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ORIGINAL ARTICLE

Safety and accuracy of a new long-term subconjunctival glucose sensor

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Abstract

Background: A new biosensor has been developed by EyeSense (Großostheim, Germany) that is placed into the conjunctiva of one eye to measure the glucose concentration of the surrounding tissue in a non-invasive manner. In the present study we investigated the correlation between glucose concentrations measured by the EyeSense implant and those determined by finger prick testing, as well as the tolerability and safety of the implant over a 16-week period.
Methods: The study was performed in 28 diabetic patients. The biosensor was inserted under local anesthesia and sterile conditions. Correlations between capillary glucose measured by laboratory methods and interstitial glucose determined by the biosensor were investigated by inducing increases and decreases in glucose values between 60 and 300 mg/dL.
Results: Most patients experienced a mild subconjunctival hemorrhage postoperatively. Except for the minor sensation of the presence of foreign body, the implants were well tolerated. Three patients lost the ocular mini insert spontaneously, whereas there was a function failure of the insert in four patients. Error grid analysis showed that the percentage of data pairs in the acceptable ranges (zone A and B) was very high (>96%). However, there was a shift from zone A to zone B during observation. This was due primarily to an increase in the lag time between capillary and interstitial measured glucose.
Conclusion: The present study demonstrates good tolerability and measurement performance of the biosensor. The reasons for an increase in the lag time are still unknown; local reactions may be involved.

Keywords: diabetes mellitus, eye, glucose measurement system, glucose sensor.

Significant findings of the study: It is possible to measure glucose concentrations using the new biosensor without the need for finger pricking.
What this study adds: The new method may improve the technology for metabolic self-control by diabetic patients.

Introduction

Self-monitoring of blood glucose is an essential component of diabetes therapy, especially in patients with type 1 and insulin-dependent type 2 diabetes. Glucose concentrations are routinely measured by electronic devices in capillary blood obtained after finger pricking. Furthermore, continuous glucose monitoring is

possible by introducing a sensor into the skin that allows interstitial glucose monitoring for approximately 5–7 days. The known disadvantages of these methods are the unpleasant procedure associated with the finger pricking tests and the high costs and limited period of measurement associated with the use of the skin sensor. Several new methods have been tested in the past to overcome these disadvantages, but an accurate and reliable method that can be handled easily by diabetic patients has not yet been developed.[1,2]

Recently, EyeSense (Großostheim, Germany) developed a biosensor that is placed in the conjunctiva of one eye and is able to measure the glucose concentration of the surrounding tissue.[3] In the present study, we investigated the objective and subjective short-term performance of this biosensor, referred to herein as the ocular mini insert (OMI).

Methods

The present study was approved by the Freiburger Ethics Committee, Germany, and was conducted according to the principles outlined by the Declaration of Helsinki. All subjects provided written informed consent prior to participation in the study. The study was registered at ClinicalTrials.gov (identifier: NCT00999856).

The OMI and hand-held photometer have been described in detail elsewhere.[3] Briefly, the biosensor is a flat disc, with a typical thickness of 200 μm and a diameter of approximately 3–4 mm. The main components of the disc are a hydrogel matrix with embedded sensor particles that contain dye-labeled dextran and dye-labeled concanavalin A (ConA). There is a fluorescence resonance energy transfer (FRET) between the dyes attached to the ConA and dextran. Glucose freely permeating the biosensor alters the FRET by binding to ConA competitively with dextran. The change in the fluorescence emitted from the OMI on an excitatory light from an LED source is measured by a hand-held photometer and reflects changes in glucose concentration. As described previously,[3] the biosensor was inserted under sterile conditions, and after application of topical anesthesia, into the temporal side of the conjunctiva using a minimally invasive procedure.

Insulin-dependent diabetic patients requiring daily finger pricking to monitor blood glucose were included in the present study. The main exclusion criteria were: pregnant or lactating women; known hypersensitivity to any of the products to be used in the study; malignancies or other severe chronic diseases, poorly controlled diabetes (HbA1c > 10%), or hypertension (> 180/100 mmHg); any ocular disease requiring topical medication or other conditions that increase the risk of ocular surgery (e.g. acute or chronic conjunctivitis or blepharitis, corneal disorders); and previous or scheduled eye surgery.

Assessment of accuracy

The EyeSense glucose-monitoring system was calibrated against blood glucose using two capillary blood glucose readings at different blood glucose levels to determine the slope of the response function. The correlation between capillary glucose measured by standard laboratory methods and interstitial glucose measured by the OMI was investigated by increasing and decreasing blood glucose concentrations in the range 60–300 mg/dL by administration of a standardized carbohydrate-rich meal and/or injection of an appropriate dose of insulin. Capillary blood samples were collected by a study nurse every 10 min and glucose concentrations were assayed by the glucose–oxidase method (Hitado Super GL; Hitado, Möhnesee, Germany). In addition, capillary blood glucose was measured using a commercially available home glucose meter to obtain immediate information on current glucose levels. Fluorescence measurements were taken by patients using the hand-held photometer at the same time as capillary blood samples were collected, at 10-min intervals, with each fluorescence measurement consisting of three consecutive readings. In total, each measurement session took approximately 4–6 h.

A loss of function of the OMI was identified if two consecutive measurement sessions showed that the change in the fluorescence caused by a change of blood glucose > 100 mg/dL was smaller than the error of the measurement. In this case, measurement sessions were stopped.

Biometric evaluation

The relationships between glucose values measured by the OMI and the laboratory were analyzed mathematically by calculation of the correlation coefficient, as well as the mean average of relative difference (MARD), and by error grid analysis. The zones of the error grid analysis (A–E) indicate how adequate the therapeutic decision taken on the basis of the measurement system result would be compared with the decision that would have been taken on the basis of the laboratory result. Zone A indicates blood glucose values within the precision tolerance (≤20% of the reference value); zone B indicates values outside the precision tolerance, but probably not resulting in any serious consequences; zone C values would lead to

overcorrection of normal or subnormal glucose levels; zone D values indicate a ''dangerous failure to detect and treat errors''; and zone E values represent an ''erroneous treatment zone''.

Results

Twenty-eight insulin-dependent diabetic patients ($n = 20$ with type 1 diabetes; $n = 8$ with type 2 diabetes) were included in the present study. The mean (±SD) age of all patients and duration of diabetes were 49.9 ± 9.5 and 17.2 ± 4.3 years, respectively. Metabolic control was stable (HbA1c 7.5 ± 1.3%) and all patients were free of serious concomitant disease.

The biosensor was inserted subconjunctivally into the right eye in the predetermined area without any complications (Fig. 1). The findings regarding tolerability and safety are given in Table 1. Most patients exhibited a small subconjunctival hemorrhage postoperatively that disappeared within 7–14 days. All patients reported the sensation of a foreign body that lasted approximately 5–10 days. This was due to the suture and was treated successfully using artificial tears. One patient showed moderate wound inflammation with vessel injection and seven patients exhibited mild conjunctivitis during the observation period. These complications were treated successfully with eye drops prednisolone (10 mg/mL) and ofloxacine (3 mg/mL). The OMI was extruded spontaneously in three patients 3, 8, and 12 weeks after implantation. The loss was not noticed by the patients themselves and there were no specific reasons determined to explain the spontaneous extrusion. Ophthalmological investigations did not indicate any local consequences of the loss of the OMI. No serious adverse events were noted during the observation period.

Table 1 Tolerability and safety of the glucose sensor after implantation

	No. patients	Duration	Treatment
Small subconjunctival hemorrhage	26	5–14 days	None
Foreign body feeling	25	5–10 days	Artificial tears
Prolonged wound healing	1	14 days	Prednisolone, ofloxacine
Conjunctivitis	7	Days	Prednisolone, ofloxacine
Loss of OMI	3		

OMI, ocular mini insert.

The typical course of a glucose measurement session is shown in Fig. 2. It is obvious that there is a close correlation between the glucose values measured in capillary blood by standard laboratory methods and the interstitial glucose values measured by the OMI. The lag time between blood and interstitial glucose

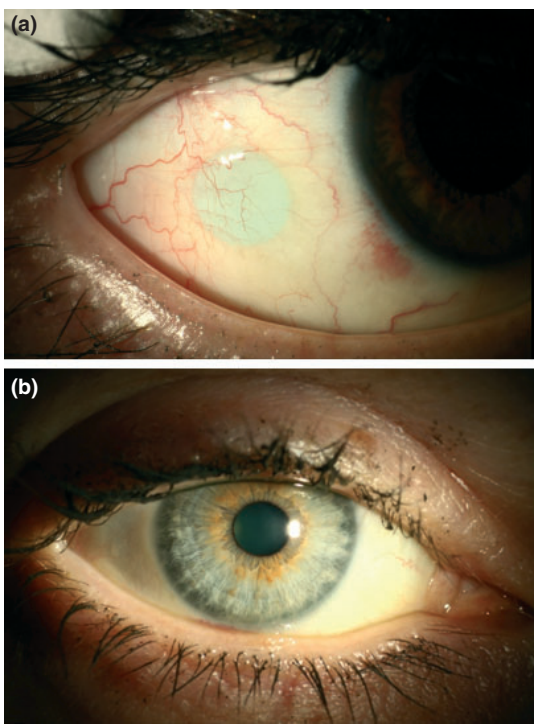

Figure 1 The biosensor 3 weeks after implantation. (a) The patient is looking towards his left. The sensor can be seen on the left (light blue tinge), far enough away from the iris. There is no reaction evident on the conjunctiva. (b) The same patient looking straight ahead. The sensor is no longer visible.

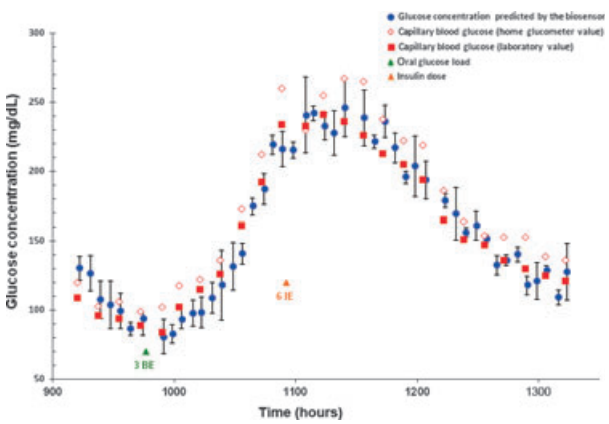

Figure 2 Typical course of a glucose measurement session in subject A20, no. 3, 12 days after implantation of the sensor. 3BE, 30 g carbohydrates; 6IE, insulin. Glucose concentrations predicted by the biosensor are shown as the mean ± SD of three consecutive photometer readings (see text for details).

was usually 5–10 min depending on the dynamics of the glucose changes.

Figure 3 shows the error grid analysis of the six measurement sessions after implantation of the OMI, with corresponding numerical results listed in Table 2. It is obvious that the percentage of values in the acceptable range (zones A and B) was very high (99.9–94.4%) throughout the 16-week observation period. However, there was a shift of the data pairs from zone A to zone B during the observation period, which is in line with a decreasing slope of the curves when calculating regression analysis and an increase in MARD (Table 2). This was due primarily to an increase in the lag time between blood and sensor-measured glucose in some patients, which, in some patients, increased from 5–10 min in week 1 to 10–20 min in week 16. Following the introduction of a lag time compensation of 10–20 min at week 16 resulted in the prevalence of data pairs in zone A increasing from 57.0% to 67.3%. The prevalence of data pairs in zone B at this time was 30.9%, whereas that in zones C–E was 1.8%. The increase in the lag time was not related to age, the duration of diabetes, or patients' HbA1c levels.

In addition to the three patients in whom the OMI was spontaneously extruded, the measurement sessions were stopped in four patients due to a loss of sensor function 4–12 weeks after implantation. Thus, observations were made in 21 patients with a functioning biosensor for the full duration of 16 weeks.

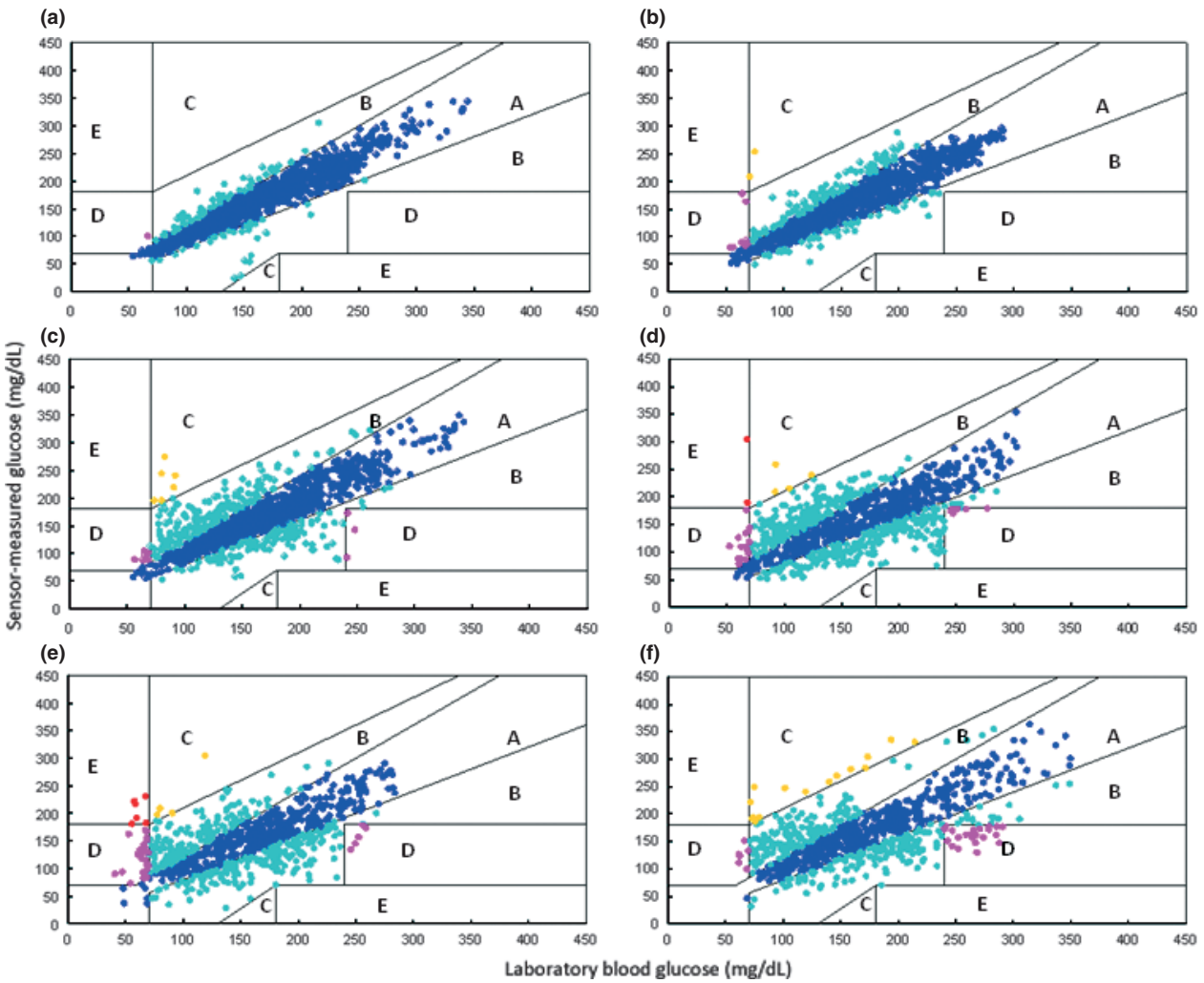

Figure 3 Accuracy of glucose measurement: error grid analysis in Weeks 1 (a), 2 (b), 4 (c), 8 (d), 12 (e), and 16 (f) showing values falling in zones A–E. The zones of the error grid analysis indicate how adequate the therapeutic decision taken on the basis of the measurement system result would be compared with the decision that would have been taken on the basis of the laboratory result. Data pairs falling in zones A and B are acceptable.

Table 2 Error grid analysis of the percentage of data pairs in the different zones and mathematical parameters regarding accuracy

	Week 1	Week 2	Week 4	Week 8	Week 12	Week 16
No. data pairs	1151	1086	1051	1008	792	802
Error grid analysis						
Zone A	91.7%	85.0%	72.4%	54.0%	54.4%	57.0%
Zone B	8.2%	14.1%	26.3%	43.1%	40.4%	37.4%
Zones A + B	99.9%	99.1%	98.7%	97.1%	94.8%	94.4%
Zones C–E	0.1%	0.9%	1.3%	2.9%	5.2%	5.6%
Mathematical parameter						
MARD	9.4 ± 4.3%	11.4 ± 7.6%	16.8 ± 1.3%	23.6 ± 13.1%	26.9 ± 13.8%	24.4 ± 13.2%

MARD, mean absolute relative difference.

Discussion

The present study shows that the newly developed biosensor is able to determine interstitial glucose concentrations in a non-invasive manner with high accuracy over a period of 16 weeks. The subconjunctival implantation of the biosensor using a minimally invasive procedure was performed without complications and local tolerability was very good.

Several devices for non-invasive glucose measurement have been investigated in the past[1,2] and the skin is usually the target for procedures using methods such as reverse iontophoresis[1] or impedance spectroscopy.[2] However, none of these devices has been able to provide consistent results over a period of time that would be long enough for daily diabetes control. The eye has also been investigated as a target for glucose measurement, usually in eye fluids.[4–7] However, glucose concentrations in tears can be significantly affected by several factors other than blood glucose, such as chemical substances or the wind.[8,9] The conjunctiva of the eye was chosen by EyeSense as an access point for glucose measurement for various reasons. First, the conjunctiva is known for its biocompatibility and is therefore well suited for the implantation of a biosensor. Second, it has very good capillary perfusion, which provides a fast glucose exchange between capillaries and tissue. This is an important prerequisite for measuring glucose concentrations in interstitial tissue for therapeutic purposes.[10] And third, unlike the skin, the conjunctiva is very transparent and the tear fluid covering the tissue provides constant hydration. The simple layer composition of the conjunctiva makes it easier to place the biosensor in to the correct and reproducible depth. The sensor is located at a safe distance from the iris and, if implanted at the level of the lower eyelid, the OMI is not visible if the patient looks straight ahead (Fig. 1).

The surgical procedure to place the OMI was performed under local anesthesia and took only a few minutes; no complications were observed. A small subconjunctival hemorrhage was seen in most patients postoperatively, but this disappeared within a few days. A minor foreign body sensation was reported by patients, which lasted 5–10 days. This was due to the suture and was treated successfully using artificial tears. One quarter of the patients developed mild conjunctivitis that was treated successfully with eye drops containing prednisolone and ofloxacine. The reasons for the spontaneous loss of the biosensor in three patients are unknown. In two patients, the OMI was lost during the night and so it is possible that unconscious manipulation (e.g. rubbing) of the eye may be responsible. However, an immediate ophthalmological investigation failed to identify and local consequences following the loss of the OMI.

To study the accuracy of the OMI, blood glucose levels were increased and decreased in the range 60–300 mg/dL. According to error grid analysis, the new biosensor showed a high accuracy during the 16-week measurement period: 94%–99% of the data pairs were found in error grid zones A and B, with only 0.1%–5.6% falling in the unacceptable zones C–E. However, evaluating error grid zones A and B in detail, a shift of data pairs from zone A to zone B was detected over the course of the study. From a clinical point of view, the significance of this finding may be not so important because zone A and B are acceptable ranges. However, this trend could indicate decreasing accuracy over time. The increasing MARD is in line with this assumption.

The shift of data pairs from zone A to zone B seems to be due to an increase in lag time. This time difference results from the equilibrium process between the vessels and interstitial tissue across the transcapillary wall, as well as the time to permeation on or into the biosensor.[11] A lag time of 5–10 min is common when measuring glucose in interstitial tissue and was also found to be appropriate at the beginning of the present study (week 1). However, in some patients there was

an increase in the lag time at the end of the study (week 16) to 10–20 min. Introducing a lag time compensation of 10–20 min at week 16, markedly increased the percentage of data pairs in zone A. We could not find any relationship between the increase in lag time and either age, duration of diabetes, or HbA1c values.

The reasons for the increased lag time in some patients phenomenon remain unknown, but the aging of the OMI and/or problems associated with glucose permeation could be involved. *In vitro* investigations of the OMI demonstrated high functionality over months;[3] therefore, it seems unlikely that the increased lag time was due to aging of the device. As shown previously, there are many local factors that may influence the measurement accuracy of an implanted biosensor, including occult hemorrhage or thrombosis formation,[12] tissue protein adsorption, localized accumulation of inflammatory cells, or fibrous encapsulation.[13,14] All these factors have to be considered in further evaluations of the new OMI in studies with longer observation periods.

In conclusion, we have demonstrated, for the first time, in the present study in diabetic patients that the new technology was able to measure glucose concentrations over a period of 16 weeks after minimally invasive surgical procedures in three-quarters of our patients. Three patients lost the biosensor spontaneously, whereas the sensor failed in another four. The tolerability and the measurement performance were good; thus, this technology has the potential to partially replace self-monitoring of glucose using finger pricking in patients who have to measure their blood glucose levels frequently. However, it should be kept in mind that the results presented herein are only preliminary owing to the small number of patients investigated ($n = 28$) and the limited period of observation (16 weeks). Thus, further studies are required to determine the long-term accuracy and tolerability of the OMI.

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Disclosure

As indicated by the author affiliations, AM, MK, and KN are full employees of EyeSense GmbH and own stock in the company. None of the other authors owns stock in the company. This study was funded by EyeSense GmbH.

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