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A miniaturized glucose biosensor for *in vitro* and *in vivo* studies

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***Abstract* — A miniaturized wireless glucose biosensor has been developed to perform *in vitro* and *in vivo* studies. It consists of an external control subsystem and an implant sensing subsystem. The implant subsystem consists of a micro-processor, which coordinates circuitries of radio frequency, power regulator, command demodulator, glucose sensing trigger and signal read-out. Except for a set of sensing electrodes, the micro-processor, the circuitries and a receiving coil were hermetically sealed with polydimethylsiloxane. The electrode set is a substrate of silicon oxide coated with platinum, which includes a working electrode and a reference electrode. Glucose oxidase was immobilized on the surface of the working electrode. The implant subsystem bi-directionally communicates with the external subsystem via radio frequency technologies. The external subsystem wirelessly supplies electricity to power the implant, issues commands to the implant to perform tasks, receives the glucose responses detected by the electrode, and relays the response signals to a computer through a RS-232 connection. Studies of *in vitro* and *in vivo* were performed to evaluate the biosensor. The linear response of the biosensor is up to 15 mM of glucose *in vitro*. The results of *in vivo* study show significant glucose variations measured from the interstitial tissue fluid of a diabetes rat in fasting and non-fasting periods.**

I. Introduction

DIABETES is a chronic metabolic disease. Patients with this disease have less control of blood glucose levels, which may result in many complications such as heart diseases, blindness, many kidney diseases; erectile dysfunction etc. Continuously monitoring blood glucose level and controlling it in the normal level is an effective way to avoid the complications. Implantable glucose biosensors are believed to be a useful tool for performing such a monitoring task. However, in developing the biosensors, several major technical issues remain to be coped with. Examples of these issues are device miniaturization, power and data wireless transmission, bio-compatible hermetic packaging, electrode fabrication, enzyme immobilization etc. Any of these involves intense study dedication, not to mention the fact that considerable works are associated with the development of the entire biosensor. Even so, many researchers have been devoting themselves to such a challenge because it is believed that implantable glucose biosensors can be significantly beneficial to diabetes patients.

Some glucose biosensor *in vivo* studies have been reported by Shults et al.[1], Atanasov et al. [2], and Beach et al.[3, 4]. The reported glucose biosensors were enzymatic (glucose oxidase based). Though the biosensors were subcutaneously implanted on dogs, the physical sizes were considered large. Among them, the most compact one was measured about 51 x 22 x 1 mm in dimension. All functionality of the biosensors was powered by battery cells. The glucose signals detected by the sensors were transmitted to an external unit by radio frequency (RF) telemetry at the frequency of 303.825 MHz.

Though most of the recent attention has been given to the development of subcutaneously implantable needle-type electrodes with wires connecting external systems [5, 6], wireless transmission of electrical power and data is still a desirable approach for implantable applications. RF wireless technologies based on class-E amplifiers can transmit electrical power and bi-directionally communicate signals through antenna coupling. The advantages of concurrently transmitting power and signal lead the technology become an essential technique in implantable device developments today. In 1992, Troyk et al. developed a close-loop class-E amplifier that successfully demonstrates data and power transmission for an implant in subcutaneous tissues [7]. Catrysse et al. reported an integrated circuit of the class-E amplifier for the applications not only in electric stimulation but also in sensing [8].

We have been interested in the development of miniaturized glucose biosensors in the past few years. The system architecture developed in-house is similar to that of the aforementioned biosensors. That is an external subsystem wirelessly linked with an implant subsystem. In the previous reports, we demonstrated that the power supply of the implant and the data communication between the two subsystems in air and in pork can be accomplished by a class-E based RF

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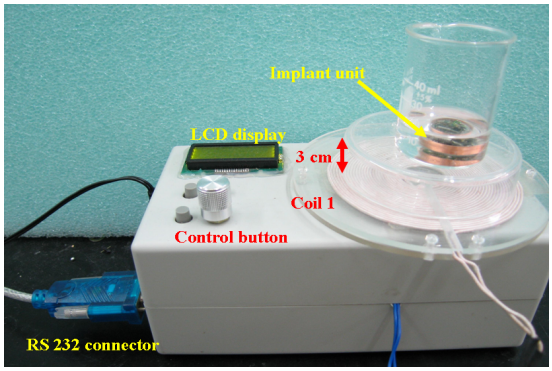

Fig. 1. The external subsystem equipped with an LCD display, control buttons, an RS-232 connection, and a coupling coil.

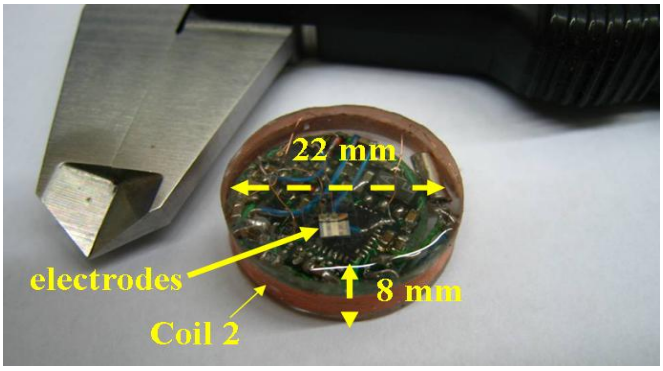

Fig. 2. The implant subsystem used to perform the *in vitro* and *in vivo* studies.

transmission module. In addition, the signal read-out functionality of the implant was evaluated by a set of commercial electrodes in a phosphate buffer solution (PBS) containing various concentrations of hydrogen peroxide. In this context, we will report an in-house developed electrode set integrated with the implant subsystem. Glucose oxidase (GOD) was immobilized on the surface of the working electrode. The glucose detection functionality of the electrode was first verified *in vitro*. Then the glucose response linearity of the biosensor was calculated from a calibration curve. Finally, the biosensor was subcutaneously implanted to an Alloxan induced diabetes rat. *In vivo* studies were performed to compare glucose levels in the interstitial tissue fluid between the fasting and non-fasting periods.

II. Materials and Methods

A. External subsystem

The external subsystem as shown in Fig. 1 is mainly composed of three parts. The first part is an interface module including an LCD display and a set of command buttons. The second part is a wireless RF transmission module with a carrier frequency at 800 kHz. Important to know is that this module consists of a modulation/demodulation circuitry. The interface module and the transmission module are coordinated by a microprocessor. The third part is a coil (coil 1) which serves as a communication coupling device. Specifically it transmits electrical power and modulated signals and receives detected glucose response signals. These detected signals are demodulated and converted to corresponding values which are then presented on the LCD and transferred to a computer through an RS-232 connection. In the computer, there is a software program equipped with an RS-232 interface module and a graphic user interface (GUI) module. The RS-232 interface module is responsible for receiving the data from the external subsystem. The GUI automatically presents the data as a function of glucose concentration.

B. Implant subsystem

The dimension (22 x 8 mm, D x H) of our implant subsystem is shown in Fig. 2. To assure being a functional counter part of the external subsystem, this implant unit was assembled with the similar circuitry modules as designed in the external. A coil (coil 2) couples over the RF signals from the coil 1. The coupled signal is processed concurrently. It is regulated to direct current electricity and decoded into command signals. The electricity supplies to all circuits in the implant and the signals command a microprocessor to perform coded tasks. In the current stage, the command is simply a value of operation potential to perform a designated detection such as 0.6 V of measuring glucoses. This is then issued to a digital to analog converter for forming such a potential. The measurement task is accomplished by an electrochemical amperometric circuitry and a set of in-house developed electrodes. The electrode set consists of a working electrode (dimension 0.05 × 0.7 mm) and a reference electrode (dimension 0.1 × 0.7 mm). Both are made of a substrate of silicon oxide coated with platinum and fabricated with micro-machining processes. Effort was made so that a glucose catalyst was immobilized onto the surface of the working electrode. In this work, a Nafion® membrane modified glucose oxidase electrode was fabricated to perform amperometry studies. From these studies, current signals, which theoretically should be proportional to the glucose concentrations being measured, are read out and then converted into digital form. The digital data are transferred to the external subsystem wirelessly via the coil 1 and a circuitry based on the technique of amplitude shift keying. Important to note is that all circuits of the implant including the coil 2 were hermetically enclosed by polydimethylsiloxane.

C. Functionality verification of glucose biosensor in vitro

Verifying the information flow that loops through the external subsystem and the implant subsystem is essential. The setup of this study was as follows. The GUI software program was launched for monitoring glucose concentration variation. The implant subsystem was placed in a beaker containing a PBS (0.1 mM, pH 7.0) so that the coils 1 and 2 were a distance of 3 cm apart as shown in Fig. 2. When the external subsystem was powered up, a pre-coded command modulated in the RF carrier was transmitted to the miniaturized implant. It then performed amperometry measurements accordingly by applying a potential of 0.6 V on the working electrode. The functionality verification included two tests: peroxide hydrogen and glucose. The peroxide hydrogen test was to verify the system functionality of

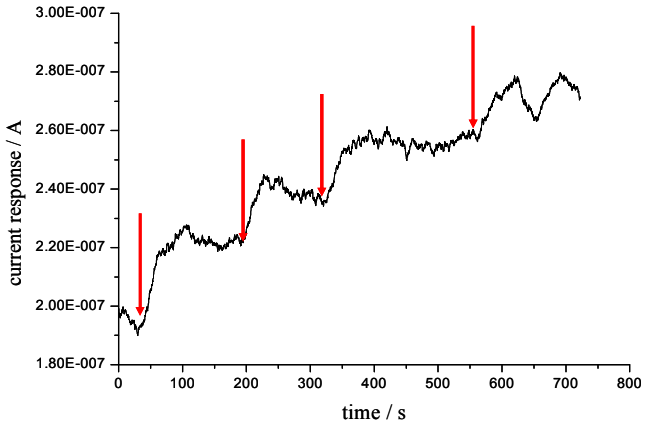

Fig. 3. Current responses of glucose. The arrows indicate the injections of 5 mM glucose to the PBS. The operation potential was 0.6 V.

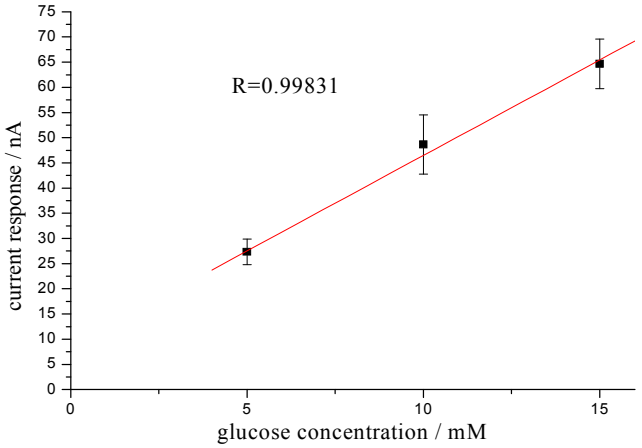

Fig. 4. Calibration curve of the glucose biosensor. The linear range is between 0 to 15 mM, and R = 0.998.

the biosensor. The GOD immobilized electrode was confirmed by the glucose test. When neither glucose nor peroxide hydrogen presented in the PBS, the current response curve should be flat. This is referred to the response baseline in this context. On the other hand, the GUI on the computer monitor would present current signals in response to the injections of glucose and peroxide hydrogen.

D. Linearity study

The experimental setup of the linearity study was as described above. The implant subsystem was immersed in a fresh PBS. It then performed the detection task upon the power-up of the external subsystem. Because glucose was absent in the PBS, the current response curve on the GUI eventually reached to a baseline. Since then glucose injections followed. Totally 20 mM of glucose was injected to the PBS with each 5 mM increase. These processes were repeated three times.

E. Fasting and non-fasting studies on a diabetes rat

The developed biosensor was also evaluated *in vivo*. This study aims on the comparison of glucose variations in interstitial tissue fluid on a diabetes induced Wistar rat between fasting and nonfasting. The rat was weighted about 450 grams and was induced by Alloxan, which is a chemical compound destructing insulin-secreting β cells through hydroxyl radicals. Usually the Alloxan effect takes place in about ten days. Our implant subsystem was subcutaneously implanted to this diabetes rat on the back near the right thigh. Special care was taken so that the electrode set incorporated with the implant was faced to the muscle tissues. The fasting and non-fasting studies performed at one week after the operation.

III. RESULTS AND DISCUSSIONS

A. Peroxide hydrogen and glucose responses in vitro

As stated above, the peroxide hydrogen test was to verify the system functionality of the biosensor. The setup was that the implant subsystem was in a PBS and the coils 1 and 2 were in a distance of 3 cm apart. A command of applying an operation voltage of 0.6 V was issued from the external subsystem to the implant subsystem via the wireless transmission module. When an injection of 0.002 mM hydrogen peroxide to the PBS, the baseline laddered up gradually indicating the signal loops through the entire system. The successive injections of peroxide hydrogen were up to 0.01 mM with 0.002 mM increase. A curve of current responses versus hydrogen peroxide concentrations was compiled. The linearity performance of the biosensor derived from the curve is R=0.999 in the glucose range between 0.002 and 0.01 mM.

In addition to the peroxide hydrogen test, we also performed the glucose tests to confirm the functionality of the GOD electrode. The study setup was identical to the peroxide hydrogen tests. Fig. 3 shows the current responses versus time, where the arrows indicate the injections of 5-mM glucose to the PBS. The first three injections of glucose laddered up the current responses. However, the response by the fourth injection was not identifiable from the diagram. This result indicates that the glucose linearity response of the biosensor is up to 15 mM with R=0.998 as shown in Fig. 4.

B. Duration glucose concentration curve studies

One of the advantages of an implantable glucose biosensor is that it can monitor blood sugar levels at various stages such as before and after exercises, nutrition or medicine uptakes etc. Thus, prior knowledge of duration glucose concentration curves is essential. To obtain such duration curves, we carried out amperometric measurements analogous to the GOD electrode verification study above, which the glucose of 0, 5, 10, and 15 mM were injected into a PBS. However, it is important to note that the duration curve study refreshed the PBS before each glucose concentration was measured. On the other hand, the electrode verification study applied glucose injections with 5 mM increment. More importantly, in the duration curve study, each current response tracing (or recording) lasted for 400 seconds at least. The results were tailored and compiled in Fig. 5, where four current response curves of 250-second recording in upward order represent for 0, 5, 10, and 15 mM of glucose,

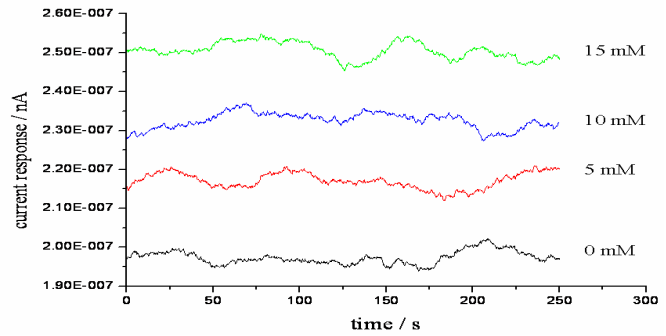

Fig. 5. Duration glucose concentration curves *in vitro* study. The curves were tailored from the four amperometric traces of glucose 0, 5, 10 and 15 mM.

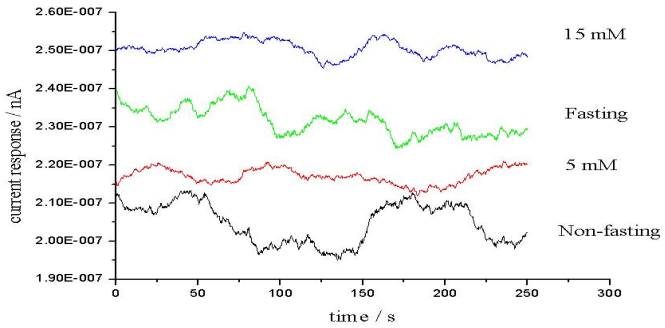

Fig. 6. *In vivo* studies. The fasting curve is at the level of 10 mM and the non-fasting curve was in the range between 0 and 5 mM.

respectively.

C. *Fasting and non-fasting glucose level studies*

It should be noted that in this study our implant subsystem was already inside the rat; and during the study the rat was free in a cage. For the fasting tracing, except for water the rat had not taken any food for 24 hours. On the other hand, the non-fasting tracing study was performed at 60 minutes later after feeding. The two glucose level tracing curves were tailored and compiled in Fig 6. For the comparison purpose, the response curves of 5 and 15 mM in Fig. 5 were duplicated and shown in Fig. 6. In this figure, a few remarks are as follows. The glucose response fluctuations were rather large for the *in vivo* studies. We believe this is likely due to the body movement of the rat. For a diabetes rat suffered starvation for 24 hours, its glucose concentration in interstitial tissue fluid was below 5 mM and can be lower down to the baseline, i.e. 0 mM. The rat's glucose concentration can be boosted up to the level of 10 mM after taking food. This is considered high because the glucose concentration in vessels can be much higher than that in interstitial tissue fluid.

IV. CONCLUSION

We have developed a miniature glucose biosensor that includes an external subsystem and an implant subsystem. An RF based wireless communication technology bridges the two subsystems not only in data transfer but also in power supply. The implant subsystem was incorporated with an in-house developed electrode set including a platinum working electrode and a platinum reference electrode. Glucose oxidase was immobilized on the surface of the working electrode. The glucose sensing functionality of the biosensor was confirmed through both peroxide hydrogen test and glucose test. The linearity performances of the biosensor in the both tests were rather satisfied. The sensing subsystem was implanted subcutaneously in an Alloxan induced diabetes rat for performing *in vivo* studies. In the non-fasting study, the rat's blood sugar level in the interstitial tissue fluid was between the range of 0 and 5 mM. After the rat was fed, the fasting glucose level curve recorded from the interstitial tissue fluid showed a significant increase to a level of 10 mM. Generally speaking, the glucose response fluctuations were large *in vivo* studies, which is likely due to the movement of the rat.

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