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Contents

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Quartz Crystal Microbalance DNA Based Biosensor for Diagnosis: A Review

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Abstract: Generally, the detection of particular DNA sequence is carried out by gel electrophoresis of DNA fragments amplified by polymerase chain reaction (PCR) using primers which are specific complementary sequence with the chosen region of DNA. Although gel electrophoresis is simple and effective for detection of PCR products, the use of carcinogenic ethidium bromide as a common stain is risky and hazardous. The DNA quartz crystal microbalance (DNA-QCM) lays the groundwork for incorporating the method for rapid DNA analysis. QCM biosensor is an extremely sensitive mass sensor, capable of measuring picogram levels of mass changes. This is a great potential market for simple, cheap, rapid, and quantitative detection of specific genes. QCM requires any non-labeling such as radio isotope, enzyme, and fluorescence tag. *Copyright © 2012 IFSA.*

Keywords: Biosensors, Quartz crystal microbalance, DNA, Immobilization, Hybridization.

1. Introduction

The biosensor technology was first developed many decades ago. The first biosensor was described by Clark and Lyons in 1962 [1] for the determination of blood glucose level. Biosensor is an analytical

device made by attaching the biological substances to a suitable transducer, which converts the biological response into electrical signal as shown in Fig. 1. A biosensor consists of a biological sensing element with a transducer to produce the signal proportional to target analyses.

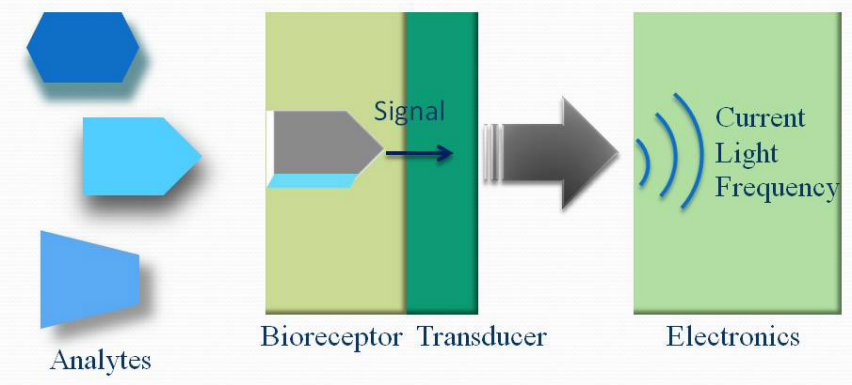

Fig. 1. General principle of biosensors.

When biological molecules interact with target analyses, there is a change in one or more physical-chemical parameter that associated with the interaction such as the generation of ions, gases, electrons, heat, or mass. The quantities of these indicating that signals are converted into the electrical signals by suitable transducer. The transducer is used to convert the biological recognition into the measurable signal that can be detected and displayed [2]. The most important characteristic of biosensor is the selectivity of bioreceptor for the specific target analyte. Therefore, the specificity of biosensor depends on the properties of the biological component which providing the selectivity and specificity for target analyte. Since the sensitivity depends on both the biological components and the transducer, the significance of reaction is related to the biological substances, target analyte, and efficiency of transducer [3].

The molecular recognition corresponds to the association of the biological element and its target molecule (analyte) through an association such as: enzyme-substrate, antibody-antigen, receptor-hormone, and complementary DNA sequencing, etc. These associations maximize the capacity of the biomolecules to recognize a unique substance among various substances [4].

The combinations of recognition-transducer systems are numerous and this explains many definitions and nomenclatures of these types of sensors. The main methods of transduction that are the most current and well developed from both a fundamental and experimental point of view are: electrochemical [5] and piezoelectric quartz crystal [6, 7].

Various types of transducer have been used in biosensor. These are the measurement of the change in electrochemical (potentiometry, amperometry or conductimetry), mass (piezoelectric crystal or quartz crystal microbalance), heat (colorimetry) and optical change (luminescence, fluorescence, reflective index, surface plasmon resonance and waveguide).

2. Quartz Crystal Microbalance (QCM)

A piezoelectric (PZ) quartz crystal or quartz crystal microbalance (QCM) biosensor [8] is an extremely sensitive mass sensor, capable of measuring subnanogram levels of mass changes, and it consists of a thin quartz disc sandwiched between a pair of electrodes. Due to the piezoelectric properties of quartz, it is possible to excite the crystal to oscillation by applying an AC voltage across its electrodes.

The term "piezoelectric" is derived from the Greek word "*piezen*" meaning "to press". Therefore, the piezoelectric effect means "pressure electricity" The theoretical foundation for using piezoelectricity was first pioneered by Raleigh in 1885, but the first investigation was performed by Jacques and Pierre Curie in 1880 [9], who observed a mechanical stress to a quartz crystal which induces an electrical potential across the crystal surface. The piezoelectric effects can be found in natural crystals but are very rare. The crystal has to go through a polarization process to obtain the piezoelectric property [10]. The piezoelectric device as a mass sensor arises from the linear relationship between the change in mass at the crystal surface and the change in oscillating frequency [10]. The vibration of piezoelectric crystals produces an oscillating electric field that the resonant frequency of the crystals depends on parameters associated with the phases adjacent to the crystal and the physical properties such as size, cut density, and shear modules [11]. The change in mass is identified by a corresponding change in the frequency of vibration of the crystal.

2.1. Gas Phase or Stepwise of QCM Biosensor

A resonant oscillation is achieved by including the crystal into an oscillation circuit where the electric and the mechanical oscillations are near to the fundamental frequency of the crystal. The fundamental frequency depends upon the thickness of the wafer, its chemical structure, its shape and its mass. Some factors can influence the oscillation frequency, like the thickness, the density and the shear modulus of the quartz that are constant, and the physical properties of the adjacent media (density or viscosity of air or liquid). As shown by Sauerbrey (1959), changes in the resonant frequency are simply related to the mass accumulated on the crystal. The fundamental frequency increases as the piezoelectric thickness decreases [12].

Thus for detection of analyses in air (the 'dip-and dry') method the frequency change is simply related to the change in mass:

$$\Delta F = \frac{-2 f_0^2 \Delta m}{A\sqrt{\mu_q \rho_q}}, \tag{1}$$

where:

ΔF = measured frequency shift (Hz);

f_0^2 = the fundamental resonant frequency of the crystal (Hz);

Δm = mass change (g);

A = area of electrode surface (cm^2);

μ_q = shear modules of quartz crystal = 2.947×10^{11} $g/cm^2 \times s^2$;

ρ_q = density of quartz crystal = 2.648 g/cm^3;

Gas phase mass sensitivity is determined by the QCM of fundamental frequency as described in equation 1. Mass sensitivity increases as the fundamental frequency of the quartz crystal increases which the fundamental frequency increases as the quartz crystal thickness decreases. Thus, one approach to increase sensitivity is to fabricate thinner crystal which in turn increases the operating frequency and sensitivity. Clearly, miniaturization can only be performed within physical limits. The size cannot be reduced below a certain value without affecting the durability of the device. However, recent fabrication designs have been tested that provide fundamental frequencies of 30 MHz [13]. The most commonly used crystals are 5–15 MHz quartz disks with a 10–16 mm diameter. For a fundamental frequency of 10 MHz and an electrode surface area of 0.22 cm^2, the gas phase mass sensitivity is approximately 1 Hz per nanogram [14].

Quartz crystal microbalance is made from piezoelectric materials which has specific dimension and orientation with respect to the crystallographic axes of material. The resonator consists of one or more pairs of conducting electrodes which are deposited by vacuum vaporization. The electric field is applied between the electrodes cause mechanical vibration or oscillation at the particular frequency in the resonator. These vibrations or oscillations will produce a reverse electrical potential which caused by the interaction coupling between the mechanical properties of the piezoelectric resonator and the electrical potential is termed the piezoelectric effect. The quartz crystal resonators are covered with metals such as gold, silver, or platinum by evaporation on the quartz surface, which are used as electrodes. There are various types of materials that exhibit the piezoelectric effect such as quartz, tourmaline, zinc oxide, or aluminium nitrite, but quartz usually used in analytical applications.

A quartz crystal resonator is cut slab from natural or synthetic crystal of the quartz. The quartz crystal plate must be cut in the specific orientation with respect to the crystal axes [15]. The properties of a quartz crystal depend on the plane in which it is cut. The AT-cut is normally employed which has an orientation of approximately 35° to the z-axis or optical axis. Since the AT-cut crystal has a temperature coefficient near zero, then the resonant frequency is stable over a wide range of temperature. Therefore, the AT-cut crystal has been used in majority of the piezoelectric work [9].

There are other terms to describe piezoelectric quartz crystal such as quartz crystal microbalance (QCM) and thickness shear mode (TSM). The AT-cut quartz crystal is a piezoelectric material which functions in microbalance mode as quartz crystal microbalance. TSM can describe the motion of crystal's vibration [15]. Quartz crystal has different vibration modes, which are grouped into flexure, extensional, face shear, and thickness shear mode. The thickness shear mode is also divided into fundamental mode and third overtone thickness shear. The thickness shear mode of vibrations is the type of vibrations that using AT-cut resonator [16]. When an appropriate alternating electrical potential is applied to gold electrode on opposite side of the quartz crystal, the crystal will oscillate at specific frequency. If increasing in mass at the interface, the oscillation frequency will decrease when the analyte binds with the coated surface. The frequency change is directly proportional to the increase in mass and correlated with analyte binding [17].

Dip and dry piezoelectric sensing (gas phase or stepwise) applications are advantageous in that the frequency signal can in some cases be interpreted through the use of the Sauerbrey relation; although, the method is sensitive to errors due to hydration, humidity, and solvent retention. The time consuming and tedious nature of the analysis scheme suggest the dip and dry method is not likely to ever become a routinely used analytical procedure. Furthermore, the application of solution phase piezoelectric sensing is far superior in that real-time data analysis can be conducted and the system can be configured for automated analysis [18].

2.2. Liquid Phase of QCM Biosensor

Quartz crystal sensing system to liquid phase measurement failed because the crystal ceased to oscillate when submerged in solution. To sense solution phase analytes either the sample was converted into a gas or a tedious dipping procedure was used [19]. These problems have been addressed by developing oscillator circuits that allow for crystal immersion in solution [20] or through the use of specially designed crystal flow through and/or batch reaction cells where solution contacts only one crystal surface as shown in Fig. 2.

The theoretical foundation for using piezoelectricity was first pioneered by Kanazawa and Gordon in 1985 [21], but the first attempt to use an acoustic device as a liquid phase sensor by Konash and Bastiaans in 1980 [22], who used as a liquid chromatography detector where one crystal face was exposed to a flowing organic solution.

Fig. 2. QCM based DNA biosensor for liquid phase system [6].

The system had poor sensitivity and reproducibility, but they demonstrated the quartz crystal could produce stable oscillations in liquid.

When a crystal is dipped into a solution or real time, the oscillating frequency depends on the solvent used. The question as to which factors determine the frequency is important for understanding the mechanism of oscillation of a crystal in solution and for its potential development as a sensor in solution [21, 23]. When an over layer is thick, the relationship between the *f* and D*m* is no longer linear and corrections are necessary. The coupling of the crystal surface to a liquid drastically changes the frequency when a quartz crystal oscillates in contact with a liquid that a shear motion on the surface generates motion in the liquid near the interface.

Thus for detection of analytes in liquid phase method which derived a relationship that expresses the change in oscillation frequency of a quartz crystal in contact with a fluid,

$$\Delta F = f_0^{3/2}\left(\frac{\rho_L \eta_L}{\pi \mu_q \rho_q}\right)^{1/2} \tag{2}$$

where:

ΔF = measured frequency shift (Hz);

$f_0^{3/2}$ = resonant frequency of the unloaded quartz crystal (Hz);

ρ_L = density of liquid in contact with the quartz crystal;

η_L = viscosity of liquid in contact with the quartz crystal;

μ_q = shear modulus of quartz crystal = 2.947×10^{11} g/cm^2×s^2;

ρ_q = density of quartz crystal = 2.648 g/cm^3;

The penetration depth of this sheer wave depends on $\left(\pi f_0 \mu_q \rho_q\right)^{-1/2}$. Kanasawa and Gordon (equation 2) [21] demonstrated stress that ΔF in solution is linear function of $(\rho_L \eta_L)^{1/2}$ except for salts and high polymer solutions. They, in fact, tested the linearity of dependence of the frequency decrease on $(\rho_L \eta_L)^{1/2}$ using many solvents selected on the basis of their different viscosity, density

and electrical conductivity. In 1994, Bruckenstein and shay observed that quartz crystal with on face exposed to dilute aqueous solutions near temperature induced frequency change by several kilohertz [23].

Kanazawa and Gordon described the frequency response in fluid phases in terms of the physical parameters of the quartz crystal and analyte solution by considering the coupling of quartz crystal shear wave to a dampened shear wave propagating into the fluid [21, 24]. The depth of the shear wave penetration varied with the square root of the bulk liquid viscosity. The decay length corresponded to the effective thickness of the liquid in motion with the quartz crystal. This liquid layer was treated as a sheet of mass attached to the quartz crystal surface. The model treated the fluid layer as continuous which no slip boundary between the quartz crystal surface and the fluid was allowed. The equation was verified by contacting a single quartz crystal surface with solutions of glucose and ethanol. The oscillation frequency in pure water was taken as a reference. The decrease in frequency varied as the solution viscosity and density product increased.

Liquid phase systems are designed using a myriad of conditions. The most common method employs the use of a contact cell where one crystal surface is adjacent to the solution. The contact cell is configured in a flow or batch mode and the solution is typically introduced using a peristaltic or syringe pump. In flow cells, the volume of solution flowing over the crystal surface is commonly less than 100 ml [25] but some flow cells have been designed for solution contact volumes of up to 7 ml [26]. No generalities can be made concerning the volume of solution in a batch contact cell which volumes range from a few microliters to nominally one liter. Alternatively, the whole crystal can be in contact with the solution phase. Here, either the quartz crystal surface is masked to avoid solution contact [27, 28] or the oscillator circuit is designed to allow the crystal to oscillate while immersed in solution [29]. Many conventional quartz crystal designs do not allow for dual electrode contact with a solution to avoid short-circuiting due to exposure to a common conductive solution. Further, with full solution contact more than just the piezoelectrically active surface interacts with the solution. This may cause calibration inconsistencies [30]. On the other hand, immersion in solution allows for detection of changes in solution conductivity. This could be advantageous, or a serious drawback, depending on the type of analysis performed.

This technique has been investigated for monitoring nucleic acid hybridization by immobilized the crystal with nucleic acid probe and detecting the mass change after hybridization with specific target sequence in solution, Fawcett et. al. [31] were the first group to describe a QCM for DNA immobilizing ssDNA onto the quartz crystal and detecting the mass change after hybridization. The advantages of this technique are not only the high sensitivity but also the label less operation. Additionally, frequency change can be followed in real time.

The drawback of quartz crystal microbalance is non-specific adsorption of molecules present in real matrices. QCM is a mass sensor which has surface for binding with any molecules, which can potentially interfere the reaction. However, previous experiments with QCM, DNA probes and hybridization of oligonucleotides clearly demonstrated that using appropriate chemistry immobilization, non-specific binding can be effectively minimized [6, 7]. Method to improve the sensitivity of the piezoelectric biosensor can be improved by using higher frequency of quartz, but this device is often difficult to operate in liquid because of fragility and frequency stability problems. Alternatively, the selectivity can be improved by improving the quality of the surface of the crystal and the immobilization method [33].

For most biosensor techniques, a biological component is immobilized on the quartz crystal surface. The signal is generated in response to adsorb recognition. The sensitivity of direct analyte detection through an immobilized ligand is limited by the number of binding sites available to interact. This is beneficial for characterization of conditions associated with a reaction but may be a limitation in terms

of detection sensitivity. One technique used to increase the sensitivity of QCM is to relate the first-derivative of the change in frequency versus time to the analyte concentration [6, 7]. Additionally, a number of analysis schemes have been proposed that do not rely on direct mass attachment to the crystal surface.

Liquid phase QCM techniques for monitoring cellular process have been described [33]. QCM biosensor integrated in a flow injection analysis (FIA) system has the advantage to work continuously and to monitor on-line the binding of the analyte A cell growth sensor was developed by Ebersole et al. to detect metabolites without immobilization of a biological entity [34]. This was the first sensor capable of detecting metabolic responses and division rates of viable cells. The authors indicated production of metabolic acids is a useful marker of cell metabolism and growth and utilized this relation in their sensor design. Detection of metabolic processes was based on adhesion of a polymer to the quartz crystal. The polymer precipitated from solution in response to titration of its carboxylate groups by the metabolic acids which converted the polymer to its isoelectric form. Moa et al. [35] reported DNA piezoelectric sensor, based on the nanoparticle amplification method, was developed for detection of *Escherichia coli* O157:H7 by using a thiolated single-stranded DNA (ssDNA) probe specific to *E. coli* O157:H7 *eaeA* gene was immobilized onto the piezoelectric sensor surface through self-assembly. Wong et al. have demonstrated DNA QCM biosensing method for real-time detection of E. coli O157:H7 in a circulating-flow system was developed in this study. The thioled surface of the Au electrode could be immobilized by many inner Au nanoparticles, then more thiolated single-stranded DNA (ssDNA) probes which were specific to E. coli O157:H7 eaeA gene could be fixed through Au-SH bonding [36]. Wu et al were developed DNA piezoelectric biosensing method for real-time detection of Escherichia coli O157:H7 in a circulating-flow system was developed in this study. Specific probes for the detection of E. coli O157:H7 gene eaeA, synthetic oligonucleotide targets (30 and 104 mer) and PCR-amplified DNA fragments from the E. coli O157:H7 eaeA gene (104 bp), were used to evaluate the efficiency of the probe immobilization and hybridization with target DNA in the circulating-flow QCM device [37].

3. QCM DNA Based Biosensor

Since the completion of the human genome mapping, interest has shifted to the study of the molecular functions of genes and how they might lead to a diseased state. Consequently, DNA biosensors have rapidly been applied to fields such as gene identification, genetic expression analysis, DNA sequencing, and clinical diagnostics. The detection and analysis of point mutations, and single nucleotide polymorphisms (SNP), is one of the main applications of DNA biosensors. However, in order to detect such mutations, optical fluorescent label or redox label must attach to target DNAs directly or indirectly, which requires the extra label binding processes and the use of hazardous materials and expensive lab equipments. Also, it takes a relatively long time to analyze the results, and is difficult to detect quantitatively the absolute amount of hybridization with those labeling because one has to normalize the data DNA has an important role in clinical diagnostics. DNA is arguably the most important of all biomolecules. The unique complementary structure of DNA between the base parts adenine/thymine and cytosine/guanine has been the basis for genetic analysis over the last few decades. The DNA techniques, including hybridization, amplification, and recombination, are all based on the double helix structure of the DNA. DNA is coiled to form a double helix (double-stranded DNA, dsDNA) composed of two strands held together by hydrogen bonds as shown in Fig. 3 that can be broken by heat or high pH. The single-stranded DNA is relatively stable, but on removal of the heat source or pH extreme, the ssDNA will reanneal into the double-stranded configuration. Reannealing between the ssDNA from different sources is called hybridization [38]. The reannealing of the dsDNA is possible because nucleotide bases will re-form hydrogen bonds only with specific complementary bases: adenine pairs with thymine, and cytosine pairs with guanine.

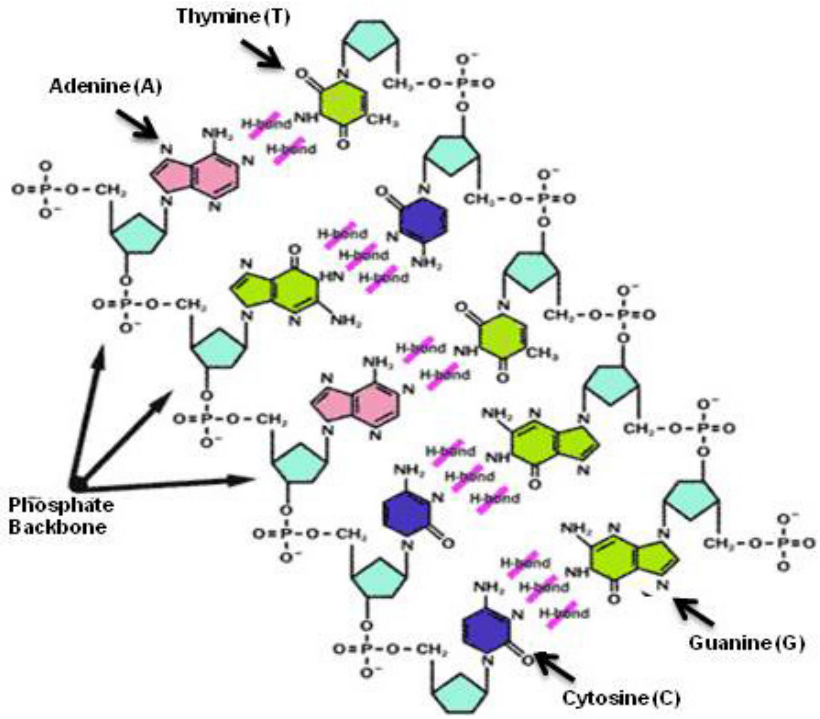

Fig. 3. Configurations of DNA and the hybridization principle.

The stability of the hybridization depends on the nucleotides sequences of both strands. A perfect match in the sequence of nucleotides produces very stable dsDNA, whereas one or more base mismatch can increase instability that can lead to weak hybridization of strand.

Molecular detection has shown a great potential for rapid identification of diseases and for food and environmental monitoring. After the recent successes in sequencing the human genome, the detection of specific DNA sequences in biological samples has been playing a fundamental role in genetic diagnostic and in the detection of pathogens in cells [39].

Generally, the detection of particular DNA sequence is carried out by gel electrophoresis of DNA fragments amplified by polymerase chain reaction (PCR) using primers which are specific complementary sequence with the chosen region of DNA. Although, gel electrophoresis is simple and effective for detection of PCR products, ethidium bromide which is a common stain is a carcinogen [40]. As an alternative way to gel electrophoresis, DNA probe has been widely used for detection of specific DNA sequences by hybridization. In this method, the probe is usually labeled with a radioisotope, enzyme, and fluorescent tag, these labeling and detection process require long and tedious steps.

The large demand for low-cost genetic assays has lead to the development of portable and easy-to-use biosensors. These systems should be able to perform the analysis in a very short time and with a very limited amount of specimen. Micro-fabricated structures, based on micro and nanotechnology can satisfy these requirements and also allow a high degree of parallelism and sensitivity.

The biosensor technologies have been intensively investigated because of their promise for rapid consuming, high specificity, and low cost. Therefore, DNA based biosensor is a promising alternative detection method by base pairing property in DNA. The specificity of DNA base pairing can be used as a bio-recognition. This biosensor has been based on electrochemical [5], and piezoelectric transducer [6, 7]. This is a great potential market for simple, cheap, rapid, and quantitative detection of specific genes. Areas of application include clinical, veterinary, environment, and food industry.

3.1. Self-assembled Monolayer (SAM) DNA Probe Immobilization

The immobilization technique is used to help the biological element attached with the sensor surface. This step not only helps for attachment between biological element and transducer, but also helps in stability for reusing if the immobilization is strong enough. The immobilization of the nucleic acid probe onto the transducer surface plays an important role in performance of DNA sensor. The immobilization step leads to well defined probe orientation, readily accessible to the target. This process depends on the nature of physical transducer and method that used for attachment of DNA probe to the surface [11].

There are various common immobilization methods for biosensor such as the using of direct physical adsorption on a solid surface, entrapment in a matrix, covalent coupling to functional groups on the electrode [41]. The covalent binding method can improve uniformity, density, distribution of bound molecules, and reproducibility of surface and instability, diffusion, aggregation, and inactivation of biomolecules. Glutaraldehyde, carbodiimide, and succinimide esters are widely used for covalent binding method [42].

The self-assembled monolayer is one of immobilization method by covalent binding. This method is rapidly grown since the discovery of these structures and ability to modify the physical and chemical property of surface. Self-assembled monolayer is molecular assembly obtained by immersion of an appropriate substrate into a solution containing molecules that specific with the surface. The term "self assembly" involves arrangement of atoms and molecules into an ordered or even aggregate of functional entities without the intervention of mankind towards an energetically stable form. SAM is distinguished from ordinary surfactant monolayer by the fact that one end of the molecule is designed to have a favorable and specific interaction with the solid surface of the substrate [6]. The self assembled monolayer have been composed from different types of simple organic molecules, such as alkanethiols and silane compounds on different substrate such as on metal oxide and gold surface substrate. The SAM system is extremely versatile and can provide a method for *in vitro* development of bio-surface.

The simple method involved in immobilizing self-assembly monolayer makes attractive strategy for achieving better control in the orientation and molecular organization of biomolecules at interfaces. SAM is easy formation of ordered, pinhole free and stable monolayer. More significantly, the easy method for SAM formation and compatibility with metal substrates such as gold or silver provided benefits for biosensor application such as only needed minimum amount of molecules. The stability and organization of the SAM depend on the forces of attraction between the immobilized molecules and the binding force between the surfaces and the binding group. Thiols on gold form very well-assembled monolayer which is highly resistant to washing due to the strong chemisorption of the sulfur atoms. There is a rapid reaction between –SH group and gold atom with formation of S-Au bond. After the initial fast adsorption of thiols, the akyl side chains assemble together to maximize the van der Waal's interaction [7].

Kaewphinit et al. [7] immobilized oligonucleotides directly on the gold quartz crystals, by forming a self-assembled monolayer, and also used biotin-oligonucleotides to immobilize them on avidin-modified gold quartz crystals. Zhou et al. [17] compared different immobilization methods (direct chemical bonding and avidin-biotin interaction) and different immobilization architectures (oriented oligonucleotide monolayer and multilayer created by self-assembling of alternating DNA and polymers). The researcher observed the biotinylated DNA films that provided fast sensor responses and high hybridization efficiencies, due to the spacer group that conferred better accessibility, and that multilayered films increased the sensor sensitivity, indicating that the complementary DNA can penetrate into the multilayer sensing film, but also increased the sensor response time because of the more difficult transport of the complementary sequence.

Probe immobilization is a fundamental step in DNA based piezoelectric biosensor development. Often, the detection limits and, in general, the analytical performances of the biosensor can be improved by optimizing the immobilization procedure on the quartz surface [17, 43]. Actually, the limitation of QCM devices is nonspecific adsorption of molecules present in real matrices, since QCM is a mass sensor and any molecule is able to bind or to be adsorbed on the surface which is a potential interference. Moreover, receptor which is DNA must be attached to the solid support retaining native conformation and binding activity. This attachment must be stable over the course of a binding assay and, in addition, sufficient binding sites must be presented to the solution phase to interact with the analyte. Many coupling strategies utilize a chemical linker layer between the sensor base (e.g., the gold layer) and the biological component to achieve these ends. Functionalized alkane thiols form stable self-assembled layers on planar surfaces and act as ideal linkers [7]. Moreover, many published papers showed that immobilization techniques based on direct adsorption or on protein A coating, resulted in appropriate sensor signals, but only crosslinker procedures using thiols or the interaction between avidin and biotinylated molecules, provided a long sensor lifetime and an increased stability against degradation during the regeneration process [44].

DNA sequences of even a few hundred base pairs have considerable molecular weights (Fig. 3). It seems likely, therefore, that the mass change associated with DNA hybridization may be detectable by employing piezoelectric devices. Campbell (1993) [45] immobilized ssDNA onto quartz crystal and detected the mass change after hybridization. Nucleic acid strands are covalently attached to the polymer-modified surface of a QCM. When immobilized probe strands are melted and bind to the complementary target strands in solution to form duplexes, the increase in mass that is detectable as a decrease of several hundred hertz in the crystal's resonance frequency relative to control crystals are observed. Furthermore, the crystals used are inexpensive and the results are quantitatively and quantitatively determined.

The steric effect on oligonucleotide immobilization onto a solid surface and DNA target hybridization in a real time DNA piezoelectric biosensor was improved in this study. It could be increased in the hybridization efficiency by added of 6 dT to the 5' end of the probes. This confirmed the studies that reported the poly dT at 5' end of probes might reduce steric hindrance in three-dimensional space also the addition of the spacer during DNA hybridization caused the increase of molecule collision as shown in Fig. 4. It is also proposed that during DNA target sequence hybridization, spacers have been shown to reduce steric interference, making the probe end closet to the surface of the device more accessible [46].

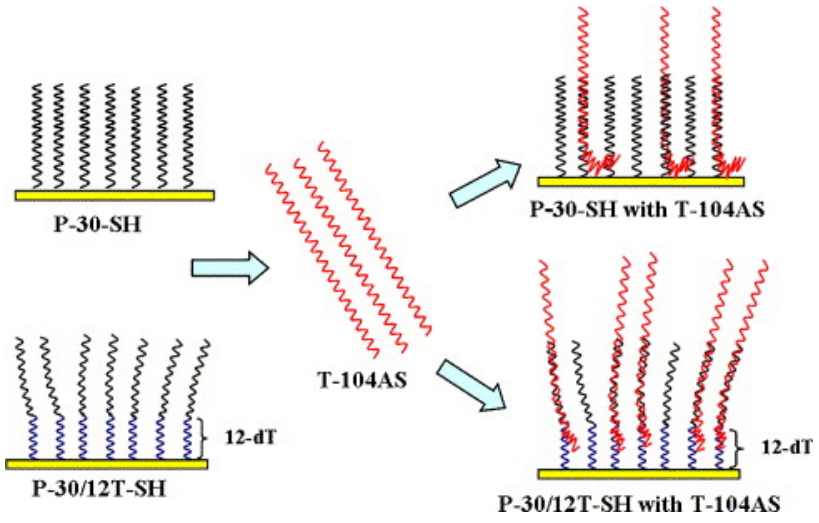

Fig. 4. Schematic representation of steric hindrance of the probe and target DNA hybridization on the QCM device [37].

The shelf-life of DNA probe on QCM could be the storage time at 4 °C for several weeks without losing the activity [43, 32].

3.2. DNA Target Hybridization

The kinetics of nucleic acid hybridization at a solid substrate-solution interface is still not well understood due to the lack of suitable methods for the continuous measurement of the hybridization process. The process is also difficult to predict from theoretical considerations, partly because the exact concentration of the immobilized nucleic acid and its availability for hybridization are unknown. Furthermore, the actual mechanism of strand association in solid-support hybridization remains obscure [47].

The kinetics and mechanism of nucleic acid single strand hybridization are widely studied only in cases where both strands are in free solution. The hybridization reaction between DNA probe and target oligonucleotide can be expressed by the following equation:

$$[probe] + [t\arg et] \longrightarrow [hybrid] \tag{3}$$

The association constant (K_a) is defined as:

$$K_a = \frac{[hybrid]}{[t\arg et][probe]} \tag{4}$$

$$= \frac{[V]}{[t\arg et][V_{\max} - V]} \tag{5}$$

The initial reaction rate of the hybridization reaction between the immobilization and detected DNA is represented as V and $V_{\max}$ which indicates the maximum initial reaction rate of the hybridization reaction. Because the amount of immobilization is very small, the hybridized DNA was also very small. Thus the equilibrium concentration can be considered as the initial concentration $[t\arg et]_0$. So the above equation can be written as:

$$\frac{[t\arg et]_0}{V} = \frac{[tagrget]_0}{V_{\max}} + \frac{1}{V_{\max} K_a} \tag{5}$$

Reciprocal plots between $[t\arg et]_0 / V$ and $[t\arg et]$ give a simple straight line. The association constant and the maximum initial reaction rate are calculated from the slope and the linear correlation respectively [48].

Hybridization in solution is believed to be a two-step process involving nucleation and zippering up. Nucleation is the rate-limiting step, and a second order reaction equation can describe the process. The nature of the hybridization reaction on solid surfaces is assumed to approximate closely to that of hybridization in solution. However, the rate of solid-phase hybridization is only about a tenth to a hundredth of that in solution. Although not systematically examined, it is suggested that efficient hybridization to DNA attached to solid supports can be impeded by several related phenomena. For example, the immobilized DNA molecules may link to solid surface at several points along the DNA chain, hence some of the attached DNA may not be accessible for hybridization. Generally, detection of single copy gene by piezoelectric quartz crystal DNA-based biosensor requires PCR for amplifying

target DNA [36]. However, PCR is still time consuming and requires expensive reagents and machines. Development of piezoelectric DNA-based biosensor for direct detection of highly repeated sequences using non-amplified genomic DNA has been recently reported. Minnunni et al. [49-51] detected satellite 13 DNA from genomic DNA of *Bos taurus*. They also applied this method for direct detection of single copy gene in non-amplified genomic DNA from *Nicotiana glauca* tobacco plant. They detected the promoter region (35S) that present in various GMOs and can be used as a marker for GMO screening [52]. They also applied this method for direct detection of sequences in nonamplified genomic DNA is described. The system relies on real-time and label-free detection of the hybridization reaction between an immobilized probe and the complementary sequence in solution. From above described, DNA piezoelectric biosensor can possibly be used for direct detection in non-amplified bacterial genomic DNA. In 2010, Kaewphinit et al. were developed the DNA-piezoelectric biosensor for direct detection in non-amplified bacterial genomic DNA of *M. tuberculosis*. The method involved in immobilization of specific synthetic biotinylated and thioled modified DNA probe that was designed from IS*6110* gene-specific for *M. tuberculosis* [6, 7].

The detection of genomic DNA target by using a QCM normally has less probability to hybridize with DNA probe due to the steric hindrance effect of DNA secondary structure and lower amount of specific DNA sequence than the detection limit of general analytical technique. The alternative solution to solve this problem was restricted enzymatic digestion of the DNA samples without any previous amplification step to separate small DNA fragments [50].

However, in non-amplified genomic DNA, the blocking oligonucleotides were added during denaturation step. This blocking oligonucleotides can prevent the re-annealing of single-stranded DNA to increase the efficiency of DNA target hybridization as shown in Fig. 5. Therefore, the blocking oligonucleotides were used for improvement of DNA target hybridization. The thermal plus blocking oligonucleotides gave the frequency change (indecreasing the resonant frequency) higher than only thermal denaturation. The thermal plus blocking oligonucleotides gave the frequency changes higher than the frequency change of thermal denaturation only [50, 51, 7].

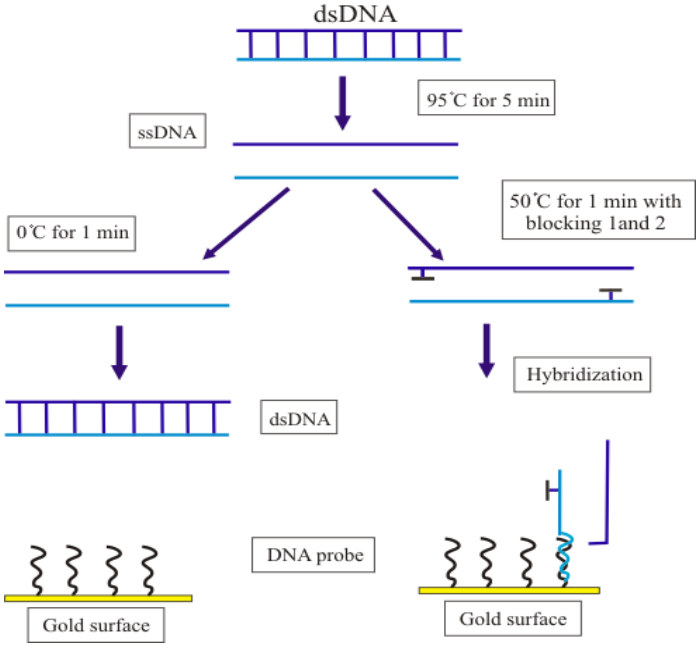

Fig. 5. Schematic diagram of denaturation method (The dsDNA was denatured to ssDNA before hybridization with the DNA probe. Left: simple thermal denaturation, 95 °C for 5 minutes and on ice for 1 minute. Right: simple thermal denaturation plus blocking oligonucleotides, 95 °C for 5 minutes and cooled down at 50 °C for 1 minute to anneal the blocking oligonucleotides) [7].

These thermal plus blocking oligonucleotides method could be improved the sensitivity by using mass enhancement (gold nanoparticle) was capped with blocking oligonucleotides by using 20 nm diameter of gold nanoparticle as optimal for the surface immobilization of QCM biosensor and the consequent sensitivity improvement because the hybridization was highest maximum value when the average diameter of nanoparticles was 20 nm and then decreased with the increasing of particle size [53]. Therefore, the frequency shift of the sensor was decreased accompanied by an increase of nanoparticle size applied in the static solution [53, 54]. In addition, gold nanoparticle modified blocking oligonuclotide deposited onto the piezoelectric sensor were depended on the DNA hybridization efficiency between the specific probe and target sequences [53, 55].

The gold nanoparticles could be attributed to the steric hindrance effect which the larger nanoparticles could not move as independently as the smaller nanoparticles and the larger nanoparticles connected to the DNA target hinder the approach of further nanoparticles [54].

Moreover, QCM could be developed a sensitivity of detection DNA based on gold nanoparticles amplification which detected *E. coli* O157:H7 cells to limit of 2.0×10^3 CFU/ml [36], 1.3×10^3 CFU/ml [57], and Chen and colleagues [56] detected Dengue virus to limit of 2 PFU/ml by gold nanoparticle signal amplified. Gill and co-works [58] detected *M. tuberculosis* by using gold nanoparticle probes for colorimetric detection to limit of 10 CFU/ml. Uludag and colleagues [59] studied the hybridisation with neutravidin capture for signal enhancement for the detection of HSV viral nucleic acids at 5.2×10^{-11} M concentration.

4. Conclusions

A biosensor is an analytical tool consisting of biologically active material used in close conjunction with a device that will convert a biochemical signal into a quantifiable electrical signal. Biosensors have many advantages, such as simple and low-cost instrumentation, fast response times, minimum sample pretreatment, and high sample throughput. A biosensor has two components, a receptor and a detector. The receptor is responsible for the selectivity of the sensor including enzymes, antibody, and DNA which this review described DNA analysis only. The detector, which plays the role of the transducer, translates the physical or chemical change by recognizing its analyte and relaying through an electrical signal. It is well known that the resonant frequency of an oscillating piezoelectric crystal can be affected by a change in mass at the crystal surface. In DNA-based QCM biosensor, hybridization detection is performed and the frequency changes resulting from the interaction between specific probes immobilized on the gold electrode of a quartz crystal and the complementary DNA strand in solution. The sensitivity can, in some cases, be improved by using crystals of higher frequencies (>10 MHz), but these devices are often difficult to operate in liquids because of fragility and frequency stability problems [32]. Alternatively, the selectivity can be improved by improving the quality of the surface of the crystal and the immobilization procedures. Actually, the principal limitation of the quartz crystal microbalance (QCM) is non-specific adsorption of molecules present in real matrices. QCM is a mass sensor and any molecule able to bind to the surface is a potential interferent. Experiments with QCM biosensor applied to DNA hybridization clearly demonstrate that non-specific binding can be effectively minimized by using appropriate immobilization chemistry [7]. These analytical systems are often founded on the immobilization of a fragment of DNA with a specific sequence (probe) and followed by monitoring and recording of the variation of the transducer signal when the complementary fragment (target) in solution hybridizes with the probe.

Most importantly, it is gratifying to see the emergence of a number of commercial organizations that are carrying out the long-awaited and much needed development of robust, reliable sensors, sample delivery and analysis systems that together will push the technology into the general laboratory and the field such as clinical diagnosis, epidemiology study, and bioterrorist weapon survey.

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