



Nanobiosensors: Diagnostic Tool for Pathogen Detection

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Abstract

Biosensors could be a thrilling alternative to the conventional systems for the detection of toxins and pathogens. Biosensors are reliable which allow the specific detection of target analyte at minimum cost. Recent advancement in the field of nanotechnology has been used to develop highly sensitive bio-sensor's chips by using multidisciplinary approach. This strategy could be seen as a key for developing bio-sensing devices and demonstrates rapid responses combined with high sensitivity and specificity. Indeed, these traits have nearly become standard attributes of this technological development and come up from the extremely high surface and small size nanostructure areas as nanotubes, nanowires and nanoparticles. In view of above, it is acceptable to state that biosensor technology has the prospective to augment the sensitivity and specificity, hasten the detection and enable high-throughput analysis.

Keywords: Nanobiosensors, diagnostic tool, pathogen detection, clinical diagnostics, quartz crystal microbalance (QCM), nanoparticles.

Introduction

What are Bio-sensors? Biosensor could be defined as a biological sensor i.e. a device which combines a biological component with a physicochemical detector component for the detection of an analyte. Recent advancement in the field of nanotechnology facilitates the material science-based approach to nanoscale synthesis of nanomaterial. Nanoparticles could be used as quantum dots and as chemical catalysts. These particles are a link between bulk materials and atomic or molecular structures. In comparison to bulk nanomaterials, nanoparticles display many unique properties. For example; it can be used for nanobiosensor to recognize a particular type of analyte through a chemical process for qualitative and quantitative analysis of target analyte. According to the behaviour and role of biosensors, there are different types such as photometric biosensor, biological biosensors, electrochemical biosensors etc.

Optical biosensors are developed according to surface plasmon resonance and are evanescent wave techniques. This type of sensor are having the properties of gold and can absorb laser light which produces electron waves (surface plasmons) on the surface of gold, while thin layer of gold are also having a high refractive index glass surface. This is highly dependent on the surface of the gold because of the specificity of angle and wavelength of incident light. Target analyte is bound to a receptor located on the surface of gold and generates a signal that can be quantified. The chip consists of a plastic cassette supporting a glass plate and microscopic layer of gold is coated

on one side of the chip. This side is used for the contact of optical detection apparatus and opposite side is used for microfluidic flow system.

The contact with the micro-fluidic system produces channels across where reagents are passed in solution. The glass sensor chip could be customized by several ways which allow easy and effective attachment of the molecules of interest. Coating of the chip is done by carboxymethyl dextran or similar compound. Binding of the molecule of the interest can effect on the refractive index and through this, biological interactions can be analysed to a high degree of sensitivity with some sort of energy; for example, dual polarisation interferometry. However, optical biosensors are working according to the variations in absorbance of the indicator compound and do not require a total internal reflection geometry.

Biosensors are often incorporated with some forms of native proteins or enzymes, which are genetically modified. This receptor protein is attached to specifically detect the analyte. The resulting signal will be analysed by fluorometer or luminometer. Biosensor for determining the concentration of the analyte cAMP (cyclic adenosine monophosphate) in cytosol is an example for this. Moreover, most of the cAMP assays require lysis of the cells before analysing the concentration of cAMP. For solving this problem, a live-cell biosensor for cAMP is developed to use without lysis of cells and having added benefits of multiple reads to understand the kinetics of receptor response.

Electrochemical biosensors are mainly based on enzymatic catalysis of a reaction and produce or consume electrons. These enzymes are known as redox enzymes. In electrochemical biosensors three electrodes are present viz. a reference electrode, an active electrode and a sink electrode. There may be an auxiliary electrode (counter electrode) also, which function as the ion source. The target analyte is engaged in the reaction that happen on the active electrode surface, and the ions that arises produce a potential which is deducted from that of the reference electrode to produce a signal; for example, potentiometric biosensor. On the contrary, there are two more type of biosensors such as thermometric biosensors and magnetic biosensors but these sensors are not used frequently and are very rare.

Bio-sensor Components: There are three different components of a biosensor. These are described below: i. the sensitive biological element (biological materials such as tissues, microorganisms, cell organelles and receptors, enzymes, antibodies, nucleic acids etc.), a biologically derived material or bio-mimic and these sensitive elements can be developed by biological engineering; ii. the transducer or the detector element (works in a physicochemical way; optical, piezoelectric, electrochemical, colorimetric etc.) that modifies the signal resulting from the interaction of the analyte with the biological element into another signal (i.e., transducers) that can be more easily measured and quantified; iii. associated electronics or signal processors which are mainly associate with the display of the results in a user-friendly way¹. This is the mainly costlier component of the sensor device; yet it is probable to produce a user friendly display consisting the transducer and sensitive element. The biochemical interactions are converted into a quantifiable electronic signal by the transducer².

Discovery of Bio-sensors and Its History: Biosensors have imperative and significant position in medical science, industries and the environment, offering regular examinations, critical monitoring, and timely diagnosis of problems and crisis points. The history of biosensors started many years back. During the year 1956, a scientist named Leland C Clark Jr., published his classic findings on the oxygen electrode³ and he is recognized as the father of biocensors. The history of biocensors is summarised in tables-1 and tables-2.

Types of Bio-sensors: The detection by biocensors depends upon the principle of alterations in different parameters like electric distribution (conductance, current and potential), light intensity, frequency, pressure etc. However, as described above in brief, details of various types of biosensors are explained below in table-3.

Electrochemical Sensor: Ions or electrons are produced or consumed in many chemical reactions. These particles in turn may cause many alterations in the electrical properties of the solution which can be used for quantifying the event. For example; for the detection of hybridized DNA⁴, DNA-binding drugs, glucose concentration⁵, electrochemical biosensors are chiefly employed.

Table-1
Major events in the history of development of biocensors³ from 1916 to 2004

Year	Biosensor development
1916	First report on the immobilization of proteins: adsorption of invertase on activated charcoal
1922	First glass pH electrode
1956	Invention of the oxygen electrode
1962	First description of a biosensor: an amperometric enzyme electrode for glucose
1969	First potentiometric biosensor: urease immobilized on an ammonia electrode to detect urea
1970	Invention of the Ion-Selective Field-Effect Transistor (ISFET)
1972	First commercial biosensor: Yellow Springs Instruments glucose biosensor
1976	First bedside artificial pancreas (Miles)
1980	First fibre optic pH sensor for in vivo blood gases
1982	First fibre optic-based biosensor for glucose
1983	First surface Plasmon resonance (SPR) immunosensor
1984	First mediated amperometric biosensor: ferrocene used with glucose oxidize for the detection of glucose
1987	Launch of the MediSense ExacTech™ blood glucose biosensor
1990	Launch of the Pharmacia BIACore SPR-based biosensor system
1992	i-STAT launches hand-held blood analyzer
1996	Glucocard launched
1996	Abbott acquires MediSense for \$867 million
1998	Launch of LifeScan Fast Take blood glucose biosensor
1998	Merger of Roche and Boehringer Mannheim to form Roche Diagnostics
2001	LifeScan purchases Inverness Medical's glucose testing business for \$1.3billion
2003	i-STAT acquired by Abbott for \$392 million
2004	Abbott acquires TheraSense for \$1.2 billion

Table-2
Biosensor development from 2005 to present

2005	A portable automated multianalyte biosensor
2006	Electrochemical deoxyribonucleic acid (DNA) biosensor was developed using an electroactive label based on human interleukine-2 gene
2007	Development of thermal immune biosensor for detecting nonylphenol in the environment
2008	Development of amperometric glucose biosensor
2009	Piezoelectric sensor was developed by Romero and co-workers for detecting methamphetamine
2010	SERS nanosensors
2011	Optical biosensors in the field of nano bioelectronics
2012	For determining organophosphate pesticides, novel automated flow based- biosensor was developed

Table-3
Principle of detection

Types of Biosensors	Determine the variations in
Electrochemical	Electric distribution
Optical	Intensity of light
Resonant	Frequency/mass deposit/resistance
Piezoelectric	Pressure, acceleration, strain or force

Depending upon the measuring of electrical parameters, electrochemical biosensors are classified as i. Conductimetric, ii. Amperometric and iii. Potentiometric.

The consequence of the immobilization and interaction of biomolecules between a pair of metal electrodes in a bulk solution can be measured using conductimetric sensors because of the effect caused by chemical and biological changes upon the conductance⁶. The overall conductivity or resistivity of the solution can be changed due to ions or electrons produced by electrochemical reactions. These changes can be quantified and standardized to an appropriate degree. Conductance measurements have comparatively lower sensitivity.

Amperometric biosensors measure current fluctuations. The biosensors depend on linear concentration and quantify alterations in the current on the working electrode resulting from the direct oxidation/reduction of the products of a biochemical reaction in direct or indirect measuring systems. Amperometric transducers have advantages of good sensitivity, speedy response, low expensive, and disposable in comparison to other analytical devices^{7, 8} and are preferentially used for the design of biosensors⁹. The amperometric transducers with best sensitivity are glassy carbon^{10, 11}, carbon paste¹² and diamond paste electrodes^{13,14}. The diamond paste are used as a transducer in the biosensor technology owing to their qualities like minimum background current, broadened potential range, little adsorption, high signal-to-noise and signal-to-background ratios.

The oxidation or reduction potential of an electrochemical reaction is measured in potentiometric type of sensor. This is based on the principle that by applying ramp voltage through an electrode in a solution, a current flow can be elicited due to electrochemical reactions. There may be differences among the voltage which elicit these reactions in particular reaction and particular species, which can be used as an indicator. These biosensors detect the accumulation of charge created by selective binding at the electrode surface¹⁵.

Optical Biosensor: Optical biosensors have been invented for quick and accurate diagnosis of contaminants, poisonous substances or medicines and even pathogenic bacteria. These sensors utilize light waves to detect the presence or alterations due to a chemical on certain biological system. It is based upon optical diffraction or electrochemiluminescence. In case of optical diffraction based devices, protein is coated on a silicon

wafer through covalent bonds. The exposure of wafer to ultra violet rays using a photo-mask may cause inactivation of the antibodies in certain areas which are exposed to the rays. After this, when the wafer chip is exposed to an analyte, Ag-Ab (antigen-antibody) binding takes place at the active regions and there forms a diffraction grating. This leads to the production of diffraction signal if it get excited through some source of light like laser. Then the signals produced through this can be quantified. Optical biosensor includes vibration (IR, Raman) and chemiluminescence phenomenon. The three important optical transducers employed in biosensors are optical fibres¹⁶, chemiluminescence and surface plasmon resonance¹⁷. The most common types of optical transducers are fluorescence and chemiluminescence transducers. Bioluminescence comes under the broad classification of chemiluminescence and these take place in living creatures such as fireflies, glow-worms, bacteria and others¹⁸.

Resonant Sensor: In resonant sensor, the change in frequency is measured which appears due to the attachment of analyte molecule with a biological element like Abs which in turn is coupled with an acoustic wave transducer. This attachment of analyte results in the mass change of the acoustic wave transducer. A binding incident can be transformed into a quantifiable signal with the help of a piezoelectric transducer, for example resonance frequency changes, mass deposit and resistance. This type of biosensor can detect two or more species simultaneously depending upon the way of development of piezoelectric transducer with a simple and multifunctional recognition element¹⁹.

Piezoelectric Biosensor: Piezoelectric sensors are based on piezoelectric effect discovered by Curie brothers. Pierre Curie and Jacques Curie recognized that piezoelectric objects like tourmaline and quartz have the capacity to convert energy of a mechanical input into an electrical output. There is a mechanical deformation and displacement of charges, when pressure is exerted over a piezoelectric object. The inner dipoles are re-orientated and a crystalline mechanical strain is observed. These charges are directly proportional to the exerted pressure (Piezoelectricity).

QCM (Quartz Crystal Microbalance) is a device which can be modified by using different schemes in order to develop different immunosensors. Immunosensors are those biosensors in which the recognition element is Ab. It is a device comprising of an Ag or Ab species coupled to a single transducer which detects the binding of the complementary species. QCM is made up of quartz and belongs to the trigonal crystal system. Quartz is a continuous framework of SiO₄ (silicon-oxygen tetrahedral) in which two tetrahedral are being shared by each oxygen molecules and gives an overall formula of SiO₂ (figure-2).

QCM is an easy, less expensive, high-resolution mass sensing practice. It is mainly based upon the alterations in the frequency of a quartz crystal resonator by mass per unit area and measures

piezoelectric effect. QCM has high sensitive solution-surface interface measurement capability and therefore in largely analytical chemistry and electrochemistry applications, evolved solution quantification potential.

In this system large variety of molecules are detected such as low mass is detected by monolayer surface coverage by using tiny molecules or polymer films while at the greater end, it is competent to detect heavy masses attached to its surface. It could be considered as a multifaceted array of biopolymers and biomacromolecules, even the entire cells. Moreover, details regarding the energy dispersing qualities of the bound surface mass can also be provided by the QCM.

The progress of piezoelectric tools is mainly associated with a set of intrinsic benefits such as i. high modulus of elasticity as compared to many metallic substances ii. piezoelectric sensors are electromechanical systems that react on compression, iii. The deflection showed by this sensing elements are almost nil, iv. this technology is insensitive to electromagnetic fields and radiation and v. some piezoelectric materials (especially gallium phosphate or tourmaline) remain highly stable even above 1000°C temperature, which facilitate the sensors to perform under unfavorable conditions also.

At the same time, piezoelectric sensors have the disadvantage that they cannot be employed for true static measurements. A static force may lead to some fixed quantity of charges on the piezoelectric material. Only those piezoelectric materials which can maintain high internal resistance can be used at elevated temperatures as, the increased temperature may lead to an added reduction in internal resistance.

Besides this piezoelectric devices can be classified into TSM (Thickness Shear Mode) Sensor, SAW (Surface Acoustic Wave) Sensor and APM (Acoustic Plate Mode) Sensor on the basis of mode of acoustic wave propagation.

Theory of QCM Device

Sauerbrey Equation: Sauerbrey investigated the association between the variation in resonance frequency and the added mass²⁰. This equation is applicable only for thin, even and purely elastic layers.

$$\Delta f = -C_f \times \Delta m \quad (1)$$

Where Δf is frequency change in Hz, C_f = Sensitivity factor of the crystal in Hz/ng/cm², and Δm = Change in mass per unit area in g/cm².

According to Sauerbrey equations, the additional mass/film deposited over the crystal has the same acousto-elastic properties as quartz and this assumption resulted in a sensitivity factor, C_f which is a fundamental property of the QCM crystal as shown in equation 2.

$$C_f = \frac{2n \times f^2}{\sqrt{\rho_q \times \mu_q}} \quad (2)$$

In this formula, n stands for number of the harmonic at which the crystal is driven, f is resonant frequency of the fundamental mode in Hz, ρ_q is the density of quartz (2.648 g.cm⁻³) and μ_q is the effective piezoelectrically stiffened shear modulus of quartz (2.947 x 10¹¹ g cm⁻¹.sec⁻²). The sensitivity factor for different crystal can be summarized as 0.0566 Hz/ng/cm² for a 5 MHz crystal, 0.0815 Hz/ng/cm² for a 6 MHz crystal and 0.1834 Hz/ng/cm² for a 9 MHz crystal at 20 °C.

Solving these equations for Δm yields

$$\Delta m = \frac{-\Delta f}{C_f} = \frac{(f_q - f) \sqrt{\rho_q \times \mu_q}}{2n \times f^2} \quad (3)$$

Where, f_q is the resonant frequency of unloaded crystal and f is resonant frequency of loaded crystal in Hz. It should be noted that in these hypothesis, the variation in frequency is a function of mass per unit area. As a result, theoretically calibration is not required for the QCM mass sensor. But, Sauerbrey equation is appropriate for uniform, rigid, thin-film deposits only. More complicated frequency-mass correlations may be shown by vacuum and gas phase thin film depositions which could not satisfy these conditions; therefore they require some calibration for providing perfect results.

Nanoparticles

Since nanomaterials have more surface to volume ratio and can provide more space for binding of antigen and antibody, the efficacy and sensitivity of sensors can be increased by immobilization of functional nanomaterials like silver and gold nanoparticles on the surface of quartz crystal. The nanoparticles possess specific optical, thermal, catalytic and electric properties drawing major attention with the advancement of Nanotechnology²¹.

Nowadays, metal nanoparticles like silver and gold nanoparticles, micelles, dendrimers, polymeric nanoparticles, quantum dots play significant role in biomedical applications. A polymeric nanoparticle is the one which is derived from a polymer. It has its application in drug delivery. Abraxane is the first polymeric nanoparticle made. A metal nanoparticle is made up of metal like Gold and Silver. These gold and silver nanoparticles have their uses in biosensors, optics and catalysts. Recent research is focused on the synthesis of nanoparticles based on gold chemistry. They are point of interest for many researchers as these are multipurpose agents with very much biomedical relevance. For example: antibody modified gold nanoparticles detect prostate specific antigen which is having million times more sensitivity than the routinely using ELISA technique²².

On the other hand, silver nanoparticles also hold great application as catalysts²³. Due to the unique ability to terminate

bacterial division, silver nanoparticles can also be used as bactericidal agents²⁴. Another application of silver NPs (nanoparticles) is a real time optical sensor. Among these three metallic substances (Ag, Au, and Cu) which show plasmon resonances in visible spectrum, silver (Ag) possess the highest competence of plasmon excitation.

Advancement in Bio-sensors for Bacterial Detection

Basic Immunosensor development: The initial step in the development of QCM based immunosensor is to achieve a thiolated crystal surface. Thiolization not only leads to surface activation but also modification in favour of antibody immobilization. Attachment of ATP (*p*-Aminothiophenol) molecules decreases the average frequency of bare quartz crystal. The modification can be done by using ATP because thiol group shows great affinity towards the gold and second, the developing SAM (self-assembled monolayer) film activates the electrode (gold) by providing partial positive charge to crystal surface because $-NH_2$ lies at right angles to the surface. This partial positive charged surface is the ideal location for antibody immobilization. The ATP modified crystal can then be exposed to the Ab so as to prepare the surface of biosensor specific to the Ag (figure-1).

Immunsensor development using AgNP (silver nanoparticles) @ATP: The process of biosensor development starts with quartz crystal surface modification with DTPA (3,3'-dithiopropionic acid). As a result, a SAM will be there on the golden electrode.

This SAM film develops partial negative charge on its surface as the $-COOH$ remains at right angles to the surface and prompt the gold electrode. The modification was performed using DTPA because thiol have increased affinity towards the gold and at the same time, the surface which is partially negative charged, due to $-COOH$ groups, also offer better site for AgNPs@ATP which are partially positive charged. When AgNPs@ATP are exerted over the modified gold electrode with DTPA, there is an attraction force building from increased affinity of the partially negative charged $-COOH$ group towards the partially positively charged $-NH_2$ group. Due to this attraction, the silver nanoparticles will be bound firmly on the gold surface. Immobilisation of silver nanoparticles provides a larger surface area along with a partial positive surface. This partially positively charged surface also offer an ideal site for partially negative charged antibody for binding to the surface thus favouring greater Ab-Ag binding (figure-3).

Immunosensor development using AuNP (gold nanoparticles) @ATP: Quartz crystal is used to wash with Piranha solution (mixture of 70% H_2SO_4 and 30% H_2O_2 (3:1). And modified with TDPA (3,3'-thiodipropionic acid). Excess of TDPA is removed by using methanol solution. AuNP@ATPs is attached to the crystal surface using DCC (N,N'-dicyclohexyle carbodimide) / NHS (N-Hydrosuccinimide) chemistry. Then, AuNP@ATPs is immobilized onto the modified crystal surface followed by Abs immobilization. The Ab-Ag interactions can then be studied (figure-4).

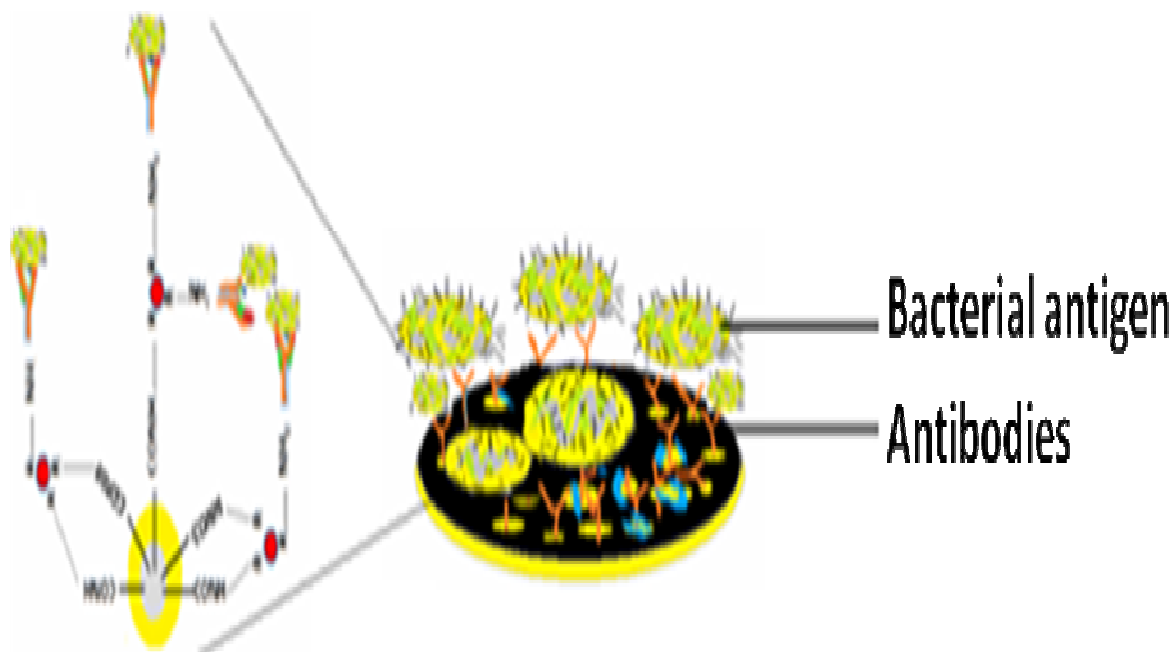


Figure-1

Schematic Diagram of Modified QCM Device Coated with Nanoparticles (Au/Ag) for Bacterial Detection with Increased Sensitivity

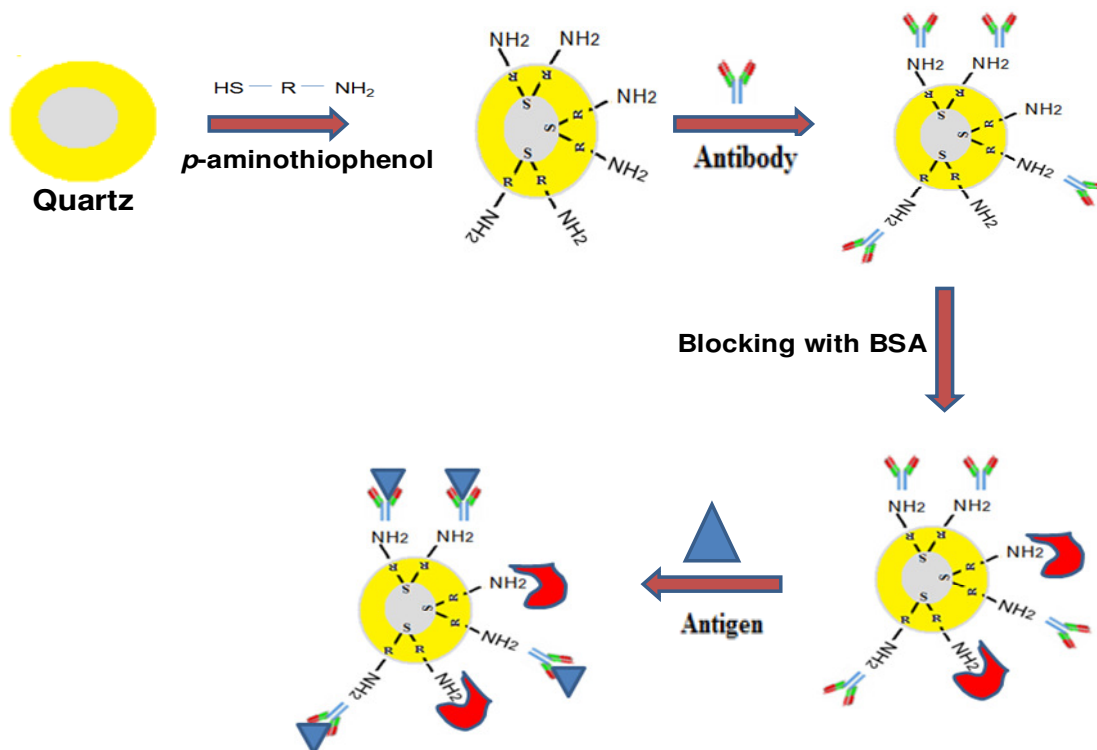


Figure-2
Schematic Diagram for Immunosensor Development for Bacterial Detection

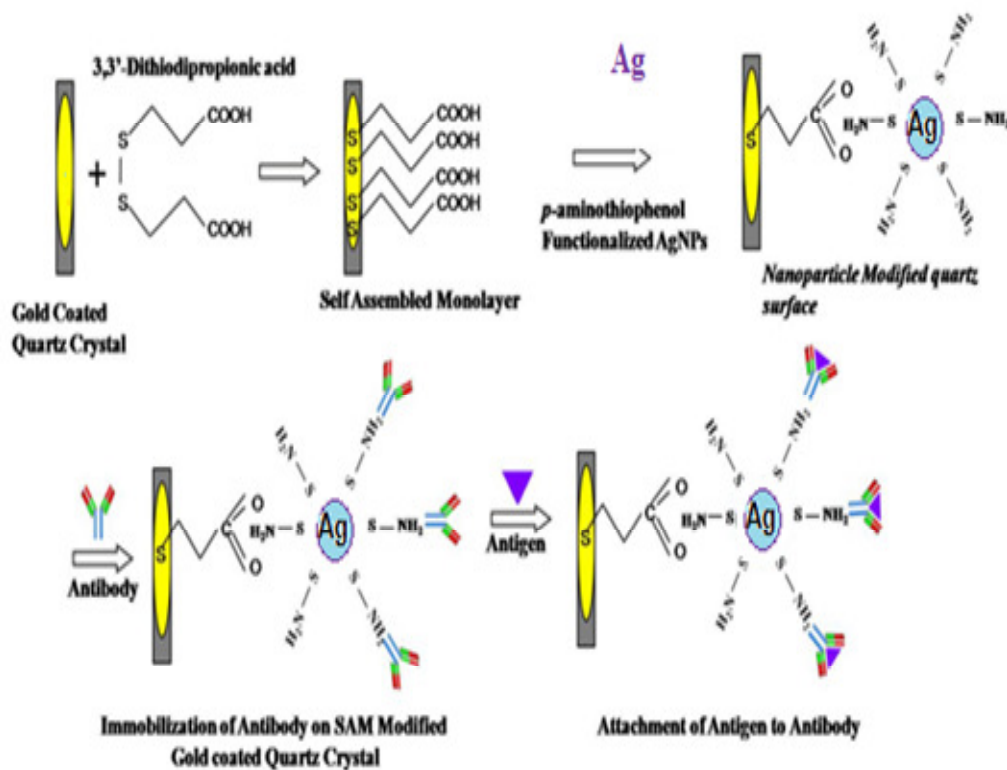


Figure-3
Schematic Diagram for Immunosensor Development using Agnp@ATP for Bacterial Detection

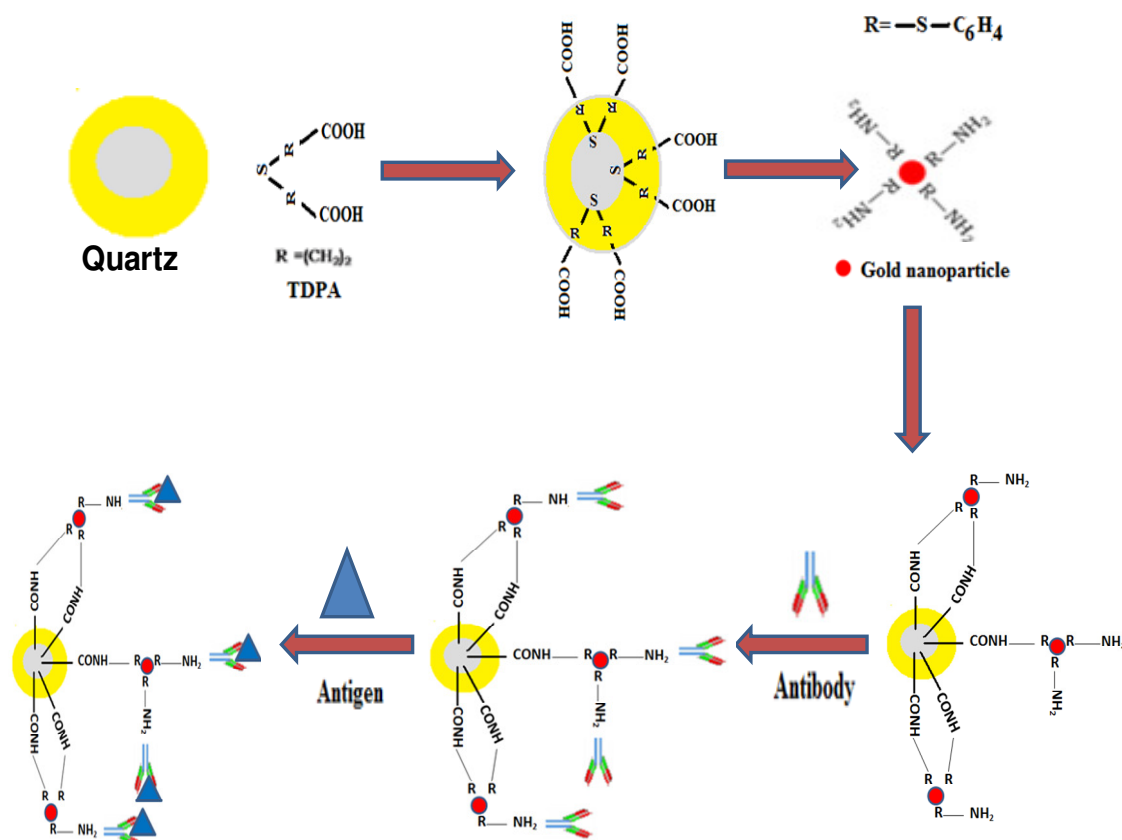


Figure-4
Schematic Diagram for Immunosensor Development using Aunp@ATP for Bacterial Detection

Conclusion

In this review we made an effort to discuss the recent developments, industrial applications of pathogen sensing systems and realized that biosensor technology is a young and growing field. It is observed that the efficacy and sensitivity of sensors can be increased by immobilization of functional nanoparticles (Gold, Silver etc) on quartz crystal surfaces. The detection limit will be increased remarkably and will form the basis of presence of minor contamination and presence of pathogens/microbes in various food products, biological fluids etc. Bashir and co workers were the first one to report bacterial detection at Microsystems in 2001²⁵. The study involves a microsystem which uses impedance spectroscopy to detect *Listeria*. Woo and co-workers also described the selective amperometric detection of *Escherichia coli* in 2001 within 40 minutes which is a very short time span²⁶, while Mura et al., 2012, demonstrated that contamination of *E. coli* in food and water could be detected in by using FITR spectrophotometry by using titania thin-film substrates as sensors²⁷.

However, lab on a chip biosensors are having ample prospects to be used in the area of pathogen detection because of the scope for miniature and automated features, similarly they are rapid and highly sensitive. The biocensors are able to detect microorganisms in various farms, post harvest and process units, logistics channels and even at the end user level. However, there are number of difficulties to be addressed before extending these labs on chip sensors for field applications²⁸. A model experiment is carried out by Wang et al., 2012 in which *E. coli* was detected at very low concentrations of 50 cfu/mL, 4 times greater than the sensitivity of detection through regular grating-coupled SPR with direct detection limit²⁹. Cheng et al., 2012 has demonstrated that nanoporous alumina-modified platinum electrode can be used for developing membrane-based electrochemical nanobiosensor which can be used for the diagnosis of *dengue type 2 virus (DENV-2)*³⁰.

The value of nanobiosensors as a diagnostic tool has long been in focus and when the advantages of nanobiosensor based approach in diagnostics were recognized during the last two,

three decades, it led to an evolution. This sensitive approach towards the diagnostic could be a breakthrough which is filling the gap and advancing the technology for the pathogen detection. This approach is certainly have several advantages over traditional methods of pathogen detection such as culture techniques, PCR (polymerase chain reaction), ELISA (enzyme linked immunosorbant assay), Flow cytometry etc. because the technology is smaller, faster, and more sensitive.

Nanobiosensors present as a detection option that can be effectively employed to specific pathogen related requirements. The prospects are low detection limit, less time consuming, economical, biocompatible, precise and accurate like features. In coming years through the application of biosensors, the speed with which water treatment plants, nursing stations, and society can respond to biosecurity threats, unsafe food and water, and medical problem will be changed considerably.

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