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Minimizing tissue–material interaction in microsensor for subcutaneous glucose monitoring

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Abstract

A new implantable electrocatalytic glucose sensor for subcutaneous glucose monitoring has been fabricated by immobilizing glucose oxidase on a chemically modified carbon fiber. The sensor was inserted subcutaneously on a male spraguely rat without any incision after dipping the microsensor in the rat's serum for 3 days. The so called "stained" microsensor, operated in the amperometric mode with an applied potential of +0.23 V versus Ag|AgCl, was able to directly measure the glucose concentration upon infusion of glucose. The results obtained were encouraging, with the response time was less than 2 s and the apparent Michaelis–Menten value at 5.1 ± 0.5 mM. The "stained" microsensor shows good stability and reproducibility with constant response spanned over 25 days. Most common interferences in glucose analysis were minimized by the outerlayer Nafion®. Hematology examinations showed minimal material–tissue interaction. Use of such mechanical devices will allow a more refined understanding towards glucose control in diabetic patients as the implanted microsensor was not effected by biocompatibility failures.

Keywords: Microsensor; Rat's serum; Subcutaneous; Hematology; Biocompatibility

1. Introduction

There are three different major groups of diabetes, i.e. gestational, non-insulin dependant (maturity-onset diabetes) and insulin dependant diabetes (juvenile-onset diabetes). In all, diabetes mellitus occurs upon absolute deficiency of insulin, which is secreted continuously by pancreatic beta cells. The secretion of insulin in response to glucose is rapid and could be triggered even by as low as 2 mM glucose (Velho et al., 1989). The secretion is stopped when insulin has brought the glucose level to its basal. This overall mechanism keeps the blood glucose level in check. Thus, the destruction of pancreatic beta cells could lead to severe insulin deficiency. Hyperglycemia is a classic symptom of diabetes mellitus but hyperglycemic condition without other classic symptoms is not dispositive of a diagnosis of diabetes mellitus. However, hyperglycemia is also an independent medical condition with other causes (WHO, 1980). Hyperglycaemia is due to uncontrolled hepatic glucose output and reduce uptake of glucose by skeletal muscle with reduce glycogen synthesis. As the renal threshold for glucose re-absorption is exceeded, glucose spills over into the urinary tract. Hence, for this very reason, it is important to monitor the glucose level in human blood.

One of the approaches to monitor the glucose concentration in human blood is by means of mechanical device, i.e. glucose sensor (Chen et al., 2002; Harrison et al., 1988). The availability of implantable glucose sensors, first introduced during the early 80 s, has improved the monitoring process (Shichiri et al., 1982). The measurement is possible because of a high transfer rate of glucose from blood vessels to the interstitial space which will result in a short lag time on changes of the blood glucose concentration and the subcutaneous tissue. It has also been reported (Jansson et al., 1988) that the subcutaneous glucose concentration is at the same level as blood glucose

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under certain conditions. Therefore, any glucose sensor for in vivo application would have to be rapid in its response. Several enzyme-based glucose sensors (Shichiri et al., 1982; Pickup et al., 1989; Ege, 1989; Dixon et al., 2002; Ward et al., 2002) have been applied for the in vivo or subcutaneous glucose monitoring with some degree of success. These sensors, upon implantation have relatively short "implantable life". The decrease in response over time is, probably, due to an inflammatory reaction which occurs at the electrode–solution interface. The material–tissue interaction upon sensor implantation is the major obstacles in developing a viable implantable sensor. Biocompability failures, i.e. fibrous encapsulation and membrane biodegradation limited the sensor's longevity and caused inflammatory skin reaction at the implantation site.

Previous work from this laboratory is mainly on fabricating microsensors for the detection of glucose (Ahmad and Ab Ghani, 2005). The mediated electrical communications between redox enzymes and the chemically modified microelectrode were shown to be possible (Ahmad and Ab Ghani, 2005). The current paper describes the fabrication of a glucose microsensor for subcutaneous monitoring. The fabricated microsensor was dipped in the rat's serum ("stained" microsensor) and its performance was compared with the microsensor without dipping in the rat's serum ("clean" microsensor). In addition, we also examine and compare the hematological aspect on each group of rats after the implantation for both types of microsensor.

2. Experimental

2.1. Materials

The 4-vinylpyridine (4VP) monomer (∼96%), mercury (Hg, RdH 10008) (99.99%) and tetramethylammonium chloride (TMAC), were from Fluka Chemika, Switzerland and used without further purification. Acetonitrile (MeCN) from Romil Chemicals, England; Araldite® epoxy resin from Ciba Geigy, Switzerland; Triton® X-100, i.e. polyethylene glycol *tert*-octylphenyl ether (PEG), 10% (w/v) and glucose oxidase (GOD) from *Asperigillus niger* (EC 1.1.3.4, catalogue no. 646 431, grade II lyophilised, 100,000 i.u.), obtained from Boehringer Mannheim GmbH, Germany were used as received. Bovine serum albumin (Fraction V, 98–99% albumin), ethyl carbamate, bovine serum, β-D-glucose, glutaraldehyde and mineral oil were obtained from Sigma Chemicals, USA and were used as received. Oxygen-free nitrogen (OFN) was obtained from Nissan-IOI, Malaysia. A stock solution of Nafion® (0.5%, w/v) was prepared in methanol. Nafion® (5%, w/v) (equivalent mass, 1100 g) in a mixture of low molecular weight alcohols and 10% water was obtained from Aldrich (Milwaukee, WI, USA). The composite graphite powder from pencil lead 2B, Mars Lumograph 100, was from Staedtler, Germany. All other reagents were of the highest grade available and were used as received.

Bovine serum was first diluted to 80% in phosphate-buffered saline (PBS) solution at pH 7.4 prior use. The stock solution of β-D-glucose was also prepared in PBS and allowed to mutarotate and left overnight at 4 °C before use. This was to establish equilibrium concentrations between α and β anomers. The glucose concentrations were reported as total glucose concentrations. The phosphate-buffered saline (PBS) solution was prepared, daily, by dissolving 2.754 g of NaCl, 2.081 g of KH_2PO_4 and 0.477 g of NaOH in 1000 mL of distilled water and then pH adjusted to 7.4 with 0.1 M NaOH. Pure water from a Milli-Q Plus of Millipore Corp., USA was used for preparation of all aqueous solutions.

2.2. Instrumentation

Most electrochemical measurements and analysis were done using either the Potentiostat/Galvanostat (Model 273A, Princeton Applied Research, USA) controlled by an IBM PS/2 model 480 PC or Autoranging Microvolt DMM (Model 197A, Keithley Ins., Cleveland, OH, USA). The pH measurement was obtained using benchtop pH/ISE meter (model 720A Orion, Boston, USA). Teflon® coated antimagnetic stainless steel tweezers style #4 (110 mm) and style #3 (120 mm) of Sigma Chemicals, St. Louis, USA, were used in the preparation of microelectrode. Magnification of microelectrode was seen under a stereomicroscope (model SMZ-2T, Nikon, Japan) and photos were taken with an attached camera (model F-601, Nikon, Japan). The micropippete puller (PB-7) and micropippete grinder (EG-4) was from Narishige, Japan. The rat's blood glucose level was also determined using glucometer (Accu-Chek® Advantage II, with test strips and complete meter, Roche Diagnostics, GmbH, Mannheim, Germany).

2.3. Microsensors fabrication

The carbon fiber (8 μm in diameter, 6.4 mm long,) was from Lot # 20590, Johnson Matthey Electronics (MA, USA) was inserted into the glass capillary (1× 90 mm GD-1, Narishige, Japan) using tweezers and was then pulled by a micropipette puller (PB-7, Narishige, Japan) with maximum weight. The protruding carbon fiber was trimmed so that the remaining of the fiber was inside the glass capillary. The microelectrodes were then polished at an angle of 45° on the micropipette grinder (EG-4, Narishige, Japan) on an extra-fine diamond abrasive plate to produce elliptical carbon disk. Electrical contact was established by filling the capillary with mercury (0.5 mL) and then with composite graphite paste. Preparation of the composite graphite paste was mentioned elsewhere (Ahmad and Ab Ghani, 2005; Zahir and Ab Ghani, 1997; Halim and Ab Ghani, 2000). A copper wire was then inserted into the paste. The upper part of the capillary was finally sealed with Araldite® epoxy resin. The microelectrode was left sitting at room temperature overnight and was then cured at 70 °C for 2 days. It was then washed with acetone, later rinsed with pure water, and finally dried in a cold air stream prior to polymer deposition.

The preparation of P4VP from 4VP was described elsewhere (Ahmad and Ab Ghani, 2005; Zahir and Ab Ghani, 1997). The GOD was then immobilized onto the polymer modified microelectrodes by immersing the microelectrode in 2.0 mL of PBS solution containing 200 i.u mL^{-1} GOD and glutaraldehyde for a period of 30 min. The dropwise addition of 10 μL of stock Nafion® solution was applied onto the GOD/P4VP elec-