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A Quantitative Look Inside the Body: Minimally Invasive Infrared Analysis in Vivo

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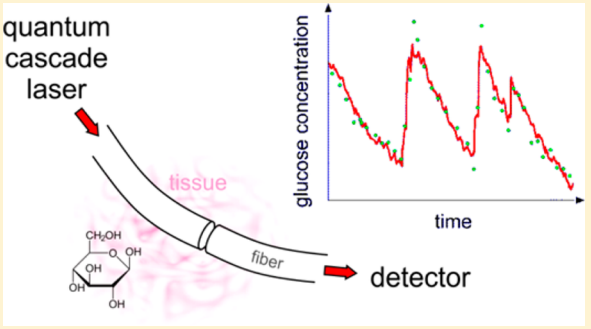
ABSTRACT: Today's minimally invasive biosensors are often based on chemical reagents and suffer from, e.g., oxygen dependence, toxic reaction products, excess analyte consumption, and/or degradation of the reagents. Here, we show the first successful analyte quantification by means of a minimally invasive sensor in vivo, which does not use chemical reactions. The concentration of glucose is determined continuously in vivo using transcutaneous, fiber-based mid-infrared laser spectroscopy. When comparing the infrared data measured in vivo with the 127 reference readings of glucose obtained in vitro, an overall standard deviation of 17.5% and a median of the absolute values of the relative deviations of 11.0% are achieved. The encouraging results open up the path toward a reagent-free long-term implant for the continuous surveillance of metabolites. In addition, the high sampling rate facilitates important research in body metabolism as well as its application outside the field of medicine such as real-time analyte sensing during fermentation.



While biosensors in general constitute a very broad field of rapidly increasing research, 85% of the world's biosensor market is dedicated to glucose detection.[1] The high and increasing prevalence of diabetes mellitus,[2,3] a disorder of the glucose metabolism, drives the large demand for glucose biosensors. Diabetes mellitus is directly linked to life-threatening situations as well as long-term complications such as retinopathy, nephropathy, neuropathy, and cardiovascular diseases. While recent metabolomics research addresses diabetes risk assessment,[4] it has long been known that for those diagnosed with diabetes the frequent determination of the concentration of glucose in blood facilitates proper therapy adjustment such as the dosage of insulin. *Continuous* glucose monitoring (CGM) has recently been shown to provide improved glycemic control[5−8] to an even greater extent. The combination of continuous glucose monitoring with automated insulin delivery systems may be considered as an artificial pancreas and promises a safe and efficient means of managing diabetes.[9−11]

Given the medical, social, and economic importance of diabetes,[3] this manuscript focuses on the example of glucose sensing. Most present-day CGM systems are applied transcutaneously in that a needle-type, reagent-loaded[12] sensor head with electrodes is inserted into the abdominal subcutaneous tissue. Small wires connect these electrodes to the read-out electronics which is placed on the outer skin. The concentration of glucose is not measured in the blood directly but in the interstitial fluid (ISF) of subcutaneous tissue, whereby the concentration of glucose in ISF is known to correlate with that of blood.

The accuracy and speed of any CGM system are essential for the prospective reliability of an artificial pancreas. However, the CGM devices currently available on the market still partly lack accuracy, reliability, and/or speed. One of the reasons for this is the working principle: Since the sensor chemistry[12] reacts with and thereby consumes glucose (mostly in forms of the glucose oxidase-based enzymatic digestion of glucose), there will be a local depletion of glucose in the immediate vicinity of the sensor. In this sense, any sensor involving a chemical reaction of glucose is deemed to be a measure for the efficient replenishment and, thus, diffusion of glucose, rather than concentration.

A nondestructive, i.e., reagent-free detection scheme fundamentally avoids this issue. Experiments have shown that the spectroscopy of molecular vibrations, and in particular mid-infrared (MIR) spectroscopy, is well suited to the reagent-free quantification of glucose owing to its sensitivity and specificity:[13−19] MIR spectroscopy measures the change in molecular dipole moment during the molecule's vibration, and it is therefore more sensitive than near-infrared spectroscopy, which assesses overtones and combination bands. The excellent signal-to-noise ratio of MIR spectroscopy outperforms classical

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Raman spectroscopy, too, whereby the latter may also suffer from background fluorescence, especially in tissue. The invention[20] and commercial availability of the quantum cascade lasers (QCL) fuels the recent renaissance of MIR spectroscopy. Using a mid-infrared quantum cascade laser[20] and a dedicated optical fiber sensor head in vitro, we were able[21−23] to reduce the noise-limited detection of glucose (noise equivalent concentration) to below 1 mg/dL (i.e., less than 1% of the typical concentration of glucose of 100 mg/dL) at an integration time as short as 4 s.

EXPERIMENTAL METHODS

For the measurements reported here, we use the setup described by Vrančić et al.:[21] Infrared radiation at a glucose absorption band (wavelength 9.7 μm) is emitted from a pulsed, nontunable QCL (pulse frequency 300 kHz, pulse length 50 ns, average power 1 mW; Alpes Laser S.A., Neuchâtel, Switzerland), sent through a silver/silver halide fiber sensor head (see also ref 24), and detected by a pyroelectric detector via a lock-in technique. A 20 μm gap between two fiber ends within the fiber sensor head acts as a miniaturized transmission cavity filled with the tissue's ISF. The fibers' small lateral dimension (~500 μm diameter) enables the cell to be filled with the glucose-containing interstitial fluid inside the tissue purely by diffusion. At the same time, the small thickness of the gap allows for sufficiently many photons to be transmitted through the cell.

Given the relatively small glucose-specific changes in infrared absorption, a simple linear relationship between the concentration of glucose and the transmission through the fiber sensor head's gap is permissible in order to calibrate the detector signal.[23] In addition to this relationship, a linear drift in time is accepted when calibrating the data.

Infrared transmission signals are detected in rats in vivo while the rats' blood glucose concentration was deliberately altered via the injection of glucose or insulin. One fiber sensor head is implanted into the neck of each anesthetized Sprague−Dawley rat (permission No. 35-9185.81/G-66/09 issued by Regierungspräsidium Karlsruhe, Germany). Midazolam/medetomidine/fentanyl (MMF, experiments #1 and #3) or ketamine (experiment #2) is chosen for anesthesia via intraperitoneal injection. Glucose or insulin is administered intravenously through the femoral vein in order to increase or decrease the blood glucose level, respectively. Following each dose of glucose or insulin, the catheter is flushed with 0.15 mL of NaCl solution. Tail vein blood sampling is employed for the reference measurements of glucose concentrations with a commercial, test strip-based blood glucose meter (AccuChek Mobile, Roche Diagnostics GmbH, Germany). The system meets the ISO 15197 accuracy goals. Clinical evaluation studies (see, for example, ref 25) have shown that the meter yields reproducible readings with an imprecision of less than 5% (coefficient of variation), which suffices for the purposes of the measurements presented in this manuscript.

Note that no precautions (such as a membrane cover) had been taken against any occlusion of the transmission cell. One analogous measurement and two similar measurements (coated fibers instead of uncoated fibers, see Results and Discussion section) were therefore excluded from further analysis if, upon implantation into rats, the gap turned out to be filled with blood or cells rather than ISF.

Furthermore, the acute physiological response to sensor implantation may result in large signal drifts in electrochemical sensing as well as, obviously, in infrared-based sensing. We therefore follow the data evaluation procedures of electrochemical sensing in that we disregard the early phase after implantation as the so-called "run-in time" and, hence, exclude the first hour of data from the calibration procedure.

RESULTS AND DISCUSSION

In the experiments evaluated, the retrospectively calibrated detector signal closely follows the reference concentrations (Figure 1). The correlation between the reference concentrations in vitro and those glucose concentrations predicted by the reagent-free infrared sensor in vivo is illustrated in Figure 2

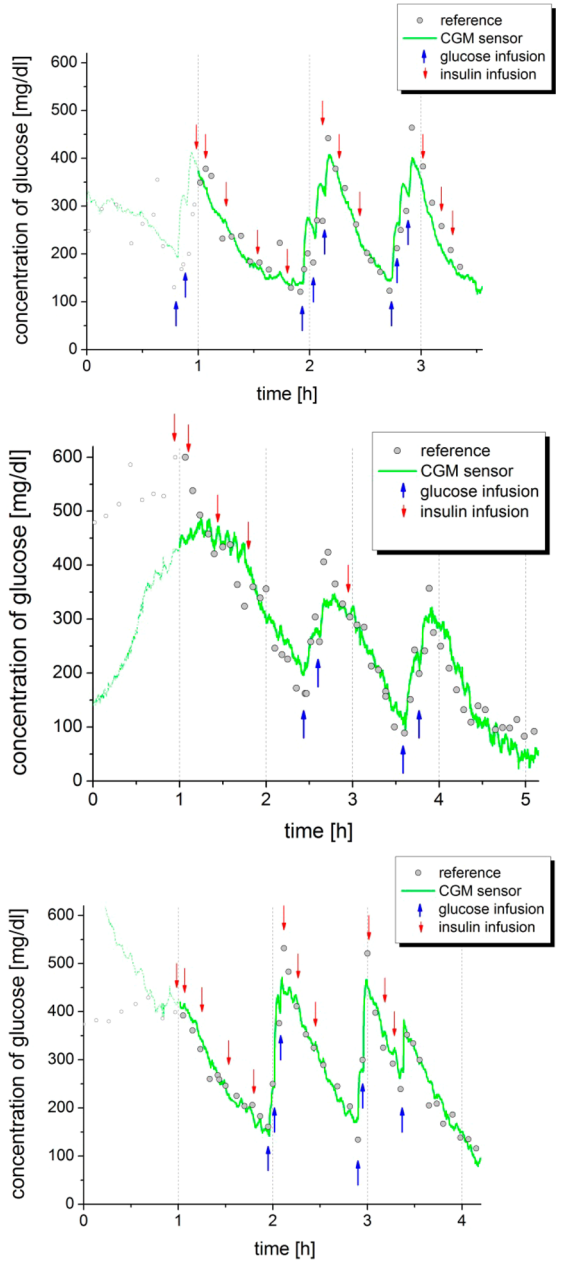

Figure 1. Concentration of glucose as a function of time as derived from the retrospectively calibrated sensor signal (green line) together with the test strip-based blood glucose reference measurements (dots) for the three different rats. Time points of administering glucose or insulin are indicated by the upward blue or downward red arrows, respectively.

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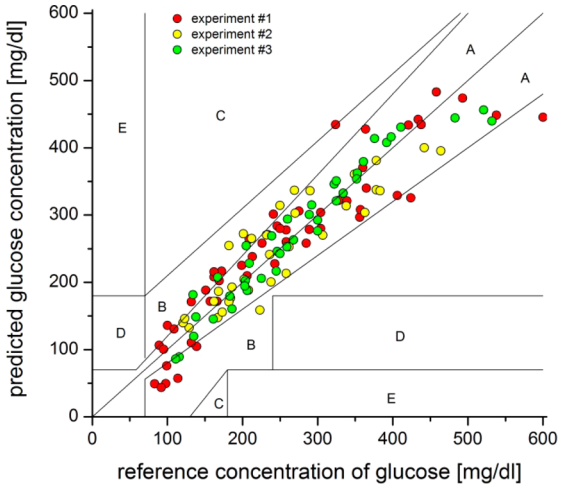

Figure 2. Comparison between the concentration of glucose as predicted by the in vivo infrared analysis of interstitial fluid and the reference concentration of blood glucose obtained from a test strip measurement in vitro at the same point in time. The domains labeled "A" to "E" refer to regions of different medical relevance, whereby values in regions "A" and "B" are considered acceptable from a medical standpoint.[26]

by the so-called Clarke Error Grid.[26] 99 out of the 127 data pairs fall into the region "A" and 27 are in region "B". These regions represent those regimes which are medically acceptable in that a moderate deviation from the most accurate value would not lead to a patient receiving the wrong treatment. One of the data pairs falls right onto the borderline between regions "B" and "C".

The square of Pearson's coefficient of correlation amounts to $R^2 = 0.875$. A standard deviation of 17.5% can be derived from the data. (No confidence levels are given since the deviations do not follow a Gaussian distribution according to Wilk-Shapiro testing ($p = 0.005$).) It is common in the field of continuous glucose monitoring to enumerate the median or mean of the absolute values of the relative deviations (ARD), which amount to 11.0% or 13.4%, respectively. These values are similar to the results obtained by in vivo studies which use the most up-to-date electrochemical sensors, whereby the DexCom G4 version A, the Abbott Diabetes Care Freestyle Navigator I, or the Medtronic Paradigm Enlite achieve mean ARDs of 20.5%, 16.5%, and 16.4%, respectively.[27]

The retrospective calibration reveals a drift of the QCL-based sensors of (22 ± 6), (-7 ± 3), and (5 ± 2) $\mu g\ dL^{-1}\ s^{-1}$ for experiments #1, #2, and #3, respectively. These results are concordant with our previous in vitro analysis[21] from which the drift was estimated to be on the order of ± 15 $\mu g\ dL^{-1}\ s^{-1}$. While the above results may serve as a demonstration of the principle for our intriguingly simple single-wavelength setup, precautions against this drift need to be taken in future experiments. One possibility is to use one or more fixed-frequency QCL(s) at wavelengths outside the glucose absorption band. Should mechanically moving parts be permissible, tunable QCLs could provide a further solution. Given the simplicity of the variations of ISF's mid-infrared spectral signatures (mainly albumin and glucose)[28] and the small number of noticeable interfering agents,[21] we speculate that only a rather limited number of wavelengths will be required.

In electrochemical sensing, the signal-to-noise level is balanced against the sampling rate so that these sensors usually provide reasonable performance, if independent data points are provided on time scales of between 1 and 15 min. In contrast, the reagent-free sensor reported here samples independent data points every 6 s, which corresponds to an improvement of 1 order of magnitude in speed with respect to even the fastest electrochemical sensors. In turn, increasing the integration time to 60 s does not provide any significant improvement in our sensors' accuracy. The signal-to-noise ratio of the reagent-free sensor at 6 s sampling time can be used to enumerate the average noise-equivalent concentration to 7 mg/dL.

The high time resolution provides an almost immediate insight into the metabolism. Very fast changes in glucose concentration can therefore be detected continuously. The data shown in Figure 1 exhibits changes in reference glucose concentration which exceed 30 mg/dL/min and which are nonetheless readily tracked by our CGM sensor. On the contrary, electrochemical sensors are usually limited to detecting rates of change in glucose concentrations of less than 3 mg/dL/min. While, admittedly, physiological changes in humans hardly exceed 3 mg/dL/min, a severe technical limitation of the detectable rates (i.e., of the bandwidth of the measurement) may lead to false readings of amplitude and phase of the measurement signal, which in this case translates to inaccurate glucose readings and a technical lag time, respectively.

On the other hand, a physiological lag time is expected between the concentration of glucose in interstitial fluid and in blood.[29,30] While no such lag time was taken into account in the analysis of our experiments described up to this point, it is interesting to examine the role of a lag time in light of the high time resolution of the infrared sensor. The data in Figure 1 shows a surprisingly fast tissue response to the intravenous injection of glucose (note that, in practice, the time difference between the infrared measurement and the tail blood sampling was well below 1 min). Indeed, when adding an artificial lag time to the sensor signal (in silico) the correlation between interstitial fluid glucose and blood glucose does not improve. We find that the correlation deteriorates so that, for example, lag times of 2, 5, or 10 min lead to a decrease of the squared correlation coefficient from 0.875 to 0.782, 0.637, and 0.504 and to an increase of the median ARD from 11.0% to 11.8%, 15.1%, or 17.9%, respectively. Hence, our reagent-free biosensor supports suggestions by, for example, Davey et al.[31] and Voskanyan et al.[32] that electrochemical sensors feature technical lag times and/or bandwidth limitations which are long enough to substantially contribute to the detected overall lag times. Thus, we hypothesize that seemingly "physiological" lag times in some published research could have been over-estimated in those cases in which the measurement is based on a glucose-consuming, electrochemical reaction.

A retrospective analysis of all the data is used to derive the results so far. However, a prospective analysis on the basis of a single-point calibration would be ideal for real-time application. Even in the absence of slow drifts, electrochemical sensors require at least two measurements to be made in order to adjust for enzyme activity or an offset in current. In turn, if no drift is present, the purely physics-based approach in reagent-free infrared sensing allows for a true single-point calibration since all impact parameters such as laser power, coupling efficiency, detector efficiency, or gap width, impact both the signal background (caused by the absorption of water, $c_{glu} = 0$) and

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the signal ($c_{glu} \neq 0$) in exactly the same way. If, for example, the first pair of normoglycemic glucose readings and drift-corrected detector signals past the run-in time is being used for a single-point calibration, the median ARD only increases to 14.2% as compared to 11.0% for the retrospective calibration.

CONCLUSION

In the research described in this manuscript, mid-infrared spectroscopy using a quantum cascade laser and a miniaturized fiber sensor head thus, for the first time, enabled the minimally invasive quantification of the concentration of glucose in interstitial fluid in vivo without using reagents. On the basis of these findings, there are three key topics that need to be addressed by future experiments: one is the addition of a membrane in order to present the penetration of cells into the gap. Second, the compensation of the sensor drift needs to addressed, e.g., by using a second laser with a different wavelength as a reference and/or a tunable quantum cascade laser. The third topic is the quest for the fiber's biocompatibility. It is known that silver/silver halide fibers are toxic in nature, which in our case does not play a major role owing to the limited duration of the in vivo experiment. However, we have recently performed experiments aiming at a passivation of the fiber by means of a polymer coating. Initial investigations with cell cultures as well as in vivo toxicity testing indicate that biotoxicity can be avoided with these coatings. Further material research also needs to address concerns with respect to protein adsorption to the fiber tips ("protein fouling").

Beyond the continuous glucose monitoring with transcutaneous MIR fibers, the approach appears to be well suited to fully implanted glucose sensing owing to its reagent-free nature, i.e., the absence of enzyme degradation or toxic chemical reaction products. A fully implantable long-term sensor in combination with an insulin pump would open up the path toward an artificial pancreas.

Meanwhile, the results reported here are available for use in biotechnology. The monitoring of metabolites during fermentation constitutes an application which appears to be particularly suited to fiber-based surveillance using MIR laser spectroscopy. It is the high sample rate of this technique in particular, which has been demonstrated in this paper in a rather harsh biological environment and which will be of particular interest for an active feedback loop during in-line control of the production processes in biotechnology. The method's large bandwidth, i.e., the capability to detect large concentration changes within short times, supports this path.

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All authors have given approval to the final version of the manuscript.

Notes

The authors declare the following competing financial interest(s): In addition to his affiliation with the Kirchhoff-Institute for Physics, W. Petrich is employed by Roche Diagnostics GmbH, Mannheim, Germany.

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