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ORIGINAL ARTICLE

First Clinical Evaluation of a New Long-Term Subconjunctival Glucose Sensor

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Abstract

Background:

To evaluate the feasibility of an implantable subconjunctival glucose monitoring system (SGMS) for glucose monitoring in humans, we investigated the *in vivo* performance of the sensor in a clinical trial with five patients.

Methods:

The new SGMS consists of an implantable ocular mini implant (OMI) and a hand-held fluorescence photometer. The implantable subconjunctival glucose sensor is composed of a fluorescence resonance energy transfer system based on Concanavalin A chemistry, embedded in a nelfilcon polymer hydrogel disk. Blood glucose changes in humans were induced by oral glucose intake and insulin injections.

Results:

The *in vivo* response of the new SGMS was tested in a first human clinical study with five diabetes patients. The OMI was well tolerated in the eyes of the patients. The SGMS exhibited high correlation coefficients (>0.88) with blood glucose changes and a good stability of the sensor response to glucose for the study period of 2 weeks. Lag times were in the range of 5–10 min. A total of 98% of all data pairs was in the clinical acceptable ranges A and B of the consensus error grid.

Conclusions:

For the first time, the possibility to measure glucose *in vivo* in the subconjunctival interstitial fluid for a period of 2 weeks was demonstrated in a human clinical trial.

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Abbreviations: (BG) blood glucose, ($C_2O_2H_3Na$) sodium acetate, ($CaCl_2$) calcium dichloride, (Con A) Concanavalin A, (K_2CO_3) dipotassium carbonate, (KCl) potassium chloride, (LED) light-emitting diode, ($MgCl_2$) magnesium dichloride, (NaCl) sodium chloride, ($NaHCO_3$) sodium hydrogencarbonate, (Na_2HPO_4) disodium hydrogenphosphate, ($NaSO_4$) sodium sulfate, (OMI) ocular mini implant, (SGMS) subconjunctival glucose monitoring system, (UV) ultraviolet

Keywords: Concanavalin A, diabetes, fluorescence, glucose monitoring, glucose sensor, long-term sensor

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Introduction

Diabetes is an epidemically growing disease affecting millions of people worldwide with severe medical and economic implications.[1–2] There is strong evidence that tight glycemic control is mandatory to prevent or at least slow down the progression of chronic diabetes complications, such as kidney failure, heart disease, gangrene, and blindness.[3] For many diabetes patients, especially those with type 1 and insulin-dependent type 2, disease management requires measuring blood glucose (BG) levels several times per day for their whole life. Hence, a long-term implantable glucose sensor is a desirable alternative to the *in vitro* BG test strips and the short-term sensor implants currently available for monitoring BG.

Our group has developed a new long-term BG monitoring system based on an ocular mini implant (OMI) placed under the bulbar conjunctiva of the patient's eye and a hand-held fluorescence photometer that can read out the sensor signal from the implant and translate it to a BG reading. The eye has been widely evaluated as a window for BG measurements, either by direct physical measurements of the glucose in the eye fluids[4–5] or by utilizing glucose specific sensors in the form of contact lenses for tear glucose evaluation.[6–11] However, the glucose concentration in the tear film is not solely dependent on the BG concentration but varies with stimulation of the tear film, e.g., by wind or chemical substances.[12–13] Therefore, our approach with a sensor implant in the bulbar conjunctiva of the eye uses the well-known interstitial fluid, with glucose values closely correlated to blood for long-term glucose monitoring.[14–15] Unlike the skin, the conjunctiva has a relatively simple structure and is mainly made up of epithelial cells.[16] With only few melanocytes present, the tissue is highly transparent and ideal for optical measurements. The high blood perfusion is an important prerequisite for short lag times between BG and interstitial glucose concentration changes. Compared to normal skin tissue, the layer composition of the different eye tissues makes it much easier to place the implant in a reproducible fashion and at the correct depth. Hence, the conjunctiva is a place very well suited for a small long-term glucose sensor.

The measurement principle of the OMI is based on the known ability of the plant lectin Concanavalin A (Con A) to bind carbohydrates such as dextran and glucose in a reversible and competitive manner.[17–19] The main advantage of employing Con A as a glucose sensitive sensor element rather than glucose-consuming enzymes such as glucose oxidase or glucose dehydrogenase is the reversible, nonconsumptive binding mechanism preventing a depletion of glucose at the sensor site. Moreover, binding of glucose to Con A occurs independent of oxygen, which is an additional advantage over the use of glucose oxidase. The Con A/dextran system has often been described in the literature of fluorescence-based affinity glucose sensors, where sensor molecules are labeled with fluorescence dyes, and respective fluorescence intensities are measured at the wavelengths of the dyes as a function of the glucose concentration.[20–31] The fluorescence-based technology has several advantages over electrode-enzymatic sensors, especially for *in vivo* sensing. First, the sensor produces no side products such as hydrogen peroxide in the tissue. Second, the equilibrium-driven binding reaction is independent of the rate of glucose diffusion to the sensor. Especially for our system, with a tiny implant under the conjunctiva of the eye, a noncontact read out of the sensor signal is mandatory for the convenience of the patient. This is ensured by use of fluorescence sensing technology.

This article provides the first *in vivo* data of a human clinical trial of the subconjunctival glucose monitoring system (SGMS) over a period of 2 weeks.

Methods

Production of the Ocular Mini Implant

Alginate micro beads were manufactured by cross-linking droplets of sodium alginate solution (Sigma Aldrich GmbH, Taufkirchen, Germany) in a calcium chloride bath. The formed particles were collected by centrifugation at 1000 × g for 20 min.

The sensor chemistry of the OMI has been described in detail elsewhere.[8] Briefly, Con A, labeled with a fluorescent dye emitting in the red spectral region (Zedira GmbH, Darmstadt, Germany), and dextran, labeled with another red emitting fluorescent dye (Life Technologies GmbH, Darmstadt, Germany), were loaded to previously-formed alginate beads by incubation of the beads in a solution of 4 mg/ml Con A and 7 mg/ml dextran. The exact nature of the fluorescent dyes used cannot be disclosed because of a patent in preparation.

Alginate beads containing the sensor chemistry were mixed with Nelfilcon™ A polymer (CIBA Vision GmbH, Grosswallstadt, Germany), an ultra-violet (UV)-polymerizable derivatized poly(vinyl alcohol). The highly viscous solution was molded into poly(methyl methacrylate) molds and cured under UV light for 1 s.

The formed implants had a thickness of 200 µm and a diameter of 4 mm. The implants were sterilized by beta radiation at a contractor (BGS Beta-Gamma-Service GmbH, Bruchsal, Gemany).

Fluorescence Detection by the Hand-held Photometer

To determine glucose concentration, the OMI is excited by an orange light-emitting diode (**LED**) from a computer-driven hand-held fluorescence photometer specially developed by Qiagen Lake Constance (Qiagen Lake Constance GmbH, Stockach, Gemany). The fluorescence photometer measures the intensity of the fluorescent light emitted back by the OMI at two wavelengths, one of which correlates with the glucose concentration, the other which acts as a reference and is independent of the glucose concentration. **Figure 1** gives a schematic presentation of the fluorescence photometer: an excitatory light from an LED source is focused by a lens system onto the OMI. The fluorescence light emitted from the implant is collected by the front lens and passes through a series of beam splitters. The shorter wavelength light is reflected onto a first photodiode; the longer wavelength light is focused onto a second photodiode. The signals from the photodiodes are amplified and digitalized. An appropriate algorithm calculates the glucose concentration from the ratio of the two fluorescence intensities.

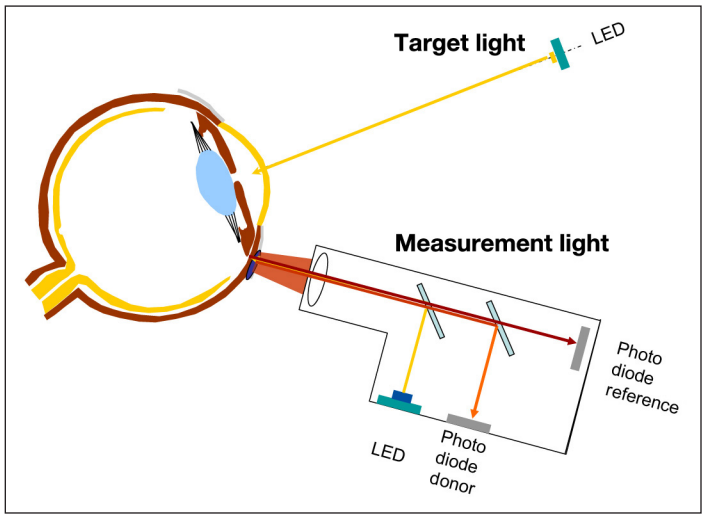

Figure 1. Schematic presentation of the optical measurement setup. An excitatory light from an LED source is focused by a lens system onto the OMI. The fluorescence light emitted back from the implant is collected by the front lens and passes through a series of beam splitters. The shorter wavelength light is reflected onto a first photodiode; the longer wavelength light is focused onto a second photodiode. For *in vivo* applications, the photometer additionally contains a patient-leading target light.

For *in vivo* applications, the photometer additionally contains a target light. Angle, distance, and height difference of the target light relative to the measurement light are individually adaptable to ensure easy and reliable positioning of the photometer in front of the OMI when looking at the target light. As a third element, the photometers front shape is ergonomically designed to give the patient mechanical guidance to place the photometer correctly in front of the face and the eye. The size of the hand-held photometer, including front shape and target light, is about 15 cm × 10 cm × 8 cm. For data communication and power supply, the hand-held photometer is connected to a personal computer via a USB 2.0 connector.

In Vitro *Sensor Test System*

For *in vitro* measurements of the fluorescence response to glucose, the OMI was stepwise submerged in solutions of 0, 50, 100, 250, and 500 mg/dl of glucose. Fluorescence readings were taken under static conditions. To determine the *in vitro* stability of the OMI response, OMIs were stored at 37 °C in artificial interstitial fluid buffer (30 mM $NaHCO_3$, 106 mM NaCl, 1.2 mM Na_2HPO_4, 1.05 mM $MgCl_2$, 0,5 mM K_2CO_3, 2.7 mM KCl, 0.6 mM Na_2SO_4, 1.5 mM $CaCl_2$, 6.5 mM $C_2O_2H_3Na$, pH 7.4), and the response to glucose was measured at 0, 1, 14, 30, 60, 120, 180, 270, and 360 days of storage. The ratio response between 50 and 250 mg/dl glucose concentration was calculated using the following relation:

$$\Delta \text{ ratio} / \Delta \text{ glucose} = \text{slope} (\text{ratio}_{50\ \text{mg/dl}}; \text{ratio}_{100\ \text{mg/dl}}; \text{ratio}_{250\text{mg/dl}}) / 200 \quad (1)$$

Ocular Mini Implant in Human Eyes

After administration of topical anesthesia, a 2.2 mm incision was made to the temporal side of the conjunctiva. The OMI was folded in a Viscoject™ 2.2 intraocular lens injector (Oculentis GmbH, Berlin, Germany) and injected under the conjunctiva. The edges of the incision were sutured. A topical antibiotic (Floxal Augentropfen, Dr. Mann Pharma, Berlin, Germany) was instilled, and administration was continued as required by the ophthalmologist. The patients were instructed for daily

self-examination of the status of the insert and their eyes (rubeosis, swelling, paresthesia) and advised to visit the ophthalmologist immediately in case of adverse events.

Figure 2 shows the eye of a patient with an OMI.

Measurement and Analysis of in Vivo Subconjunctival Glucose Monitoring System Response

After implantation of the OMI, the hand-held fluorescence photometer was adjusted to the patient by individually aligning the position of the target light relative to the measurement light and afterwards setting the individual reference intensity threshold. The correct threshold setting guarantees that the excitation beam illuminates the OMI at the center and at the distance of the focal length of the collecting lens during the measurement.

The patients self-triggered the OMI measurements by positioning the photometer's front shape to their face and looking in the direction of the target light. Valid measurements were recorded after reaching the individually set reference threshold, ensuring a reproducible positioning. Typically, 20 readings were collected and averaged. The software calculated the ratio of donor to reference fluorescence.

The correlation between capillary glucose measured by a Hitado Super GL easy system (Hitado GmbH, Moehnesee, Germany) and interstitial fluid glucose measured by the OMI was investigated by inducing an increase and decrease of BG values by oral intake of carbohydrates (ACCU-CHEK® Dextro oral glucose tolerance test, Roche Pharma AG, Grenzach-Wyhlen, Germany) or subcutaneous insulin injection (patient-specific insulin) in a range of 50–450 mg/dl.

In a typical measurement cycle, capillary blood was sampled at a frequency of 10 min. Fluorescence measurements by the hand-held photometer were performed by the patients at the same time interval, with each fluorescence measurement consisting of 3 consecutive readings, which were averaged. One measurement session lasted about 4–6 h.

Five insulin-dependent diabetes patients (three male, two female; three with type 1, two with type 2; average age of 50 ± 9 years) were included in the study. In total, the five patients performed 12 measurement sessions within 2 weeks wearing time of the OMI. The first measurement visits were carried out 1 or 2 days after implantation of the OMI.

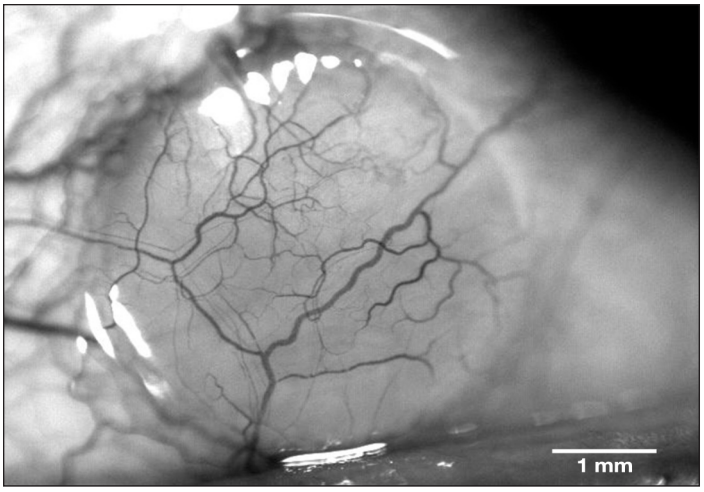

Figure 2. Ocular mini implant in patient's eye.

The EyeSense glucose monitoring system was individually calibrated against BG at each measurement day, using two capillary BG readings at different BG concentrations to determine the slope of the response function.

For lag time analysis, the time axes of the fluorescence and BG readings were shifted against each other. Shifts of 5, 10, 15, and 20 min were calculated and the correlation coefficients determined for each data set—10 min lag time shift giving the highest overall correlation.

The study was conducted in accordance with the Declaration of Helsinki and International Organization for Standardization (ISO 14155) and was approved by local ethics committees. All patients provided written, informed consent.

Ocular Mini Implant Removal From Human Eyes

After administration of topical anesthesia, a small incision was made adjacent to the OMI position, and the OMI was explanted using suitable forceps. The edges of the incision were sutured. A topical antibiotic (Floxal Augentropfen, Dr. Mann Pharma, Berlin, Germany) was instilled, and administration was continued as required by the ophthalmologist. A follow-up visit was performed 1 week after OMI explantation.

Results

In Vitro *Performance and Stability of the Subconjunctival Glucose Monitoring System*

In vitro performance and stability of the OMI under physiological conditions were validated by measuring the response to changing glucose concentrations.

Figure 3A depicts the fluorescence ratio measured at glucose concentrations 0–500 mg/dl. The corresponding linear calibration curve has a coefficient of variation of 0.98. The time constant of the sensor is 2 min, based on a first order kinetic model. The excellent performance of the sensor system is facilitated by use of alginate spheres, whose low solids content facilitates a high mobility of the sensor molecules. This leads to an efficient breakup of the fluorescence resonance energy transfer mechanism upon glucose binding, providing a high sensitivity of the Con A/dextran system. **Figure 3B** displays the excellent stability of the ratio response, calculated according to equation (1), under physiological conditions with a decrease of only 20% up to 1 year.

In Vivo *Response of the Subconjunctival Glucose Monitoring System in Five Diabetic Patients*

The implantation procedure was generally well tolerated. Four patients postoperatively showed a minor subconjunctival hemorrhage, which disappeared within a few days. All patients reported a "foreign body feeling" which lasted about 5–10 days and was attributed to the suture.

The patients performed the fluorescence measurements quickly and easily. A total ofOf all measurements, 95% wereas finished within 12.5 s, including the alignment of the photometer in front of the eye.

The correlation between capillary glucose measured by a standard laboratory method and interstitial glucose measured by the OMI was investigated. Results of typical time courses of glucose measurement sessions of all five patients are given in **Figure 4**. Close correlation between the glucose values measured in capillary blood and the interstitial glucose values measured by the OMI can be observed with the individual correlation coefficients ranging from 0.92 to 0.97. Lag times between BG and interstitial glucose lay within 5–10 min, depending on the dynamic of glucose changes.

Figure 5 depicts the BG time courses of two measurement visits from the same patient, measured 2 days (5a) and 2 weeks postimplantation (5b). After 2 weeks *in vivo*, the response of the OMI was still accurate.

The correlation coefficient of the pooled data of the 12 visits in the study was 0.93, pointing out the high accuracy of the SGMS response over the 2 weeks study duration. In **Figure 6**, the pooled data pairs of all patients are plotted in the consensus error grid.[32] A total of 71.3% of all data pairs was within zone A, 98.0% of all data pairs fell within the combined zones A and B, and 2.0% fell in zone C. No data pairs were found in zones D or E. Introducing a general lag time compensation of 10 min, the fraction of data pairs in zone A increased to 81.6%.

The explantation of the OMI at the end of the study was easy and well tolerated. All conjunctivas healed well.

Discussion

The objective of this clinical study was to demonstrate for the first time the feasibility of an implant placed in the conjunctiva of the eye in diabetes patients for glucose monitoring.

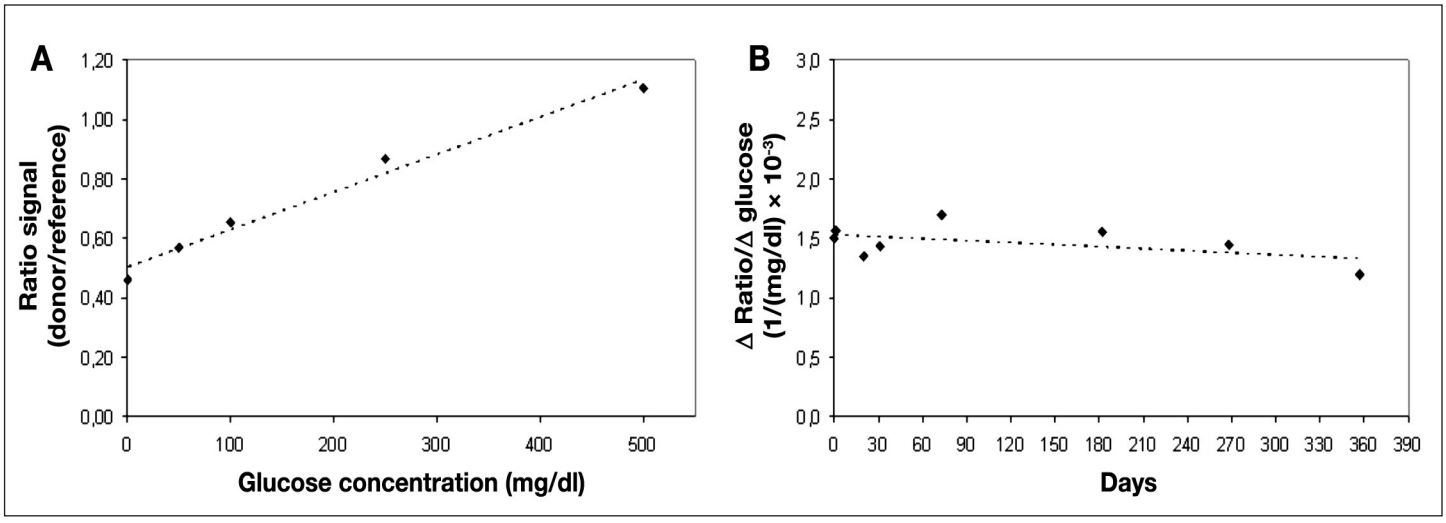

Figure 3. *In vitro* response of the OMI to physiological glucose concentrations **(A)** and *in vitro* stability of the response over 1 year **(B)** under physiological conditions (37 °C, physiological buffer).

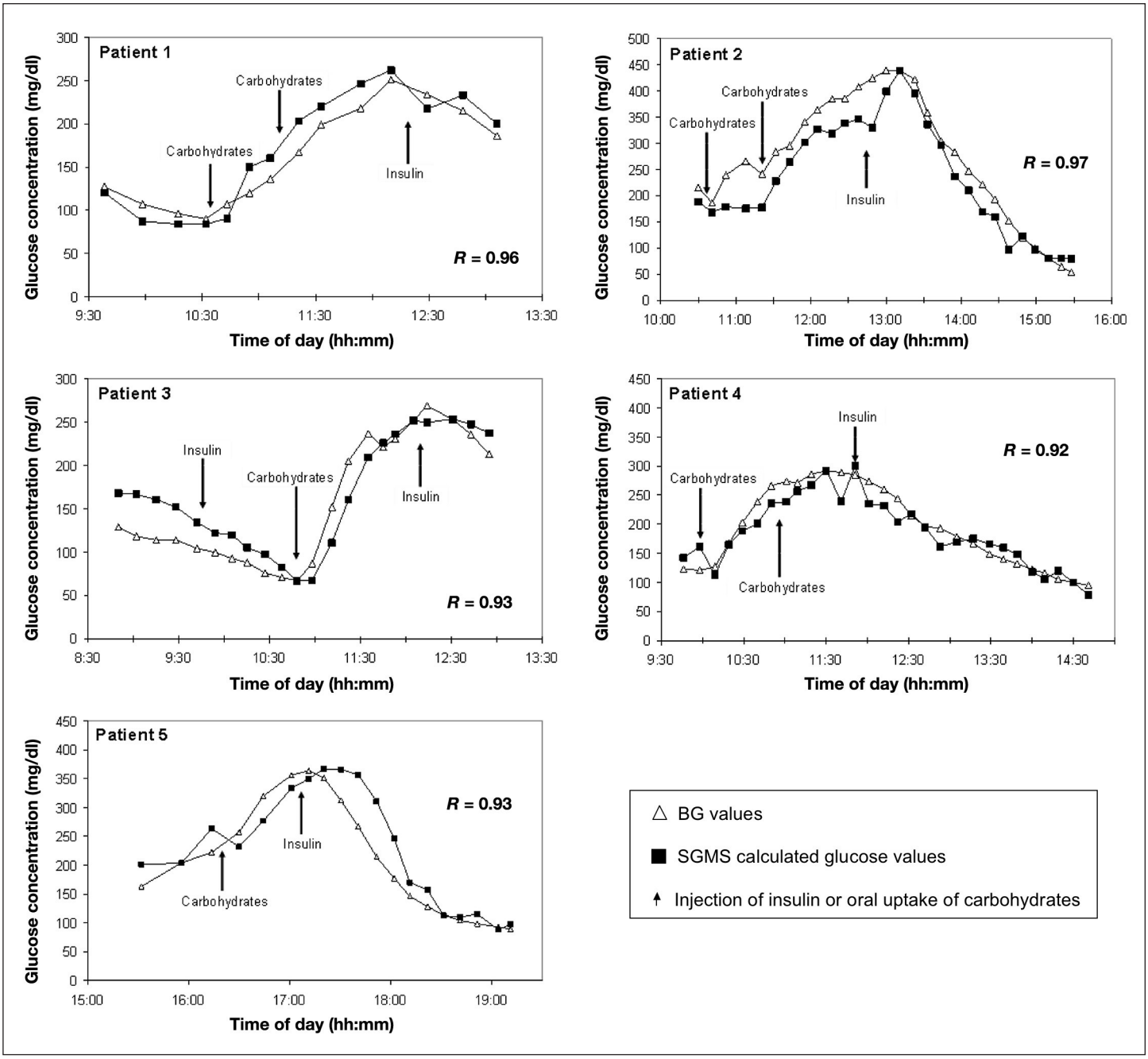

Figure 4. *In vivo* performance of five individual OMIs in eyes of human patients in the first week after implantation. *R* denotes correlation coefficient between blood glucose and SGMS signal.

The OMI was implanted in the temporal region of the conjunctiva and did not affect the vision in any way. Implantation was performed under local anesthesia and took only a few minutes. Aside from a small subconjunctival hemorrhage and a temporary foreign body feeling, no postoperative problems occurred. The foreign body feeling is most likely attributed to the suture and might therefore be reduced by improvements, such as an implantation procedure without suturing, which would use an injector leading only to a very small hole in the conjunctiva.

The biocompatibility of the implant and the components was previously tested in several preclinical studies. There was no indication for any local or systemic toxic effects (unpublished data), and the biocompatibility was shown now to be very good also in humans. Main functional components of the system are dye-labeled Con A and dextran. Regarding toxicity, Con A is the most important compound. The biotoxicity of Con A for *in vivo* glucose sensing has been reviewed in detail by Ballerstadt and colleagues.[33] The main conclusion is that low doses of Con A used in glucose biosensors and

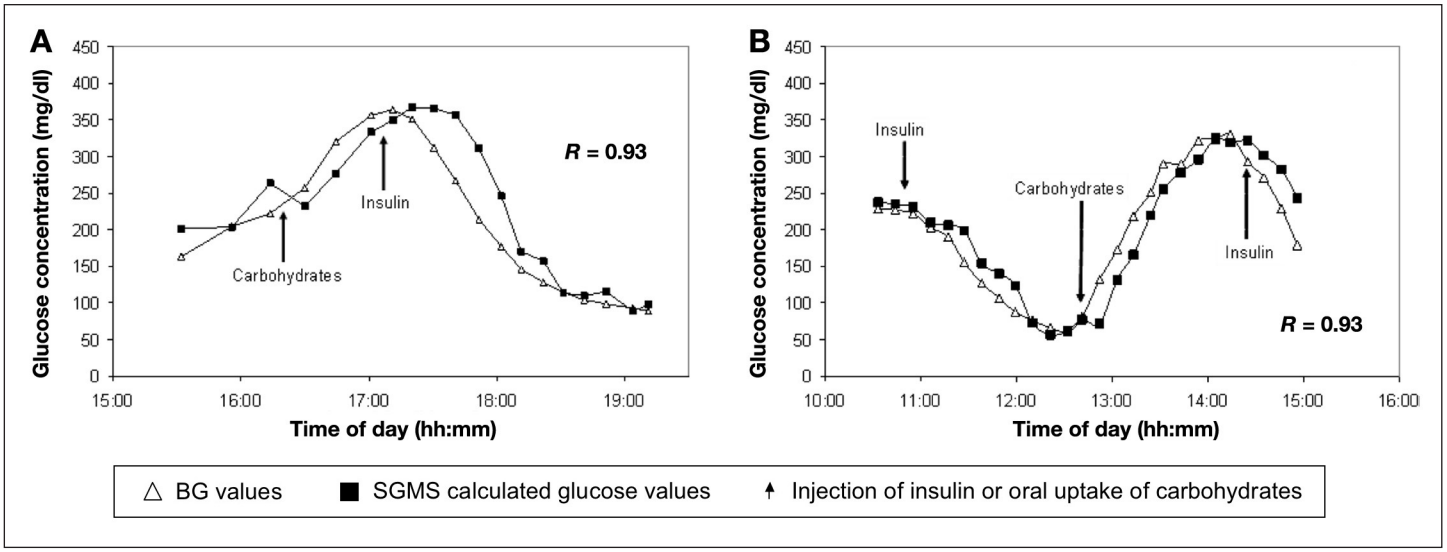

Figure 5. *In vivo* performance of the OMI from patient 5 at day 2 **(A)** and day 14 **(B)** after implantation. *R* denotes correlation coefficient between blood glucose and SGMS signal.

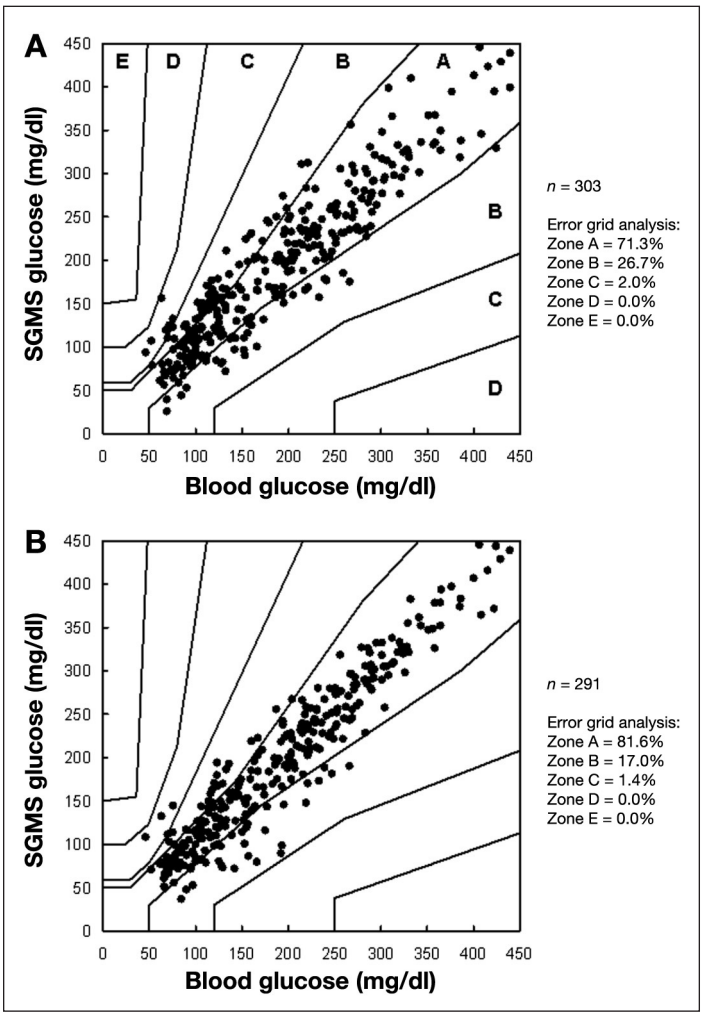

Figure 6. Consensus error grid plot of pooled data pairs of 12 measurement visits of five patients without **(A)** and with 10 min lag time compensation **(B)**.

administration of Con A in interstitial fluid are suitably safe. This also applies to the subconjunctival glucose sensor presented here.

In terms of glucose monitoring results, the study revealed a good correlation between the glucose values predicted by the SGMS and those obtained by the reference method. No decrease in glucose responsiveness was observable during the study duration of 2 weeks. Together with the very good *in vitro* stability shown in **Figure 2,** with only 20% decrease in response during 1 year, the new SGMS clearly shows the opportunity for minimally invasive, long-term glucose monitoring with implantation periods of up to 1 year or longer.

A short lag time of 5 to 10 min was detected between BG and interstitial glucose changes, which and is well within the range published for interstitial fluid glucose measurements.[34] A consensus error grid analysis of the data pairs showed that 71% of the data waswere in zone A, and 98% of all data pairs were in the clinically acceptable zones A and B. The positioning of the hand-held photometer in front of the patient's eye revealed to be crucial to obtain reproducible readings. With the guidance provided by the photometer in this study, it was still possible to hold the photometer at different angles and distances in front of the eye and get valid readings. This variance in the positioning led to a decrease in the accuracy of the glucose prediction. As the measurement is self-performed by the patients, appropriate guidance measures for precise positioning of the photometer in front of the eye will likely lead to more precise readings

and hence a higher percentage of data pairs in zone A of the error grid.

Further clinical studies with optimized guiding methods for the photometer, a higher number of patients, and longer observation periods are underway.

Conclusions

We evaluated the feasibility of a new glucose monitoring system comprising a subconjunctival OMI and a prototype hand-held fluorescence photometer for minimally invasive glucose monitoring in a first human clinical study with five diabetes patients. The sensor response was studied under highly dynamic BG changes induced by oral intake of carbohydrates or subcutaneous injections of insulin and was well maintained over a study period of 14 days. The new SGMS exhibited an excellent correlation coefficient of 0.93. A total of 98% of all data pairs was in the clinically acceptable zones A and B of the consensus error grid. Overall, for the first time in a human clinical study, the new SGMS shows the opportunity for long-term, noninvasive glucose monitoring in the management of diabetes.

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Disclosures:

Achim J. Mueller, Peter Herbrechtsmeier, Monika Knuth, and Katharina S. Nikolaus are full-time employees of EyeSense GmbH and own stocks from EyeSense AG.

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