphenylethylene derivative 3 (TPE3) to form AIE-SiO_2 NP nanocomposite. The binding of the aptamer to the target PSA could induce a stiff aptamer configuration, making the PA away from the surface of SiO_2 NPs. This made the AIE molecules, TPE3 aggregate on the SiO_2 NP surface and emit high fluorescence as shown Fig. 11. This biosensor achieved a detection limit up to 0.5 ng/mL (Kong, 2017).

The point-of-care testing overcomes the traditional technique which is highly expensive, immovable and needs analytical equipment such as gas chromatography-mass spectrometer. To overcome this difficulty, screen printing technique for the fabrication of potent biosensor and chemical sensor was introduced which offered low cost and could be produced in bulk. Till date primary substrate was an organic film for screen printing of Screen printed electrodes (SPEs). Instead of using such organic film, alumina ceramic and nylon sheet approach was developed and yielded the disposable electrochemical immunosensor which was based on SPEs for the detection of PSA. SPEs on sheet of vegetable parchment were produced which was durable, appropriate, low cost and offered wide area for screen printing. The graphene nanosheets (GS) and horseradish peroxidase (HRP)-labeled signal antibody functionalized with gold nanoparticles (HRP-Ab2/Au NPs)

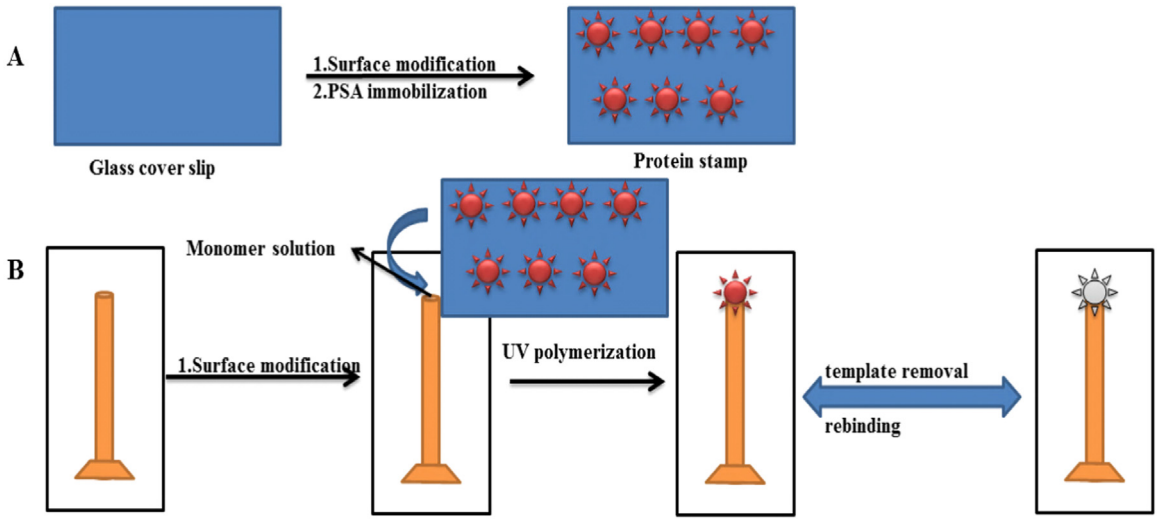

Fig. 10. Schematic representation of microcontact imprinting of PSA onto the capacitive biosensor (A) Preparation of protein stamp on glass cover (B) Preparation of the capacitive gold electrodes and microcontact imprinting via UV polymerization.

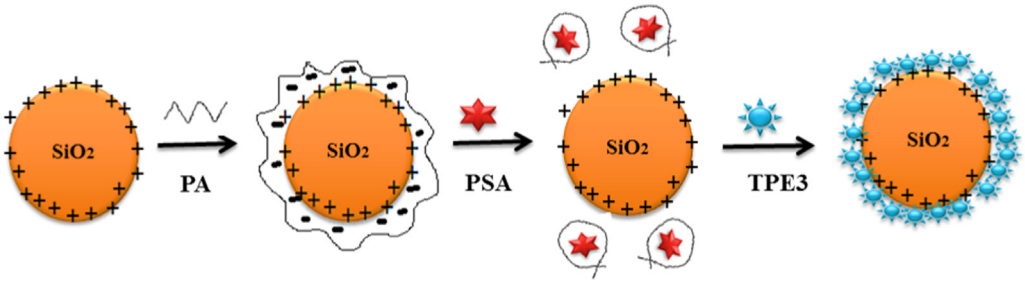

Fig. 11. Schematic representation of AIE-SiO_2NP based label free fluorescent aptasensor for the sensitive "turn on" detection of PSA.

fabricated on disposable and highly sensitive electroanalytical immunosensor was developed. GS was used to enhance the conductivity and stability of this immunosensor due to its fast electron carrying capacity and offered a good biocompatibility. Au NPs could not only provide an extended surface area for the immobilization of HRP-Ab2 but also increased the electro reduction among HRP and H_2O_2 to amplify the electrochemical signal on the sandwich immuno-complexes modified SPEs. The sensitivity of this biosensor was 2 pg/mL^{-1}. Apart from these, several biosensors were developed to detect PSA (Wu, 2007).

4.3. Biosensors for cancer antigen 125

Ovarian cancer is associated with elevated level of cancer antigen (CA) 125, although CA125 is also linked with cancers of the uterus, cervix, pancreas, liver, colon, breast, lung, and digestive tract. Although, menstruation and pregnancy are non pathological conditions, but they can also give rise to elevated CA 125. Almost 90% of women showed an increased level of CA 125 in ovarian cancer. About 50% of pateints will have a normal CA 125 level in stage I of ovarian cancer. Human chorionic gonadotrophin (HCG), alpha-fetoprotein (AFP), and lactate dehydrogenase (LDH) are other biomarkers, which are linked to ovarian cancer. CA 125 is a valuable biomarker for cancer diagnostics and also for other aspects of cancer progression (Scholler and Urban, 2007). There are several reports available to detect CA 125 from which some of the recent reported biosensors are discussed below.

Recently, CdZnTeS quantum dots (QDs) with a high electrochemiluminescence (ECL) were produced for strong ECL signal. The sandwich-structured ECL immunosensors for the tracking down of alpha-fetoprotein (AFP) and cancer antigen 125 (CA125) were engineered with direct ECL image analysis. The lower detection achieved with this approach was 0.5 fg/mL to 20 ng/mL and from 0.1 mU/mL to 500 U/mL respectively for AFP and CA 125. This approach led to fast biomedical screening and clinical diagnosis (Liang, 2018).

For inexpensive, simple and accurate detection, the electrochemical immunosensor specific to CA 125 based ZnO nanorods (NRs)-Au NPs nanohybrids was developed by researchers, which was immobilized with anti-CA 125 antibody. In this approach nanohybrid (ZnO NRs-Au NPs) based matrix was synthesized by hydrothermal and sputtering methods and employed for the detection of CA 125. This engineered matrix offered a supportive platform for immobilization of anti CA 125 towards its active site. This biosensor has a promising role in the detection of CA 125 (Gasparotto, 2017).

The graphene quantum dots (GQDs) were immobilized on a functionalized glass chip and an immunoassay for the detection of CA 125 was developed by employing the chemiluminescence resonance energy transfer to graphene quantum dots. The horse radish peroxidase (HRP) catalyzes the yielding of reactive oxygen species i.e. ROS from H_2O_2 which oxidized luminol into the singlet dianion by generating excited electrons. This electron jumps from excited energy state to ground state giving chemiluminescence and the emitted blue light is detected using fluorescence plate reader, in the presence of CA 125. In absence of CA 125 there will be electrostatic repulsion between the Ab–HRP and the capture antibody (cAb) which makes HRP labeled antibody far away from GQDs and dianion resulting in no strong interaction between GQDs and the dianion. This biosensor showed limit of detection of 0.05 U/mL for CA 125 in a buffer solution (Al-Ogaidi, 2014).

4.3.1. Biosensor for the detection of CA 15-3

The CA 15-3 is main biomarker for breast cancer and is widely used clinically in advanced breast cancer. In patients with breast cancer, CA 15-3 concentrations are elevated by 10% in Stage I cancer, 20% in Stage II, 40% in Stage III, and 75% in Stage IV. At present, CA 15-3 levels are considered along with tumor size, cancer stage, and negative risk factors (i.e., Her-2 and Estrogen receptor (ER) / Progesterone receptor (PR) status) in deciding the treatment protocols. Other conditions that can cause CA 15-3 levels to increase are endometriosis, pelvic inflammatory disease, hepatitis, pregnancy, and lactation (Barak, 1988).

In conventional, detection of CA 15-3 immunoassay the methods that are mentioned in literature are: radioimmunoassay, high performance liquid chromatography (HPLC) electrophoretic immunoassay and fluorescence immunoassay. These methods are associated with some disadvantages like expensive, labor-intensive, highly time consuming and much more complex. So several approaches are made for rapid and accurate methods of CA 15-3 detection which are discussed below.

One approach mentioned the electropolymerization of films made with amine-substitution through the benzene rings of monomers such as aniline, o-phenylenediamine (oPDA) and p-phenylenediamine (pPDA) with definite charged monomers (4-Styrenesulfonic Acid Sodium Salt, negatively charged; and dopamine, or 3-hydroxytyraminium chloride, positively charged) for modification of protein imprinting materials for CA15–3 detection as shown in Fig. 12. On gold surface the protein CA 15–3 was adsorbed, which was enclosed by the polymeric matrix with the proteolytic enzyme that cleaves the protein peptide bonds and leads to destruction of imprinted proteins or peptide. The surface without template and non imprinted served as control material. This biosensors showed the limit of detection of CA15–3 by 0.05 U/mL. This biosensor offers point-of-care diagnostic tool for cancer screening and give results in less than 30 min (Gomes, 2018).

In another approach where the researchers reported electrochemical immunosensor for the tumor marker for CA 15–3 which was placed on the use of miniaturized grapheme oxide (RGO) and copper sulfide (CuS) based on the screen printed graphite electrode. This electrode had excellence activity against the oxidation of catechol with best active potential of 0.16 V. For the detection of CA 15–3 this electrode was modified with anti CA 15–3 antibody which reduced catechol after binding with CA 15-3. This biosensor showed the linear response in the range of 1.0–150 U/mL. The lowest concentration of CA 15–3 that could be detected using the biosensor was 0.3 U/mL. This nanocomposite based detection offers a great scope in the detection of cancer (Amani, 2018).

The use of gold nanoparticle for coating on specific antibody offers greater surface area for improved reaction site and enhanced electrical conductivity that can give signals for the sensor which may be read utilizing electrochemical impedance spectroscopy (EIS) and

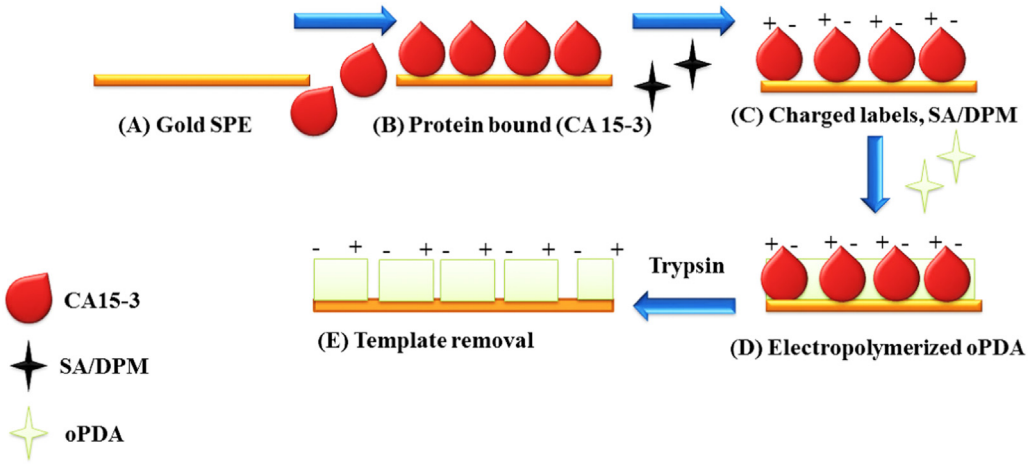

Fig. 12. Simplified representation of the synthesis of the imprinted material. (A) Au-SPE; (B) CA15–3 adsorption on Au-SPE surface; (C) Charged monomers labelling; (D) Electropolymerization of PDA; and (E) binding site formation by template removal with Trypsin.

potentiostat. This approach used gold nanoparticle coated specific antibody on screen printed electrode for the detection of CA15–3 concentration in human serum sample involving antigen-antibody reaction. The immunosensor permitted a consistent detection of CA 15–3 concentration upto 5–75 U/mL (Selwyna, 2013).

4.3.2. Biosensor for the detection of CA 19–9

Cancer antigen 19–9 (CA 19-9) have a molecular weight of 1000 kDa. This CA 19–9 is an isolated lewis antigen of MUC1 glycoprotein. The first CA 19–9 was detected in the serum of pancreatic and colon cancer patients in 1981. It has a normal range in human serum up to 37 U/mL. Recently this antigen has become a standard biomarker for the diagnosis of pancreatic cancer. So many recent developments have focused this type of antigen for its early detection by using biosensor discussed below (Kaneko, 1999).

The conventional measurement technique for the recognition of CA 19–9 mainly includes electrochemiluminescent immunoassay (ECLIA) which offers high sensitivity with broader detection range. But such type of biosensors always include multi-step reaction process which consume more time. To overcome such multi-step reaction process, a one step detection immunoassay for CA 19–9 was developed which offered a narrow detection level than ECLIA. This demonstrated one step immunoassay for CA 19–9 involving biosensor based imaging ellipsometry (BIE) in which anti CA 19–9 monoclonal antibody (ligand) was covalently immobilized on the carboxy terminal exterior to make sensing layer. Once sensing layer was obstructed by BSA, CA 19–9 can be identified at a minimal concentration of 10.0 U/mL with premium reproducibility and adequate specificity (Huang, 2011).

Another electrochemiluminiscence immunoassay based biosensor developed for the detection of CA 19–9 in serum, included capture probe, i.e., antibody labeled with Fe_3O_4 nanoparticle and a signal amplifier using graphene/CdTe quantum dots bioconjugate. The capture probes (CA 19–9 Ab1/Fe_3O_4) was synthesized by immobilizing CA19–9 antibody on magnetic nanoparticles (dextran-Fe_3O_4). The ECL signal probe amplifier was achieved (CA 19–9 Ab2/CdTe-G), by joining the CA 19–9 antibody (CA 19–9 Ab2) to the exterior of graphene/CdTe quantum dots (CdTe-G). The detection of CA 19–9 was achieved by the conjugation of capture probes (CA 19–9 Ab1/Fe_3O_4) and signal amplifier (CA 19–9 Ab2/CdTe-G). The limit of detection of CA 19–9 was 0.002 pg/mL (Gan, 2013).

Another nanocomposite approach was used for the detection of CA 19–9 in which glucose derived Carbon Quantum Dots (CQDs) were utilized as reducing material and the fabrication of CQDs/Au nanocompositewas done. The CA 19–9 monoclonal antibodies were immobilized with a horse radish peroxidase enzyme (Ab-HRP) over the nanocomposite. The Ab-HRP was attached to the exterior of CQDs/Au nanocomposite with a peptide that was influenced by the carboxylic and amine active groups. The CA 19–9 antigen was caught by additional monoclonal antibody that was covered on the surface of the microtiter wells. The formed sandwich, topped with antibody– antigen–antibody enzyme intricate had variable fluorescence properties that were recognized under excitation and emission of wavelengths of 420 and 530 nm. The lower detection limit with this biosensor was 0.007 U/mL (Alarfaj, 2018). Other than medicine, there are various applications of nanobiosensors, especially fluorescence based biosensors (Girigoswami and Akhtar, 2019).

5. Conclusion

The biomarker detection related to various diseases are significant for the control of progression of a disease. They are not only used to diagnose or monitor disease, but also to deliver a prognostic method for treatment. One of the leading goal of biosensor is point of care (POC) diagnostic of the disease. The point-of-care-testing (POCT), or diagnostic testing is done on site, and is a field in which biosensors could have a major impact, letting the patient and medical staff to get results quickly and definitely in a cost effective manner. In any case, to move biosensors toward POC gadgets, multitarget discovery of numerous biomarkers is essential. Moreover, POCT and multibiosensors, while conveying biosensor innovation to the patient's bedside, must keep up the precision and reliability of the laboratory. The development of laboratory on a chip (LOC) technology showed a promising role in this area. The real impacts of nanotechnology on biosensor advancement include nanomaterials, as they can't just facilitate diagnosis and tracking of cancer cells, can also deliver drug to target sites with accuracy and take into account more delicate imaging frameworks that can identify disease at a prior stage. In the following 5–10 years, it is sure that nanotechnology will revolutionize disease diagnosis and treatment. Incorporating nanomaterials in biosensors will enable us to recognize infection at an early stage, enhance disease imaging, and help in analysis/visualization and advance drug delivery with limiting adverse reactions. The biomarkers of diseases like cancer, CVD, diabetes etc. found in body fluids are very minute in concentration and it is very important to sense that level at an early stage to prevent the progression of the disease. Biosensor innovation can possibly give quick, precise outcomes, while keeping up cost adequacy.

Future scope

The *ex vivo* or *in vivo* genetically engineered protein that are fused

with the cells makes researchers to sense the levels of hormone, toxins and drugs continuously as well as noninvasively by the use of biophotonics or other physical priniciples with the help of engineered biosensors. Nanomaterials enhance mechanical, electrochemical, optical and attractive properties of biosensors and are able to create single particle biosensors with high throughput biosensor arrays.

Biological molecules have uncommon structures and functions, and to decide how to completely utilize the structure and capacity of nanomaterials and biomolecules to manufacture single atom multifunctional nanocomposites, nanofilms, and nanoelectrodes, is as yet an incredible task to be explored. The processing, characterization, interface issues, accessibility of top notch nanomaterials, fitting of nanomaterials, and the mechanisms governing the conduct of these nanoscale composites on the surface of electrodes are additional difficulties for the presently existing methods.

Future work should center around clearing up the instrument of collaboration among nanomaterials and biomolecules on the surface of electrodes or nanofilms and utilizing novel properties to create another generation of biosensors. In any case, nanomaterial-based biosensors indicate incredible appealing prospects, which will be comprehensively connected in clinical diagnosis, food analysis and environmental monitoring in coming future.

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Conflict of interest

The authors declare no any conflict of interest.

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