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A Novel Approach for Frequent Testing of Physiological Glucose Levels in Diabetes Patients Using NanoBiosensors

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Abstract: ***Nanotechnology is an advanced scientific technique that provides more accurate and timely medical information for diagnosing disease. Diabetes mellitus (DM) is a commonly seen chronic disease, which seriously threatens the health of human beings. Nanotechnology is a focal point in diabetes research. The use of nanotechnology in the development of glucose sensors is a prominent focus in non-invasive glucose monitoring systems. Nanotechnology can now offers new implantable or wearable sensing technologies that provide continuous and extremely accurate medical information. Electrochemical biosensors, as exemplified by the blood glucose monitor, have the potential to revolutionize medical diagnostics and patient monitoring. They offer cheap, automated and easy-to-handle platforms which combine sample processing and analysis to give a read-out in minutes. Nano biosensors are enormously attractive medically, as they offer a rapid, portable, inexpensive and highly sensitive test that can be performed at the point-of-care by a non-specialist user. The purpose of this is to throw more light on the recent advances and impact of nanotechnology on biomedical sciences to cure diabetes. Nanomedicine, the application of nanotechnology to medicine, has already offered some new solutions, and many biosensor manufacturing companies are trying to develop sensors using nanotechnology that offers some new solutions in treating diabetes mellitus.***

Keywords- **Nanotechnology, Glucose Sensors, Glucose Monitor, Wearable sensing technologies.**

I. INTRODUCTION

Diabetes mellitus, often simply referred to as diabetes is a group of metabolic diseases in which a person has high blood sugar, either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced. This high blood sugar produces the classical symptoms of polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger. There are three main types of diabetes:

Type 1 diabetes: results from the body's failure to produce insulin, and presently requires the person to inject insulin

Type 2 diabetes: results from insulin resistance, a condition in which cells fail to use insulin properly, sometimes combined with an absolute insulin deficiency.

Gestational diabetes: is when pregnant women, who have never had diabetes before, have a high blood glucose level during pregnancy. It may precede development of type 2 DM.

Other forms of diabetes mellitus include congenital diabetes, which is due to genetic defects of insulin secretion, cystic fibrosis-related diabetes, steroid diabetes induced by high doses of glucocorticoids, and several forms of monogenic diabetes. Self-monitoring of Blood Glucose (BG) is a critical part of managing diabetes. However, present procedures for obtaining such information are invasive, painful, and provide only periodic measurements. Development of a painless and automatic approach would represent a significant improvement in the quality of life for people with diabetes. In addition, results from the Diabetes Control and Complication Trial Research Group , and UK Prospective Diabetes Study showed that a tight glucose control regimen, which uses frequent glucose measurements to guide the administration of insulin or oral hypoglycemic agents, leads to a substantial decrease in the long-term complications of diabetes; however, there was a 3-fold increase in hypoglycemic events. Moreover, as many as 7 BG measurements per day were not sufficient to detect a number of severe hypoglycemic and hyperglycemic events. The development of glucose biosensor for frequent testing of physiological glucose levels in diabetes patients is crucial for confirming the treatment effectiveness and to avoid diabetic emergency. The developed biosensor provides a means to obtain painless, automatic, and noninvasive glucose measurements. The biosensor also provides automatic and frequent measurements that could provide detailed information on glucose patterns and trends that might identify opportunities for improved Blood Glucose (BG) control. Automatic readings also provide the opportunity for an alarm to be sounded in response to values below a user-selected alert level or as a result of rapid declines in the measured glucose values. These alarms could provide a method to reduce the risk of hypoglycemia and make intensive therapy for diabetes safer and acceptable to more patients.

II. NANO BIOSENSORS

Medical diagnostics is an industry that is expanding rapidly, with the global biosensor market expected to exceed $US18 billion by 2018. A biosensor is a

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compact analytical device, often considered as a "lab-on-a-chip", which facilitates the detection and quantification of a target analyte, at the point-of-care (POC). This confers several key advantages over laboratory-based means of analyte detection: biosensors are rapid, cost-effective and operate at the bedside or in the home without the need for specialist users or equipment. As such, biosensors can save lives and tackle medical problems sooner by providing a faster diagnosis. Biosensors prove particularly useful, in addition to their application in hospitals and GP surgeries, (a) in the developing world, where cheap disease detection without the need for specialist clinicians is lacking and, (b) in the developed world, for self-monitoring of diabetes, cholesterol levels etc[46]. A biosensor in its most simple form may be described as a device comprising three parts: a biological recognition system, a transducer, and a signal processing display. Interaction of the analyte of interest with the biorecognition element is converted to a measurable signal by the transducer, before conversion to the readout or display. The demand for reliable and user-friendly medical diagnostic tools that can be used at the point-of-care need to be well established. Point-of-care may refer to use in a clinical environment, field use by a clinician or other operative, or self-use by a patient. Commercial point-of-care diagnostic devices are currently available for amperometric and potentiometric electrochemical biosensors, e.g. glucose home blood sugar monitors, iStat Portable Clinical Analyzer (amperometric and potentiometric,). However, till date, no nano biosensors have made the transition from successful research laboratory trials to mass produced point-of-care diagnostics. In this research paper, we outline the main considerations associated with the transition of an impedimetric Nano biosensor from research laboratory to widespread use. The so-called "finger stick" systems (invasive) which take a drop of blood typically from the finger and measure blood glucose level are among the most dominant methodologies. In medicine, it is also known that the presence of detectable levels of glucose in the urine is an indicative of diabetes. Hence, an accurate detection of glucose levels in urine using biosensors would facilitate a reliable, non-invasive approach for proper monitoring of the glycemic state of diabetes patients. It is known that normal concentration of glucose in urine is in the range of 0.0–0.8 mM. However, in case of diabetes, patients normally contain glucose concentration of 2.8 mM in urine at highest blood glucose level [1]. Renal glycosuria is also the excretion of glucose into the urine. The appearance of glucose in urine is reflected in the concept of a renal threshold for glucose (RTG) excretion. In some, but not all, textbooks the concept of RTG excretion is propagated with the threshold specified at 10 mM [1]. According to this concept, no glucose should be detectable in urine at sub-threshold blood glucose levels. If the RTG is so low that even normal blood glucose levels produce the condition, it is referred to as renal glycosuria. At a lower glucose concentration in urine, it is not possible to distinguish between diabetes and renal glycosuria. But at a higher concentration of glucose in urine, the sensor is capable to detect diabetes. Moreover, this biosensor will be useful for patients with known glycemic state, who requires frequent monitoring of blood glucose concentration various times per day in order to manage diet and therapy. Hence such a non-invasive, painless and convenient approach would provide the additional benefits of eliminating the danger of infection resulting from multiple skin punctures. Furthermore, the degree of enzyme immobilization on mediators is crucial for the development of high performance glucose sensor, capable to detect glucose concentration with wide linear range, high selectivity and fast sensitivity. Since the glucose level in bloods from diabetic patients easily goes up to 15–20 mM and the linear range of detection is dependent on the amount of immobilized enzymes, it becomes necessary to develop biosensors having wide linear range of detection through optimization of enzymes immobilization on zinc oxide (ZnO) nanostructures-mediated electrodes. Among various immobilization strategies [2][3][46], physical adsorption is commonly chosen owing to its simplicity, high selectivity and relative low cost [5–7]. In general, the degree of enzyme immobilization is a function of surface area of mediators which is determined by the structural morphology of a nanostructure. However, little work on the glucose biosensor based on aspect ratio (AR) controlled ZnO NRs except our previous work on a cholesterol biosensor [8] has been reported. A higher AR means a greater specific surface area per volume, thusmore enzyme immobilization with higher AR. As well varieties of materials were investigated for the preparation of biosensors possessing good specificity, selectivity, and rapid response [9–20]. Among all, ZnO is one of the unique materials for fabrication of facile, biosafe, and low cost biosensors. Especially, nanostructured ZnO owing to its large specific surface area and high isoelectric point (IEP ~9.5) makes it appropriate for absorption of low IEPs proteins or enzymes such as glucose oxidize (GOx, IEP 4.2) at pH 7.4. Moreover the ample available ZnO nanostructures including nanorods [21–24], nanotubes [25–27], nanowires [28–30], nanoparticles [31–33], nanonails [34–36], nanocombs [37], nano-flake [38] and hollow nanospheres [39] have drawn substantial attention for their applications in glucose biosensors. However, most of the research groups mentioned above not only used gold (Au) electrodes but also synthesized nanostructures on substrates separated and transferred them onto electrodes, which needs additional processes such as

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preparation of nanostructures containing solution, coating, network-forming for tight adhesion, and drying [40]. Furthermore, to our best knowledge, no work has been reported on the effect of AR of ZnO NRs on the amount of enzymes immobilization and the performance of glucose biosensors. Here, we report a simple technique for the fabrication of highly selective, ultrafast, reliable and wide linear-range detecting glucose biosensors based on the AR-controlled ZnO NRs grown directly on Si/Ag electrodes in solution and a potential use for the detection of glucose in urine.

III. EXPERIMENTAL METHODS

Modern scientific instruments, from gene sequencers to particle accelerators, generate unprecedented amounts of data. Other contributors to the data tsunami include point-of-sale terminals, social networks, the World Wide Web, mobile phones (equipped with cameras, accelerometers, and GPS technology), and electronic health records. These sensors, instruments, and other information sources and, indeed, simulations too produce enormous volumes of data that must be captured, transported, stored, organized, accessed, mined, visualized, and interpreted in order to extract knowledge and determine action. [41] The fundamental principle behind biosensing is the conversion of a specific biorecognition event into a measurable signal. A wide variety of bioreceptors can be employed to detect a diverse range of analytes. The signal may be transduced in a variety of ways; electrochemically, optically or mechanically. In the case of Nano biosensors, a high-affinity interaction between the analyte and bioreceptors, which are often antibodies, gives rise to alterations in the electrical properties of the sensor surface. Therefore, the layer-by-layer construction and the nanostructure of the biosensor surface must be finely-tuned in order to achieve specific, measurable and robust analyte detection within complex patient samples. Amperometric glucose biosensors will be fabricated using aspect-ratio (AR) controlled zinc oxide nanorods (ZnO NRs) grown directly on Si/Ag electrodes. The performance in terms of selectivity, response time, linear range and repeatability will be studied, showed in fig 1. The performance is also studied in repect to immobilization on the well-aligned ZnO NRs arrays and direct electron conduction between the NRs and the electrodes. Furthermore the biosensor will be studied for a promising application for detecting glucose in urine, which is useful for detecting renal glycosuria.

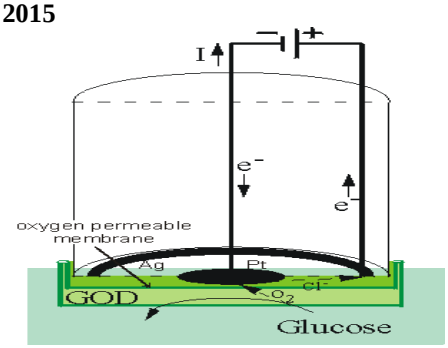

Fig 1. Schematic diagram of a simple amperometric biosensor. A potential is applied between the central platinum cathode and the annular silver anode.

This generates a current (I) which is carried between the electrodes by means of a saturated solution of KCl. This electrode compartment is separated from the biocatalyst (here shown glucose oxidize, GOD) by a thin plastic membrane, permeable only to oxygen. The analyte solution is separated from the biocatalyst by another membrane, permeable to the substrate(s) and product(s). This biosensor is normally about 1 cm in diameter but has been scaled down to 0.25 mm diameter using a Pt wire cathode within a silver plated steel needle anode and utilizing dip-coated membranes

IV. REAGENTS

Glucose oxidase Type VII (GOx, EC 1.1.3.4 from Aspergillus niger, 100 U/mg), nafion (5 wt.% in lower aliphatic alcohol and water mixture), bovine serum albumin (BSA >98%), glucose (d-(+)-99.5%), ascorbic acid (AA), uric acid (UA), l-cysteine (l-cys), glutamate (GA), Bradford reagent (for 1–1400 g/ml protein), Urea, lactic acid, citric acid, creatinine, glycine, NaHCO3, CaCl2, NaCl, MgSO4, K2HPO4, Na2SO4, NH4Cl, Zinc nitrate hexahydrate (Zn(NO3)2·6H2O, 99%), hexamethylenetetramine (HMTA, 99%) and phosphate buffered saline (PBS, pH 7.4) need to be purchased from Sigma–Aldrich and used without further purification. Artificial urine was prepared which contains urea, lactic acid, citric acid, creatinine, glycine, NaHCO3, CaCl2, NaCl, MgSO4, K2HPO4, Na2SO4, and NH4Cl. The final pH was setto 7.0 and glucose was added latter when CV was measured at different concentration of glucose.

V. SYNTHESIS AND CHARACTERIZATION

In a typical synthesis process, an equal molar of ZnNO3·6H2O (0.01 M) and HMTA (0.01 M) was dissolved in 50 mL of distilled water and transferred into a Pyrex glass bottle followed by heating in a laboratory oven for 1 h at 90 ◦C after suspending the seeded substrates upside down. To control AR, the ZnO NRs were synthesized with varying reaction time of 2, 4, 6, 8, 10, 12 and 14 h. After the reaction, the electrodes were rinsed with DI water to remove impurities before

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characterization. The value of AR was calculated simply by dividing the average length with the average diameter of the individual ZnO NRs. The morphology and crystallinity of as-synthesized ZnO NRs was examined by field emission scanning electron microscopy (FESEM) and X-ray diffractometer (XRD) measured with Cu-K radiations (= 1.54178 ´ A)˚ in the range of 20–60◦ with 8◦/min scanning speed respectively.

Glucose Biosensor Fabrication and Measurements

The biosensor fabrication process includes: preparation of Si/Ag electrodes by sputtering silver on Si substrate, sputter deposition of ZnO seed layer on the Si/Ag electrode, direct growth of ZnO NRs on the Si/Ag electrode (0.04 cm2), and immobilization of GOx enzyme by physical adsorption. For the GOx immobilization step, we have used a drop-casting method followed by drying at 4 ◦C for overnight. After 12 h, the GOx-modified electrodes were washed with PBS to remove the loosely adsorbed GOx. Finally, a droplet of 0.5% Nafion was applied to form a membrane. After immobilizing GOx enzymes on the ZnO NRs, the as shown in fig2 immobilization degree was determined by Bradford assay.

Fig 2. Schematic of glucose detection via electrochemical method by using ZnO NRs modified electrode

Cyclic voltammetric (CV) and amperometric experiments were performed using an electrochemical analyzer connected to a personal computer. All experiments were carried out using a conventional three-electrode system with the Si/Ag/ZnO NRs/GOx/Nafion electrode as the working electrode, a platinum wire as the counter electrode, and Ag/AgCl with saturated KCl solution as the reference electrode. All the potentials in this work were with respect to Ag/AgCl reference electrode and the electrochemical measurements were carried out at room temperature (25 ± 2) in 0.01 M PBS (pH 7.4). Each cyclic voltammetry was performed in a solution of 10 ml volume between −0.40 and +0.80V (vs. Ag/AgCl). In steady state amperometric experiments of glucose, the applied potential was set at +0.60V (vs. Ag/AgCl (sat'd KCl) reference) with magnetic stirring.

VI. RESULTS AND DISCUSSION

A. Aspect Ratio Controlled Zno Nanorods and Immobilization Efficiency

The immobilization percentage (%) increases linearly with increase in AR of ZnO NRs, attributed to the greater specific surface area of ZnO NRs with higher AR. In order to investigate the electrochemical activity of the GOx in the Si/Ag/ZnO NRs/GOx/Nafion, the current response of the electrode was explored.

B. Cyclic Voltammetry

To study the electrochemical behavior of the Si/Ag/ZnO NRs/GOx/Nafion electrodes with different ARs (5, 30 and 60), the CV measurements were carried out at different scan rates from 20 to 200 mV/s in the presence of 1 mM glucose in 0.01 M PBS

C. Effect of Nafion Membrane

To evaluate the importance of nafion membrane coating, the CV measurements of electrodes with and without coating was carried out after 20 times usages

D. Amperometric Response

The amperometric response (at +0.60V) of Si/Ag/ZnO NRs/GOx/Nafion electrodes with different ARs (5, 30 and 60) to the successive addition of glucose in 0.01 M PBS (pH 7.4) is measured, The apparent Kapp M which gives an indication of the enzyme–substrate kinetics is calculated from the Lineweaver–Burk equation 1/i = (Kapp M /imax)(1/C) + (1/imax), where i is the current, imax is the maximum current measured under saturated condition, and C is the glucose concentration. The Kapp M of glucose biosensors is calculated with AR = 5, 15 and 60, respectively, representing the GOx affinity to glucose with a respect to AR, which provides electron communication features that enhance the direct electron transfer through the ZnO NRs to the Ag electrodes. Performances in terms of higher sensitivity, lower Kapp M , wider linear range, and faster response time need to be Compared with the previously reported nanostructures-based glucose sensors. In this work, we will find the glucose biosensor with ZnO NRs having good aspect ratio (AR) that shows the best result in terms of sensitivity, linear range, and ultrafast response time.

E. Interference Study and Storage Stability of Biosensors

It is well known that some electro active species in serum, such as ascorbic acid (AA), l-cysteine (l-cys) may influence the performance of glucose biosensor. These

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endogenous interfering species being structurally similar to glucose were capable of simultaneously oxidized along with glucose at the electrode surface, hence, give interfering electrochemical signals. It is important to study the anti-interference ability of the fabricated glucose biosensors The long-term storage stability of the fabricated biosensor electrodes were also investigated by storing the sensors and studying the response intermittently after 7, 15, and 45 days

F. Urine Glucose Analysis

The practical application of the biosensor with best AR (best performing biosensor) was examined in an artificial urine sample for its response towards glucose in the presence of other interfering species. Initially, the biosensor performance in urine at different pH ranging from6.0 to 8.0 was calibrated and optimized (pH 7.0). CV measurements of the biosensor carried out in different concentrations of glucose in artificial urine (pH 7.0) at a scan rate of 100 mV/s.

VII. CONCLUSION AND SUMMARY

In summary, we will synthesize ZnO NRs of different ARs directly on Si/Ag electrodes in solution and use for the fabrication of glucose biosensors. The biosensor with good AR will exhibit the best result in PBS, i.e. sensitivity, linear response, and response time. The performance properties of glucose biosensors using ZnO nanostructures need to be examined for the best performance. The biosensor also needs to show the promising application for the detection of glucose in artificial urine. Thus, such an approach would avert the requirement of drawing blood for analysis, often done on a routine basis for certain types of diabetic patients. In some diabetes patients, whose blood glucose concentration must be monitored various times per day in order to manage diet and therapy, such a non-invasive, painless and convenient approach would provide the additional benefits of eliminating the danger of infection resulting from multiple skin punctures. Furthermore the fabricated biosensor also needs to show for a promising application for selectively detecting glucose in urine, which is useful for detecting renal glycosuria. Additionally studies need to be conducted in regard to the surface areas, fabrication cost and performance of ZnO NRs that is suitable for commercial applications.

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