Lipoprotein Sensor: A Piezoelectric Quartz Crystal Device

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ABSTRACT

Among biosensor devices, piezoelectric has been highly recognized due to their small size, inexpensive, requires a small volume of sample, high sensitivity, high specificity, rapid response, reproducibility and ease of portable multiple specific sensor array fabrication. The piezoelectric quartz crystal (PQC) biosensor is well known in its ability to measure or detect a small mass change by using the piezoelectric effect of the quartz crystal. This paper focuses on the piezoelectric immunosensor for lipoprotein particle measurement. It reviews current technology used for the measurement as a tool for diagnosis and monitoring of patients with or at high risk of developing coronary heart disease.

Keywords: Piezoelectric quartz crystal; Biosensor; Lipoprotein particles

1. INTRODUCTION

Biosensor is a measuring system that composes of two major parts: a recognition part and a transducer part. The recognition part involves biological sensing elements or receptor molecules that lend the sensor specific to a target analyte [1]. A variety of biological substance (recognition part) can be used including antibody, affinity ligand, isolated receptor, enzyme, organelles, microorganism, cell, tissue, oligonucleotide, molecular imprinting polymer (MIP) [2]. When biological substances interact with the target analytes, there is a change in one or more physico-chemical parameters such as generation of ions, gases, electrons, second messenger formation, increase or decrease in enzyme activity, heat or mass [1]. The transducer can be used to convert these properties into electrical signal, as shown in figure 1. There are four main types of transducers: electrochemical transducer, optical transducer, thermal transducer and piezoelectric (Mass sensitive) transducer [3].

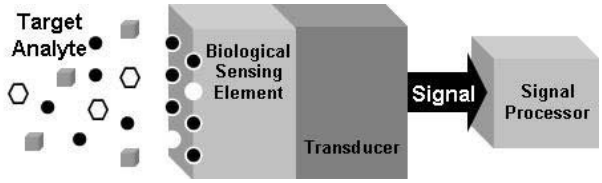

Fig.1: *Biosensor Configuration*

Among all type of transducers, piezoelectric device is relatively simple. There are many type of mass sensitive sensors such as thickness shear mode (TSM) sensor, surface acoustic wave (SAW) sensor, flexural plate wave (FPW) sensor and thin-film bulk acoustic wave resonator (FBAR) [4]. TSM is the most commonly used in the biosensor application at the last few years.

The present article is concerned with the development of the most popular piezoelectric, thickness shear mode for the reliable lipoprotein particle measurement purposes. We start with the brief historical piezoelectric theory and immobilization technical overview. Then the application, of piezoelectric device was described in many fields. Next we review about are lipoprotein and its important role in coronary heart disease development. Finally, we discuss the future technical direction of the piezoelectric for lipoprotein particle measurement.

2. PIEZOELECTRIC SENSORS - THEORY

Piezoelectric device, often named quartz crystal microbalance (QCM), shows a very high sensitivity for detecting the target analyte that is placed to the surface of the device and generates the resonant frequency change. The linearly downward resonant frequency shift is generated by increasing of mass on the device [5]. The piezoelectricity is discovered by Jacques and Pierre Curie in 1880 [1]. They discovered that a mechanical stress applied to surfaces of various crystals, including quartz, Rochelle salt and tourmaline [6], generated a corresponding electrical potential to the crystal whose magnitude was proportional to the applied stress. This behavior is called "piezoelec-

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tric effect". Moreover, lithium niobate, oriented zinc oxide, aluminium ni-tride, and tantalite are discovered as the piezoelectric materials [4]. However, the quartz (silicon dioxide) crystal is the common crystal type that has been used in analytical works. This device can be used to measure the target analyte in gas phase and in aqueous solution [7] [8]. It is insoluble in water and resists to high temperature with no loss of piezoelectric properties. It composes of positively and negatively charge ions that separate when it is applied to stress. In nature, it has a resonant frequency which depends on its chemical, size, shape and mass. The piezoelectric quartz crystal is the sandwiched, sliced quartz wafer placed between a pair of electrodes [9]. The electrodes are prepared by conductive material thermal evaporation applied on to the quartz surface, as shown in Figure 2.

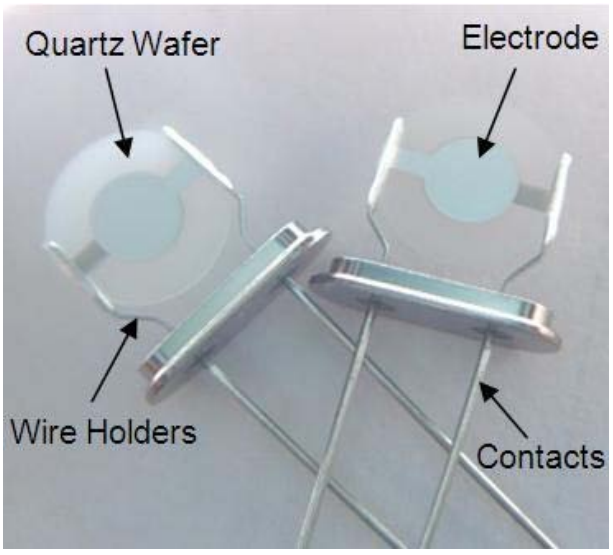

Fig.2: *Piezoelectric Quartz Crystal Structures*

Quartz crystal can be cut in different angles which gives different quartz crystal types with specific properties. AT-cut crystal has been used in piezoelectric analytical work. It refers to quartz wafers cut at $+35°15'$ angle from z-axis. It has a zero temperature coefficient. Therefore this quartz has the stable property in wide temperature range [3].

When the quartz crystal is putted on to an electronic oscillator, as shown in Figure 3 and the AC voltage is applied to pair of electrodes. The crystal can be made to oscillate at its resonant frequency via the piezoelectric effect [10]. There are many types of electrode such as gold, platinum or silver with different properties. In research application, gold electrode has been most popular used because it has long term frequency stability and reusability. However, it has high price per unit. On the other hand, silver electrode is inexpensive but possessing continuous oxidation overtime producing ineffective measurement [11]

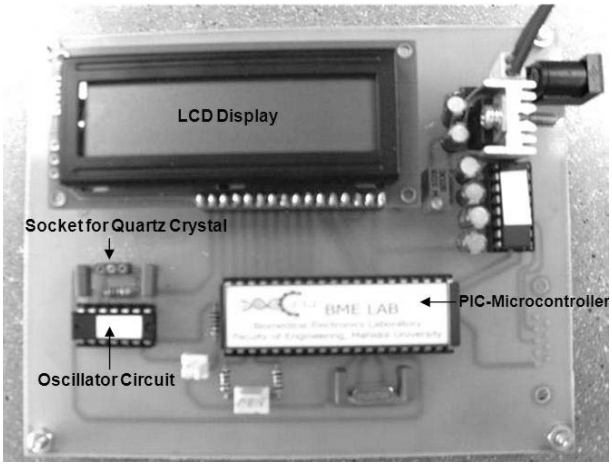

Fig.3: *Oscillation Frequency Counting Device (In-house developing by Department of Biomedical Engineering, Faculty of Engineering, Mahidol University)*

[12].

In 1959 Sauerbrey developed an equation that used to calculate the mass change on PQC in gas phase [4] [13].

$$\Delta f_m = -[2 \times (f_0)^2 \times \Delta m]/[A \times (\rho_q \mu_q)^{1/2}] \quad (1)$$

Where Δf_m is the change in the fundamental frequency, in Hz; f_0 is the resonant frequency of crystal, in Hz; Δm is mass change, in g; A is electrode surface area, in cm^2; ρ_q is density of quartz crystal, 2.648 g/cm^3; μ_q is shear modulus of quartz, 2.947×10^{11} $g/(cm \times s^2)$.

In 1980, the new PQC in aqueous solution phase was developed. The frequency shift (Δf_L) related to changes in viscosity and density of the solution. The frequency shift of quartz crystal that contacted with the Newtonian liquid (density ρ_L, viscosity η_L) is expressed by the Kanazawa's equation [4] [10] as shown below.

$$\Delta f_L = -[(f_0)^{3/2} \times (\rho_L \eta_L)^{1/2}]/[A \times (\pi \rho_q \mu_q)^{1/2}] \quad (2)$$

In experiment, the frequency shift of quartz crystal in the liquid phase system can be the results of the frequency shift of the mass (Δf_m) and the frequency shift of the viscous load (Δf_L). The change of the series resonant frequency (Δf_s) has been reported by Martin *etal* [10] as shown below.

$$\Delta f_s = \Delta f_m + \Delta f_L$$

$$= \frac{-2 \times N(f_s)^2 \times \Delta m}{A \times (\rho_q \mu_q)^{1/2}} - \frac{N^{1/2} \times (f_s)^{3/2} \times (\rho_L \eta_L)^{1/2}}{A \times (\pi \rho_q \mu_q)^{1/2}} \quad (3)$$

Where N is the overtone or harmonic number =1, 3, 5, ...

3. IMMOBILIZATION METHOD

Immobilization is the important step in fabricating a biosensor device. This technique used for the immobilized the biological substance on the electrode surface of quartz crystal via the physical or chemical fixation method that depends on the biological type and purpose of study. There are many factors to consider when choosing an immobilization method such as the density of functional molecule, low non-specific protein adsorption, long term stability, reusability and the orientation of the biological substance, particularly the active site [14] [15]. This paper will concentrate on the protein (antibody) immobilization method. Numerous immobilization methods have been attempt with the QCM immunosensor including, physical adsorption via protein A and protein G, entrapment, avidinbiotin binding [16] and covalent attachment via the saline compounds [15] [17]. Proteins contain a number of reactive immobilized site by which they can be immobilized on to the electrode surface such as the primary amine from lysine, (α)-amino groups, thiols from cysteine, cystine, methionine, and carbohydrate group of proteins. In case of antibody immobilization, antibody should be immobilized in the optimal concentration on the electrode surface in the proper orientation to obtain the highest possible capture capacity of the target analyte. More recently, the antibody immobilization using the covalent attachment method via self-assembled monolayer (SAM) has been developed, due to the apparent simplicity and the improvement of protein stability [15]. This method enabled the antibody to immobilize on the functionalized surface in a single layer that was suitable for attaching with the antigen.

4. APPLICATION OF PIEZOELECTRIC QUARTZ CRYSTAL DEVICE

At present, the quartz crystal has been applied in wide area. The qualitative and quantitative result can be showed by using this device. The major application of this device involved as the biological recognition such as protein peptide and nucleic acid. Therefore, there are many type of QCM based biosensors categoriged by type of biological elements. QCM immunosensors determine the antigen-antibody binding and the limit sensitivity is about a few μ g/ml [18]. Enzyme-based QCM uses to measure the product of the substrate conversion via the enzyme by using the electrochemical reaction [19], called as electrochemical quartz crystal microbalance (EQCM). Nucleic acid-based QCM uses to measure the formation of complementary single stranded nucleotide via hybridization method [46]. The detection sensitivity of this sensor is about a few nanograms DNA. Cell-based QCM uses to detect the growth rate of cell on the QCM surface. Moreover, QCM can be applied to the drug discovery field for investigation of the drug-target interaction. Recently, QCM sensors are developed to determine the concentration of the target analyte in clinical analysis, food and drink analysis, and environmental pollution control [20] as shown in Table 1.

5. LIPOPROTEINS

Lipoproteins are macromolecular complexes of lipids and proteins. A major function of lipoproteins is to transport lipid through the blood vessel. They are classified on the basis of their density in preparative ultracentrifuge into several classes; chylomicron, very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), low density lipoprotein (LDL) and high density lipoprotein (HDL). All classes have the same structure; triglyceride (TG) and cholesteryl ester (CE) form a hydrophobic core surrounded by a layer of amphipathic phospholipid (PL) and apolipoprotein (apo). A small proportion of lipoprotein cholesterol is unesterified (free cholesterol (FC)) and located on their surface [21]. VLDL, LDL and HDL levels in serum have close relationship with atherosclerosis. High levels of VLDL and LDL are associated with increased progression of atherosclerosis and risk of coronary heart disease (CHD), while high level of HDL is protective [39] [41]. Moreover, oxidized Low Density Lipoprotein (ox-LDL) has been shown to play an important role in the atherosclerosis pathogenesis [42]. The elevated level of circulating oxidized-LDL is associated with CHD too. CHD is the leading cause of mortality in the United States, Europe, and the vast majority of Asian countries including Thailand; it is the top three causes of mortality [43]. Therefore, VLDL, LDL, HDL and ox-LDL measurements have been utilized as a tool for diagnosis and monitoring of patients with or at risk of developing CHD together with the measurement of cholesterol, triglyceride and other cardiac markers.

6. LIPOPROTEIN MEASUREMENT

At present, LDL and HDL levels are measured in the lipid and protein form. The cholesterol (LDL-C, HDL-C) and apolipoprotein (apo B-100, apo A-I) concentrations that carried on LDL and HDL particles are the only lipid and protein that have been routinely measured. In part of VLDL and LDL-C, they are measured by using Friedewald calculation (LDL-Cholesterol = Total Cholesterol - (HDL-Cholesterol + Triglyceride/5)) [44]. Ox-LDL is measured by using indirect method such as measures in conjugated diene and lipid peroxides form [45] [46]. Many analytical techniques including ultracentrifugation, electrophoresis, precipitation methods, homogeneous enzymatic colorimetric assay, chromatography, HPLC and GC-MS are developed to separate and quantify the LDL-C, HDL-C and ox-LDL

Table 1: *Application of Piezoelectric Quartz Crystal Device*

Target Analyte	**Biological Substance (Recognition site)**	**Immobilization Method**	**Detection Limit**	**Reference**
Clinical Analysis Virus				
African swine fever virus protein	Antibody	Physical adsorption Covalent coupling via DSP	1 μg/ml	[18]
African swine fever virus antibody	Protein of virus	Streptavidin-biotin	0.2 μg/ml	[18], [22]
Human herpes virus	Antibody	Protein A	5×10^3 cells/ml	[23]
Hepatitis virus genomic DNA	DNA probe	SAM of biotin-avidin	8.6 pg/L	[24]
Bacteria				
Staphylococcus aureus	Antibody	SAM	5×10^5 cells/ml	[25]
Vibrio cholerae	Antibody	Physical adsorption	10^5 cells/ml	[26]
Parasite				
Schistsoma japonicum antibody	*Schistsoma japonicum* antigen	SAM via MPA	Titer 1:800	[27]
Clinical Chemical				
C-reactive protein	Antibody	HEMA/MMA copolymer	37.8 μg/ml	[28]
α-estrogen	α-estrogen receptor	SAM via cystein	20 μM	[29]
Albumin	Albumin MIP	SAM	60 ppm	[30]
α-Thalassemia	Oligonucleotide probe	AM of biotin-avidin	nr	[31]
Point mutation detection of TP53 gene	Oligonucleotide probe	SAM of biotin-avidin	nr	[1]
Food and Drink Analysis				
Salmonella enteritidis	Antibody	SAM via MPA	10^5 cells/ml	[32]
Pseudomonas aeruginosa	Antibody	SAM via sulfo-LC-SPDP	1.3×10^7 CFU/ml	[33]
Escherichia coli	Antibody	SAM via cysteamine	3×10^5cells/ml	[34]
Haloacetic acids	Trichloacetic acid-MIP	SAM	20 μg/l	[35]
Chloramphenicol	Antibody	SAM via MPA	5×10^{-6}M	[36]
Environmental Monitoring				
Dioxin	Antibody	SAM via cysteamine	0.1 ng/ml	[37]
Gas (CO_2, NH_3, SO_2, H_2S)	TMAF	Physical adsorption	nr	[38]

$*$nr: value not reported

DSP:3,3'-dithio-bis(propionic acid n-hydroysuccinimide ester, SAM: Self-assembly monolayer, MPA: 3-mercaptopropionic acid, Sulfo-LC-SPDP: Sulfosuccinimidyl 6-[3-(2-pyridyldithio)propionamido] hexanoate, MIP: Molecular imprinted polymer, TMAF: Tetramethylammonium fluoride tetrahydrate

[[47] [50]. Apolipoprotein is commonly determined by using various immunoassay techniques such as radioimmunoassay, radial immunodiffusion, enzyme-linked immunosorbent assay (ELISA), and immunoturbidimetry assay [51].

The amount of lipoprotein particles present cause atherosclerosis (VLDL, LDL, ox-LDL) and anti-atherosclerosis (HDL) activity, not the amount of cholesterol and apolipoprotein that they carry. Therefore, direct lipoprotein particle measurement is more effective than cholesterol or protein measurement. Because of the cholesterol and protein levels

Table 2: *Various Types of Lipoprotein Sensors*

Transducer	Target Analyte	Biological Substance (Recognition site)	Immobilization Method	Reference
Electrochemical				
- Amperometric	LDL	Anti Apo B-100	Adsorption on Au nanoparticles	[53]
	LDL-C	Horseradish peroxidase (HRP) /cholesterol oxidase	Entrapment	[54]
	Apolipoprotein E	Anti-Apo E	Site-directed attachment	[55]
	Apolipoprotein E	ss-oligonucleo-tide of Apo E	Adsorption	[56], [57]
- Electrochemilu minescence	LDL	Apo B-100	SAM	[58]
Optical	LDL, HDL	Anti-LDL, Anti-HDL	Protein A	[59]
- Surface plasmon	LDL, ox-LDL	Heparin	SAM	[60], [61]
	LDL	Anti-LDL	SAM	[62]
- Ellipsometry	Apo B-100	Anti-Apo B-100	Entrapment	[63]
	LDL, IDL, HDL	Proteoheparan sulfate	Adsorption	[64], [65], [66]
Piezoelectric				
- QCM	LDL	Cholesterol modified dextran (CMD)	SAM	[67]
	VLDL/chylomicron	Transthyretin (TTR)	Adsorption	[68]
	LDL	Dextran sulfate	SAM	[69]
	Apolipoprotein E	Biotinylated 23-mer probes	Streptavidin-biotin	[56]
- Magnetoelastic	LDL	Dextran sulfate	SAM	[70]
- Microcantilever	LDL, ox-LDL	Heparin	SAM	[71]

are vary in lipoprotein particles [52]. At the same lipoprotein cholesterol level, varying particle amounts are present due to the particle size effect. For this reason, LDL-C and HDL-C values are often misleading; normal levels of them mask an increased or decreased LDL or HDL particles amount, respectively. Ultracentrifugation can measure the amount of lipoprotein particle directly but it is costly, requires relatively large volumes of serum, time-consuming, used technical skill and requires many steps for measurement. Currently, nuclear magnetic resonance (NMR) spectrometry is the most popular method that use for measure the lipoprotein particle amount and size directly [52]. However, this method is inappropriate in routine laboratory because it requires the complicated sample preparation step, costly, time consuming and requires high technical skill.

7. LIPOPROTEIN SENSOR

The aforementioned disadvantages can be overcome by biosensor based technology. Currently, lipoprotein sensors for lipoprotein and apolipoprotein measurement are developed by using various types of biosensor as shown in Table 2. Development of biosensor technology coupled with immunoassay, called "immunosensor" [72] is a successful technique for the detection of lipoprotein, for example LDL and HDL [53][59]. A piezoelectric biosensor device has important attractive properties such as small in size, rapid with high throughput, high sensitivity and specificity. Piezoelectric immunosensor is based on adsorbed recognition sites where selective binding cause a mass change which is identified by corresponding change in the acoustic parameter.

Therefore, lipoprotein immunosensor based on piezoelectric technology will be developed. This sensor will be used for the direct lipoprotein particle measurement. Specific antibody will be employed to capture the ligands on lipoprotein particle as shown in Figure 4. Thus, the frequency change from the mass change obtaining from the antigen-antibody complex formation will be recorded. In addition, the other type of receptor molecules such as specific lipoprotein (VLDL, LDL, HDL, ox-LDL) receptor can be used

[73] [75]. Moreover, lipoprotein sensor technology can fabricate the lipoprotein sensor array that can measure the amount of multiple lipoprotein classes at the same time.

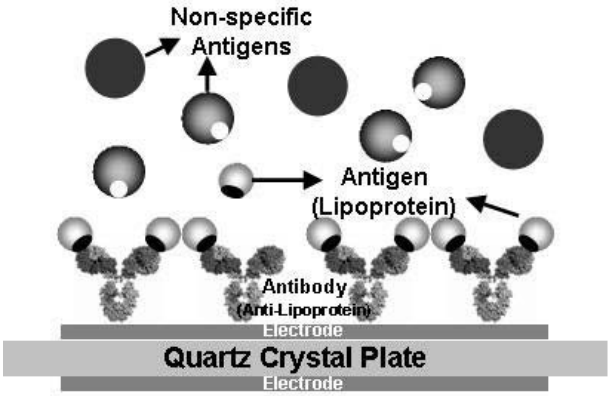

Fig.4: *Lipoprotein Immunosensor*

8. CONCLUSION AND FUTURE DIRECTION

This review enumerates the lipoprotein sensor that can be prepared by using the piezoelectric sensor technology. The antibody concentration and incubation time will be optimized to enhance the signal response from antibody immobilization method. Moreover, the optimization studies will helpul in reducing the cost and time required for the piezoelectric sensor preparation by reducing the amount of antibody and increasing the binding activity. The stability and regeneration ability of this sensor will be studied.

The novel lipoprotein sensor procedure should be verified for its metrological traceability that can be performed the property of the result of measurement whereby it can be related to state references, usually national or international standard, through an unbroken chain of comparisons all having state uncertainty. Additionally, the method valida-tion in term of accuracy, precision, working range, sensitivity, and specificity will be characterized regarding its validation before it will be introduced into routine use. Moreover, this sensor reliability will be studied by estimation the measurement uncertainty. These results can be used to judge the traceability, quality, reliability and consistency of the analytical results that fit for intended use.

9. ACKNOWLEDGMENT

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