

APPLICATIONS OF ENZYME BIOSENSORS IN DIABETES

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Abstract— This review gives details about the application of glucose biosensors in Diabetes. Diabetes is a serious health hazard which also leads to other chronic diseases. Increasing Diabetes cases drive market. It is mostly prevalent in affluent societies due to their lifestyle. Many biosensors have been designed for diagnosis of Diabetes, but still researches are going on to design a classical biosensor. Since last 30 years, only short term glucose measurement in skin are developed, long term use in Diabetic patient of glucose sensor is still not available. In glucose biosensor, nanotechnology based biosensors are most widely used. The behavior of implantable glucose sensors are disappointing in-vivo. Alternative glucose sensing techniques which do not involve tools that break the skin or physically enter the body are still under experiment.

Index terms- Diabetes, Glucose biosensor, Blood glucose monitor, Glucose oxidase, Glucose dehydrogenase, Amperometric, Quinoprotein glucose dehydrogenase.

I. INTRODUCTION

This review article mainly focuses on the latest advances in the development of glucose biosensors, many of them are electrochemical and few are optical (Davis and Seamus Higson, 2009). Diabetes has become a major threat in growing population due to irregular feeding habit, Life style and lack of exercise. It is a metabolic disorder, in which pancreas doesn't produce sufficient insulin (Adams, DD 2008). It is one of the most duel disease faced by medical approach. About 5-6% of the population in North America is affected by diabetes mellitus which produces a large amount of mortality. From the overgrowing population, it is recently figured out that about 18.2 million persons have diagnosed diabetes and another 5.2 million are undiagnosed (Derek Le Roith et al. 2005).

Prolong diabetes leads to cardiovascular diseases (Clark et al. 1962) gangrene, diabetic nephropathy, diabetic neuropathy (Fujoka et al.), diabetic retinopathy and erectile dysfunction. Besides all these, diabetic patients also have slow wound healing. There are three types of diabetes:

1. Diabetes Mellitus (type-1 diabetes)

(National Diabetes Data Group 1979) - it mostly affects the young and occurs due to under production of insulin by pancreas.

2. Diabetes Insipidus (type-2 diabetes) - it mainly affects middle aged or older people and occurs due to deficiency of Anti Diuretic Hormone(ADH), secreted by neurohypophysis of pituitary gland.

3. Gestational diabetes- it affects pregnant ladies and there is 2-4% desultory of foetus being affected.

According to American Diabetes Association, it is advised that patient with type-1 diabetes should monitor their blood glucose level at least 4 times a day (American Diabetes Association, 1994) and with type-2 at least twice a day.

From the diagnostic point of view many biosensors (Newman et al. 2004) are developed. The vigorous attempts are pointed towards biosensors instruments (Turner et al. 1988) talented of testing multiple parameters for home diagnostic and point of care market. It is expected that by 2015, the biosensors throughout the world will cross over US\$ 12 billion. Years from years the major key factor for the development of glucose biosensors include friendly design biosensors, diabetic population growth and point of care applications. Currently over 40 blood glucose meters are available in the market. The very large companies like Roche Diagnostics, Life Scan, Abbott and Bayer are fair number from an ocean player in the glucose biosensors market. Asia Pacific grasps the highest growth potential for biosensors.

II. GLUCOSE BIOSENSOR TECHNOLOGY

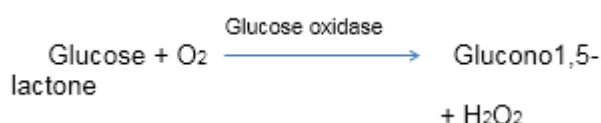
Biosensor is an analytical device which contains a biologically sensitive material which when interact with a specific analyte that produces physical, chemical or electrical signals. There are many strategies developed regarding to glucose biosensor but most common are embellished glucose oxidase, which involves piercing the skin to draw blood, and then the blood is applied to a chemically active disposable test strip.

Personal (Home) Glucose monitoring/First Generation Biosensor:

The first successful blood glucose biosensors for home use was a mediated device based around a disposable

screen-printed sensor design/Finger stick measurement method (Elizabeth H. Holt. 2008). The home glucose monitoring enables the patients to monitor their glucose level regulatory so as to keep control over their diet. Enzyme based glucose biosensors are amperometric biosensors which are based on redox reactions. It includes following three enzymes:

1. Glucose oxidase: The simplest type of glucose biosensor contains a paper strip impregnated with glucose oxidase enzyme, HRP [Horse Reddish Peroxidase] and chromogen. Here oxygen acts as a co substrate. When a drop of blood is placed on the strip, the blood glucose gets oxidized to H₂O₂ (Hatzinikolaou et al. 1996) and the peroxidase produced oxidizes weakly colored chromogen (Toluidine) to a highly colored dye. The intensity of the color of the dye is directly proportional to the glucose concentration and can be measured by using a portable reflectance meter (Clemens, A.H., 1971).



This method is known as GOD-POD method (Witt et al. 2000). It is extensively used because of its good stability and is inexpensive.

2. Glucose dehydrogenase: It requires NAD⁺ as a cofactor instead of oxygen as a substrate. In this case NADH is produced. It was discovered by Laurinavicius et al. 1999.



3. Quinoprotein Glucose dehydrogenase (GDH): It requires orthoquinone cofactors. The soluble Quinoprotein was pyrroloquinoline quinine(PQQ) which acts as a cofactor (Katz and Willner,2001)

All of these have different advantages and drawbacks:

□ Glucose oxidase is inexpensive but requires O₂ as a co substrate, so when O₂ is depleted, performance also decreases.

□ Glucose dehydrogenase is independent of O₂ and well established probe for glucose monitoring and also good at low glucose concentration. But its biggest drawback is that cofactors are expensive and unstable.

□ PQQ-GDH is very efficient enzyme system but it is costly. Nanotechnology based biosensors/ Second generation biosensor Nanotechnology is considered as an impulsive growth in biosensor field.

□ Glucose biosensor based on chitosan-prussian blue-multiwell carbon nanotubes (M.Diaconu et al. 2010)-hollow Pt-Co nanochains formed by one step electrode position discovered by Iijima (1991).

This biosensor was constructed by electrochemical deposition of Cs-PB-MWNT's-H-Pt-Co composite on

a gold electrode as a mediator, after deposition glucose oxidase (GOD) was immobilized on the film, Then nafion was dipped on GOD/ Cs-PB-MWNT's-H-Pt-Co membrane, which maintain the stability of modified electrode. The introduction of H-Pt-Co Nano chains have improved the performance of the glucose biosensors as they not only facilitate electron transfer but also have catalytic activities for glucose oxidation. The biosensor provides high loading capacity for glucose oxidase. It is widely used biosensors because of their unique properties like: high sensitivity, electrical conductivity, mechanical strength, chemical and long term stability, quick response and good reproducibility (W. Zhao and I.Baravik)

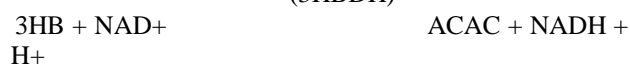
□ Glucose biosensors based on glucose oxidase (GOx) immobilized in Ag-Au sol. Ag-Au nanoparticles are produced in Sodium bis (2 ethylhexyl)-sulphosuccinate-cyclohexane reverse micelle system. GOx is mixed with Ag-Au nanoparticles and cross linked with Polyvinyl Butyral medium by Glutaraldehyde then this mixture was coated on platinum electrode.

III. ELECTROCHEMICAL BASED BIOSENSOR

For monitoring the concentration of ketone 3-beta-hydroxybutyrate (3 HB) for potential diabetic patient management (Lei Fang et al.). It consist of enzyme -3-hydroxy butyrate dehydrogenase (3HBDH) immobilized on thick film of iridium modified working electrode detecting NADH which is the reaction product of 3HB and NAD⁺ (Albers et al.1997)

The blood ketone testing for quantifying 3-betahydroxybutyrateis essential for diagnosing and monitoring ketoacidosis for diabetic patient management. Three blood ketones produced by liver are 3HB, Acetoacetate (ACAC) and acetone out of which ACAC and 3HB are major ketones. In a normal individual the ratio between 3HB and AcAC is approximately 1:1 whereas in Diabetic ketoacidosis it may be as high as 10:1. Therefore detection of 3HB is important.

(3HBDH)



3HB will be detected by detecting NADH by a spectrophotometer.

IV. TOMATO SKIN BIOSENSOR

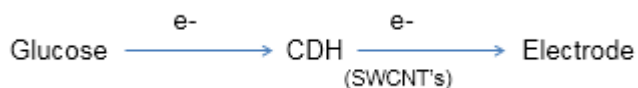
In this type of biosensor, the enzyme GOx is immobilized on tomato skin which is then kept on surface of O₂ electrode. The glucose concentration was quantified by the changed of dissolved oxygen. The response of the biosensor has linear relationship with a concentration range of 1-30 mmol/l. The tomato skin biosensors are cheap, highly sensitive, have simple fabrication and fast response time.

V. NYLON NET BIOSENSORS

In this biosensor GOx is immobilized on nylon net with glutaraldehyde as cross linking reagent and O₂ electrode. Nylon net is polymer compound i.e. cross linked by amide bonds. They have high mechanical strength and resistance to microbial attack. But its one major drawback is that it is inert and due to its inertness, it is unable to bind enzymes without specific treatment. There are number of methods for activating nylon net, such as hydrolyzing it with cyclohexyl isocyanides / glutaraldehyde before immobilizing enzyme or by a simple and low cost method using O-alkylation with Di-methyl sulphate(DMS) and 2-lysine as a spacer to activate nylon net and glutaraldehyde to link the enzymes.

VI. THIRD GENERATION BIOSENSOR

It is based on cellobiose dehydrogenase (CDH) from *Corynascus thermophilus* and single walled- carbon nanotubes.



SWCNT'S- Oxidatively shortens single walled carbon nanotubes.

VII. TEAR GLUCOSE

It is a potential approach for the non-invasive estimation of blood glucose. For this calcium alginate, poly-2-hydroxyethylmethacrylate and polyurethane foam were taken as an absorbent. It is a quantitative model. This model explains the sampling of tear fluids with glucose concentration into a sampling material.

VIII. CONCLUSION

The biosensors become a dominating field of research worldwide. Due to growing population and modern lifestyle, the various health issues comes under biosensor from which one is Diabetes. In medical applications, glucose biosensor becomes a wide division of a global biosensor market. Diagnosis by biosensor is made when patient has symptoms of diabetes like chronic hyperglycemia which is the gold standard of the disease. The glucose biomass has many practical approaches at industrial level, biotechnology, drug discovery / therapeutics key market driver and restraints, pharmaceuticals etc. The development of glucose biosensors have made the life of patients suffering from diabetes smooth and easy. Micro fabrication technology has immense importance in designing newer biosensors. In near future in-vivo glucose sensing for diabetes management will be developed (Shichiri et al. 1982). That would be progressed towards non-invasive monitoring. There is a need of future development in tissue fluid sample and extraction technique which enables to measure

glucose outside the body. The researchers have developed many glucose biosensors but now also they haven't succeeded to develop an ideal glucose biosensor.

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