REVIEW OF PIEZOELECTRIC BIOSENSORS IN MEDICINE

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ABSTRACT:
Piezoelectric sensors have been developed based on profoundly investigated topics and theories in mass, electricity, and viscoelasticity and with commercially available devices like quartz crystal microbalance. Biosensors based on piezoelectric principles show significant advantage over other biosensors in the field of sensitivity, versatility, low cost, label free, and simplicity.
This paper has the purpose of presenting the use of piezoelectric biosensors for medical applications in terms of immunosensors and genosensors for rapid detection of viruses, bacteria, proteins, and DNA/RNA hybridization. Accent is on their low-cost potential and high correlation with biochemical diagnostics and molecular biology based diagnostics.

1. INTRODUCTION

Piezoelectric sensors have been developed based on profoundly investigated topics and theories in mass, electricity, and viscoelasticity and with commercially available devices like quartz crystal microbalance. Piezoelectric sensors show significant advantage over other sensors in the field of sensitivity, versatility, low cost, label free, and simplicity. Biomedical area utilizes them in terms of immunosensors and genosensors for rapid detection of viruses, bacteria, proteins, and DNA/RNA hybridization [1].
In the bio-recognition processes of bio-affinity recognition and bio-metabolic recognition use binding of one chemical species with another of a complementary structure. In bio-affinity recognition, the binding is very strong and the transducer detects the presence of bound receptor-analyte pair. Basic processes regarding this method are receptor-ligand and antibody-antigen binding. In bio-metabolic recognition, analyte and other co-reactants have to be chemically altered in order to form product molecules. Interaction of antibodies with their corresponding antigens is the field of antibody-based chemical biosensors, commonly referred to as immunosensors. The high specificity and affinity of antibodies towards complementary ligand molecules does not necessarily lead to an electronically measurable change. The piezoelectric effect in crystalline substances is a useful property for detection of analytes. Piezoelectric immunosensors are among most sensitive analytical instruments which are capable to detect antigens in picogram quantities. Also, they have a potential to detect antigens in gas and liquid phases [2].
Piezoelectric crystals vibration under the influence of an electrical field, and the relationship between the resonant frequency changes and the mass of molecules adsorbed or desorbed from the surface area of the crystal are the basic properties that make the transduction mechanism in piezoelectric crystals efficient. Piezoelectric biosensors can be coupled to different biomolecules, such as: antibodies, nucleic acids and bio-mimetic receptor, mainly aptamers [2].

2. MEDICAL APPLICATIONS

Piezoelectric biosensors offer an important advantage of real-time, label-free transduction, so they are used for studying of a broad range of biomolecular interactions. The crucial aspects in development of biosensors are:

- Immobilization and amplification procedures
- Advantageous features of the piezoelectric category of biosensors [3].

Table 1: Piezoelectric biosensors in medicine [4].

Target	**Piezoelectric sensor**	**Real matrix**	**Correlation with reference methods**
Total IgE and allergen-specifi c IgE	AT-cut gold- or silver-coated (10, 20 and 25 MHz)	Human serum (28 samples)	Fluorescence AllergoSorbent Test (correlation, 0.98)
Human chorionic gonadotropin	AT-cut 10 MHz, gold	Human serum and urine (60 samples)	Radioimmunoassay (correlation, 0.92)
Ceruloplasmin	AT-cut, 9 MHz, gold	Human serum	ELISA (correlation, 0.98)
Anti-sperm antibody	AT-cut, 9 MHz, gold	Human serum (six samples)	ELISA (average relative deviation, 6 %)
Carcinoembryonic antigen	AT-cut, 9 MHz, gold	Human serum (fi ve samples)	Chemiluminescence immunoassay (average relative deviation, 9 %)
AFP	AT-cut 10 MHz, gold	Human serum (fi ve samples)	ELISA (correlation, 0.98)
Dengue virus	10 MHz, gold	Simulated serum and clinical serum specimens (15 samples)	ELISA
AFP, CEA, PSA, and CA125	AT-cut 10 MHz, gold	Human serum composite samples (seven)	Chemiluminescence immunoassay (average relative deviation, <10%)
Total PSA	AT-cut, 9 MHz, gold	Human serum (four samples)	Chemiluminescence immunoassay (average relative deviation, 6%)
Rheumatoid arthritis autoantibodies	AT-cut, 5 MHz, gold	Human serum (32 samples)	ELISA (diagnostic values are given)

Hepatitis B surface antigen	SAW	Whole blood (three samples)	Whole blood (three samples
Staphylococcal enterotoxin B	Gold, 16.5 MHz	Spiked urine	Not applied
Carcinoembryonic antigen	Gold, 9 MHz	Human serum (six samples)	Chemiluminescence immunoassay (average relative deviation, <8%)
PSA	Gold, 20 MHz	75% human serum	Commercial ELISA kits
AFP	Gold, 10 MHz	Spiked serum	RIA method (correlation, 0.975)

2.1. PIEZOELECTRIC IMMUNOSENSORS

First piezoelectric immunosensor was introduced in 1972 by Shons and his research group. Besides the classical usage of antibody-antigen interaction and the important advantage of piezoelectric transduction of being a mass balance, elimination of the need for labeling or use of secondary antibodies, there are other fundamental factors.

Piezoelectric immunosensors can be used for cancer biomarker detection in human serum. An immunosensor was developed in order to detect prostate-specific antigen (PSA) by using monoclonal anti-PSA antibodies immobilized onto SAM-coated sensor chips. Gold nanoparticles amplification and a particulate study was done to determine the ideal buffer composition in terms of additives, salts and detergents for minimization of matrix effect on human serum analysis. PBS buffer containing 200 µg/ml BSA, 0.5 M NaCl, 500 µg/ml dextran and 0.5% Tween 20, eliminated 98% of the interfering signal. Gold nanoparticles added to the buffer as signal amplification tools gave detection limit of 0.29 µg/ml PSA in 75% human serum with a linear dynamic detection range of up to 150 ng/ml. These boundaries were more than adequate for detection of physiological concentrations of PSA (cut-off value of 4ng/ml). This work showed that these biosensors are an efficient tool for biomedical applications, and it demonstrated the main advantages such as repeat usability of sensor chips, utilization of label-free detection method, short assay time and comparison of analytical characteristics of the developed biosensor with some commercial ELISA kits for same target molecule [5].

Piezoelectric biosensor based on immunosensing was developed for a competitive label-free immunoassay of 2,4-dichlorophenoxyacetic acid by direct measurement in liquid solution. The piezoelectric crystals used in this experiment were modified with 2,4-dichlorophenoxyacetic acid (2,4-D) using coupling procedures based on self-assembled monolayers of suitable thiocompounds on the surface of gold electrodes of the crystal. The piezoelectric biosensor has been placed in a flow-through cell and the affinity binding of two monoclonal antibodies against 2,4-D on the modified piezoelectric crystals was studied in real time without any additional labels. As stated in the title, this is an immunosensor which is the mostly used application of piezoelectric biosensors [6].

Detection of presence of different odors even at trace levels of them is the characteristic of human olfactory system. Quantification of particular samples is based on conventional panel molecules. Trace level of odorous molecules can be detected by other analytical instruments. This artificial nose consists of a sensor in which the isolated olfactory receptor proteins (ORPs) from bullfrogs (Rana spp.) coated onto the surface of a piezoelectric (PZ) electrode, similar to the mechanism of human olfaction. The piezoelectric crystal served as a signal transducer. The results provided information about rapid (about 400 s), reversible, and long-term (up to 3 months) stable responses to different

volatile compounds like n-caproic acid, isoamyl acetate, n-decyl alcohol, β-ionone, linalool, and ethyl caporate. Sensitivity was in range from 10-6 to 10-7 g, which is fully correlated with the olfactory threshold values of human noses. Forming a typical fingerprint for each odorant and response to different odorants requires an array of six sensors consisting of five fractionated ORPs and one referenced phospholipid probe [7].

2.2. PIEZOELECTRIC GENOSENSORS

A nucleic acid biosensor is an analytical device incorporating a sequenced oligonucleotide, even a modified one, or a complex structure of nucleic acid, either integrated within or intimately associated with a signal transducer. Nucleic acid biosensors are used for detection of DNA/RNA fragments or biological or chemical species. Most of them are based on high specificity of hybridization of complementary strands of DNA or RNA, and these biosensors are termed genosensors. Probe, immobilized to the transducer surface is a biorecognition molecule and it recognizes the target DNA, and the transducer is the component that converts the biorecognition event into a measurable signal. In genosensors, detection of hybridization events is carried out through different recognition technologies, such as label-free method of piezoelectric and surface plasmon resonance transduction to labeling methods like electrochemical techniques [3].

Medicine bares close interaction with genetics in determination of diseases with the use of genotypes, polymorphisms or other genetic markers. There are a lot of methods used for monitoring of genotypes and polymorphisms, and they include: fluorescence in situ hybridization (FISH), flow cytometry (FCM), real time-quantitative reverse transcription polymerase chain reaction (RT-PCR) and restriction fragment length polymorphism (RFLP). These methods are punctual but they have limitations in terms of long-time needed for analysis, poor precision and high cost. Clinical diagnostics does not require massive data accumulation provided by gene chips, alternative technologies offer results in relatively fewer measurements. Piezoelectric transduction is very attractive because of its simplicity, low instrumentation costs, and possibility for real-time and label-free detection.

First nucleic acid detection based on the interaction of nucleic acids with the use of acoustic wave was described in 1988 by Fawcett and his research group. Single-stranded DNA was immobilized onto a quartz crystal and mass was detected after hybridization. HPV PCR samples can be analyzed by a self-designed adjustable metal-clamping piezoelectric sensor. The adjustable metal clamping system has the potential of overcoming some of the drawbacks of traditional piezoelectric sensor assemblies, that lead to an uneven stress distribution on the edge of a piezoelectric sensor which further affects the stability of sensor frequency. This work enable a detailed study concerning the effect of temperature on the PCR sample under analysis. PCR sample denaturation needs high temperature, followed by ice-cold bath. This means that the PCR product detection by DNA sensor requires the addition of PCR products in an ice bath: however, when the PCR product in an ice bath is added to the balance buffer at room temperature sensor well, a process of temperature change occurs upon mixing. The results indicate that small volumes of PCR products in ice bath require more attention. The entire experiment is shown in Figure 1 [8].

A highly sensitive piezoelectric HBV DNA biosensor, based on sensitive mass-transducing function on quartz crystal microbalance and the specialty of nucleic acid hybridization reaction has been developed. HBV nucleic acid probe was immobilized onto the gold electrodes of a 9 MHz AT-cut piezoelectric quartz crystal with the polyethyleneimine adhesion, glutaraldehyde cross-linking (PEI-Glu) method or the physical adsorption method. The coated crystal with the PEI-Glu method to immobilized HBV nucleic acid probe showed the better results than the physical adsorption method with respect to sensitivity reproducibility and stability. The frequency shifts of hybridization have better linear relationship with the amount of HBV DNA, when the amount was in range 0.02–0.14 μg/ml. The crystal could be regenerated nearly five times without perceptible decrease of sensitivity [9].

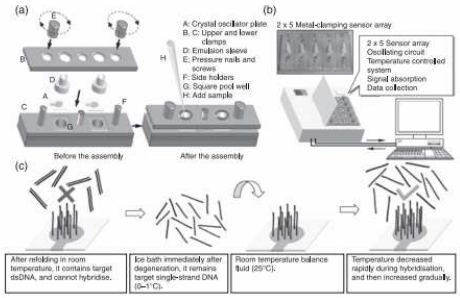

Figure 1: Biosensing by metal-clamping piezoelectric sensor [7].

Biotinylated 23-mer probes were immobilized on the streptavidin coated gold surface of a quartz crystal; streptavidin was covalently bound to the thiol/dextran modified gold surface. Investigation was done on hybridization of the immobilized probe with a synthetic oligonucleotide and the absence of non-specific adsorption was verified using a non-complementary oligonucleotide. Regeneration of single strand with 1mM HCl enables performing many cycles of measurement on the same crystal. The same reaction was done with real DNA samples extracted from bacteria and amplified by PCR. In this study, a fragment of Aeromonas hydrophila's gene was the PCR product used. This piezoelectric biosensor was capable of distinguishing samples containing gene or not, so the pathogenicity of different Aeromonas strains isolated from different specimens, was determined. Experiments using non-specific samples confirmed the absence of adsorption or non-specific effect on the quartz crystal if the procedure was done by the book [10].

Detection of point mutation in a human gene is possible by coupling of PCR with a piezoelectric biosensor. Biotinylated 23-mer probes were immobilized on the streptavidin coated gold surface of a quartz crystal; streptavidin was covalently bound to the thiol/dextran modified gold surface. The hybridization of the immobilized probes with a short sequence (23 mer) complementary, non-complementary and mismatched DNA was investigated: the device was able to distinguish the different synthetic oligonucleotides. As in one of previously mentioned experiment, single strand DNA regeneration with 1mM HCl enables multiple cycles on same crystal. The same hybridisation reaction was then performed using real samples of human DNA extracted from blood and amplified by PCR, following a standard procedure for genetic detection of the polymorphism of the apolipoprotein E (apoE) gene. The procedure has proven successful because it enabled distinguishing the sequences present in different samples, which differ only in one base, so it was possible to distinguish between different groups of genotypes with apoE typing. Testing of "blank" samples confirmed the absence of adsorption or non-specific effects on the quartz crystal treated with the experimental procedure [11].

3. CONCLUSION

Piezoelectric biosensors are highly sophisticated sensors with broad applications. They are based upon interactions similar to those of an antigen and an antibody. Because there is a lot of types of piezoelectric biosensors, it is possible to talk about multiple advantages. The advantages are most certainly their ability to perform quantitative analysis of samples without any additional steps, label-free recognition, low expense and short time needed to perform the analysis. Piezoelectric biosensors can be used in all environments, depending on their type. That is very important because the human body presents multiple challenges related to the environments. The basis of detection is change of frequency that can be accurately measured by the use of piezoelectric materials. Usage of piezoelectric biosensors in conjunction with nanoparticles gives novel applications. Nanoparticles are

becoming more and more used in the field of detection of various molecules. As mentioned in the application part, there are a lot of applications, from basic ones related to antigen-antibody reactions, to development of an artificial olfactory system. This provides us with a perspective that piezoelectric biosensors can be further developed and reach outstanding performances. Biosensing based on piezoelectricity is simplest and cheapest and piezoelectric materials are able to detect biomolecules down to nanodimensions. This has significantly approved diagnostics and made it easier. Monitoring of hard tissues in orthopaedic application and monitoring of growth of neo-tissues to scaffolds or artificial organs is complicated. This is a possible field of application for piezoelectric biosensors. A dual response biosensor which was developed in 1997 but it is still not widely used in biomedical applications. Nanomaterials in conjunction with piezoelectric biosensors can be used in this field. Improving dual response to multiple response is very important field in which piezoelectric biosensors can be developed. Finally, it is possible to say that even though these sensors have somewhat complicated both composition and signal obtaining, they have a huge development perspective in both medical and industrial field of application.

4. LITERATURE

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