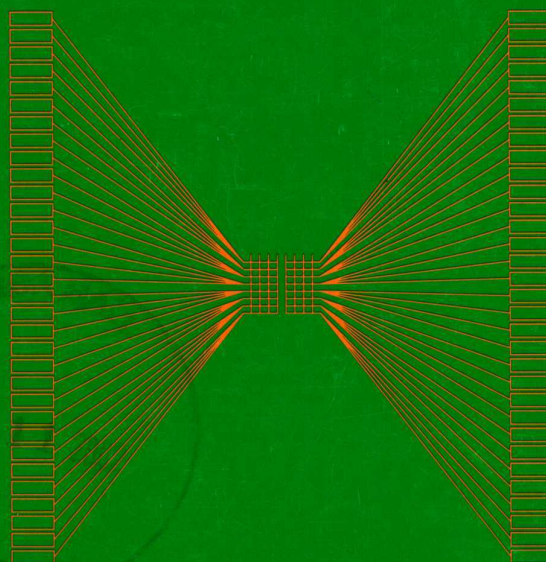


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Publication information: *Biosensors & Bioelectronics* (ISSN 0956-5663). For 2002, volume 17, issues 1-12 are scheduled for publication. Subscription prices are available upon request from the Publisher or from the Regional Sales Office nearest you or from this journal's website (<http://www.elsevier.nl/locate/bios>). Further information is available on this journal and other Elsevier Science products through Elsevier's website: (<http://www.elsevier.nl>). Subscriptions are accepted on a prepaid basis only and are entered on a calendar year basis. Issues are sent by standard mail (surface within Europe, air delivery outside Europe). Priority rates are available upon request. Claims for missing issues should be made within six months of the date of dispatch.

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Periodicals postage is paid at Rahway, NJ. *Biosensors & Bioelectronics* (ISSN 0956-5663) is published 12 times per year from January to December by Elsevier Science Ltd., The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, UK. The US subscription price is \$1113.00 per year.

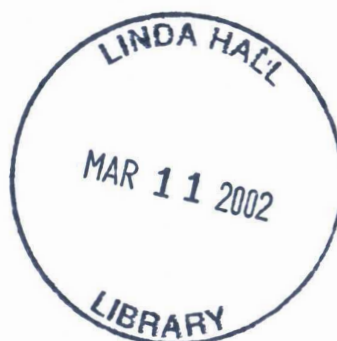
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Printed in Great Britain by Page Bros, Norwich, England



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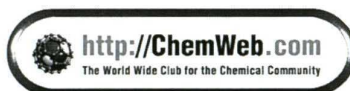
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A new amperometric glucose microsensor: in vitro and short-term in vivo evaluation

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Received 19 February 2001; received in revised form 1 August 2001; accepted 6 August 2001

Abstract

For biosensor fabrication, it is important to optimize materials and methods in order to create predictable function in vitro and in vivo. For this reason, we designed a new glucose sensor ('revised protocol') that utilized an outer permselective membrane made of amphiphobic polyurethane which allows glucose passage through hydrophilic segments. An inner polyethersulfone membrane, stabilized with a trimethoxysilane, provided specificity. Before application of the inner membrane, it was necessary to etch the platinum electrode with a radio frequency oxygen plasma. The revised protocol sensors ($n = 185$) were compared with sensors fabricated with an earlier ('original') protocol ($n = 204$) which used an outer polyurethane without hydrophilic segments and a complex inner membrane of cellulose acetate and Nafion. The function of revised protocol sensors was more predictable in vitro as evidenced by a much lower variation of glucose sensitivity than the original protocol sensors. Revised and original protocol sensors were nearly linear up to a glucose concentration of 20 mM. In vitro interference from 0.1 mM acetaminophen was minimal in both groups of sensors and would be expected to represent about 2% of the total sensor response at normal glucose levels for revised protocol sensors. Prolonged testing of the revised protocol sensors for 11 days during immersion in buffer revealed stable sensitivities (day 1: 6.12 ± 1.34 nA/mM; day 3: 6.33 ± 1.40 ; day 8: 7.13 ± 1.39 ; and day 11: 7.56 ± 1.47 ; sensitivity for day 1 vs. each other day: not significant) and no critical loss of glucose oxidase activity. The response of the revised protocol sensors ($n = 7$) to intraperitoneal glucose was tested in rats approximately one day after subcutaneous implantation and the sensors tracked glucose closely with a slight lag of 3–6 min. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Diabetes mellitus; Glucose sensor; Subcutaneous; Polyurethane; Acetaminophen; Implantable

1. Introduction

A number of studies have demonstrated that in persons with diabetes, the risk for renal, retinal and neural complications is directly related to the magnitude of chronic elevations of blood glucose (DCCT/EDIC Research Group, 2000; Diabetes Control and Complications Trial Research Group, 1993; Klein et al., 1989; Shichiri et al., 2000; Turner, 1998; UK Prospective Diabetes Study Group, 1998). In addition, cost-benefit

projections from these studies performed in multiple countries suggest that intensive glycemic care is cost-effective (Herman et al., 1997; Herman and Eastman, 1998). A promising method of improving glycemic control is use of a minimally-invasive implantable glucose microsensor which would provide to the user continuous glucose data and early warning for extremes of glycemia.

However, several obstacles have precluded widespread use of an implantable real-time sensor. One important issue encountered during the fabrication of sensors is the need for optimization of materials and methods in order to obtain predictable in vitro and in vivo function. Predictable and reliable function in vivo

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is almost certainly contingent on consistent fabrication techniques. Although we have studied in detail implantable glucose sensors with an outer polyurethane coat in rats (Thome-Duret et al., 1998, 1996), in dogs (Poitout et al., 1992) and in humans (Moatti-Sirat et al., 1994; Poitout et al., 1993), we and others have experienced suboptimal fabrication yield. For this reason, we now address a different method to regulate entry of analytes, the use of a polymer with intrinsic permeability to glucose and oxygen, applied with a technique that leads to a dense, pore-free membrane. We hypothesized that use of such a membrane as the outer sensor coat will lead to a higher yield and more predictable functional characteristics. In this report, we present *in vitro* data in which different permselective membranes are compared and short term *in vivo* experiments in which glucose is administered.

2. Materials and methods

2.1. Sensor fabrication and testing

With regard to sensor dimensions, a 178 μm diameter platinum wire, jacketed in polytetra-fluoroethylene, served as the indicating electrode. A 50 μm diameter silver reference electrode wire (which was later chloridized electrolytically) was coiled around the platinum wire except for a 2–8 mm long measurement cavity toward the distal extremity of the sensor where the PTFE jacket had been removed (see diagram in Fig. 1). The diameter of the sensor was 250 μm distal to the

reference coil and 350 μm at the level of the reference coil. The length of the sensor indwelled under the skin of animals was 20–25 mm.

For all sensors, three membranes were deposited in the measurement cavity around the circumference of the electrode. From innermost to outermost membrane, the functions are to prevent passage of interfering compounds, to convert glucose to hydrogen peroxide, and to regulate influx of glucose and oxygen.

Two hundred and four sensors were fabricated using methods reported previously (Bindra et al., 1991) and these sensors are termed 'original protocol sensors'. The inner membrane for these sensors, applied directly to the platinum electrode, consisted of a complex, 11 layer system of cellulose acetate and Nafion, as described earlier (Bindra et al., 1991; Moatti-Sirat et al., 1994). For 185 sensors fabricated with a simpler 'revised protocol', the inner membrane consisted of three layers of 10% (w/w) sulfonated polyethersulfone (PES, Amoco Polymers, Alpharetta, Georgia 30202), dissolved in dimethylacetamide (DMAC). The PES polymer was similar to that used by Churchouse and Vadgama (Churchouse et al., 1986).

In the process of investigating inner membrane methodology, we also addressed the issue of surface preparation of the platinum electrode before application of the PES inner membrane. For this pilot study, we used sensors with the platinum indicating electrode and Ag/AgCl reference electrode but without the enzyme or outer polyurethane layers. Two methods of platinum surface preparation (prior to coating with PES) were compared. Some sensors were treated only

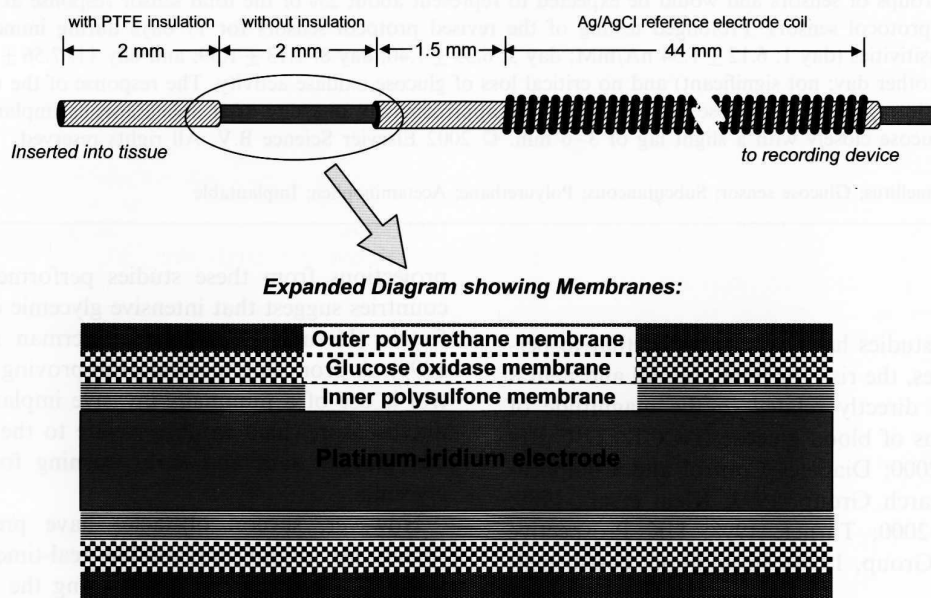


Fig. 1. A drawing of the implantable glucose sensor, showing linear sensor dimensions (diameter dimensions are discussed in text) and membrane system.

with a chemical cleaning solution (70% ethanol). In addition to the ethanol application, others received a radio frequency oxygen plasma etch at a vacuum of 0.8 Torr for 2 min at a frequency of 13.5 MHz and a power setting of 125 W, using a Plasmod RF plasma chamber (March Instruments, Concord, CA, 94520). Sensors were then tested in acetaminophen, 0.1 mM and in freshly-prepared hydrogen peroxide, 25 μ M. It has been our prior experience that acetaminophen is the most difficult interferant to block (Moatti-Sirat et al., 1994) and so this compound was used exclusively for interference testing in all studies reported here.

Sensors whose electrode surface was treated only with ethanol yielded a much larger response to acetaminophen than those that underwent plasma treatment (6.98 ± 2.47 ($n = 9$) versus 0.78 ± 0.48 nA ($n = 15$), $P < 0.02$). The treatments yielded similar responses to H_2O_2 of 10.77 ± 2.34 nA versus 10.92 ± 1.01 (P not significant). Our interpretation of these findings was that, in order to avoid interference from acetaminophen, it was necessary for the platinum surface to have a fresh surface etch so that the PES would adhere well and form a continuous, defect-free jacket. In view of these results, the revised protocol for fabricating sensors included an oxygen plasma etch, as described, immediately before inner membrane application.

Another problem that became apparent during the development of the inner membrane procedure was attack (partial dissolution) of the inner membrane material, PES, by the DMAC in which the outer polyurethane was dissolved. For this reason, it was necessary to add a layer of silane in order to stabilize the PES layer and prevent its attack by DMAC. For this purpose, we used *N*-2 aminoethyl, 3-aminopropyltrimethoxy silane (AATS, Aldrich). The AATS solution was made as 20% AATS (v/v), 72% ethanol (w/w) and 8% deionized water. The AATS was applied between the second and third dip coats of PES. Separate experiments (data not presented) verified that sensors with the AATS layer led to substantially less interference by acetaminophen than those without AATS.

Each sensor was cured for 2 h at 40 °C after each coat of the PES and oven-baked for 30 min at 150 °C after the dip coat of AATS. For all sensors, glucose oxidase mixed with bovine serum albumin and glutaraldehyde served as the intermediate layer as previously described (Bindra et al., 1991).

For all sensors, the polyurethane outer membrane material was applied by manual loop coating (meniscus-coating) over the measurement cavity and over the coiled reference electrode. The 204 original protocol sensors were coated with Tecoflex polyurethane (concentration 4.5% w/w, Thermedics Inc, Woburn, MA) as previously described (Bindra et al., 1991). The solvent for this polyurethane was tetrahydrofuran. For the revised protocol, 185 sensors were coated with a differ-

ent polyurethane, an amphiphobic polymer which had also been used as a glucose-and oxygen-permeable immunoisolation membrane for the purpose of enclosing transplanted cells (Polymer Technology Group, Berkeley, CA. Ward et al., 1993; Ward and White, 1991, 1995). The requirements for islet cell enclosure polymers and for implantable glucose sensors are similar to the extent that both must allow permeation of glucose and oxygen. Earlier studies also showed that glucose permeability can be predicted by the degree of water uptake by the polymer (Ward et al., 1993). In the present study, the amphiphobic polyurethane was prepared as an 8% w/w solution dissolved in DMAC, a low vapor pressure solvent. This polyurethane is not a hydrogel and has a water uptake of less than 2% at 24 h. In addition to testing in glucose standards, sensors were also tested in acetaminophen that was injected into the stirred final glucose test solution (20 mM), yielding an acetaminophen concentration of 0.1 mM.

Before glucose was added to the solutions, sensors were observed during a run-in period for at least 40 min to reduce the background current to very low levels. During this period, sensors were polarized at the usual potential (0.65 V) in phosphate-buffered saline (PBS).

Linearity of the sensor response to glucose in vitro was tested in two ways: by linear regression analysis (correlation coefficient, r ; and coefficient of determination, r^2) and by an analysis of residuals at each glucose standard (difference between the sensor value predicted for each standard level (assuming strict linearity) and the actual sensor reading) (Analytical Methods Committee, 1994).

Original and revised protocol sensors were also compared with regard to the issue of repeatability of fabrication. For this analysis, the variance of the in vitro sensitivities for each group were compared. In order to adjust variances for differing means, each value was expressed as a residual: (individual sensitivity value – mean sensitivity)/mean sensitivity. The ratio of variances of the residuals (F test, original vs. revised) was then compared.

In addition, a study was undertaken with the revised protocol sensors to address the issue of in vitro stability over time. The sensors, destined to be implanted for 3–7 days in humans, were studied in ascending glucose standard curves periodically for 11 days ($n = 8$ sensors). During this time, they were continuously polarized at 0.65 V and immersed in phosphate-buffered saline that was thermostatically controlled at 37 °C.

2.2. In vivo studies with revised protocol sensors

Using revised protocol sensors, studies were also performed in order to assess their ability to monitor rising and falling glucose in vivo. The study was carried out in an AAALAC-approved veterinary research facil-

ity, Legacy Clinical Research and Technology Center in Portland, OR. The study was approved by the Legacy Institutional Animal Care and Use Committee and by the Legacy staff veterinarian.

After sterilization in ethylene oxide at 38 °C, sensors were implanted in the dorsal subcutaneous region of Sprague–Dawley rats (weight 300–450 g) under general anesthesia with 2% isoflurane. The sensors were inserted while indwelled in a 21 gauge steel trocar. The trocar was then removed, leaving the sensor in place. The sensor was secured with cyanoacrylate glue (Vet-bond, World Precision Instruments, Inc Sarasota, Florida 34240) applied to the shaved skin and the animal was fitted with a light harness to protect the sensor and secure the tether. Recording wires were routed through a swivel tether through the ceiling of the cage to the external recording device. Animals were observed until they recovered from anesthesia. Subsequently, glucose administration studies were performed approximately one day after sensor implantation (mean: 30 h after implantation).

During *in vivo* sensor assessment, animals were re-anesthetized with 2% isoflurane. Animals were given dextrose 1 g/kg body weight intraperitoneally (IP) after a 10–20 min baseline period. A single drop of blood was obtained every 10 min from tail nicks (the arterialized tail was kept warm with towels heated to approximately 50 °C and assayed in duplicate using a Sure-Step glucose meter (Lifescan Division, Johnson and Johnson, Milpitas, CA). The study was continued for 150 min. Sensor current was continuously recorded by a digital data logger (Hobo, Onset Computers, Pocasset, MA 02559) or by the Armines Electronic Control Unit (ECU), a device designed for glucose sensor data recording, as previously reported (Aussedat et al., 1997). The filtering functions of the ECU were not used for this study. After the study ended, animals were euthanized by CO₂ narcosis, in accordance with the 1993 Report of the American Veterinary Medical Association Panel on Euthanasia.

The sensor output current was converted into an estimate of blood glucose by a two point calibration (one point at baseline and one point at the peak glucose). During rising and falling glucose, the temporal relationship between sensor output and blood glucose levels was analyzed by Polynomial curve-fitting (4–6th order) for each curve using Sigma Plot Scientific Graphing program (SPSS Science, Chicago, IL, 60606). In order to calculate the sensor lag, measurements of the temporal difference between the two curves were taken along 10 equally-spaced intervals from the lowest to highest parts of the curves. Lead/lag data are reported as the mean of the 10 measurements. The sign preceding the reported mean was used to indicate whether there was a lead (+) or lag (–).

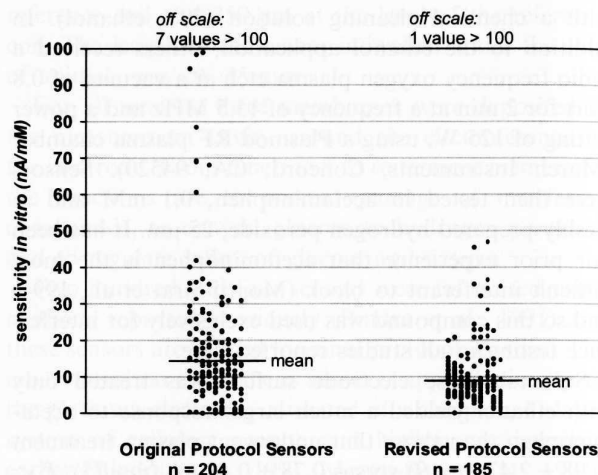


Fig. 2. A comparison of sensitivities of sensors fabricated with the original protocol versus a revised protocol using an amphiphobic polyurethane. For both groups, the data presented include all sensors fabricated consecutively over a 13 month period. Note marked variability of sensitivity in the original protocol group and less variability in the revised group.

2.3. Statistics

Since most data were distributed in a non-parametric fashion, the Mann–Whitney *U* (Wilcoxon) test was used for most comparisons. The *F* test was used to compare variance of residuals.

3. Results

3.1. *In vitro* repeatability of sensors fabricated by original protocol versus revised protocol

Data for 389 glucose sensors (204 original protocol sensors and 185 revised protocol sensors) fabricated consecutively over a 13 month period (beginning of August 1999 to the end of August 2000) were analyzed. All sensors had sensitivity measured as the rise in sensor output per unit rise in glucose as glucose was raised from 0 to 5 mM. The revised protocol sensors demonstrated much less variation of sensitivity than the original protocol sensors as depicted in Fig. 2. The variance of residuals was 0.74 for the revised protocol sensors versus 2.73 for the original protocol sensors, a highly significant difference ($P < 0.001$, *F* test).

Sensor output was measured at multiple glucose levels (0, 5, 10, 15, and 20 mM) in many sensors (of intermediate sensitivity) in both groups in order to assess linearity. Mean correlation coefficient *r* and coefficient of determination *r*² were 0.996 and 0.993 for revised protocol sensors (*n* = 141); and *r* and *r*² of 0.996 and 0.992 for original protocol sensors (*n* = 89). For the residual analysis linearity test (Analytical Methods

Committee, 1994), it was found that there was a slightly greater residual for the highest (20 mM glucose) standard than for the next lower (15 mM) standard, indicating very mild non-linearity (original protocol: 1.74 vs. -0.96 nA; revised protocol: 1.89 vs. -1.6 nA).

A typical example of a response of a sensor (revised protocol) to stepwise increases in glucose and acetaminophen in vitro is shown in Fig. 3. We also examined the relationship between in vitro sensitivity and linearity in original protocol and revised protocol sensors. For this analysis, a sensitive metric that detects subtle degrees of non-linearity was used: the percent loss of slope at the highest glucose region (15–20 mM) as compared with the lowest region (0–5 mM). Larger values denote large degrees of non-linearity. Although the correlation was relatively weak, there was a significant correlation between sensitivity and this measure of non-linearity (for original sensors: $r = 0.31$, $P = 0.021$, $n = 55$; for revised sensors: $r = 0.2209$, $P = 0.0375$, $n = 89$). Stated differently, the sensors that were the most sensitive to glucose demonstrated more of a tendency to be non-linear.

The response to acetaminophen was measured in 94 sensors in the original protocol group and 54 sensors in the revised protocol group. For this test, acetaminophen was injected into the stirred solution that was used for the final glucose standard test (20 mM), yielding an ultimate acetaminophen concentration of 0.1 mM. The incremental response to acetaminophen was low and averaged 1.39 ± 0.32 nA (original) and $1.04 \pm$

0.46 nA (revised, P not significant). In addition, a calculation was performed in order to provide an estimate of the expected percentage error caused by a large dose of acetaminophen taken at a normal glucose level. For this calculation, the interference was expressed as the percentage of the total response at a normal glucose level (5 mM) which was accounted for by acetaminophen. For original protocol sensors, $3.94 \pm 1.45\%$ was accounted for by acetaminophen versus $2.00 \pm 0.55\%$ for revised protocol sensors (P not significant).

Data obtained during the 11 day incubation is shown in Fig. 4. For the first 3 days, there was no change in the sensitivity to glucose (day 1 sensitivity: 6.12 ± 1.34 ; day 3: 6.33 ± 1.40 nA/mM). Thereafter, there was a trend toward a slight rise at day 8 (7.13 ± 1.39) and at day 11 (7.56 ± 1.47) but neither change was significant using paired Mann–Whitney U (Wilcoxon) signed rank tests.

3.2. In vivo study

The mean in vivo study data is shown in Fig. 5. The temporal relationships between blood glucose and sensor-estimated glucose were analyzed during ascent and descent of glucose. During ascent, there was a modest lag of the sensor output versus blood glucose (-4.71 ± 1.46 min, $P < 0.05$ for null hypothesis that lead/lag = 0). During the descending glucose phase the animals showed a trend towards a slight lag

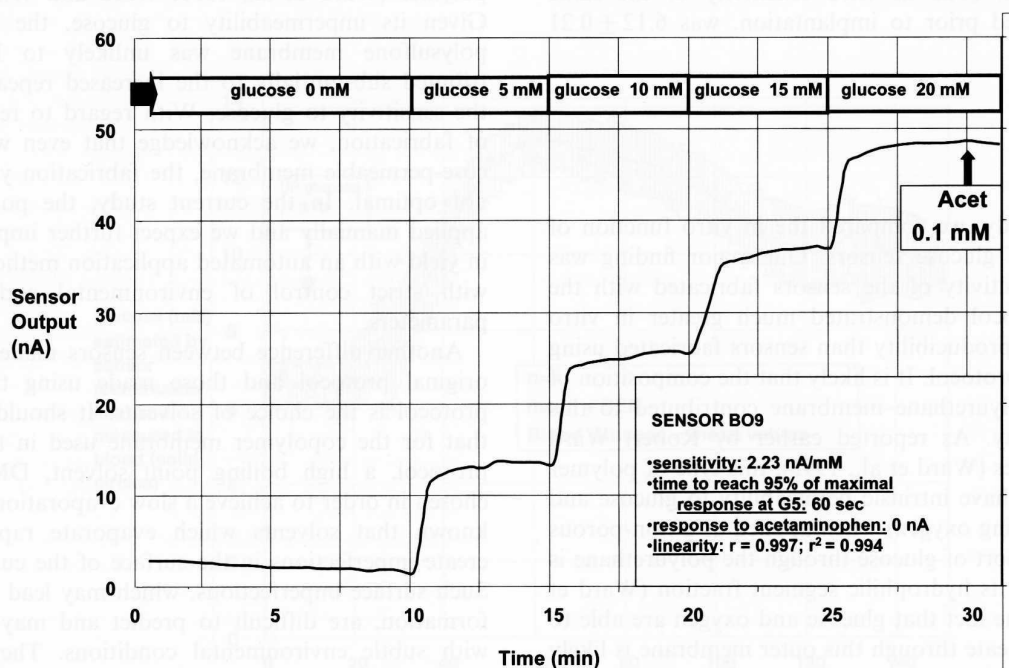


Fig. 3. A representative example of a single sensor prepared with the revised protocol, using PES and silane as the inner membrane and an amphiphobic polyurethane as the outer membrane. The sensor was tested in vitro in ascending glucose levels from 0–20 mM. Acetaminophen was added during testing in 20 mM glucose. All testing was carried out in a closed, stirred cell at 37 °C.

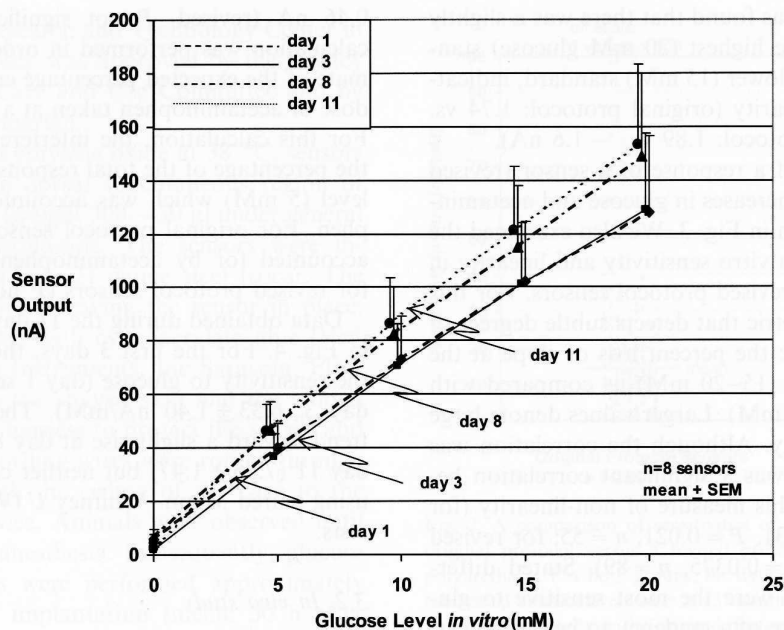


Fig. 4. In vitro tests of eight sensors studied at repeated intervals during prolonged incubation in phosphate-buffered saline (PBS) at 37 °C for 11 days during continuous polarization (0.65 V). No significant change in slope (sensitivity) was noted at day 3, 8 or 11 as compared with day 1.

(-4.21 ± 3.05 min, P not significant, same null hypothesis). The sensitivity of the sensors in vivo was calculated during the ascending glucose phase during the two point calibration procedure and was 1.64 ± 0.09 nA/mM. (The in vitro sensitivity of the same sensors, tested prior to implantation, was 6.12 ± 0.21 nA/mM).

4. Discussion

In this study, we compared the in vitro function of two types of glucose sensors. Our major finding was that the sensitivity of the sensors fabricated with the revised protocol demonstrated much greater in vitro functional reproducibility than sensors fabricated using the original protocol. It is likely that the composition of the outer polyurethane membrane contributed to this reproducibility. As reported earlier by Robert Ward and colleagues (Ward et al., 1993), this type of polymer is known to have intrinsic permeability to glucose and gases (including oxygen) when applied as a non-porous layer. Transport of glucose through the polyurethane is regulated by its hydrophilic segment fraction (Ward et al., 1993). The fact that glucose and oxygen are able to directly permeate through this outer membrane is likely to have contributed to its improved functional predictability. The original polyurethane is thought to be devoid of hydrophilic segments and it is likely that permeation of glucose through such a membrane occurs

via physical defects. Taken together, these findings verify and extend the earlier finding that the influx of hydrophilic compounds such as glucose can be regulated by the presence of hydrophilic groups in a dense polymer (Ward et al., 1993; Ward and White, 1991). Given its impermeability to glucose, the new inner polysulfone membrane was unlikely to have contributed substantially to the increased repeatability of the sensitivity to glucose. With regard to repeatability of fabrication, we acknowledge that even with a glucose-permeable membrane, the fabrication yield is still not optimal. In the current study, the polymer was applied manually and we expect further improvements in yield with an automated application method coupled with strict control of environmental and chemical parameters.

Another difference between sensors made using the original protocol and those made using the revised protocol is the choice of solvents. It should be noted that for the copolymer membrane used in the revised protocol, a high boiling point solvent, DMAC, was chosen in order to achieve a slow evaporation rate. It is known that solvents which evaporate rapidly often create imperfections in the surface of the cured solute. Such surface imperfections, which may lead to pinhole formation, are difficult to predict and may well vary with subtle environmental conditions. They may be responsible for suboptimal repeatability of sensors made with the original method where rapid solvent evaporation can occur. It should be mentioned that imperfections in polymer surfaces from which a solvent

has been rapidly evaporated were noted a number of years ago by Vadgama and colleagues (Churchouse et al., 1986).

For the purpose of eliminating the effects of interfering substances, we applied three layers of polyethersulfone (PES) to the platinum electrode. The formulation of PES we used is similar to that utilized by Churchouse and Vadgama (Churchouse et al., 1986). However, we found that application of the PES to the platinum electrode was not sufficient to exclude interferants such as acetaminophen. In order to avoid PES attack by DMAC, we found it necessary to apply a stabilizing layer of AATS between the second and third layers of PES. We also found that a preceding oxygen plasma etch of the underlying electrode cleans and roughens the platinum surface. This etch increases the adherence and coating uniformity of PES and reduces the interference by acetaminophen to acceptable levels.

The PES and silane appeared to effectively eliminate *in vitro* interference from acetaminophen in the completed glucose sensor, as did the inner membrane of the original protocol sensors. The PES/silane inner membrane has the advantage of being simpler to apply and more amenable to manufacturability than that used in the original protocol. The polyethersulfone used in the current study is known to be biocompatible and has been widely-used for many years as a hemodialysis membrane (Schneider and Streicher, 1985; Streicher and Schneider, 1985). We observed a mean interference current of about 1 nA during testing in 0.1 mM acet-

aminophen when PES and silane were used. For the sake of perspective, this degree of interference from acetaminophen represented 2.0% of the sensor output at a normal glucose level of 5 mM. Thus, at an actual glucose value of 5.0 mM in the presence of 0.1 mM acetaminophen, the sensor would be expected to yield a glucose estimate of 5.1 mM. Since of the fact that the absolute interference is constant over the range of glucose levels, the percentage error is less at higher glucose levels. Thus, we would expect a 2% error at 5 mM to translate into a 1% error at 10 mM and a 0.5% error at 20 mM. We chose the acetaminophen concentration of 0.1 mM because this is a serum level that can be achieved during high doses of oral acetaminophen in humans (Lifshitz et al., 1999; Rygnestad et al., 2000). The situation in an *in vivo* setting is probably even more favorable given previous work showing that acetaminophen interference measured in subcutaneous tissue is less than that measured concurrently in serum. The explanation for this finding is based on the fact that acetaminophen is rapidly cleared from the circulation and does not reach high levels in mammalian tissue (Zhang et al., 1994). In terms of clinical use of the glucose sensor, we acknowledge that the *in vitro* tests of interference must be verified during *in vivo* tests in animals and/or humans.

Other research groups have successfully used a variety of compounds to reduce the effect of interfering compounds, including polytetra-fluoroethylene (Vaidya and Wilkins, 1993), the perfluorinated ionomer Nafion

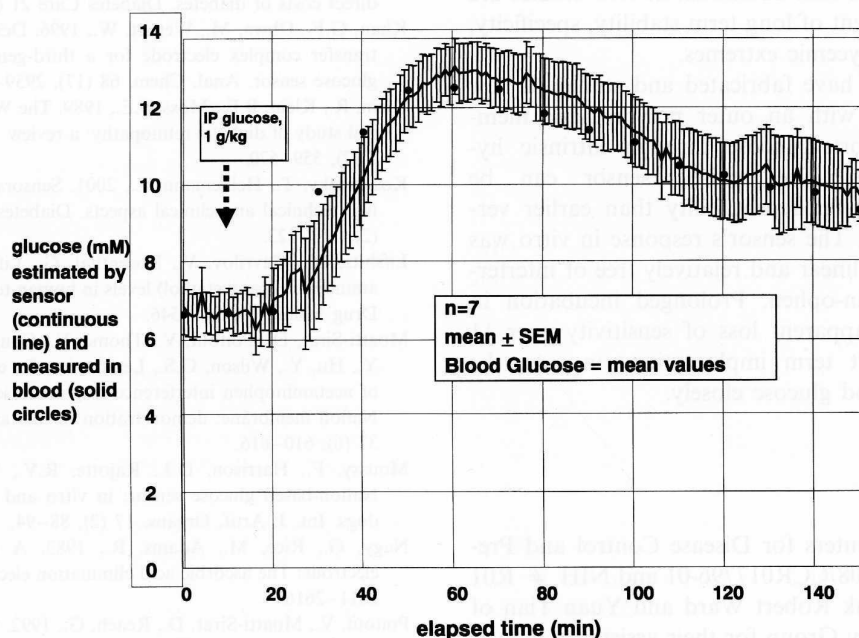


Fig. 5. Sensor output data during IP administration of glucose in rats. There is a satisfactory tracking of blood glucose level with a tendency toward a slight lag both during ascent and descent of glucose.

(Vaidya et al., 1995), polyphenylenediamine (Moussy et al., 1994), epoxy (Sriyudthsak et al., 1996), the charge transfer complex TTF-TCNQ (Khan et al., 1996), and a horseradish peroxidase-based film (Csoregi et al., 1994). In order to reduce the interfering effect of ascorbate, Nagy and colleagues applied a separate layer of ascorbate oxidase to sensors in order to oxidize ascorbate to an inert form (Nagy et al., 1983). A review of methods of measuring glucose continuously (electrochemical and other) has recently been published (Koschinsky and Heinemann, 2001). We also studied the response of continuously-polarized sensors during prolonged incubation at 37 °C. There was very little change in sensitivity to glucose for the first 3 days. After this time period, a slight, but non-significant rise in sensitivity was noted between 8 and 11 days (see Fig. 4). These results suggest that a critical loss of glucose oxidase did not occur, and that its immobilization with glutaraldehyde was satisfactory for this time period. Other investigators have reported no change (Wilkins et al., 1995) or a slight rise (Harrison et al., 1988) in sensitivity during prolonged incubation of amperometric glucose sensors.

We also tested sensors fabricated using the revised protocol in short term studies in rats that received an intraperitoneal dose of glucose. The data showed satisfactory tracking of frequently-measured blood glucose values. There was a modest sensor lag of 3–6 min behind blood glucose, which is unlikely to create substantial measurement error. The in vivo study reported herein was intended to be a simple feasibility test of the sensor. It is obvious that additional in vivo studies are needed for assessment of long term stability, specificity, and detection of glycemic extremes.

In summary, we have fabricated and studied a new microsensor made with an outer polyurethane membrane that transports glucose through intrinsic hydrophilic segments. The revised sensor can be fabricated with greater repeatability than earlier versions of the sensor. The sensor's response in vitro was found to be nearly linear and relatively free of interference from acetaminophen. Prolonged incubation in vitro revealed no apparent loss of sensitivity over 11 days. During short term implantations in rats, the sensor tracked blood glucose closely.

Acknowledgements

Supported by Centers for Disease Control and Prevention grant # R08/CCR017796-01 and NIH # R01 DK55297. We thank Robert Ward and Yuan Tian of Polymer Technology Group for their assistance in synthesizing the sensor membrane material and Dr Louis Homer for statistical discussions.

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