Biochip for minimally invasive blood glucose monitoring

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ABSTRACT

The most common techniques for blood glucose sensing are based on enzymatic glucose oxidation techniques because of being highly sensitive and specific to the glucose. But, the techniques need oxygen environment and the measurement accuracy is affected by the sufficiency of oxygen. In this paper, a new microsystem for minimally invasive monitoring of blood glucose is proposed. The system proposed here for monitoring of blood glucose is composed of integrated microneedle array and glucose sensors. The microneedle array is out-of-plane and employs a bi-mask technique to facilitate sharp tips, a cylindric body that has high strength and side ports. The biosensor is based on a new mediator electrochemical approach. This approach eliminates the need for oxygen and reduces the response time. This system can sample blood and analyze the sample and can be used in monitoring blood glucose of diabetes. The experiment results demonstrate the excellent response of the microsensor up to about 80 mM of glucose. The needles are small enough in size to prevent user discomfort. It is also suitability for use by children owning to no pain and requirement of a less blood sample. Similarly, this device can be used in monitoring of performance enhances in athletes. In addition, the miccrosystem can be extended to other applications through employing different reagents specific for a particular analyte such as test for toxic exposure, identification of disease conditions.

Keywords: monitoring of blood glucose, microneedles, biosensor, BioMEMS

Introduction

Over the past decade or so, progresses in the fabrication of Micro-Electro-Mechanical Systems (MEMS) based on the fabrication techniques developed for integrated microelectronics circuitry offer exciting opportunities to advance the field of medicine, particularly in the area of analysis and therapy. A technology generally referred to as bio-MEMS, and is one of the most promising fields in the development of new MEMS devices [1][2]. In particular, biological microsystems using MEMS and microfluidic technologies, such as metabolic monitors, glucose level determination for diabetics and continuous delivery of insulin to a diabetic patient, are among the more prominent. A large amount of research has been applied to the development of new diagnostic appliance. Among them, the monitoring of blood glucose has been paid more attention. The development of sensors for glucose monitoring has been the focus of much research recently.

Diabetes mellitus is a mainly cause of death and disability in the world. The World Health Organization expects the number of people with diabetes worldwide to reach 300 million by 2025 [3]. Diabetes mellitus is a chronic disease characterized by varying or persistent hyperglycemia. The body cannot maintain blood glucose levels. Normal levels of blood glucose range between 3-8 mmol/L, for diabetics the range is 2-20 mmol/L [4]. Beyond this area can result in long term complications such as blindness, kidney disease and circulatory problems. Frequent and accurate glucose monitoring is needed if the disease is to be effectively brought under control.

Some techniques are used to sense glucose such as electrochemical, optical, piezoelectric, thermoelectric and acoustic techniques [5]. Among them, the electrochemical technique is no doubt the most reliable and clinically usable [5][6]. A electrochemical biosensor is based on the use of a biological molecular recognition element such as glucose oxidase (GOx) which is an enzyme specific for glucose that catalyzes the oxidation of glucose to gluconic acid [7]. Electrochemical glucose biosensors have been developing three generations, based on hydrogen peroxide electrode (H2O2-based) or oxygen electrode-based (O2-based), mediator instead of oxygen to transfer electrons from the reduced enzyme to the electrode and direct electron transfer between the enzyme and an electrode with special properties [5].

Those techniques above can be classified as invasive and noninvasive methods. For instance, implanted glucose sensor [8-10] is invasive, painful and inconvenient. And the response of implantable glucose sensors may produce a notable change when exposed to a biological environment because of either materials of sensors themselves or tissue embracing it or both [11][12]. In addition, the biocompatibility of the implantable glucose sensor is also especially considered.

Conventional self-testing finger-prick method is also invasive and requires the blood sample of several microliters which is then transferred to the test strip or cartridge for each glucose measurement, a painful and inconvenient procedure with poor patient compliance. The less painful armsticks for glucose test have been also employed. But, the microvasculature of the arm may not be sufficient to produce microliter-sized blood samples reliably, even though a conventional lancet is used [13].

Since the invasive methods are painful and inconvenient with poor patient compliance, noninvasive method was also proposed using Optical techniques [14][15]. Optical techniques to monitor glucose are truly noninvasive. The optical techniques, such as absorption spectroscopy [16], polarimetry [17], Raman spectroscopy [18], being investigated for monitoring glucose. The advantages of this method are obviously, painlessness and no tissue trauma. However, natural variations in skin pigmentation, erythema,

water content (edema), high scattering coefficients and skin layer thickness have limited its precise and application.

To facilitate the patient compliance for daily monitoring of blood glucose, a high accurate, painless and minimally invasive glucose monitor for the detection of blood glucose for diabetes mellitus patients would be highly beneficial Because both invasive and noninvasive methods have their limitation. Minimally invasive monitoring technologies have received the most attention due to the large potential market.

The structure and physiology of the skin make the technical realization of transdermal glucose monitoring a difficult challenge. Some techniques are being used for glucose monitoring, such as minimally invasive harvesting of interstitial fluid (ISF) [19], transdermal extraction, fluorescence-based sensors, which are generally coupled to a fluid collection technique [20].

The techniques of Micro-Electro-Mechanical Systems (MEMS) offer tremendous possibilities to minimally invasive methods. The blood sampling with painless and minimal tissue trauma was achieved when emergence of MEMS-based microneedles and microneedle array [21][22].

Unlike most of the noninvasive optical approaches, the oxidase-based techniques for glucose sensing have the advantage of being highly specific to the glucose because the approaches have to be in contact with the sample. The approaches to monitor glucose level through monitoring interstitial fluid or blood had been explored [23] [24]. But the former must be a high sensitivity since interstitial fluid has a glucose concentration range that is orders of magnitude smaller than that of blood glucose. Moreover, local changes in the blood flow or metabolic rate affect glucose levels in the ISF, such that changes in glucose levels here are not always consistent what is happening in blood [25].

The approaches employing glucose oxidize-based sensors possess highly sensitive and specific to the analyte of interest and eliminate many of the potential interferences common with optical techniques. But at the same time, the exogenous chemicals have to be introduced to the body or sample. In addition, these chemicals may be susceptible to degradation over time via consumption, photo-bleaching or denaturation in long-term use.

Recently, Dr. Birss' group has developed a new mediator-based electrochemical macro glucose sensor, which eliminates the need for oxygen and reduces the response time and potentically should be very stable in-vivo owing to no formation of hydrogen peroxide in the enzymatic reaction [26][27]. The material used to immobilize the enzyme is composed of iridium and iridium oxide. The biosensor by Birss' group needs a bigger specific area to electrodes than the one for micro sensor. The applicability of this technique in micron scale needs further research.

The development of glucose sensors based on enzymatic glucose oxidation has been made [28]. Most of them are focused on electrode fabrication, but blood sample part is rarely developed and integrated.

In this paper, we propose a minimally invasive integrated microsystem for monitoring of blood glucose comprised integrated microneedle array and glucose sensors. The glucose sensor is based on a new mediator-based electrochemical approach[27].

Microsystem for monitoring of blood glucose

In order to determine appropriate dimensions and structures of microneedles, an understanding of skin anatomy is required. The skin is composed of three primary layers, stratum corneum, viable epidermis and dermis. The dermis is in close contact with blood vessels and nerves. The viable epiderm is approximately 50-100 μm thick, is composed of living cells but void of blood vessels and contains few nerves. The stratum corneum, the outermost layer of skin, is 10-15 μm thick. It is primarily made of dead tissue and provides the primary barrier to penetration or transport. This signifies that a microneedle which can penetrate the stratum corneum (10-15μm) but is shorter than 50-100μm, can provide pathways for drug delivery without pain, and the needles should reach the dermis for blood samples.

The small dimensions of microneedles therefore can provide painless minimal invasion, and reduce damage to the skin. The opportunity of infection is also considerably decreased.

Because of the small dimensions of microneedles, the fluid flow is quite small, but high needle density can compensate and increase the fluid flow rate which is a current issue with microneedles. Therefore, the issue about fluid flow rate of microneedles has to be considered. It is difficult for in-plane needle designs to obtain high fluid flow rate, but for out-of-plane needles, this issue about fluid flow rate can be compensated and solved through high needle density. The blockage problem must be taken into consideration when designing microneedle structures. For example, blockages are likely to occur when the inlet or outlet is on the top of the needle. In this paper we present a Microneedle array structure that attempts to address these issues.

The microsystem for monitoring of glucose by electrochemical method includes Microneedle array and a glucose sensor composed of a reaction chamber and enzyme metal microelectrodes. Fig.1 shows the schematic drawing of the chip.

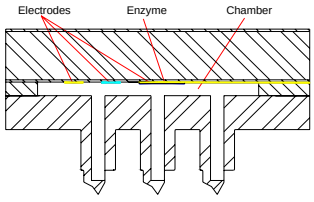

Figure 1 The Structure of chip for monitoring of glucose

Fabrication

The process flow of microsystem for monitoring of blood glucose is shown in Fig. 2. The tip and body of the needle

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are formed by employing a bi-mask technique. The process can be referred to [28]. A silicon wafer is thermally oxided following by the metal electrodes are evaporated and etched (Fig. 2ak). The deposition of SiO_2 and pattern to open electrodes are carried out (Fig. 2bl). The formation of microelectrodes is performed (Fig. 2cm). Finally, the needle array and biosensor are bonded using a fast curing expozy (Fig. 2dn).

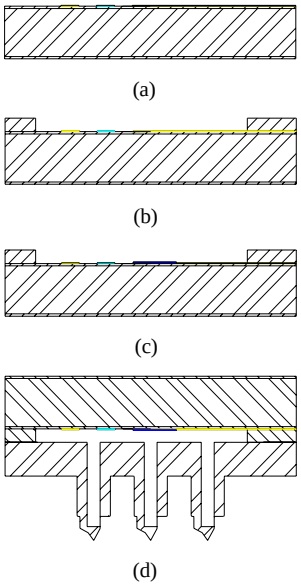


Figure 2: Process flow of the microsystem

The biosensor is not reliable without a reliable stabile enzyme membrane. As a result, the enzyme has to be immobilized reliably in a membrane. The characteristics of this membrane are very important for the function of biosensor. There are several methods for enzyme immobilization [29]. The sol-gel method is one of the approaches used for entrapping biological species in a gel matrix. Because it has some good properties such as excellent thermal stability, chemical inertness, resistance to photochemical and electrochemical degradation, excellent mechanical properties to keep its physical integrity during application in solvent, and prevention of enzyme leaching due to effective caging [30], the sol-gel method is used in this paper for enzyme immobilization.

Experiment and discussion

A cyclic voltammetry experiment is usually the initial experiment to investigate an electrochemical system because it can provide a great deal of experiment information and insight into both the kinetic and the thermodynamic details of an electrochemical system [31].

The validation of microworking electrode need to be verified because the glucose sensor proposed by Birss group is macro sensor with macro electrodes. The evaluation of performance of microworking electrodes by comparing the cyclic voltammetry of microworking and macroworking electrodes. Fig 5 shows very good agreement in the functionalities of the two electrodes.

SiO_2 and pattern to open electrodes, the reference electrode is chloridized and the enzyme is immobilized to the working electrode. A cyclic voltammetry process is required to convert the iridium nanoparticles to the iridium oxide nanoparticles after coating the working electrode with the enzymatic mixture. The micosensor of microsystem is shown in fig. 3 and Fig. 4 shows the structure of the micromachined needle array of microsystem.

The formation of electrodes of micro biosensor is as followed: The working and count metal electrodes are formed by a lift-off process after electron beam evaporation of Ti/Ni/Au. The reference electrode (Ti/Ni/Ag) is also constructed in the similar process. After the deposition of

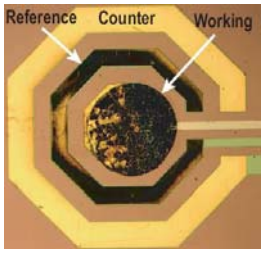
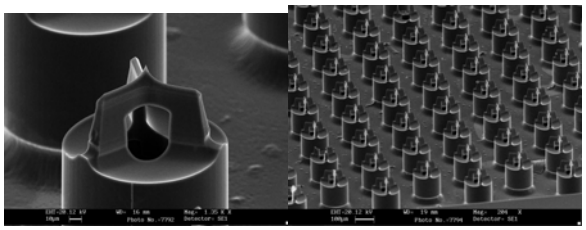
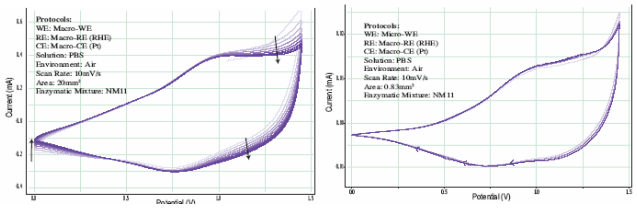


Figure 3 The fabricated glucose sensors of microsystem



(a) a single microneedle (b) microneedle array
Figure 4 the microneedle array of microsystem

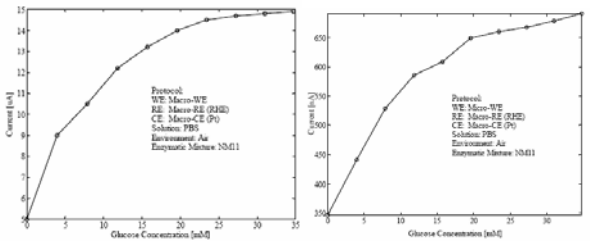


(a) CV of a macro-WE (b) CV of micro WE.
Fig. 5 Cyclic voltammogram of working electrodes

Fig 6 illustrates results of amperometric experiments using macro-working and micro-working electrodes with the same enzymatic mixture (NM11). From the figures, the measured currents are very different because the areas of the micro- and macro-working electrodes are much different. But, the curve patterns are very similar.

Performance of microsensor was measured by an amperometric glucose test using the commercial potentiostat. Fig. 7 illustrates the results of the experiment, demonstrating

the excellent response of the microsensor up to about 80 mM of glucose[32].



(a) a macro-working electrode (b) a micro-working electrode

Fig. 6 the calibration plot of output current versus glucose concentration

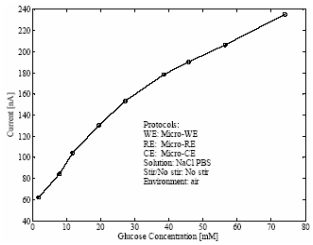


Fig. 7 A calibration curve of the output current of a microsensor versus glucose concentration.

The microsystem proposed here is an integrated system of microneedle array and the glucose microsensor. The microneedle array employs a bi-mask technique to facilitate sharp tips, a cylindric body that possesses high strength and side ports which minimize the potential for clogging. The microneedle is out-of-plane structure, having high needle density. Moreover, it has a low flow resistance owing to the cylindrical channel and side ports. The microneedle has an extremely sharp tip and the knife-like edges allow easy penetration of the skin. The biosensor is a unique miniaturized glucose sensor which not only does not require oxygen for reaction with glucose, but does not produce hydrogen peroxide in an environment with low oxygen concentration environment. In addition, the biosensor has a high sensitivity for detecting glucose levels in a wide linear range, up to about 80 mM of glucose. The microsystem can be used in monitoring blood glucose of diabetes. It is also especially suitability for use by children since it requires a less blood sample and the microneedle is small enough in size to prevent user discomfort. The microsustem employs a one-step process. That is, processes on sampling and analysis are performed in a device, thus minimizing blood required and possible mess and avoiding the demands to transfer blood from a skin puncture to a test strip, unlike finger strip method. Similarly, this device can be also used in monitoring of performance enhances in athletes. The athletes can regulate physical training and food consumption appropriately according to the measurement of indicators through monitoring glucose levels in the blood. Apparently, through the reagents immobilized in the micropool are changed specific for a particular analyte, the miccrosystem can be extended to the other application, such as testing for toxic exposure, monitoring of therapeutic drug levels, and identification of disease conditions.

Conclusions

A miniaturized system comprised of microsampling and assay components is implemented. This novel minimally invasive system for painless blood monitoring incorporates microneedle array capable of reliably taking a very small sample of whole blood painlessly and a unique miniaturized glucose sensor which has a high sensitivity for detecting glucose levels in a wide linear range.

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